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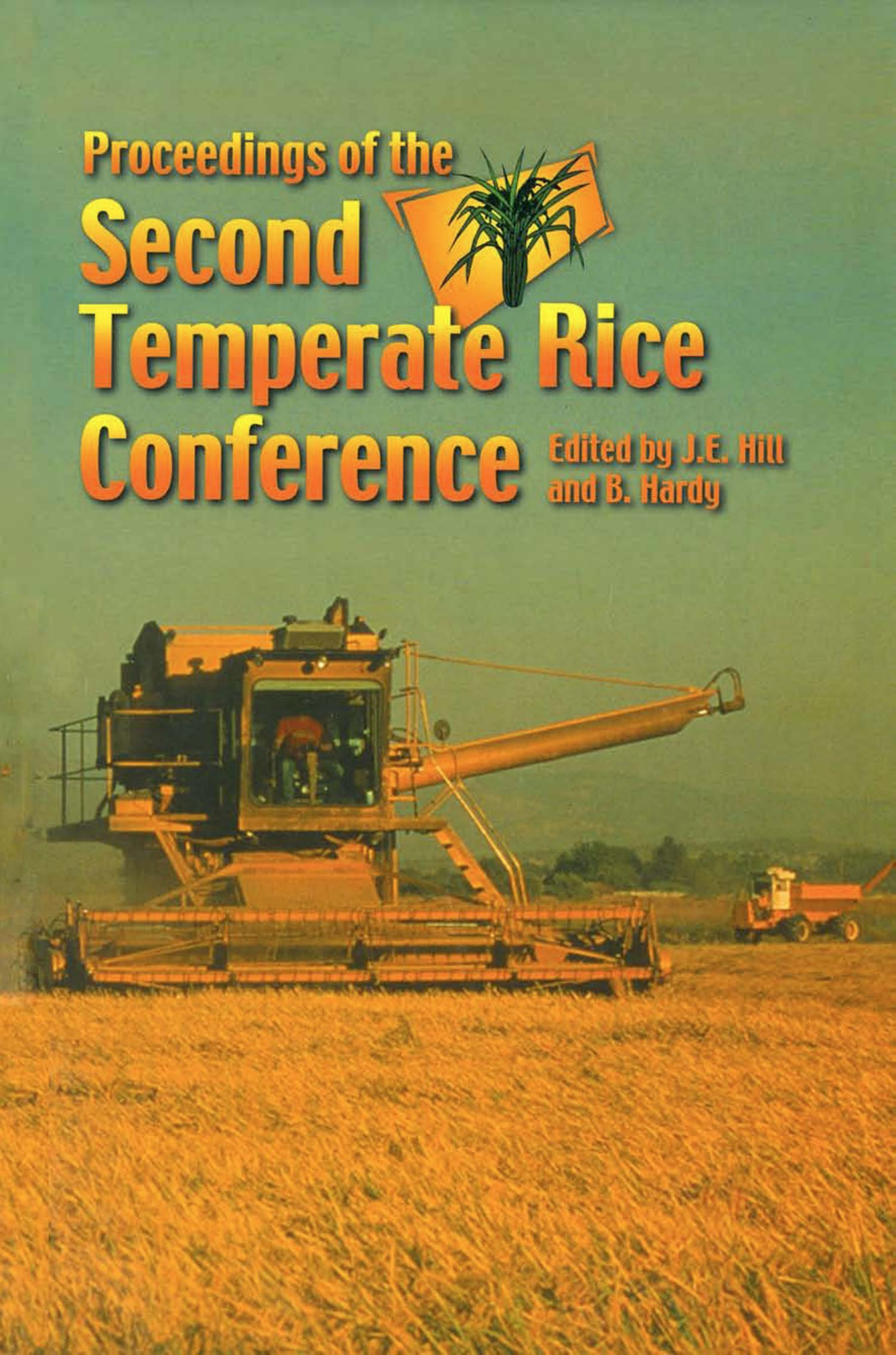

RICE BIOTECHNOLOGY

# Public germplasm development at a new crossroads: biotechnology and intellectual property

P.G. Lemaux

> "The greatest service which can be rendered to any country is to add a useful plant to its culture."
>
> Thomas Jefferson

Historically, federal and state monies have been used to produce new cultivars of many crop species through classical breeding techniques. The public-sector varieties that arose from these efforts were moved easily to commercial fields, often with no practical need or thought for legal protection. With the advent of more costly genetic practices used to create new varieties, including biotechnology, this paradigm is shifting. This is because, although the potential payoff for these technologies is substantial, up-front development costs are high. For companies and organizations to recoup these high costs, intellectual property protection for key elements and technologies is sought well before products enter the field. This early protection strategy has led to situations in which the requisite intellectual property rights needed to move products to the marketplace are not available to those who developed the new product. To be able to market new products, companies have made acquisitions and entered into consolidations to gain "freedom to operate" in practicing the new inventions. Often, control over protected technologies, genes, regulatory sequences, and germplasm is critical to success during the bargaining phases of the acquisition or merger. This trend toward trading key elements and technologies has practical implications for public-sector efforts to produce improved germplasm. Is the oft-used practice of not protecting publically developed varieties in the best interests of the farmer, the researcher, or the taxpayer? Companies that have the required freedom to operate in certain areas could obtain unprotected cultivars, developed with public money, make needed licensing agreements with other private entities holding pivotal "pieces," introduce new value-added genes, and then sell the improved germplasm at a premium back to the original developers of the varieties. If, on the other hand, the publically developed cultivars were protected through patents or plant variety protection, the scenario could be different. In this case, the company wanting to use a developed variety as the basis for a new cultivar would be obligated to negotiate with the original developers of the germplasm. This might necessitate that the company wishing to develop the new cultivar work more closely with the developers of existing and future germplasm. Concomitantly, the developers of the germplasm would have to be vigilant about protecting it and growers would have to be encouraged to use the protected germplasm over unprotected varieties. Without having legal control over key technologies and genes and/or germplasm that can be used as "bargaining chips" with pivotal industrial players, the commercial sector will either not "play the game" or will play the game with little input from the original germplasm developers. Public-sector agriculturalists must take an active role in investing in the development and protection of key elements of the new cultivars if they are to continue to participate in future germplasm development using biotechnology.

During the agricultural revolution, plants traveled with people as sources of food and began to adapt to new environments, which led to incredible diversity in germplasm. This genetic diversity has become, in the eyes of many, the world's most valuable raw material (Fowler 1994). As we enter the "age of biotechnology," in which genes, which form the basis for biodiversity, are also the basis for manipulating organisms, the fate of the human race is tied even more closely than ever to this biological diversity and its control. Questions of ownership and control over biological resources will be the basis for political and economic skirmishes and will influence how effectively this valuable resource is used and conserved.

The manner in which these issues are resolved will affect the way in which the benefits of diversity are shared. To appreciate fully how control mechanisms arose for biological resources, a brief consideration of plant domestication is informative.

## History of germplasm development

In the United States, seed collection and control emerged through the U.S. Department of Agriculture (USDA), which was charged with making agriculture more productive. Farmers played an active role in developing this genetic material to suit their own needs. They saved the seed for their own use and their neighbors' since there were no good commercial sources of seed (Juma 1989). Legal protection was not sought because it was difficult to enforce; unlike other inventions, such as the electric light bulb, plants were able to reproduce themselves!

The arrival of the train in the late 1800s changed the distribution of agricultural products since now they could be shipped to distant markets. In addition, the railroad served as an extension service, distributing improved crop varieties.

With the introduction of double-cross hybrids of maize in the 1930s, the role of farmers changed (Fitzgerald 1990). They were now suppliers of raw materials to commercial breeders, who were able to acquire control over the new seeds they created.

This was a paradigm shift in the relationship of farmers to their seed because now breeders maintained control over the inbreds, not the farmers. And hybrids did not breed true, so saving seed was not successful. This led to the concept of a proprietary product—this was *nature's own patent,* similar in many ways to a legal patent.

This situation was revolutionary. The use of genetics by commercial breeders to create desirable germplasm meant that breeders had tools to use that were not available to farmers. Farmers could continue to "fine-tune" germplasm for their particular uses, but the major advances and rapid progress would be within the research establishment. This seems comparable in many ways to the situation in which public germplasm breeders find themselves today with the advent of biotechnology, which is practiced in the laboratory away from the breeders' fields and is practiced with tools that are not available to breeders.

## Plant Patent Act of 1930

The advent of the hybrid offered germplasm protection for certain crops. The burgeoning nursery business, however, still relied on the discovery of chance mutations for new varieties of fruit trees, berry bushes, and roses, which were clonally propagated. Once sold, however, these varieties could be easily multiplied and sold by competitors. The Plant Patent Act of 1930 (PPA) was directed toward protecting this germplasm of vegetatively propagated plants in order to capture economic and legal control over a variety.

The PPA excluded seed-bearing plants, however, because Congress would not allow patents on foods. However, in the 1950s and '60s, breeding of crop species became more of a rational and scientific process and often competing seed companies would take varieties, developed by others through years of breeding, multiply them, and sell them at cut rates. The seed industry wanted protection because other forms of protection (hybrids, breeder/grower contracts) were not reliable.

Proving patentability of plant varieties seemed problematic in terms of patent law. The move toward finding a solution was aided by the corn blight of the late '60s and early '70s (Fowler 1994). A reason for its severity, put forward by the seed companies, was that the seed industry needed some protection for its "creations" in order to invest in creating varied germplasm.

## Plant Variety Protection Act of 1970

The formulation of the Plant Variety Protection Act, passed in 1970, permitted the protection of new varieties through the USDA (USDA Agricultural Marketing Service 1996). This avenue was thought to open up public breeding efforts to focus on issues such as the corn blight while companies pursued more lucrative areas. This was the genesis of the split between public and private efforts at germplasm improvement.

By 1980, the seed industry for certain crops was becoming big business and the biotechnology industry was entering the agricultural arena. In this industry, the ability to raise funds for research was tied directly to securing patent position and protection. At this point, agricultural companies were moving commodities internationally and they wanted protection for their commercial opportunities that went beyond the capabilities of the U.S. Congress or the USDA.

### Utility patent option

Some "players" sought utility patent protection; they asked the courts to reinterpret existing patent law to expand the applicability of those laws to living organisms. In the 1970s, General Electric fashioned a powerful test case on the patentability of life forms (Diamond v. Chakrabarty 447 US303 1980). The case was won in the Supreme Court by General Electric through the creation of an oil-eating bacterium, which was judged to be of human manufacture. In 1985, the *ex parte* Hibberd ruling confirmed that seeds, plants, and tissue culture materials could be patented (*ex parte* Hibberd, 227 U.S. Patent Quarterly 443, BPA1 1985). This set the stage for modern patent rights over biological organisms.

### Contrasts between PVP and utility patent protection

This time chronicle outlined what the options are for plant protection today: plant variety protection (PVP), which is mediated through the USDA PVP office, and utility patents, which are adjudicated through the courts. In both cases, the invention must be novel. PVP requires that the cultivar be distinctive; the distinctiveness can be satisfied if any trait, regardless of its importance, differs from that of all previous cultivars.

For utility patents, close "look-alike cultivars" do not generally afford patent protection for both cultivars, under the "doctrine of equivalents," which states that, if the product accomplishes the same thing and in substantially the same way, there is infringement. In this case, the first patented cultivar may exclude patent protection on the "look-alike cultivar." In addition, if the invention is predictable to one "skilled in the art," it will not be patentable.

Another difference between the two forms of protection has to do with the extent of coverage. Under PVP, the seed and plant of a cultivar are protected; with utility patent protection, a broader array of tangibles can be protected, such as seeds, whole plants, plant parts, cultivars, hybrids, genes or physical traits, methods of plant regeneration, and other biotechnological processes and products.

Another significant difference is that PVP has farmers' exemptions and research exemptions for creating new commercial cultivars, whereas utility patents have neither of these exemptions. Research exemptions for noncommercial purposes are available for both.

In 1994, there was an amendment to the PVP, called PVP(a). This extends the coverage of the protection to cultivars that are "essentially derived" from the original protected variety. This includes genetically engineered traits as long as the fundamental variety itself is not substantially changed. Certificates prior to 1994 are not "grandfathered" in under PVP(a). So, a variety protected before 1994 could be used by another entity and changed through genetic engineering but could not be prosecuted by the breeder. With post-1994 varieties, this could not be done without violating the breeder's PVP certificate; however, the entity making the change in the PVP variety could obtain a patent on the new variety. It is important to note that, to date, this has not been challenged in the courts.

### Examples of protection in the marketplace

How would this type of legal protection play out in the marketplace? For example, what if a rice variety, which was developed in the state of California, was used by a biotechnology company to insert a new gene for herbicide tolerance. Does the new herbicide-tolerant variety belong solely to the company that inserts the gene? Or does the organization that developed the original variety have some rights over the germplasm?

In this example, public-sector breeders have spent years developing the new rice variety with traits that are desirable for growers. Conversely, another industry has spent years and millions of dollars developing the tools to make fine-tuned changes using genetic engineering. Obviously, both components, the original germplasm and the potential utility of the new technologies, are important; either component by itself is less useful.

The question is, Who has rights over the new variety with the engineered trait? It depends on the method by which the original germplasm was protected. If the cultivar were protected by PVP, the addition of a new trait through genetic engineering would create a new variety for which new protection could be sought under PVP(a).

The unanswered question of law is whether the new plant variety made by inserting, for example, a herbicide tolerance gene into a PVP(a)-protected variety would infringe the original PVP. This issue of an "essentially derived" variety is an attempt by Congress to expand PVP protection to PVP varieties that already exist, but the extent of the legal protection afforded by this expansion has not yet been tested in the courts.

If the original plant variety was not protected with PVP, then there is little legal recourse, although clearly in many cases considerable resources have been invested in developing the cultivars. It is probably not in the best long-term interests of the company adding the new traits to use the original cultivar without reaching some "gentleman's agreement" with the holders of the original variety, but legally they can do so.

If, on the other hand, the original cultivar was protected using a utility patent, something not done frequently in public-sector germplasm development, it would be an infringement for a company to change the cultivar through genetic engineering; a license to change the original invention would be needed. Even if the new trait is being added to the utility-patented cultivar, the result is the same since patented new improvements are often subordinate to a dominating patent.

The bottom line is that protection afforded by a utility patent is much broader and safer than that afforded by PVP, but it is also much more costly. In the U.S., the number of plant patents, plant variety protection certificates, and utility patents has increased dramatically in the last 25 years. For example, the number of PVPs issued for new varieties of field crops, grasses, and vegetables climbed from 153 in the early 1970s to 992 in the early 1990s. By the end of 1994, 286 utility patents had been issued for plants or plant parts (Barton and Siebeck 1994). The trend in the biotechnology arena is clearly toward utility patents because of the high cost of product development.

A different approach to sharing in the "fruits" of the improvement between the cultivar developers and "trait introducers" is to transfer the ability to replicate or modify the cultivar, subject to a contract that restricts what the recipient can do with the cultivar unless the cultivar developers are involved and potentially benefit from the changes. This can be in the form of a material transfer agreement. Another key element in genetic engineering is the gene. The U.S. Patent and Trademark Office received 4,000 patent requests for the nucleic acid sequences making up genes in 1991, and 500,000 in 1996 (Enriquez 1998). As a legal agreement, the licensee can be expressly prohibited from altering the cultivar through any means unless agreement is reached with the developer of the cultivar. This agreement, however, is only as good as the licensee's compliance with it since it is very difficult to enforce the ban as it is easy for plant material to change hands.

## Summary

In summary, the public sector has been an important player in the development of new plant varieties. The varieties so developed were moved to the commercial field with little thought for legal protection until the 1970s. However, the advent of biotechnology has resulted in methods that require substantial up-front investment. This has led to increasing intellectual property protection of the key elements necessary for product development so that companies can recoup the substantial costs of product development.

For key product development elements, this protection is sought long before products enter the field to ensure that the requisite intellectual property rights needed to "practice the invention" are available at the time of commercialization. This practice is unprecedented in the agricultural sector and has led to a new paradigm for production agriculture.

It will be difficult for individual commodity groups to capture the tools needed to practice biotechnological inventions. Producers of commodities will need to work with public- and private-sector researchers to move these technologies into their cultivars. Commodity groups also need to protect the cultivars they develop so that they will have bargaining power in making trades with the requisite players in biotechnology.

In the short term, companies have many "vendors" from which to obtain germplasm and therefore some "vendors" will be involved in developing the first genetically engineered cultivars and some will not. In the long term, however, biotechnological inventions must interact with the providers of germplasm on a large scale. The public sector can be involved in providing elite lines into which private-sector breeders can introgress their new traits. In addition, public-sector efforts might gain the freedom to operate to focus on traits from which the private sector will realize insufficient financial gain. These traits could then be "piggy-backed" with more lucrative traits that are pursued in the private sector.

If this path is not followed, the genetic diversity of the germplasm used by the private sector and its suitability for certain environmental niches will be compromised. This does not seem like a good strategic move for agriculture. The corn blight of the late 1960s was a dramatic demonstration of this! A working partnership between the two sectors, in which both parties gain something from the partnership, seems to be the most rational and prudent strategy.

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## Notes

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RICE BIOTECHNOLOGY

# Biotechnology and rice production: providing choices

K.S. Fischer

The International Rice Research Institute (IRRI) and the national agricultural research systems (NARS) have served the rice-growing community for almost 40 years as providers of international public goods of benefit to rice farmers and consumers worldwide. Their success is in the discovery and use of the most modern science to develop, and freely deliver, a diverse array of high-yielding rice varieties to small rice growers. The impact of their released varieties, in terms of area covered and sustained food for the poor, is unparalleled.

In the next 40 years, Asia will need much more rice than what is produced today. There will be less land for rice cultivation and thus a need to increase yields and the intensity of rice production in both irrigated and less favorable rainfed systems. At the same time, the globalization of the rice market will create a greater demand for improved grain quality and new food products for rice in the largely urbanized population. The large multinational private sector plans to enter this growing market and contribute to satisfying some of these demands.

The public-sector rice science community has fostered a tradition of sharing its improved rice germplasm and cultivars freely with both the private and public sector. To date, the private sector has played only a small role in the development and delivery of rice technology (in the tropics). The rapid advancement in modern science (biotechnology) and the overwhelmingly large investment by the private sector in it mean that the balance of rice research between the public and private sector will change. The private sector can bring to rice farmers the added value of its investment in "traits" and its knowledge of the seed business. For this, the private sector requires protection of its intellectual property (IP). The public-goods international (and national) research centers must evolve, yet maintain their essential role in developing and sharing diverse germplasm for sustainable production. The public sector must also continue to develop varieties for the heterogeneous, less favorable environments where most of the poor farm. To do so, the NARS and international rice research centers such as IRRI require the "freedom to operate" to use the most modern science.

This paper proposes several options that IRRI is exploring to gain access to modern science to provide a choice of improved germplasm to NARS (and ultimately to farmers). One option will explore a research and commercialization arrangement that builds on the strengths of IRRI, the NARS, and the private sector. Another case study examines a consortium approach to license a "biotech toolkit" for specific constraints of a noncommercial nature, and a third examines IRRI's role in functional genomics to ensure the freedom to operate in rice gene discovery. All examples are explorative and will require substantive discussions with all partners and approval by the IRRI Board of Trustees before becoming operational. These three options are not the only possibilities; other options will need to be added and explored as all parties seek ways to use the new science to the benefit of all rice farmers in the developing countries.

Varietal improvement occupies center stage in rice research programs in all public national and international rice research systems. The success of the varietal improvement program has depended on a culture of free exchange of germplasm among scientists. Since 1975, the International Network for Genetic Evaluation of Rice (INGER), formerly the International Rice Testing Program (IRTP), has facilitated the safe, global exchange of rice germplasm. INGER has facilitated the global exchange and evaluation of more than 42,000 breeding lines and varieties since 1975. More than 5,300 unique breeding lines and varieties from INGER have been used in national breeding programs to improve local varieties. Out of this shared germplasm, 423 entries from around 50 countries and from the International Center for Tropical Agriculture (CIAT), International Institute of Tropical Agriculture (IITA), and IRRI have been used directly as 625 varieties in 66 countries.

Evenson and Gollin (1997) studied the release of indica and japonica varieties from 1962 to 1991. They found that, although IRRI is an important producer of the crosses from which releases subsequently were made, 75% of the varieties released resulted from material exchanged among rice scientists in the INGER nursery. In 1994, R. Evenson (personal communication) estimated that each released variety contributes US$2.5 million annually to the global economy. Therefore, the annual contribution to the world economy of the 423 modern varieties released worldwide through the free exchange among the INGER members is $1.05 billion per year. This figure does not include the value of more than 1,200 varieties used for national breeding programs from INGER parental lines.

In addition to these economic benefits, the free flow of germplasm has increased the level of diversity in modern cultivars. For example, only three varieties released before 1965 had more than four ancestors. Two hundred and twenty-two varieties released through INGER after 1976 can be traced to five or more ancestors and 75 varieties have more than 15 ancestors. The origin of 1,709 modern rice varieties in Asia can be traced to 11,592 cultivars (Evenson and Gollin 1997).

Thus, maintaining a system for the free and safe exchange of diverse genetic materials from different NARS, and giving the national systems a choice of germplasm from which to select appropriate material, has served rice farmers well. It remains an important goal for IRRI.

IRRI's Policy on Intellectual Property Rights (IPR) guides our use of germplasm and our emerging role with the private sector. For germplasm held in the genebank, IRRI adheres to the Convention on Biological Diversity and our policy states:

*"IRRI adheres to the principle of unrestricted availability to the rice genetic resources it holds in trust, including related information"* and *"IRRI will not protect the rice genetic resources it holds in trust by any form of intellectual property protection."*

IRRI shares materials in the germplasm bank under a material transfer agreement (MTA) endorsed by the chairman of the Consultative Group on International Agricultural Research (CGIAR) (see Appendix 1).

For improved rice germplasm, the essential statements in the IPR policy are:

- *"All breeding materials, elite germplasm, and parental lines of hybrid rice that are derived from conventional breeding will be made freely available."*
- *"IRRI will provide breeding lines, elite germplasm, and parental lines of hybrid rice to both public and private sectors on the understanding that: (a) the material is not intended for exclusive use by any single organization; (b) IRRI retains the right to distribute the same material to other organizations; (c) the use of IRRI materials will be publicly recognized when a derived variety or hybrid is released."*

Currently, IRRI uses an MTA agreement for inbred lines for hybrids (A, B, and restorer lines) for the private sector (see Appendix 2). All other materials are shared freely on request.

The current IPR policy for inventions and material derived from biotechnology states:

- *"In negotiating collaborative arrangements for the development of products and technologies derived from biotechnology, IRRI will seek to ensure free access to the products of the research."*
- *"To make advanced biological technologies and techniques available to developing nations, IRRI may, but only to the extent necessary, and for a limited period, accept limitations on distribution of the derived and associated materials."*
- *"To ensure availability to developing nations of advanced biological technologies or biological materials such as microbial strains, IRRI will, exceptionally, apply for intellectual property protection of the technologies or materials or provide them to a collaborator on a restricted basis, but only after a specific judgment that such arrangements best serve IRRI's client—developing nations' farmers."*
- *"In obtaining and exercising any form of intellectual property rights over biological material, IRRI will seek in good faith to notify and consult with the nation or nations from which the material came."*

Clearly, these very broad guidelines do not address specific applications in a changing world of intellectual property (IP) and the full impact of the emerging role of the private sector in rice science in Asia. In addition, a recent external review of IRRI pointed out that some aspects of our policies could make IRRI lose its competitive advantage as a research institution. The review noted that much of IRRI's IP is potentially valuable as collateral for exchange with others who possess patented technology that we need to fulfill our public mission.

IRRI is currently reviewing its policy on IP and on partnership with the private sector. The following section addresses some of the ideas and concepts that are being developed for discussion with our stakeholders to develop appropriate policies and guidelines.

## The changing environment

Recent advances made in hybrid rice technology (in the tropics) and in biotechnology and the emergence of multinational companies with interest in seed, physical inputs, and knowledge systems have resulted in increased commercial interest in rice.

John Barton (personal communication, 1999) notes three factors that affect the rate of involvement of the private sector in rice. The first is the potential growth of a substantial seed market through more privatization of national seed industries. The second is that biotechnologies, which are already being used in other crops and markets (i.e., *Bt,* herbicide resistance), are becoming relevant for rice. The third is the change that must take place in the legal environment, including seed legislation and intellectual property. Of these, the most problematic for the use of biotechnology by the private sector is the legal environment. Most nations have agreed to strengthen their IP systems to comply with the Trade-Related Aspects of Intellectual Property Rights Agreement (TRIPs) by 2000. However, there will be considerable variation in the system that each country adopts as law, with varying degrees of protection for the private sector interested in transgenic crops. It seems that most Asian countries will develop plant variety protection (PVP) that maintains farmers' and breeders' rights and this will not provide protection for IP of genes. Also, many countries' patent laws may not allow protection of varieties or plants (and in some cases any life form), which again may affect the use of IP in some cases.

The "traits" that are now available or are potentially available for use in rice are considerable (Table 1). They can be grouped into those traits that increase the efficiency of the inputs into rice production and lower costs and those that modify the grain (output) to increase value and profits.

With the considerable interest of the private sector in rice in Asia, the public sector needs to evolve and develop appropriate strategies that complement rather than compete with the private sector, and yet maintain the freedom of choice of improved cultivars for rice farmers. But not all sectors of the rice market will be able to pay for the new technology. IRRI and the public sector need to ensure that all farmers have access to the benefits of new technology. Where appropriate, that may require a patent to protect our own IP; in other cases, it may mean that "we seek IP in order to gain

**Table 1. Traits available in rice that affect the production of the crop (input traits) and the quality and use of the product (output traits).**

| Input traits | Output traits |
|---|---|
| Herbicide tolerance | By-products |
| Insect resistance | Nutritional quality (Zn, Fe, vitamin A) |
| Virus resistance | Cooking quality |
| Disease resistance | Amylose content |
| Salt tolerance | Aroma |
| Drought tolerance | |
| Submergence tolerance | |
| Yield processes | |

access to technology from others." IRRI and the public sector need to choose carefully the area of research for which biotech tools are required. Where necessary, IRRI needs to seek collaboration with the owners of the technology.

## Targets for IRRI biotechnology research

In today's popular discussion of biotechnology, it is easy to overlook the important contribution already made by the enabling techniques of biotechnology. The use of embryo rescue has broadened the rice gene pool with the application of genes from wild rice now in commercial cultivars. In addition, genes for a large array of characters have been mapped and marker-aided selection for some is now being practiced by NARS. Also, genes have been pyramided for resistance to bacterial blight (e.g., *Xa4, Xa21, xa5,* and *xa13*) and for blast (*Pi1, Pi2, Pi4,* and *Pi3*) and germplasm with these genes is now being shared with NARS.

In view of the uncertainty that remains over the widespread use of transgenic rice, IRRI has selected strategic targets for genetic modification as determined by constraints at the farm level and the lack of progress from conventional approaches.

Over the past three decades, considerable change has taken place in the way rice is being cultivated. And to meet the new demand for rice in the next 20–40 years, more change must take place. Our targets for biotechnology must be in light of these changes that we associate with (1) a shift in importance of rice pests—disease, insects, and weeds; (2) changes in inputs (particularly the supply of water); and (3) increased productivity of rainfed rice. These targets have been documented elsewhere (see IRRI 1999b).

A more recent report by Savary et al (2000) provides more focus with respect to pests. Their studies are based on a unique set of field data (pest injuries determined in more than 800 farmers' fields in five countries in Asia) that demonstrate the loss from profiles of pest injury in various production situations. Their study indicated a total loss of 37% caused by pests over all production situations. Of this total, the major pest is weeds, accounting for more than 20% of the losses. Although there are interactions among the various pests in any production situation, Savary et al (2000) estimate the maximum individual losses of the other pests as sheath blight, 6%; blast, 5%; brown spot, 5%; whiteheads, 3.2%; and deadhearts, 0.1%.

However, Savary et al (2000) note that the methodology did not account for potential yield losses from the breakdown of resistance in currently grown cultivars, thus underscoring the role of "maintenance" breeding for such pests as bacterial blight, blast, and planthoppers. They also noted that such region-wide analysis underscores the devastating effect of local and more sporadic diseases such as viruses (i.e., tungro).

Of these major pests, the role and need for biotech approaches can be summarized as follows:

*Sheath blight.* This disease will increase with further intensification. There is no adequate source of resistance in the rice gene pool and there is some evidence that direct seeding may provide a more conducive environment. As such, it is a major target for the use of antifungal defense genes through transformation.

*Blast and bacterial leaf blight.* These diseases have been managed through conventional breeding, and more recently through pyramiding and marker-aided selection. The focus has been on irrigated rice varieties. In rainfed rice, it has been more difficult to obtain durable resistance. In the rainfed systems, the complex genes controlling adaptation and acceptance (quality) are often lost in the backcrossing process used to introduce disease resistance to increase productivity. Thus, the use of genetic transformation, using known rice genes, to add resistance to the rainfed cultivars while maintaining their adaptive and acceptance traits is an important target.

*Stem borer.* Under present practices, the loss of yield to stem borer is low. Part of the low yields are due to the compensatory capacity of high-tillering rice cultivars grown at current yield levels. However, the losses from stem borer will probably increase as those compensatory mechanisms are reduced as in the case of the new plant type rice designed by IRRI to raise the yield ceiling, or by increasing the present management level that maximizes the use of tillers. Although the levels of resistance from the rice gene pool are moderate, the potential for management with *Bt* or other toxins appears to be relatively high. However, the environmental issues associated with the use of those toxins in Asia require careful study. (For IRRI's approach to the use of *Bt* in rice in Asia, see IRRI 1997).

*Weeds.* The dominance of yield loss from weeds, the evolving practice of direct seeding, and the increased competition for water highlight the need for intensive research efforts for integrated weed management. Cultivars of most major food crops are increasingly being either transformed to be resistant to herbicides or selected for resistance. The private sector is now developing rice that is resistant to herbicide. IRRI does not intend to develop herbicide-resistant rice at present but recognizes its responsibility to objectively evaluate the consequences of the introduction of herbicide-resistant rice along with all other integrated weed management strategies. (For IRRI's approach to herbicide use in Asia, see IRRI 1999a).

In addition to these targets for reducing yield loss from pests and reducing the use of pesticides, IRRI's biotech work also targets production under less favorable environments of soil and water and the nutritional quality of rice in the diets of the poor. Examples follow.

*Micronutrient deficiency of iron and zinc.* Deficiency of these nutrients is widespread in Asia and it predominantly affects women and children because of their greater physiological need. IRRI collaborative research identified large genetic variation for Fe and Zn concentration in rice grain. These high-iron and -zinc traits can be combined with high-yielding agronomic traits. Improved rice was identified that almost doubled the Fe and Zn in the grain (Senadhira et al 1998). Micronutrient in the grain can be further increased by using molecular marker technology to improve selection efficiency and gene transformation of the high-iron trait. Because of the high consumption of rice in developing countries, the extra iron and zinc would have a meaningful impact on human nutrition and health. IRRI has an active program in improving nutritional quality by conventional and biotechnology methods.

*Salinity.* More than 12 million ha of rice lands in Asia are now affected by salinity, and another potentially productive 9.5 million ha are unused because of salinity. The development of rice with enhanced tolerance of salinity would make these saline lands productive, but the selection efficiency of segregating populations for tolerance is low. As a result, little progress has been made in varietal improvement. IRRI has focused on developing molecular-marker-assisted selection (MAS) techniques for different mechanisms of salt tolerance (salt exclusion and tissue tolerance). This method enhances tolerance by pyramiding different mechanisms to produce durable salt-tolerant rice.

*Water-limiting rainfed environments.* The increasing competition for water outside of agriculture and the need to improve rainfed rice (which accounts for almost half of the area planted to rice) highlight the importance of new approaches to improving productivity under water-limiting conditions. IRRI is focusing on functional genomics and gene discovery in rice for drought.

*Yield.* Raising the yield of irrigated rice remains a major challenge to meet the demand for rice in the next 20 years. IRRI's focus to date has been on

- Using the hybrid technology developed by the Chinese for temperate rice and adapting it to tropical conditions.
- Designing and developing a new plant type (NPT) with characters to raise the yield ceiling.
- Exploring biotech approaches to enhance basic yield processes.
- Apomixis.

In each of these targeted areas of impact, IRRI has ongoing research in biotechnology approaches, usually in collaboration with other laboratories in Asia (NARS) and advanced research institutes. However, as in many public institutions, the application and use of these biotech products (in some cases the IP of others) are not well defined. The following section will outline the approaches that IRRI is considering with respect to IP.

## Possible options for gaining access to biotech tools for rice science

For some years, IRRI has been asked by the NARS members of the Asian Rice Biotechnology Network (ARBN) to assist in obtaining IP for their use. IRRI has stated that, as a matter of policy, it shall try to acquire IP in such a way that the IP and/or its derivatives (e.g., transgenic rice plants produced at IRRI) are freely available to NARS and the public sector in rice-growing countries. Furthermore, IRRI had undertaken to negotiate whenever possible for permission to release these products to farmers rather than to restrict us to research use only. Table 2 summarizes the options available to IRRI and NARS to obtain IP and some examples explored to date.

Our experience identified several difficulties with some of these approaches to acquire IP agreements on behalf of the network members, such as

- the slow pace of negotiations and heavy administrative responsibilities,
- the problem of having to exclude certain countries from the agreements and the linkage of deals to segmentation of a market that is not IRRI's to segment,
- the time spent on deals that might not ever lead to a product and bilateral deals between the IP holder and NARS that undercut IRRI's slower approach, and
- the legal repercussions if NARS renege on their sublicensing agreements.

We are exploring other options, the first of which is a triangular arrangement for a research agreement between IRRI and the IP owners, and for a use agreement between NARS and the IP owners. The principles of this option are that the private sector needs access to improved rice germplasm in order to add value to its IP; IRRI needs to continue to develop and deliver diverse and improved germplasm—with and without added IP—to provide choices to NARS; and the NARS need to negotiate with the owners of the IP to provide the best choices of seed for their farmers.

**Table 2. Options for acquisition of IP by IRRI and NARS, with some examples.**

| Realistic options | Roles | | Example |
|---|---|---|---|
| | IRRI | NARS | |
| Gift | + | + | *Bt* gene from Novartis |
| License | + | + | AFLP[a] from key gene, *Xa21* gene |
| Cross license | + | + | |
| IP substitution | + | + | |
| Partnerships | – | + | |
| Market segmentation | – | + | |
| Concessions | – | + | |
| Consortium, etc. | + | + | *Bt* gene from Plantech |

[a] AFLP = amplified fragment length polymorphism.

In this triangular agreement, IRRI would offer its germplasm and technical skills to "add value" to the IP of the private sector on a nonexclusive, freely distributed basis, but, on the understanding by the NARS recipients, that further commercialization of such materials will be subject to an agreement by them and the appropriate owners of the IP. Figure 1 shows a schematic of this option. We believe that such a model meets the needs of the various partners. The NARS can benefit from this option by having access to improved and divergent germplasm with and without IP traits and the private sector can forge the best relationship with the ultimate supplier of the locally adapted germplasm (the NARS) for its commercial use.

We are in the process of developing the concepts of this option and discussing them with the NARS and with the private sector. Since the negotiation for the use of the IP will be between the NARS and the owners of the IP, this option assumes an enhanced capacity by the public sector for negotiations. The CGIAR has developed a Central Advisory Service (CAS) for IP rights at ISNAR for NARS and CG centers. The option also assumes that there will be an adequate regulatory mechanism in the NARS to manage the IP in the released varieties.

The second option is to form a consortium among NARS to gain access to sufficient IP for a basic toolkit for biotech application. The need to have access to IP depends on whether the technology of interest is patented in each country (or whether there are implications for trade). Thus, it may be legal for many developing countries to use the current IP technology if it is not patented in their country. However, for other countries, there are, and will be, patents that restrict the use of basic transformation methods that are needed to address noncommercial targets. Thus, we are exploring the option of a basic toolkit for transformation. This approach will probably be most appropriate when several competing, well-established technologies are available for any one step of the transformation process. The consortium of research and breeding institutes would agree on a framework for the negotiation with the holders of the IP for the best arrangement to ensure freedom to operate for each step of the technology in each of the member countries. If necessary, the consortium would fund

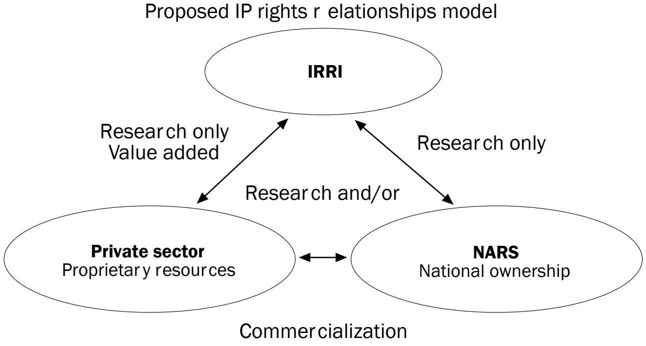

**Fig. 1. Schematic of a triangular IP relationship for research and use among IP owners, IRRI, and NARS (W. Padolina, personal communication).**

public research on those components for which they could not obtain freedom to operate at reasonable costs.

A summary of the ownership of the IP (as lodged in the United States) associated with some of the transformation technology of relevance to rice is given in Appendix 3. Table 3 summarizes possible approaches in order for a consortium to gain access to the appropriate technology to address noncommercial targets. This table is a summary only and is not inclusive of all approaches. However, it does provide a framework around which a consortium of stronger public-sector institutes could provide a basic toolkit for biotech application. For example, the CaMV 35S promoter is widely used in genetic engineering of plants to provide constitutive expression of genes in most cells. The commercial release of transgenic plants containing the CaMV 35S promoter requires a license from Monsanto, which holds the relevant patents. However, research at IRRI has identified two rice genes that could act as a replacement for the CaMV 35S promoter. Also, IRRI, in collaboration with advanced research institutes, is exploring the development of a selectable marker for use by the public sector.

There are also opportunities for a public-sector consortium to obtain IP from the small private-sector companies or the universities that own considerable IP. (One example of this approach was a consortium of public-sector institutes for the research on and use of a *Bt* gene developed by Plantech Company of Japan [J. Bennett, personal communication, 1999].)

A third area that IRRI is exploring in IP options is in functional genomics. The scientific and economic returns anticipated from rice genomics have stimulated the formation of partnerships between the public and private sector. The Rice Genome Project has a funding commitment and active participation from the governments of Japan, the U.S., the European Union, and major rice-producing countries in Asia (China, Thailand, Korea, and possibly India). Many private companies have also accumulated sizable sequence databases on rice and other related cereals. More recently, Craig Venter of Celera Genomics announced that the rice genome could be completely sequenced much ahead of the public effort and that the rice DNA sequence database could be made accessible at a cost of $30 million. These events underscore the fact that complete sequencing of the rice genome will be a reality soon and that timely access to the database may depend on the degree of ownership by the public and private sector.

Although genome sequencing has a clear end-point, functional genomics has a wide-open agenda for research and commercial applications. Functional genomics requires both DNA sequence information and biological variation as manifested by mutant lines, elite genetic stocks, and germplasm. With the strong need for understanding biological functions and practical linkage to breeding, functional genomics provides more opportunities for smaller research institutions such as NARS to participate. While there has been a lot of discussion on the ownership of DNA sequences, little attention has been given to defining the value of mutant stocks needed to discover new traits. One possible strategy to ensure broad applications of the mutants is to publish the characteristics and utility of mutants in the public domain and release

**Table 3. Toward a public-sector toolkit for genetic engineering of rice.**

| Step/component | Options (owners) | Public-sector opportunities | Best option for toolkit |
|---|---|---|---|
| Transformation protocol | 1. *Agrobacterium*<br>2. Electric discharge gun<br>3. Carbon whiskers<br>4. Helium inflow gun | Probably none on basic technologies but scope for development of more efficient protocols | Negotiate with all owners but develop more efficient protocols for specific rice varieties |
| Environmentally friendly selectable marker system | 1. Phosphomannose isomerase<br>2. *cre/lox* recombinase<br>3. *FRT/flp* recombinase phosphotransferase | Devise novel environmentally friendly selectable marker system | Negotiate with all owners but pursue development of a novel system |
| Constitutive promoter | 1. CaMV 35S<br>2. Ubiquitin | Isolate specific promoter from rice | Isolate own promoters |
| Terminator | 1. Nos<br>2. CaMV 35S | Isolate terminator from rice | Isolate own terminator |
| Stem borer resistance | Codon-corrected *Bt* gene | 1. Isolate appropriate promoters for use with *Bt* gene<br>2. Isolate naturally occurring stem borer resistance genes | Negotiate with Monsanto on sharing of IP but pursue gene isolation |
| Major disease resistance | 1. *Xa21*, Davis<br>2. *Xa1*<br>3. Resistance gene analogs | Isolate other naturally occurring bacterial blight resistance genes | Negotiate with all owners but pursue gene isolation |
| Sheath blight resistance | 1. Chitinase<br>2. Thaumatin | Isolate appropriate promoters | Negotiate with owners on sharing of IP |

these mutants under proper material transfer agreements. IRRI will explore the formation of a consortium with support to provide information that will benefit all parties—public and private—interested in using the genetic resources. It is anticipated that these genetic resources will be shared with the private sector, such that the public-sector consortium members can lever access to appropriate gene products developed by the private sector.

A fourth option undertaken by IRRI is to register our trade name and trademark. IRRI and IRRI's early IR varieties are well known in the rice world. Recently, IRRI has trademarked its logo (IRRI) and its nomenclature for varietal products (IR) in the Philippines. Trademark protection has been sought to ensure that any IRRI product, now fully available in the public domain, is used in accordance with its original intent. We are examining the benefits of such a limited approach to IP protection in the major rice-growing countries.

Our policy also indicates that IRRI would explore a defensive patent if it were in the best interest of poor clients. At this stage, IRRI has no plans for such action, but is in the process of auditing all of its potential IP. Any discussions on patent application that would enhance our capacity in bargaining for needed IP would be done in full consultation with our NARS partners.

## Summary

In addition to the many legal difficulties in commercializing " traits" owned by the private sector, rice is a "cultural" crop with strong involvement by the government in all aspects, including varietal approval and seed laws. It is possible that the legal environment for rice may be more stringent than that for other transgenic crops. Yet biotechnology does provide answers to some of the most intractable problems and ultimately can benefit rice farmers. Given this uncertainty, IRRI is attempting to develop mutually rewarding arrangements whereby the best technology is used to give farmers the most profitable rice in a safe and sustainable environment.

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## Notes

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## Appendix 1. Material transfer agreement for germplasm material.

IRRI
International Rice Research Institute
DAPO Box 7777, Metro Manila, Philippines

### Material Transfer Agreement (MTA)

IRGC Request No.: ____________

The material contained herein is being furnished by the International Rice Research Institute (IRRI) under the following conditions:

**Designated Germplasm**

IRRI is making the material described in the attached list available as part of its policy of maximizing the utilization of genetic material for research. The material was either developed by IRRI, or was acquired prior to the entry into force of the Convention on Biological Diversity, or if it was acquired after the entering into force of the Convention on Biological Diversity, it was obtained with the understanding that it could be made freely available for any agricultural research or breeding purposes.

The material is held in trust under the terms of an agreement between IRRI and FAO, and the recipient has no rights to obtain intellectual property rights (IPR) on the germplasm or related information.

The recipient may reproduce the seed and use the material for agricultural research and breeding purposes and may distribute it to other parties provided the recipient is also willing to accept the conditions of this agreement.[1]

The recipient, therefore, hereby agrees not to claim ownership over the germplasm to be received, nor to seek IPR over that germplasm or related information. He/she further agrees to ensure that any subsequent person or institution to whom he/she makes samples of the germplasm available is bound by the same provision and undertakes to pass on the same obligations to future recipients of the germplasm.

IRRI makes no warranties as to the safety or title of the material, nor as to the accuracy or correctness of any passport or other data provided with the material. Neither does it make any warranties as to the quality, availability, or purity (genetic or mechanical) of the material being furnished. The phytosanitary condition of the material is warranted only as described in the attached phytosanitary certificate. The recipient assumes full responsibility for complying with the recipient nation's quarantine/biosafety regulations and rules as to import or release of genetic material.

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[1] This does not prevent the recipient from releasing or reproducing the seed for purposes of making it directly available to farmers or consumers for cultivation, provided that the other conditions set out in the MTA are complied with.

Upon request, IRRI will furnish information that may be available in addition to whatever is furnished with the seed. Recipients are requested to furnish IRRI performance data collected during evaluations.

The material is supplied expressly conditional on acceptance of the terms of this agreement. The recipient's acceptance of the material constitutes acceptance of the terms of this agreement.

**Dr. M.T. Jackson**
Head, Genetic Resources Center

NAME OF REQUESTOR ____________________

COMPANY ____________________

ADDRESS ____________________

____________________

**Appendix 2. Material transfer agreement for hybrid lines of IRRI.**

IRRI
International Rice Research Institute
DAPO Box 7777, Metro Manila, Philippines

### Material Transfer Agreement

The material contained herein is being furnished by the International Rice Research Institute (hereafter IRRI) under the following conditions:

1. IRRI is making the germplasm described in the attached list available as part of its policy of maximizing unrestricted distribution and utilization of genetic material for research. The germplasm was developed by IRRI.
2. The recipient may reproduce the seed and use the material for agricultural research and breeding purposes and may distribute it to other parties provided that any recipient is willing to accept the conditions of this agreement.
3. Recipients are free to release for commercialization IRRI research products in the form they are provided. If released for commercialization without obtaining intellectual property rights (IPR), IRRI requests notification and acknowledgment. Recipients are not to apply for any form of IPR of IRRI research products without the written permission of IRRI. Moreover, while IRRI recognizes the validity of IPR, it reserves the right to distribute all material in accordance with paragraph 1 above.
4. IRRI makes every effort to produce rice seeds and distribute information of the highest quality. However, IRRI makes no warranties as to the safety of the material, nor as to the accuracy or correctness of any data provided with the material. Neither does it make any warranties as to the quality, viability, or purity (genetic or mechanical) of the material being furnished.
5. The phytosanitary condition of the material is warranted only as described in the attached phytosanitary certificate. The recipient assumes full responsibility for complying with the recipient nation's biosafety regulations and rules on importation or release of genetic material.
6. Upon request, IRRI will furnish information that may be available in addition to whatever is furnished with the seed. Recipients are requested to acknowledge use of these materials in the release of any variety .
7. The supply of this material is subject to the acceptance of the terms and conditions contained in this agreement. The recipient's retention of the material constitutes such acceptance.

SIGNATURE OF SENDER

**Appendix 3. Ownership of some of the transformation technology in rice and implications for the public sector.**

| Component | Owner | Availability | Public toolkit |
|---|---|---|---|
| *Transformation protocol* | | | |
| *Agrobacterium* | Basic owner: Max Planck Institute, Cologne | For research purposes | Required for efficient transformation of rice |
| | Additional IP for cereals: Japan Tobacco | For research purposes, MTA | |
| Electric discharge gun | Agricetus | Not available | |
| Carbon whiskers | Zeneca | Not available | |
| Helium inflow gun | DuPont | For research purposes, BioRad | Required for cultivar-independent transformation of rice |
| *Promoter* | | | |
| CaMV 35S | Monsanto | | |
| Maize ubiquitin | | For scientific purposes, University of California-Berkeley (P. Quail) | |
| Tapetum specific | PGS/AgrEvo | For scientific purposes | Required for efficient hybrid rice production |
| *Terminator* | | | |
| Nos | | | |
| CaMV 35S | Monsanto | | |
| *Selectable marker gene* | | | |
| Hygromycin phosphotransferase | Novartis | For scientific purposes | |
| Phosphinothricin amino transferase (BASTA sel) | PGS/AgrEvo | For scientific purposes | |
| Phosphomannose isomerase (mannose sel) | Novartis | For scientific purposes, MTA (B. Lee) | Environmentally friendly |
| *SU1* gene (negative selection with sulfonurea herbicide precursor R7402) | DuPont | For scientific purposes | |
| *Reporter gene* | | | |
| Gus | CAMBIA | For scientific purposes, MTA (R. Jefferson) | |

**Appendix 3 continued.**

| Component | Owner | Availability | Public toolkit |
|---|---|---|---|
| Green fluorescent protein | Columbia University | For scientific purposes, buy from Clontech; for other purposes, discuss with Columbia University | |
| *Useful genes* | | | |
| *Cre/lox* system | DuPont | For scientific purposes, MTA | |
| *Xa21* | University of California-Davis | For scientific purposes, MTA | |
| *Bt crylA(b)* | Mycogen, Monsanto, and others | For scientific purposes | Required for deepwater rice |
| *Concepts* | | | |
| Resistance gene analog | Monsanto | For scientific purposes | Required for disease resistance |
| Codon correction concept | Monsanto | | Required for *Bt* gene |
| Gene stacking | PGS/AgrEvo | | Required for durable *Bt* deployment |
| *Vectors* | | | |
| PBR322 | | | |
| PMOG22 | Mogen, Leiden | For scientific purposes | |
| *Transposons* | | | |
| *Ac-Ds* from maize | University of Cologne | Freely available (P. Starlinger) | |
| En-I (Spm) | Max Planck Institute, Cologne | For scientific purposes, MTA (H. Saedler) | |

RICE GENOMICS

# Implications of rice genomics research for temperate breeding programs

D.J. Mackill

Since the development of molecular markers for mapping genes in plants in the 1980s, rice has been one of the prominent beneficiaries of molecular genetic technology. Its small chromosomes with high gene density and its ease of transformation relative to other cereals has ensured that it will continue to profit from future efforts in plant genomics. The use of molecular markers to map genes controlling qualitative and quantitative traits has provided a powerful tool for understanding the genetic basis of economic traits. In addition to gene identification and isolation, molecular markers are being applied to cultivar identification and classification. Japonica cultivars grown in temperate regions present special challenges because of the limited polymorphism of molecular markers. All of these activities will benefit enormously from the sequencing of the entire rice genome. Large-scale identification of important genes combined with more efficient transformation methods will facilitate the direct transfer of the superior alleles of multiple genes into adapted cultivars. Rapid progress in functional genomics will ultimately change the way rice breeding is done. However, elucidating of the function of all rice genes and the complex interaction between loci and between alleles of the same locus is a long-term undertaking. For the foreseeable future, direct manipulation of genes to develop superior cultivars will still require the steady production of elite germplasm through conventional breeding methods.

Rice has become the model species for the study of grass genomes because of the low amount of DNA, limited duplication of genes, and high gene density (Moore et al 1993). Rice will thus be the first agricultural species with a completed genome sequence. This will facilitate large-scale study of gene function by new approaches pioneered in yeast (Lashkari et al 1997) and *Arabidopsis* (Ruan et al 1998). In this chapter, the progress of rice genome mapping over the past decade will be reviewed and applications specific to temperate rice will be highlighted. The limitations of genome mapping will be discussed and future trends that will help overcome these limitations will be briefly mentioned.

## Mapping major genes

The application of restriction fragment length polymorphism (RFLP) markers to plant genetics resulted in the first molecular map of the rice chromosomes (McCouch et al 1988). A major objective of the genome mapping projects has been the “tagging” of genes controlling important economic traits with molecular markers. There has been a rapid increase in publications reporting the tagging of major genes controlling economic traits in rice with various types of markers. Most of these studies have focused on resistances to diseases and insects.

The ability to map genes in a wide range of crosses with molecular markers has a tremendous impact on rice genetics research. Molecular markers greatly facilitate the determination of allelism of newly studied genes with known genes. In the past, this could only be accomplished by making crosses with a range of testers—trisomics, marker stocks, or other accessions with known genes of a similar phenotype. The map position of a gene can now be determined in almost any cross if there is sufficient polymorphism for molecular marker analysis. For major genes, this can be done quite rapidly with the bulk segregant analysis method (Michelmore et al 1991), where two or four bulks based on the two phenotypes are screened with markers spanning the 12 rice chromosomes.

Blast resistance in the tropics provides an excellent example of how valuable these marker maps have been. Early work was hampered by the apparently large number of genes in indica cultivars, and the inability to compare genes identified in different studies because of the use of different isolates. In our early work at IRRI, we decided to use near-isogenic lines (NILs), developed using six backcrosses to the susceptible indica parent CO39, to facilitate the identification of genes in indica rice (Mackill and Bonman 1992). This laborious process did allow isolation of individual genes in a susceptible background, and facilitated the mapping of these genes (Yu et al 1991). Molecular markers, however, can now be used to identify the relationship of unknown genes to those already mapped. This has resulted in a rapid accumulation of information on the race specificities of the different blast resistance genes. More importantly, genetic markers can now be used to combine disease resistance genes with different specificities in a single line (Huang et al 1997).

Molecular markers are particularly attractive in selection for traits that are expressed at a late stage in the life cycle, enabling the detection of these traits in the seedling stage, so that backcrossing can be carried out. Wide compatibility and restorer genes are extreme examples, where the trait is only observed in the maturity stage of the test cross of the plants. These traits would normally represent a formidable challenge for a backcrossing program, but molecular markers for the major wide compatibility gene (Liu et al 1997) and various restorer genes (Bharaj et al 1995, Akagi et al 1996, Ichikawa et al 1997, Yao et al 1997, Zhang et al 1997) have made this feasible.

## Mapping quantitative trait loci (QTL)

The identification of the loci underlying quantitative traits has been one of the most enticing applications of genome maps. The importance of quantitative traits in plant breeding stimulated wide interest and study. However, geneticists were limited to statistical descriptions of variation with little or no application to applied breeding. With the advent of DNA markers, breeders suddenly possessed the means to determine the number of factors controlling a trait, their approximate position on the genetic map, and their relative importance in the ultimate expression of the trait. The number of publications on QTLs in rice has increased dramatically since the first major study of Wang et al (1994) on partial resistance to blast. Most of these studies have focused on agronomic traits, particularly those related to yield.

QTL studies suffer many limitations. Large population sizes are necessary for accurate detection of loci with smaller effects (Lande and Thompson 1990), and resolution is often poor, with loci being assigned to intervals of 10 cM or more. Another problem is that loci identified in one mapping population may not be important in other populations that breeders would use. For some traits, multiple studies have accumulated, and comparisons can be made for different populations. Many QTLs do seem specific to only one cross, but in some cases loci in the same region of the map are observed in almost all studies of a particular trait (Mackill 1999). These widely expressed loci may be attractive targets for marker-assisted selection (MAS) or map-based cloning.

Since mapping populations must contain sufficient polymorphism for finding markers spread over the entire genome, most researchers have focused on indica × japonica crosses. Tanksley and Nelson (1996) have pointed out the disadvantages of using such “balanced” populations for QTL mapping studies. Once useful loci are identified, they are generally in a background that is agronomically undesirable, and they may not be expressed sufficiently when backcrossed into a more suitable genetic background. Tanksley and Nelson (1996) proposed the advanced backcross QTL (ABQTL) method, following the multiple-backcross strategy of Wehrhahn and Allard (1965). An exotic or wild species is crossed with an agronomically superior elite line. The resulting $F_1$ is backcrossed to the same parent. The resulting $BC_1F_1$ plants (commonly 30–60 plants) are backcrossed again. $BC_2F_1$ or selfed generations are evaluated for various agronomic traits.

This approach is now being used with wild species to identify loci that confer enhanced performance. For example, two loci identified from the wild species *Oryza rufipogon* contributed 17% and 18% to yield improvement, even though the wild parent was inferior to the recurrent parent in yield (Xiao et al 1998). ABQTL populations are currently under development in temperate rice populations.

## Constraints to gene mapping in japonica rice

Nearly all temperate rice cultivars belong to the japonica subspecies. Short- and medium-grain cultivars belong to the temperate japonica, while the long-grain cultivars grown in the United States, Europe, and Australia belong to the tropical japonica subspecies (Mackill 1995). Unfortunately, the level of polymorphism is usually too low within the japonicas for genetic mapping with RFLPs. Redoña and Mackill (1996) were able to use RAPD (randomly amplified polymorphic DNA) markers to construct a genetic map in a temperate × tropical japonica cross. They mapped seedling-vigor QTLs with this map. Microsatellite markers should prove even more useful for mapping within japonica crosses. Considerable polymorphism has been observed among japonica cultivars for microsatellite markers (Akagi et al 1997). Although closely related lines are still similar, temperate japonica cultivars from diverse sources appear to have sufficient polymorphism for mapping (Mackill et al 1996). One interesting observation is that the genetic diversity among japonica rice is not uniformly distributed among the rice chromosomes. Redoña and Mackill (1996) found that polymorphic RAPD markers were abundant on chromosomes 10 and 11 but relatively sparse on chromosomes 1 and 2. This is in contrast to published RFLP maps based on indica × japonica or wild species crosses. Our preliminary data on microsatellite markers indicate that japonica cultivars possess higher gene diversity on chromosomes 10 and 11 than on chromosomes 1 and 2. For indica cultivars, gene diversity was similar for these chromosomes. One practical implication of this observation is that it may be more difficult to find microsatellite markers for genes on chromosomes 1 and 2. We have already found it difficult to identify polymorphic microsatellite markers among California japonica cultivars on chromosome 2, where a QTL for resistance to stem rot was mapped.

## Conclusions

The above discussion highlights some of the applications of genome maps that are relevant to temperate environments. However, some limitations may hinder the widespread application of marker-assisted selection in rice breeding programs. Of particular importance is the limited polymorphism found in japonica rice. Current initiatives in rice genomics will help overcome many of these constraints. The complete sequence of the rice genome will lead to an abundance of markers for gene mapping and selection. The sequence will also facilitate fine-scale mapping and positional cloning of important genes, including QTLs for agronomic traits. Once genes are isolated, they can be manipulated or modified directly, linked with other genes on the same construct, and reintroduced into desirable genotypes.

Functional genomic approaches such as DNA microarrays and deletion or insertional mutants will facilitate the identification of many important genes. However, these approaches cannot identify many of the important alleles that result in variability for agronomic traits. Thus, genetic mapping using molecular marker technology will complement the assignment of function to rice genes identified through the sequencing project. Ultimately, this will result in a much more scientific approach to rice breeding. For the foreseeable future, however, most approaches will depend on conventional breeding approaches that can be enhanced through MAS.

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# The second phase of the Japanese Rice Genome Research Program: toward unraveling the complete sequence of the rice genome

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With its long history of rice cultivation, Japan is in a good position to consolidate a centralized program for rice genome analysis. The Rice Genome Research Program began with the ultimate aim of understanding the genome structure of rice. During its first phase (1991-98), we catalogued about 17,000 expressed genes in rice, constructed a high-density linkage map with 2,275 DNA markers, and established a YAC-based physical map covering about 70% of the genome. With these basic tools, we embarked on the second phase of the program in April 1998 with three main objectives: complete genome sequencing, gene functional analysis, and application of markers to breeding. A PAC genomic library has been constructed as the core of genome sequencing, anchoring of expressed sequence tags (ESTs) on a YAC-based physical map is in progress to provide more markers, and a sequence-ready physical map is being produced by ordering PAC clones with polymerase chain reaction primers from mapped DNA markers and ESTs. Using the clone-by-clone shotgun sequencing strategy, our initial target is to complete the sequence of chromosomes 1 and 6 as part of a collaboration with the International Rice Genome Sequencing Project. A complete sequence of the rice genome is expected to give way to integrated analysis of structure, function, expression, and metabolic pathways that will provide more efficient strategies for the improvement of rice and other cereals.

Rice is the essence of culture in Japan. Rice cultivation began more than 2,000 years ago. The crop's significant status is reflected by the tremendous progress in rice-related studies worldwide in the last 100 years. In Japan, rice research began with basic morphological studies and gradually developed into substantial aspects of physiology and ecology, and eventually into broader and practical studies on genetics and breeding. The recent emergence of advancements in molecular biology led to a comprehensive and coordinated program on rice genome analysis. Rice is considered a model crop for genome analysis because of several factors, such as its small genome and well-developed system for transformation, known synteny with other cereals, and enormous availability of molecular genetic resources. At the Rice Genome Research Program (RGP), our principal objectives are directed toward an ultimate evaluation of the rice genome structure and function of genes composing the genome. In the process, we hope to elucidate complex genetic traits previously demonstrated as phenotypic or physiological mutants. We present here the accomplishments of the RGP during its first phase and the second phase currently in progress.

## The first phase of the RGP: an overview

Figure 1 summarizes the overall research strategy of the RGP. The three major goals during the first phase of the program were the construction of (1) a catalog of all genes in rice, (2) a genetic map using DNA markers, and (3) a physical map by ordering cloned genomic DNA fragments.

A large-scale cDNA analysis was carried out to catalog all the expressed genes in rice including genes involved in growth and development, and tissue- and stress-specific genes (Yamamoto and Sasaki 1997). A total of 21 cDNA libraries were prepared from various tissues and organs (root, shoot, panicle, and callus) under different culture conditions and developmental stages. The resulting approximately 40,000 cDNA clones were partially sequenced, translated into amino acid sequence, and searched for similarity with known genes from rice and other organisms. With this strategy, we were able to identify the similarity of 25.7% of all analyzed clones to known genes registered in the protein identification resources (PIR) database. The remaining sequences that showed no similarity to known genes probably correspond to novel genes in rice whose functions need to be further analyzed. The redundancy of each 5′-sequence relative to all other isolated 5′-sequences was evaluated and about 17,000 expressed sequences were catalogued, representing more than one-third of the total genes in rice.

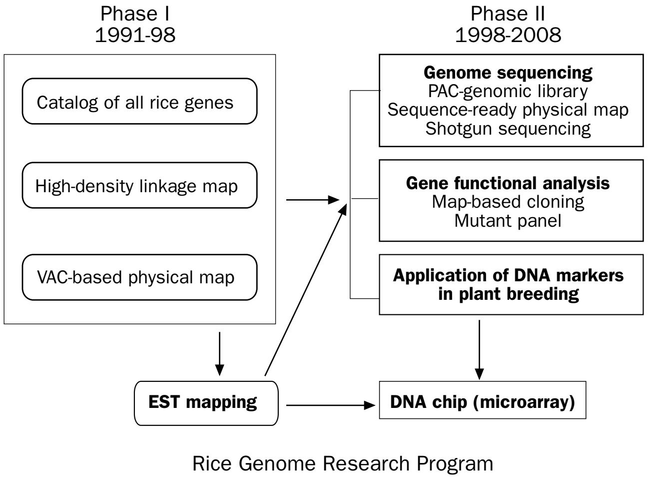

**Fig. 1. The overall research strategy of the Rice Genome Research Program.**

The sequenced cDNA clones were the main resources used in the construction of a genetic map by restriction fragment length polymorphism (RFLP). The map was constructed using a single $F_2$ population derived from a cross between a japonica variety (Nipponbare) and an indica variety (Kasalath) with 2,275 markers of mostly rice cDNA and a total genetic distance of 1,521.6 cM in the Kosambi function (Harushima et al 1998). Possibly one of the most saturated genetic maps of any cereal crop, this high-density linkage map has been useful for understanding the distribution of single-copy genes, gene families, and isozymes. Furthermore, this map has been used to clarify the synteny between rice and wheat (Kurata et al 1994), identify duplicate segments between rice chromosomes 11 and 12 (Nagamura et al 1995), detect segregation distortions in an indica-japonica rice cross (Harushima et al 1996), compare genetic distance and order of DNA markers in five japonica-indica crosses (Antonio et al 1996), and map several quantitative trait loci (QTL) in rice (Yano et al 1997).

The DNA markers in the high-density linkage map were used in ordering cloned DNA fragments to reconstruct the whole genome. A genomic library was constructed using yeast artificial chromosome (YAC) as the vector. This library consisted of about 7,000 clones with an average insert size of 350 kb that corresponds to 6 rice genome equivalents (Umehara et al 1995). The markers along the genetic map were used to screen the YAC library using colony Southern hybridization and polymerase chain reaction (PCR) screening. The selected YACs carrying the corresponding markers were then ordered along the 12 rice chromosomes. As a result, a physical map with 407 YAC contigs and a total length of 306 Mb covering about 70% of the entire rice genome has been developed (Saji et al 2001). This physical map has been used to clarify the order and orientation of multiple markers with the same locus position in the genetic map, and the structure of many regions in the chromosomes with high recombination frequency. It has also provided an indispensable tool for map-based cloning of many agronomically and biologically important genes known only by their phenotypes.

## The second phase of RGP

The second phase of RGP unfolded in April 1998 with three major objectives: (1) complete sequencing of the rice genome, (2) gene functional analysis, and (3) application of DNA markers in plant breeding.

### Genome sequencing

The main thrust of the second phase of RGP is to completely sequence the rice genome and to reveal all genetic information in rice. A clone-by-clone shotgun sequencing strategy was adopted. We constructed a genomic library in a P1-derived artificial chromosome (PAC) as the core for high-throughput genome sequencing (Baba et al 1998). This genomic PAC library consisted of approximately 71,000 clones with an average insert size of 112 kb and 16 times genome equivalent. To develop more markers for ordering the PAC clones, we have an ongoing EST mapping project that also aims at determining the map position of most rice sequences obtained from large-scale cDNA analysis (Wu et al 1999). This mapping strategy involves selecting unique rice ESTs by sequencing 3′-UT regions of cDNA clones and screening the YAC library by three-dimensional PCR using the clone-specific primers. So far, it has resulted in more than twice the number of markers from the combined genetic and EST maps. Using the PAC genomic library and the mapped DNA markers as well as ESTs, we are now constructing a sequence-ready physical map of the rice genome. A PCR screening system using STS primers of DNA markers and ESTs mapped on each chromosome was established to speed up the mapping process. Screening a total of 34,560 PAC clones from our library is now under way. The ordered PAC clones are also being analyzed by fingerprinting to confirm contig formation. The gaps between contigs are being filled by chromosome walking using the end-sequence of aligned PAC clones.

The ordered PAC clones then undergo high-throughput genome sequencing. For the initial part of the project, we are concentrating our efforts on chromosomes 1 and 6. A shotgun library is constructed from each ordered PAC clone (Aoki et al 1998). The template preparation step for genome sequencing includes PAC DNA purification by CsCl density-gradient centrifugation, quantification of *Escherichia coli* contamination by TaqMan-PCR, sonication and size selection by agarose gel electrophoresis, subcloning of shotgun fragments in the pUC18 vector, and transformation using *E. coli* DH10B (Gibco, BRL). We are constructing 2-kb and 5-kb shotgun libraries for each PAC clone for genome sequencing. The 2-kb library directly undergoes PCR and the nonamplified clones as well as the 5-kb clones undergo plasmid isolation to obtain templates for sequencing. We are using the Big-Dye primer chemistry for sequencing in ABI377 and ABI3700 DNA sequencers. Sequence data are assembled into the contig by Pred/Phrap and gap regions are filled by the primer-walking method. Our initial sequencing efforts will concentrate on gene-rich regions of chromosomes 1 and 6 in order to identify as many genes as possible early in the project.

The completed sequence is then annotated by similarity search using BLASTN, RGP EST Database, NRP, BLASTX, and LTR of retrotransposons. To predict the coding region of the gene, we used GENSCAN for the maize and *Arabidopsis* protocol and the SplicePredictor to determine the splice sites. All of our sequence data are released to our database INE (Integrated Rice Genome Explorer) and can be accessed through our Web site at http://www.staff.or.jp. Our database incorporates all genomic information for the 12 rice chromosomes such as the genetic map, physical map, EST map, and ordered PAC contigs.

## Gene functional analysis

The functional genomics aspect of the program is aimed at relating the genome structure and organization to plant function at the cellular and organismal level. Foremost among these studies is the use of mapping information for isolation of agronomic genes in rice already in progress since the first phase of the program. With the map-based cloning strategy, several disease-resistance genes in rice have been isolated such as the *Xa1* gene resistant to *Xanthomonas oryzae*, the causal pathogen of bacterial blight (Yoshimura et al 1998), and *Pib* gene against rice blast caused by the fungal pathogen *Magnaporthe grisea* (Wang et al 1999). We have also isolated and characterized an agronomic gene for controlling plant height, the rice Dwarf 1 (*D1*) gene whose recessive mutant allele confers the dwarf phenotype (Ashikari et al 1999). Isolation of the photoperiod sensitivity gene is also in progress. Several QTLs for heading date have been identified, such as *Hd1* and *Hd3* in chromosome 6 and *Hd6* in chromosome 3. These QTLs will also be isolated by map-based cloning strategies.

The use of endogenous transposable elements is another approach we used to clarify the function of unknown genes identified by a large-scale EST analysis (Hirochika 1997). This strategy is also known as the gene-knockout system by insertional mutagenesis using retrotransposons, especially *Tos17*. Mutants carrying transposed *Tos17* copies are being developed, including a system for characterization by PCR using one primer for the ends of *Tos17* and another for the gene of interest to define the function of the gene.

Gene expression profiling is also being developed using DNA microarray for genome-wide analysis of expression patterns for pathways or networks of genes under specific environmental conditions or various developmental stages. These combined strategies for functional analysis will be used to clarify many of the most important attributes of rice genes, ranging from their transcription and translation to the subcellular localization of their products and to their genotype and mutant phenotypes as well.

### Applications in plant breeding

Application of all the genomic information for the development of improved rice varieties is also a major goal of the program. To develop rice varieties with important agronomic traits, studies on the use of DNA markers for marker-aided selection are in progress at various experimental research stations in Japan. The DNA markers are also being used for molecular genetic studies of other crops including vegetables, forage crops, fruit trees, and forest trees. The completion of the genome sequence of rice is also expected to pave the way for new breeding strategies in rice and other cereal crops as well.

## The International Rice Genome Sequencing Project

Rice genome analysis is useful for understanding genetic information not only in rice but also in other major cereal crops as staple food for the entire world population. For this benefit, the immediate release of sequence information is imperative. The status of rice genome sequencing in Japan has served as a stimulus for other countries such as the United States, European countries, and other Asian countries to establish a similar research approach. The International Rice Genome Sequencing Project was therefore established with the common goal of sequencing the entire rice genome. By sharing materials and adopting a common sequencing strategy, it is expected that this cooperative research program will hasten the release of sequence information to the public domain. The collaboration was originally projected for a period of ten years but may be completed in a shorter time frame with the development of advanced sequencing technology and renewed funding support from government agencies. The participating countries and their chromosomes of interest for sequencing are as follows: chr. 1—Japan (mainly) and Korea (some regions); chr. 2—U.K. and Canada; chr. 4—China; chr. 5—Taiwan; chr. 6—Japan; chr. 8—India; chr. 9—Thailand; chr.10—USA; and chr. 12—France. Details of this collaborative project can be viewed through the World Wide Web at http://www.staff.or.jp/Seqcollab.html.

## Clone accessibility

All DNA materials including mapped cDNA clones and YAC filters are available from the DNA Bank of the National Institute of Agrobiological Resources (NIAR) and can be viewed through the World Wide Web at http://bank.dna.affrc.go.jp.

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## Notes

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# Bird species occurring in rice fields and surrounding habitats at Merin Lagoon watershed, Uruguay

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As part of a comprehensive study of bird pest management in rice, we censused birds at four locations in the Merin Lagoon rice-growing region of eastern Uruguay. Study sites were randomly selected and included six different habitat types: rice field (all stages), grassland, irrigation canal, forest, wetland, and wet meadows. Study localities averaged 400 ha and we visited each location 13 times from March 1994 to February 1995 (624 total observation hours). The rice habitat was the most used (more species seen more frequently). Altogether, we identified 121 bird species from 17 orders. Passerines, or perching birds, represented 43% of the total. The chestnut-capped blackbird (*Agelaius ruficapillus*) was the most common species, and was often present in large flocks feeding on the rice crop. Of the species recorded, 71% breed in the study area. Migratory species represented 15% of the total. Several species classified as rare or endangered were also recorded. The rice crop environment, including surrounding habitats, represents a critical resource for avian populations.

Rice is the third most valuable export commodity of Uruguay. Since 1989, rice area has doubled and is now 200,000 ha. Most production (72%) is concentrated in the eastern part of the country (Rocha, Treinta y Tres, and Cerro Largo Departments) where the crop is planted in a wetland environment modified recently for rice cultivation. This environment features special ecological characteristics, is very rich in bird species, and has much value for conservation.

The objective of this study was to determine what bird species occurred in rice crops and surrounding habitats and to determine the frequency of occurrence on a monthly basis throughout the year.

## Materials and methods

The study area comprises the Uruguayan part of Merin Lagoon. The observation sites were chosen randomly from a list of rice farms within the zone. The four chosen localities were Río Branco (Cerro Largo Department), Arrozal 33 (Treinta y Tres), Cebollatí, and Chuy (both in Rocha). Six habitat types or environments were defined:

1. Rice fields.
2. Prairie, road border, and levee: areas where rice is no longer grown that have been seeded or have naturally converted to grassland or pasture, also includes grassy borders along the roads and the irrigation canals/levees.
3. Irrigation canals and levee borders: irrigation canals and ditches—an environment rich in aquatic vegetation, aquatic vertebrates, and invertebrates.
4. Forest: natural forest or artificial woods of *Eucalyptus* sp.
5. Wetlands and estuary: inundated areas surrounding the crops—small areas in most cases.
6. Wild bushes: *Panicum* sp. and *Eryngium* sp., generally small areas between the rice crop and other habitat types.

Bird observations were made from March 1994 to February 1995 and each locality was visited 13 times. The counts commenced at daylight and totaled 624 observation hours. At each locality, we covered an average of 400 ha in a vehicle by using roads as sampling transects.

Bird species were identified with binoculars, with a spotting scope, or by vocalizations. For bird identifications, we followed Arballo and Cravino (1999). We tabulated observations by locality, date, bird species present, and environment. We also noted the presence of nesting in rice fields.

For each habitat type, we calculated the frequency of occurrence of bird species by dividing the number of times the species was detected by the total number of visits to the habitat type. The frequency of occurrence of each species in the various environments permitted insights into the species' use of the different habitats.

## Results and discussion

Altogether, 121 species from 17 orders were identified (Table 1). Passerines (order Passeriformes) represented 43% of the total. The overall frequency of occurrence by number of species present peaked in September, when the rice fields were in stubble (Fig. 1). Overall, species occurrence was greatest in the rice-field environment.

Throughout all of the habitat types, the 10 most frequently observed species were *Agelaius ruficapillus*, followed by *Furnarius rufus, Pitangus sulphuratus, Circus buffoni, Platalea ajaja, Myiopsitta monachus, Egretta thula, Anumbius annumbi, Ciconia maguari*, and *Amazonetta brasiliensis.*

In rice-field habitats, the species observed most frequently was *Agelaius ruficapillus,* which was recorded on every visit and which occurred in large flocks that have a substantial economic impact on the rice crop (Rodríguez et al 1998). Other common species were *Circus buffoni* (100%), *Ciconia maguari* (100%),

**Table 1. Bird species within the study area by frequency of occurrence (percentage of field days in which a species was found within a certain habitat type), n = 13 visits = 100%. Bird identification according to Arballo and Cravino (1999).**

| Species | Rice | Irrigation canals | Prairie | Forest | Wetland | Wild bushes |
|---|---|---|---|---|---|---|
| *Rhea americana* | 62 | 0 | 15 | 0 | 8 | 0 |
| *Nothura maculosa* | 31 | 0 | 31 | 8 | 0 | 0 |
| *Phalacrocorax brasilianus* | 0 | 54 | 0 | 0 | 0 | 0 |
| *Syrigma sibilatrix* | 31 | 0 | 31 | 0 | 0 | 0 |
| *Ardea cocoi* | 23 | 46 | 0 | 0 | 0 | 0 |
| *Egretta alba* | 85 | 54 | 0 | 0 | 8 | 0 |
| *E. thula* | 85 | 54 | 8 | 0 | 15 | 0 |
| *Bubulcus ibis* | 54 | 8 | 46 | 0 | 0 | 0 |
| *Butorides striatus* | 0 | 0 | 0 | 0 | 23 | 0 |
| *Nycticorax nycticorax* | 8 | 0 | 0 | 8 | 15 | 0 |
| *Tigrisoma lineatum* | 8 | 15 | 0 | 0 | 8 | 0 |
| *Botaurus pinnatus* | 31 | 15 | 0 | 0 | 0 | 0 |
| *Mycteria americana* | 46 | 15 | 8 | 0 | 8 | 0 |
| *Ciconia maguari* | 100 | 38 | 8 | 0 | 8 | 0 |
| *Cathartes aura* | 8 | 0 | 8 | 0 | 0 | 0 |
| *C. burrovianus* | 38 | 0 | 8 | 0 | 0 | 0 |
| *Theristicus caerulescens* | 38 | 0 | 15 | 0 | 0 | 0 |
| *Phimosus infuscatus* | 92 | 15 | 23 | 0 | 0 | 0 |
| *Plegadis chihi* | 77 | 23 | 8 | 8 | 23 | 0 |
| *Platalea ajaja* | 31 | 62 | 23 | 23 | 31 | 0 |
| *Chauna torquata* | 69 | 0 | 15 | 0 | 8 | 0 |
| *Dendrocygna bicolor* | 46 | 0 | 0 | 0 | 0 | 0 |
| *D. viduata* | 77 | 0 | 8 | 0 | 8 | 0 |
| *Calloneta leucophrys* | 38 | 46 | 8 | 0 | 0 | 0 |
| *Amazonetta brasiliensis* | 77 | 62 | 15 | 0 | 0 | 0 |
| *Anas flavirostris* | 38 | 8 | 0 | 8 | 0 | 0 |
| *A. georgica* | 23 | 31 | 0 | 0 | 0 | 0 |
| *A. versicolor* | 23 | 15 | 0 | 0 | 15 | 0 |
| *Netta peposaca* | 31 | 8 | 0 | 0 | 0 | 0 |
| *Elanus leucurus* | 0 | 23 | 8 | 0 | 0 | 0 |
| *Rostrhamus sociabilis* | 23 | 54 | 23 | 8 | 0 | 0 |
| *Circus buffoni* | 100 | 0 | 31 | 0 | 31 | 31 |
| *C. cinereus* | 23 | 0 | 15 | 0 | 0 | 8 |
| *Buteogallus meridionalis* | 8 | 0 | 0 | 0 | 0 | 0 |
| *Rupornis magnirostris* | 0 | 0 | 23 | 23 | 0 | 0 |
| *Polyborus plancus* | 46 | 0 | 31 | 15 | 0 | 0 |
| *Milvago chimango* | 54 | 0 | 54 | 8 | 0 | 0 |
| *Falco sparverius* | 15 | 0 | 15 | 8 | 0 | 0 |
| *F. femoralis* | 23 | 0 | 0 | 0 | 0 | 0 |
| *Aramus guarauna* | 15 | 46 | 0 | 0 | 0 | 0 |
| *Aramides ypecaha* | 0 | 15 | 15 | 15 | 15 | 31 |
| *Pardirallus sanguinolentus* | 23 | 0 | 0 | 0 | 0 | 0 |
| *Gallinula chloropus* | 38 | 0 | 0 | 0 | 8 | 0 |
| *Jacana jacana* | 0 | 8 | 0 | 0 | 8 | 0 |

*continued on next page*

**Table 1 continued.**

| Species | Rice | Irrigation canals | Prairie | Forest | Wetland | Wild bushes |
|---|---|---|---|---|---|---|
| *Himantopus melanurus* | 54 | 23 | 0 | 0 | 0 | 0 |
| *Vanellus chilensis* | 62 | 8 | 46 | 8 | 15 | 0 |
| *Pluvialis dominica* | 46 | 8 | 15 | 0 | 8 | 0 |
| *Charadrius collaris* | 8 | 0 | 8 | 0 | 0 | 0 |
| *C. modestus* | 15 | 0 | 0 | 0 | 0 | 0 |
| *Gallinago paraguaiae* | 38 | 31 | 8 | 8 | 23 | 0 |
| *Tringa flavipes* | 15 | 0 | 0 | 0 | 8 | 0 |
| *T. solitaria* | 0 | 8 | 0 | 0 | 0 | 0 |
| *Calidris melanotos* | 8 | 0 | 0 | 0 | 8 | 0 |
| *Larus maculipennis* | 69 | 38 | 15 | 0 | 8 | 0 |
| *Columba picazuro* | 46 | 0 | 0 | 15 | 0 | 0 |
| *C. maculosa* | 8 | 0 | 8 | 0 | 0 | 0 |
| *Zenaida auriculata* | 15 | 0 | 62 | 0 | 0 | 0 |
| *Columbina talpacoti* | 8 | 0 | 23 | 8 | 0 | 0 |
| *C. picui* | 31 | 0 | 54 | 15 | 0 | 0 |
| *Leptotila verreauxi* | 0 | 0 | 0 | 8 | 0 | 0 |
| *Myiopsitta monachus* | 62 | 0 | 23 | 85 | 0 | 0 |
| *Guira guira* | 31 | 0 | 8 | 31 | 0 | 0 |
| *Speotyto cunicularia* | 8 | 15 | 46 | 0 | 0 | 0 |
| *Podager nacunda* | 23 | 0 | 8 | 0 | 0 | 0 |
| *Choroceryle amazona* | 0 | 23 | 0 | 0 | 0 | 0 |
| *C. americana* | 0 | 31 | 0 | 0 | 0 | 0 |
| *Melanerpes candidus* | 0 | 0 | 0 | 8 | 0 | 0 |
| *Colaptes melanochloros* | 0 | 0 | 8 | 15 | 0 | 0 |
| *C. campestris* | 8 | 0 | 8 | 23 | 0 | 0 |
| *Cinclodes fuscus* | 8 | 8 | 0 | 0 | 0 | 0 |
| *Furnarius rufus* | 77 | 0 | 92 | 54 | 0 | 0 |
| *Schoeniophylax phryganophila* | 0 | 0 | 0 | 8 | 0 | 0 |
| *Asthenes hudsoni* | 0 | 0 | 0 | 0 | 8 | 0 |
| *Spartonoica maluroides* | 8 | 0 | 0 | 0 | 0 | 0 |
| *Phacellodomus striaticollis* | 0 | 0 | 0 | 0 | 0 | 23 |
| *Anumbius annumbi* | 23 | 0 | 62 | 69 | 0 | 8 |
| *Serpophaga nigricans* | 0 | 8 | 8 | 0 | 0 | 0 |
| *S. munda* | 0 | 0 | 0 | 8 | 0 | 0 |
| *Pyrocephalus rubinus* | 15 | 0 | 15 | 15 | 0 | 0 |
| *Lessonia rufa* | 0 | 0 | 8 | 0 | 0 | 0 |
| *Xolmis cinerea* | 0 | 0 | 8 | 8 | 0 | 0 |
| *X. irupero* | 15 | 0 | 46 | 0 | 0 | 0 |
| *Heteroxolmis dominicana* | 0 | 0 | 0 | 0 | 0 | 8 |
| *Satrapa icterophrys* | 0 | 15 | 8 | 0 | 8 | 0 |
| *Machetornis rixosus* | 31 | 8 | 38 | 38 | 0 | 0 |
| *Hymenops perspicillatus* | 0 | 0 | 0 | 0 | 8 | 0 |
| *Pitangus sulphuratus* | 54 | 38 | 69 | 46 | 0 | 0 |
| *Tyrannus melancholicus* | 0 | 0 | 8 | 0 | 0 | 0 |
| *T. savana* | 23 | 0 | 31 | 31 | 0 | 0 |
| *Phaeoprogne tapera* | 62 | 0 | 15 | 8 | 0 | 0 |

*continued on next page*

**Table 1 continued.**

| Species | Rice | Irrigation canals | Prairie | Forest | Wetland | Wild bushes |
|---|---|---|---|---|---|---|
| *Tachycineta leucorrhoa* | 38 | 0 | 46 | 0 | 0 | 0 |
| *Notiochelidon cyanoleuca* | 8 | 0 | 0 | 8 | 0 | 0 |
| *Hirundo rustica* | 8 | 8 | 0 | 0 | 0 | 0 |
| *Troglodytes aedon* | 0 | 0 | 23 | 38 | 8 | 0 |
| *Polioptila dumicola* | 0 | 0 | 0 | 8 | 0 | 0 |
| *Turdus rufiventris* | 8 | 0 | 0 | 8 | 0 | 0 |
| *T. amaurochalinus* | 15 | 0 | 0 | 8 | 0 | 0 |
| *Passer domesticus* | 15 | 0 | 23 | 8 | 0 | 0 |
| *Anthus lutescens* | 8 | 8 | 0 | 0 | 0 | 0 |
| *A. hellmayri* | 38 | 0 | 8 | 0 | 0 | 0 |
| *A. correndera* | 0 | 0 | 0 | 0 | 0 | 0 |
| *Icterus cayanensis* | 8 | 0 | 0 | 8 | 0 | 0 |
| *Agelaius flavus* | 15 | 0 | 0 | 8 | 0 | 8 |
| *A. thilius* | 100 | 0 | 0 | 0 | 15 | 15 |
| *A. ruficapillus* | 54 | 23 | 62 | 69 | 0 | 15 |
| *Leistes superciliaris* | 38 | 0 | 15 | 0 | 8 | 0 |
| *Pseudoleistes virescens* | 8 | 0 | 31 | 15 | 8 | 15 |
| *Amblyramphus holosericeus* | 46 | 0 | 0 | 0 | 15 | 8 |
| *Molothrus badius* | 31 | 0 | 23 | 31 | 15 | 0 |
| *M. rufoaxillaris* | 23 | 0 | 0 | 8 | 0 | 0 |
| *M. bonariensis* | 46 | 0 | 62 | 15 | 0 | 0 |
| *Paroaria coronata* | 0 | 0 | 23 | 38 | 8 | 0 |
| *Sporophila collaris* | 8 | 0 | 0 | 0 | 8 | 8 |
| *Poospiza nigrorufa* | 8 | 0 | 8 | 8 | 0 | 0 |
| *Zonotrichia capensis* | 0 | 0 | 31 | 15 | 0 | 0 |
| *Ammodramus humeralis* | 0 | 0 | 23 | 0 | 0 | 0 |
| *Embernagra platensis* | 31 | 0 | 0 | 0 | 0 | 15 |
| *Sicalis flaveola* | 77 | 0 | 54 | 54 | 0 | 0 |
| *S. luteola* | 0 | 0 | 31 | 15 | 0 | 0 |
| *Carduelis magellanica* | 0 | 0 | 31 | 0 | 0 | 0 |
| *C. carduelis* | 0 | 0 | 8 | 0 | 0 | 0 |

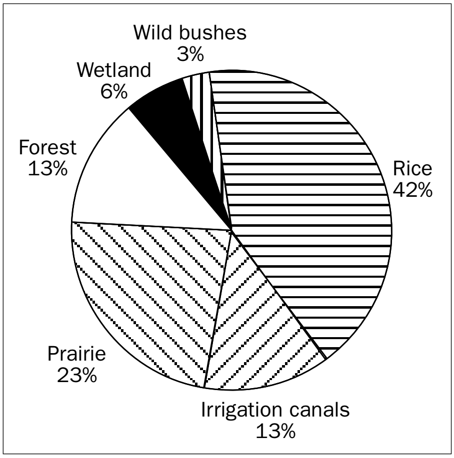

**Fig. 1. Percentage of species occurrence by habitat type in rice fields in Uruguay.**

*Phimosus infuscatus* (92%), *Egretta alba* (85%), *Egretta thula* (85%), *Plegadis chihi* (White-faced Ibis) (77%), *Dendrocygna viduata* (White-faced Tree-Duck) (77%), *Amazonetta brasiliensis* (77%), *Furnarius rufus* (77%), and *Sicalis luteola* (Grassland Yellow-Finch) (77%).

Many of the species recorded are seasonal in their occurrence. These migratory species can be assigned to three categories (Arballo y Cravino 1999): non-nesting summer visitors from the northern hemisphere, winter visitors from southern South America, and summer-nesting species.

Non-nesting summer visitors were *Mycteria americana, Pluvialis dominicana, Tringa flavipes, Tringa solitaria, Calidris melanotos,* and *Hirundo rustica.*

Winter visitors from South America were *Charadrius modestus, Cinclodes fuscus,* and *Lessonia rufa.*

Summer-nesting species were *Butorides striatus, Rostrhamus sociabilis, Podager nacunda, Phyroscephalus rubinus, Tyrannus savana, Tyrannus melancholicus, Phaeoprogne tapera, Tachicinetta leucorrhoa,* and *Notiochelidon cyanoleuca.*

Some of the species recorded are considered rare or scarce for Uruguay (Gore and Gepp 1978). These include *Botaurus pinnatus* (31% of occurrence inside the crops and 15% on terreplains), *Callonetta leucophirys* present throughout the study and sometimes nesting in the area, *Cathartes burrovianus* (38% of occurrence in crops, 8% on grassland), *Asthenes hudsoni*, and *Spartanoica maluroides*. The latter two species occurred in May in the rice-field environment. *Columbina talpicoti*, considered a species of uncertain distribution (Gore and Gepp 1978), was observed regularly throughout the area, sometimes in flocks of 10 to 15 individuals. *Sporophila collaris*, a species with irregular movement patterns, was recorded year-round. Our observations of these last two species confirm the previous findings of Arballo (1990).

Other species categorized as scarce for the region but present in the rice habitat were *Circus cinereus, Melanerpes candidus, Anthus lutescens, Icterus cayanensis,* and *Serpophaga munda.*

Of the 121 species observed during the study, 71% nest in the various habitat types of the study area. Those species recorded nesting in the rice-field habitat were *Gallinula chloropus, Agelaius ruficapillus, Tigrisoma linneatum, Botaurus pinnatus, Dendrocygna bicolor, D. viduata, Netta peposaca,* and *Pardirallus sanguinolentus*. After the conclusion of this study, the area was still visited by other species, thus increasing this list of species to 145 (92.3% of the total for these environments).

We recorded seven species of particular importance from the conservation point of view. *Agelaius flavus* and *Sporophila palustris* found on wild bushes are considered endangered. *Heteroxolmis dominicana* is classified as vulnerable. Four others are considered almost endangered: *Rhea americana, Spartonoica malluroides, Limnoctites rectirostris* (on wild bushes of "caraguatal"), and *Polystictus pectoralis* (in the transition zone between wild bushes and natural forest).

Our observations clearly demonstrate the value of the rice-field ecosystem (the rice crop itself and the associated habitat types) for a diverse avifaunal community. Conservation planning for enhancing biodiversity should incorporate the rice-field environment as an integral component of the landscape. Continued responsible management of the rice agroecosystem will contribute to the preservation of many bird species.

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## Notes

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# Integrating agronomic management practices with waterfowl populations in rice fields: opportunities and mutual benefits

C. van Kessel, J. Eadie, W. Horwath, F. Reid, J.E. Hill, and J. Fleskes

Rice production in California is largely concentrated in the Sacramento Valley. Before the land was converted into rice production, the valley was largely composed of interconnected wetlands that were inundated in the winter months when most of the rainfall occurred. Numerous ducks, geese, swans, and shorebird species winter in the Sacramento Valley as they migrate each fall from the northern regions of the western part of the United States and Canada. Following the conversion of the wetlands into rice production in the early 20th century, the winter habitat for waterfowl changed significantly.

California rice production is considered to be one of the highest in the world and grain yields of 12 t $ha^{-1}$ paddy rice are no exception. High grain yields are always associated with high residue yields as the harvest index for rice remains close to 0.5. Whereas in the early days residues were burned in the fall or spring, new regulations will reduce the area that can be burned to 25% of the total area used for rice production. As off-site use for rice straw remains limited, on-site disposal is often the only option left for farmers. To accelerate the decomposition process of the residue, almost half of the total rice production area in California is reflooded during the winter. Once the straw is dispersed across the field in the fall, the fields are flooded in late October or early November and drained again in early spring to allow preparations for seeding.

By providing alternative habitat during the winter, the rice fields attract large numbers of waterbirds, which use the fields mainly to forage and roost. When many birds visit rice fields, the extra disturbance they induce may increase the rate of decomposition of the rice straw. Early findings from an enclosure study suggest that indeed waterfowl enhanced decomposition. Because waterfowl forage on rice seeds and on weed seeds present in the rice fields, the waterfowl population may also affect the size of the weed seed bank. Depending on tillage practices, a reduction in weed herbicide use could also be anticipated. Along similar lines, the invertebrate population in the soil would also depend on the size of the waterbird populations that visit the winter-flooded fields. The effect of a reduced invertebrate population on nutrient cycling remains unknown.

Fully integrating rice agronomic practices with waterbird biology remains a largely unexplored area. Although rice management practices and the ecology of wildlife in rice fields have been studied independently, integrated research should be conducted to understand the influence of management on rice production and the health and reproductive capacity of waterbirds. The classical agronomic research design will have to be replaced with a design that can verify how the frequency of waterfowl visits to rice fields affects agronomically significant parameters such as the rate of decomposition of rice residues, pest occurrence, and grain yield.

An interdisciplinary research project with participation of agronomists, soil scientists, and wildlife biologists began in 1999. A large landscape-scale-driven project has been designed to quantify and predict the mutual benefits of producing rice in association with the waterfowl population.

Recent changes in California rice-field management have fundamentally altered the habitat available to wildlife. The Rice Straw Burning Act of 1991 (AB1378) reduced the rice area that can be burned each year and increased the use of alternative straw disposal methods such as winter flooding of rice fields, often in conjunction with some form of straw manipulation (rolled, baled, disked, or chopped). New developments in harvest technology (e.g., stripper-header harvesters) have added further to the variety of rice management practices currently operating in California that may affect waterbird foraging success.

Efforts to evaluate the agronomic efficiency of alternative rice management (ARM) practices have generally proceeded independently of efforts to evaluate the benefit of those practices to wildlife. Initial efforts from 1992 to 1998 were supported by the California Energy Commission, Bureau of Reclamation, Ducks Unlimited, and the Central Valley Habitat Joint Venture. The latter group recognized that the priority of funding should be, in order, to study (1) agronomy, (2) waterfowl ecology, (3) water issues, (4) effects on other species (such as shorebirds, endangered species, or anadromous fish). Agronomic concerns focus on issues such as the effect of winter flooding and high straw loads on nutrient availability, carbon buildup, and weed, disease, and insect pests. Wildlife concerns, in contrast, focus on the value of rice fields as a foraging or roosting habitat and on the potential effect of rice management practices on the quality of this habitat. Recently, there has been growing recognition of the value of rice lands to waterbirds and cooperative ventures between rice growers and wildlife agencies and organizations are increasing. However, a fully integrated effort to develop management practices that maximize the value of ARM to both farmers and wildlife is needed.

An interdisciplinary research program to evaluate the factors that limit rice production and waterbird sustainability in California seeks to integrate both agronomic and natural resource functions of ARM practices. The research comprises five components with linkages as indicated below (Fig. 1).

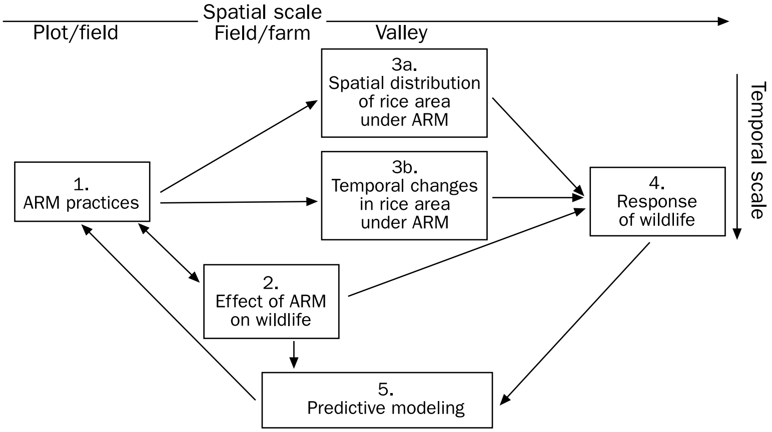

**Fig. 1. Linkages in alternative rice management (ARM) practices.**

## Component 1: ARM and rice production

Rice producers in California face recently imposed legislative changes in production practices. Alternative rice residue management practices that incorporate rice straw into paddy soils and winter flooding are currently being adopted in California because of the legislative restriction of open-field burning mandated by AB1378. These changes may alter the sustainability of rice production unless producers can adequately manage nitrogen in soil with continuous flooding and incorporated rice residues. Nitrogen-use optimization must achieve both efficient use of fertilizer N inputs and soil organic N. The influence of the soil organic fraction on soil fertility in rice-cropping systems is rarely considered, although soil organic matter (SOM) has been identified as the single most important indicator of soil quality in agricultural systems (Doran and Jones 1996). Previous studies indicate that soil organic N is the most important source of plant-available N for rice in California, representing 50–80% of the total N assimilated by the crop (Broadbent 1979, Mikkelsen 1987). The effect of plant residues and winter flooding on N immobilization into organic fractions of rice systems has received little attention, especially in California. The implementation of residue incorporation with winter flooding has been found to reduce straw waste for seedbed preparation and provide needed habitat for migratory waterfowl. Incorporation of 9–10 t $ha^{-1}$ of rice straw each year to soils with virtually continuous flooding may alter the composition and nature of SOM fractions, which in turn may have important agronomic implications for N availability by affecting the rate of N sequestration by SOM.

The immobilization of N into soil organic matter represents a substantial sink for fertilizer and crop residue N inputs in terrestrial soils. Field trials, using the stable isotope $^{15}N$, have shown that from 20% to 40% of fertilizer N remains behind in organic forms after the growing season in temperate-zone agricultural soils (Kelley and Stevenson 1996). The organic N stabilized in humic fractions generally resists microbial attack and is not readily available for plant uptake (Stevenson 1994). The long-term availability of the immobilized N is not often determined because of the lack of an adequate methodology. In California, rice-cropping systems have begun to use residue incorporation and winter flooding management on a routine basis. These changes in management have prompted the need for an understanding of the role of SOM in regulating the immobilization and mineralization of N in submerged soils, and the improvement of N-use efficiency in rice.

Soils continuously cropped to rice and flooded have been shown to differ in SOM composition and N availability compared with soils that have had fewer annual crops and longer aerated fallow periods. Rice yield declines have been seen in long-term experiments with continuously flooded double- and triple-cropped rice in the tropics. These yield depressions have been attributed to a declining effective-N supply while total soil N and C levels were maintained or increased (Cassman et al 1995). Organic components of soils subjected to long-term intensive rice culture were higher in total soil N, phenolic compounds, and humic acid-N and had a greater proportion of total soil organic N as humic acid-N than those soils that were less intensively cropped and flooded (Olk et al 1996). In California, residue incorporation and winter flooding may similarly increase phenolic accumulation and N sequestration into humic materials and result in lower N-use efficiency of added fertilizer N.

## Component 2: ARM and wildlife

Harvested rice fields in the Sacramento Valley provide the bulk of food resources for large populations of wintering ducks, geese, and swans, even with the presence of tens of thousands of acres of managed wetlands on national wildlife refuges, state wildlife areas, and private duck clubs. Good estimates of the amount of rice remaining in conventionally harvested fields are available (Miller 1987, Miller et al 1989), but estimates for the newer strip-harvested fields are only preliminary. In either case, little is known about the efficiency with which waterfowl forage in the different rice habitats and thus the usable proportion of rice seed in harvested fields is not known. Some may be unavailable because of the presence of straw, which covers seeds and makes discovery and consumption by waterfowl difficult. Furthermore, the proportion available may vary by rice management. For example, some fields are conventionally harvested (standing stubble) or strip-harvested (standing straw) and left with no additional treatment; some stripped fields are swathed or mowed, and either harvest type may be chopped or baled; many fields are disked/plowed; and many are still burned. All of these treatments may be left dry or flooded. Thus, waterfowl have a variety of rice-field habitat types to choose from and foraging efficiency may vary in each, making certain treatments more valuable for waterfowl management purposes (Day and Colwell 1998).

Reciprocal benefits to rice growers of attracting waterfowl also need further investigation. Research on small study plots in California indicated that waterfowl activity significantly reduced both the amount and average diameter of surface straw residues in flooded rice fields (Bird et al 1998 a,b). C, N, and lignin concentrations in the surface straw residue were also reduced on plots with duck activity, as were densities of invertebrates. These results suggest that considerable agronomic benefits may result for growers through attracting foraging waterfowl.

## Component 3: ARM and its spatial distribution

Resource agencies, agricultural interests, conservation organizations, and local governments require up-to-date information on the location and extent of seasonally flooded rice fields and wetlands for use in planning and evaluating land-use actions and effects on wildlife (Kempka et al 1996, Andree et al 1998). However, the highly variable nature of seasonally flooded habitat for wildlife makes it difficult to quantify and monitor. Flooded habitat changes in response to precipitation, management decisions, availability of water, and agricultural markets. Differences are observed between years and within a single fall/winter season (Spell et al 1995).

## Component 4: ARM and the response of waterfowl

Despite the loss of more than 90% of California's wetlands since the beginning of the 20th century, about 60% of Pacific Flyway and 18% of North American waterfowl winter in the Central Valley; millions more migrate through or nest there (U.S. Fish and Wildlife Service [USFWS] 1978, Gilmer et al 1982, Canadian Wildlife Service and USFWS 1986). The amount, distribution, and quality of waterfowl habitat in the Central Valley have changed markedly during the past decade because of changing agricultural practices and habitat conservation efforts of the Central Valley Habitat Joint Venture (CVHJV) and others. For example, the area of rice fields flooded after harvest in the Sacramento Valley increased 250% (from 60,000 to 150,000 acres) between 1985 and 1995 because of efforts of farmers and other conservationists to replace rice-straw burning with an economical and wildlife-friendly alternative. Waterfowl sanctuary in the Sacramento Valley provided by flooded rice fields increased nearly 700% (from 6,000 to 40,000 acres) during the same period because much of this additional flooded area was not hunted (CVHJV Technical Committee 1996). In contrast, flooded crop-land habitat important to waterfowl declined about 50% in the San Joaquin Valley because of efforts to reduce water use and agricultural wastewater (Barnum and Euliss 1991). Managed wetland area increased 36% in the Sacramento Valley (from 49,021 to 66,675 acres) but only 9% in the northern San Joaquin Valley (from 66,207 to 72,207 acres) during 1985-95 (CVHJV Technical Committee 1996).

Managers will need current information on waterfowl distribution, movement patterns, and habitat use throughout the wintering period to understand how waterfowl have responded to habitat changes and to estimate the area, distribution, and flooding regimes of habitats needed to support waterfowl populations in each Central Valley basin. For instance, to estimate waterfowl use-days and habitat requirements in each basin (USFWS 1978) at the desired Central Valley wintering populations of 4.7 million ducks and 865,000 geese and swans (CVHJV Implementation Board 1990), CVHJV planners assumed that waterfowl distribution would be similar to that observed during 1973-77 midwinter surveys and that waterfowl populations in each basin would gradually build during fall, peak at midwinter in early January, and gradually decline to desired summer breeding levels (Heitmeyer 1989a). CVHJV goals for wetland and agricultural habitats in each basin were developed assuming that waterfowl would increase their use of wetlands as wetland habitat was increased (Heitmeyer 1989a). Once waterfowl are in place, assumptions regarding their distribution, movements, and habitat use should be evaluated. Managers can then determine whether habitat goals and management strategies of the CVHJV and other programs need to be modified to ensure long-term viability of conservation programs and the wildlife populations they support.

When fully implemented, the CVHJV will affect activities on 950,000 acres of wetlands and agricultural lands in the Central Valley at a capital cost of more than $528 million and an annual cost of about $38 million (CVHJV Implementation Board 1990). It is crucial that farmers and managers of conservation programs such as the CVHJV have the information necessary to understand how wildlife respond to landscape-scale changes so that their large investments can provide the maximum sustained benefit for the natural resources.

## Component 5: ARM and predicting the effect on rice production and waterfowl population

Recent studies have indicated that the implementation of alternative rice management practices can have important benefits for waterbird conservation (Elphick 1998, Elphick and Oring 1998). To date, however, research on the relationships between waterbirds and rice-field management has been hampered in two ways. First, most research has been descriptive in nature. It is important to move into the realm of prediction by using mathematical models that blend ecological theory with practical information obtained in the field to estimate what will happen under different management scenarios. The second limitation on current knowledge is that different investigators have worked largely in isolation, coming together only occasionally at meetings to discuss their research. By integrating modeling research with studies of agronomy and waterbird behavior, we can effectively address the concerns of farmers, wildlife managers, and conservation biologists and develop recommendations on rice management to satisfy all interested parties.

Through rigorously designed large-scale surveys and experimental manipulations, flooded fields can support significantly greater densities of water birds (24 species) than fields left unflooded (Elphick 1998, Elphick and Oring 1998). Many of these species are thought to have undergone significant declines because of the conversion of Central Valley wetlands into agricultural lands (e.g., Heitmeyer et al 1989). Results indicate that agricultural flooding may provide the key to reversing these declines in waterbird populations.

Research to date has focused on understanding how the management of individual fields influences bird use. This information is useful for directing management at a local scale and needed to provide an accurate picture of the large-scale consequences of different management options.

Studying large-scale questions is difficult without enormous resources and considerable inconvenience to landowners. Given the information available, the most effective way to address these problems is through modeling. A modeling approach allows us to integrate the results from various studies to assess the effects of different management regimes without having to conduct the large-scale experiments that would otherwise be necessary. Once the modeling is completed, field tests should be conducted to validate the results.

The modeling approach should explicitly incorporate a spatial and temporal scale. Most agronomic analyses, of necessity, take place at a small spatial scale, usually in experimental field plots. However, wildlife responses typically occur at considerably larger scales, usually in fields or valley-wide. Studies will be integrated at the plot level with studies of changing rice management practices at the valley level. By examining wildlife response to both field-level management practices and valley-wide changes in the extent and location of rice area under ARM, the effect of ARM practices on wildlife at both small and large spatial scales will become known.

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AGRONOMY

# Influence of air temperature on rice population, length of period from sowing to flowering, and spikelet sterility

J.R. Alvarado

Because low temperature is relatively common in Chile during the rice growth period, the objective of this research was to understand the relationship among air temperature and rice population, length of period from sowing to flowering, and spikelet sterility in two Chilean varieties, Diamante-INIA and Oro. Planting date trials were carried out from the 1989-90 to 1997-98 seasons at the Quilamapu Regional Research Center (36°34′S). The experiment used a split-plot design with three replications. A linear relationship between average temperature and rice population was found for the first 5 d from sowing. The rice population varied from 104 to 224 plants m$^{-2}$ ($R^2 = 0.64$, $P \leq 0.01$) for Diamante-INIA and from 77 to 224 plants m$^{-2}$ for Oro ($R^2 = 0.5$, $P \leq 0.05$). The length of the period from sowing to flowering showed a linear relationship with average air temperature. The period varied from 91 to 129 d for Diamante ($R^2 = 0.68$, $P \leq 0.01$) and from 89 to 126 d for Oro ($R^2 = 0.5$, $P \leq 0.01$). Spikelet sterility showed a quadratic relationship with temperature, and the best curve fitting was found when the temperature was averaged over 5 d at 50% flowering ± 2 d. In Diamante ($R^2 = 0.88$, $P \leq 0.01$), sterility varied from 7.8% to 65.3% and in Oro ($R^2 = 0.68$, $P \leq 0.01$) from 6.4% to 39.3%.

The rice crop began as a commercial activity in Chile during the 1937-38 season in an area known as the "soil rice area" located in the irrigated Central Valley between 34°S and 36°36′S. Rice soils are characterized by poor drainage because of high clay content and/or the presence of a waterproof horizon. Rice is cultivated under flooded conditions from sowing to maturity. Seeds are pregerminated and sown in direct form. In the past few years, the area has stabilized around 30,000 ha with an average yield of 4.3 t ha$^{-1}$.

In the rice area, the climate is temperate and low temperature is relatively common throughout the growing period. Cold affects rice production mainly at germination and during flowering. The consequences of cold temperature are plant losses (increasing in the growing period), yellowing of leaves, and increasing spikelet sterility. Therefore, cold tolerance in all stages of growth is one of the goals of the national rice breeding program.

The objective of this study was to understand the relationship between air temperature and the following variables: rice population, length of the growth period from sowing to flowering, and spikelet sterility.

## Materials and methods

Data were obtained from planting date trials carried out from the 1989-90 to 1997-98 seasons at the Quilamapu Regional Research Center (36°34′S), Instituto de Investigaciones Agropecuarias, Chile. The planting dates ranged from 27 September to 23 November (Table 1). Data from the two main Chilean varieties were used: Diamante-INIA, a late variety with translucent long grain, and Oro, a medium-early variety with bold and short grain and high white belly presence. The experimental design corresponded to a split plot with three replications, where the main plot was the planting date and the subplot the variety. In the 1996-97 and 1997-98 seasons, the main plot was the variety.

Air temperature data were taken from the Agrometeorological Station, University of Concepción. The rice population was obtained by counting the rice plants with at least three leaves during four seasons for Oro and five seasons for Diamante-INIA, on an area of 0.50 × 0.50 m. The date of flowering was taken when the rice had 50% of plants with flowering (CIAT 1983). For spikelet sterility, five panicles per plot were selected and spikelets set were counted in the laboratory. The best fit among rice population, temperature, and spikelet sterility was determined over a period of 5 d: for population, 5 d after sowing; for spikelet sterility, 2 d before to 2 d after flowering.

**Table 1. Date of sowing of varieties Diamante-INIA and Oro in different seasons.**

| Crop seasons | Planting date | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| 1989-90 | 29 Sep | 9 Oct | 23 Oct | 6 Nov | 20 Nov |
| 1990-91 | 27 Sep | 9 Oct | 23 Oct | 6 Nov | 20 Nov |
| 1991-92 | 28 Sep | 10 Oct | 24 Oct | 7 Nov | 21 Nov |
| 1992-93 | 28 Sep | 10 Oct | 24 Oct | 7 Nov | 21 Nov |
| 1993-94 | 28 Sep | 8 Oct | 22 Oct | 5 Nov | 19 Nov |
| 1994-95 | 28 Sep | 10 Oct | 24 Oct | 7 Nov | 21 Nov |
| 1995-96 | 3 Oct | 11 Oct | 26 Oct | 9 Nov | 23 Nov |
| 1996-97 | 27 Sep | 11 Oct | 25 Oct | 8 Nov | 22 Nov |
| 1997-98 | 30 Sep | 10 Oct | 24 Oct | 7 Nov | 21 Nov |

Regression analysis was made using Microsoft Excel and following the procedure of Little and Hills (1991).

## Results and discussion

### Air temperature

The climate of the Chilean rice area is a temperate, Mediterranean type, with large differences between the minimum and maximum temperature. The 1995-96 and 1997-98 cropping seasons had an average of 17.8 and 16.8 °C, respectively, in the period of growth from October to March (Table 2). The first one was considered a good season and the second one a cold season. The 1996-97 season had a good average temperature, but was a dry season, and the rice crops suffered because of a lack of water. According to Sthapit et al (1997), who cited different authors, a temperature below 20 °C can be considered as a chilling temperature and may result in some symptoms in rice such as short plants, prolonged plant duration, poor panicle exsertion, leaf yellowing, and spikelet sterility.

### Rice population

The rice population varied from 104 to 224 plants m$^{-2}$ with a range of air temperatures from 10.9 to 19.3 °C for Diamante-INIA and from 77 to 224 plants m$^{-2}$ with temperatures ranging from 10.9 to 18.8 °C for Oro. The minimum temperature in the experiment is similar to the minimum temperature for germination and seedling emergence, 10 to 12 °C, reported by Yoshida (1981) and Tinarelli (1989). Oro emergence was lower than that of Diamante-INIA with colder temperature. These data suggest that Oro is more susceptible to chilling during the germination and seedling stage. Grau et al (1987) reported that Diamante-INIA was less susceptible to cold than Oro in these development stages. The best adjustment between temperature and rice population (number of plants) was a linear relationship in Diamante ($R^2 = 0.64$ at $P \leq 0.01$) and Oro ($R^2 = 0.50$ at $P \leq 0.05$) (Fig. 1). These results agree with those of Yoshida (1981), who affirmed that temperature has a large influence on germination, especially in the first week during the seedling stage. Sthapit (cited by Sthapit and Witcombe

**Table 2. Monthly average temperature (°C) during different rice seasons.**

| Crop season | October | November | December | January | February | March | Average |
|---|---|---|---|---|---|---|---|
| 1989-90 | 13.7 | 17.0 | 18.3 | 20.3 | 19.1 | 17.0 | 17.5 |
| 1990-91 | 12.7 | 15.0 | 18.3 | 19.1 | 19.7 | 16.5 | 16.9 |
| 1991-92 | 13.1 | 15.8 | 16.2 | 20.8 | 19.1 | 18.3 | 17.2 |
| 1992-93 | 12.1 | 16.3 | 17.7 | 20.6 | 21.4 | 18.9 | 17.8 |
| 1993-94 | 13.5 | 15.2 | 18.3 | 20.3 | 19.6 | 18.3 | 17.5 |
| 1994-95 | 13.9 | 16.0 | 18.6 | 19.4 | 19.2 | 17.1 | 17.3 |
| 1995-96 | 13.2 | 16.2 | 21.1 | 19.2 | 19.2 | 18.0 | 17.8 |
| 1996-97 | 13.9 | 17.2 | 18.8 | 20.0 | 19.2 | 19.8 | 18.2 |
| 1997-98 | 12.3 | 15.0 | 17.7 | 19.6 | 19.0 | 17.2 | 16.8 |

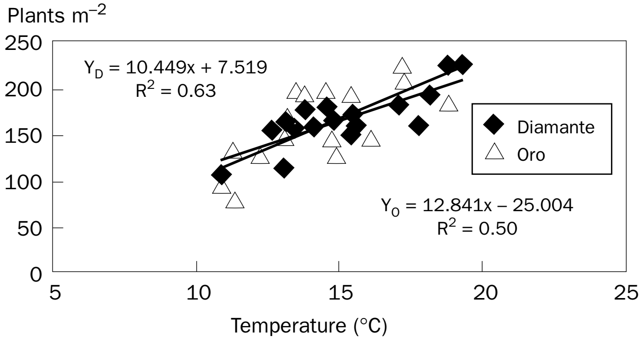

**Fig. 1. Relationship among rice population, plants m$^{-2}$, and average air temperature during 5 d after planting date for varieties Diamante-INIA and Oro. $Y_D$ = Diamante regression curve, $Y_O$ = Oro regression curve.**

1998) added that a common symptom of chilling damage is a poor and delayed germination rate. Rice establishment is affected by many factors such as soil preparation and water depth. The results in this experiment could have been affected by these uncontrolled factors among seasons. With uniform conditions, $R^2$ could be greater than that obtained in the experiment. Early planting date (September and the first days of October) showed a greater chilling risk than normal and late sowing dates.

## Sowing to flowering period

Tinarelli (1989) related delayed flowering to temperature lower than 20 °C during the change from the vegetative to reproductive period. Shibata (1979) related delayed heading to "when rice plants are subjected to low temperatures in the period 45–35 d before heading." Kaneda, cited by Satake (1976), said that delayed heading is a common cool injury. The results showed that the length of the period from sowing to flowering had a negative linear relationship with average air temperature (Fig. 2). The period varied from 91 to 129 d for Diamante ($R^2 = 0.68$ at $P \leq 0.01$) and from 85 to 120 d for Oro ($R^2 = 0.50$ at $P \leq 0.01$). When data on temperature accumulation and degree days were analyzed by comparison, results showed that Oro needed less accumulation of temperature than Diamante-INIA to reach the flowering stage. The range for Oro was 1,666.7 to 1,954.6 °C and for Diamante it was 1,752.6 to 2,116.9 °C.

The delay in flowering produced an increase in the risk of greater cold during this stage because the temperature decreased in February and March when compared with January (Table 2).

## Spikelet sterility

Cool weather can cause panicle sterility by interfering with pollen grain formation (Peterson and Jones 1975). The critical temperature for inducing spikelet sterility varied from 10 to 15 °C (Tinarelli 1989). The best curve for fitting air temperature and spikelet sterility was found when the temperature for 5 d during flowering was averaged (the day of 50% flowering and 2 d before and 2 d after). Regarding this,

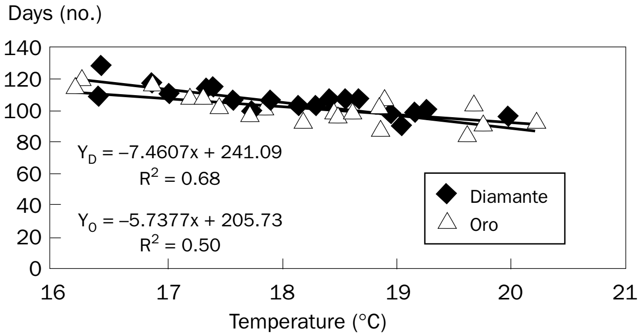

**Fig. 2. Relationship between average air temperature and length of period from sowing to flowering. $Y_D$ = Diamante regression curve, $Y_O$ = Oro regression curve.**

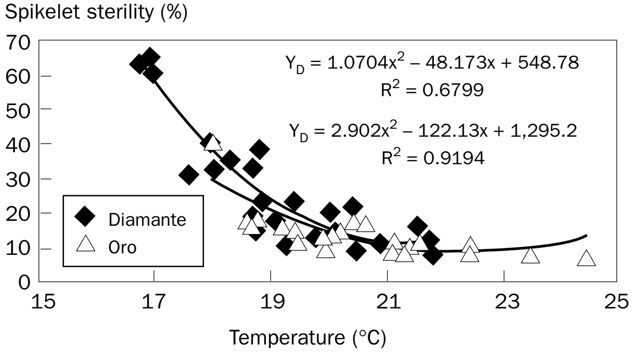

**Fig. 3. Relationship between spikelet sterility and 5-d average air temperature during flowering for varieties Diamante-INIA and Oro. $Y_D$ = Diamante regression curve, $Y_O$ = Oro regression curve.**

Tinarelli (1989) stated that the presence of cold for more than 3 or 4 d increased the probability of spikelet sterility. Yoshida (1981) and Lee (1979) reported that the most sensitive stage for increasing sterility is the meiotic stage, but heading or flowering is the second most sensitive stage for low temperature (Yoshida 1981). In our experiment, we studied only the relationship between temperature and spikelet sterility at the flowering stage and the two varieties showed a good quadratic relationship with temperature. In Diamante ($R^2 = 0.88$ at $P \leq 0.01$), sterility varied from 7.8% to 65.3%, and in Oro ($R^2 = 0.68$ at $P \leq 0.01$) it ranged from 6.4% to 39.3%. Oro showed less sterility probably because of its greater earliness, which allowed it to escape from low temperature during flowering.

It is possible to affirm that average temperature under 20 °C for 5 d during flowering time increased the probability of obtaining spikelet sterility greater than 10% to 12%, values considered normal in rice production.

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# Nitrogen fertilizer management: from standard recommendations to decision-making aids—the case of rice cultivation in the Camargue area of France

J.M. Barbier and J.C. Mouret

Nitrogen fertilization recommendations for flooded rice fields are similar worldwide. In the French rice-growing area of Camargue (43°N), recommendations are to (1) split one-half to two-thirds of total N before flooding (basal application) and between half and one-third at panicle initiation (topdressing) and (2) for the first basal application, incorporate the nitrogen into the soil followed by a continuous seasonal flooding. These recommendations are justified to minimize nitrogen losses through leaching, volatilization, and denitrification.

Surveys were conducted in the Camargue area to identify farmers' fertilization management practices. The surveys revealed that split applications were highly adopted by farmers whereas the second recommendation was less widespread. This paper explains why Camargue farmers cannot adopt it. Taking into account farmers' constraints, the authors question the local relevance of these recommendations. By looking closely at how the rice-farming system functions, we found that these recommendations led farmers to sow late. In the Camargue, sowing rice before 20 April is usually constrained by cold temperature, but rice must be sown before 10 May to avoid late flowering and cold temperature-induced floret sterility. Rainfall, which is highly probable during this period, can delay sowing and increase the time between fertilization and sowing. This paper argues for a new approach to N fertilization taking into account the complete technical sequence from land preparation to sowing. We modeled farmers' decision-making processes for rice cultivation and, using decision-support software, we compared dates of sowing with and without basal fertilizer application for the last 25 years' climatological data (this allowed us to conduct a risk analysis). Experiments and modeling related to the effects of no basal nitrogen application and the date of sowing on potential yields should allow us to propose more suitable recommendations to farmers.

Nitrogen-use efficiency is only 20% to 40% of applied fertilizer in paddy soils (De Datta and Patrick 1986). This is due to losses through various physical, chemical, and biological processes: ammonia volatilization, denitrification, leaching, and lateral drainage, to name some of the most important ones.

## The "normative" approach

### Scientific knowledge and technical recommendations

In France, similar results have been obtained. Without the application of nitrogen fertilizer, yields from 4 to 6 t $ha^{-1}$ can be achieved (on an annual basis). When fertilizer is applied, the apparent absorption coefficient[1] of the fertilizer has been estimated at 20% to 25% (Barbier et al 1989). A production of 50 kg grain $kg^{-1}$ of applied N constitutes a theoretical upper limit (De Datta and Patrick 1986). In France, yield increases vary from 10 to 20 kg grain $kg^{-1}$ N applied (Barbier et al 1989). Thus, N supplied by soil, irrigation water, and atmosphere is considerable in Camargue, from 80 to 130 kg N $ha^{-1}$ or approximately two-thirds of seasonal uptake.

Several years of agronomic surveys and rice field experiments in the Camargue have shown a stable relationship between yield and soil organic matter content (Barbier et al 1994).

The literature is full of experimental results and recommendations to advise farmers how to improve N-use efficiency in paddy fields. All these results are consistent for both Asian (Vergara 1984) and temperate European rice (Russo et al 1990). These results show that

- N fertilizers should be incorporated into the reduced soil zone to lessen nitrogen losses through ammonia volatilization, and nitrification-denitrification. As a consequence, from one-half to two-thirds of total N is often incorporated into the soil before planting.
- Following N incorporation, the fields must be flooded to prevent alternate soil drying and wetting, which transforms $NH_4$ to $NO_3$ to $N_2$, resulting in potentially high losses to the atmosphere.
- The total amount of N must be correctly estimated before application, taking into account the growing season (dry or wet), native soil fertility, and the characteristics of the specific variety. The correct timing of applied N has a marked effect at tillering, panicle initiation, and heading, all contributing to yield.

### Field-level constraints

In a preceding article, we emphasized the difficulties farmers face in implementing current N fertilizer recommendations (Barbier et al 1994). We took into consideration the ecological conditions (soil and climate) of the Camargue area as well as the se-

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[1] Quantity of nitrogen removed from the soil in a fertilized plot minus quantity of N removed without N fertilizer application divided by amount of fertilizer applied.

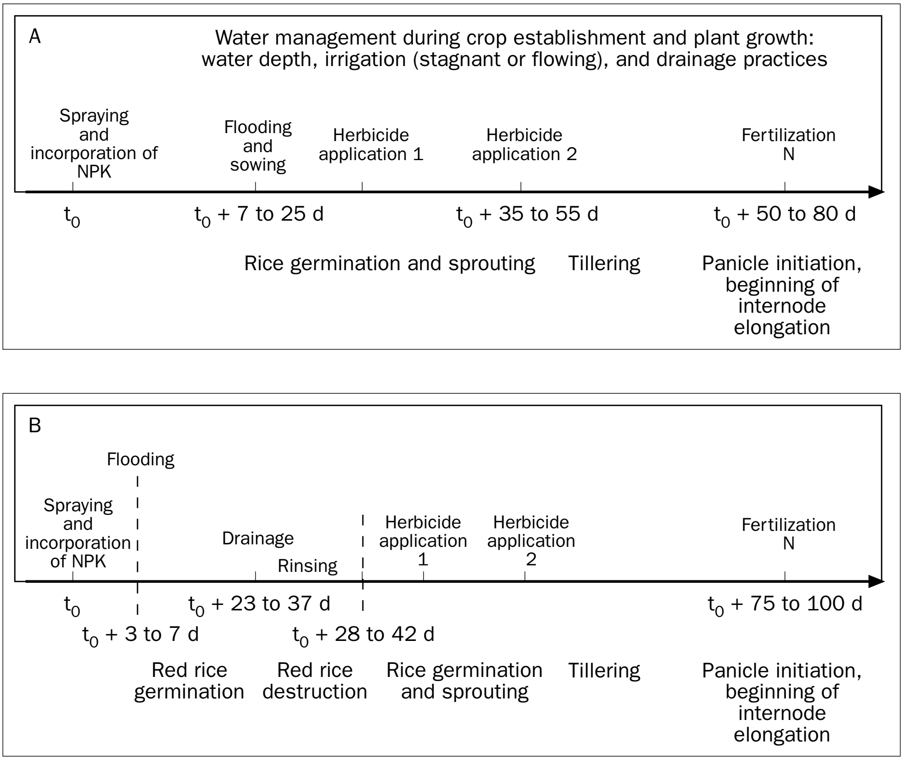

**Fig. 1. Main features of the cropping scheme commonly practiced in the Camargue area during the vegetative growth of rice: (A) usual cultivation, (B) cultivation with red rice control strategy. Dates of sowing are from 20 April to 10 May.**

quence of operations farmers use to manage their crop. By doing so, N fertilization techniques were replaced in the context of a "logical, ordered combination of crop management practices (including flooding, sowing, weeding, and others) applied to a set of fields cultivated with a given plant species and achieving a target yield." We assumed that this target yield could vary among farmers and from field to field. This "objective-oriented analysis" allowed us to highlight farmers' strategies and to explain the diversity of farmers' practices in fertilizer management. We identified two major sets of constraints (Fig. 1):

1. The time separating the incorporation of N, P, and K fertilizers and crop stand establishment is long. This is due to (1) a long duration between fertilizer application and flooding-sowing (seeds are mostly broadcast into the floodwater and drilling is rare) and (2) poor germination and low plant vigor. After sowing, temperatures are close to the minimum requirement (assumed to be 12–13 °C); as a consequence, rice seedling growth is poor, sometimes plant emergence is completely inadequate, and resowing becomes necessary. Plant stand density is highly variable and unpredictable from one field to another (from 50 to 500 plants m$^{-2}$). Late sowing is not recommended

because the maturation period of Camargue's varieties (from 140 to 160 d) may result in spikelet sterility. Hence, delays of up to 30 d may occur from one field to another based on the soil conditions needed for proper basal N application, thus adding 3 to 4 wk to the season. Furthermore, during this phase, N requirements are very low.

2. During the time separating fertilizer application and crop establishment, farmers are compelled to drain and dry their fields several times. The reasons are
   - To avoid seed burial and the uprooting of plantlets caused by wave action during frequent windy periods. Seed burial reduces germination and emergence because of anoxic conditions around the seeds and newly elongating roots.
   - To facilitate rooting of rice through soil reoxygenation. In anoxic conditions, shoot growth is promoted while root growth is reduced, resulting in poor anchorage.
   - To limit the proliferation of harmful algae and rice midge (Choronomidae).
   - To control weeds, especially wild red rice. Herbicides for wild rice are not effective; therefore, farmers have adopted cultural methods. Among them is early plowing, followed by seedbed preparation and flooding to encourage wild rice germination (irrigation starts in mid-March instead of mid-April). After 3 to 4 wk of alternate or permanent flooding, fields are drained and wild rice seedlings are destroyed by using nonselective herbicides or tillage. Rice is subsequently sown after reflooding. Figure 1 shows that, in this case, basal N is applied before the onset of the first irrigation.
   - Finally, the chemical control of barnyardgrass and *Cyperus* sp. requires farmers to partially or totally drain their fields after sowing, depending on the need for foliar coverage of the herbicide used.

### The limits of experimentation

Most of the current recommendations on N management originate from trials with transplanted rice in which N is incorporated into the soil just before flooding and transplanting. Following transplanting shock, the demand for N is higher than in direct-seeded rice because of the larger plant size. In the Camargue area, rice was transplanted in the 1960s and '70s and the current recommendation for basal N was relevant. Despite the shift from manual transplanting to mechanized direct broadcasting in the 1970s, N fertilizer recommendations remain unchanged.

On-farm and on-station trials were conducted from 1987 to 1991 to assess the effects of plus or minus basal N compared with other methods of N timing on rice growth, development, and yield (Barbier et al 1994). We compared two splits with the same total N (150 kg ha$^{-1}$): (1) two-thirds basally incorporated before flooding and sowing and one-third broadcast into the water at panicle initiation and (2) one-half broadcast at the beginning of tillering and one-half at panicle initiation. No significant difference for yield was found.

We concluded that it was necessary to reevaluate N fertilizer recommendations for the Camargue. To achieve these goals in a reasonable time, especially because of rapidly changing economic conditions, we decided to use decision-making aids to provide advice to farmers.

## The decision-making aid approach

### Theoretical position

This approach deals with decision-making tools for agricultural managers in a fast-changing economic environment with uncertainties and risk. Uncertainties include market prices, new agricultural policies and rules, and forthcoming constraints related to protection of the natural environment as well as the reliability of our technical recommendations. Uncertainty is distinct from risk. For risk, the probability for forthcoming events can be determined, for example, climate, for which the probability of a specific climatic event (assuming that long-term data are available) can be calculated. With this approach, both uncertainty and risk can be taken into account. Therefore, the objective of this approach is to build new sources of information with the decision-makers (farmers) to help them choose among different possible solutions. To reach this objective, we followed several principles:

- Establish a direct relationship between farmers and technical advisers to build a common representation of the functioning of the farming enterprise. Technical recommendations for farmers' specific situations can be adjusted at this early stage. However, the objective of this approach was to identify a range of possible solutions to a problem and simulate its effects on a given set of performance criteria (for example, yields, economic returns, rate of manpower deployment, etc.) taking into account environmental changes (for example, yearly climatic variability or evolution of market prices).
- Supply farmers with information enabling them to consider a broad range of possible decisions likely to satisfy their personal goals and not just to optimize the functioning of the farm system by maximizing economic returns. As a consequence, the performance criteria may vary among farmers.
- Use the approach of the "limited rationality" theoretical framework (Simon 1978), which recognizes that decision-makers (farmers) have difficulty in forming a clear view of all possible alternatives and their consequences. Thus, decisions have to be made with "limited" knowledge.

### Application at the farm level

These principles were applied to the specific area of the farm enterprise related to rice growing. By doing so, it was expected that

- A better comprehension of current farmers' practices concerning fertilizer management would be achieved.
- N management would be studied within the context of other rice cultivation practices so that its importance relative to other practices could be discussed.

- Alternative methods of cultivation would be tested under a wide range of scenarios to help farmers in decision making.

To facilitate modeling, the inquiry is carried out using a precise framework in three steps as follows:

- Step 1. Identification and characterization of resources such as land and water, equipment, and labor force for specific tasks. For example, a tractor fitted with iron wheels can be used only in wet conditions, a given skilled worker may be assigned exclusively to water management, and the daily labor force and its variation over the year must be estimated.

  A farm map must be developed assuming that all land units are not equivalent for rice production. The spatial division of land must be identified by the farmer as "blocks" or homogeneous units of land management (1) not supporting the same cropping pattern or sequence of technical operations, for example, fields with rice monoculture versus fields with a rice-wheat rotation; (2) with different cultivation, for example, fields in which heavy rainfall may stop land preparation for several days; (3) where the performances of cultivation operations are different, for example, the time required for plowing a heavy clay versus a sandy soil; and (4) with different starting points for a specific operation, for example, the timing of plowing the lowest-elevation fields rich in clay.
- Step 2. Identification of the operational rules regarding the use of farming resources (labor force and equipment) in space and time. The farmer must complete a sequence of tasks going from the harvest of the preceding crop to the harvest of the next crop. The objective is to identify more or less stable rules concerning
  - spatial priorities for the starting location and the specific tasks and
  - the temporal succession of tasks in a specific block of fields. These may refer to
    - a rigid time indicator, for example, "secondary tillage starts the first of March,"
    - an indicator relative to the state of progress of the preceding task, for example, "secondary tillage starts when 50% of the total rice-growing area has been plowed,"
    - an indicator relative to the state of the environment, for example, "sowing does not start unless the daily minimum temperature rises above 10 °C during two consecutive days."
- Step 3. Identification of the conditions for cultivation and assessment of implement performances depending on the type and power required as well as the block where they operate. It is important to identify the climatic features, which may affect a given task. In Camargue, rainfall, wind, and temperature are important parameters to be considered. The threshold values, which induce a break in the implementation of a task, must be assessed, for example, wind speed above 50 km $h^{-1}$ means that land leveling must stop because the laser does not work properly.

- Step 4. Identification of the main and subsidiary objectives of farmers at any time. A subsidiary objective is a checkpoint situated at a specific moment when the farmer evaluates the state of progress of his cultivation operations and compares it to the schedule planned for the achievement of the main objective. For example, the state of progress of primary tillage (plowing) is generally evaluated at the beginning of spring, but, if at a given time farmers believe that they are late (because they have not finished with this task), the scheduled program for the following month may be changed.

### Implementing the procedure for predicting sowing dates of rice

In the case we consider here, the date of sowing of rice has been chosen as the farmers' main objective. All the technical operations carried out from the harvesting of the preceding crop until the sowing of the next crop are performed to achieve this goal. Sowing dates are crucial for the success of rice productivity; thus, farmers have set deadlines they do not want to overshoot. Farmers for whom high yields are not an objective will set the completion of seeding on their farm for 20 May, whereas others, who aim at high yields, want to complete the sowing of the earliest varieties on 5 May and that of the latest on 30 April.

Throughout this clarification phase, the knowledge of agronomists (experts) is simply used for the inquiry and not to formulate opinions. At this stage, we assume that the expert knowledge is in concert with the farmers' circumstances and goals. A "model for action" or strategic work organization is developed. This means that farmers are asked to clarify management rules for normal conditions. Usually, the question is, "What kind of organization do you want to put into practice?" It is obvious that the farmers' personal "model for action" includes anticipation, which should lessen the effects of nonregular events.

So, the implementation of this methodology leads to the identification of farming resources and rules of time allocation to different blocks. These rules are based on "indicators" that are farmer-specific. All these rules, which reveal farm functioning, can be easily put in simple sentences of the following type:

if "checked indicator(s)," then "action"

At the end of this phase, a model has been built that represents the usual farmer's work organization. This is followed by a computerized application called OTELO (Attonaty et al 1990, Papy et al 1988), compatible with applying such rules. OTELO deals with work organization at the farm level for a given season, enabling an evaluation of the risks for various combinations of operations and the means of production used to implement them (labor force and equipment).

In this example, we assessed the risks of failure regarding the expected sowing dates of rice. The success or failure in reaching this objective was analyzed as a consequence of a specific organization and means of production at the farm level. An initial simulation used climatic data of the most recent year, then compared the results with the dates of sowing registered by the farmer, thus enabling validation of the model. If discrepancies appear between estimated and observed dates, the model must be reviewed. After validation, simulations over a wider range of climatic conditions were undertaken, then possible (virtual) changes in available resources or organizational rules were simulated and the results assessed with the farmer.

## Results

Table 1 and Figure 2 show the results obtained for one case study. Table 1 presents the resources allocated to the successive rice cultivation operations and their performances. Figure 2 shows a simplified "model for action" for this particular farmer, including rules of time-succession of the different tasks, climatic indicators that stop the implementation of some tasks, and rules of priority between blocks of fields.

Figure 3 shows the field layout of this rice farm and the elapsed time between fertilizer (NPK) application and flooding date. This helps to understand the work organization pattern presented in Figure 2. Because of a limited number of available days for tillage, the low fields (clayey soils with low drainage capacity) are awarded priority for tilling and fertilizer incorporation. On the other hand, flooding starts with the highest fields and moves downward because (1) topographical position and soil type require irrigation of elevated fields with a maximum head of water and (2) the irrigation stations are situated at the high points of the farm and leakage from ground channels is avoided by first irrigating the fields that are located nearby. These constraints explain why the time elapsing between fertilizer application and flooding-sowing is so long in the block of fields situated in the low-lying parts of the farm territory.

This same work organization pattern was simulated based on the date of rice sowing considering 25 different climatic scenarios (25 years). It shows that the farmers' objective sowing deadline (10 May) is overshot in 19 cases. This high rate of failure is due to (1) farmers hesitating to apply basal N too early because of the extended period between fertilizaton and flooding and (2) because rainfall is likely to occur during N incorporation, thus creating a temporary cessation of this operation. Figure 4B presents the results of the same simulation when no nitrogen fertilizer is applied before sowing, P and K fertilizers are applied in February-March when the working calendar is not tight, and N is topdressed at tillering and panicle initiation. The results in achieving the main objective (to complete sowing rice before 10 May) are much better.

In light of these results, the current recommendations calling for basal N fertilizer management must be reevaluated because the number of days between N incorporation and flooding to minimize N losses cannot be reduced by farmers unless they invest in costly irrigation systems (for example, building concrete canals). Underwater or wet cultivation can also be considered but this requires further investigation on machinery. Additionally, there is no significant difference in yield when N is applied before sowing or split in topdressing, whereas late sowing greatly increases the risk of spikelet sterility, poor grain filling, and low yield. Obviously, to completely assess the risks to the farmer, a simulation model for predicting flowering and harvesting

**Table 1. Assignments of farming resources to the successive cultivation tasks from land preparation to sowing of rice (farm with 205 ha of rice and 16 ha of winter wheat).**

| Cultivation tasks | Tractors (total number and type used, horsepower)[a] | | | | | | Implements | | | | | | Workers (total number and individuals' specific assignments) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total no. | 80 hp | 105 hp | 130 hp | 185[b] hp | 185[c] hp | Plow | Rotovator | Spreader | Disk | Rotary tiller | Land plane + laser | Number | Worker 1 | Worker 2 | Worker 3 | Performance ($ha\ d^{-1}$) |
| Primary tillage | 2 | | | | × | × | × | | | × | | | 2 | 0 | 0 | 0 | 12–20 |
| Secondary tillage | 2 | | | × | 0 | 0 | | × | | × | | | 2 | 0 | 0 | 0 | 20–25 |
| Land leveling | 1 | | | | 0 | 0 | | | | | | × | 1 | 0 | 0 | 0 | 14–16[b]<br>25–30[c] |
| Harrowing | 1 | | | × | | | | | | × | | | 1 | 0 | 0 | 0 | 25–30 |
| NPK broadcasting | 1 | × | | | | | | | × | | | | 3 | × | × | × | 35–40 |
| NPK soil incorporation | 2 | | | × | 0 | 0 | | × | | | × | | 2 | × | | × | 25–30 |
| Flooding | | | | | | | | | | | | | 1 | | × | | 12–14 |
| Sowing | 1 | | × | | | | | | × | | | | 3 | × | × | × | 35–40 |

[a] Key × = tractors, implements, or workers necessarily assigned to this task, 0 = tractors, implements, or workers to be chosen among the tasks marked. [b] When the preceding crop is not rice. [c] When the preceding crop is rice.

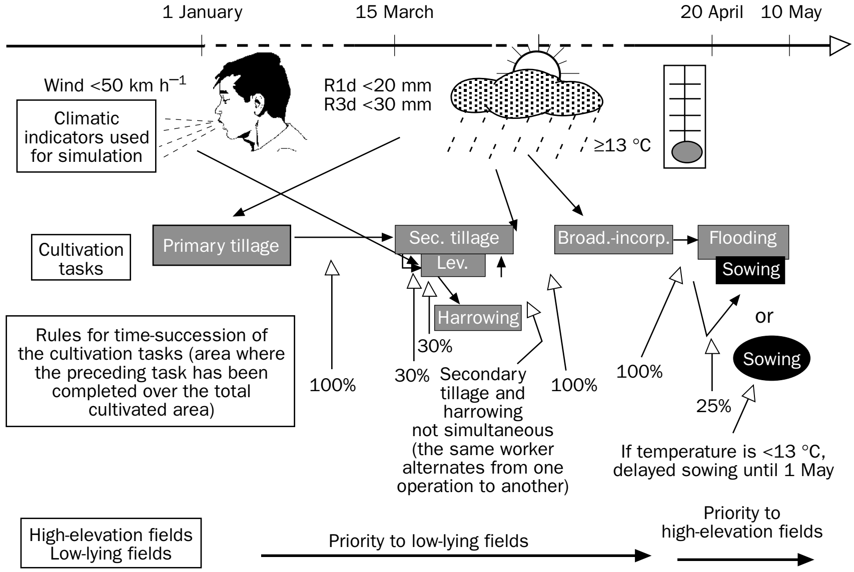

**Fig. 2. A simplified farmer's model for action (same farm as in Table 1). R1d = daily rainfall, R3d = total rainfall on 3 consecutive days, Sec. tillage = secondary tillage, Lev. = land leveling, Broad.-incorp. = NPK broadcasting-incorporation.**

dates as well as the final yield would be helpful. For a given date of sowing and a given variety, expected yields over a wide range of climatic conditions from flowering to maturity (using the same 25 years) could be calculated and the risk of a yield reduction because of a shortage of N could be compared for topdressing versus basally applied N.

## Conclusions

This study demonstrated that technical recommendations focused too narrowly on a specific cultivation operation can produce advice not consistent with the objectives of profit maximization and risk minimization. Advice must take into consideration the complete set of cultivation operations at the farm level, at which they may be regarded as tasks that mobilize farming resources and farmers' knowledge and skills. Yield maximization at the field level is seldom consistent with profit maximization at the farm level.

We used a simulation software to analyze the consequences of farmers' work organization and farm structure. This tool enabled us to consider the farm as a whole system and to introduce interannual climatic variation and its effects on farm operations and thus assess the risks involved in different types of farm organization or structure. The implementation of this procedure reveals the "indicators" used by farmers and improves their understanding of the basis for decisions. These indicators mobilize farmers' knowledge developed from their own experience, exchanges with a net-

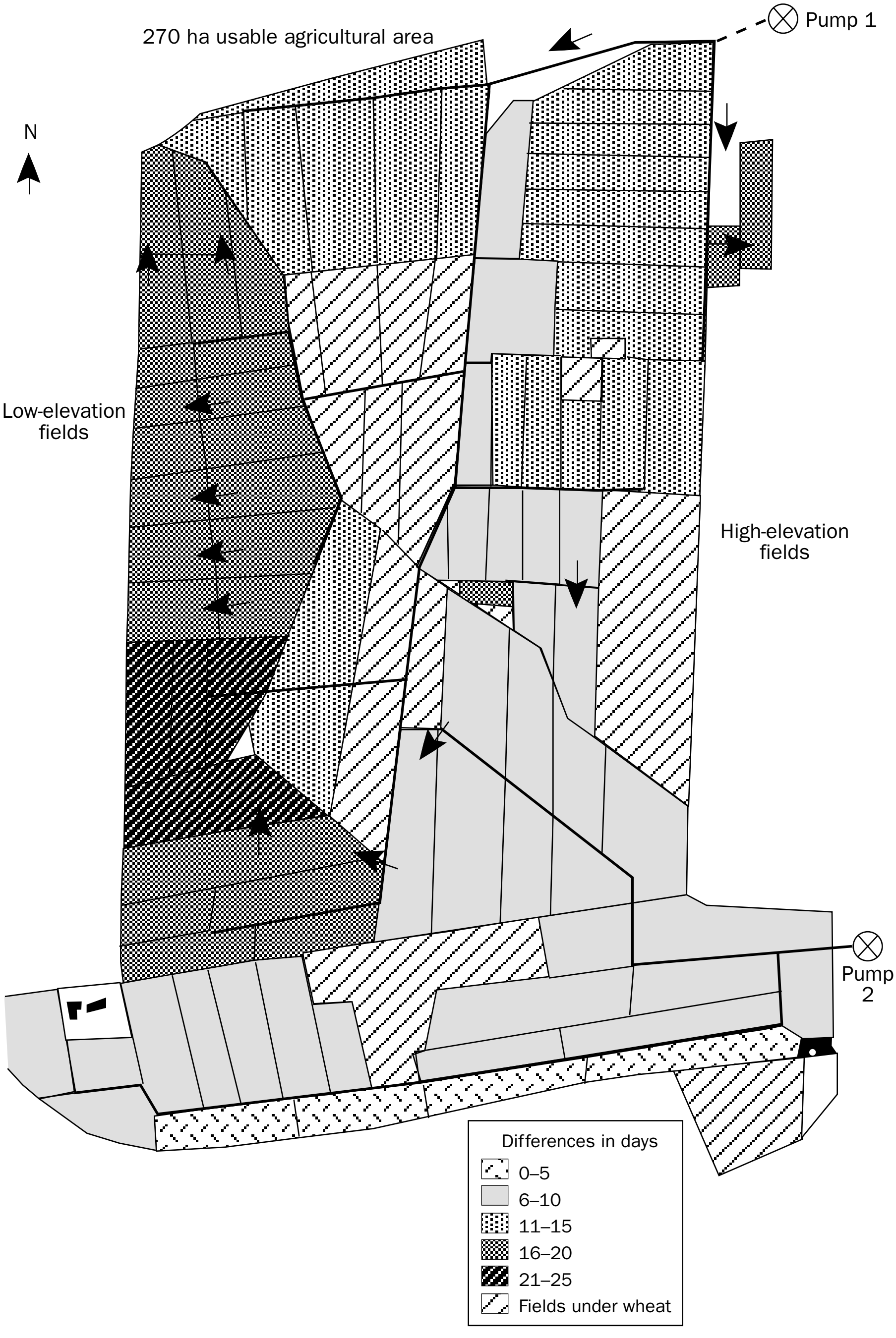

**Fig. 3. Field layout of a rice farm showing differences between fertilizer (NPK) application dates and the dates of the start of submersion for the 1994 season.**

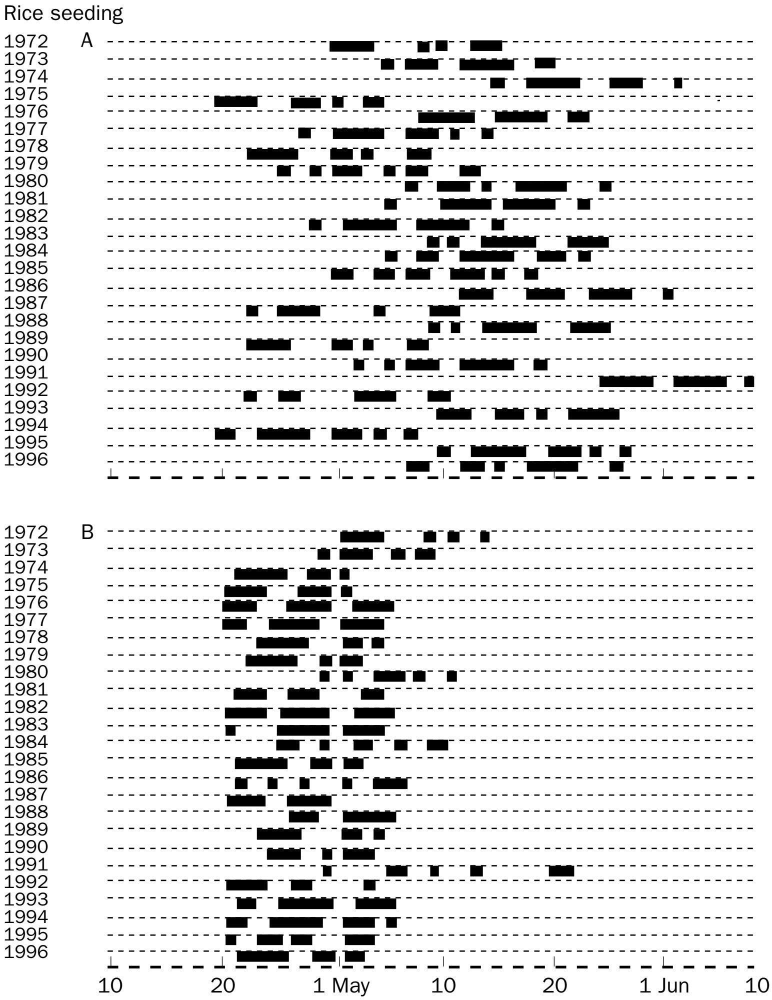

**Fig. 4. Results of simulations of rice sowing dates according to two modes of application of N fertilizer (black broken lines represent days of sowing): (A) soil incorporation before sowing and (B) topdressed after sowing. Simulations are based on the real climatic characteristics of 25 successive years (1972-96). Table 1 and Figure 2 present farming resources and a farmer's model for action.**

work of neighbors, and advice from various experts. Little research has been done and little aid has been provided for farmers for the choice of these indicators; however, they are central to farmer decision making. Focusing on these indicators may provide researchers with new fields of investigation and can guide technical recommendations.

For N fertilizer management, we argue that the lack of significant and stable differences between basal incorporation and topdressed application is not surprising because N-use efficiency in flooded rice fields is very low and, as a consequence, the yields achieved with various programs of applications do not exhibit high differences (Stutterheim and Barbier 1995). On-farm and on-station trials should serve to provide agronomic recommendations and to broaden the scope of farmers' decision making. Demonstrations that rice can be grown without the application of N before sowing have made it possible to be more flexible with certain farm calendars and to change farmers' thinking about rice-field N management.

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AGRONOMY

# N uptake and agronomic efficiency for rice production under the dry-seeded delayed-flood system in Italy

M. Biloni, S. Bocchi, and M. Tabacchi

Dry seeding with delayed flooding at the 3–4-leaf stage has become more and more widespread in Italy since the mid-1980s. Some conditions are needed to dry-seed rice such as good weather, a dry and drying field at sowing, a leveled soil surface for further water management, and well-prepared soil (plowed, leveled, fertilized, and harrowed) to place the seed at no more than 2–3-cm depth. Some of these conditions (i.e., fields that dry fast at sowing to support tractors moving on them, fine soil preparation) are found more easily in the area of light soils located south of Milan and in Novara Province and northeast Pavia Province. Although several studies were conducted to pinpoint the correct strategy for N management in dry-seeded delayed-flood rice, little information comes from experiments conducted in Italy.

The present field experiment aimed at evaluating N uptake by the plant, leaf N content, grain yield, and agronomic efficiency in typical conditions for dry-seeded rice in Italy. Two Italian varieties with different growth patterns were grown. Four preplant N applications (0, 60, 120, and 180 kg N ha$^{-1}$) and three split applications at 120 kg N ha$^{-1}$ were considered. Split N was applied at sowing, at preflooding, and at panicle differentiation. A split-plot design with three replications was adopted. The study was carried out at two sites in Pavia Province in 1996 and 1997.

Grain yield was higher with split N applications, mainly with fertilizer topdressed at midtillering. High preplant-only N application led to good grain yield but at a higher N rate. N accumulation in the biomass was significantly affected by N application and ranged from 90 to 230 kg N ha$^{-1}$. Agronomic efficiency (AE) varied from 5.0 to 38.5 kg grain kg$^{-1}$ N depending on location, amount of N, and application timing. Higher AE corresponded to N applied in splits at sowing and preflooding or at preflooding and panicle differentiation, with some differences between years. Leaf N content at 52 and 85 days, after sowing in the best-yielding treatments was always high. Thus, farmers' leaf N may be a useful parameter for optimizing grain yield.

Dry-seeded delayed-flood rice cultivation is quite common in some areas such as the southern United States (Bollich et al 1994). In north Italy, this technique was adopted at the beginning of the 20th century to reduce problems caused by flooding (Poli 1913). It was soon abandoned because of the diffusion of transplanting from about 1915 to 1960, with a peak during the 1950s when in Pavia Province more than 70% of rice was transplanted. Transplanting was economically useful for growing forages during winter, which allowed cattle to be fed. Direct seeding became the common system because of the increase in labor cost with the use of herbicides to control weeds, and the reduction in the number of farms with cattle. Broadcast seeding into the flooded fields was the main system. Dry seeding was reintroduced in the mid-1980s and is now adopted on about 30,000 ha (about 13% of the total Italian rice area). Water seeding in flooded fields using tractors with metal wheels (no use of airplanes occurs in Italy) is still the main sowing method and it allows higher yield than dry seeding with delayed flooding, but in some areas it is more convenient to grow rice with the latter method. These areas correspond to the zone located south of Milan and in Novara Province and northeast Pavia Province, where light soils dry fast during spring and allow movement of tractors with conventional wheels and uniform seeding and seedling emergence. Dry-seeded delayed-flood rice is also cultivated wherever farmers face water shortages in spring, soil lightness, low levels of soil organic matter, algal and aquatic weed infestation, and variable plant density because of difficult seedling establishment. The disadvantages of these conditions are greatly reduced when adopting dry seeding. Some conditions are needed to sow rice in dry soil such as nonrainy days, a dry and drying field at sowing, a leveled soil surface for further water management, and well-prepared soil (plowed, leveled, fertilized, and harrowed) to place the seeds uniformly at 2–3-cm depth.

Nitrogen fertilization has been a major constraint in dry-seeded delayed-flood rice cultivation and it has been studied extensively. In several trials, split applications of N fertilizer provided higher yield than all N applied at once (Patnaik and Broadbent 1963, Sims et al 1965, Prasad and De Datta 1979, Wilson et al 1989, Moletti et al 1990, 1992). Other investigators found a higher production with all N in a single preflood application (Reddy and Patrick 1976, Heenan and Bacon 1987, Bollich et al 1994, Spanu and Pruneddu 1997, Wilson et al 1998). In some experiments, no differences were reported between the two N application strategies (Westcott et al 1986) or with midseason application preflooding (Patrick and Reddy 1976, Wilson et al 1998). In some cases, N distribution at internode elongation, at panicle initiation, or at panicle development was ineffective, but Bollich et al (1994) found a yield reduction caused by midseason applications. Preplant N application was evaluated in several trials with different results. In most cases, it was not effective (Brandon and Wells 1986, Humphreys et al 1987, Ebelhar and Welch 1992). In others, it was useful if split with topdressings (Patnaik and Broadbent 1963, Patrick and Reddy 1976, Moletti et al 1990, 1992). In some cases, all N applied at sowing, incorporated into the soil, could give higher yield (Patrick and Reddy 1976, Spanu and Pruneddu 1997).

Many soil-crop-environment parameters are involved in determining nitrogen-driving yield and this might be the cause of the different responses to fertilizer application in the rice trials described. One is the seeding method. Westcott et al (1986) found no yield difference in dry- or wet-seeded rice. In Italy, Moletti et al (1990) found a clear interaction between N strategies and seeding method. They stated that with dry seeding more nitrogen and delayed split applications were needed. Variety characteristics need to be considered since plants respond differently to N, based on plant source-sink relation (Sims et al 1965, Hall et al 1967, Wells and Johnston 1970, Prasad and De Datta 1979, De Datta 1986, Bollich et al 1994). Tall traditional varieties usually need delayed midseason N application. Late varieties commonly need more split applications. Soil mainly governs the fate of applied nitrogen (Prasad and De Datta 1979, De Datta 1995). Generally, it was found that light-textured soils need more splits. Nitrogen application with respect to flooding timing was also evaluated. Large N losses were found with N applied just after flooding. Mengel and Wilson (1988) carried out an experiment to clarify the effect of topdressed N application on field flooding timing. Early applications were less effective than the ones close to flooding and delayed distribution in flooded fields had a very low effect on yield. Heenan and Bacon (1987) confirmed these results.

In Italy, the fertilization of dry-seeded delayed-flood rice fields usually occurs using preplant nitrogen applications. Split applications are common but a large amount of N is still applied at sowing. In recent studies, Moletti et al (1989, 1990) proposed applying 0% to 30% of N at sowing and 35% to 50% of N at both the 3–4-leaf stage and the end of tillering. Little information has been reported on other experiments related to N application in dry-seeded rice in Italy. The present situation causes farmers to adopt the fertilization strategy they are most confident of. Since farmers usually converting to dry seeding are typically flooded-rice growers, they tend to apply most N at preplant, without considering the very different soil conditions. The purpose of the present study was to verify the importance of the preplant N application in Italian rice fields, to check the effects of a two-split application, to pinpoint the best topdressing application strategy, and to improve N efficiency through an increase in agronomic efficiency. A reduction in N losses and increment in yield were taken into account to improve the economic benefit for farmers and environmental sustainability.

## Materials and methods

A field experiment was carried out in 1996 and 1997 at two locations: Castello d'Agogna (location 1) and Mortara (location 2) in northern Italy. The soil of the two locations is slightly different (Table 1): sandy-loam at Castello d'Agogna and sandy at Mortara.

Two Italian rice cultivars were considered because of their relevance in Italy and their widespread cultivation (the area cultivated with the two is about 12% of the total). Loto is an early cultivar with a long A kernel, according to the European Classification (white kernel length and width of 6.24 and 2.84 mm, respectively), mainly cultivated after postemergence red rice control. Drago is a medium-late cultivar with a long A kernel (white kernel length and width of 6.51 and 2.70 mm, respectively) and high yield.

**Table 1. Soil characteristics of the experimental sites.**

| Characteristic | Unit | Loc. 1 Castello d'Agogna | Loc. 2 Mortara |
|---|---|---|---|
| Sand | % | 69 | 85 |
| Silt | % | 24 | 12 |
| Clay | % | 7 | 3 |
| Texture class | | Sandy-loam | Sand |
| pH (w) | – | 5.1 | 6.1 |
| Org. C | % | 1.10 | 0.79 |
| Org. matter | % | 1.82 | 1.36 |
| Total N | % | 0.84 | 0.71 |
| C/N | – | 12.6 | 11.6 |
| CEC | meq 100 $g^{-1}$ | 10.50 | 6.69 |
| $P_2O_5$ | ppm | 111 | 532 |
| $K_2O$ | ppm | 101 | 79 |

**Table 2. Nitrogen application methods (amount of N applied in each split in kg N $ha^{-1}$).**

| Treatment no. | PP[a] | PF | PD |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 60 | 0 | 0 |
| 3 | 120 | 0 | 0 |
| 4 | 180 | 0 | 0 |
| 5 | 60 | 60 | 0 |
| 6 | 60 | 0 | 60 |
| 7 | 0 | 60 | 60 |

[a] PP = preplant, PF = preflooding, PD = panicle differentiation.

Seven different N applications were compared. N was applied at four levels (0, 60, 120, and 180 kg N $ha^{-1}$) and at three crop stages: sowing (preplant, PP), preflooding (PF), and panicle differentiation (PD) (Table 2). Fertilizer was broadcast and incorporated into the 10-cm soil layer at PP, was applied onto the dry soil surface the day before flooding at PF, and was applied into a flooded soil at PD. A split-plot design with three replications was adopted with the cultivar in the main plot and N application method in the subplot. The plot size was about 30 $m^2$. The same design was adopted at both locations and in both years. The experiment is part of a larger trial with more treatments. Here, only the treatments present in both years and at both locations are considered.

Rice was sown in rows at the end of April in dry soil. P and K were applied preplant just before sowing at 80 and 150 kg $ha^{-1}$ of $P_2O_5$ and $K_2O$, respectively. N was applied as prilled urea before harrowing (PP application), at 40 days after sowing (DAS) (PF application), and at 80 DAS (PD application).

Yield was estimated using a plot combine. Grain was dried in a plot dryer with independent cells, weighed, and reported at 14% grain moisture. Plant samples were gathered during crop growth (at 38, 52, 85, 111, and 145 DAS) by cutting plants at the soil surface, dividing them into leaf, culm, and panicle, and drying these in an oven. Samples were weighed and then ground in a Retsch KG-type SK3 mill. Nitrogen content was analyzed by near-infrared reflectance. Total aboveground crop N (NTOT) was calculated summing the amount in leaves, culms, and panicles.

Agronomic efficiency (AE) was calculated as the ratio between the yield difference in the fertilized and unfertilized plot and the total applied N. All the data were analyzed statistically using MSTAT software.

## Results and discussion

### Yield

Drago generally yielded more than Loto. Average grain yield was 7.4 and 6.2 t ha$^{-1}$ for Drago and Loto, respectively. Variety did not interact significantly with year, location, and N treatment for yield. Although rainfall was greater and temperature cooler in 1997, year had a small effect on grain yield. Year and N application method interaction affected yield (Fig. 1). With preplant N applications, the observed pattern was similar in the two years (the higher the N rate, the higher the yield). With split applications, the results varied. In 1996, yield was higher when N was not missing at PF and in particular when application at PP and PF was considered. In 1997, yield was higher when PD application was not missing and in particular when fertilizer was applied at PF and PD. In each year, applying 120 kg N ha$^{-1}$ in two splits meant higher yield than the preplant N application of 180 kg ha$^{-1}$.

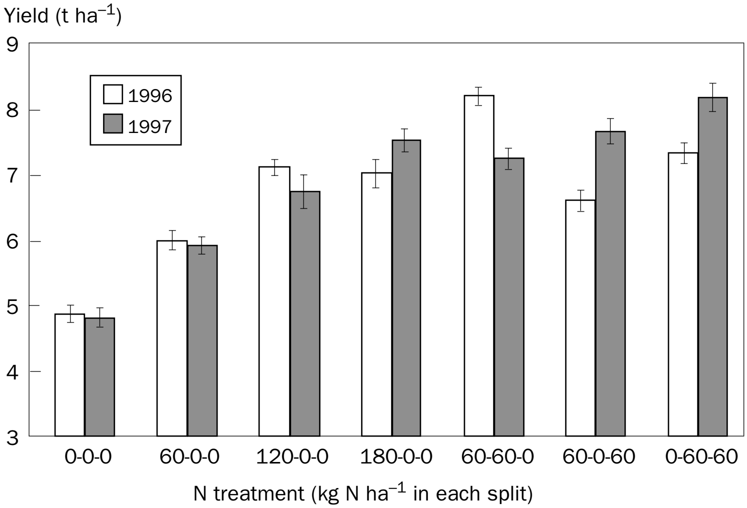

**Fig. 1. Yield as affected by year and N application method.**

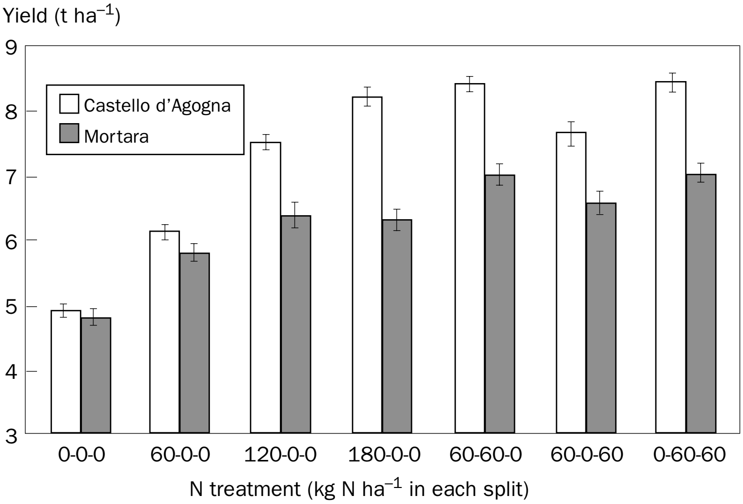

**Fig. 2. Yield as affected by location and N application method.**

The location effect on grain yield interacted with N treatment (Fig. 2). Increasing N rates, from 0 to 180 kg preplant N ha$^{-1}$, led to increasing yield at location 1. At location 2, yield was generally lower and reached a ceiling at 120 kg N ha$^{-1}$. Differences between locations might be explained by soil analysis. Higher sand content and lower organic matter and CEC at location 2 might have caused more N losses and less fertilizer efficiency. With split applications, the same pattern was observed at both locations, with location 1 always yielding more than location 2. At 120 kg N ha$^{-1}$ applied half at PP and half at PD at both locations, no differences were observed for 120 kg N ha$^{-1}$ applied all at PP. The other two split treatments with a split at PF generally yielded the same at each location. At location 1, these two-split treatments at 120 kg N ha$^{-1}$ yielded no differently than did 180 kg N ha$^{-1}$ all at PP, whereas, at location 2, these two-split treatments yielded more than did 180 kg N ha$^{-1}$.

## N uptake

Final aboveground N content in the biomass ranged from 90 to 230 kg ha$^{-1}$. N uptake was affected by N application, year, and location, but not by variety. In 1997, final crop N content was generally higher than in 1996 (Fig. 3). As with yield, the higher the preplant N rate, the higher the N uptake. Split applications generally led to high N accumulation. In 1996, at 120 kg N ha$^{-1}$ all split applications tended to accumulate more N in the crop than all N applied at PP, whereas, in 1997, this occurred only with N split at PF and PD. The highest N uptake was observed with split applications at PF and PD.

## Agronomic efficiency

AE was affected by location and year by N application interaction. Overall minimum and maximum recorded values were 5.0 and 38.5, respectively. At location 1, higher

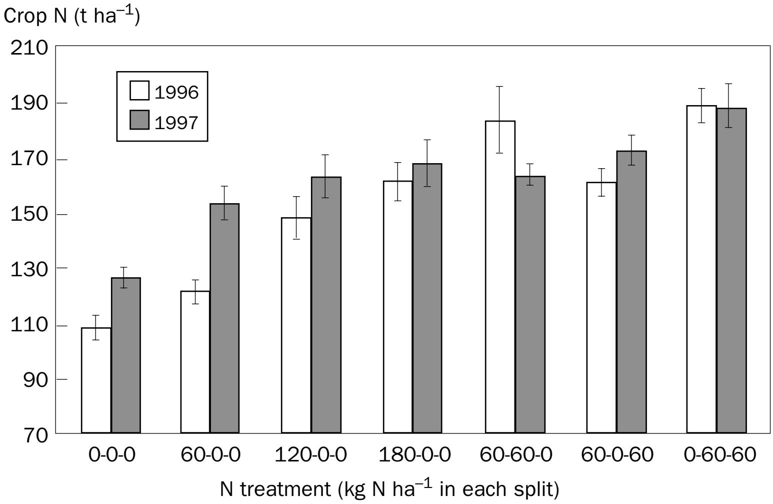

**Fig. 3. Total crop N as affected by year and application method.**

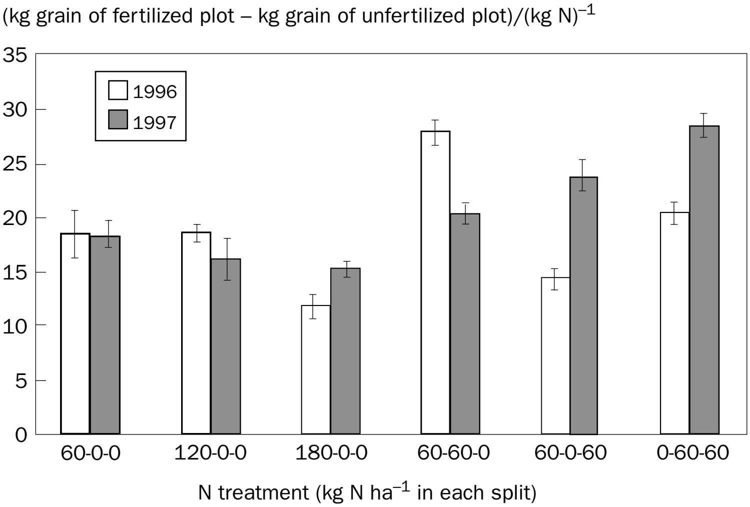

**Fig. 4. Agronomic efficiency as affected by year and N application method.**

values were generally recorded. Average AE was 23.7 and 15.3 Δ kg grain kg$^{-1}$ N at locations 1 and 2, respectively. In 1996, AE was about 19 Δ kg grain kg$^{-1}$ N at 60 and 120 kg preplant N ha$^{-1}$ and 12 Δ kg grain kg$^{-1}$ N at 180 kg preplant N ha$^{-1}$, whereas, in 1997, AE decreased from 18 Δ kg grain kg$^{-1}$ N at 60 kg preplant N ha$^{-1}$ to 15 Δ kg grain kg$^{-1}$ N at 180 kg preplant N ha$^{-1}$ (Fig. 4). For yield in 1996, AE was higher when PF N application was not missing than in 1997, when PD N application was not missing. The best split application usually led to higher AE than did N applied all at PP.

**Fig. 5. Leaf N content at 52 days after sowing. Bars indicate the lowermost and uppermost observed values and blocks indicate the leaf N intervals of the best-yielding varieties in both years and at both locations.**

### Leaf N content

Leaf N content (LN) was characterized by a large range of variation when comparing different sampling dates or different treatments at the same sampling date. LN pattern during the season was similar in 1996 and 1997. LN ranged from 2.60% to 4.07% at 38 DAS. At 52 DAS, it increased to 4.85% but decreased afterward. The lowest values were observed at 145 DAS, when the LN ranged from 0.86% to 1.68%. The differences over years between the maximum and minimum LN were 1.47%, 1.13%, 1.24%, 1.15%, and 0.78% at 38, 52, 85, 111, and 145 DAS, respectively. In several cases, these intervals were as large as more than 50% of the minimum measured value at that sampling date, demonstrating a consistent LN variation caused by N fertilizer. No significant difference was recorded between varieties.

Among N application treatments, the best-yielding cases generally showed intermediate LN during the season but a peculiar situation was observed at 52 and 85 DAS. At these two sampling dates, the best-yielding treatments were characterized by high leaf N content, whereas, during the early growing stages and during ripening, high N content did not necessarily lead to the highest yield. In particular at 52 DAS, the best-yielding varieties had a range of N content close to the uppermost observed value even if the observed LN intervals were different at the two locations and in the two years (Fig. 5). In 1997, at location 1, the range of best-yielding varieties was intermediate between the overall minimum and maximum observed values but it was still close to the uppermost value at 85 DAS (data not shown).

## Conclusions

The present work aimed at understanding the effect of different N application methods on two widely cultivated varieties under dry-seeding and delayed-flood systems. Many previous studies demonstrated the low importance of N application at PP. Nevertheless, in previous studies in Italy, preplant N assured good seedling vigor mainly in sandy soils with low CEC and organic matter content. The data showed that, even if AE was low with N applied all at PP, yield could be high, especially with high N. N application at PF was always important for increasing yield. Further studies are needed to clarify the effect of midseason application since different results were obtained in 1996 and 1997. Nevertheless, a two-split application at PP and PF or at PF and PD at 120 kg N $ha^{-1}$ was as effective as or more effective than an application all at once of 180 kg N $ha^{-1}$. Split application should be encouraged especially in sandy soil with low CEC and organic matter content. The costs of fertilizer application with a tractor and equipment remain to be evaluated since they might negate the advantage of a split application.

N uptake was significantly affected by N application, mainly at PF and PD. Final N content in the biomass and final grain yield had a similar pattern but correlation was not very good ($r = 0.37$). Final N content and final grain yield may be due to different causes, that is, final N content may be more linked to the time from the last N application to biomass harvesting, and grain yield may be linked to many more factors during the entire season.

LN followed a characteristic pattern during the crop season, increasing up to PF at 52 DAS and decreasing afterward. At 38, 111, and 145 DAS, the best-yielding varieties were not characterized by the highest LN. At 52 and 85 DAS, the best-yielding varieties tended to have more N in the crop. Thus, farmers should choose the N application method that maximizes crop N content during this period. Among the treatments considered in this study, a higher N content at 52 DAS was achieved with a split application at PP and PF or at PF and PD and with 180 kg preplant N $ha^{-1}$.

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## Notes

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# Effect of straw residue management practices on soil bulk density in two flooded Vertisols: implications for nutrient cycling in rice

J.A. Bird, W.R. Horwath, C. van Kessel, J.E. Hill, and A.J. Eagle

Soil bulk density is rarely considered when assessing nutrient cycling in agricultural systems. This study was conducted to determine whether the gravimetric or volumetric basis expression of soil biological and chemical properties is the most appropriate and relevant method for examining N and C cycling in agronomic systems research. The effect of long-term applications of alternative rice straw management practices on soil bulk density, total soil C and N, and microbial biomass N and C was investigated in the top 15 cm on two contrasting Vertisols (a fine, smectitic, superactive, thermic Sodic Endoaquert and a fine, mixed, superactive, thermic Xeric Duraquert) in the Sacramento Valley of California during 1997 and 1998. Soil bulk density was variable seasonally at the clay-rich Maxwell site (51% clay) and decreased in all treatments after spring flooding from 1.04 (0.12) to 0.64 (0.12) g $cm^{-3}$ at crop maturity in 1998. At the lower clay Biggs site (35% clay), soil bulk density decreased after spring flooding from 1.15 (0.09) to 0.99 (0.11) g $cm^{-3}$ at crop maturity in 1998. Incorporation of residues and winter flooding decreased bulk density both during and after the growing season. Soil microbial biomass C and N increased under straw incorporation compared with burning (257.5 vs 171.5 mg C $cm^{-3}$ and 49.7 vs 37.1 mg N $cm^{-3}$, respectively) and were less variable seasonally and among treatments when expressed volumetrically rather than gravimetrically. Total soil N and C were not affected by straw management treatments, but, when expressed volumetrically, they showed large fluctuations seasonally yet resulted in less variability among treatments. When examining nutrient cycling effects in agricultural soils that experience seasonal and treatment-induced changes in soil bulk density, it is necessary to express data on a volumetric basis to evaluate effects of treatments on nutrient cycling.

Soil bulk density is rarely considered when assessing nutrient cycling in agricultural systems. Clay-rich rice soils with high shrink-swell capacity demonstrate large fluctuations in bulk density after flooding and tillage (Sharma and De Datta 1985). The expression of soil chemical and biological properties needs to be relevant to the purpose of the investigation when examining the effects of agronomic practices on nutrient cycling in rice. This is especially important when management practices that are evaluated also affect bulk density.

While most studies examining soil nutrient cycling express soil chemical and biological properties on a gravimetric or concentration basis, recent studies have considered the use of a volumetric basis to be more agronomically relevant (Reganold and Palmer 1995, Tiessen et al 1982). In soil-plant relationship studies, Mehlich (1980) proposed that chemical data are most relevant when they are based on the same volume from which plant roots obtain nutrients and water. In studies examining soil quality, Dorin and Parkin (1996) contend that gravimetric basis expressions of soil chemical and biological data may not be valid or ecologically relevant. Nutrient data have been reported on a volumetric basis adjusted for horizon thickness (weight/area-solum depth) (Aguilar et al 1988, Tiessen et al 1982). For studies examining total soil organic matter budgets, Ellert and Bettany (1995) believed that haphazard differences in soil thickness and bulk density require the use of equivalent soil masses. Still other options have been reported, including using adjusted volumetric expressions for soil chemical and biological properties (i.e., data adjusted to one treatment's soil bulk density) (McCarty et al 1998). The debate will likely continue regarding the best method for expression of soil nutrients; however, one method may not be universally acceptable for all conditions and investigations.

Typical cultural practices of rice production in California involve spring tillage and subsequent shallow flooding during crop growth. These practices can promote substantial changes in soil bulk density over the season in the top 15 cm of soil similar to the effect seen from soil puddling in the tropics (Sharma and De Datta 1985). This is especially evident in Vertisols, which represent 32% of rice area in California and are widely cultivated in rice production areas worldwide (Flach and Slusher 1978, Virmani et al 1982). Rice root growth in paddy varieties is highly concentrated in the top 15 cm of the soil (Teo et al 1995, Shukla and Sen 1975). Therefore, variations in bulk density will have a major effect on the availability and uptake of nutrients by the crop.

Alternative rice straw residue management practices are currently being adopted in California because of the restriction of open-field burning mandated by the California Rice Straw Burning Reduction Act (AB 1378, 1991). Historically, burning has been the dominant rice residue management practice and has contributed to air pollution problems in the region. The most commonly used alternative method of residue management is stubble-disk incorporation with winter flooding. Alternative rice straw management practices (flooding, straw residue incorporation) may affect soil C and N cycling and the subsequent availability of N to the rice crop. At the same time, alternative straw management can affect the soil bulk density similarly to spring tillage and crop flooding. These changes in bulk density may not only be seasonal, but may result in long-term changes in the physical properties of the soil. In the tropics, long-term incorporation of rice straw residues (10 y) resulted in a decrease in soil bulk density along with an increase in the N-supplying power of the soil compared with a control (Bellakki et al 1998). It is clear that the potential for changes in physical, chemical, and biological properties caused by alternative straw management methods needs to be evaluated. Furthermore, these effects need to be considered in relevant and appropriate units for meaningful interpretations of plant nutrient uptake, N cycling in soil, and microbiological nutrient dynamics.

This study was conducted to determine whether the gravimetric or volumetric basis expression of soil biological and chemical properties is the most appropriate and relevant method for examining N and C cycling in agronomic systems research. We investigated N and C cycling under alternative straw disposal options and addressed the effect of these practices on soil bulk density, total soil C and N, and microbial biomass N and C pools. These data are evaluated and compared on a gravimetric and volumetric basis.

## Study area and methods

We examined the effects of alternative rice straw management methods at two field sites. Both sites are ongoing, long-term rice straw residue management trials located in Maxwell, California, and at the University of California Rice Experiment Station in Biggs. Maxwell and Biggs have large field-scale plots (Maxwell 0.75 ha, Biggs 0.3 ha) representing different rice straw residue management practices used in California. The Maxwell field study began in the fall of 1993 and the Biggs study in the fall of 1994. The Maxwell soil is classified as a fine, smectitic, superactive, thermic Sodic Endoaquert (Soil Survey Staff 1998). The soil at Biggs is classified as a fine, mixed, superactive, thermic Xeric Duraquert (Soil Survey Staff 1998). Notable differences between the soils include clay content (higher at Maxwell, 51%, vs 35% at Biggs), total N, exchangeable K, and electrical conductivity (EC) (Table 1).

The field experiment is a split-plot design with four replications. Winter flooding (F) and nonflooding is the main treatment and straw management (C, burn, incorporate, roll, and bale/remove) is the split-plot treatment. Treatments at both field sites include (1) burned residue with winter flooding, (2) burned residue without winter flooding, (3) incorporated residue with winter flooding, (4) incorporated residue without winter flooding, (5) rolled residue with winter flooding, (6) rolled residue without winter flooding, (7) baled residue with winter flooding, and (8) baled residue without winter flooding.

The winter-flooded treatments were submerged after fall tillage in November and plots were drained in early March before field preparation and spring planting. Winter-flooded plots were initially flooded to a 15-cm depth and varied between 5 and 15 cm in depth. In incorporated plots, residual straw was swathed, forage chopped, and straw stubble-disked. The rolled plots were tilled with a 7-m-long open-cage

**Table 1. Selected chemical properties of soils used in this study (0–15-cm depth).**

| Soil parameter[a] | Unit | Maxwell | Biggs |
|---|---|---|---|
| Texture | | | |
| Clay | % | 51 | 35 |
| Silt | % | 44 | 48 |
| Sand | % | 5 | 17 |
| pH | | 6.6 | 4.7 |
| EC | dS $m^{-1}$ | 1.4 | 0.4 |
| CEC | cmol $kg^{-1}$ | 42 | 30 |
| SAR | | 7.5 | <1 |
| Total C | g $kg^{-1}$ | 19.5 | 12.3 |
| Total N | g $kg^{-1}$ | 1.7 | 1.0 |
| P (Olsen) | mg $kg^{-1}$ | 11.3 | 11.1 |
| Exchange K | mg $kg^{-1}$ | 305 | 72 |

[a] EC = electrical conductivity, CEC = cation exchange capacity, SAR = sodium absorption ratio.

roller. Winter-flooded rolled plots were tilled after submergence. In baled plots, straw was swathed, baled, and removed. The plots were managed following agronomic practices typical for California rice production.

Soil samples, to 15-cm depth, were taken from plots using an intact core sampler (5-cm diameter) that allows for bulk density determinations for each sample. Soil samples were taken every 2 mo throughout the year from all treatments in 1998 at both field sites. In 1997, soil samples were taken from the burned and incorporated treatments at the Maxwell site. Soils sampled were stored moist at 4 °C in capped, butyrate plastic tubes and subsampled within 7 d for extractable inorganic N and microbial biomass C and N. Large visible pieces of crop residue were removed from the soil before analyses. Microbial biomass C and N were determined using the chloroform-fumigation incubation (CFI) method with a 10-d chloroform period as described by Horwath and Paul (1994). Microbial biomass C was calculated without use of the control and adjusted using a $K_C$ value of 0.78 (Horwath et al 1996). Microbial biomass N was calculated by subtracting the ammonium flush after fumigation and a 10-d incubation from initial extractable ammonium levels and adjusted using a $K_N$ value of 0.58 (Voroney and Paul 1984). Field-moist soil samples were subsampled and extracted with 2 N KCl for exchangeable inorganic N using a 5:1 extractant to dry soil ratio. Inorganic N was determined colorimetrically using an automated N analyzer (LACHAT, Mequin, WI) (Bremner 1965). Carbon dioxide concentrations evolved at the end of the 10-d incubation were determined using an infrared gas analyzer. Gravimetric water content was determined by drying soil subsamples at 105 °C for 24 h.

The remaining soil was air-dried at 35 °C, ground using a ball-mill grinder, and sieved to pass through a no. 40-mesh sieve. Total C and N were determined using the Dumas dry combustion method using a Carlo-Erba CHN gas analyzer. All data are expressed as least squares means with standard errors of indicated treatment means. Statistical analyses were performed using SYSTAT (1997).

## Results

### Soil bulk density

Figures 1 and 2 show soil bulk density in the top 15 cm of the soil, measured during the 1998 growing season for Maxwell and Biggs, respectively. For all eight straw management treatments, soil bulk density was measured at both field sites in 1998, except in November 1998, when only the straw-incorporated and burned treatments were measured. In 1997, only the straw-incorporated and burned treatments were measured at Maxwell (Fig. 3). The soil biological and chemical data presented here were taken in 1997 from Maxwell. Soil bulk density at the clay-rich Maxwell site (51%) decreased in all treatments after spring flooding from a mean of 1.04 (0.12) g cm$^{-3}$ to 0.64 (0.12) g cm$^{-3}$ at crop maturity in 1998. The lower clay Biggs site (35% clay) was affected less, but did decrease slightly after spring flooding from 1.15 (0.09) to 0.99 (0.11) g cm$^{-3}$ at crop maturity in 1998 (Fig. 2).

Fall and winter applied straw residue treatments affected soil bulk density both during and after the growing season at Maxwell. Soon after drainage of the winter-

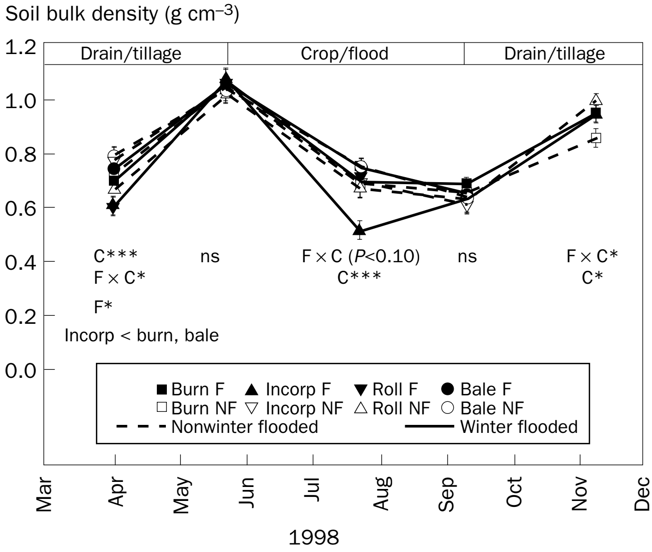

**Fig. 1. Soil bulk density in top 15 cm of soil (g cm$^{-3}$) at the Maxwell site in 1998 after 5 y of straw management treatments. Least squares means and standard error (n=16) for all dates except Nov. 1998 (n=12). Significant treatment effects of winter flooding (F) and straw management (incorp, burn, bale, and roll) (C) are indicated for each date. *, **, *** = significant at the 0.05, 0.01, and 0.001 probability levels. ns = nonsignificant.**

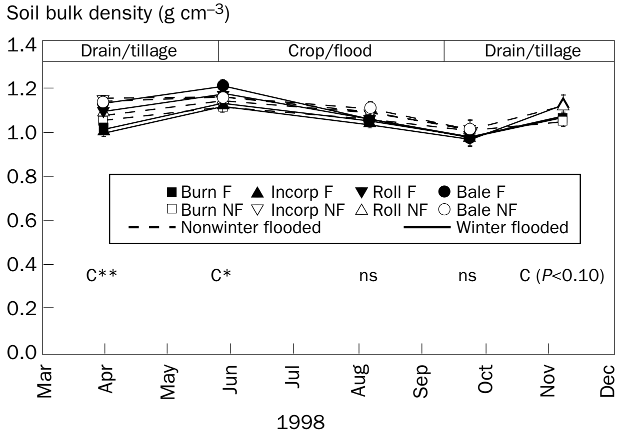

**Fig. 2. Soil bulk density in top 15 cm of soil (g cm$^{-3}$) at the Biggs site in 1998 after 4 y of straw management treatments. Least squares means and standard error (n=16). Significant treatment effects of winter flooding (F) and straw management (incorp, burn, bale, and roll) (C) are indicated for each date. *, **, *** = significant at the 0.05, 0.01, and 0.001 probability levels. ns = nonsignificant.**

flooded plots, the winter-flooded treatments had lower bulk densities than the nonflooded plots in the incorporated and rolled plots (Fig. 1). Additionally, in April 1998, the baled treatments were denser in the top 15 cm of soil than the incorporated and rolled treatment. These differences were not apparent before planting when all treatments had similar bulk densities in the top 15-cm depth.

At Biggs, residue management had less effect on soil bulk density in the top 15 cm than at Maxwell (Fig. 2). Soon after drainage in April, no significant difference was seen between the winter-flooded and nonwinter-flooded treatments. The baled and rolled treatments (1.13 (0.02) and 1.12 (0.01) g cm$^{-3}$, respectively) had significantly higher bulk densities than the incorporated and burned treatments (1.04 (0.02) and 1.03 (0.01) g cm$^{-3}$, respectively) just after drainage in the spring. In contrast to Maxwell, at Biggs the baled plots (1.18 (0.01) g cm$^{-3}$) had greater soil bulk density than the incorporated and burned treatments (1.12 (0.02) and 1.13 (0.01) g cm$^{-3}$, respectively) just before seeding.

During the cropping period in summer 1998, soil bulk density in the incorporated, winter-flooded treatment was significantly less than in the other treatments midway through the season at Maxwell. This difference was not apparent just before harvest in September. The incorporated, winter-flooded treatment consistently had lower soil bulk densities than the burned and incorporated, nonwinter-flooded treatments in summer 1997 (crop maturity) (Fig. 3). In summer 1998, soil bulk density was similar among all treatments at Biggs in the 0–15-cm depth.

After application of the straw management treatments in fall 1998, incorporated plots were slightly more dense in the top 15 cm at Maxwell ($P < 0.04$) and Biggs

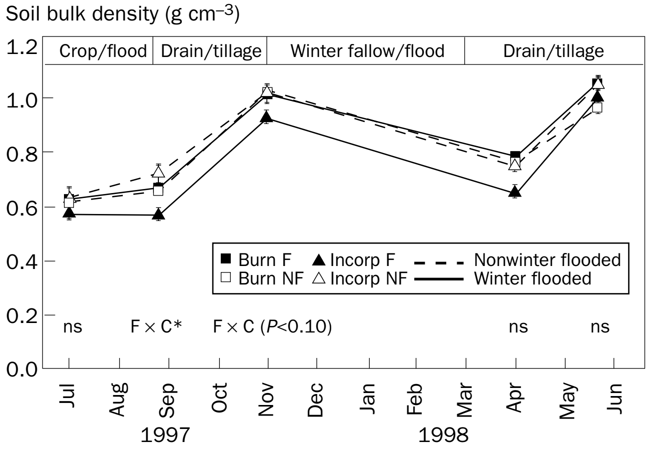

**Fig. 3. Soil bulk density in top 15 cm of soil (g cm$^{-3}$) at the Maxwell site in 1997 in burned and incorporated straw management treatments. Least squares means and standard error (n=12). Significant treatment effects of winter flooding (F) and straw management (C) are indicated for each date. *, **, *** = significant at the 0.05, 0.01, and 0.001 probability levels. ns = nonsignificant.**

($P$ <0.10). These measurements were made approximately 10 d after fall tillage was applied in the incorporated plots.

## Microbial biomass N and C

Estimates of the N and C contents in the soil microbial biomass were determined at Maxwell throughout the 1997 season (July 1997-May 1998). Figure 3 illustrates the corresponding soil bulk densities for these samples. These data are shown on a volumetric basis (g cm$^{-3}$) and on a concentration basis (µg g$^{-1}$) in Figures 4 and 5 for N and C, respectively. Straw management treatments influenced microbial biomass N and C throughout the season. Overall, volumetric and gravimetric expression of microbial biomass showed similar effects among the treatments. Gravimetric basis units amplified differences among the treatments and resulted in greater seasonal variation (Figs. 4A and 5A).

Microbial biomass N (MBN), as calculated on a volumetric basis (Fig. 4B), was relatively constant throughout the year; however, when expressed on a gravimetric basis, biomass N was much more seasonally variable (Fig. 4A). For example, MBN expressed on a gravimetric basis in the incorporated, winter-flooded treatments during the cropping season was much greater (108.7 (15.0) µg g$^{-1}$ and 105.6 (5.61) µg g$^{-1}$, respectively) than in the fall (54.9 (3.3) µg g$^{-1}$) and following spring (62.9 (4.4) µg g$^{-1}$). Amplification of the seasonal trend in gravimetrically expressed MBN is similar for the other three treatments and for microbial biomass C (MBC) measurements (Fig. 5) when calculations were expressed on a gravimetric basis. We found that MBN and MBC values varied much more dramatically within a sampling date

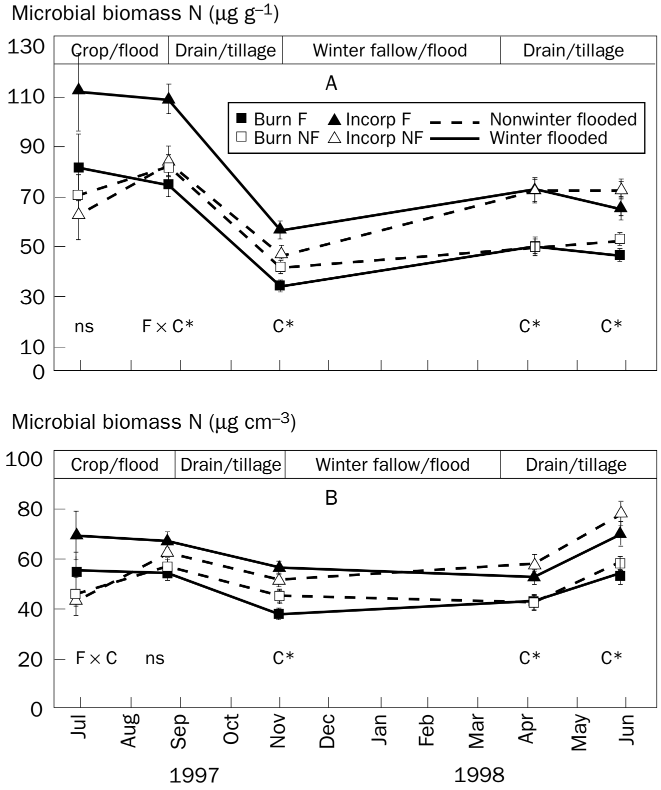

**Fig. 4. Total soil microbial biomass N at the Maxwell site (0–15-cm depth) during the 1997-98 season in burned and incorporated straw management treatments expressed on (A) gravimetric basis (µg g$^{-1}$ soil) and (B) volumetric basis (µg cm$^{-3}$ soil). Least squares means and standard error (n=12). Significant treatment effects of winter flooding (F) and straw management (C) are indicated for each date. *, **, *** = significant at the 0.05, 0.01, and 0.001 probability levels. ns = nonsignificant.**

when considered on a gravimetric basis than when considered on a volumetric basis. Trends were similar, however, with incorporated plots having a greater MBN and MBC after 5 y of straw treatment effects. The method of expressing soil biomass did not affect statistical differences found in MBC; however, with MBN, comparisons of methods resulted in different conclusions for samples measured during the 1997 cropping season.

## Total C and N

All straw management treatments showed similar total C and N amounts after five seasons (0–15-cm soil depth). Total C and N contents were determined on soil samples (0–15-cm depth) from the Maxwell site throughout the 1997-98 season. Gravimetric

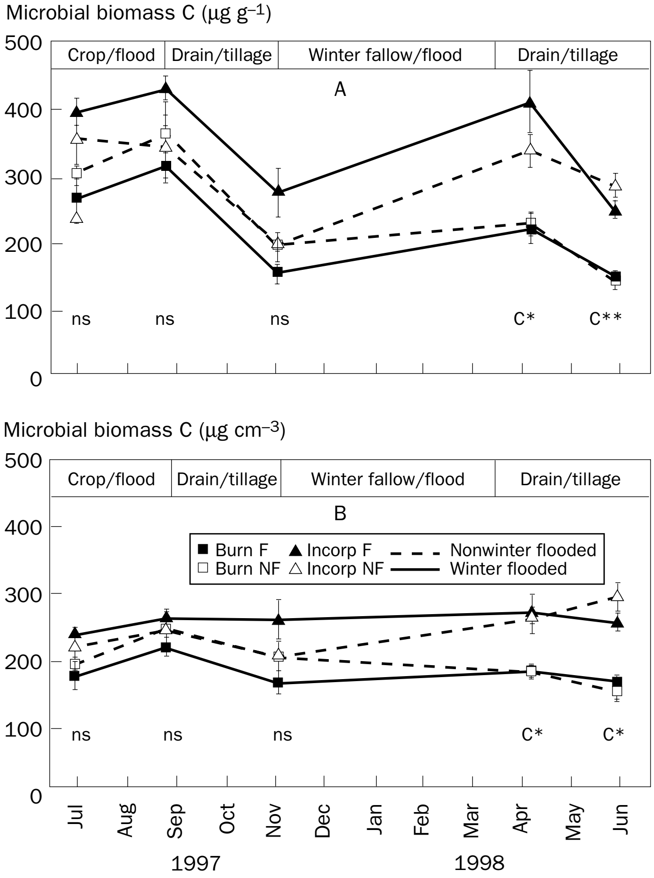

**Fig. 5. Total soil microbial biomass C at the Maxwell site (0–15-cm depth) during the 1997-98 season in burned and incorporated straw management treatments expressed on (A) gravimetric basis (µg $g^{-1}$ soil) and (B) volumetric basis (µg $cm^{-3}$ soil). Least squares means and standard error (n=12). Significant treatment effects of winter flooding (F) and straw management (C) are indicated for each date. *, **, *** = significant at the 0.05, 0.01, and 0.001 probability levels. ns = nonsignificant.**

expression of these data as concentrations showed a similar C and N amount per unit weight over the season (Figs. 6A and 7A). After conversion to a volumetric expression, total soil N and C in the top 15 cm varied seasonally, but showed less variability among treatments for a specific sampling date (Figs. 6B and 7B).

Total soil C and N, when expressed volumetrically, varied considerably over the season. Total soil N ranged from 9.8 (0.1) µg $cm^{-3}$ at midseason to 15.4 (0.3) µg $cm^{-3}$ before winter fallow. Similarly, the trend for soil bulk density was from values of 0.65 (0.01) g soil $cm^{-3}$ at crop maturity to 0.99 (0.02) g soil $cm^{-3}$ after straw treat-

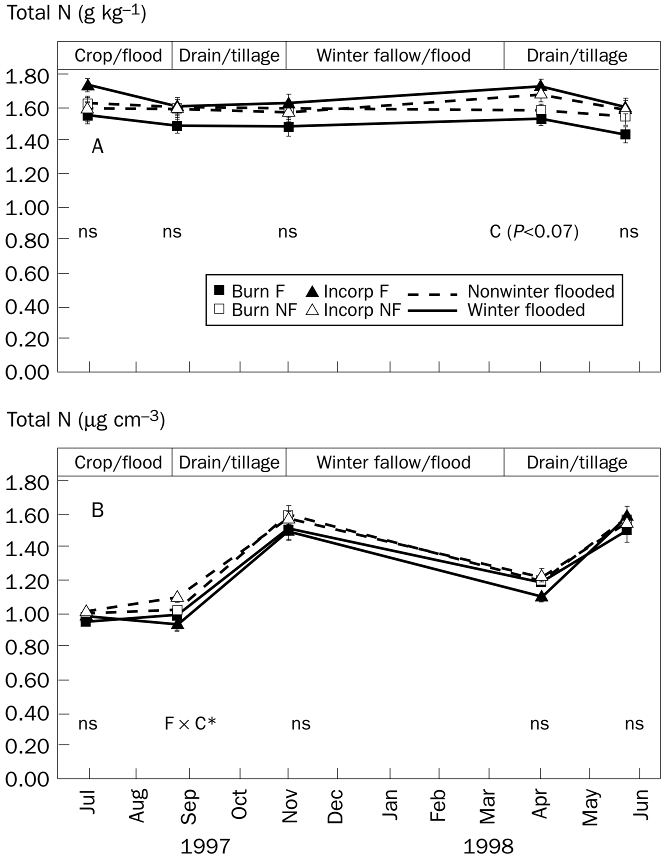

**Fig. 6. Total soil N at the Maxwell site (0–15-cm depth) during the 1997-98 season in burned and incorporated straw management treatments expressed on (A) gravimetric basis (g kg$^{-1}$ soil) and (B) volumetric basis (µg cm$^{-3}$ soil). Least squares means and standard error (n=12). Significant treatment effects of winter flooding (F) and straw management (C) are indicated for each date. *, **, *** = significant at the 0.05, 0.01, and 0.001 probability levels. ns = nonsignificant.**

ments were applied in the fall. Significant effects of straw management treatment on total soil C and N expressed volumetrically were not seen below a *P* value of 0.05 except for total N at crop maturity in 1997 (Fig. 6B).

## Discussion

Soil bulk density on the clay-rich Maxwell soil was variable seasonally and affected by straw disposal method and winter flooding. During the flooded cropping season, soil bulk densities were lowest (0.65–0.75 g cm$^{-3}$) and, during the drying/tillage periods, surface soil bulk densities approached 1.1 g cm$^{-3}$. Soil bulk density decreases in

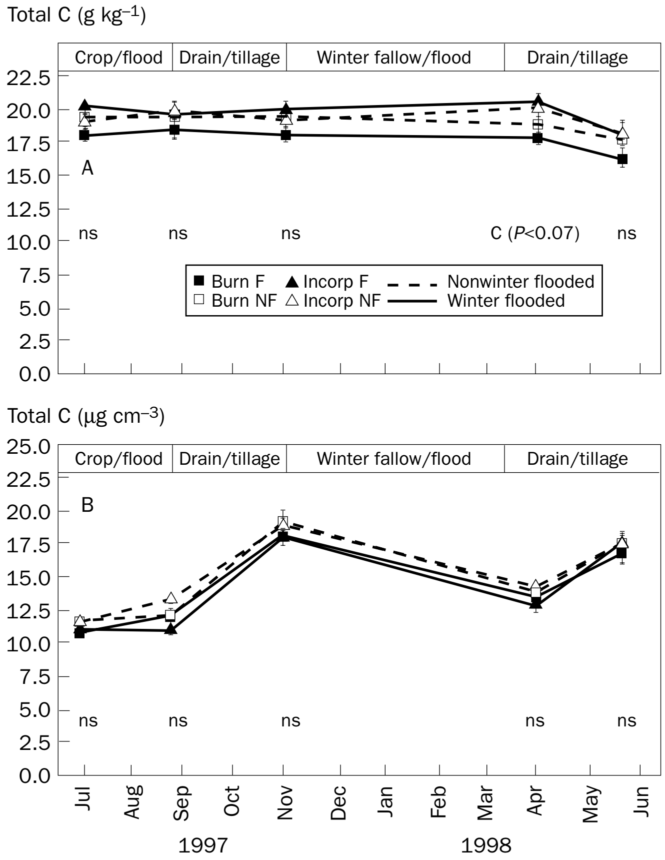

**Fig. 7. Total soil C at the Maxwell site (0–15-cm depth) during the 1997-98 season in burned and incorporated straw management treatments expressed on (A) gravimetric basis (g $kg^{-1}$ soil) and (B) volumetric basis (µg $cm^{-3}$ soil). Least squares means and standard error (n=12). Significant treatment effects of winter flooding (F) and straw management (C) are indicated for each date. *, **, *** = significant at the 0.05, 0.01, and 0.001 probability levels. ns = nonsignificant.**

some Vertisols after flooding because of the swelling nature of the clay minerals. Rao et al (1978) reported a bulk density change of almost 60% when a dry Vertisol was saturated with water.

We found that winter-flooded plots were less dense than nonwinter-flooded plots at the Maxwell site during spring dry-down because of greater moisture contents. Fall incorporation of straw residues with winter flooding resulted in the lowest bulk density during the growing season at the Maxwell site in 1997 and 1998. Fall incorporation with winter flooding created substantial changes in soil structure that affected soil bulk density many months later during the growing season. Long-term incorporation of rice straw residues (10 y) has been shown to decrease soil bulk density from 1.49 to 1.26 g cm$^{-3}$ compared with a control on a Typic Chromustert in India (Bellakki et al 1998).

In our study, the effect of straw incorporation on reducing the soil bulk density was exacerbated by subsequent winter flooding. After submergence of the winter-flooded plots, the swelling effect of the soil was enhanced by the already reduced structure because of fall tillage. A similar combined effect on lowering soil bulk density was seen in the spring after tillage and flooding in all treatments examined. This interaction of flooding and tillage/incorporation was also seen in the comparison of soil bulk density because of puddling in a smectitic Haplaquoll (Sharma and De Datta 1985). Sharma and De Datta (1985) reported lower soil bulk density 60 d after puddling/flooding (0.67 g cm$^{-3}$) compared with no tillage (0.89 g cm$^{-3}$) in the top 15 cm of the soil.

At the lower clay Biggs site (35%), soil bulk density decreased slightly after spring flooding from 1.15 (0.09) to 0.99 (0.11) g cm$^{-3}$ at crop maturity in 1998. We found no effects of winter flooding on soil bulk density at the Biggs site, but did find that the rolled and baled plots were slightly more compact than the burned and incorporated plots before planting. Soil bulk density at the Biggs site clearly responded differently than at the Maxwell site because of the lower clay content and shrink-swell potential of the soil.

Microbial biomass has been shown to be a sensitive indicator of differences in nutrient dynamics in agronomic research (Anderson and Domsch 1989, Powlson et al 1987). Volumetric expression of biomass showed a more constant C and N pool held in the biomass (Figs. 4B and 5B). Values for microbial biomass over the course of the season reported in recent literature have shown wide fluctuations (Bossio et al 1998, Franzluebbers et al 1995). In a German Luvisol under wheat production (0–10-cm depth), MBC ranged from 363 to 180 μg g$^{-1}$ and MBN ranged from 66 to 35 μg g$^{-1}$ over the course of 1 year (Joergensen et al 1994). All of the studies cited have values expressed on a concentration basis.

Soil microbial C and N were greater in the incorporated straw treatments than in the burned treatments. This effect was apparent when expressing MBN and MBC on a gravimetric or volumetric basis. Volumetrically expressed MBN and MBC were less variable seasonally and among treatments for a specific sampling date. MBC measurements made using phospholipid fatty acid profiles (PLFA) at Maxwell in winter 1994 and 1995 showed no differences among treatments in 1994 but greater total PLFA (nmol g$^{-1}$ soil) was found in incorporated plots than in burned ones (Bossio and Scow 1998). Our results are also in accordance with other long-term studies examining the effects of straw residue additions on soil MBN and MBC (Dalal et al 1991). Long-term incorporation of straw residues (18 y) at two sites showed greater MBN (50% and 46%) and MBC (45% and 37%) compared with annually burned barley residues using data adjusted for equal soil mass (i.e., lesser depth used in denser soil) (Powlson et al 1987). McCarty et al (1998) found increases in MBN and MBC on a volumetric basis after 3 y of no tillage (top soil layers); however, these differences were reduced after adjusting values to reflect differences in bulk density between treatments.

Soil bulk density and microbial biomass expressed on a gravimetric basis show opposite trends both seasonally and within treatments. Therefore, when these data are expressed volumetrically, the result is less variability seasonally and among treatments. The significance of less variability is difficult to assess given the scarcity of MBN and MBC data reported on a volumetric basis in the literature. More importantly for this study, however, volumetrically expressed microbial biomass can be related directly to crop rooting volume and the active site of straw decomposition.

Total N and C in the top 15 cm of the soil at the Maxwell site were similar for all straw management treatments. In contrast to soil microbial measurements, volumetrically expressed total C and N were more seasonally variable than soil N and C on a concentration basis. It may be reasonable to conclude that a less dense soil is lower in total N and C; however, the microbial biomass may be less limited by surface area and more responsive to substrate availability. Similarly to MBN and MBC, total N and C expressed volumetrically were less variable among treatments for a specific sampling date than gravimetric expressions.

Total soil N and C, when considered in relation to nutrient availability to crops or microorganisms, may need to be examined volumetrically. This is especially important for soils that display seasonal and treatment-induced changes in soil bulk density in the zone of maximum crop uptake and residue decomposition. Caution is necessary, however, when looking at seasonal changes in nutrients closely tied to soil bulk density (e.g., total soil C and N) if only part of the profile is sampled. Volumetric measurements for the entire solum depth are needed when assessing changes in total C and N in a soil profile over time.

When examining nutrient cycling effects in agricultural soils that experience seasonal and treatment-induced changes in soil bulk density, it is necessary to express data on a volumetric basis to evaluate effects of treatments on nutrient cycling. This is especially important in flooded rice soils because of the prevalence of high shrink-swell soils and the concentration of roots and decomposition in the top layer of the soil. Without the perspective of nutrients on a volumetric basis, it would not be possible to relate soil nutrient dynamics to plant uptake or decomposition potentials *in situ* in a system that differs in bulk density seasonally and among treatments.

Generally, the use of volumetric soil expressions in soil biology and biochemistry would allow for more universally applicable results. Researchers could compare data from differing soils, systems, and climates more easily if units were all expressed on a volumetric basis. Additional information on soil depth sampled and horizon distribution would enable researchers to more thoroughly and precisely describe treatment effects. The volumetric expression of soil nutrient data allows for the comparison of total nutrient budgets as long as the entire soil profile is measured to a depth where the soil property is found in abundance.

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## Notes

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AGRONOMY

# Effect of root damage on rice growth and yield response

Cai Kunzheng, Luo Shiming, and Duan Shunshan

Field experiments with different varieties under different root cutting (root was cut from one, two, or three sides of the rice plant and no cutting) at the heading stage were conducted on their effect on yield and yield components. The results showed that root cutting increased root activity significantly at the ripening stage in general, irrespective of varieties and treatments. One side cutting caused a yield increase in four varieties (Guang-Lu-Ai 10.2%, Pei-Za-72 4.6%, Feng-Ai-Zhan 1 12.8%, Te-San-Ai 11.4%) and a decrease in one (Er-Qing-Ai by 18.2%). The difference was mainly associated with a difference in grains per panicle and percentage filled grains. Two side cuttings (T2) and three side cuttings (T3) caused a yield increase in two varieties (Guang-Lu-Ai and Te-San-Ai) and a decrease in three (Er-Qing-Ai, Pei-Za-72, Feng-Ai-Zhan 1). It can be concluded that light root cutting at the heading stage has no significant effects on rice yield. It can even increase the yield of some varieties. This may be because root cutting can increase root activity at maturity, which is important for improving the ability to absorb water and nutrient and delay the plant from senescence.

Root systems of plants play an important role in the absorption of water and nutrients, in the storage of organic matter and nutrients, and in the synthesis of some hormones. Therefore, it is suggested that, for rice cultivation, more roots produce higher yields and the development of a large root system and its maintenance are essential factors for increases in grain yield. However, an excessively large root system reduces shoot growth because of the competition for carbohydrates between roots and shoots. To determine whether a large root system is beneficial to yield, we conducted a field experiment on root cutting at the heading stage to study its effect on yield and yield components. Five varieties were chosen for early season and late-season experiments: Guang-Lu-Ai (short stalk) and Er-Qing-Ai (tall stalk) for the early season and Pei-Za-72 (hybrid rice), Feng-Ai-Zhan 1, and Te-San-Ai for the late season.

Thirty-day-old seedlings were transplanted at 20 × 20-cm spacing with two seedlings per hill. The root systems of treated blocks were cut vertically with 20-cm

**Table 1. Effect of root cutting on root activity at the heading stage (μg α-NA $g^{-1}$ DW $h^{-1}$). α-NA = α-naphthylamine.**

| Treatment | Variety[a] | | | | |
|---|---|---|---|---|---|
| | Guang-Lu-Ai | Er-Qing-Ai | Pei-Za-72 | Feng-Ai-Zhan 1 | Te-San-Ai |
| Control | 43.74 b | 87.52 b | 96.4 c | 59.2 b | 84.2 b |
| T1 | 48.32 b | 111.78 a | 108.2 b | 73.4 a | 89.3 ab |
| T2 | 111.42 a | 109.94 a | 123.5 a | 70.2 a | 93.4 a |
| T3 | 101.71 a | 102.32 a | 109.5 b | 68.4 a | 90.5 a |

[a] In the same column, numbers followed by a different letter indicate a significant difference (*P* = 0.05).

depth and 1 cm away from the stalk on one side (T1), two sides (T2), or three sides (T3) of the rice plant, respectively, at the heading stage, and no cutting as the control (CK). The block size is 15 $m^2$. All treatments are arranged according to a randomized complete block design with three replications. Root activity, rice yield, and yield components were analyzed and recorded at maturity.

Table 1 shows that root cutting at the heading stage increased root activity at the ripening stage in general, irrespective of varieties and treatments, probably as a compensation by the remaining roots.

Cutting at one side (T1) caused a yield increase for four varieties and a yield decrease for one (Table 2). The yield of Guang-Lu-Ai increased by 10.2%, Pei-Za-72 by 4.6%, Feng-Ai-Zhan 1 by 12.8%, and Te-San-Ai by 11.4%. The only yield reduction was for Er-Qing-Ai, which decreased by 18.2%, mainly because of the decrease in grains per panicle and percentage filled grains. Cutting on two sides (T2) and three sides (T3) caused a yield increase for two varieties (Guang-Lu-Ai and Te-San-Ai) and a yield decrease for three (Er-Qing-Ai, Pei-Za-72, and Feng-Ai-Zhan 1).

We conclude that light root cutting at the heading stage has no significant effects on rice yield. It can even increase the yield of some varieties.

## Notes

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**Table 2. Effect of root cutting at the heading stage on yield and yield components of different rice varieties, Guangzhou, China.**

| Variety and treatment | Effective panicles[a] ($10^4$ $m^{-2}$) | Grains per panicle | Filled grains (%) | 1,000-grain weight (g) | Yield (t $ha^{-1}$) | Yield difference (%) |
|---|---|---|---|---|---|---|
| Guang-Li-Ai | | | | | | |
| Control | 300.0 a | 90 a | 86.4 a | 25.9 a | 6.0 b | 0 |
| T1 | 312.5 a | 94 a | 88.9 a | 25.5 a | 6.7 a | 10.2 |
| T2 | 282.5 b | 95 a | 88.5 a | 25.8 a | 6.1 b | 1.4 |
| T3 | 292.5 b | 94 a | 87.0 a | 25.4 a | 6.1 b | 0.6 |
| Er-Qing-Ai | | | | | | |
| Control | 270.0 a | 155 a | 85.1 a | 23.8 a | 8.5 a | 0 |
| T1 | 260.0 a | 143 b | 78.0 b | 23.9 a | 6.9 b | –18.2 |
| T2 | 250.0 a | 144 b | 83.2 a | 23.3 a | 7.0 b | –17.8 |
| T3 | 270.0 a | 133 c | 84.2 a | 24.3 a | 7.3 b | –13.3 |
| Pei-Za 72 | | | | | | |
| Control | 243.8 a | 255 a | 71.0 a | 19.2 a | 8.5 a | 0 |
| T1 | 233.3 a | 267 a | 74.5 a | 19.1 a | 8.9 a | 4.6 |
| T2 | 247.3 a | 241 b | 71.4 a | 19.3 a | 8.2 a | 3.1 |
| T3 | 225.0 b | 234 b | 66.7 b | 19.2 a | 6.7 b | –20.4 |
| Feng-Ai-Zhan 1 | | | | | | |
| Control | 246.5 ab | 214 a | 63.1 b | 20.6 a | 6.9 a | 0 |
| T1 | 247.3 ab | 191 b | 78.6 a | 20.7 a | 7.7 a | 12.8 |
| T2 | 236.3 b | 171 c | 64.4 b | 19.9 a | 5.2 b | –24.5 |
| T3 | 267.8 a | 189 b | 52.9 c | 20.2 a | 5.4 b | –21.2 |
| Te-San-Ai | | | | | | |
| Control | 207.5 b | 172 a | 66.9 b | 28.9 a | 6.9 b | 0 |
| T1 | 220.0 a | 169 b | 73.3 a | 28.2 a | 7.7 a | 11.4 |
| T2 | 227.5 a | 166 b | 69.3 ab | 28.4 a | 7.4 ab | 7.7 |
| T3 | 212.5 a | 180 a | 66.1 b | 27.5 b | 7.0 b | 0.8 |

[a] In the same column, numbers followed by a different letter indicate a significant difference ($P = 0.05$).

# An overview of temperate rice production, technology, and development in New South Wales, Australia

W.S. Clampett, L.G. Lewin, R.L. Williams, G. Batten, H.G. Beecher, J.M. Lacy,
M. Fitzgerald, and M. Stevens

Rice growing in New South Wales, Australia, is a highly productive, efficient, and innovative industry, producing 1.3 million t from around 150,000 ha. Yields of 8 to 12 t $ha^{-1}$, depending on variety, management, and seasonal conditions, are among the highest in the world.

The temperate environment provides both opportunities and limitations for rice establishment, growth, yield, and grain quality. Management technology and practices to maximize yield and minimize limitations are seen as a basic building block for current and future success.

Rice growing, a major user of irrigation water, is being continually challenged to improve its efficiency of water use and to minimize groundwater pollution. The conventional water-use limit and soil classification techniques are being complemented by electromagnetic assessment to identify and isolate preferential recharge areas.

Variety improvement continues to play a major role in improving productivity, making more efficient use of resources, particularly water, and improving grain quality and developing niche markets. Seedling vigor, reproductive cold tolerance, duration of growing period, yield potential, and grain quality are important objectives of the Australian breeding program.

Crop establishment, a significant limit to productivity, continues to rely largely on aerial seeding of pregerminated seed into permanent floodwater. Innovative rice growers are seeking to reduce seedling drift losses by distributing dry seed onto the soil followed by permanent flooding.

Nitrogen continues to be the major nutrient applied, mainly before sowing. Topdressing at panicle initiation remains a widely used practice to fulfill nitrogen needs. The near-infrared rice tissue test now uses N content (%) and fresh weight to calculate N uptake at panicle initiation as the basis for topdressing recommendations. The software program *maNage rice* provides the opportunity to assess risks and the potential for extra yield compared with extra costs of topdressing. Near-infrared soil analysis shows promise as a preflood soil-testing tool. More intensive rice rotations and increased yields are resulting in increased phosphorus and, to a lesser extent, zinc fertilizer use.

Weeds are a major cost area in which herbicide resistance, increased use of MCPA, emerging weeds, and new herbicides are important developments. Insect pest investigations focus on reducing inputs for chironomid control and developing cultural techniques for managing aquatic snails and oligochaetes.

Water depth management is a major component of crop establishment, weed control, and cold protection at reproductive stages. Cold stress at early pollen microspore has been identified as a major yield loss factor in nearly all rice seasons, with deepwater protection at this time an important management practice.

Grain quality, as determined by whole grain, cleanliness, trueness to specification, taste, and cooking characteristics, remains a keystone for an industry that targets the premium end of niche markets. Crop draining and harvest management have important effects on quality. Improving the early evaluation of quality in the breeding program and understanding the processes in the developing plant that influence the expression of quality attributes are being investigated.

Rice growing in New South Wales (NSW), Australia, is a highly productive, efficient, and innovative industry, producing an average of 1.36 million t from around 150,000 ha for an overall average of 9 t ha$^{-1}$ over the last three harvests (Fig. 1). It represents almost all rice currently grown in Australia. Yields generally range from 8 to 12 t ha$^{-1}$, depending on variety, management, and seasonal conditions, and are among the highest in the world (Fig. 2).

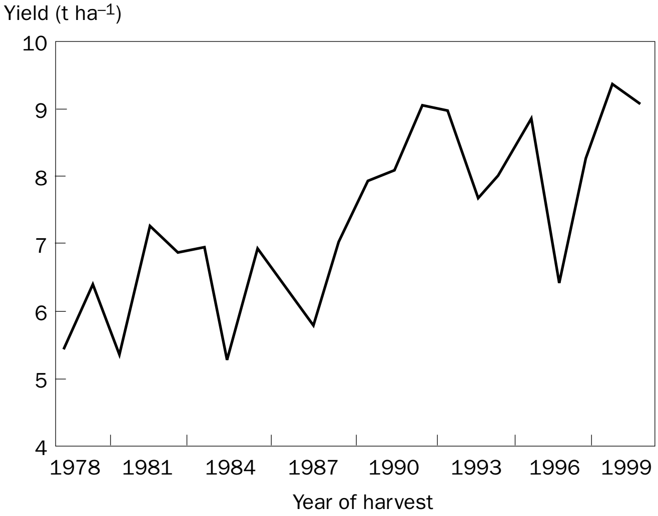

**Fig. 1. Mean rice yields in New South Wales, Australia, 1978-99 (Ricegrowers' Co-operative Limited 1999a).**

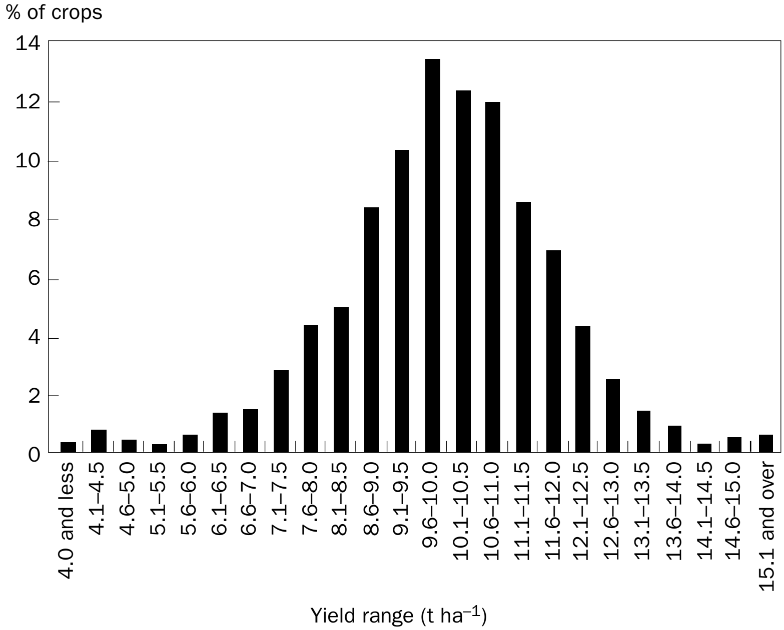

**Fig. 2. Distribution of yields for semidwarf medium-grain varieties Amaroo and Namaga, 1999 (Ricegrowers' Co-operative Limited 1999b).**

Rice growing in New South Wales is carried out on the fine-textured soils of the riverine plain of southwest NSW between 34 and 36 °S, primarily in the irrigation areas and districts, and river frontage land of the Murrumbidgee and Murray River valleys. One crop is grown per year over the spring, summer, and autumn period from October to March. The crop-growing period varies from 160 to 180 d for full-season varieties, such as Amaroo, down to 135 to 155 d for short-season varieties, such as Jarrah. Rice is grown as a fully ponded crop, depending on the method of crop establishment, with irrigation water providing 90% or more of the total water use of 12 to 16 megaliters (ML) ha$^{-1}$.

The temperate environment provides both opportunities and limitations for rice establishment, growth, yield, and grain quality. The importance of management technology and best management practices to maximize opportunities and minimize limitations, within a framework of sustainable resource use and productivity, is seen as a basis for current and future success.

## Climate

High levels of solar radiation and generally favorable temperatures provide the basis for high yield potential. This is offset by the variability of these conditions, which affects yields through floret fertility, total biomass production, and, to a lesser but important extent, crop establishment.

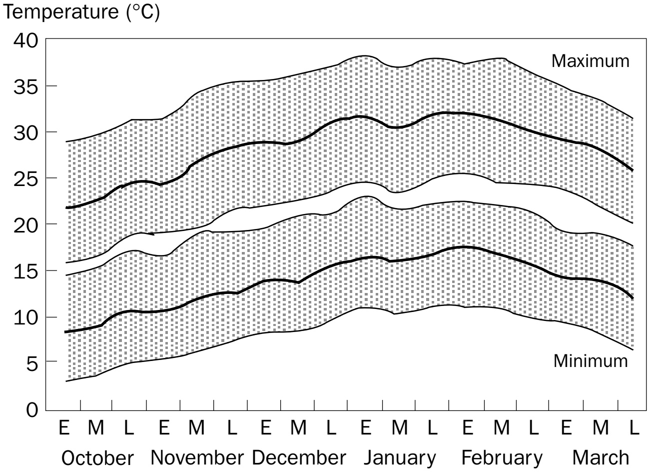

**Fig. 3. Comparison of mean maximum and minimum temperatures (10-d means) with 10 and 90 percentiles at Griffith, New South Wales, Australia (Erskine and Smith 1983). E = early, M = mid, L = late.**

Management practices aim at optimizing the potential provided by the climate, while minimizing the effect of variability on yields. Adherence to best management practices for establishment, sowing time, nitrogen fertilizer management, water depth, draining, and harvest is all related to this aim. Temperature variability of 10-d means (Fig. 3) between the 10 and 90 percentiles and the 50 percentile (mean) shows a normal variation in the range of ± 5 to 7 °C (Erskine and Smith 1983).

Solar radiation levels average 19 to 23 MJ $m^{-2}$ in October and March, reaching a mean of 29 to 30 MJ $m^{-2}$ in December. Rainfall over the 6-mo growing period averages around 200 mm, whereas evaporation averages around 1,400 mm.

## Soils and water

A major challenge is identifying excessively permeable soils so their use for rice growing can be avoided and accessions to regional water tables reduced. In the past, the deep percolation component of rice water use of some soils growing rice has exceeded 6 ML $ha^{-1}$ per rice crop. Water-use targets suggest that this component should be around 2 ML $ha^{-1}$ or less.

Soils used for rice growing are mainly part of the variable riverine/aeolian deposition system of fine-, medium-, and coarse-textured layers. Their suitability for rice growing has traditionally been assessed by textural classification of the surface and the immediate underlying soil layers, with the depth of suitable clay layers being a major criterion. This textural classification has been of qualified success in identifying excessively permeable soils and avoiding their use for rice growing, but has been limited by the intensity of sampling in identifying unsuitable underlying soil conditions and in the inability of textural classification to identify the permeability of some clay soils.

This system has been complemented by the use of measured water supply to rice fields to identify those fields that use in excess of target water-use criteria. This, too, has been useful as a broad secondary indicator, but is limited by the accuracy of measuring the water supply and is open to abuse.

Over the past 5 years, electromagnetic (EM) induction technology (Geonics EM-31) (Beecher et al 1997) has shown great potential as a tool for identifying within-field variability due to textural discontinuities within a rice field. On the basis of such textural differences, soils can be identified that do not meet the current criteria to a degree of intensity and accuracy not previously possible.

Importantly, the technique cannot only assess a whole field quickly and relatively cheaply but can also, with the associated targeted drilling specifically, identify and isolate preferential recharge areas. Previously, when a field was seen as too permeable (i.e., field water use was excessive), the whole field could be removed from rice growing. The EM-31 technology allows recharge areas to be isolated by redesigning the rice field layout or the recharge area to be treated to reduce permeability.

The EM-31, when mounted on a four-wheel-drive motor bike, increases the density of data sampling from 1 site per 4 ha (random drilling) up to 100 points $ha^{-1}$. This, combined with global-positioning and computer-based mapping systems, allows the delineation of differences in what superficially appears to be a "uniform" field or landscape. A reduced number of sites can then be investigated based on EM values.

Extra information such as water table depth, cropping history, surface soil condition, vegetation profile, drainage patterns, and available information from existing bore holes may be needed to aid in the interpretation of the EM response within a field. Targeted soil sampling and rice land assessment allowing for field variability can then be made on the basis of apparent electrical conductivity values (ECa) measured to infer the likely level of groundwater recharge. Figure 4 shows season-long cumulative infiltration for four sites (EM values) identified from an EM-31-surveyed rice field.

McNeil (1992) concluded that ground conductivity meters will find increasing use for the ". . .location of suitable sites for evaporation basins and subsequent monitoring for leakage, classification of land for irrigation suitability, and mapping of recharge and discharge areas in addition to a range of potential uses."

Beecher et al (1997) showed the potential for using EM-31 instruments combined with global-positioning systems to provide spatial detail on variation in soil properties across rice fields and a methodology on assessment of rice soil using the current soil suitability criteria.

Electromagnetic surveys are undertaken by commercial service providers who complete the EM-31 survey, data manipulations, and subsequent drilling program to an agreed set of guidelines. The guidelines provide for hard-copy maps and survey interpretation along with electronic copies of the raw data to be provided to the agencies that regulate rice land approvals.

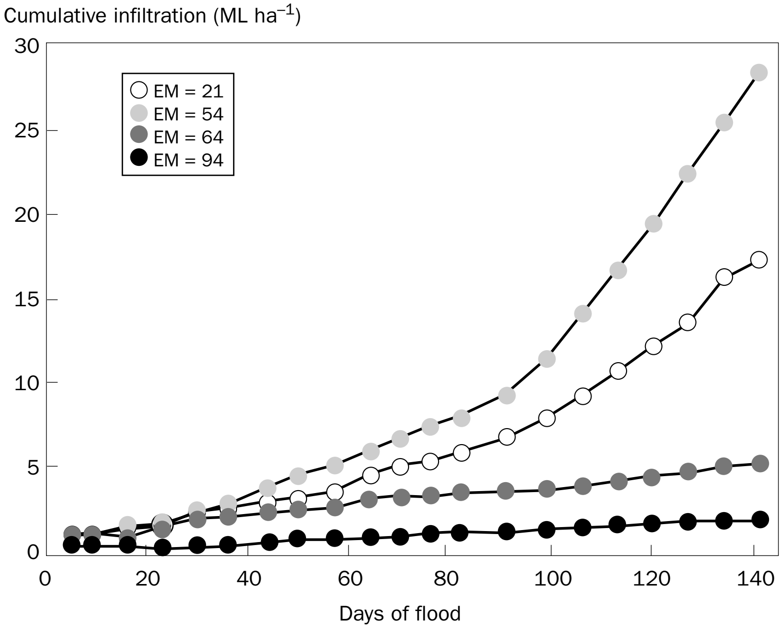

**Fig. 4. Season-long cumulative infiltration for four sites (electromagnetic, EM, values) identified from an EM-31-surveyed rice field.**

Irrigation companies and other regulatory agencies already use commercial EM-31 surveys routinely to assess new fields for rice growing and existing lands that are suspect because of a lack of either previous soil information or measured water use. The use of EM-31 surveys will probably become a mandatory feature of soil suitability classification in the future.

Some growers are already using data obtained from such surveys as a step in the purchase of rice-growing lands.

## Varieties and plant breeding

McDonald (1994) and Brennan et al (1994) described the cultivar profile for the NSW rice industry for the period up to 1993. Some changes in the suite of cultivars have occurred since that time (Table 1) with the release of some new cultivars and others no longer produced.

Improved yield potential remains an important objective to further enhance water-use efficiency to keep pace with the continuing decline in the cost/price ratio and to improve the efficiency of production. The yield performance of newer cultivars is reflected by the commercial yield in the high-yielding 1997-98 season (Table 2). Illabong remains the highest-yielding cultivar, perhaps because of its larger grain. There are also some regional differences in yield performance and some cultivars (e.g., Doongara, Kyeema) are not recommended for production in the southern areas.

**Table 1. New South Wales rice cultivars in production since 1993.**

| Cultivar type and name | Year released | Year production ceased | Parents | Breeders |
|---|---|---|---|---|
| Medium grains | | | | |
| Amaroo | 1987 | – | Calrose/M7 | L.G. Lewin, A.B. Bakeney, R.F. Reinke |
| Bogan | 1987 | 1997 | Calrose/M7 | L.G. Lewin, A.B. Bakeney, R.F. Reinke |
| Jarrah | 1993 | – | M7//M7/Somewake | R.F. Reinke, L.G. Lewin, A.B. Blakeney |
| Namaga | 1997 | – | Calrose/Baru//Calrose///M7 | L.G. Lewin, R.F. Reinke, A.B. Blakeney, L. Welsh |
| Japanese quality | | | | |
| Millin | 1995 | – | M7//M7/Somewake | R.F. Reinke, L.G. Lewin, A.B. Blakeney |
| Koshihikari | Introduced | – | | |
| Opus | 1999 | – | Bogan/Koshihikari | L.G. Lewin, R.F. Reinke, A.B. Blakeney, M. Fitzgerald |
| Arborio style | | | | |
| Illabong | 1993 | – | Saint Andrea/M7 | R.F. Reinke, A.B. Blakeney, L.G. Lewin |
| Long grain | | | | |
| Pelde | 1982 | 1997 | YR13.89.11/Bluebelle | L.G. Lewin, R.A. Hartley, A.B. Blakeney |
| Langi | 1994 | – | YR73/M7//Pelde | L.G. Lewin, R.F. Reinke, A.B. Blakeney |
| Long grain–firm | | | | |
| Doongara | 1989 | – | Calrose/Bluebelle//Jojuitla | L.G. Lewin, A.B. Blakeney, R.F. Reinke |
| Long grain–fragrant | | | | |
| Goolarah | 1991 | 1996 | Della/Kulu | R.F. Reinke, A.B. Blakeney, L. Welsh, L.G. Lewin |
| Kyeema | 1994 | – | Pelde//Della/Kulu | R.F. Reinke, L.G. Lewin, A.B. Blakeney |

Seedling vigor remains an important problem in many seasons. Specific selection techniques have been described to assist the development of early vigor. These have included the use of slant boards (Li and Rutger 1980) and other techniques under controlled conditions, but there has not been significant progress for this character.

Cold tolerance also remains an important objective. The effects of cold were clearly demonstrated in the 1995-96 season when cold in the reproductive period significantly depressed yields. Recent work in the Cooperative Research Center for Sustainable Rice Production (Lewin and Davidge 1999) is beginning to describe the physiology and some aspects of genetic control of cold tolerance. Cultivar Millin is

**Table 2. Rice (paddy) yields in the New South Wales rice-producing regions for the 1997-98 season. Yield is t ha$^{-1}$ at 14% moisture. The regions are MIA (Murrumbidgee Irrigation Area), CIA (Coleambally Irrigation Area), EMV (Eastern Murray Valley), and WMV (Western Murray Valley).**

| Variety | MIA | CIA | EMV | WMV | NSW av |
|---|---|---|---|---|---|
| Amaroo | 9.9 | 10.1 | 9.5 | 8.5 | 9.6 |
| Namaga | 10.8 | 10.4 | 10.0 | 9.5 | 10.1 |
| Jarrah | 9.0 | 5.7 | 8.4 | 8.1 | 8.2 |
| Millin | 8.2 | 8.8 | 8.5 | 7.8 | 8.3 |
| E. Millin | 9.8 | 8.5 | 10.0 | 8.2 | 9.5 |
| Illabong |  |  | 10.8 | 9.4 | 10.7 |
| Koshihikari | 6.2 |  | 6.3 | 6.8 | 6.6 |
| Langi | 9.6 | 9.8 | 8.7 | 7.8 | 9.4 |
| Doongara | 9.0 | 9.3 |  |  | 9.1 |
| Kyeema | 7.7 | 8.2 | 8.8 |  | 8.0 |
| Area average—all varieties | 9.7 | 9.7 | 9.4 | 8.5 | 9.4 |

apparently the most tolerant of the NSW cultivars, but more improvement for this character is possible.

Reduced duration is also an important characteristic for NSW rice cultivars to save water, reduce recharge, and improve efficiency of production. Progress has been made in reducing duration, but this has not necessarily resulted in improved efficiency of water use (Williams et al, this volume).

Six cultivar types, grown to meet specific market criteria, are produced in the NSW rice area. Reinke et al (1999) provide details. Selection for improved quality within each variety class remains a cornerstone of the improvement program. The aim is to develop objective tests for each quality characteristic and significant progress has been made with this objective. Objective tests are now used for the appearance characteristics of length and width, chalkiness, and color in addition to the range of tests for cooking quality. Many of these tests are regularly used at early generations of the selection program.

## Rotations

Traditionally, organic nitrogen accumulation from a 3–5-year subterranean clover rotation between rice crops has played a major part in the supply of N to rice crops. Over the years, the legume rotation has become less important because of increased rice after rice, more intensive cropping rotations, the higher N rates for semidwarf varieties, and more intensive rice growing. Survey data (J. Lacy, personal communication) show that pasture is still a major component as around 40% of rice crops followed some legume phase, whereas more than 30% of crops followed a previous rice crop. Table 3 provides the percentage of three broad rotation categories.

**Table 3. Average percentage of three broad rotation systems for 5 seasons (1994-98).**

| Preceeding rotation crop | Murrumbidgee (%) | Murray Valley (%) |
|---|---|---|
| Clover pasture (2–3 y) | 42 | 38 |
| Other nonrice crops | 22 | 29 |
| Rice crop | 35 | 31 |

## Crop establishment

Crop establishment, a significant limit to productivity, continues to rely largely on aerial seeding of pregerminated seed into permanent floodwater. Innovative rice growers are seeking to reduce seedling drift losses by distributing dry seed onto the soil followed by permanent flooding or a flushing irrigation and permanent flooding.

Target recommendations aim for 150–300 plants $m^{-2}$. In recent years, seeding rates for aerially sown rice have tended to increase from around 130 kg $ha^{-1}$ up to 150–160 kg $ha^{-1}$.

Turner and Lewin (1994) found that some 70% of rice crops had poor establishment each season. Surveys over three crop seasons estimated that about 7% of the total crop was affected. Poor establishment can vary widely between regions and seasons. Beale (1998) found that 15–20% of the Western Murray Valley rice crop area had poor establishment versus 7–14% in the Eastern Murray Valley. Poor establishment and low or absent plant stands were caused by floating seedlings, ducks, bloodworms, snails, algae, and/or cold weather.

Aerial sowing of pregerminated seed into flooded fields continues to be the most popular method of sowing with 90% plus of the area sown. Aerial sowing increased markedly following the release of bensulfuron-methyl in 1998. Traditional drill sowing, where dry sowing is followed by flushing until permanent water is applied at the 3–4-leaf stage, continues at around 5%. The newest trend, in an attempt to reduce seedling losses, is to sow dry seed on the soil surface, followed by early intermittent irrigation and then a permanent flood or permanent flood after seeding. This technique, which also reduces establishment costs, is convenient, has a reduced workload, and is managed as for aerial-sown crops after permanent flooding. Seeding rates are often increased by 10% over normal rates. Table 4 shows the changes in crop establishment over the past 15 years.

**Table 4. Methods of crop establishment over the past 15 years.**

| Rice season | Aerial-sown | Dry-sown/flooded (%) | Drill-seeded |
|---|---|---|---|
| 1985-86 | 52 | Nil | 48 |
| 1988-89 | 65 | Nil | 35 |
| 1993-94 | 94 | Trace | 6 |
| 1998-99 | 89 | 5 | 6 |

## Nitrogen management

Nitrogen continues to be the major nutrient applied, although increasing use of phosphorus and, to a small extent, zinc has occurred in recent years as a result of more intensive rotations. Total nitrogen rates vary from 50 to 200 kg N ha$^{-1}$, with 40–80% applied before permanent flood, normally as urea, but including a small amount of anhydrous ammonia. Most postpermanent-flood N is applied at panicle initiation (PI).

The widespread use of PI topdressing reflects a strategic approach to nitrogen application, using PI topdressing as a low-risk method of tailoring N fertilizer to seasonal needs of the crop, and to a lesser extent the lack of a reliable preflood soil test.

The near-infrared rice tissue test continues to be widely used at PI, with around 1,500–1,800 crops tested each year, or 45% of rice farms. Developed in 1987, it initially used a combination of N concentration (as % total aboveground biomass) and shoot counts m$^{-2}$ as an index of plant biomass. In 1996-97, the shoot counts were replaced by the more direct measure of plant biomass by using measured fresh weight at PI. The relationship between shoot numbers and biomass varied too much between crops and seasons to be reliable. Farmers carry out most of the crop sampling and use wet newspaper as a wrapping to maintain tissue turgidity from the time of field sampling until the sample is weighed.

The combination of tissue % nitrogen and biomass weight provides a measure of total plant N uptake at PI, which is the basis of topdressing recommendations. This is used in a paper-based system of recommendations (Lacy et al 1998) that provide yield responses to topdressing. The cost-benefit return of the extra yield and extra costs of topdressing relative to the risk must be assessed by the rice growers themselves. Rice growers generally tend to fertilize to maximize total yield rather than to analyze the specific cost-benefit relationship of topdressing.

The software program *maNage rice* (Angus et al, this volume) was developed to help rice growers obtain a more detailed assessment of tissue test results for each individual rice field. It uses the temperate rice yield model (TRYM) developed at Yanco. Extra inputs include date of sowing, PI date, likely water depth at early pollen microspore, costs of fertilizer and application, expected rice returns, and historic temperature data to provide a risk assessment of likely yield response and profit.

There is a need for a reliable preflood soil nitrogen test rather than relying on PI N as the topdressing application is more critical in determining yield potential. An exciting development is that near-infrared soil analysis shows promise as a preflood soil-testing tool. Preliminary work indicates that good correlations arise from using the relationship between soil N and total N uptake at PI.

## Weed control

Weeds are a major cost of rice growing, representing some 20–25% of the total costs of production. Herbicide resistance, increased use of MCPA, emerging weeds, and new herbicides are important developments.

Barnyardgrass (*Echinochloa* spp.) and to a lesser extent silvertop grass (*Leptochloa fusca*) are the major grass weeds, and the main weeds of drill-sown rice crops. In aerial-sown rice, grass weeds are of increasing importance as more intensive rotations and rice after rice have become popular over the past 15 years.

Molinate is the main grass herbicide used, but increasing use is being made of thiobencarb because of its efficacy on *Cyperus difformis* and despite the narrower application window. Molinate, used only as a 96% EC formulation, is normally applied at 2.4–3.6 kg ai ha$^{-1}$ (Clampett and Stevens 1998).

Rates for thiobencarb in drill-sown rice, used as an EC formulation, vary from 4 kg ai ha$^{-1}$ when used alone to 3 kg ai ha$^{-1}$ in a tank mix with propanil at 1.5 kg ha$^{-1}$. In aerial-sown rice, total thiobencarb rates vary from 3 to 3.4 kg ai ha$^{-1}$.

In the 1998-99 season, the grass herbicide clomazone, a carotenoid biosynthesis inhibitor for the control of *Echinochloa* spp. and to suppress *Leptochloa fusca* at higher rates, was released for commercial use. Clomazone is applied to flooded basins at 192–288 g ai ha$^{-1}$.

In aerial-sown rice, and in systems where the field is flooded soon after sowing, the aquatic weeds *Cyperus difformis*, *Damasonium minus*, and *Sagittaria montevidensis* become important competitors. Since 1988, bensulfuron-methyl has been the most widely used herbicide on these weeds. In recent years, however, as resistance to this herbicide became more obvious, the use of MCPA sodium salt has become more widespread and, to a lesser extent, the use of thiobencarb has also increased. Bensulfuron-methyl is used at 30–50 g ai ha$^{-1}$, whereas MCPA sodium salt is normally used at 0.675 kg ai ha$^{-1}$.

In the past 5 to 15 years, *Alisma lanceolatum* and *A. plantago-aquatica* have continued to emerge as problem weeds in some rice fields.

In 1998-99, a new herbicide, benzofenap, was used to control the aquatic weeds *A. lanceolatum, A. plantago-aquatica*, and *S. montevidensis* and suppress *C. difformis*. It is applied at 0.6 kg ai ha$^{-1}$.

Since the development of resistance to bensulfuron-methyl, much effort has gone into developing herbicide programs that maximize the use of the commercial herbicides available. Sequential and split-rate treatments involving the strategy of two modes of action for each aquatic weed and the use of MCPA sodium have been developed. Taylor and Clampett (this volume) described these.

## Insects and other pests

Five invertebrate pests regularly cause crop damage. Four of these primarily affect the establishment phase of the crop. Chironomid midge larvae, known as bloodworms, are the most serious and widespread establishment pests, and can cause a 95% plant loss in aerially sown crops if not controlled. The most damaging species is *Chironomus tepperi* Skuse, although other species are also implicated in crop damage. Control is achieved by using insecticides, which are usually applied twice in the first 3 wk after sowing. The recent introduction of fipronil seed treatments (Stevens et al 1998) has reduced insecticide inputs for bloodworm control by more than 75%.

The snail *Isidorella newcombi* (Adams & Angas) affects approximately 15% of the crop each season, feeding on plant root systems from establishment until late tillering. Serious outbreaks early in the season are often linked to repeat cropping, since snails that enter dormancy after one crop is drained can survive until the following spring. Breaking the cycle of repeat rice crops destroys dormant snails because they cannot survive in dry soil over summer.

The oligochaete *Eukerria saltensis* (Beddard) is the only introduced pest affecting rice production in NSW. Dense infestations attract large flocks of ibis birds that trample seedlings into the mud. *Eukerria* infestations also increase turbidity, thus reducing photosynthesis in submerged seedlings, and mobilize soil nutrients into the water column, thus promoting dense algal blooms. Dispersive clay soils are the worst affected, particularly when rice is grown after an irrigated clover pasture. Management is mainly cultural—shallow water on fields with little or no slope, combined with rapid flooding and sowing. Scare guns are used for bird management.

The leafminer *Hydrellia michelae* Bock is a sporadic pest during later establishment, and is effectively controlled with trichlorfon. Armyworms (*Leucania convecta* (Walker)) are occasionally a problem in the months leading up to harvest. Stevens (1997) has reviewed the biology of NSW rice pests.

## Deep water at early pollen microspore

Cold stress at early pollen microspore (EPM) has been identified as an important yield loss factor in nearly all rice seasons. Comparisons between deep and shallow water depths at EPM in conjunction with a range of varieties and nitrogen rates continue to reinforce the value of deep water as an essential management practice, particularly where high nitrogen rates are aiming for high yields.

Simulation modeling of responses to minimum temperatures at EPM indicates that yield losses can occur up to a temperature threshold near 19 °C at EPM (Williams, unpublished data). In 1998 early sowings, where EPM temperatures averaged around 19 °C, no yield response occurred in deepwater (20 cm) versus shallow (5 cm) treatments, whereas later sowings and similar levels of plant biomass in deep water at EPM gave yield responses of up to 4.7 t $ha^{-1}$ more than in shallow water treatments (Fig. 5).

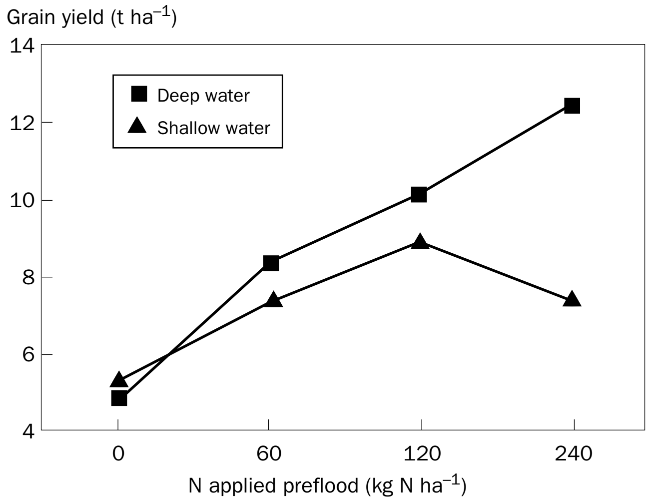

**Fig. 5. Yield response to nitrogen rate under deep (20 cm) versus shallow (5 cm) water depth at early pollen microspore on Amaroo rice.**

Given that the 10-d mean minimum temperatures during this critical stage are around 16–17 °C, and that 10-d means below 19 °C are likely 70–75% of the time, the benefit of deepwater protection will occur most of the time.

Recent survey data indicate that 51% of growers are achieving a high level of protection with the recommended minimum 20-cm water depth and a further 29% are achieving a moderate level of protection (Lacy, unpublished data). Minimum temperatures in water are 6–9 °C above the temperature in the air.

## Grain quality

Grain quality, as determined by whole grain, cleanliness, trueness to specification, taste, and cooking characteristics, remains a keystone for an industry that targets the premium end of niche markets, through the marketing of a branded product to specification. Crop draining and harvest management have important effects on quality. Improving the early evaluation of quality in the breeding program and understanding the processes in the developing plant that influence the expression of quality attributes are being investigated.

Three aspects of quality are receiving particular attention: % whole grain (%WG) or head rice, chalk, and cooking quality.

### % Whole grain (WG)

Variability of %WG yields between and within seasons, particularly in medium grains, has caused concern for many years. Best management practices to maximize %WG have sought to maximize grain yield and quality within the limits imposed by variety and environment. The practices aim to promote crop uniformity, place grain ripening

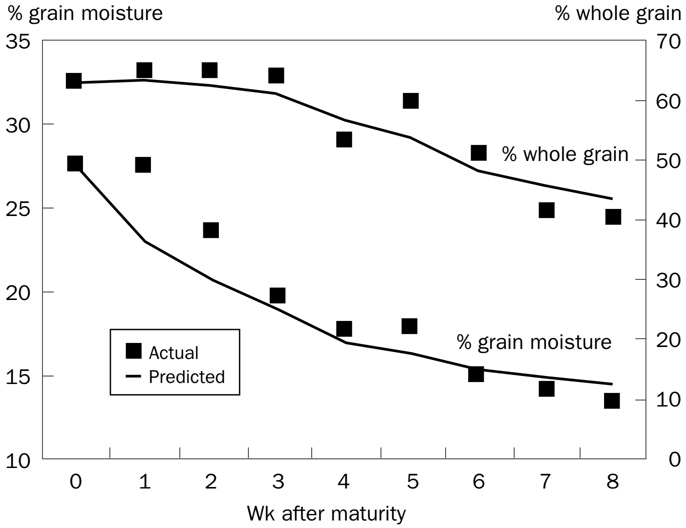

**Fig. 6. Comparison of actual and predicted % grain moisture and % whole grain for an individual crop of medium-grain Amaroo rice.**

and drying stages within a period of moderate weather, and encourage harvesting as soon as possible after physiological maturity. Despite a %WG appraisal premium, adoption and acceptance of the recommended practices have been variable.

A sequential grain-harvesting project (Clampett et al 1999) sampled some 430 crops each week for 8 to 10 wk from physiological maturity onward. The data showed that the main difference between crops that maintained a comparatively high %WG versus those with low %WG was the rate of grain drying after physiological maturity. The rate of grain drying drove the rate of whole-grain decline, both of which could be simulated using climatic, soil moisture, and grain moisture data (Fig. 6).

It was found that all medium-grain crops have a potentially high whole-grain yield of around 65%, which remains relatively stable until the grain moisture drops below 20–22%. After this, the rate of %WG decline is directly related to daily evaporative demand in excess of 2 mm $d^{-1}$, the effect of which will be modified by the presence of soil surface moisture. Below 20–22% grain moisture, rainfall can cause a major decline in %WG, in excess of 30% in 1 wk. Long-grain varieties respond differently and need further investigation.

The project not only reinforced the current recommended practices but also provided a more objective basis for their recommendation and adoption. The study shows rice growers a way forward to improving the quality of delivered rice grain, but they are often constrained by the low extra returns relative to the extra costs of compliance and difficulties of harvesting at 20% grain or higher.

## Chalky rice

High temperatures during certain stages of grain development increase the likelihood that rice grains will contain chalky areas. Chalky grain decreases yield because the grains are softer and more likely to break during milling, and it decreases quality because it is deemed visually displeasing. We aim to understand why chalk occurs and to identify the processes that are sensitive to high temperatures and that ultimately lead to chalk. The information from this will allow the breeding program to select crossbred lines that tolerate the environmental factors that lead to chalk.

The most likely cause of chalk in our region is an interval of high temperatures during grain development. When this occurs, grains located in the inferior position on the panicle are likely to exceed the amount of chalk stipulated for market acceptability.

In chalky areas of a rice grain grown at high temperature, starch granules are shaped and packed differently compared with the shape and packing in translucent areas. This indicates that high temperatures affect the process of starch synthesis and organization, which therefore affects the shape and the packing of the starch granules. Starch synthesis occurs by the action of several different enzymes. The enzyme activity is lowered greatly by high temperatures, the rate of starch deposition is lowered by high temperatures, and the resulting starch structure is altered. The difficulty is separating the factors that lead to chalk from the effects of high temperature on starch synthesis, but progress is being made. Lines that are tolerant of and susceptible to forming chalk at high temperatures have been identified, and work is under way to understand the cause of chalk at the molecular level. At this stage, it seems that chalk is a bad case of heat exposure.

## Cooking quality

Different varieties have different capacities to synthesize starch of a particular structure and a particular amount and complement of proteins. Environmental conditions (climate + nutrition) during grain development can affect the extent to which the grain can express its genetic capacity. Any manipulation of the nutritional or cultural regime and any spells of excessively high or low temperatures alter cooking quality.

Rapid visco analysis (RVA) is the most useful tool for evaluating cooking quality and theory suggests that amylose content exerts the main influence on cooking quality. However, varieties with the same amylose content differ markedly in cooking quality and this is related to both the starch structure and the content and complement of grain proteins. How each part of the viscosity curve translates into quality is not fully understood. Research is under way to understand the effect of different starch structures and different complements of proteins on cooking quality, and to enable us to predict cooking quality from RVA.

## Yields

Rice yields in the region reflect both the opportunities and limitations of the environment, as modified by management practices, for variation of yield within and between seasons. Figure 1 shows seasonal yield variation over the past 22 years and this strongly reflects the broad effect of weather conditions on floret fertility, biomass development, and, to a lesser extent, establishment. The distribution of yields in a high-yielding season as shown in Figure 2 highlights the effect of management in all its aspects—sowing time, establishment, nutrition, weed and pest control, water depth control, and draining—to minimize the effect of seasonal variation. Table 2 provides further details on varietal yields.

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AGRONOMY

# An adaptive and objective rice growth staging system

P.A. Counce, T.C. Keisling, and A.J. Mitchell

The large area of rice (*Oryza sativa* L.) production worldwide is critical to the well-being of large numbers of the world's people. Undefined and conflicting descriptions of rice developmental stages make comparison and application of research results difficult. The use of time (days after emergence, d after planting, d after flooding, d after anthesis) or thermal time such as degree-day units in lieu of developmental stage descriptions is only accurate in controlled environmental conditions. Consequently, an objective growth staging system with enumeration adapted to the development of the plant would improve communication among scientists, farmers, and educators. We propose a rice developmental staging system with four growth stages during seedling development (unimbibed seed, radicle emergence, coleoptile emergence, and prophyll emergence), the number of vegetative stages equal to the number of completely expanded leaves (excluding the prophyll) on the main stem, and ten reproductive growth stages based on discrete morphological criteria (from panicle initiation to maturity). Assigning rice growth stages based on discrete morphological criteria will result in unambiguous growth stage determination (i.e., two people staging the same plant will, using this system, arrive at the same growth stage). This is because the system exploits the presence or absence of distinct morphological criteria in a symbolic logic dichotomous framework that permits only "yes" or "no" answers. The system can, however, be expanded to fit the needs of various workers to include quantitative criteria if such criteria are needed for certain applications. Consequently, an unequivocal rice growth staging system is presented that is based on objective morphological criteria and that has enumeration adapted to the rice plant's development.

Rice (*Oryza sativa* L.) grain is a food staple for more people than food obtained from any other single plant species. Major portions of the human population of China, Southeast Asia, India, parts of Africa, and parts of South America get more than half of their daily dietary calories from rice. Consequently, rice research and its application potentially affect the well-being of a large part of the world's human population.

Crop growth staging systems are meant to be an aid in information transfer for crop management. They can also aid scientists and others in observing, recording, and communicating critical crop growth data. Yet, for rice, the most important single plant species for human nutrition, there is no widely used growth staging system. Three published systems exist for expressing rice developmental stages: Zadoks et al (1974), IRRI (1980), and the BBCH system (Lancashire et al 1991). The Zadoks et al (1974) system is an adaptation of Feeks' (1941) scale but broken into 10 subdivisions. The IRRI (1980) scale is a 10-point system (0 to 9). The BBCH system (Lancashire et al 1991) is an adaptation of the Zadoks et al (1974) scale. Consequently, the BBCH and Zadoks et al scales are 99-point systems with both discrete and continuous criteria for plant growth stage determination. The very nature of arbitrarily assigning numerical crop growth stages makes them subject to individual judgments rather than discrete and objective criteria. Haun (1973) described a developmental system for wheat (*Triticum aestivum* L.) that has been applied to rice.

One of the growth staging systems with the most long-lived usefulness and employment is the system presented by Fehr and Caviness (1979) for soybean (*Glycine max* L.). The concept of leaf age was recognized by D'Arcy Thompson, Bonnet, Kepler, Leonardo da Vinci, and perhaps Aristotle (Jean 1994). But Fehr and Caviness recognized that the basic developmental pattern for soybeans was leaf age and incorporated that knowledge into their soybean growth staging system. Fehr and Caviness also allowed the enumeration of the vegetative growth stages to be determined by the plant rather than being imposed arbitrarily upon it. Their system uses objective, determinable morphological criteria (with the possible exceptions of growth stages R3 and R4) for soybean development. Consequently, two people independently examining one plant for soybean growth stage will arrive at the same answer.

A crop growth staging system based on plant morphogenesis, with each stage differentiated from another dichotomously, would remove the major limitation of other systems. The dichotomy referred to is the symbolic logic framework for phrasing questions. Only discrete criteria are employed because criteria determined by quantitative measurement (length, width, etc.) are plastic and consequently productive of equivocal growth stage determinations. An objective morphological criterion is either present or absent; thus, for the question of whether a plant is at a given growth stage, the answer is either yes or no.

Several systems are in place to express various stages of rice development. Some are adapted to a small area of land over which conditions are consistent enough to make applicable assumptions about plant growth and development. These assumptions do not hold true over a larger area of land. In Arkansas, Louisiana, Mississippi, and Texas rice-growing areas, the differentiation of the panicle into branches coincides with the first internode to elongate much more than its own width. Therefore, in

Arkansas, this stage of growth is called internode elongation (IE). By splitting the culm in half lengthwise, the differentiating panicle is visible along with the first elongated internode. This is the stage of development when the nitrogen demand by the developing panicle is relatively high and the rice crop is responsive to midseason nitrogen fertilization. The green ring is the dark green circle found by slicing across the vertical axis of the rice culm just below the closely stacked initiated final leaves of the culm and the initiating panicle. Now, logically, supplying nitrogen at the green ring would provide adequate nitrogen for the soon-to-be differentiating panicle. Both the green ring and IE are easily visible without magnification and farmers, salesmen, extension personnel, and researchers are familiar with the term "green ring" in Louisiana and IE in Arkansas, so these terms are valuable. The problem with both terms is that they are indirect guides to the actual events of interest—panicle initiation and differentiation. In some cases, panicle initiation and differentiation can occur without green ring or IE. Clearly, what is needed is a system for expressing the actual event rather than the proxy. In Texas, the terms "panicle initiation" and "differentiation" are employed for the sake of scientific precision. Moreover, although panicle initiation is an event at a point in time, panicle differentiation is a process through time. Also, what is differentiating—panicle branches, florets, ovules? Informally, the term "panicle differentiation" refers to differentiation of the panicle branches.

The power of the local jargon and systems is that they are concise and only growth stages needed for management decisions are identified. Consequently, local systems are simple and short and rely on visible markers. However, when new management decisions are required, the short local systems must often be expanded to address the new problems. Also, research results that would be useful to farmers, educators, and researchers across large geographical areas are often impossible to apply because of imprecise growth stage expression, which may mean one thing in one area but another elsewhere. Consequently, a precise, objective, and adaptive rice growth staging system is needed for present application and future needs of farmers, educators, and researchers.

The primary value of an objective and adaptive crop growth staging system is enhanced communication among farmers, consultants, crop insurers, scientists, teachers, and extension personnel. The central feature that allows meaningful communication is the ability to objectively determine the growth stage. The dichotomous feature assures that different people staging the same rice plant will deduce the same crop growth stage. The visibility of criteria is also critical to the system. It is more convenient in the field to observe things visible to the naked eye or with a small hand lens (approximately 10X magnification) rather than requiring a microscope. Consequently, plant morphological features visible with magnification less than or equal to 10X were favored as growth stage criteria.

## Morphogenesis

Rice morphological development can be divided into three stages: seedling, vegetative, and reproductive. These three stages are described in detail below. The late vegetative stage and the early reproductive stage occur simultaneously. The seedling and reproductive stages progress through a series of unique (occurring only once during development) events, whereas the vegetative stage progresses through an iterative sequence of leaf developmental events. Thus, the phyllochrons are true examples of gnomonic development (Jean 1994).

### Seedling development

To germinate, a dry rice seed must imbibe water. In germinating seeds, either a coleoptile or a radicle may emerge first. In dry-seeded conditions, the radicle emerges first, but in water-seeded conditions the coleoptile may emerge first. However, in some lines of rice, the coleoptile emerges before the radicle in dry-seeded culture. A prophyll (rudimentary leaf) emerges from the coleoptile. This is followed by the emergence of the first complete leaf (one with a blade and sheath) above the prophyll. In anoxic conditions, such as water-seeded rice, the emergence of the radicle may occur after the emergence of the first complete leaf.

### Vegetative development

Vegetative growth occurs in distinct biological units of developmental time. Terms that have been used to describe this developmental time include "leaf age," plastochron, and phyllochron. Leaf age is a somewhat loose term used to refer to leaf number on a rice culm. The time interval between successive leaf initiations is called a *plastochron* (Langer 1972). The problem with determining the plastochron is that the initiated leaves are not visible without microscopy. A *phyllochron* is the time interval between the same leaf developmental events of successive leaves (Rickman and Klepper 1995, Wilhelm and McMaster 1995, Nemoto et al 1995). The period between leaf collar formations on successive leaves is one measure of a phyllochron that is easily determined without magnification. We chose to employ successive collar formations for the vegetative growth stage determinations.

Vegetative development also occurs in distinct structural units called phytomers. A phytomer is the unit of vegetative development consisting of a leaf (blade and sheath) and its subtended node, internode, nodal roots, and tiller bud (Hoshikawa 1989). Each complete (i.e., containing both sheath and blade) leaf on the main stem beginning with the first true leaf can be marked as having completed elongation with collar formation. Note that this is also the completion of one phytomer or vegetative building block.

### Reproductive development

Reproductive development begins when the shoot apex initiates panicle structures. Subsequently, the panicle branches differentiate, the florets differentiate including male and female organs, as the panicle enlarges concurrently with the rest of the rice culm, megasporogenesis occurs, the panicle emerges, anthesis occurs, the caryopsis elongates, the grain fills with starch, the grain ceases to fill, and the grain dries down. Very detailed wheat apex developmental information is provided by McMaster (1997) and detailed development of the rice plant is provided by Hoshikawa (1989) and by a later volume translated from Japanese (Matsuo and Hoshikawa 1993).

## Materials and methods

The primary methods leading to the development of the system were (1) observing rice plants as they developed, (2) studying the literature on plant development and growth staging, and (3) reflectively discussing these. Discussions were held with scientists from several disciplines to elucidate rice growth stages that were important to their discipline. Literature searches were made and an objective, adaptive rice growth staging system was presented and discussed on 4 March 1998 at the Rice Technical Working Group in Reno, Nevada, USA (Counce 1998). Also, a workshop was held on 3 September 1998 in Stuttgart, Arkansas, to demonstrate the system and to query participants about it. Several points raised in the discussion session required further clarification either by literature searches or through experimental observation. The following experiments and observations were used to provide clarification where needed.

### Seedling growth stages

Laboratory, growth chamber, and greenhouse experiments with water imbibition and seedling germination were used together with several years of field observations to delineate seedling stages. The procedure was to take 100 rice seeds of different cultivars and seed lots and place them in aerated tap water at a constant temperature of 25 °C. Each day, seedlings with visible radicles and coleoptiles were counted.

### Vegetative growth stages

Seeds of Carolina Gold, Drew, Guichao2, Lemont, M11-131, M202, and Qiguizao rice were planted in 1998 at the Rice Research and Extension Center near Stuttgart, Arkansas, in May, July, and August on a Crowley silt loam soil (Typic Albaqualfs). Table 1 lists these cultivars and some of their characteristics. Beginning on 21 May 1998, plants were tagged when the prophyll leaves began to emerge from the coleoptiles. The date of collar formation for the first true leaf was subsequently noted. Collar formation for each successive leaf on the main stem was then noted. A group of the same varieties grown at the Stuttgart location was also grown at the Northeast Research and Extension Center near Keiser, Arkansas, on a Sharkey silty clay loam (Vertic Haplaquept), planted in May 1998. On 23 July 1998, Drew, Lemont, and M202 were planted at the site near Stuttgart and notes were taken as for the earlier planting. On 8 August 1998, Drew was planted at the site near Stuttgart and notes were taken as for the earlier plantings. Observations on selected plants were made Monday through Saturday until anthesis occurred. One day per week, plants were not observed and

**Table 1. Characteristics of rice used in field experiments conducted at the Rice Research and Extension Center at Stuttgart, Arkansas, and at the Northeast Research and Extension Center near Keiser, Arkansas, in 1998.**

| Cultivar | Characteristics |
|---|---|
| Carolina Gold | 300-year-old U.S. rice cultivar |
| Drew | Arkansas long-grain rice released in 1997 |
| Guichao2 | South China rice cultivar with round grains and nonsticky texture |
| Lemont | Texas long-grain rice released in 1982 |
| M11-131 | Line developed from cross of Lemont and Bond |
| M202 | California medium-grain rice cultivar |
| Qiguizao | South China long-grain rice cultivar |

leaf emergence noted on the subsequent day was marked to denote that the actual date of collar formation could have been either of two days.

In August 1998, a farmer's field of rice near El Campo, Texas, was visited to make observations on plants from a ratoon rice crop. In the growth chambers and greenhouses at Stuttgart and Keiser, experimental observations on main stem and tiller leaf number development over time have been made since 1989.

### Reproductive growth stages

Rice panicle and grain development has been examined since 1989. In 1997, individual panicles and grains were examined over the course of their development to propose the staging system. Confirming observations were made in 1998 in the field.

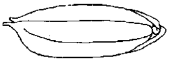
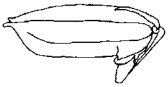
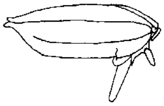
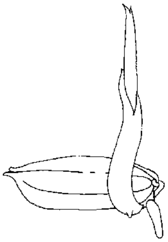
## Description of the rice crop growth staging system

Table 2 presents the seedling growth stages, illustrations, and morphological criteria for each growth stage. The stages outline the morphological development described above.

Table 3 presents the vegetative growth stages, illustrations, and morphological criteria for each growth stage. Figure 1 provides vegetative structures. The vegetative growth stages allow us to exploit the iterative nature of vegetative development. Each leaf is succeeded by an additional leaf until the formation of the final leaf on a culm, the flag leaf. A phyllochron usually varies from 3 to 8 d (80–115 DD10 units). Late vegetative developmental stages ($V_{F-4}$ to $V_F$) and early reproductive developmental stages (R0 to R2) occur simultaneously.

Table 4 presents the reproductive growth stages, illustrations, and the morphological criteria for each growth stage. Stages R0 through R3 refer to the developing panicle, whereas growth stages R4 through R8 refer to the individual florets or grains on a panicle. If any florets or grains on a main stem panicle have passed into the next R stage, then the plant is determined to be at that R stage, that is, if one grain on a panicle has a caryopsis filling the cavity of the lemma and palea (the "hull"), then the

**Table 2. Rice seedling growth stages with morphological markers.**[a]

| | Growth stage | | | |
|---|---|---|---|---|
| Morphological criteria | S0<br>Dry, unimbibed seed | S1<br>Emergence of coleoptile[b] | S2<br>Emergence of radicle[b] | S3<br>Emergence of prophyll leaf from coleoptile |
| Illustration |  |  |  |  |

[a] The sequence of seedling growth stages normally encountered in aerobic seedbed conditions (whether direct-seeded or transplanted rice culture) may change under anaerobic (such as under water-seeded culture) conditions. Under anaerobic conditions, the coleoptile emerges first. This table is for aerobic conditions only, which apply to most of the rice grown in the world. [b] The prophyll leaf is the first leaf to emerge, but it lacks a blade and consists only of the leaf sheath.

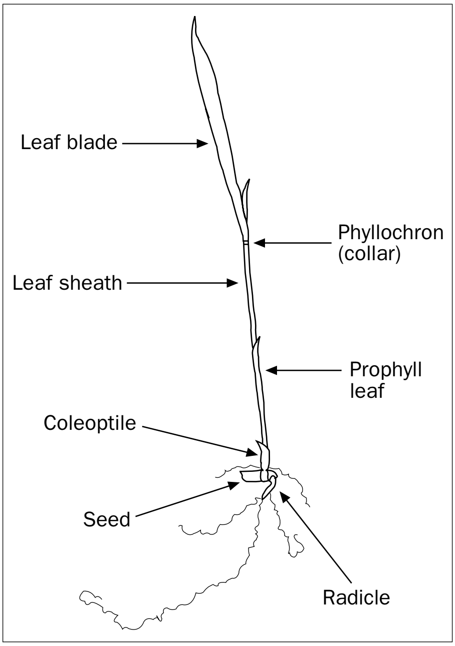


**Fig. 1. Vegetative structures of the rice plant.**

**Table 3. Rice vegetative growth stages with morphological markers for a rice cultivar with 13 true leaves on the main stem.**[a]

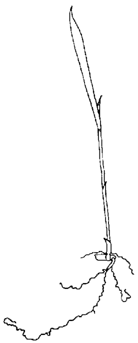
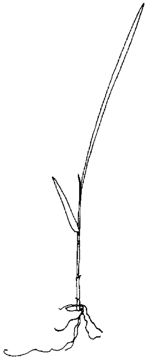
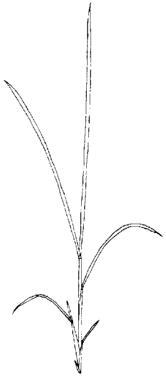
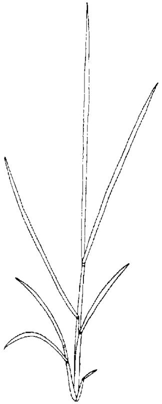
| | Growth stage | | | | |
|---|---|---|---|---|---|
| Morphological marker | V1<br>Collar formation on first complete leaf (leaf 1) on main stem | V2<br>Collar formation on leaf 2 on main stem | V3<br>Collar formation on leaf 3 on main stem | V4<br>Collar formation on leaf 4 on main stem | V5<br>Collar formation on leaf 5 on main stem |
| Illustration |  |  |  |  |  |

**Table 3. continued**

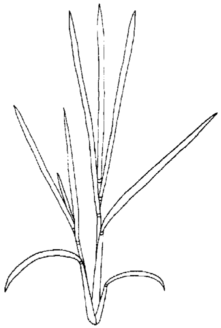
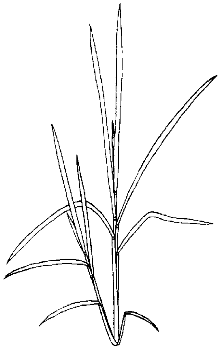
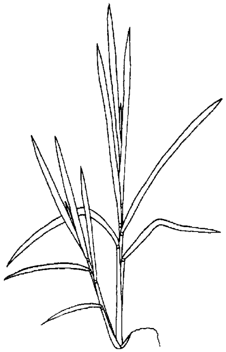
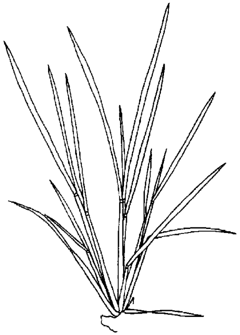
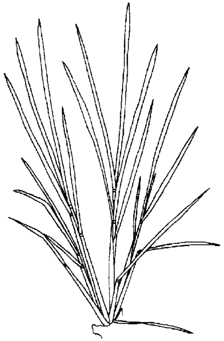
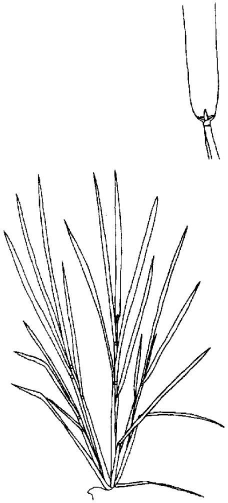
| | Growth stage | | | |
|---|---|---|---|---|
| Morphological marker | V6<br>Collar formation on leaf 6 on main stem | V7<br>Collar formation on leaf 7 on main stem | V8<br>Collar formation on leaf 8 on main stem | V9 ($V_{F-4}$)[b]<br>Collar formation on leaf 9 on main stem |
| Illustration |  |  |  |  |

**Table 3. continued**

| Morphological marker | Growth stage | | | |
|---|---|---|---|---|
| | V10 $(V_{F-3})^{b}$ Collar formation on leaf 10 on main stem | V11 $(V_{F-2})^{b}$ Collar formation on leaf 11 on main stem | V12 $(V_{F-1})^{b}$ Collar formation on leaf 12 on main stem | V13 $(V_{F})^{b}$ Collar formation on leaf 13 (flag leaf) on main stem |
| Illustration |  |  |  |  |

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[a]The number of vegetative growth stages varies with the number of true leaves on the main stem. [b]VF denotes flag leaf and it follows that $V_{F-n}$ denotes the nth node before the flag leaf.

**Table 4. Rice reproductive growth stages with morphological markers.**

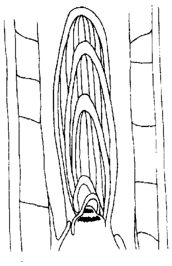
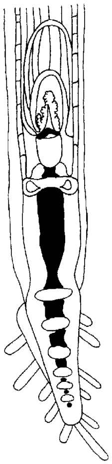
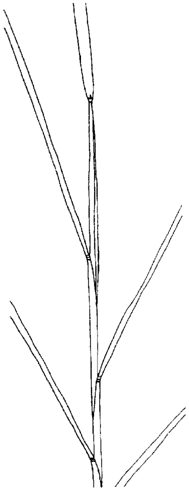
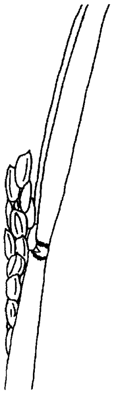
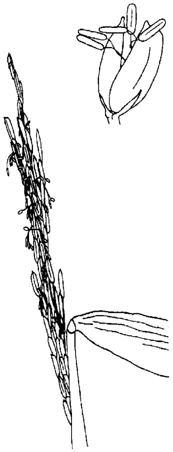
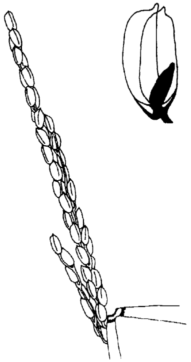
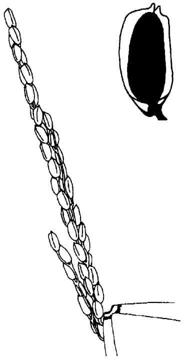
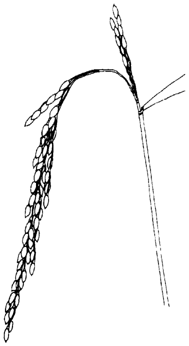
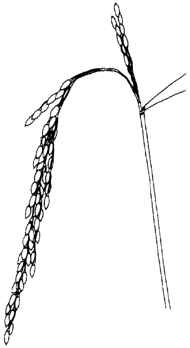
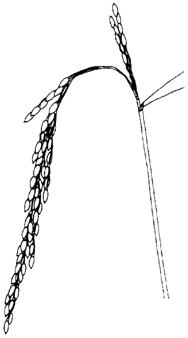
| | Growth stage | | | | |
|---|---|---|---|---|---|
| Morphological marker | R0<br>Panicle growth initiated | R1<br>Panicle branches have formed | R2<br>Flag leaf collar formation | R3<br>Panicle exsertion from boot, tip of panicle is above collar of flag leaf | R4<br>One or more florets on the main stem panicle have reached anthesis |
| Illustration |  |  |  |  |  |

**Table 4. continued**

|  | Growth stage |  |  |  |  |
|---|---|---|---|---|---|
| Morphological marker | R5<br>At least one caryopsis on the main stem panicle is elongating to the end of the hull | R6<br>At least one caryopsis on the main stem panicle has elongated to the end of the hull | R7<br>At least one grain on the main stem panicle has a yellow hull[a] | R8<br>At least one grain on the main stem panicle has a brown hull[b] | R9<br>All grains that reached R6 have brown hulls |
| Illustration |  |  |  |  |  |

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[a] The grain turning yellow along with the panicle branch indicates that the branching tissue has died and transport of sugars and amino acids through the phloem is no longer possible. Other authors have chosen to use the terms "physiological maturity" or "cessation of dry matter accumulation." We avoid these terms because, except for grains with black layer formation, such a determination is difficult or impossible to make with any known morphological marker. [b] The brown hull indicates that the grain has dried enough to be harvestable.

growth stage is R6. Usually, the first grains to be at the criteria stages of development will be the grains at the top of the panicle because the upper grains initiate growth before lower grains do.

### Rice developmental time line

Normal morphological developmental events are juxtaposed with the growth stages in Figure 2. Since some growth stages are defined by certain morphological criteria, they occur simultaneously (R0 and panicle initiation, R4 and the beginning of pollination, etc.). Other events occur near the time or may not occur such as elongation of the first elongating internode. The term "physiological maturity" is included to show when that undefined term with no criteria may occur. Seed viability may occur at around the same time.

### Expansion of the system to include quantitative criteria

The system can be expanded to include quantitative criteria if so desired. Continuous criteria for growth stage determination could include growth stages for prophyll elongation completion, which could be known as V0, but which has no discrete criterion. Microsporogenesis in some situations is an important event for crop management. The criterion for determining microsporogenesis is continuous and not discrete. It normally occurs during the R2 growth stage. This could be a growth stage denoted by $R2_{ms}$. The criterion for growth stage $R2_{ms}$ would be continuous, not discrete: elongation of the sheath of the flag leaf to the midpoint in the height of the leaf sheath of the preceding leaf (which is at vegetative growth stage VF) and reproductive growth stage R2. Microsporogenesis is often, but not always, associated with the criterion proposed for $R2_{ms}$. To be certain of microsporogenesis, microscopic examination of the anther is required.

Expansion of the system to include quantitative or microscopic criteria may be useful for some applications. The meaningfulness of such an expansion will be of limited value outside a small area in most cases.

### Enumeration of a crop

The growth stages refer to individual plants, main stems, or grains. It is useful to have a way to quantitatively express the stage of development for a field. Fehr and Caviness (1979) and others suggested enumeration of individuals in a population and, if more than 50% had advanced into the next growth stage, then the crop was reckoned to be in the next growth stage. Individual grain maturation in the panicle can occur in more than one grain simultaneously. To enumerate a field within seedling, vegetative, or reproductive growth stages, take the average of the growth stages for the plants staged. Note that field enumeration cannot be averaged across growth stage divisions, that is, seedling, vegetative, or reproductive. The decimal part of the average indicates the tendency of the plants on average to advance to the next growth stage. For instance, if three plants were staged from one field and one plant was at V5, one at V7, and one at V8, the growth stage would be V6.7.

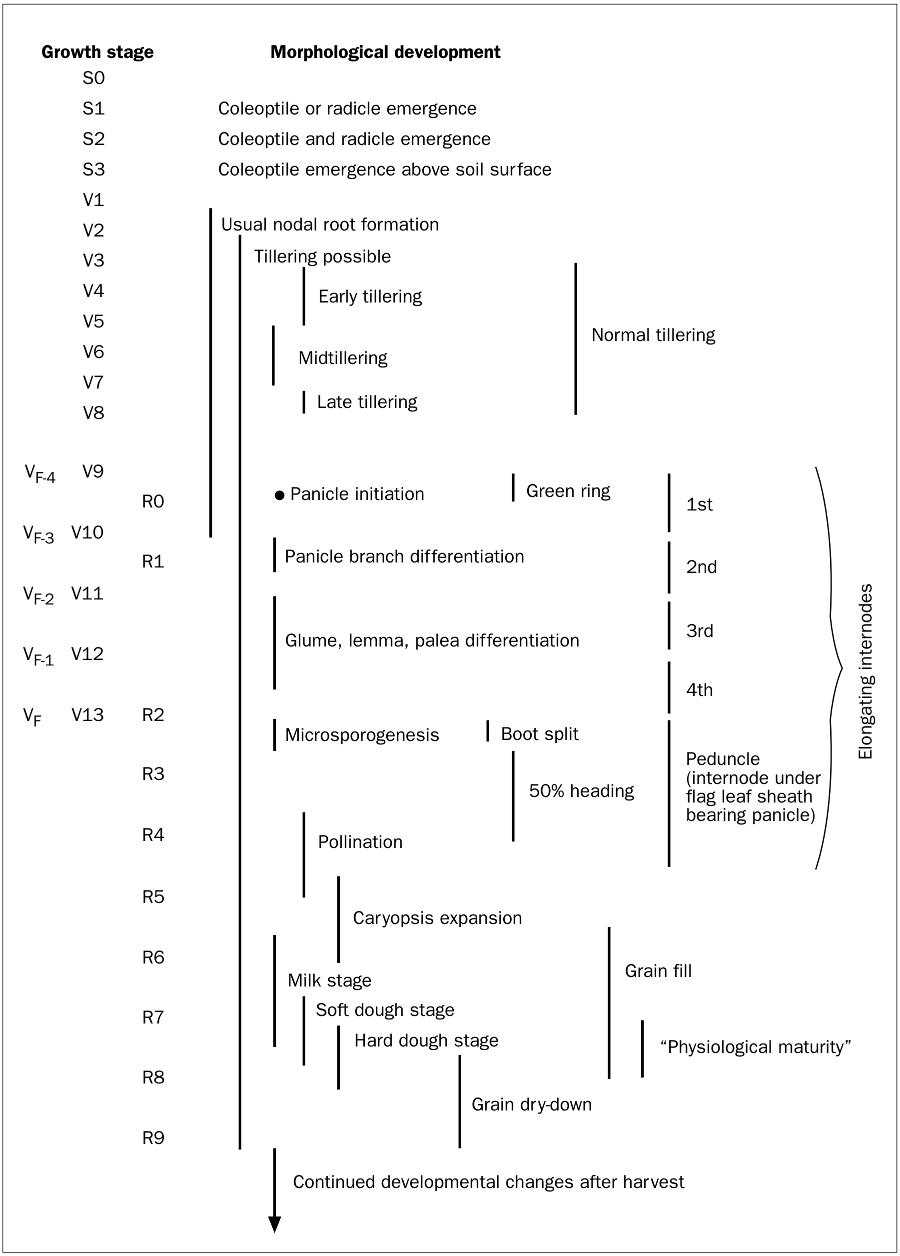

**Fig. 2. Rice developmental time line.**

## Conclusions

A uniform system for expressing a developmental rice growth staging system employing dichotomously applied objective morphological criteria with enumeration adapted to the development of the plant has been presented. The system can be used to determine stages of growth in an objective, unequivocal manner so that communication of rice research results and producer practices can be facilitated. The system is described in detail in Counce et al (2000).

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## Notes

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# Response of rice to midseason nitrogen applications in Uruguay

E. Deambrosi and R. Méndez

Farmers plant rice in dry soil surface conditions in Uruguay. According to rainfall, flushing (one or two) is required to prevent water stress before establishing the permanent flood 40–60 d after planting.

Nitrogen split applications are recommended, according to type of soil, rotation system, time of seeding, and planting method. When N is needed, tillering and panicle initiation are the appropiate times to apply it.

Three experiments were conducted to improve the efficiency of N split applications. In the first one, using cultivar Bluebelle, treatments consisted of different timing of N applications at tillering related to irrigation (–9 to +3 days, before or after flushing). According to the Tukey range test (0.05), there were no significant differences in dry matter yield and N uptake at panicle initiation (PI) among timing treatments. It rained at least once in the application period in both years. The response of two rice cultivars of different growth duration (one japonica and one indica type) to different timing of N split applications at tillering and PI was examined across two growing seasons. The responses of the varieties in 1998 and 1999 were different. Within one year, the cultivars' responses to N timing treatments differed. Weather conditions and diseases affected the responses.

Management and breeding are continually changing and their co-evolution creates new opportunities for improving rice production. Advancing yield potential should be coupled closely with improving systems, practices, and tools for management (IRRI 1994). Three factors should be taken into account: efficiency in the use of nutrients, efficiency in the use of water, and the instability of weather conditions. As yield potential increases, different rates or timing from present applications may be required. Interference among neighboring plants may differ if cultivars of distinct tillering and root growth capability are planted.

Farmers plant drilled or broadcast rice in dry soil surface conditions in Uruguay. According to rainfall, irrigation flushing (one or two) is required to prevent water stress before establishing the permanent flood 40–60 d after planting.

Field research work demonstrated the advantages of applying nitrogen one, two, or three times according to type of soil, rotation system, land preparation, planting method, time of seeding, disease history, and climatic conditions, but no more than 70 kg N ha$^{-1}$ is used. When split applications are needed, tillering and panicle initiation are the appropriate times to apply N. The number of panicles m$^{-2}$ depends on seeding rate, percent emergence, and tiller number. There is a close correlation between the number of tillers and the amount of N absorbed during this period. Spikelets per panicle is determined during the early reproductive growth stage; the number of spikelets observed at maturity is the difference between the number of differentiated primordia and the number of those that degenerate (Mae 1997).

Three experiments were conducted to improve the efficiency of N split applications. The objective of the first was to determine N uptake at panicle initiation (PI) when the nutrient was applied at tillering, as urea, varying the number of days before or after flushing. To study the effects of different timing of N split applications at tillering (T) or at PI was the objective of the second and third experiments, respectively.

## Materials and methods

### Experiment 1

A complete randomized block design (CRBD) with 10 treatments and 4 replications was used. In treatments 1–7, 10 kg N ha$^{-1}$ was applied at planting and 23 kg N ha$^{-1}$ topdressed at tillering; in treatment 8, 33 kg N ha$^{-1}$ was applied at planting; in treatment 9, 10 kg N ha$^{-1}$ was applied only at planting and a check (treatment 10) with no N was included. Treatments consisted of different timing of applications related to irrigation: –9 (T1), –6 (T2), –3 (T3), –1 (T4), 0 (T5 on water), +1 (T6), and +3 (T7) days before or after flushing. Long-grain cultivar Bluebelle was broadcast-planted in the two growing seasons (1993-94 and 1994-95). Whole aboveground plant samples were taken at random from $0.3 \times 0.3$ m$^2$ on each plot at PI. Main stem and tiller dry matter production were determined. The samples were washed and dried in an oven at 60 °C for 48 h, ground in a stainless-steel Wiley mill, and analyzed for N content by Kjeldahl digestion.

### Experiments 2 and 3

The response of two rice cultivars of differing growth duration to different timing of N split applications was examined across two growing seasons. El Paso 144, indica type, and INIA Tacuarí, cold-tolerant long-grain japonica type (Blanco et al 1994), the most extensively planted cultivars in Uruguay, were used. Cultivar El Paso 144, with longer growth duration, needs 1,577 temperature-units, base 10, from emergence to maturity, and INIA Tacuarí needs 1,492 (adapted from Méndez and Roel 1997, 1998). El Paso 144 begins tillering earlier (195 temperature-units, base 10, from emergence to tillering initiation, vs 216 for INIA Tacuarí), but INIA Tacuarí reaches panicle initiation, flowering, and maturity in a shorter time.

A CRBD design with 4 replications was used with a split-plot arrangement of treatments. Cultivars were drill-planted in the large plots and N split applications were located in the subplots.

In experiment 2, treatments consisted of applications of the same nitrogen rate (23 kg N $ha^{-1}$) at 5-d intervals after the development of the fifth leaf.

Whole aboveground plant samples were taken at random from 0.16 $m^2$ on each plot at PI. Dry matter (DM) production was determined. The samples were washed, dried in an oven at 60 °C for 48 h, ground in a stainless-steel Wiley mill, and analyzed for N and P content and uptake.

In experiment 3, treatments consisted of applications of the same nitrogen rate (23 kg N $ha^{-1}$) at 5-d intervals after internode elongation. In the last season, a check without N application was included in both experiments.

Plant samples were taken from two drill-rows 16 cm wide and 30 cm long on each plot at harvest to study grain yield components. The number of panicles per unit area was counted and 15 randomly selected panicles were chosen to determine empty and filled grains. The rice plants of the nine center rows of each plot were harvested; the grain was weighed and moisture was determined and corrected to 13%. Simple correlation analyses between certain variables were performed.

## Results and discussion

### Experiment 1

The combined analysis of 2 y showed that application treatments significantly affected total, main stem, and tiller DM production and N uptake (Table 1). N uptake

**Table 1. Effects of treatments on dry matter (DM) yield and N uptake (experiment 1).**

| Treatment[a] | DM yield (t $ha^{-1}$)[b] | | | N uptake (kg $ha^{-1}$) |
|---|---|---|---|---|
| | Main stem | Tillers | Total | |
| 9 dbf | 2.5 ab | 1.0 a | 3.6 a | 42.4 a |
| 6 dbf | 2.7 a | 0.9 ab | 3.6 a | 41.8 a |
| 3 dbf | 2.5 ab | 0.8 abc | 3.3 ab | 36.5 abc |
| 1 dbf | 2.4 abc | 0.7 abc | 3.2 ab | 38.7 ab |
| On water | 2.6 ab | 0.7 abc | 3.3 ab | 35.2 abc |
| 1 daf | 2.4 abc | 0.7 abc | 3.0 abc | 36.5 abc |
| 3 daf | 2.2 abc | 0.7 abc | 2.9 abc | 38.0 ab |
| 100% basal | 2.5 abc | 0.8 abc | 3.3 ab | 36.5 abc |
| No topdressing | 1.9 bc | 0.6 bc | 2.5 bc | 29.7 bc |
| No N | 1.8 c | 0.5 c | 2.2 c | 26.1 c |
| Prob. | 0.0 | 0.001 | 0.0 | 0.000 |
| CV (%) | 16.2 | 38.3 | 14.0 | 21.2 |
| Mean | 2.3 | 0.7 | 3.1 | 36.1 |

[a]dbf = days before flushing, daf = days after flushing. [b]Mean separation test, Tukey (0.05). Numbers followed by different letters are significantly different.

varied in a trend similar to that of total DM yield. Total N absorbed in treatments 1 and 2 (–9 and –6 d before flushing) was significantly different only from that of checks 9 (no topdressed N) and 10 (no N). According to the Tukey range test (0.05), there were no significant differences among timing treatments (1 to 8). It must be taken into consideration that it rained at least once in the period of application in both years (68.3 mm in 1993-94 and twice, 4 and 15 mm, in 1994-95) and rainfall could incorporate the N urea into the soil, thus preventing N losses.

### Experiment 2

A decrease in tiller DM was found in 1998 for INIA Tacuarí when the N split application was delayed after 10 d (342 temperature-units, base 10) following the development of the fifth leaf (probability 0.013). No significant differences were detected for El Paso 144 in the same growing season (De los Santos and Jacques 1999).

Figure 1 presents mean temperatures, average of 10 d. Colder temperatures were registered in the last two growing seasons in comparison with the average of 28 y (1972-99).

Dry matter yield (t ha$^{-1}$) and N and P uptake (kg ha$^{-1}$) were significantly affected by treatments in the two cultivars in 1999 (Table 2). Nitrogen was applied the same calendar days for both varieties and El Paso 144 began tillering 3 d earlier than INIA Tacuarí. An increase was observed until 5 d after tillering (292 temperature-units, base 10), followed by a decrease in the three dependent variables. During the period tillering + 10 d to tillering + 20 d, strong wind and rainfall occurred and 50% of the days presented minimum temperatures below 15 °C; this apparently affected the response of the crop to N application.

Treatments also significantly affected the number of panicles m$^{-2}$ (Table 2). El Paso 144 had maximum production at 5 d after tillering and INIA Tacuarí at 15 d after tillering.

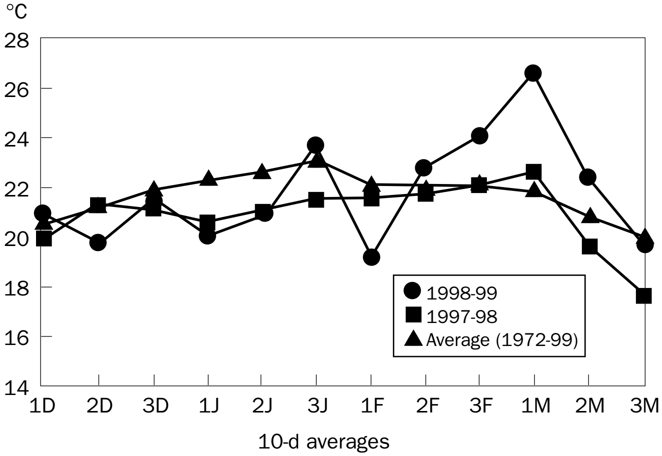

**Fig. 1. Mean temperatures (average of 10 d). D = December, J = January, F = February, M = March.**

**Table 2. Dry matter (DM) yield and N and P uptake at panicle initiation (experiment 2, 1999).**

| N application treatment (d after tillering)[a] | DM yield (t ha$^{-1}$) | | N uptake (kg ha$^{-1}$) | | P uptake (kg ha$^{-1}$) | | Panicles m$^{-2}$ | |
|---|---|---|---|---|---|---|---|---|
| | Tacuarí | EP 144 | Tacuarí | EP 144 | Tacuarí | EP 144 | Tacuarí | EP 144 |
| Check | 3.2 | 3.3 | 49.5 | 50.7 | 6.4 | 6.9 | 539 | 625 |
| 0 | 3.8 | 3.8 | 59.8 | 60.6 | 7.8 | 8.7 | 558 | 647 |
| 5 | 4.1 | 4.3 | 68.6 | 67.6 | 8.9 | 9.4 | 566 | 766 |
| 0 | 2.6 | 3.5 | 55.8 | 54.8 | 7.4 | 7.8 | 617 | 596 |
| 15 | 3.6 | 3.5 | 58.6 | 59.0 | 7.4 | 7.7 | 621 | 591 |
| 20 | 4.0 | 3.7 | 64.8 | 59.0 | 8.3 | 7.6 | 550 | 531 |
| Prob. | 0.08 | 0.001 | 0.02 | 0.005 | 0.03 | 0.000 | 0.02 | 0.000 |
| CV% | 11.4 | 7.2 | 11.9 | 9.2 | 11.6 | 7.5 | 6.1 | 8.8 |
| Mean | 3.7 | 3.7 | 59.5 | 57.7 | 7.7 | 8.0 | 575 | 626 |

[a]Tillering occurred at 5 d for INIA Tacuarí and 8 d for El Paso 144.

**Table 3. Simple correlation coefficients between certain variables (1999, experiment 2).**

| Variables[a] | | INIA Tacuarí | | El Paso 144 | |
|---|---|---|---|---|---|
| X | Y | r | Prob. | r | Prob. |
| DM yield | Panicles m$^{-2}$ | –0.18 | ns | 0.60 | 0.001 |
| DM yield | Filled grains panicle$^{-1}$ | 0.55 | 0.005 | –0.55 | 0.005 |
| DM yield | Filled grains m$^{-2}$ | 0.45 | 0.03 | –0.12 | ns |
| DM yield | Grain yield | 0.60 | 0.001 | 0.06 | ns |
| N uptake | Panicles m$^{-2}$ | –0.08 | ns | 0.49 | 0.01 |
| N uptake | Filled grains m$^{-2}$ | 0.53 | 0.007 | –0.12 | ns |
| N uptake | Grain yield | 0.56 | 0.004 | 0.04 | ns |

[a]DM = dry matter at panicle initiation.

Simple correlation analysis showed different trends and significances for the cultivars (Table 3). For short growing season cultivar INIA Tacuarí, filled grains panicle$^{-1}$, filled grains m$^{-2}$, and grain yield were positively and significantly correlated with DM yield at PI. Nitrogen uptake at PI was also positively and significantly correlated with filled grains m$^{-2}$ and grain yield. Neither DM yield nor N uptake at PI were related to the number of panicles m$^{-2}$. On the other hand, filled grains panicle$^{-1}$ was negatively and significantly correlated with DM yield at PI in El Paso 144; the number of panicles m$^{-2}$ was positively and significantly correlated with DM yield and N uptake at PI for the long growing season cultivar.

## Experiment 3

Only INIA Tacuarí was affected significantly by PI treatments in 1998. Grain yield and the number of panicles m$^{-2}$ decreased when N was applied late (Fig. 2A,B). These

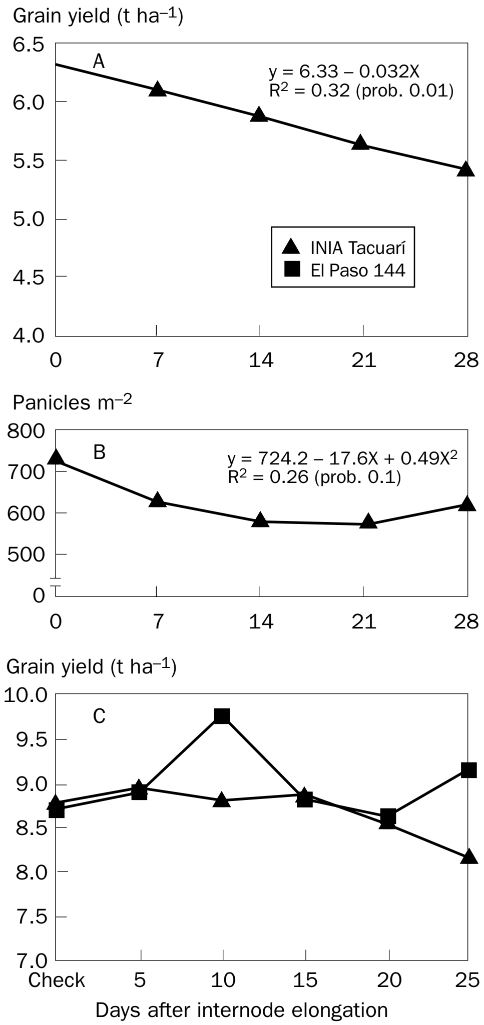

**Fig. 2. Effects of timing of N at panicle initiation on 1998 INIA Tacuarí grain yield (A), on 1998 INIA Tacuarí number of panicles $m^{-2}$ (B), and on 1999 grain yields for INIA Tacuarí and El Paso 144 (C).**

results suggest that delayed applications of N cannot help the most recently formed tillers to develop panicles (De los Santos and Jacques 1999). Méndez and Deambrosi (1995) found an increase in the number of panicles m$^{-2}$ of cultivar Bluebelle at harvest as a result of applications of N at PI, suggesting an effect on the survival of tillers.

Figure 2C presents the grain yield response of the cultivars to PI treatments in 1999. In this case, the long growing season El Paso 144 increased grain yield with N applications, but INIA Tacuarí did not. Figure 1 shows that low temperatures occurred in January and February; these weather conditions probably affected more the performance of the short growing season variety, which was in the sensitive reproductive stage at that time.

Treatments significantly affected the number of potential grains panicle$^{-1}$ (prob. 0.04), filled grains panicle$^{-1}$ (prob. 0.07), and stem rot severity index (adapted from Ou 1972) at harvest (prob. 0.05) in cultivar INIA Tacuarí. When N was applied, grain yield was negatively correlated with the degree of disease severity (r = –0.41, prob. 0.07).

Only grain yield varied significantly in response to treatments for El Paso 144 (prob. 0.01); no significant differences were found in grain yield components. The number of panicles m$^{-2}$ was negatively correlated with filled grains panicle$^{-1}$ (r = –0.43, prob. 0.06). The degree of disease severity (stem rot) was negatively correlated with filled grains m$^{-2}$ (r = –0.44, prob. 0.05).

## Conclusions

### Experiment 1

There were no significant differences (Tukey 0.05) in total DM production (main stem + tillers) at PI among N timing treatments at tillering. There were no significant differences (Tukey 0.05) in nitrogen uptake (kg N ha$^{-1}$). Only two treatments (applications 9 and 6 d before flushing) were significantly different (Tukey 0.05) for treatments 9 (no N topdressed) and 10 (no N).

### Experiments 2 and 3

The responses of INIA Tacuarí (cold-tolerant, japonica type) and El Paso 144 (indica type) differed in the two growing seasons.

Within 1 y, the cultivars' responses to N timing treatments were different. Increasing biomass or sink size through N applications did not necessarily contribute to increased yield in Uruguay.

Weather conditions and disease occurrences may affect the expected responses to nutrient application.

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## Notes

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AGRONOMY

# The potential of near-infrared reflectance (NIR) of soil as a prerice soil nitrogen test

B.W. Dunn, H.G. Beecher, A.B. Blakeney, G.D. Batten, and R.L. Williams

Rice yields in southeastern Australia have been limited because of the lack of a suitable soil nitrogen test. Growers may be hesitant to apply large rates of nitrogen preflood as high N status is associated with increased susceptibility to cold damage during the reproductive phase, as well as with increased lodging. Growers prefer to risk underfertilizing preflood and to topdress at panicle initiation after a plant tissue test. A preflood soil N test would thus be of great benefit to the Australian rice industry.

This study investigates the usefulness of near-infrared reflectance (NIR) in estimating the soil's potential to supply N to a rice crop. In this study, the soil's NIR spectra are calibrated against the soil's incubation ammonium level, the N uptake of the plant tops at panicle initiation (PI N uptake), and rice grain yield.

The results indicate that NIR spectra measurements of soil sampled immediately before permanent flood can be calibrated well against the soil's incubation ammonium level and PI N uptake, and to a lesser extent against grain yield. The best calibration was achieved for PI N uptake in experiment 2, with a calibration SE = 6.3 and $R^2 = 0.83$ and validation set of SE = 6.1 and $R^2 = 0.80$. This raises the hope that NIR soil tests can be used successfully in the industry to determine soil N supply status preflood. The rapid nature of the test means that a farmer could receive a recommendation within 5 d or less of sampling.

Rice growing in southeastern Australia has intensified in recent years, leading to a high dependence on nitrogen fertilizer to achieve high yields. This has occurred simultaneously with the adoption of semidwarf rice varieties that have a higher yield potential. It is common for N fertilizer to be applied at rates from 60 to 300 kg N ha$^{-1}$ for semidwarf rice varieties, with 110 kg N ha$^{-1}$ being the industry average. In the Australian rice industry, 90% of all rice grown is aerial-sown and once the fields are flooded they are not drained until crop maturity, providing an ideal situation for a rapid soil N test.

High N status crops are more sensitive to low temperatures at early pollen microspore. Devastating yield losses occur when cold interacts with high N status crops. Water management can reduce the risk of cold damage, but achieving a correct N status in the crop is very important. Nitrogen applied before flooding is more than twice as efficient in producing grain yield as N applied to the crop at panicle initiation (Beecher et al 1994). It is a common practice for farmers to use a conservative preflood nitrogen rate and to topdress at panicle initiation (PI) after a near-infrared reflectance (NIR)-based nitrogen tissue test (Batten et al 1994). The barrier to adoption of higher rates of preflood nitrogen is the lack of a suitable soil test to enable growers to avoid the risks associated with overfertilization.

Near-infrared reflectance has been shown to be useful in determining soil moisture, organic carbon, and nitrogen content. Dalal and Henry (1986) studied the spectral reflectance of soil in the 1,100 to 2,500 nm range. They found that, for finely ground air-dried soil, using multiple wavelengths together with multivariate statistics enabled reliable prediction of moisture, organic carbon, and total N in soils. Meyer et al (1995) reported that NIR could satisfactorily estimate soil clay content, organic matter, total N, and N mineralization in this decreasing order of reliability. The system was considered suitable for rating the soils into four N mineralization categories that correspond with average N release rates of 40, 60, 80, and 100 kg ha$^{-1}$, and is used to routinely test soil by a fertilizer advisory service (Meyer 1996).

This study investigates the ability of NIR to estimate the soil's potential to supply N to a rice crop as measured by (1) the soil's incubation ammonium levels, (2) the N uptake of the plant tops at panicle initiation (PI N uptake), and (3) rice grain yield. Incubation ammonium levels have been investigated in past attempts to devise prerice soil tests, although without much success. PI N uptake is a good indicator of the amount of nitrogen supplied by the soil to the crop.

## Materials and methods

### Experimental sites

The soil investigated was taken from two rice-based experiments conducted at Yanco Agricultural Institute in southeast Australia (34°37′S, 146°25′E). Both experiments were located on a red-brown earth (Dr 2.23, Northcote 1979), which is typical of many soils used for rice growing in southeastern Australia.

*Experiment 1: a crop rotation experiment involving seven crop and pasture sequences.* Beecher et al (1994) described in detail the design and management of the first experiment. The soil samples were taken to 10-cm depth on 15 October 1990, before sowing rice into a cultivated seedbed. This was the fifth year of the rotation experiment, when all sequences were returned to rice. After sowing, the rice was flush-irrigated three times before permanent flood on 16 November 1990.

*Experiment 2: a pasture species and phase length experiment.* In the second experiment, four legume pasture species and a ryegrass control were grown for periods of 1, 2, or 3 y. Each sequence finished in spring 1994, at which point a rice crop was grown on all treatments (Dunn and Beecher 1998). The rice crop was sown into an undisturbed seedbed on 4 October 1994 and flush-irrigated three times before permanent flood on 1 November 1994. Soil samples were taken to 10-cm depth on the day before permanent flood.

## Soil and plant analysis

Soil samples from both experiments were air-dried in a glasshouse for a period of 3 to 4 wk, then crushed and ground in a centrifical grinder with a 2-mm sieve to give a very fine particle size. The soils were then stored in plastic bags at room temperature.

To determine soil incubation ammonium levels, 20-g subsamples of ground soil were added to 100 mL of distilled water in tightly sealed 250-mL bottles and then incubated at 30 °C for 14 d (Waring and Bremner 1964). At the end of the incubation, 100 mL of 4M KCl was added and the bottles shaken for 6 h. The extract was analyzed for ammonium using a colorimetric method (Bacon et al 1986, adapted from Pym and Milham 1976).

N uptake at panicle initiation was calculated as the product of plant tissue N concentration and plant tissue weight. The latter was measured from a 1-$m^2$ quadrat and converted to a per-hectare basis.

## Soil NIR analysis

To prepare for NIR analysis, 70-g subsamples of soil were placed in 125 × 225 mm × 60 μm clip-top plastic bags. Each bag was folded to present a clear face in a coarse-grain cell, with a 12-mm-thick piece of foam placed behind the sample to hold it firm against the glass before the back of the cell was put in position. A NIR Systems Model 6500 Scanning Spectrophotometer was used to obtain soil spectra at 2-nm intervals between 1,100 and 2,500 nm wavelengths over three-fourths of the length of the cell. Each spectrum was the average of 100 scans of the soil.

The soil NIR spectra from both experiments were calibrated against the soil incubation ammonium content, N uptake at panicle initiation in the rice crop, and rice grain yield. From the original 50 samples in experiment 1 and 45 samples in experiment 2, subsets of 17 and 15 samples, respectively, that covered the range for each constituent were removed and used as the validation set. A different validation subset was identified for each constituent. The remaining samples were used as the calibration set.

Both sets of calibration spectra were transformed using a second-derivative mathematical formula before calibration development. In creating calibrations, partial least squares regression (PLS) and multiple linear regression (MLR) methods were tested using NSAS software. In all of the calibrations tested, MLR using four wavelengths gave superior results. This is the method used in all the results displayed.

## Results

Both experiments gave good calibrations for the soil incubation ammonium, with the validation set being much better for experiment 2 than for experiment 1 (Table 1).

For both PI N uptake and grain yield, soils from experiment 2 gave much lower standard errors and higher $R^2$ values than those for experiment 1 for both the calibration and validation sets (Figs. 1 and 2). The best calibration was achieved for PI N uptake in experiment 2, which was soil sampled immediately before permanent flood.

**Table 1. Calibration results (standard error of calibration, SEC, and $R^2$) and validation results (standard error of prediction, SEP, and $R^2$) for incubation ammonium, panicle initiation (PI) N uptake, and grain yield from the two experiments.**

| Item | Calibration | | | Validation | |
|---|---|---|---|---|---|
| | Range | SEC | $R^2$ | SEP | $R^2$ |
| Incubation $NH_4$ ($\mu$g $g^{-1}$) | | | | | |
| Exp. 1 | 20–85 | 8.26 | 0.81 | 11.30 | 0.47 |
| Exp. 2 | 56–95 | 5.24 | 0.77 | 4.18 | 0.74 |
| PI N uptake (kg N $ha^{-1}$) | | | | | |
| Exp. 1 | 35–107 | 12.90 | 0.55 | 11.90 | 0.50 |
| Exp. 2 | 24–75 | 6.35 | 0.83 | 6.06 | 0.80 |
| Grain yield (t $ha^{-1}$) | | | | | |
| Exp. 1 | 4.9–12.1 | 1.3 | 0.6 | 1.7 | 0.4 |
| Exp. 2 | 5.2–9.6 | 0.8 | 0.7 | 0.7 | 0.7 |

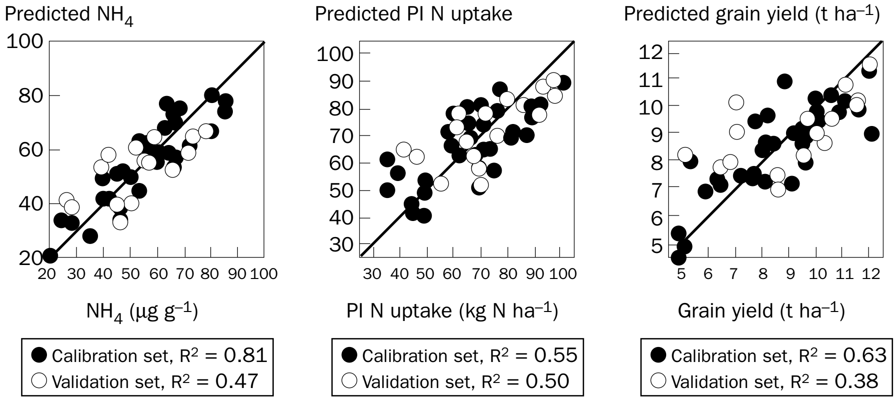

**Fig. 1. The incubation ammonium, panicle initiation N uptake, and grain yield calibration and validation data for experiment 1.**

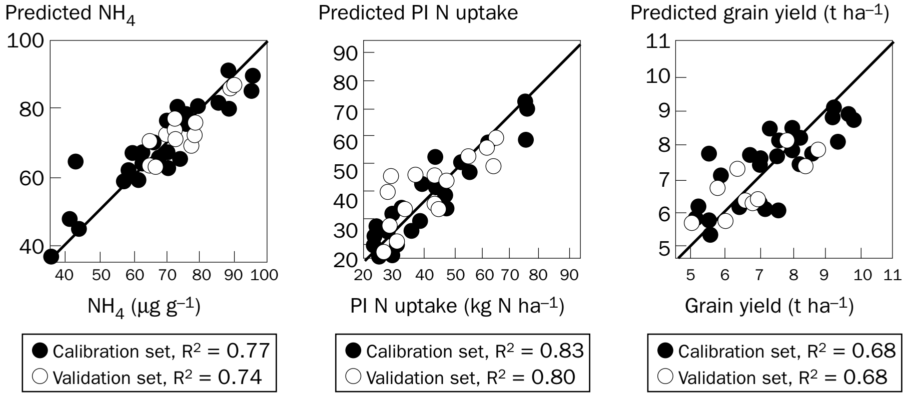

**Fig. 2. The incubation ammonium, panicle initiation N uptake, and grain yield calibration and validation data for experiment 2.**

## Discussion

The soils in experiment 2 were sampled immediately before permanent flood, which allowed no time for nitrogen transformations in the soil and losses from the root zone. In experiment 1, the soils were sampled at sowing and received three flush irrigations at a time of increasing temperatures before permanent flood was applied a month later. Boerema (1974) found that N losses were high during the flushing of combine-sown rice. He suggested that these losses may be attributed to nitrification/denitrification that occurs during the alternating wetting and drying cycles. This is considered to be a major reason for the poor calibration results obtained for PI N uptake and grain yield for experiment 1.

Both experiments were carried out on the same soil type within close physical proximity to each other. With the soil color and texture being similar, it is expected that a calibration for other elements, such as nitrogen-related constituents, would be more successful than if a large range of soil types were used.

The incubation soil ammonium test has been tried in the past as a prerice soil nitrogen test with poor results. As well as giving inconsistent results when calibrated to grain yield, the soil samples have to be taken at least 3 wk before fertilizer application because of the 2-wk incubation, with many soil N changes potentially occurring in the field during this period. The NIR soil test offers hope as it is rapid and has potential to air-dry samples quickly; the turnaround time from sampling to the farmer receiving recommendations could be 5 d or less.

Calibrating a soil N test to PI N uptake has many advantages. By using PI N uptake, the N supply potential of the soil is expressed. Calibrating against yield is less precise because of cold-induced floret sterility and other environmental factors that affect yield after PI. Nitrogen uptake at PI is currently being used by RiceCheck and maNage Rice (based on the computer crop growth model TRYM) to determine how much N to apply to rice crops at PI. Predicted PI N uptake of a soil could be an input to maNage rice, resulting in paddock-specific preflood N recommendations for any sowing date, variety, and water depth combination. These factors, combined with the results from the soil NIR calibrations we developed, suggest that calibrating the NIR soil test to PI N uptake of the crop may be beneficial.

## Conclusions

Preliminary results appear encouraging, although the results presented in this chapter are based on a relatively small number of samples, all on the same soil type. Calibrating a soil N test to PI N uptake is more effective than calibrating against grain yield and would fit well into the crop growth model TRYM.

Further research is currently being carried out to determine the potential of the NIR soil N test using 100 sites across a large range of soil types in the rice-growing regions of southeast Australia. The development of a commercial soil nitrogen test would be a desirable outcome.

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# Rice yield and nitrogen-use efficiency as affected by alternative residue management practices and winter flooding

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Air and soil quality issues have prompted the examination of alternatives in rice straw management and disposal. Burning is the traditional method of disposal in many countries and, in California, legislation now requires phasing out of this practice. Alternatives such as incorporation and removal are being tested. Winter flooding has been proposed as a method to enhance straw decomposition and provide waterfowl habitat. This study looks at the effects of alternative rice straw management practices (burn, incorporate, roll, or bale/remove) and winter flooding (winter flood and no winter flood). The effects on yield, N uptake, and N-use efficiency are reported. At current N fertilization levels, where other nutrients are sufficient, yield is unaffected by straw or winter flood treatments. By year three, straw retention increases N uptake. Winter flooding further increases N uptake in straw-retained treatments. The increased available soil N resulting from residue retention leads to luxury consumption and decreased N-use efficiency.

Rice straw management has a significant impact on soil organic matter and nutrient cycling, thus affecting sustainability. In many places, rice straw is disposed of by burning (Becker et al 1994, Miura and Kanno 1997), but alternatives to burning are desirable because of air pollution concerns. Incorporation of straw into the soil may improve soil quality and nutrient supply. In California's single-crop, temperate rice system, winter flooding has been proposed as a method to increase decomposition of the retained rice straw, also restoring winter wetland habitat for waterbirds (Elphick and Oring 1998).

Nitrogen is the most limiting nutrient in rice-cropping systems worldwide (Mikkelsen 1987, Cassman et al 1996) and the most difficult to manage in rice because of the complexity of the N cycle and opportunities for loss (Mikkelsen 1987, Buresh et al 1989). The addition of N as fertilizer and within residue can significantly increase rice yields (Verma and Bhagat 1992, Adachi et al 1997) and residual effects of fertilizer N application are notable.

The nutrients in incorporated straw are not available to the crop in the first one or two growing seasons (Verma and Bhagat 1992), but as the system comes into equilibrium the nutrients are mineralized. Since N requirements of microorganisms that decompose organic matter in flooded soils are lower than in aerated soils (DeDatta 1981), lower net immobilization of N occurs in flooded soils than in aerobic, well-drained soils with incorporation of high C residue (Mikkelsen 1987).

High potential for losses in the flooded rice system contribute to the low N-use efficiency of rice compared with that of other major crops (Keeney and Sahrawat 1986). Nitrogen is lost from rice fields through volatilization during transpiration (da Silva and Stutte 1981, Mikkelsen 1987), nitrification-denitrification (Buresh et al 1989, George et al 1993), leaching (Tripathi et al 1997), and ammonia volatilization from the soil (Keeney and Sahrawat 1986). These losses tend to be more pronounced in the absence of residue, depending on soil pH (Broadbent and Tusneem 1971), and burning also results in significant losses of N. Reducing losses would increase both soil and fertilizer N-use efficiency and reduce the environmental damage caused by denitrification and leaching of $NO_3$ (George et al 1993).

Residue incorporation in rice has been studied in greenhouse/pot experiments (Azam et al 1991), rice-wheat rotations (Verma and Bhagat 1992), and systems with multiple rice crops per year (Cassman et al 1996). However, long-term research on straw management in temperate systems is limited and the effects of winter flooding in these systems have not been studied. The purpose of this study is to examine the effects of rice straw management and winter flooding on yield, N uptake, and N-use efficiency at two long-term field sites.

## Materials and methods

Two field sites were established on different rice-growing soils in northern California. The 28-ha site on a commercial rice farm near Maxwell, Colusa County, was established in the fall of 1993 and the 10-ha site at the Rice Research Station near Biggs, Butte County, in the fall of 1994. Some key differences between the soils of the two sites were higher clay content at Maxwell (51% vs 35%), higher pH at Maxwell (6.6 vs 4.7), and higher exchangeable K at Maxwell (305 vs 72 mg $kg^{-1}$). Treatments were laid out in a split-plot design with the main plots arranged in a randomized complete block design replicated four times. The main-plot treatments were winter flooding and no winter flooding. Subplot treatments were the four postharvest straw management practices (burn, incorporate, roll, and bale/remove). With the exception of the rolled treatment, which was flooded before rolling, all winter-flooded plots were flooded after the fall straw treatment, then drained in late March. Following seedbed preparation and application of fertilizer, the fields were flooded and rice variety M202 was aerially seeded.

During each growing year of the study, microplots were established within each main plot that received no fertilizer N. They were moved to a different location within the larger plot each year. On these zero-N microplots, phosphorus was applied as triple-superphosphate at the same level as in the N-fertilized plots.

At final harvest, plants in a 1-m$^2$ area of the zero-N microplots and of the main plots were cut just above the soil surface, weighed for yield determination, separated into grain and straw components, dried to constant mass at 60 °C, and weighed again. Grain yield was corrected to 140 g kg$^{-1}$ water content. The dried samples were coarse-ground with a Wiley mill and then ground into a fine powder using a ball mill. Samples were analyzed for total C and N by complete combustion with a Carlo Erba 1500 CHN.

Physiological nitrogen-use efficiency (PNUE) was calculated as kg grain (dry) kg$^{-1}$ aboveground N uptake. Nitrogen-use efficiency (NUE) was calculated as kg grain (dry) kg$^{-1}$ N supply, where N supply is equal to N in the zero-N plot plus N fertilizer applied. Since the extractable N in the spring is largely lost through denitrification, and that in the fall was very small, available soil N could be estimated solely by the total N in the zero-N crop.

Analysis of variance (ANOVA) was performed using the PROC GLM procedure in SAS (SAS Institute Inc. 1989). Because of the split-plot design, the flood-by-block error was used as the error term for the winter flooding treatment. Contrast statements compared treatment means and sets of treatment means when the ANOVA indicated treatment effects. The effects of flooding within the two straw-retained treatments were analyzed using a contrast statement when there was a significant straw-by-flood interaction. Repeated measure analysis of variance over the four- (Biggs) or five- (Maxwell) year term evaluated treatment effects over time.

## Results and discussion

Although year-by-year differences occurred in grain yield, grain yields at Maxwell averaged 11.3 t ha$^{-1}$, with no effect of winter flooding or straw treatment throughout the five-year period. At Biggs, the average grain yield over the four-year period was 9.1 t ha$^{-1}$. Winter flooding again had no significant effect, but a significant straw treatment effect appeared; the yield in the bale/remove plots was significantly lower than yields in the other three treatments. A greater response to fertilizer N application was observed at Maxwell than at Biggs. Fertilizer N application increased yields at Biggs by 30% over the zero-N treatment, ranging from 17% to 41%, whereas, at Maxwell, application of fertilizer N resulted in an average yield increase of 105%, ranging from 66% to 161%. One key difference between the bale/remove treatment and the others that would contribute to the significant straw treatment effect at Biggs is the removal of K with the straw, which is not removed when residue is burned. Potassium was at marginal levels at Biggs and was likely also a contributing factor to the lower yield and the decreased N response at Biggs. The variation in pH between Maxwell and Biggs may also contribute to the yield differences (Broadbent and Tusneem 1971).

In the first two years, grain yields of zero-N microplots were unaffected by straw management, but a significant straw effect appeared in years three through five at Maxwell (Table 1). Yield was greater in straw-retained (incorporated or rolled) versus straw-removed plots (baled or burned). Winter flooding also significantly in-

**Table 1. Rice grain yield (zero-N plots) as affected by winter flooding and straw management, Maxwell, Colusa County, California.**

| | Year | | | | | |
|---|---|---|---|---|---|---|
| Treatment | 1994 | 1995 | 1996 | 1997 | 1998 | All years |
| | | | (t ha$^{-1}$) | | | |
| Winter flood | | | | | | |
| Burn | 4.1 | 7.1 | 5.9 | 5.8 | 3.1 | |
| Incorporate | 3.9 | 5.8 | 8.4 | 8.8 | 5.3 | |
| Roll | 4.1 | 6.1 | 7.7 | 7.5 | 5.4 | |
| Remove/bale | 4.0 | 6.9 | 5.8 | 5.5 | 3.4 | |
| No flood | | | | | | |
| Burn | 4.4 | 6.2 | 5.8 | 6.5 | 3.5 | |
| Incorporate | 4.2 | 6.8 | 6.6 | 6.8 | 4.3 | |
| Roll | 4.0 | 4.9 | 5.8 | 6.9 | 4.7 | |
| Remove/bale | 3.4 | 6.8 | 5.3 | 5.7 | 3.6 | |
| Analysis of variance[a] | | | | | | |
| Straw | ns | ns | *** | *** | *** | ** |
| Flood | ns | ns | ns | ns | ns | ns |
| Straw × flood | ns | ns | *** | ** | ns | ns |
| Contrasts | | | | | | |
| Retain vs remove | ns | ns | *** | *** | *** | *** |
| Flood in incorporate | ns | ns | * | *** | ns | ns |
| Flood in roll | ns | ns | ** | ns | ns | ns |

[a] *, **, *** = significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = not significant.

creased zero-N plot grain yields in incorporated (27% increase) and rolled (19% increase) treatments. High on-site variability contributed to insignificant treatment effects in zero-N grain yields at Biggs, although trends similar to those at Maxwell were beginning to appear.

Total plant N uptake mirrored grain yield in the zero-N plots at Maxwell (Table 2). In years three through five, residue retention increased N uptake by 29 kg ha$^{-1}$ in the winter-flooded plots and by 9 kg ha$^{-1}$ in the non-winter-flooded plots. Total N uptake at Biggs was similar, but not statistically significant. Though not reflected in yield, total N uptake in the N-fertilized plots at Maxwell also reflected the increase in soil N supply with residue retention (Table 3). In years three through five, residue retention increased average N uptake by 16 and 7 kg ha$^{-1}$ in the winter-flooded and non-winter-flooded treatments, respectively.

As indicated by the grain yield increase following fertilizer N application, N was a limiting nutrient at both sites. The yield and N uptake in the zero-N fertilizer treatments show that straw and winter flooding treatments both affect soil N supply. Straw retention initially immobilizes available N, thus contributing to lower yields in N-limiting conditions (Azam et al 1991, Kludze and Delaune 1995), though this was not statistically significant in this study. The initial negative yield effect becomes

**Table 2. Total rice plant N uptake (zero-N plots) as affected by winter flooding and straw management, Maxwell site, Colusa County, California.**

| Treatment | 1994 | 1995 | 1996 | 1997 (kg ha$^{-1}$) | 1998 | All years |
|---|---|---|---|---|---|---|
| Winter flood | | | | | | |
| Burn | 55 | 89 | 66 | 67 | 31 | |
| Incorporate | 55 | 70 | 96 | 102 | 66 | |
| Roll | 50 | 82 | 88 | 89 | 68 | |
| Remove/bale | 50 | 89 | 65 | 64 | 41 | |
| No flood | | | | | | |
| Burn | 59 | 81 | 68 | 76 | 38 | |
| Incorporate | 51 | 95 | 76 | 79 | 57 | |
| Roll | 53 | 64 | 69 | 84 | 54 | |
| Remove/bale | 44 | 91 | 65 | 70 | 52 | |
| Analysis of variance[a] | | | | | | |
| Straw | ns | ns | *** | *** | *** | ** |
| Flood | ns | ns | ns | ns | ns | ns |
| Straw × flood | ns | ns | ** | *** | ns | ns |
| Contrasts | | | | | | |
| Retain vs remove | ns | ns | *** | *** | *** | *** |
| Flood in incorporate | ns | ns | * | *** | ns | ns |
| Flood in roll | ns | ns | ** | ns | ns | * |

[a] *, **, *** = significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = not significant.

positive as the straw decomposes and the N in the straw along with the initially immobilized N become available to the crop (Adachi et al 1997, Tripathi et al 1997, Verma and Bhagat 1992). After a certain period of time, the straw retention can reduce fertilizer N requirements, as is seen by the reduced yield response to fertilizer N when straw is retained (Fig. 1). Straw retention also increases soil organic matter content (Mahapatra et al 1991) and microbial biomass in the soil (Azmal et al 1997), suggesting that the long-term effects on overall soil fertility are more important than simply the addition of N in the residue itself (Azam et al 1991). Winter flooding further enhanced the N supply of the soil in residue-retained treatments.

Residue retention significantly decreased PNUE by years three through five at Maxwell (Table 4). During these three years, these treatments yielded an average of 4.7 kg less grain kg$^{-1}$ total N uptake than residue removal. NUE was also negatively affected by straw retention at Maxwell (Table 5). Each kg of available N produced 42.4 kg grain at Maxwell and 32.9 kg grain at Biggs.

By increasing the soil N supply without a corresponding decrease in fertilizer N application, NUE was negatively affected by straw retention. The increased N supply led to luxury consumption. A slight decrease in harvest index was also observed, so that the extra available N produced more straw than grain. Optimization of fertilizer

**Table 3. Total rice plant N uptake in N-fertilized treatments as affected by winter flooding and straw management, Maxwell site, Colusa County, California.**

| Treatment | Year | | | | | |
|---|---|---|---|---|---|---|
| | 1994 | 1995 | 1996 | 1997 (kg ha$^{-1}$) | 1998 | All years |
| Winter flood | | | | | | |
| Burn | 162 | 179 | 203 | 176 | 141 | |
| Incorporate | 155 | 195 | 218 | 194 | 174 | |
| Roll | 129 | 181 | 212 | 189 | 158 | |
| Remove/bale | 160 | 154 | 198 | 173 | 160 | |
| No flood | | | | | | |
| Burn | 175 | 196 | 187 | 189 | 147 | |
| Incorporate | 176 | 193 | 212 | 191 | 160 | |
| Roll | 156 | 151 | 195 | 182 | 159 | |
| Remove/bale | 154 | 181 | 203 | 185 | 143 | |
| Analysis of variance[a] | | | | | | |
| Straw | ns | ns | * | ns | * | ** |
| Flood | ns | ns | ns | ns | ns | ns |
| Straw × flood | ns | ns | ns | ns | ns | ns |
| Contrasts | | | | | | |
| Retain vs remove | ns | ns | * | ns | ** | ns |

[a] *, ** = significant at the 0.05 and 0.01 probability levels, respectively; ns = not significant.

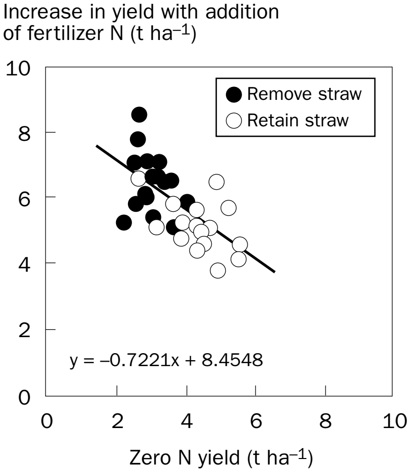

**Fig. 1. Yield response to addition of fertilizer N as affected by residue retention or removal, Maxwell, 1998 (year five of straw management study).**

**Table 4. Physiological nitrogen-use efficiency as affected by winter flooding and straw management, Maxwell, Colusa County, California.**

| Treatment | Year | | | | | All years |
|---|---|---|---|---|---|---|
| | 1994 | 1995 | 1996 | 1997 | 1998 | |
| | (kg grain kg$^{-1}$ total plant N)[a] | | | | | |
| Winter flood | | | | | | |
| Burn | 68 | 60 | 62 | 74 | 76 | |
| Incorporate | 68 | 60 | 54 | 65 | 66 | |
| Roll | 64 | 59 | 56 | 71 | 67 | |
| Remove/bale | 67 | 63 | 59 | 74 | 72 | |
| No flood | | | | | | |
| Burn | 62 | 58 | 63 | 74 | 73 | |
| Incorporate | 60 | 55 | 53 | 67 | 67 | |
| Roll | 67 | 66 | 60 | 69 | 67 | |
| Remove/bale | 61 | 54 | 58 | 72 | 70 | |
| Analysis of variance[b] | | | | | | |
| Straw | ns | ns | ** | ** | *** | * |
| Flood | ns | ns | ns | ns | ns | ns |
| Straw × flood | ns | * | ns | ns | ns | ns |
| Contrasts | | | | | | |
| Retain vs remove | ns | ** | ** | *** | ** | |

[a] Calculated using grain moisture content of 140 g kg$^{-1}$. [b] *, **, *** = significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ns = not significant.

**Table 5. Nitrogen-use efficiency as affected by winter flooding and straw management, Maxwell and Biggs sites.[a]**

| Treatment | Year | | | | |
|---|---|---|---|---|---|
| | 1994 | 1995 | 1996 | 1997 | 1998 |
| | (kg grain kg$^{-1}$ available N) | | | | |
| Maxwell site | | | | | |
| Burn[b] | 40 a* | 36 ab | 44 a | 49 a | 52a |
| Incorporate | 39 a | 37 a | 39 c | 43 b | 48 bc |
| Roll | 41 a | 35 ab | 41 b | 45 b | 46 c |
| Remove/bale | 39 a | 32 b | 43 ab | 49 a | 50ab |
| Biggs site | | | | | |
| Burn | | 36 a | 34 a | 32 a | 37 a |
| Incorporate | | 37 a | 32 a | 29 b | 32 b |
| Roll | | 36 a | 34 a | 30 b | 33 ab |
| Remove/bale | | 31 b | 32 a | 28 b | 32 b |

[a] Nitrogen-use efficiency calculated as kg grain kg$^{-1}$ available N, which is N in zero-N plants plus fertilizer N applied. [b] Winter-flooded and nonflooded treatments averaged together because there were no flooding effects. *If within same row and site, numbers followed by the same letter are not significantly different by Duncan's multiple range test.

N application rate combined with residue retention may increase the efficiency of the use of N because of a reduction in losses. In this system, residue retention, in combination with winter flooding, reduces the needed fertilizer N inputs, thus contributing to more sustainable rice production.

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## Notes

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# Root weight affects radiation-use efficiency in rice

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Recent studies showed that root biomass in different rice varieties can range from 2 to 5 t $ha^{-1}$ at flowering, with less root growth generally associated with earlier flowering varieties. This chapter reports how radiation-use efficiency (RUE) is affected by whether roots are included or excluded from the calculation of biomass.

In 1998, four rice cultivars of different duration were grown in a replicated field experiment at the Yanco Agricultural Institute to investigate the effect of root weight and length of growing season on RUE. Plots were sampled every 2 wk from sowing to maturity to determine leaf area index, aboveground biomass, and root biomass. RUE was calculated for each plot based on two methods of expressing biomass and three time intervals. RUE for total biomass ($RUE_T$) was estimated from the relationship between total biomass (shoot plus root weight) and intercepted radiation. RUE for aboveground biomass ($RUE_{AG}$) was estimated from the relationship between aboveground biomass and intercepted radiation. The three time intervals were sowing to flowering, flowering to maturity, and sowing to maturity.

The longest duration variety, Amaroo, partitioned twice as much biomass to the roots as the shorter duration varieties and had the highest $RUE_T$. There were greater differences among the varieties for $RUE_T$ than for $RUE_{AG}$ and $RUE_T$ was highly correlated with increases in root weight.

Research to increase the yield potential of rice is a high priority for providing food for the increasing world population. In Australia, the absence of serious pests and diseases, along with the high levels of solar radiation and full irrigation, means that average rice yields are relatively high (9–10 t $ha^{-1}$), having risen strongly since the introduction of semidwarf varieties in the mid-1980s. Many farmers produce yields close to the potential of 14–15 t $ha^{-1}$. Further yield improvement needs to be focused on more fully using the environmental resources. There is a concern that yield potential cannot continue to increase using existing techniques. The approach reported here is to identify very high yielding varieties (VHYV) that combine high radiation-use efficiency (RUE), intercepted photosynthetically active radiation (IPAR), and harvest index (HI, the ratio of grain to aboveground biomass):

$$\underset{(\text{g m}^{-2})}{\text{Yield}} = \underset{(\text{g MJ}^{-1})}{\text{RUE}} \times \underset{(\text{MJ m}^{-2})}{\text{IPAR}} \times \underset{(\text{g g}^{-1})}{\text{HI}}$$

Much of the increased yield potential associated with semidwarf varieties has been achieved through increases in harvest index (Nishiyama 1993), but the limit for increases in HI is likely being approached. An alternative approach to yield improvement is to increase RUE for biomass production, while maintaining HI.

The partitioning of assimilates between vegetative biomass and grain is normally expressed on the basis of aboveground biomass alone, or adjusted for root weight using a constant factor such as 10% of the total plant dry matter at anthesis (Kiniry et al 1989). Ignoring changes in root growth is unjustified because the requirements for attaining high rice yield include high root activity in addition to faster dry matter accumulation during grain ripening and effective translocation of carbohydrates from all previously accumulated biomass to grain (Nishiyama 1993). Although considerable research has been dedicated to understanding RUE, most has ignored root growth as a component of total biomass. The objective of this research was to evaluate the effect of root biomass on the estimates of RUE among long- and short-season varieties.

## Materials and methods

In the 1997-98 season, four rice varieties were grown in a field experiment at the Yanco Agricultural Institute, Australia (35°S, 146°E). The four varieties represented different origins and growth durations (Table 1) and were grown in a randomized complete block design experiment with three replicates. The experimental details are described by Angus et al (1999) and this chapter reports aspects related to RUE.

**Table 1. Varieties and their maturities, yields, harvest index, canopy characteristics, and root growth.**

| Variety | Origin | Days from sowing to flowering | Grain yield (t ha$^{-1}$) | Harvest index (%) | Measurements 87 days after sowing | | |
|---|---|---|---|---|---|---|---|
| | | | | | Extinction coefficient | Intercepted radiation (MJ m$^{-2}$) | Root biomass (g m$^{-2}$) |
| Jarrah | Australia | 95 | 10.7 | 50 | 0.31 | 484 | 186 |
| Amaroo | Australia | 113 | 11.7 | 44 | 0.33 | 569 | 331 |
| Barkat | India | 94 | 10.4 | 52 | 0.44 | 654 | 131 |
| H473 | Hungary | 87 | 7.7 | 48 | 0.54 | 753 | 161 |
| LSD[a] ($P<0.05$) | | | 0.78 | 3.5 | 0.06 | 124 | 120 |

[a] LSD = least significant difference.

Each plot was sampled every 2 wk from sowing to maturity to determine leaf area index, aboveground biomass, and root biomass. Aboveground biomass was measured from a sample of two rows of 1.0 m cut at ground level from each plot. At maturity, six rows of 1.0 m were sampled to determine yield. All plant samples were dried and weighed, and measurements were made of leaf area index and specific leaf weight. At maturity, postanthesis growth and RUE were estimated. At each time of sampling, two soil cores (15 cm diameter and 15 cm deep) were taken from where the plants had been sampled and processed as described by Angus et al (this volume). Roots were washed free of soil and decaying organic matter, oven-dried, and weighed.

At weekly intervals from establishment to full canopy cover, photosynthetically active radiation (PAR) was measured above and below the plants using a probe to estimate percent interception in each plot. An exponential curve was fitted to the observations of interception over time to estimate daily interception (IPAR) based on the product of shortwave radiation and the proportion intercepted by the canopy. Radiation interception and RUE are based on incoming shortwave radiation, which is approximately double PAR. After full canopy cover, interception was estimated to be 95%. The canopy extinction coefficient ($k$) for each plot was calculated from the proportion of PAR intercepted by the canopy and the leaf area index, L:

$$k = (1/\mathrm{L}) \ln (1 - \mathrm{IPAR})/\mathrm{PAR}$$

RUE was estimated by the slope of the regression of accumulated IPAR and accumulated biomass for each plot. In total, six estimates of RUE were calculated. These were based on three time intervals: sowing to flowering, flowering to maturity, and sowing to maturity. For each of these intervals, one estimate of RUE was for total biomass, $\mathrm{RUE_T}$, while the other was for aboveground biomass, $\mathrm{RUE_{AG}}$. The estimates were compared by analysis of variance using the different sampling dates and blocks as two different strata of replication.

## Results and discussion

The four varieties tested different in phenology, grain yield, and harvest index (Table 1). The longest duration variety, Amaroo, had a significantly higher yield than all the other varieties at 11.7 t ha$^{-1}$, whereas the shorter duration variety H473 had the lowest yield at 7.7 t ha$^{-1}$. The extinction coefficient, intercepted radiation, and root biomass are reported for 87 d after sowing at about the panicle initiation stage. Varieties Amaroo and Jarrah intercepted less PAR than the other varieties and had significantly lower values of extinction coefficients possibly because of their more erect leaves (Table 1). However, even though Amaroo and Jarrah intercepted less PAR early in the season than Barkat and H473, they produced more biomass. Amaroo accumulates approximately twice as much root weight as the other varieties.

In the following sections, radiation-use efficiency is presented on the basis of aboveground biomass, as in previous studies (Kiniry et al 1989), and on the basis of total biomass, including root biomass.

## Full-season growth and radiation interception

The relationships between aboveground biomass and IPAR for the four varieties were linear, with values of $R^2$ from 93% to 98% (Table 2). The relationships between total biomass and IPAR were also linear, with similar values for $R^2$. The short-season variety H473 had smaller values of $RUE_{AG}$ and $RUE_T$ than the other varieties. $RUE_T$ appeared to be greater for the longer duration varieties (r = 0.99 between days to flowering and $RUE_T$).

## Pre- and postflowering growth and radiation interception

There were no significant differences between varieties in $RUE_{AG}$, either before or after flowering (Table 3), even though there were significant differences for the whole season. The reason may have been partly because of the variation in the data for the shorter phases. For example, the coefficient of variation of $RUE_{AG}$ increased from 6% for the whole season to 15% for the period from sowing to flowering and the period from flowering to maturity (not shown). The improved accuracy of measuring whole-season $RUE_{AG}$ is confirmed by the value of $R^2$, which increased from 83% as an average of all $R^2$ for the shorter periods (Table 3) to 95% for the longer periods (Table 2).

When $RUE_T$ is separated pre- and postflowering, the rank order of varieties changes (Table 4). Up to flowering, Amaroo had a 39% higher $RUE_T$ than the mean of Barkat and H473. However, after anthesis, $RUE_T$ decreased in all varieties, with Amaroo declining by 67%. $RUE_T$ was estimated less precisely after flowering ($R^2$ = 84) compared with before flowering ($R^2$ = 98).

The weight of roots increased with an increase in tiller number, attaining maximum values at head emergence, thus confirming Murata and Matsushima (1975). Amaroo's large number of tillers early in the season (54 d after sowing, DAS) was

**Table 2. Radiation-use efficiency (RUE) for aboveground ($_{AG}$) and total ($_T$) biomass accumulation for the full growing season of four rice varieties. The values of $R^2$ refer to the linear relationships between biomass production and intercepted radiation.**

| Variety | Aboveground biomass | | Total biomass | |
|---|---|---|---|---|
| | $RUE_{AG}$ (g $MJ^{-1}$) | $R^2$ | $RUE_T$ (g $MJ^{-1}$) | $R^2$ |
| Jarrah | 1.00 | 93 | 1.14 | 93 |
| Amaroo | 1.02 | 96 | 1.25 | 94 |
| Barkat | 1.02 | 98 | 1.13 | 98 |
| H473 | 0.85 | 94 | 0.96 | 93 |
| LSD[a] (*P*<0.05) | 0.10 | | 0.14 | |

[a] LSD = least significant difference.

**Table 3. Radiation-use efficiency for aboveground biomass for the periods from sowing to flowering and from flowering to maturity. The values of $R^2$ refer to the linear relationships between biomass production and intercepted photosynthetically active radiation for the corresponding periods.**

| Variety | Preflowering | | Postflowering | |
|---|---|---|---|---|
| | $RUE_{AG}$ (g $MJ^{-1}$) | $R^2$ | $RUE_{AG}$ (g $MJ^{-1}$) | $R^2$ |
| Jarrah | 1.08 | 73 | 0.79 | 75 |
| Amaroo | 0.96 | 82 | 0.86 | 91 |
| Barkat | 0.90 | 96 | 0.96 | 97 |
| H473 | 0.84 | 74 | 0.70 | 74 |
| LSD[a] ($P$>0.05) | ns[b] | | ns[b] | |

[a]LSD = least significant difference. [b]ns = nonsignificant.

**Table 4. Radiation-use efficiency for total biomass production for the periods from sowing to flowering (pre-$RUE_T$) and from flowering to maturity (post-$RUE_T$). The values of $R^2$ refer to the linear relationships between biomass production and intercepted photosynthetically active radiation for the corresponding periods.**

| Variety | Preflowering | | Postflowering | |
|---|---|---|---|---|
| | $RUE_T$ (g $MJ^{-1}$) | $R^2$ | $RUE_T$ (g $MJ^{-1}$) | $R^2$ |
| Jarrah | 1.37 | 99 | 0.88 | 75 |
| Amaroo | 1.52 | 98 | 0.85 | 91 |
| Barkat | 1.13 | 95 | 0.97 | 97 |
| H473 | 1.06 | 99 | 0.82 | 74 |
| LSD[a] ($P$>0.05) | 0.278 | | ns[b] | |

[a]LSD = least significant difference. [b]ns = nonsignificant.

associated with a smaller tiller weight, which increased the number of potential primordia for root growth. Amaroo is the only variety that decreased in tiller number from 54 to 82 DAS, suggesting an excess of tillers at 54 DAS (Table 5).

This research shows that the longest season variety, Amaroo, had the highest preflowering $RUE_T$, and the largest root system. As a result, it was difficult to separate the effect of duration from root growth on RUE. It is hypothesized that the longer growth duration and more active tiller production gave rise to a larger potential root size in Amaroo. It is also hypothesized that the larger number of roots initiated acted

**Table 5. Tiller density and individual tiller weight at 54 and 82 d after sowing (DAS).**

| Variety | Tillers $m^{-2}$ 54 DAS | Tillers $m^{-2}$ 82 DAS | Tiller wt (g) 54 DAS | Tiller wt (g) 82 DAS |
|---|---|---|---|---|
| Jarrah | 480 | 556 | 0.16 | 1.19 |
| Amaroo | 1,123 | 862 | 0.11 | 0.50 |
| Barkat | 614 | 804 | 0.16 | 0.66 |
| H473 | 586 | 598 | 0.21 | 0.80 |
| LSD[a] ($P$>0.05) | 398 | ns[b] | 0.059 | 0.282 |

[a]LSD = least significant difference. [b]ns = nonsignificant.

as a positive feedback mechanism, increasing the RUE of the crop for the time that the roots were actively growing.

The mechanism by which active root growth could stimulate the $RUE_T$ of a crop remains unclear. Possible mechanisms include greater water or nutrient uptake of the plant by a relatively young root system, root signals stimulating canopy growth, and the greater sink demand of the root system stimulating crop growth. It may therefore be possible to stimulate greater aboveground growth by mimicking root signals through external applications of appropriate chemicals, by developing small root systems that produce large root signals, or by altering the root to shoot ratio.

## Implications for estimating RUE

This chapter shows that the estimated value of RUE depends on the timing of observation and whether roots are included in the calculation of biomass. Since the production of root biomass requires twice as much assimilate as the same amount of shoot biomass (Passioura 1983), $RUE_T$ before flowering may be underestimated in this analysis.

Although a correlation between $RUE_T$ and duration existed among these varieties, the relationship should be tested across a greater number of varieties of varying phenology. To further enhance the accuracy of the estimates of $RUE_{AG}$ and $RUE_T$, particularly following anthesis, the frequency of interception measurements and biomass samplings must be increased.

Understanding the mechanisms responsible for partitioning biomass between the shoots and the roots is vital to realizing whether a reduction in root weight could increase aboveground biomass and hence yield potential. In wheat, RUE increased from 1.08 g $MJ^{-1}$ for old cultivars to 1.31 g $MJ^{-1}$ for modern cultivars; this appears to relate to reduced investment in root biomass by modern cultivars (Siddique et al 1989).

## Implications for screening RUE

The influence of root growth on $RUE_T$ will affect strategies for indirectly measuring $RUE_T$. Related studies involving canopy temperature, stomatal conductance, $^{13}$C-isotope discrimination, and carbon exchange rate have been used to relate assimilation to grain yield in wheat (Fisher et al 1998) and are now being applied to rice. This study shows that, when using these techniques in rice, it is important to consider the role of active root growth in assimilation. Such research is vital to understanding whether greater root weight is necessary for crop efficiency and to assist in the selection of varieties to increase yield.

The results presented here support the speculation of Kiniry et al (1989) that variability in the shoot to root ratio is a major factor contributing to the variability of RUE. However, the results showing increased root growth of Amaroo, with no corresponding reduction in shoot growth, do not support suggestions that yields can be increased if assimilates are not allocated to root growth (Burns 1980, Passioura 1983, Richards 1991). This research suggests that increases in yield potential may come from maintaining the current high harvest index and increasing biomass through increasing $RUE_T$.

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AGRONOMY

# Predicting nitrogen, plant moisture, and yield in rice using hyperspectral remote sensing

G.J. Fitzgerald and R.G. Mutters

Hyperspectral remote sensing is a promising new tool for precision agriculture offering the capability to predict crop parameters and within-field variability. This technology has not been previously applied to rice in California. It provides hundreds of narrow spectral bands from the visible blue to the mid-infrared (367–2,328 nm), potentially allowing discrimination of physical and chemical crop characteristics. In this preliminary study, the objective was to demonstrate that % plant nitrogen, % plant moisture, and yield could be predicted using hyperspectral remote sensing. TRW Aerospace acquired a hyperspectral image of a rice field from a light aircraft flown at low altitude 2 wk before harvest in 1996. Best-fitting equations were derived from image and field data using stepwise multiple regression analysis that found the best correlation (highest $R^2$) of spectral bands to each of the crop parameters measured. From these equations, predictive images were generated showing areas of varying values for % nitrogen, % moisture, and yield. Plots of actual versus predicted data indicate that % plant nitrogen had the highest correlation ($r^2 = 0.76$), followed by yield (0.71) and % plant moisture (0.60). Thus, data indicate that hyperspectral remote sensing has the potential to predict crop parameters important to rice production.

Hyperspectral imaging is similar to multispectral imaging like that performed by Landsat$^{TM}$ and other sensor platforms but differs in three major ways: (1) there are hundreds of bands; (2) the bands are contiguous, allowing essentially continuous spectral coverage from about 400 nm (visible blue) to about 2,300 nm in the infrared; and (3) the bands are very narrow, generally 5–20 nm wide, allowing differentiation of very fine spectral features.

In agriculture, knowledge of crop status is required to make important management decisions, especially if corrective actions need to be taken. Detection of a problem early in the season and in the centers of fields, which are difficult to scout, is important for treating the problem in a timely manner and where it occurs. Hyperspectral remote sensing offers the potential to detect fine differences in important crop variables. The location of pests and weeds and their identification, crop chlorophyll (nitrogen) status, crop moisture, yield, and soil characteristics are a few of the possible uses of hyperspectral remote sensing (Green et al 1998a,b, Shibayama and Akiyama 1991). If images can be acquired at an appropriate time of the season and turnaround time reduced, farmers could benefit from this technology.

In rice, knowledge of grain moisture could help growers determine harvest date and control grain quality. Harvest should occur when grain moisture is about 21–24%. Nitrogen content can predict protein content, an important factor in grain quality. Protein content plays a role in cooking time, texture, and grain tenderness, which are important consumer issues, particularly in Asian markets. Yield prediction could allow growers to map yield in their fields, thus providing a powerful management tool. It could also allow growers to prepare in advance for marketing, harvesting equipment needs, and storage and drying facilities.

The purpose of this study was to demonstrate that remotely sensed hyperspectral data could correlate to the field-measured crop parameters % plant nitrogen, % plant moisture, and yield.

## Methodology and results

Field data (% plant N, % plant moisture, and yield) were collected as part of an ongoing straw management study by the UC Davis Rice Project. Subsamples from the field plots were dried, ground, and analyzed for % nitrogen using a Fisions NCS 1500 elemental analyzer. Percent moisture was determined gravimetrically from weighing before and after drying to 60 °C. Yield was determined by weighing dried grain collected from each plot with a harvester.

A hyperspectral remotely sensed image of rice straw management experimental plots near the Biggs rice research station in the Sacramento Valley of California was acquired on 17 September 1996, 2 wk before harvest (Fig. 1). The image was acquired by TRW Aerospace using a TRWIS III hyperspectral instrument flown over the rice field in a small jet at low altitude (1–2 km). The data set had 335 bands (285 usable in analysis) with a spectral resolution of 5.85 nm from 367 to 2,328 nm. Spatial resolution was 1.5 m. Figure 1 shows band 70 (732 nm) in the visible red. Treatment plots, levees, the central irrigation canal, and sampling paths are visible in the image, which represents an area of 383 × 467 m. Vertical lines are not straight because the image was not georectified.

ENVI software (Research Systems, Inc., Boulder, Col.) running on a Silicon Graphics workstation was used to process the image data set. The data were not radiometrically corrected so analysis proceeded on the raw, uncorrected data. The processing steps were (1) select regions of interest (ROI), (2) "clean up" the data, (3) calculate normalized difference vegetation index (NDVI)-like relationships and perform multiple regression analysis, (4) generate predictive images, and (5) validate predictive images to field-collected data. Statistical analyses were performed on a Power Macintosh with Microsoft Excel and DataDesk statistical analysis software (Data Description, Inc.).

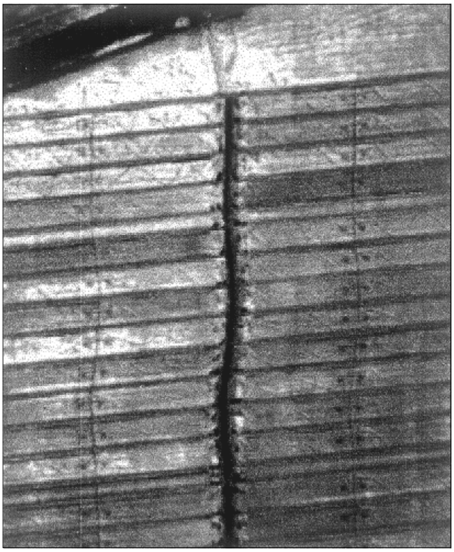

**Fig. 1. Image of Biggs straw management site, band 70 (732 nm) showing central irrigation canal (thick black line), levees (horizontal straight lines), walkways (2 thin vertical lines to right and left of irrigation canal), and sampling areas (small pairs of dots in each plot).**

Areas in the image were selected (ROI) that corresponded to the field plots where field data were collected (Fig. 2). Levees, ditches, and areas not disturbed by walkways or sampling areas (thin dark vertical lines and small dark dots in Fig. 2) were excluded. These ROI contained the means of pixels from each plot that were then used for analysis.

Some of the bands had an obvious right/left illumination problem (Fig. 3A). This was removed by applying principal components analysis available within the ENVI software to the entire data set (minimum noise fraction analysis). A new data set containing images ranked according to variance (high to low) was produced. The first band contained the high-variance illumination noise (Fig. 3B). A new data set was regenerated by discarding the illumination and low-variance random noise bands (Fig. 3C). Figures 3A-C show the original image, illumination noise band, and corrected image, respectively, for one band only.

Images similar to an NDVI were produced from data using the selected ROIs by applying the equation $(b1 - b2)/(b1 + b2)$ to data where $b1$ = a band in the infrared and $b2$ = a band in the visible. These data were regressed against the field data for % plant N, % plant moisture, and yield from each plot. Forward stepwise multiple regression was performed to identify which combinations of NDVI images correlated best to the field data. Bands were added to the analysis based on whether they maximized the multiple regression coefficient ($R^2$) and maintained the $P$ value below 0.05.

**Fig. 2. Regions of interest (ROI) selected to avoid disturbed and noncrop areas (levees, irrigation canal, walkways, and sampling areas).**

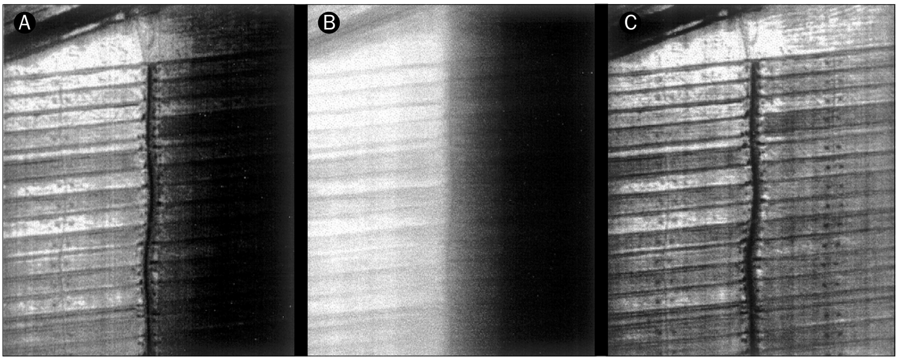

**Fig. 3. The original image in (A) shows uneven illumination (961-nm band shown as an example). (B) shows that the first principal components (MNF = minimum noise fraction) band contains the illumination data. The final corrected image in (C) was produced by removing MNF band 1 and MNF bands 16–285 (noise) from the data set.**

The highest correlation ($R^2$) resulted for percent moisture (0.74), followed by percent nitrogen (0.66) and plot yield (0.64).

The best-fitting equations produced from the multiple regression analysis were entered into the ENVI software and new images derived that were then density-sliced and colored (represented here as different levels of gray). In Figures 4A-C, water is black, dark gray represents the area of highest values (greatest % N, % moisture, and

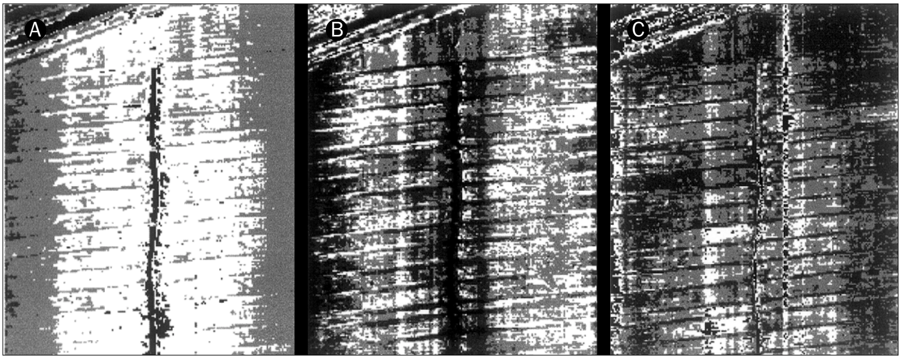

**Fig. 4. (A-C) Predictive images for % N, % moisture, and yield derived from multiple regression equations. Vertical lines and patterns indicate noise in the data set. Black = water, white = low values, light gray = medium values, dark gray = high values.**

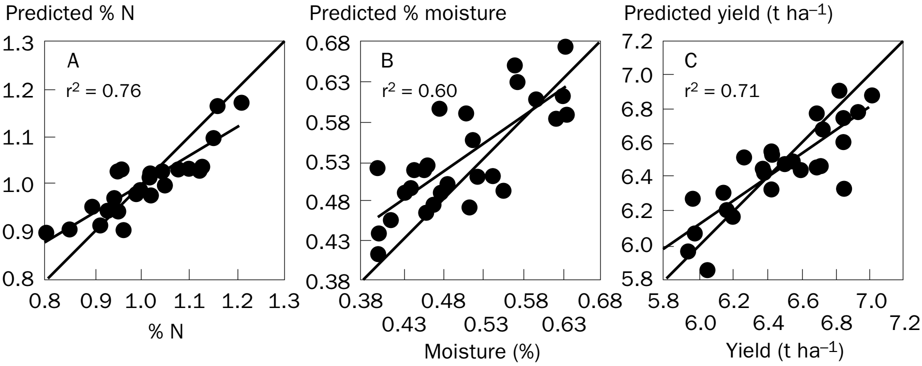

**Fig. 5. (A-C) Actual versus predicted values for % plant N, % plant moisture, and yield based on predictive images in Figure 4. The lines at 45-degree angles show a theoretical perfect fit between the predicted and actual data. The other lines are linear line fits to the data.**

yield), followed by light gray and white. In the yield image, the highest values occur on the levees and may indicate weeds (not visible in the gray-scale images). It appears that the highest yield and % N areas (dark and light gray) within the images (Figs. 4A, C) occurred along the right and left edges of the field. The vertical gray patterns in Figures 4A-C indicate that there is still noise in the data that could be removed with further image processing.

To test whether the predictive images represented actual field values for the three variables, the ROIs (Fig. 2) were applied to the predictive images and mean values were compared with actual field plot data (Figs. 5A-C). The 45-degree line represents a theoretical perfect fit between the actual and predicted points. The model predicted values less than actual (points below the line) for high values for the three variables and greater than actual (points above the line) for low values. This was probably because of additional uneven illumination in the image caused by instrumentation. The other lines are linear fits to the data and they have $r^2$ values of 0.60 to 0.76 for the predictive images.

## Discussion

One of the main drawbacks of this study was the use of nonradiometrically corrected image data. Ideally, this analysis should have been performed on data corrected to % reflectance, but this was not possible. This would be required if the data were to be compared with other images acquired on different dates or with other images of rice in different areas. However, since this data set was not compared with other image data, it was reasonable to use for analysis.

Noise was introduced into the data set probably because of instrumentation. A visual inspection of the 335 bands showed that there was an obvious uneven right-to-left illumination in many of the bands, as shown in Figure 3. Since principal components analysis transforms data into new variables and ranks the data from highest to lowest variance, systematic noise can be identified and removed (Anonymous 1997, Eastman and Fulk 1993, Afifi and Clark 1990). Thus, this procedure allowed the data to be "cleaned up" and analysis to proceed. As the vertical lines in the predictive images show in Figure 4, however, there was still noise in the data set that would need to be removed in further analysis beyond this preliminary study.

Producing predictive images is a good way to visualize data but has the disadvantage of being somewhat subjective since the colors (or gray scale) are selected based on ranges of values chosen by the operator. Thus, the values predicted by the images from the ROI were plotted against the actual field-measured data in Figure 5. This shows more objectively the relationships between these values and the validity of the methods used to predict % plant N, % plant moisture, and yield. The linear correlation coefficients of 0.60 to 0.76 indicate good fits and show that this methodology and hyperspectral technology have the potential to be useful as a tool for precision crop management.

New satellites will place a hyperspectral instrument into orbit in the next few years. Although this technology is still in the research stage, it should become available commercially within the next decade.

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# Growth and yield of rice under a water storage-type deep-irrigation method

Y. Goto, M. Saito, T. Nakamura, K. Sugai, S. Nakamura, and T. Kato

In Tohoku District of Japan, river water is abundant because of water from melting snow and rainfall in the rainy season, until summer, when a shortage in water supply often occurs. To delay the runoff into the sea of the river water using paddy fields, we are developing a water storage-type deep-irrigation method for rice. We designed a water-managing system taking into consideration the following points: (1) to increase the water storage function of the paddy field, the water is held as deep as possible using the current levee (the maximum water depth is 25–30 cm); (2) considering the steady high yield of rice in Tohoku District, the water depth at the meiotic stage is made 25–30 cm to protect young panicles from low temperature; (3) the water depth is increased continuously until mid-July, when water is abundant, assuming the water supply is steady, and the depth is controlled to have only one peak. In this study, we compared the growth and yield of rice cultivated by the water storage-type deep-irrigation method and conventional cultivation method over four years. Yields under the water storage-type deep-irrigation method were not inferior to those under conventional cultivation.

In Tohoku District of Japan, snow-melt runoff is maximum in April and river discharge from the time of rice planting to late July is abundant because of water from melting snow and rainfall during the rainy season. However, river discharge is reduced in August, and we often suffer from a shortage of urban and industrial water. Under such conditions, we tried to increase water storage by holding more irrigation water in the paddy field; otherwise, that water would run directly off into the sea during May to July. Thus, the water runoff into the sea may be delayed and the shortage of river water during the summer season may be alleviated.

Recently, the functional benefit of the paddy field for environmental protection has been drawing attention around the world. In particular, the paddy field has been summarized as having two functions in water mediation (Sekiya 1992, Minami 1993). One is the function to control runoff of rainfall derived from the structure of the paddy field and the other is the ponding function during the rice cultivation period (Goto 1996).

The maintenance of sufficient domestic food production is important, and such environmental protection measures cannot be taken if yield would decrease markedly. Instead, we should develop a cultivation method that protects the environment, while producing a yield similar to or higher than that previously obtained. Furthermore, to obtain a stable yield consistently, we must also consider how to avoid cold weather damage in Tohoku District.

To meet the above requirements, we propose to manage water depth with the pattern described later and to develop a new water management system based on an agrohydraulic study. In this study, the growth and yield of rice were examined over 4 years.

## Materials and methods

This study was conducted in the paddy field on the campus of Miyagi Agricultural College in the central area of Miyagi Prefecture in Tohoku District. To make soil and other environmental conditions uniform, 10 t ha$^{-1}$ of compost was mixed into an experimental paddy field (25 × 10 m) and, after the first puddling, experimental plots were partitioned with a concrete U-shaped drain. High-analysis compound fertilizer was applied at 50 kg N ha$^{-1}$ as the basic fertilizer and, after puddling and leveling, three seedlings each were planted by hand, with 30-cm space between rows and 15-cm hill space within rows. The cultivar used was Sasanishiki.

The two water management treatments were the conventional plot and deep-irrigation plot.

### Customary plot

The water management method for cultivation of Sasanishiki in the central area of Miyagi Prefecture was conducted as follows according to Goto et al (1995).

Young seedlings were transplanted 1–15 May and middle seedlings 10–25 May. From the time of transplanting to the rooting stage, water depth was adjusted to 5–7 cm to prevent damage by transpiration from leaves. During the tillering period, water depth was controlled at 2–3 cm, which is the best for raising water temperature. The field was irrigated intermittently from mid- to late June and drained from late June to early July. Early in July, the field was irrigated sufficiently to fill the cracks at the field surface, twice at 6-d intervals. Then, intermittent irrigation was repeated. However, at the heading stage (around 10 August), a reduced ponding depth (about 3 cm) was maintained for 10 d. About 20–30 d after anthesis, water was drained.

In the conventional plot, the water level was managed adjusting to the growth of middle seedlings, according to the water management method described above.

### Deep-irrigation plot

The method of water management for the deep-irrigation plot was used considering the following based on our preliminary experiments. To increase the water storage function of the paddy field, the water level was made as deep as possible using the current levee. According to the Standards of the Agricultural Structure Improvement Project, the maximum water depth was adjusted to 25–30 cm. Water depth was adjusted at the panicle differentiation stage, particularly at the young microspore stage, to 25–30 cm to prevent cold damage (Kobayashi and Satake 1979). The water depth was kept maximum at this stage.

Agrohydraulically, to obtain the maximum stable water supply, we considered a water depth-increasing curve with a single peak. Water depth was designed to increase until mid-July, when the discharge of water was abundant and stable because of water from melting snow and rainfall during the rainy season. Water depth was increased with the goal of developing a high-yielding canopy under deep-irrigation management as well as achieving good weed control.

### Examination of growth and yield

One hill consisted of three plants. Fifteen hills, three from each of five rows, were examined for plant age in leaf number, tiller number, and plant height. To avoid directly stepping into the paddy field, a bridge was made at the time of measurement. To examine yield, two 60-$m^2$ subplots, A and B, were included in each plot. Four or five units of plants, each consisting of 10 hills (2 hills from 5 rows), were harvested from each subplot, and the number of panicles and total number of florets were counted. Brown rice with more than 1.8 mm grain thickness was regarded as ripened brown rice, and the weight of brown rice was measured at 15.5% grain moisture.

## Results and discussion

Despite variability in annual runoff, we managed water depth according to the schedule described in the "Materials and methods" section. The daily water requirement was 1–3 cm in depth.

### Increase in plant height and tiller number

Plant age in leaf number was similar in both plots. Plant height increased rapidly after the start of deep irrigation. For example, the increase in plant height in 1995 in the deep-irrigation plot was 10 cm more than that in the conventional plot at 30 d after transplanting (30 DAT), and this difference remained thereafter (Fig. 1).

The rate of increase in tiller number from about 20 DAT to 40 DAT was lower in the deep-irrigation plot than in the conventional plot in each year. In 1995 (Fig. 2), tiller number was maximum around 6 July (49 DAT), and the maximum tiller number per hill was 24.2 in the deep-irrigation plot, which was about 70% of that in the conventional plot (35.2).

The final tiller number per hill in the deep-irrigation plot (17.6) was not significantly different from that in the conventional plot (17.7).

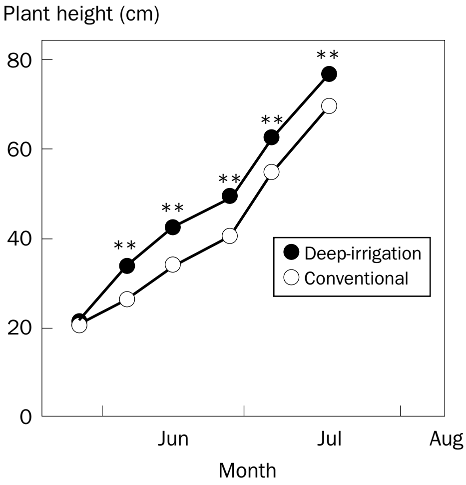

**Fig. 1. Changes in plant height of the deep-irrigation and conventional plot. ** = significant at *P* <0.01.**

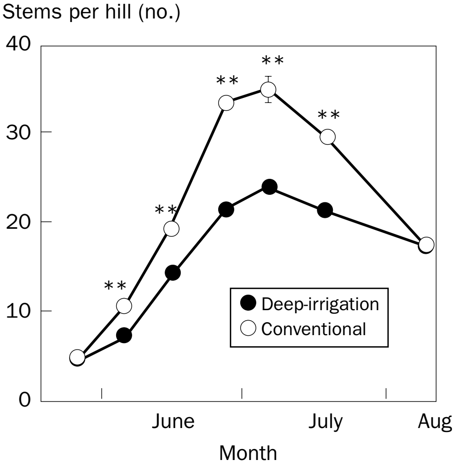

**Fig. 2. Changes in number of stems per hill of the deep-irrigation and conventional plot. ** = significant at *P* <0.01.**

**Table 1. Yield of brown rice over 4 years (t $ha^{-1}$).**

| Year | Deep-irrigation plot[a] | Conventional plot[a] | Average yield in central area of Miyagi Prefecture |
|---|---|---|---|
| 1994 | 5.38 ± 0.12 | 5.23 ± 0.15 | 4.98 |
| 1995 | 5.27 ± 0.13 | 5.37 ± 0.09 | 4.96 |
| 1996 | 5.74 ± 0.17 | 6.36 ± 0.14 | 5.04 |
| 1997 | 5.64 ± 0.12 | 5.76 ± 0.07 | 5.15 |

[a] Brown rice with more than 1.8 mm grain thickness was regarded as ripened brown rice.

## Yield

Table 1 shows that there was no difference between the brown rice yields in the deep-irrigation and conventional plots, suggesting that yield would not be reduced by using the water storage-type deep-irrigation method. The yield in the deep-irrigation plot was higher than the average yield in the central area of Miyagi Prefecture in each year.

The appearance of the canopy of the rice plants during the cultivation period suggested that a further increase in yield is possible. Actually, it might be possible to increase the density and size of the canopy during the ripening period. In general, in the deep-irrigation method used as a technique for increasing yield, efforts have been made to increase the number of grains per panicle and maintain a high percentage of ripened grains. In some studies, the number of grains per panicle tended to be increased by the deep-irrigation method (Kiriyama and Nakatani 1987, Furuya et al 1991). However, in the study of Goto et al (1996) in which fertilizer and water management were similar to those used in the present study, the number of grains per panicle in the deep-irrigation method was slightly, but not significantly, higher. The water depth at the time of deep irrigation in the present study was deeper than that in other reports and the period of deep irrigation was also longer. This might affect yield in the present study, but, to increase the number of grains per panicle, growth during the panicle differentiation stage should be promoted.

Therefore, we are now conducting studies mainly to improve the method of fertilization, including the use of availability-controlled fertilizer. In the future, we intend to combine stable high-yielding factors with the water storage-type deep-irrigation method.

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# Research on cold tolerance in Australia: focusing on nitrogen-cold interactions and genotypic variation

T.A. Gunawardena, T.C. Farrell, S. Fukai, F.P.C. Blamey, and R.L. Williams

Cold-induced spikelet sterility is a major threat to the production of stable high yields in the Australian rice industry. This chapter surveys two strands of research into understanding and preventing such cold damage.

The first aims to determine the mechanisms involved in cold damage, particularly in its interaction with the nitrogen (N) status of the crop. One series of experiments was conducted under controlled-environment conditions. It showed that a combination of high N and low temperature (18/13 °C) during early pollen microspore reduced engorged pollen production per anther. This reduction was not observed, however, when tiller number was reduced to two per plant. The small number of engorged pollen resulted in reduced intercepted pollen number and reduced pollen germination on the stigma. The results show that there must be at least 20 germinated pollen grains on each stigma to achieve 90% spikelet fertility. A field trial confirmed the negative correlation between high tiller production and the number of engorged pollen.

The second strand of research aims to identify genotypes that are tolerant of cold. Genotypic variation identified in a controlled-environment experiment was confirmed by field trials. Varieties tolerant of low temperature intercepted more pollen grains on stigma at low temperatures than did other varieties.

Midseason cold damage is a major threat to the Australian rice industry for maintaining its status as one of the highest yielding rice industries in the world. Average rice yields in 1996 were 25% lower than in the previous year because of temperatures 4 °C below average during pollen development (Williams and Wensing 1998). Low minimum temperatures (<12 °C) during microspore development dramatically increased spikelet sterility (Peterson et al 1974, Satake 1976).

This chapter surveys two strands of research into the problem of midseason cold damage. The first investigates the mechanisms of cold damage, particularly in crops where the damage is exacerbated by high N status (a problem noted by Heenan

1984 and Amano and Moriwaki 1984). This interaction was investigated across genotypes in a series of controlled-environment and field experiments at The University of Queensland and at the Cooperative Research Centre for Sustainable Rice Production, Yanco Agricultural Institute, New South Wales (NSW).

The second strand of research investigates genotypic variation. It is hoped that efficient screening of genotypes will contribute to developing cold-tolerant lines. While genotypic variation has long been known to exist (Satake 1969), this chapter discusses recent experiments in both controlled environments and the field that have identified seven varieties that are more cold-tolerant than current commercial varieties in Australia. This research was carried out at Yanco Agricultural Institute, NSW.

## Cold-nitrogen interaction: controlled-environment trials

To investigate the effect of the interaction of cold and N status on spikelet sterility, rice plants (cv. Amaroo) were grown in individual pots in a glasshouse, using three replicates. Two rates of N, 0 and 150 kg ha$^{-1}$, were applied at the three-leaf stage. In one treatment with 150 kg N ha$^{-1}$, only the first two tillers were retained, with all subsequent ones being removed as they appeared. In the other, with 150 and 0 kg N ha$^{-1}$, no tillers were removed. Plants were initially grown in a glasshouse with minimum temperature exceeding 20 °C. Low (18/13 °C) and high (28/23 °C) day/night temperature treatments were imposed for a period of 7 d starting from the pollen mother cell stage, after which the plants were returned to an ordinary glasshouse with favorable temperatures.

In this experiment, low temperature significantly reduced the number of engorged pollen grains and increased spikelet sterility (Table 1). When plants were exposed to low temperatures and limited to two tillers, however, spikelet sterility decreased. The results therefore suggest that the greater negative impact of low temperatures on high N crops resulted from increased tillering in these crops.

There was a significant positive correlation between engorged and intercepted pollen ($r = 0.89$) and between intercepted and germinated pollen on each stigma ($r = 0.88$). These correlations indicate that a large number of engorged pollen per anther is the key to successful fertilization. There was a significant negative correlation between pollen germination and spikelet sterility ($r = 0.77$). It appears that 20 germinated pollen grains on each stigma are sufficient to result in 90% spikelet fertility.

## Cold-nitrogen interaction: field trials

The interaction of cold and nitrogen status was further investigated in a field experiment. Rice seeds (cv. Amaroo) were sown in early, middle, and late October. The replicated trial was sown in three separate bays with water depths of 20 cm at panicle initiation (PI). In all three sowings, half of the plots were fertilized with 150 kg N ha$^{-1}$ at the three-leaf stage, whereas the other half received no fertilizer at this time. Half of each plot was topdressed with 150 kg N ha$^{-1}$ at PI, whereas the other half received no additional N at PI.

**Table 1. The effects of nitrogen application, tiller removal, and temperature imposed at the microspore development stage for 7 d on the engorged pollen number per anther, number of tillers per plant, and spikelet sterility in rice. Main treatment effects and not their interactions are shown.**

| Treatment | Engorged pollen anther$^{-1}$ | Tillers plant$^{-1}$ | Spikelet sterility[a] (%) |
|---|---|---|---|
| Nitrogen (kg N ha$^{-1}$) | | | |
| 0 + 10 | 649 | 1.00 | 18.0 |
| 150 + 10 | 520 | 4.53 | 35.9 |
| 150 + 10 (detillered) | 733 | 2.00 | 23.0 |
| LSD ($P = 0.05$) | 184 | 0.49 | ns |
| Temperature (day/night °C) | | | |
| 18/13 | 379 | 2.43 | 43.0 |
| 28/23 | 888 | 2.59 | 8.3 |
| LSD ($P = 0.05$) | 126 | 0.32 | 7.5 |
| Interaction | ns | ns | ** |

[a]ns = not significant at $P = 0.05$, ** = significant at $P = 0.01$.

**Table 2. Engorged pollen number per anther and tiller number m$^{-2}$ of rice as affected by sowing time (early, middle, and late October) and nitrogen application at the three-leaf and panicle initiation stages.**

| Nitrogen (kg ha$^{-1}$) | | Engorged pollen anther$^{-1}$ | | | Tiller number m$^{-2}$ | | |
|---|---|---|---|---|---|---|---|
| 3-leaf stage | Panicle initiation | Early | Middle | Late | Early | Middle | Late |
| 0 | 0 | 1,124 | 1,011 | 1,190 | 479.2 | 731.6 | 364.1 |
| 0 | 150 | 1,047 | 1,043 | 1,083 | 658.3 | 569.7 | 683.2 |
| 150 | 0 | 1,070 | 1,005 | 1,088 | 807.8 | 755.0 | 621.2 |
| 150 | 150 | 788 | 742 | 1,040 | 779.8 | 838.6 | 684.5 |
| LSD ($P = 0.05$) | | 84 | | | 82.6 | | |

For the early and middle sowings, the application of 150 kg N ha$^{-1}$ at both the three-leaf stage and PI significantly reduced engorged pollen number (Table 2). Tiller number was greater with high N application for the early and mid sowings, resulting in a significant negative correlation between tiller number and engorged pollen number (r = 0.73). Sowing early in the season extended the crop establishment period, thus producing more tillers than with the late sowing. However, this experiment was conducted in an average year in which reduction in engorged pollen number at early sowing was associated with high N application rather than cooler temperatures. In contrast, four cold days (<15 °C) occurred through the microspore development period of the late-sown crop, thus increasing spikelet sterility, even though the reduction in engorged pollen number was rather small.

## Variety testing in a controlled environment

Thirty-four varieties with known levels for tolerance for low temperature were selected from diverse origins for testing in a temperature-controlled experiment planted at Yanco Agricultural Institute in August 1998. Each pot was established in the glasshouse under warm conditions (32/26 °C) and then three day/night temperature treatments (32/26, 25/15, and 27/13 °C) were imposed from 1 wk after PI to head emergence. Following head emergence, the plants were returned to the warm glasshouse until harvest. Visual estimates of spikelet sterility were made from the panicles at physiological maturity.

This experiment confirmed that significant genotypic variation exists for cold tolerance. The results confirmed the known level of tolerance for some varieties (Table 3). Seven varieties were identified as more cold-tolerant than current commercial varieties. This identification was based on lower estimates of spikelet sterility in the coldest room. The visual estimates of sterility were highly correlated with measured sterility (r = 0.89).

## Variety testing in the field

Two approaches have been formulated for investigating cold tolerance in the field. The first involves using multiple sowing dates to increase the likelihood of some plants being exposed to low temperatures, while the second involves planting trials in cooler rice-growing regions. The first approach was followed in a trial at Yanco Agricultural Institute in the 1998-99 rice season.

In this trial, a subset of 30 varieties from the glasshouse trial was grown. Two replicates were sown on nine occasions every 10 d from early October to late December. Each 3-m plot was sampled at flowering and at physiological maturity to deter-

**Table 3. Visual estimates of spikelet sterility as a result of cold treatment (27/13 °C) imposed from 1 wk after panicle initiation to head emergence.**

| Variety | Origin | Known level of tolerance | Visual estimate of sterility (%) |
|---|---|---|---|
| Sasanishiki | Japan | Sensitive | 79 |
| Nipponbare | Japan | Sensitive | 81 |
| Doongara | Australia | Sensitive | 84 |
| M103 | America | Tolerant | 16 |
| Hitomebore | Japan | Tolerant | 17 |
| Millin | Australia | Tolerant | 24 |

mine anther length, total pollen number, spikelet sterility, grain yield, and harvest index. Those plants that were exposed to a cold event during their early pollen microspore stage were sampled 10–15 d later when their anthers were freshly exposed. At this time, a selected number of spikelets were removed for measurement of pollen number intercepted by the stigma.

This field experiment was exposed to very little cold damage, as the 1998-99 rice season included one of the warmest January-February periods on record. Although the first 3 wk in January were 6 °C above average, the three December-sown trials received cold damage at the critical pollen development stage. From 17 to 20 February 1999, four days had a minimum night temperature of 12 °C. Plants that were at the critical early pollen microspore stage flowered in early March. Measurements of engorged pollen grains intercepted on the stigma of varieties confirmed the observed tolerance in the glasshouse of M103, Hitomebore, and Millin. The susceptible Japanese varieties Sasanishiki and Nipponbare had a significantly lower pollen number on the stigma than the tolerant varieties.

## Conclusions

Cold-tolerant genotypes have been identified in temperature-controlled rooms and confirmed in the field. Further cold-tolerance research should aim at revealing the mechanisms involved through screening varieties and understanding the interaction of N and low temperature. Results suggest that engorged pollen number is the key feature that affects spikelet fertilization. Engorged pollen production is affected by agronomic practices such as time of planting and N application. Results of this study will assist in progressive research in Australia on cold tolerance and the long-term sustainability of the rice industry.

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# Computer-assisted design of side-inlet rice irrigation systems

S. Hefner, J. Hester, and C. Robertson

Side-inlet irrigation, a type of multiple-inlet irrigation, delivers water to each basin of a rice field at the same time. The uniform delivery of water to each rice basin is critical to the success of an efficient system. In 1998, an EPA 319 project site was established to demonstrate the positive benefits of computer-designed side-inlet irrigation systems. The USDA-NRCS Side Inlet computer program uses survey data, well flow, and basin area to accurately design the number and size of lay-flat polyethylene tubing outlets needed to uniformly distribute water. Two side-inlet fields (10.1 and 18.6 ha) were compared with one 15.8-ha cascade field. Irrigation flow meters and electric power supply meters were used to monitor irrigation application amounts throughout the season. Irrigation requirements for both of the side-inlet fields were substantially less than for the cascade field. Electric meter readings on the 10.1-ha field indicated that 39% less energy was required for irrigation. A 60% reduction was experienced on the 18.6-ha side-inlet field, where the water-holding capacity of the soil was somewhat superior to that of the cascade field. These results indicate that an accurately designed and well-managed side-inlet system is significantly more efficient than traditional cascade irrigation methods.

Side-inlet rice irrigation is gaining acceptance with rice farmers in the U.S. southern rice belt. This irrigation practice, which applies water in each basin of a field individually by a lateral supply line, conserves water and increases profits. By comparison, a conventional cascade irrigation system has a single water inlet at the upper end of the field. Each basin is filled to capacity before water flows into an adjacent basin.

Side-inlet rice irrigation provides greater control of water and is easier to manage (Hill et al 1991). The benefits of the side-inlet system include water conservation, lower energy cost, and reduced cold-water damage to rice. With this type of multiple-inlet irrigation system, runoff from rice fields is reduced substantially. Farmers report improved weed control and nitrogen fertilizer retention. This is due in part to more timely initial flooding and uniform application of water (Hill et al 1991).

Using multiple inlets to irrigate rice is not a new concept. Lateral basins and ditches have been used before in other rice production areas. Crenwelge et al (1986) reported 36% less inflow required and a 79% reduction in runoff with multiple-inlet irrigation. Aluminum aboveground gated pipe was yet another tool used to distribute water from the well into each basin, but was labor-intensive, expensive, and inefficient. In the late 1980s, producers began using lay-flat polyethylene tubing to irrigate crops, including rice.

One of the key aspects of a successful side-inlet system is partitioning the water equally among the rice basins. Early efforts relied upon trial and error when punching outlets in the lay-flat polyethylene tubing, and with each season applied experience to refine the systems.

The USDA-NRCS irrigation team began developing a systematic approach to designing side-inlet systems using field-specific technical information gathered onsite. A computer program was developed to assist with side-inlet designs. The program requires key information about field topography and well output before a system can be designed. Both of these factors influence the pressure in the lay-flat polyethylene pipe. The computer program uses this information to monitor tubing internal pressure and designs uniform systems that safeguard against ruptures. The designer can use this computer program to consider all the parameters that affect lay-flat polyethylene systems. The side-inlet design will specify the tubing diameter and tubing thickness, as well as the number, size, and spacing of holes required in the lateral tubing to balance the water for each basin. In addition, the area contained in each basin affects the size and/or number of outlets in the section of lay-flat polyethylene tubing servicing that basin. For precision-graded fields with straight levees, calculating the area of each basin is straightforward. However, aerial photographs or global-positioning surveys are required to obtain accurate area measurements for ungraded fields with contour levees.

## Methods

A side-inlet demonstration was established near Morehouse, Missouri, to compare water use between traditional cascade and side-inlet irrigation systems. Figure 1 contains the schematic diagram of the side-inlet demonstration, illustrating the field orientation, irrigation method, and size. Side-inlet field A (10.1 ha) and field B (18.6 ha) were compared with cascade-irrigated field C (15.8 ha). The soil texture of each field was a combination of a sharkey silty clay loam and a sharkey clay. The cooperators indicated that the water-holding capacity of side-inlet field B was superior to that of the other fields in the demonstration.

Each side-inlet system was designed using the USDA-NRCS Side Inlet computer program. Well output for each field was measured with a McCrometer irrigation flow meter. The area of each rice basin was determined using a hand-held global-positioning system receiver. A surveying instrument was used to determine elevation differences on the field berm where the lay-flat polyethylene tubing was placed. The lay-flat polyethylene tubing (30.5 cm, 10 mil) was installed in a small trench on the

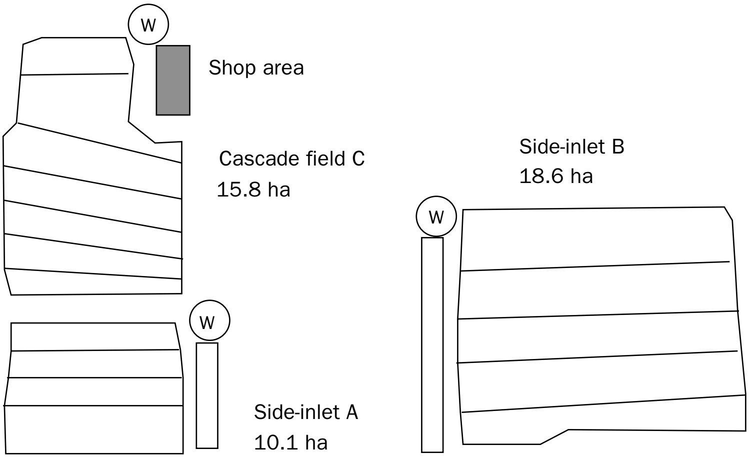

**Fig. 1. Schematic diagram of the 1998 side-inlet demonstration site near Morehouse, Missouri. W = water.**

berm. The computer program specified the number and size of the outlets in the tubing. Outlets (holes) were made by puncturing the tubing with a specialized tool that creates specified hole sizes.

Irrigation flow instruments and electric utility meters were used to measure irrigation inflow on an event-by-event basis. Both methods were used to record inflow on the cascade irrigation field. Inflow for fields A and C was determined with an irrigation flow meter and with an electric utility meter. Inflow for field B was determined with only an irrigation flow meter. Because area in each field differed, inflow data were adjusted to an equivalent area basis. Permanent flooding began within 1 d for each field. All levees were spaced on a 6.1-cm elevation interval. No levee gates were installed on field B; water distribution was solely dependent upon the lay-flat polyethylene tubing design.

## Results and discussion

Irrigation requirements for both side-inlet fields were substantially less than for the cascade field (Table 1). Electric meter readings from side-inlet field A showed that 39% less energy was required to irrigate field A than cascade field C. Flow meter readings from side-inlet field B, where the water-holding capacity of the soil was superior to that of field C, indicated that 60% less inflow was required. A major factor accounting for the difference involved harvesting rainfall. Cooperators reported 20.32 cm of rain at the demonstration site. In side-inlet field A, gate settings were set at 7.5–10 cm above the desired flood depth and the levees in field B were able to collect all precipitation. Setting the gates above the flood level to harvest rainfall also minimizes chemical and nutrient runoff.

**Table 1. Effects of irrigation method on irrigation energy and volumetric requirements.**

| Field | Cumulative energy requirement season$^{-1}$ (Joules ha$^{-1}$) | Cumulative volumetric requirement season$^{-1}$ (cm) |
|---|---|---|
| Side-inlet A | $1.75 \times 10^8$ | – |
| Side-inlet B | – | 0.13 |
| Cascade C | $2.88 \times 10^8$ | 0.33 |
| Savings | 39% | 60% |

Because the flow rates have been measured and each basin is filled simultaneously, estimating the pumping time necessary to add a specified volume of water to the field is more straightforward. The estimated pumping time is especially convenient with electric pumps equipped with timers. This information is included in each irrigation water management plan and is also an important factor responsible for improved water conservation.

## Conclusions

Each side-inlet irrigation system in this demonstration required less irrigation inflow than the cascade system. The amount of irrigation applied for the side-inlet system ranged from 39% to 60% less than the amount required by the cascade system. Observations from other computer-assisted side-inlet designs have also been monitored (data not shown). Savings in application rates have consistently ranged from 30% to 50%.

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# Reassessment of fertilizer recommendations under alternative rice straw management practices

W.R. Horwath, C. van Kessel, J.A. Bird, A.J. Eagle, and J.E. Hill

Two long-term studies located in the Sacramento Valley of California were begun to assess changes in available soil N and rice yield as affected by alternative straw management practices. The main-plot treatment was winter flooding versus no winter flooding with burning, rolling, incorporation, and straw removal as subplot treatments. During the winter months, available soil N was the highest in the winter-flooded plots (10 kg N $ha^{-1}$). In all treatments, most of the N available in the winter was present in the form of $NH_4^+$, converted into $NO_3^-$ in the spring and likely lost via denitrification during the spring planting and flooding operation. The effect of straw incorporation was addressed with a fertilizer N rate trial consisting of N rates ranging from 0 to 180 kg fertilizer N $ha^{-1}$. When rice straw residue was incorporated and no fertilizer N was applied, a yield increase of 1,500 kg $ha^{-1}$ was achieved compared to burning the straw after 5 years of rice residue incorporation. Maximum economic yield was achieved at 90 kg fertilizer N $ha^{-1}$ when residue was incorporated and at 120 kg fertilizer N $ha^{-1}$ when the straw was burned. These results clearly show the value of residue incorporation in increasing soil nitrogen availability and that fertilizer applications could be reduced.

Alternative rice residue management practices that incorporate rice straw into paddy soils and winter flooding are currently being adopted in California (USA) because of the legislative restriction of open-field burning mandated by the California Rice Straw Burning Reduction Act (AB 1378, 1991). These changes may alter the sustainability of rice production unless producers are able to adequately manage N in soil with continuous flooding and incorporated rice residues. Nitrogen-use optimization must achieve both efficient use of fertilizer N inputs and soil organic N. Since most soil N resides in soil organic pools, rice growers must begin to address management activities that affect soil organic matter and other related soil quality issues.

Although the influence of the soil organic matter fraction on soil fertility in rice-cropping systems is rarely considered, soil organic matter (SOM) has been identified as the single most important indicator of soil quality in agricultural systems

(NRC 1993). The effect of plant residues and winter flooding on N immobilization into organic fractions of rice soils has received little attention, especially in California. The implementation of residue incorporation with winter flooding has been found to reduce straw waste for seedbed preparation and to provide needed habitat for migratory waterfowl. Incorporation of 9–10 Mg $ha^{-1}$ of rice straw each year into soils with virtually continuous flooding may alter the composition and nature of SOM fractions; this in turn may have important agronomic implications for N availability by affecting the rate of N sequestration by SOM (Cassman et al 1995).

The immobilization of N into SOM is an important pathway responsible for storing N in rice soils as well as in all agroecosystems. The organic N stabilized in organic fractions of soil generally resists microbial attack and is important for sustaining long-term N availability (Stevenson 1994). The long-term availability of the immobilized N is not often determined because of the lack of an adequate methodology. In California, rice-cropping systems have begun to use residue incorporation and winter flooding management on a routine basis. These changes in management have prompted the need for an understanding of the role of SOM in regulating the immobilization and mineralization of N in submerged soils, and the improvement of N-use efficiency in rice.

In the tropics, soils continuously cropped to rice and flooded differ in SOM composition and N availability compared with soils that have had fewer annual crops and longer, aerated fallow periods (Olk et al 1996). Rice yield declines have been seen in several long-term experiments with continuously flooded double- and triple-cropped rice in the tropics. These yield depressions have been attributed to a declining effective-N supply while total soil N and C levels were maintained or increased (Cassman et al 1995). In California, residue incorporation and winter flooding may similarly affect SOM composition and result in lower N-use efficiency of added fertilizer N. In this chapter, we report on ongoing studies to determine the influence of rice straw incorporation on N availability in California rice-cropping systems.

## Materials and methods

The two main field sites used in this study are ongoing rice straw residue management trials located in Maxwell, California, on soil classified as a Willows clay, and in Biggs, California, on a Stockton adobe. The Maxwell (established in 1993) and Biggs (established in 1994) sites have large field-scale plots representing different rice straw residue treatments including burning, incorporation, winter flooding, and winter fallow without flooding (Maxwell 1.3 ha, Biggs 0.5 ha). Table 1 shows the soil properties of these three soils.

The experimental design was a split-plot design. Winter flooding and nonflooding were main plots and straw treatments (burn, incorporate, roll, and bale/remove) were subplots. Straw and flood treatments were imposed immediately following the rice harvest in the fall. All straw treatments were completed before flooding except the winter-flooded roll. At Maxwell, straw in the incorporated plots was swathed, chopped with a forage chopper, and then chisel-plowed and/or stubble-cultivated. In the

**Table 1. Characteristics of the soil (0–15 cm) from each experimental site.**

| Parameter[a] | Maxwell | Biggs |
|---|---|---|
| Classification | Willows clay | Stockton adobe |
| Clay (%) | 51 | 35 |
| Sand (%) | 5 | 17 |
| pH | 6.6 | 4.7 |
| CEC (meq 100 $g^{-1}$) | 42.0 | 30.0 |
| Total N (%) | 0.17 | 0.10 |
| Total C (%) | 1.95 | 1.23 |
| P (ppm bicarbonate) | 11.3 | 11.1 |
| Exchangeable K (ppm) | 305 | 72 |
| S (ppm) | 159 | 63 |
| Ca (meq $L^{-1}$) | 1.6 | 1.2 |
| Mg (meq $L^{-1}$) | 2.1 | 1.0 |
| EC (mmhos $cm^{-1}$) | 1.4 | 0.4 |
| SAR | 7.8 | <1.0 |
| Na (meq $L^{-1}$) | 10.2 | 0.9 |

[a]CEC = cation exchange capacity, EC = electrical conductivity, SAR = sodium absorption ratio.

nonflooding roll treatment, straw was rolled using a V-groove roller. The winter-flooded roll treatment was cage-rolled after flooding. In the straw removal plots, the straw was cut at ground level (below the disease line) and then swathed, baled, and removed. At Biggs, straw in the incorporated plots was chopped with a flail ground chopper and incorporated by chiseling, followed by disking. A cage roller was used in the rolled treatments for both the winter-flooded and nonflooding treatments.

Winter-flooded treatments were flooded in October or November and drained in mid-February to early March. Water levels fluctuated during the winter but ranged mostly from 5 to 15 cm deep. Spring field operations began in April and were the same for all treatments. Rice (variety M-202) was planted in May. Available soil N was determined before and after planting in 1997. In 1998, an N rate trial was established at the Maxwell site and consisted of N rates ranging from 0 to 180 kg N $ha^{-1}$. Phosphate (triple-superphosphate) was applied to these plots at the same rate as for the main plots.

## Results and discussion

This research was specifically intended to examine the factors that influence N availability in rice soils subjected to winter flooding and straw incorporation. Previous studies indicated that soil organic N is the most important source of plant-available N for rice in California, representing 50–80% of total N assimilated by the crop (Broadbent 1979, Mikkelsen 1987). The immobilization of N into SOM represents a substantial sink for fertilizer and crop residue N inputs in terrestrial soils. Field trials using the stable isotope $^{15}$N have shown that from 20% to 40% of fertilizer N remains behind in organic forms after the growing season in temperate-zone agricultural soils (Kelly and Stevenson 1996). The organic N stabilized in humic fractions generally resists microbial attack and is not readily available for plant uptake (Stevenson 1994).

Available soil N was affected by winter flooding in our studies. Winter flooding significantly enhanced soil inorganic N levels before planting in 1997 (Fig. 1). About 75% of the inorganic N was present as nitrate as a result of field draining and seedbed preparation activities. The majority of the nitrate would mostly likely be lost through denitrification activity (Aulakh et al 1992). The increased level of available soil N as a result of winter flooding continued through the growing season at both Maxwell and Biggs (data not shown). At both Maxwell and Biggs, there was a significant interaction between flooding and straw incorporation, indicating an increase in available soil N as a result of straw incorporation and winter flooding.

As a result of straw incorporation, yield of rice grain has increased over time on zero fertilizer N plots. During the initial 2 years after rice straw incorporation, rice grain yield decreased on the straw-incorporated plots, most likely because N was initially immobilized by the fresh residue input (see Eagle et al, this volume). The significant increase in rice grain yield after the second year has continued through the

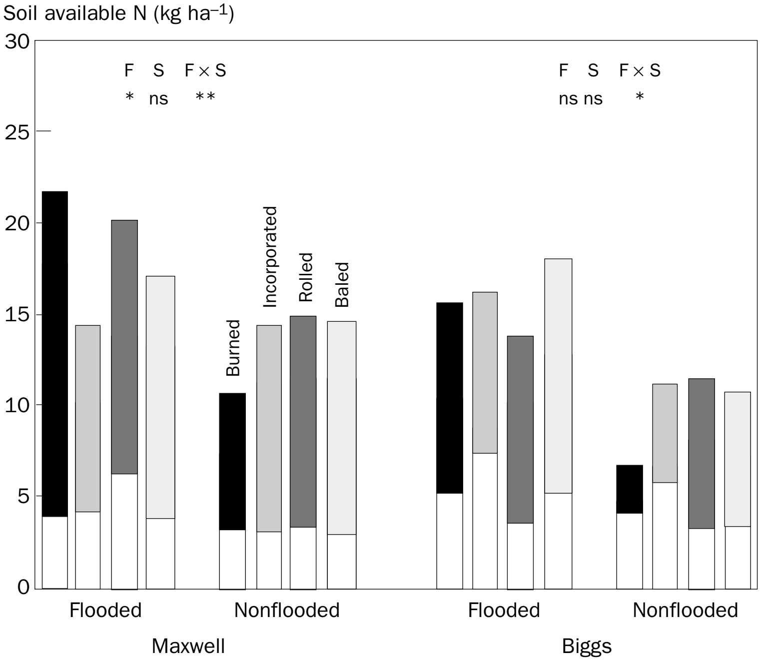

**Fig. 1. Available soil N before planting during 1997 at Maxwell and Biggs. Available N was determined on KCl extracts of soil (unpublished data). F = flooding, S = straw incorporation, ns = nonsignificant, * = significant at 95% level of probability, ** = significant at 99% level of probability.**

fifth year of straw incorporation and is expected to increase or maintain itself as long as rice straw is incorporated. The increase in rice grain yield is probably a result of a rise in soil organic matter that has increased the potentially mineralizable pool of soil N (Doran and Parkin 1994).

The increase in N availability is produced largely by creating larger pools of available soil N. This requires an increase in organic matter and/or an increase in the availability or mineralizability of existing soil N. Both processes are probably occurring in the rice residue incorporation plots. An increase in SOM occurs through the accumulation of plant and microbial decomposition products. Lignin and its phenol building blocks have been suggested as a major source of materials for humic substances (Flaig et al 1975). This mechanism for N stabilization has important implications for rice production in California and other rice systems because of the high contribution of soil to plant-available nitrogen and the recent adoption of alternative rice straw management practices that are similar to those reported to reduce rice yield and nitrogen availability in the tropics.

An N rate trial at the Maxwell site has shown the benefits of rice straw incorporation in California. The trial consisted of nitrogen rates ranging from 0 to 180 kg N $ha^{-1}$. We found that the residue incorporation treatments at 0 kg nitrogen rate had a yield increase of 1,500 kg rice $ha^{-1}$ over that found on burned treatments (Fig. 2). Maximum economic yield was achieved at 90 kg N $ha^{-1}$ in the residue-incorporated plots. In the burned treatment, maximum economic yield was achieved at 120 kg N $ha^{-1}$. These results show the value of residue incorporation in increasing soil nitrogen availability.

The immobilization of nitrogen into SOM represents a substantial sink for fertilizer and crop residue nitrogen inputs in soils. The long-term availability of the immobilized nitrogen is not often determined because of the lack of an adequate methodology. In California, rice-cropping systems have begun to use residue incorporation and winter flooding management on a routine basis and so far have shown the positive effects of rice straw residue incorporation. These results do not support the hypotheses suggested to explain the yield decline in tropical rice systems. However, rice systems in the tropics and in California are sufficiently different and it is not expected that these systems would behave similarly. These observations have prompted the need for an understanding of the role of SOM in regulating the immobilization and mineralization of nitrogen in submerged soils and in improving N-use efficiency in rice. In addition, the reassessment of fertilizer practices is an important issue in rice-cropping systems that affects the occurrence of weeds and disease, such as water grass and blast. Therefore, ongoing experiments to determine N availability from straw incorporation are essential for evaluating fertilizer nitrogen recommendations and for managing pests in alternative rice residue management systems.

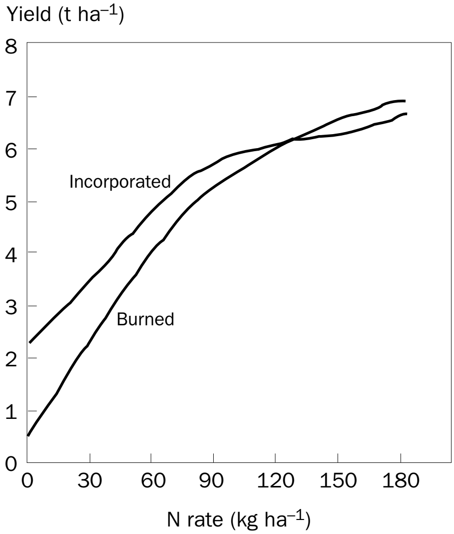

**Fig. 2. Results from the fertilizer rate trial done in the Maxwell rice residue study during 1998 (unpublished data).**

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## Notes

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# Rice stubble management in southern Australia: a review— But where is the biology?

C.A. Kirkby

Burning is the most common way of disposing of rice stubble in Australia. However, largely because of pollution problems, this will probably change, with stubble retention in the field becoming the major way of dealing with stubble. Ceasing or reducing burning together with returning large amounts of stubble to the soil will probably have a dramatic effect on soil biology and issues dealing with the effect of burning and stubble retention on the important area of nitrogen management and the associated areas of organic matter and soil microbial ecology are discussed.

In the past, most farmers and agronomists regarded crop stubbles as an unwanted by-product. Phrases such as "stubble trouble" and "stubble management by the match" have perhaps best exemplified attitudes toward crop stubbles, as they probably still do for the majority of farmers. At a recent field day at the Yanco Agricultural College held to announce the release of a new rice variety, rice growers were surveyed and 70% still practiced burning as their only or preferred stubble management method. On the other hand, a small number of farmers view crop stubble as a valuable resource. Seven percent of growers from the same survey said that they never or rarely burned their stubble, giving reasons such as "to build up the soil organic matter or return nutrients to the soil" as their principal reason for doing so. They also said that they rarely experienced any problems when retaining their stubble. This divergence of attitude and practice is one reason that stubble management remains a controversial topic.

Burning faces more and more opposition and regulation as air pollution levels from many sources increase. In the European Union, stubble burning is banned and, in some states of the United States, local laws enforce stubble retention (Carpenter et al 1992). But reducing pollution is only one reason to retain stubble. Some other reasons are obvious, such as to prevent erosion by wind and water. Others are perceptions, for example, that the retention of stubble will increase soil organic matter. Others are driven by economic theory, that at least half the crop production effort results in residues. This residue production, although unavoidable, involves a major input of resources in the form of labor, fuel, water, and nutrients. Therefore, if residues are not used to their fullest, a major part of the production effort is wasted. The International Rice Research Institute estimates that, to meet future demand, world rice production must increase markedly in the next 25 years (IRRI 1989). This is only possible, however, if the soil and water resource base is maintained and inputs are used more efficiently. Thus, the real question could be, What are the costs of not retaining crop stubble?

## Nitrogen

The influence of stubble management on the nitrogen economy under rice is by far the most intensively researched aspect of stubble management in Australia. This is not surprising when we consider statements such as “nitrogen is the single most important input limiting rice production worldwide” (Becker and Ladha 1997). Rice can use inorganic nitrogen derived from both mineral and organic N sources, but it is recognized that the efficiency of mineral N fertilizers is generally low because of large losses as N gases (Buresh and De Datta 1991). Yoneyama and Yoshida (1977) found that rice obtained only 18% of its nitrogen from fertilizers, 76% from the soil store, and the remaining 6% from decomposing straw. Multiple split aplications of mineral N reduce N losses and increase N-use efficiency and grain yield (Cassman et al 1994) but increase operating costs. Thus, residues acting as slow-release fertilizers should be able to supply nitrogen to the following crops. However, unlike mineral fertilizers, such organic residues must decompose before their nitrogen becomes available in a mineral form. In addition, soil microbes must use native soil nitrogen during this decomposition process, which leads to temporary nitrogen immobilization (Chu and Knowles 1966, Pinck et al 1946). The use of organic matter to achieve temporary N immobilzation may lead to a reduction in nitrogen losses by leaching (McGill and Myers 1987), which could be of particular benefit in flooded rice soils. Achieving such synchronization of organic matter decomposition and N mineralization is understandably difficult and has led to confusing results, even from the same author.

In a rice after rice experiment, Bacon (1990, 1991) found that, on plots where stubble had been incorporated for three successive years, there was 21% more soil $NH_4N$ and 22% more N uptake by rice than on plots where stubble had been burned. The stubble-incorporation plots outyielded the stubble-burn plots by an average of 0.35 t $ha^{-1}$. He concluded “that reasonable yield of annual rice crops could be maintained with a combination of early stubble incorporation plus heavy N applications.” Beecher et al (1994) also compared stubble incorporation and burning for rice after rice crops, but this time they concluded that the stubble management technique had little effect on rice growth or grain yield. In wheat after rice experiments, Bacon and Cooper (1985a,b) found that sowing wheat directly into stubble gave the highest wheat yield, followed by sowing into burned plots, whereas sowing into stubble-incorporated plots gave the lowest yield. In further work on wheat after rice, Bacon et al (1988) reported that tillage was the determining factor. They concluded that no-till treatments gave the highest yield and that burning or retaining stubble had no effect. The degree of stubble decomposition and N immobilization was undoubtedly a factor in all of these experiments. Unfortunately, no attempt was made to measure the level of decomposition or the microbial population and respiration rate at periods through the successive crop stages, particularly at planting and early seed growth.

## Organic matter, microbes, and soil fertility

The importance of soil organic matter (SOM) to soil physical, chemical, and biological health and resultant soil productivity is well established (Larson et al 1972, Martin and Stott 1984, Stevenson 1982). Soil organic matter is a potential major source of plant nutrients: 95% of soil N, 40% of soil P, and 90% of soil S are associated with the SOM (Smith et al 1992). Studies have shown that plants receive 76% of their nitrogen (Yoneyama and Yoshida 1977) and 75% of their phosphorus (McLaughlin et al 1988a) from soil stores, not from sources added that season. Clear evidence also shows that soil microbes are crucial to this nutrient cycling and to maintaining soil productivity (McLaughlin et al 1988b,c, Elliott and Papendick 1986).

### Organic matter pools

Fresh plant residue and the indigenous SOM have often been divided into different pools according to supposed degree of microbe use (Smith et al 1992, Harper and Lynch 1981a). This is much better than looking at organic matter purely from the point of view of C:N ratio. These pools obviously decompose at different rates even though some elements in the different pools may have the same C:N ratio (Smith et al 1992). Figure 1 shows a typical SOM diagram.

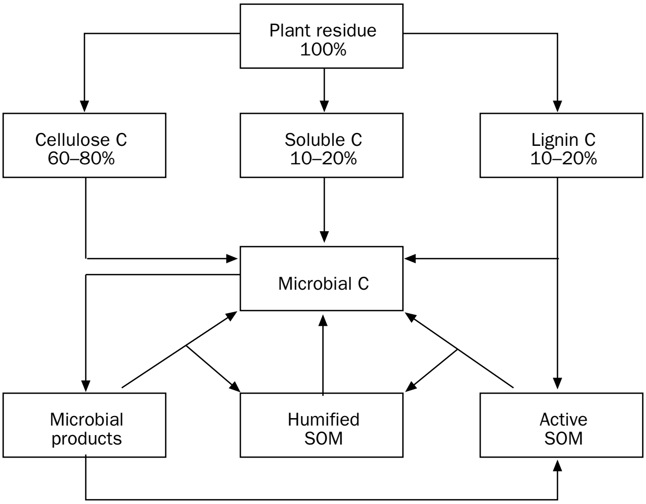

**Fig. 1. Plant residue and soil organic matter (SOM) pools (modified from Smith et al 1992 and Harper and Lynch 1981).**

This approach, however, does not take into account the substantial pool of charcoal carbon that has been detected in many Australian soils (Skjemstad et al 1996). This charcoal-like material, sometimes called char, is likely to be found in substantial quantities in agricultural soils that are subject to regular burning. An extensive survey, however, has yet to be done. Although this material contains aromatic rings, it can be very unreactive and survive for long periods of time (Skjemstad et al 1992). This material has proved to be very difficult to speciate and quantify using wet chemistry. Figure 2 shows the near magnetic resonance (NMR) spectra of charcoal formed from wheat straw and the charcoal-like organic matter remaining after a Vertisol has been photooxidized for 4 h. There is no evidence of reactive groups attached to the charcoal aromatic ring, whereas the aromatic ring of the material remaining after 4 h of photooxidation clearly still has reactive groups (carbonyl and carbohydrates) attached.

It is often stated that the Walkley and Black (1934) method, or a modification of it (Kerven et al 1999), does not determine charcoal in soils (e.g., Piper 1944). Skjemstad et al (1998), however, have thrown serious doubt on this approach. Figure 3 shows that chromic acid attack on charcoal is particle-size-dependent and, in soils containing finely divided charcoal, this method will determine charcoal as effectively as any other form of SOM.

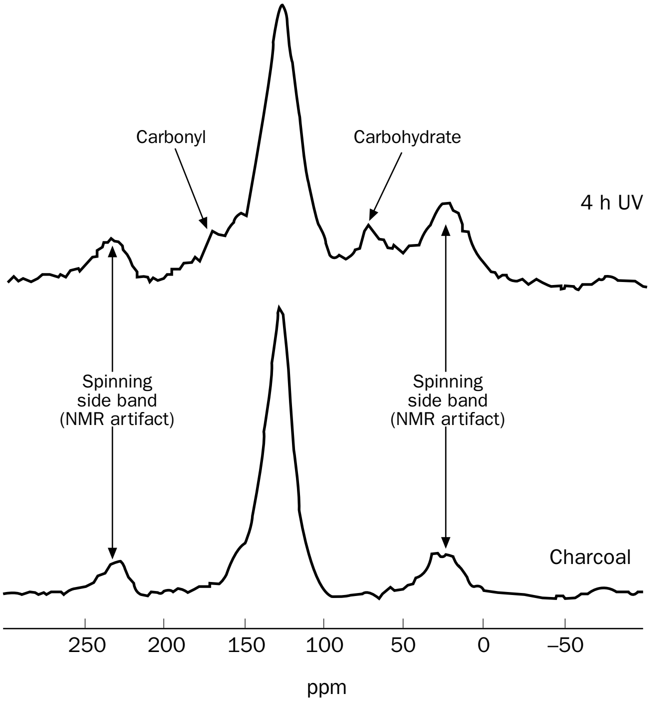

**Fig. 2. Solid-state CP/MAS $^{13}$C NMR spectra of charcoal formed from wheat straw and spectra of the organic matter from the silt fraction from a Vertisol after 4 h photooxidation (from Skjemstad et al 1988).**

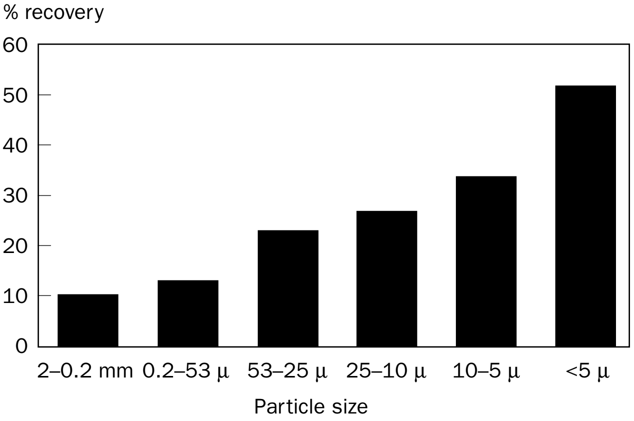

**Fig. 3. Recovery of organic carbon from charcoal made from wheat straw and with different particle size by the Walkley and Black method (from Skjemstad et al 1988).**

This material is not just of academic interest. If traditional wet chemistry shows a soil to contain 2% organic carbon and half of this is char, then the soil in fact contains only 1% active carbon and is not in as good a state as it first appears. Several workers (Toth and Milhan 1975, Toth et al 1981, Moss and Cotterill 1985) have also shown that this material affects herbicide and pesticide efficiency. Toth et al (1981) found that ash from rice stubble rendered at least 60% of the thiobencarb and molinate applied to control Japanese millet biologically unavailable because of adsorption. This could mean that far larger quantities of herbicide are being applied than would be the case without stubble burning.

## Measuring decomposition

Clearly, if stubble decomposition, through retention, is to become a major way of disposing of stubble, it must be measured conveniently. Litterbags have been used, but they are limited by the need to collect stubble, weigh it, and return it to the field. Furthermore, having stubble clumped in relatively large masses inside a bag and not intimately mixed with the soil as well as the possible loss of material upon collection may not accurately represent field decomposition. We have modified the particulate organic matter (POM) method of Cambardella and Elliott (1992). Dispersed soil samples are passed through a 53-mm sieve. The organic matter passing through the sieve, the POM fraction, is considered to be intimately associated with the mineral fraction of the soil, whereas the remaining organic matter is considered to be independent organic material. The non-POM fraction in particular has been linked to increases in yield (Grace et al 1995) and an increase in soil microbial activity (Conti et al 1997). If soil plus stubble samples are taken immediately after incorporation or when the stubble is placed on the surface and then at suitable periods thereafter and subjected to POM analysis, then the rate of decomposition in the field can be followed. Table 1 shows the total carbon, POM carbon, and non-POM carbon of the 0–5-cm soil layer

**Table 1. Effect of stubble treatment on total and particulate organic matter (POM) carbon pools (from Pankhurst et al 1977).**

| Stubble treatment[a] | Total C% | POM C% | Non-POM C% |
|---|---|---|---|
| Stubble incorporated | 0.70 | 0.22 | 0.48 |
| Surface stubble (with DD) | 0.89 | 0.25 | 0.64 |
| Stubble burned | 0.60 | 0.25 | 0.35 |

[a]DD = direct drill; other treatments included conventional cultivation.

from three treatments of a stubble management trial conducted at Cowra, New South Wales, Australia. Details are given in Pankhurst et al (1997).

The higher the non-POM C value, the higher the level of undecomposed organic material; thus, surface stubble > stubble incorporated > stubble burned, as is to be expected. The low non-POM C levels from the stubble-incorporated treatment indicate how cultivation can lead to a lowering of some soil carbon pools, probably by stimulating soil microbial action. The higher value of POM C in the stubble-burned treatment is an artifact caused by the presence of a large proportion of charcoal-like material that was observed using light microscopy. Unfortunately, we were unable to have NMR analysis done on these samples to confirm this.

Another possibility is to follow the decomposition of a discrete chemical fraction of the stubble. As Figure 1 shows, cellulose/hemicellulose (holocellulose) accounts for 60–80% of the weight of stubble and its decomposition accounts for the main loss in weight during the first season that stubble is returned to the soil (Broadbent 1954). Harper and Lynch (1981b) showed that the decomposition rates of cellulose and hemicellulose were similar and together mirrored total weight loss during the first season (see Fig. 4). Losses in lignin were small and total weight loss can be calculated from the increase in percentage lignin in recovered samples. In addition, holocellulose loss closely mirrored the ability of the straw to produce acetic acid (see Fig. 4). Decreased yields of cereals have been closely linked to the production by microorganisms of phytotoxins, notably acetic acid. Thus, following the decomposition of holocellulose also enables us to follow the potential of the stubble to produce a well-known phytotoxin.

As well as measuring the decomposition rate of stubble in the field, it is useful to have a measure of a soil's decomposition potential. For this, we have used the cotton strip assay method (Harrison et al 1988) with modifications to the statistical treatment of the data as discussed by O'Brien (1995). Cotton strips are buried in the soil, where they lose tensile strength because of decomposition. The more tensile strength lost, the greater the decomposition. Figure 4 shows results from the same trial as Table 1. The cotton buried in the stubble-burned plots has clearly not decomposed as much as the cotton in the stubble-retained plots. As these tests were done under standard conditions in the laboratory, this indicates that the stubble-burned soil does not have the same decomposing potential as the other two. That is, burning has reduced the capacity of this soil to continue decomposing. The sites were established

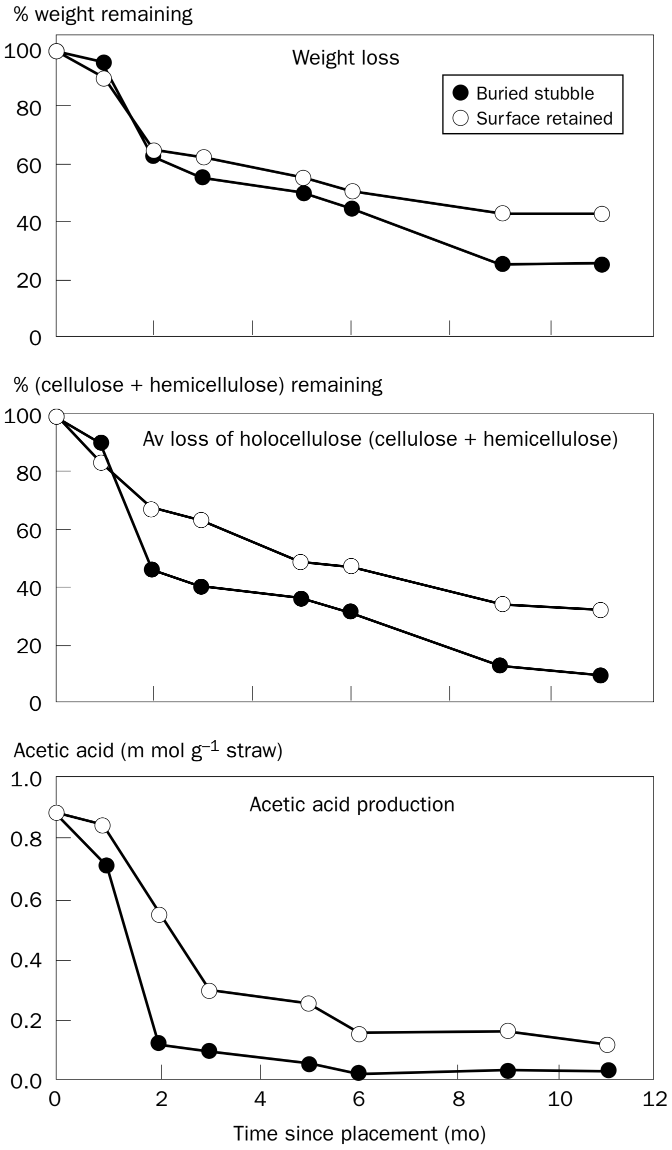

**Fig. 4. Percent weight, holocellulose loss, and acetic acid production from oat straw under field conditions (adapted from Harper and Lynch 1981).**

in 1980, but we were not called in until 1996. Unfortunately, we do not have any previous data; therefore, how long it would take the stubble-burned plots to "recover" if stubble retention were begun on them is not known.

### Residue effects on soil microorganisms

Another obvious measurement needed is the effect of stubble on the microbial community. Until relatively recently, this has been hindered by our inability to culture, and so study directly, all but a very small percentage of the estimated number of fungi and bacteria that inhabit the soil. The past decade, however, has witnessed the development of metabolism-based biochemical and molecular techniques for directly characterizing the structure and function of microbial communities without requiring isolation and culturing of individual species. Bossio and Scow (1995) analyzed the impact of rice straw retention and burning on carbon-use patterns using Gram-negative microplates (Biolog, Inc.). They found that 22 substrates were separated along environmental axes, with ten of these being used faster in rice straw-incorporated plots than in stubble-burned plots. As the inoculum density varied little across the treatments, this indicated that the metabolic diversity was greater under the incorporated treatment than in the burned treatment. Greater metabolic diversity has been linked to higher decomposition rates.

Metabolism-based methods are simple and easy to use and are thus likely to offer much for understanding differences in microbial community functioning. However, they require that the organisms be metabolically active in solution culture conditions that are usually quite different from those in the natural environment. Biochemical methods that characterize microbial community structure on the basis of the cell constituents such as fatty acids (GC-FAME) and phospholipid fatty acids (PLFA) can be applied to whole soils obtained directly from the field without any need for culturing (Cavigelli et al 1995, Fostgaard et al 1993). Figure 6 shows GC-FAME spectra from the same 0–5-cm soils referred to in Table 1 and Figure 5 (Pankhurst et al 1997). The increased fatty acid extracted from stubble-retained plots indicated that

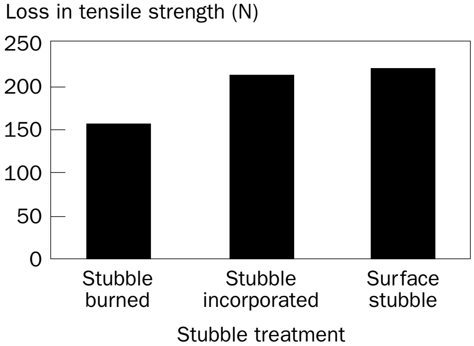

**Fig. 5. Effect of stubble treatment on the soil's decomposing potential as measured by the loss in tensile strength of cotton buried in the soil (from Pankhurst et al 1977).**

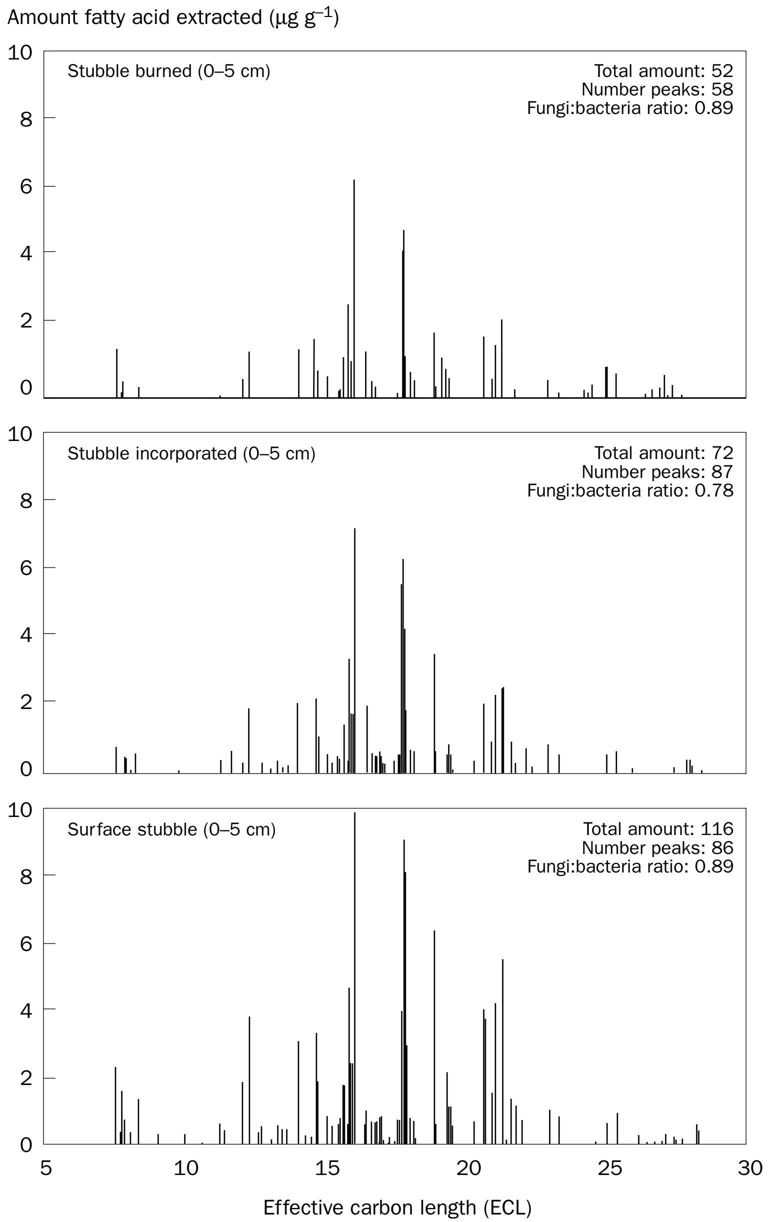

**Fig. 6. GC-FAME spectra of soil from different stubble treatments collected from Cowra, August 1997 (from Pankhurst et al 1997).**

these soils have a higher biomass, thus confirming traditional fumigation-incubation biomass estimations (Jenkinson and Powlson 1976, data not shown, details in Pankhurst et al 1997). The increased number of peaks indicated that a greater level of microbial diversity existed in the stubble-retained soils, whereas the soils from the stubble-incorporated plots had a lower fungi:bacteria ratio. This could be important in understanding why surface-stubble treatments often have higher levels of fungal diseases than stubble-incorporated treatments. The fungal:bacteria ratios are calculated by using signature fatty acids to estimate their numbers according to the method of Zelles et al (1992).

Figure 7 shows the GC-FAME difference spectra from stubble-burned plots and stubble-incorporated and surface-stubble plots. These graphs represent stubble retention effects in terms of modifying the microbial community composition when compared with burning. The amount of fatty acid present increased, except for the areas around effective carbon lengths of 24–25 and 26–27.

Table 2 shows data obtained from the GC-FAME spectra for the 5–10-cm layer of soils referred to in Figure 6. The reduction in the amount of fatty acid extracted

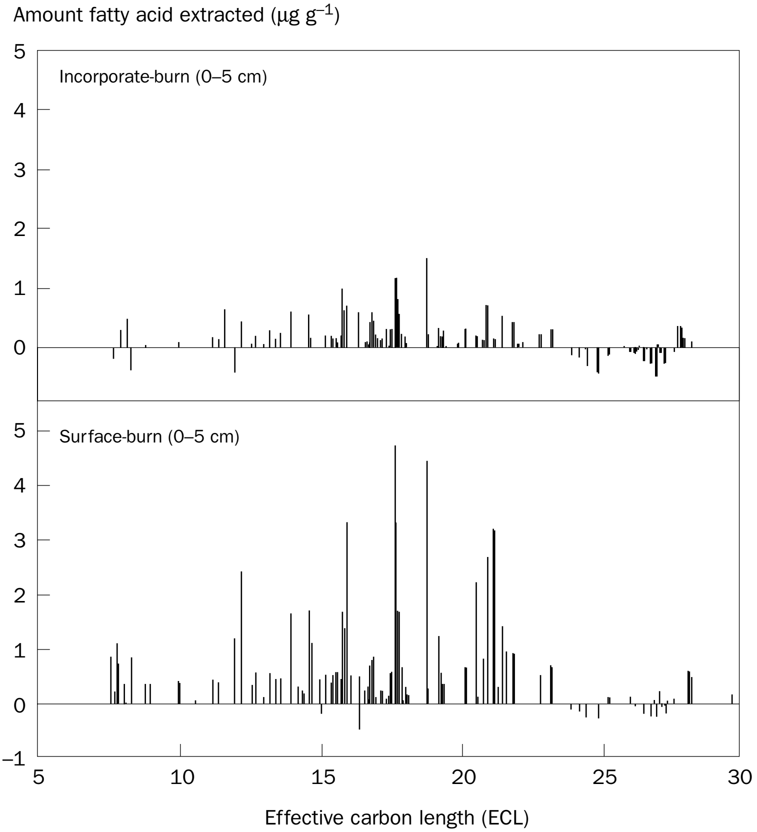

**Fig. 7. Changes in GC-FAME spectra caused by stubble retention compared with burning (details in Pankhurst et al 1977).**

**Table 2. Synopsis of GC-FAME data from 5–10-cm layer of soil from different stubble treatments collected from Cowra, August 1997.**

| Stubble treatment | Amount of fatty acid (µg $g^{-1}$ soil) | Number of GC-FAME peaks | Fungi:bacteria ratio |
|---|---|---|---|
| Burned | 35.2 | 45 | 0.95 |
| Incorporated | 43.4 | 79 | 0.45 |
| Surface-retained | 48.4 | 60 | 0.49 |

Source: Pankhurst et al (1997).

compared with that of the corresponding 0–5-cm layer corresponds to the decrease in microbial biomass in this layer as estimated by the fumigation-incubation method of Jenkinson and Powlson (1976, data not shown). The higher number of peaks from the stubble-retained soil indicated a higher level of microbial diversity, especially from the stubble-incorporated treatment compared with a decrease in the fungi:bacteria ratio in the stubble-burned treatment. The large difference in the number of peaks of the surface-retained treatment (which was also direct-drilled compared with conventional cultivation for the other two treatments) for the 0–5-cm layer (86 peaks) versus the 5–10-cm layer (60 peaks) indicates that the formation of distinct microbial layering was promoted in the soil compared with conventional cultivation, with an abundance of fungi occurring in the 0–5-cm layer. Whether this is related to the higher fungal disease levels often associated with surface-stubble retention is not known.

## What next for straw management?

Burning is by far the most common and preferred option for stubble management. It is often seen as an essential management tool to improve weed, insect, and particularly disease control (Martin 1939, Williams and Wicks 1978, Webster et al 1976). However, pressure from the broader community and problems regarding sustainability suggest that alternatives will have to be found in the future. Off-farm alternatives such as energy production, manufacturing, and construction or use as a commercial livestock feed are probably not alternatives in Australia, at least in the near future. The primary nonburn alternative is and will continue to be retention in the field. Apart from the economic aspects, many of the problems associated with this practice are related to the incomplete decomposition of the straw at the time the next crop is planted. As the decomposition process is governed by microbiology, it is important to know in detail what is occurring in the field if we are to have any prospects of manipulating it. Looking at the quality of the straw purely in terms of its C:N ratio has given mixed results. Identifying the principal components (lignin, cellulose, soluble carbon compounds, etc.) as well as which organisms attack these and what constraints there are to the decomposition of each group seems to hold more promise. In the literature, nitrogen has often been identified as a limiting element; however, others such as potassium and some trace elements are also occasionally mentioned, and this area probably deserves more investigation. Some commercial "miracle" sprays claim to speed up the decomposition process, but whether these claims could be substantiated under a rigorous scientific examination is unknown. Some growers have achieved very good results and it would seem possible to concoct mixtures that contain organisms for each stubble type for a given soil type. Whether this can be done economically and whether it would create extra disease problems are questions waiting to be answered.

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AGRONOMY

# Effects of no-tillage transplanting cultivation with polyolefin-coated fertilizers on rice: root system, yield, and yield components of Hohohono-ho

Y. Kujira, T. Yagi, K. Tanaka, M. Hikitsu, H. Umemoto, and K. Kitada

The effects of no-tillage transplanting cultivation with polyolefin-coated fertilizer (POCF) on the root system, yield, and yield components of paddy rice cultivar Hohohono-ho are discussed. Root dry weight below 10 cm in the soil layer in no-tillage was lower than in conventional tillage. Nitrogen-use efficiency was 72%, 44%, and 41% for POCF application to the nursery box with no-tillage, chemical fertilizer with no-tillage, and conventional tillage, respectively. This farming method may be a better way to reduce nitrogen losses.

It is common knowledge that slow-release fertilizers have application for horticultural plants, turfgrass, and high-cash crops under certain conditions (Hauck 1985). Even though they are somewhat expensive, however, polyolefin-coated fertilizer (POCF) recently began to be used even for common crops such as rice, maize, and others in Japan. In the United States and Canada, this fertilizer has been used primarily to fertilize horticultural crops (Fuller 1991). POCF does not injure seeds and plant roots and can supply all the nutrients necessary for rice during the growing period; thus, tillage for fertilization is unnecessary and seeding can be done together with fertilization. Serious losses of applied fertilizer nitrogen occur in soil by leaching, denitrification, and volatilization. Reduction of nutrient losses by controlling their release into the soil solution and groundwater and into the atmosphere will surely help protect the global environment. No-tillage transplanting cultivation will allow savings on other major field operations, thus significantly reducing the costs of rice farming (Gandeza et al 1991). In this research, we discuss this farming method and its effects on the root system, yield, and yield components of Japanese rice cv. Hohohono-ho.

**Table 1. Experimental design in the paddy rice field in 1997.**

| Tillage or no-tillage[a] | Basal (kg ha$^{-1}$) N | $P_2O_5$ | $K_2O$ | Topdressing N (kg ha$^{-1}$) | Nitrogen supply |
|---|---|---|---|---|---|
| Tillage | 0 | 100 | 110 | 0 | No N supply |
| Tillage | 20 | 100 | 110 | 50 | Control |
| No-tillage | 0 | 100 | 110 | 0 | No N supply |
| No-tillage | 20 | 100 | 110 | 50 | Control |
| No-tillage | 20 | 100 | 110 | 50 | Supply LPSS100[b] in the nursery bed |
| No-tillage | 20 | 100 | 110 | 50 | After legume[c] |

[a]Planting density: 19.0 m$^{-2}$ (tillage plot), 18.4 m$^{-2}$ (no-tillage). Transplanting date 5 May 1997, heading stage 24 July 1997, harvesting 1 Sept. [b]Polyolefin-coated slow-release fertilizer (40% urea). [c]LPSS100 was supplied in the nursery bed.

## Materials and methods

Japanese paddy rice (*Oryza sativa* L.) cultivar Hohohono-ho was transplanted on 7 May 1997 and grown in the field of the Ishikawa Prefectural Research Agricultural Center at a planting density of 18.4 m$^{-2}$. Fertilizers were applied as basal dressing at 20, 100, and 110 kg ha$^{-1}$ of N, $P_2O_5$, and $K_2O$, respectively, and 50 kg N ha$^{-1}$ was applied as topdressing except for the no-nitrogen plot. Polyolefin-coated fertilizer (LPSS100, contains 40% urea as slow-release fertilizer) was applied in the nursery box as nitrogen fertilizer. Table 1 describes the experimental plots in detail. Roots were sampled at the position of interhill, under the hill, and interrow on 6 August and 1 September. Root dry weight at each 10 cm of soil layer was measured by core sampling using a steel sampling tube of 53 mm inner diameter and 400 mm depth. Each soil core with root was washed out using a Hydroelute Root Washing Unit (Gillison's Ltd., USA). After harvest (1 September), yields and yield components were measured.

## Results and discussion

The number of stems was small during the early stage but it increased rapidly after heading under no-tillage cultivation. The percentage of fruitful culms was better than under conventional tillage. Yield of the no-tillage plot was 4.5 t ha$^{-1}$ and it was lower (87%) than under conventional tillage because of a decrease in both unhulled rice grains m$^{-2}$ and 1,000-grain weight (Table 2).

Nitrogen-use efficiency was 72%, 44%, and 41% for POCF application to the nursery box with no-tillage, application of chemical fertilizer with no-tillage, and conventional tillage, respectively (Table 3).

**Table 2. Yield and yield components of Hohohono-ho.**

| Yield[a] (t ha$^{-1}$) | Panicle no. (m$^{-2}$) | Unhulled rice no. (panicle$^{-1}$) | Ripened grain (%) | Total unhulled number (×1,000 m$^{-2}$) | Brown rice weight (g 1,000 grain$^{-1}$) | Harvest index (%) |
|---|---|---|---|---|---|---|
| 4.2 | 279 | 78.2 | 75.5 | 21.9 | 23.4 | 81 |
| 5.2 | 351 | 78.1 | 78.4 | 27.3 | 23.4 | 100 |
| 3.5 | 267 | 66.1 | 84.0 | 17.7 | 23.1 | 68 |
| 4.0 | 330 | 62.1 | 76.3 | 20.5 | 23.6 | 77 |
| 4.5 | 297 | 89.1 | 71.9 | 24.3 | 22.8 | 87 |
| 4.5 | 296 | 86.7 | 83.1 | 25.6 | 23.0 | 87 |

[a] Brown rice (seed selection by specific gravity, density = 1.06).

**Table 3. Nitrogen-use efficiency in different farming methods.**

| Plot | Fertilizer | N-use efficiency (%) |
|---|---|---|
| No-tillage | POCF[a] | 72 |
| No-tillage | Chemical fertilizer | 44 |
| Tillage | Chemical fertilizer | 41 |

[a] Application to the nursery box. POCF = polyolefin-coated fertilizer.

Total root dry weight hill$^{-1}$ at the interhill was the largest in the experimental plot with no-tillage and no N application. Root dry weight below 10 cm in the soil layer in no-tillage was lower than in conventional tillage (Table 4).

No-tillage transplanting cultivation with POCF and single basal fertilization of Hohohono-ho, a recommended cultivar in Ishikawa Prefecture, may be a better way to reduce nitrogen losses.

The rooting system of rice plants plays an important role in maximizing crop yields in lowland production systems. Under intensified agriculture, the rhizosphere volume available for root growth and proliferation is limited because of the high plant population rates. Therefore, management of roots is as important as that of aboveground plant parts, whereas it is well known that root thickness in rice and the rooting pattern (spreading angle) are genetically controlled (Kujira et al 1996), but root weight and total root weight and total length (density/unit area) are influenced by soil, water, and fertilizer management.

Hohohono-ho has good grain quality and a deeper root length than other Japanese cultivars (Kujira et al 1999). This may be an adapted cultivar for direct-seeding cultivation. In addition, no-tillage is a better way to control methane emission. We have observed that application of gypsum in the no-tillage paddy field with POCF

**Table 4. Stratification of root dry weight in the soil layer.**

| Plot | Stratification of root dry weight | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Interhill | | | | Interrow | | | |
| | TRDW[a] | 0–10 cm (mg) | 10–40 cm | Under 10 cm (%) | TRDW | 0–10 cm (mg) | 10–40 cm | Under 10 cm (%) |
| 1 | 61.5 | 25.0 | 36.5 | 59.5 | 63.0 | 27.0 | 36.0 | 57.1 |
| 2 | 137.0 | 98.0 | 39.0 | 28.5 | 102.5 | 42.0 | 60.5 | 59.0 |
| 6 | 151.5 | 102.5 | 49.0 | 32.3 | 54.5 | 34.0 | 20.5 | 37.6 |
| 7 | 60.5 | 39.0 | 21.5 | 35.5 | 147.0 | 100.0 | 47.0 | 32.0 |
| 8 | 103.5 | 65.0 | 38.5 | 37.2 | 66.0 | 53.5 | 12.5 | 18.9 |
| 10 | 118.0 | 91.0 | 27.0 | 22.9 | 130.5 | 100.0 | 25.5 | 19.5 |
| LSD (*P* = 0.05) | 18.9 | 15.6 | 10.0 | 9.9 | 21.1 | 17.8 | 11.6 | 11.6 |

[a]TRDW = total root dry weight per plant. n = 2 (sampling date 6 Aug. 1997). LSD = least significant difference.

reduced methane emission by 35% (for both compost and POCF application) to 78% (POCF application) compared with the control (conventional tillage) (Kujira et al 1996). Methane reduction because of $CaSO_4$ appeared to be greatest during the mid growing season in rice (Lindau et al 1994). For our research, we conclude that no-tillage transplanting cultivation of Hohohono-ho may be a better way to reduce nitrogen losses, but additional chemical fertilizers need to be supplied to increase both unhulled rice grains $m^{-2}$ and 1,000-grain weight to have a stable high yield.

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# Development and use of a crop management database to evaluate rice crop performance in New South Wales, Australia

J. Lacy, W. Clampett, and J. Nagy

In the 1980s, New South Wales (NSW) Agriculture developed the objective crop management and collaborative learning extension program *Ricecheck* aimed at improving yields and grain quality. *Ricecheck* provided objective recommendations and learning tools to rice growers. An important learning tool of *Ricecheck* is the use of crop records. These records were used to develop and validate the *Ricecheck* recommendations, to measure the level of on-farm adoption, and to provide reports to individual rice growers and the industry on crop performance. However, the system of data collection and method of analysis were a barrier to further progress.

Concurrently, the rice near-infrared (NIR) tissue test (TT), which assessed responses to topdressed nitrogen fertilizer at panicle initiation (PI), provided the basis for building a system of collecting, recording, processing, analyzing, and reporting data from a large range of crops across all rice-growing regions. The TT recorded a wide range of management and crop growth data from sowing to PI on a computer database.

To enhance data collection and crop evaluation, a new system was developed incorporating the approach of the old system and the rice NIR TT database. The additional requirement was to collect crop data from PI to harvest, and develop an efficient system for entering, analyzing, and reporting results. This would improve *Ricecheck* and enable more crops to be evaluated. This chapter describes this development and the results achieved.

*Ricecheck,* the crop management and collaborative learning system based on crop checking, has been used in the New South Wales (NSW) rice industry since 1986 (Lacy and Clampett 1998a,b). It aims to improve yields and grain quality. The *Ricecheck* program provides a package of seven key recommendations (or checks) targeting high yields. Another check targets improved quality. Farmers strive to implement the seven Key Checks to increase yields. As part of the learning process, farmers are encouraged to record their management of the checks by completing crop record cards.

The benefit of recording crop data is that crop growth and management can be related to yield and grain quality. These data can then be analyzed to compare management practices among farmer crops and indicate how high yields were achieved. The records allow farmer management practices to be compared with the *Ricecheck* recommendations and to determine the adoption of the recommendations. Another benefit of records is that poorly adopted recommendations or checks can be quickly identified, thus providing timely signals to extension, research, and the rice industry as a whole on problems requiring further investigation.

The record card data were previously entered onto a spreadsheet. Analysis of data for individual crops and varieties was carried out and reports prepared and delivered to cooperating farmers. A major barrier to increasing the numbers of crop records, however, was that the collection of data was slow and entry of data onto a spreadsheet and the analysis were labor-intensive, while the preparation of individual farmer reports was carried out manually (Lacy and Clampett 1998a). It was also difficult to generate a large and representative number of complete record books from all rice-growing districts. These difficulties highlighted the need to improve the system of data collection and report generation. The key was to link with the rice near-infrared tissue test (NIR TT).

The rice NIR TT is used to provide recommendations on nitrogen requirements at the panicle initiation (PI) stage. As part of the test, farmers supply information on sowing and establishment up to PI, which is recorded on a rice NIR TT data sheet. Approximately 1,500 crops are tested each year. The rice NIR TT data sheet presented a great opportunity to simplify the data collection method and increase crop numbers by modifying this data sheet to incorporate all the records needed for *Ricecheck* up to panicle initiation.

## Development of the database: stage 1

In 1993-94, a new format using the 1993-94 rice NIR TT as the basic building block began as follows:

1. Review and modification of the rice NIR TT crop data sheet to record sowing to PI data to ensure that it was compatible with the *Ricecheck* approach and record the essential data for both the tissue test and *Ricecheck* crop evaluation.
2. Preparation of a new data sheet—"crop evaluation data sheet 2: PI to postharvest"—to record the data from PI topdressing through flowering to harvest and delivery. This form was sent to each participating rice grower with the rice TT recommendations for topdressing.
3. After harvest, the data sheet 2 crop information was entered onto the computer dBase IV software, which included all information provided by rice growers on the rice NIR TT crop data sheet.
4. Once all the crop data were entered in the database, the data were summarized in the *Ricecheck* key check format and related to yield performance.

5. An individual crop evaluation report was prepared for each crop. This report outlined the achievement of the crop in each of the key check recommendations and compared the crop's performance with that of other high- or low-yielding crops.
6. Summary reports based on variety, district, and/or industry crop performance were prepared and, along with the crop evaluation report, mailed to each participating rice grower.
7. Further analysis of the database was undertaken to investigate the effects of other factors and interactions with yield. In this way, check adoption trends, problems, and achievements could be identified and targeted for further action.

## Development of the database: stage 2

After experience with the database developed in the 1994-95 and 1995-96 seasons, it became apparent that the database had several limitations. The entry of the PI to harvest data was very slow. The software program dBase III plus on DOS was in itself a slow system. Each year, the database template had to be constructed to accommodate the PI to harvest data.

In 1996, a better and faster database was employed. dBase was updated for Windows, with the ability to use the same template each year so that new data could be added automatically. A significant time saving was gained from the new program as it was able to enter and print individual crop performance onto the *Ricecheck* crop evaluation reports.

The new program had the ability to produce five types of reports:

1. The "statistics on high- and low-yielding crops," which show the performance for each check and other selected checks on the basis of selected percentage groupings, such as top 10% yields and lowest 10% yields.
2. The "*Ricecheck* adoption statistics" showing the results for each check and farmer adoption of each check variety and each growing area.
3. A "list of crops" in order of decreasing yield for each variety showing farmer names and check results for all records of each valley or district.
4. The "analysis of interactions," which compares any one of 60 management factors with yield graphically and numerically.
5. The "*Ricecheck* crop evaluation report," which shows individual crop results for each check, indications of the achievement of the check, a comparison with other farmers with low and high yields, and the overall farmer adoption of the check.

**Table 1. Number of crops evaluated.**

| Year | Number of crops evaluated |
|---|---|
| 1993-94 | 672 |
| 1994-95 | 752 |
| 1995-96 | 668 |
| 1996-97 | 569 |
| 1997-98 | 587[a] |
| Total | 3,248 |

[a] Very early panicle initiation prevented many farmers from near-infrared testing and hence the opportunity to evaluate crops.

## Results and use of the database

The results from the database can be divided into yield evaluation, adoption of the check recommendations, crop evaluation, and research and extension issues. Table 1 shows the excellent farmer participation in the evaluation of crops from the 1993-94 to 1997-98 rice-growing seasons.

### Yield evaluation

*Key checks and yield. Ricecheck* is based on the principle that, as the adoption of the seven Key Checks or recommendations increases, yields increase. Figure 1 shows the relation between the number of Key Checks achieved and the yield of the main medium-grain variety Amaroo from 1994 to 1998 based on 1,396 crop records where all seven Key Checks were recorded. The results confirm that adopting more checks results in higher yield.

*Top and average yields.* The database option "statistics on high- and low-yielding crops" shows the check performance for high, average, and low yields for any variety. This is important for the development of the *Ricecheck* recommendations. The check performance for high yields can be compared to the existing recommendations. This provides the opportunity to modify existing check recommendations to improve management and targeting of higher yields.

Check data comparing average Amaroo variety yields with the top 10% yields are shown in Table 2 for the 1994 to 1998 harvests. The results show that the top-yielding crops have better achievement of each of the seven key check recommendations. The top crops were sown a little earlier, achieved better plant numbers with fewer weak areas, and had better weed control and higher nitrogen topdressing rates.

*Single-check yield comparisons.* The database option "analysis of interactions" has the ability to compare any one of 60 major factors with yield and produce graphs of the results. Figure 2 is an example of one of the graphs comparing yield with sowing date for the Murrumbidgee Irrigation Area (MIA). The use of graphs at farmer meetings is an excellent tool for promoting discussion and farmer learning and motivating farmers to improve practices.

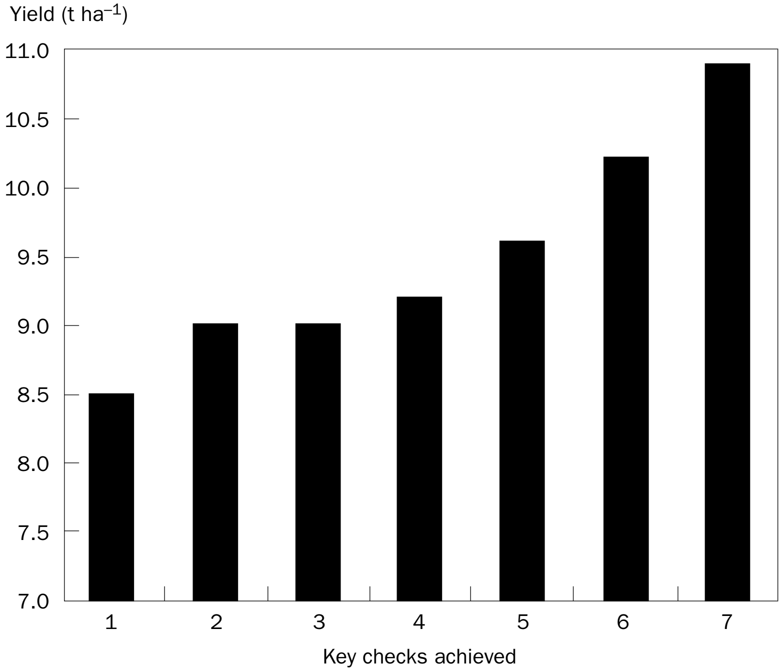

**Fig. 1. Amaroo yield response to checks adopted 1994-98.**

## Adoption of the checks

One of the aims of *Ricecheck* is to improve the adoption of the Key Checks since the higher the adoption, the higher the yields. Figure 2 shows adoption trends for variety Amaroo from 1994 to 1998. Overall adoption of the checks is good, with the exception of nitrogen uptake at PI and early pollen microspore (EPM) water depth checks. On an individual year basis, the 1998 harvest year had the best adoption for five of the checks.

The checks for which adoption has improved over the past 5 years are bank height, establishment plant number, and recommended nitrogen topdressing rate. Bank heights have increased as farmers realize the importance of deep water at the EPM stage.

Sowing rates have increased significantly to help improve establishment. More farmers are using the recommendations from the rice NIR TT or from the "manage rice" program to improve decisions for nitrogen topdressing.

Adopting of the nitrogen uptake at PI check, though poor, has improved slightly. Sowing date has remained static. Late announcements of water allocations often prevent farmers from sowing in the recommended check window. The adoption of good weed control had been falling until 1998. This is surprising given the increasing problems with resistance to bensulfuron methyl. However, the temperatures for crop establishment and weed control were excellent in 1997-98 and control was assisted by

**Table 2. Average and top 10% yielding crops for 1994 to 1998. NIR = near-infrared, PI = panicle initiation, EPM = early pollen microspore.**

| Check | 1994 | | 1995 | | 1996 | | 1997 | | 1998 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Av | Top 10% | Av | Top 10% | Av | Top 10% | Av | Top 10% | Av | Top 10% |
| Yield (t $ha^{-1}$) | 9.5 | 11.5 | 9.9 | 12.1 | 7.0 | 9.7 | 9.4 | 11.4 | 10.1 | 12.8 |
| Sowing date | 13 Oct | 12 Oct | 12 Oct | 10 Oct | 12 Oct | 8 Oct | 13 Oct | 12 Oct | 12 Oct | 10 Oct |
| Bank height (cm) | 40 | 42 | 42 | 46 | 43 | 45 | 43 | 41 | 47 | 51 |
| % Lasered[a] | – | – | – | – | – | – | 49 | 50 | 63 | 95 |
| Plant numbers $m^{-2}$ | 163 | 171 | 177 | 183 | 172 | 172 | 167 | 180 | 192 | 202 |
| Weak areas (%) | 7 | 4 | 5 | 2 | 4 | 3 | 6 | 4 | 1 | 1 |
| % Good weed control | 74 | 86 | 76 | 91 | 70 | 69 | 66 | 83 | 91 | 95 |
| Fresh weight (g $m^{-2}$) | 2,676 | 2,839 | 3,327 | 3,422 | 3,225 | 3,020 | 2,996 | 3,245 | 3,549 | 3,199 |
| NIR % N | 1.3 | 1.4 | 1.7 | 1.5 | 1.9 | 1.7 | 1.3 | 1.4 | 1.5 | 1.6 |
| Nitrogen uptake (kg N $ha^{-1}$) | 81 | 91 | 132 | 121 | 114 | 118 | 90 | 102 | 122 | 118 |
| PI (kg N $ha^{-1}$) | 61 | 59 | 37 | 57 | 33 | 43 | 50 | 45 | 40 | 56 |
| EPM water depth (cm) | 20 | 20 | 20 | 21 | 20 | 21 | 19 | 20 | 19 | 18 |
| % Paddocks correct | 64 | 68 | 62 | 77 | 63 | 77 | 65 | 72 | 51 | 33 |
| % Checks achieved | 41 | 49 | 60 | 70 | 60 | 67 | 57 | 66 | 68 | 66 |

[a] Only 2 years of data.

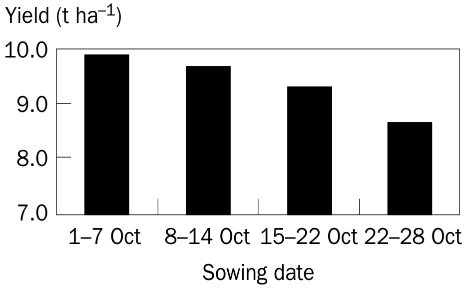

**Fig. 2. Effect of sowing date on yield for the Murrumbidgee Irrigation Area, 1994-98.**

the increased use of herbicide-resistance control options. The adoption of deep water at the cold-sensitive EPM stage stayed static until a fall in 1998. This was the result of two factors. High temperatures at the PI to EPM stage prevented farmers from raising water levels and uncertain water allocations, particularly in the Murray Valley, led to many farmers running much lower water levels than normal.

## Evaluation of farmer crops

*Ricecheck* is a collaborative learning system based on crop checking that is aimed at improving farmer yields. An important aspect of adult learning is participation and feedback. As part of the learning process, farmers like to compare themselves with others. The *Ricecheck* crop evaluation report provides feedback to each participating farmer as to how his crop compared with the *Ricecheck* Key Checks and with other farmers. The reports are produced by the computer program for each growing district, for each variety, and for each farmer crop. The reports are sent to farmers after harvest and after entry of all data sheet records and yield analysis. Table 3 shows an example of a *Ricecheck* crop evaluation report for 1998 for one crop from the Western Murray Valley for the medium-grain variety Namaga. In the report, the crop data are compared with the achievement of the Key Checks, the lowest 25% yields, and the highest 25% yields. The Table 3 example shows that the crop achieved four of the seven Key Checks.

## Issues for extension and research

One of the great benefits of the database is that weaknesses in management at a farmer or industry level can be clearly identified and hence targeted by research and extension providers. The results show that adoption of the Key Checks averages 60% to 70%. This is creditable, but shows that room for improvement exists.

Evaluation of the adoption of individual checks shows poor adoption of the nitrogen uptake at PI check and only 50% to 60% adoption of the EPM water depth check. These are both potential barriers to increasing yields. A recent survey of EPM water depths carried out in the 1998-99 season supports the *Ricecheck* results. There is a need to investigate the barriers to the adoption of both checks.

**Table 3. Ricecheck Crop Evaluation Report 1998-MVW.**
Report from the R&D Project "Performance Evaluation of Commercial Rice Crops"

| | | |
|---|---|---|
| GROWER NAME: | | |
| Address: | | Farm Number: |
| Variety: NAMAGA | Field Name: | RCL Sample Number: |

NB: The results in this Report are based on information provided by rice growers. The accuracy is that of the data provided.

| Ricecheck MANAGEMENT AREA | YOUR RESULTS | | Results for lowest 25% yield | Results for highest 25% yield | % of all growers achieving Key Checks |
|---|---|---|---|---|---|
| | CROP DATA | Achievement of each Key Check | | | |
| YIELD TARGET<br>Minimum of 10 tonne/hectare | Yield<br>9.9 t $ha^{-1}$ | NO | 8.4 t $ha^{-1}$ | 11.4 t $ha^{-1}$ | 57 |
| FIELD LAYOUT<br>Key Check 1—well-constructed banks of a minimum height of 40 cm | Bank height<br>42 cm | YES | 48 cm | 37 cm | 64 |
| SOWING TIME<br>Key Check 2—aim to sow between 1 and 15 October | Sowing date<br>4 Oct 1997 | YES | 15 Oct | 7 Oct | 59 |
| CROP ESTABLISHMENT<br>Key Check 3—aim to establish 150–300 plants $m^{-2}$ | Seedling number<br>113 $m^{-2}$ | NO | 144 | 250 | 75 |
| Weak area with less than 5% weak areas | 0% | YES | 1% | 0% | 100 |

**Table 3. continued**

Report from the R&D Project "Performance Evaluation of Commercial Rice Crops"

| Ricecheck MANAGEMENT AREA | YOUR RESULTS | | Results for lowest 25% yield | Results for highest 25% yield | % of all growers achieving Key Checks |
|---|---|---|---|---|---|
| | CROP DATA | Achievement of each Key Check | | | |
| CROP PROTECTION<br>Key Check 4—prevents economic yield loss from weeds | Good weed control<br>NIL | YES | 100% | 100% | 95 |
| CROP NUTRITION<br>Key Check 5—preflood nitrogen: apply sufficient nitrogen to achieve 2,500–3,700 g m$^{-2}$ fresh weight and 1.4–2.0% N to give 90–130 kg N ha$^{-1}$ uptake at PI | Fresh weight<br>3,511 g m$^{-2}$<br>NIR | YES | 3,267 g m$^{-2}$ | 3,592 g m$^{-2}$ | 52 |
| | 2.01% N | NO | 1.9% N | 2.0% N | 57 |
| | N Uptake<br>161 kg N ha$^{-1}$ | NO | 126 kg N ha$^{-1}$ | 158 kg N ha$^{-1}$ | 44 |
| Key Check 6—PI nitrogen: at PI, topdress with nitrogen according to the NIR tissue test recommendation | Deviation from NIR test recommendation<br>0 kg N ha$^{-1}$ | YES | –12 kg N ha$^{-1}$ | 24 kg N ha$^{-1}$ | 50 |
| WATER MANAGEMENT<br>Key Check 7—achieve a water depth of 20–25 cm during early pollen microspore | EPM water depth<br>30 cm | NO | 20 cm | 17 cm | 56 |

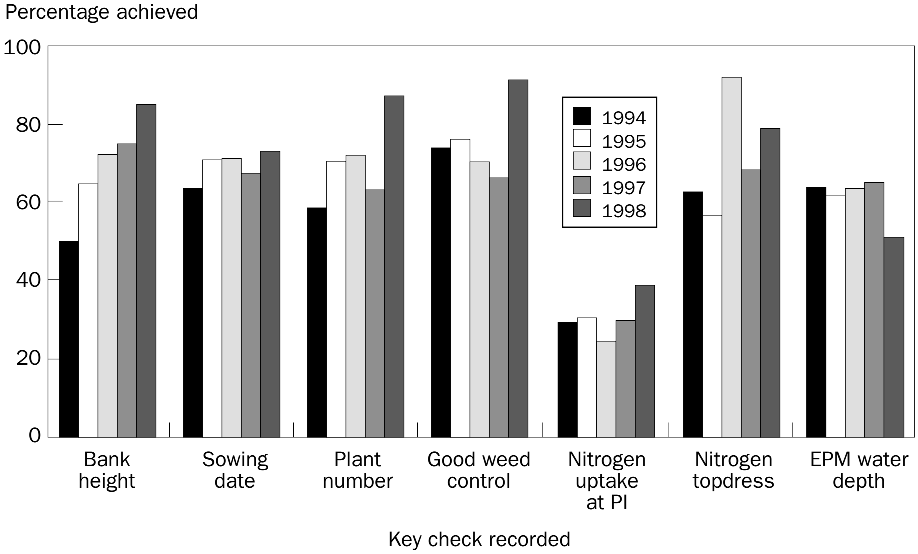

**Fig. 3. Adoption of key checks, 1994-98. PI = panicle initiation, EPM = early pollen microspore.**

The Table 2 comparison of average yields and top 10% yields showed that for most checks the top crops are performing just a little better for each check. Thus, a small lift in performance by average-yielding farmers may make a big difference in yields. Extension programs need to highlight this.

The database can provide excellent survey information on crop management. Trends in crop rotations, establishment, fertilizer rates and products, and water use are useful for identifying potential problems.

## Future development

The *Ricecheck* database is already providing benefits to growers and industry with feedback on improving yields. The database also includes several very high yielding (12 t ha$^{-1}$ and more) crops; this provides opportunities for further analysis and further checks and potential for retargeting higher *Ricecheck* yields. Another issue is improved grain quality. The data sheets already collect crop management records relating to quality. The missing link is the grain quality data for each crop. It is planned to add crop grain quality to the database, which will allow feedback and crop comparisons on grain quality and provide linkage to crop management. Quality assurance is becoming a significant issue relating to food safety and market access. The data sheets could be broadened to provide the records needed for quality assurance as a convenient alternative to separate vendor declaration forms. A "one-stop-shop" data sheet and database linking productivity, grain quality, and quality assurance records for farmer and industry use are the development planned in the next two years.

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AGRONOMY

# Relationship between root amount and sterility caused by cool temperature at the critical stage in rice cultivars differing in cool tolerance

T. Nakamura, Z. Zhang, M. Chiba, Y. Goto, and I. Nishiyama

Cultivar Sasanishiki (moderately susceptible to cool temperature) and cultivar Hitomebore (very tolerant of cool temperature) were grown under water culture conditions at three levels of nitrogen. Root/shoot dry matter ratio and root dry matter/spikelet number ratio were lower at the higher levels of nitrogen. Sterility caused by cool temperature at the critical stage was higher at the higher levels of nitrogen. As there was a negative correlation between sterility and root/shoot ratio or root/spikelet ratio within a single cultivar, the relative amount of root to shoot or spikelet is considered to be an important factor for sterility in rice plants. In addition, more tolerant cultivars have a smaller change in sterility in response to the change in root/shoot ratio or root/spikelet number ratio than more susceptible cultivars. There was a high negative correlation between number of ripened pollen grains and sterility. These facts strongly suggest that some physiological factors in roots might be involved in determining sterility by affecting the number of ripened pollen grains in rice plants, and these factors might be different within cultivars.

Increasing nitrogen fertilizer causes a higher percentage of sterility in rice plants when they are exposed to cool weather conditions at the booting (critical) stage (Hayashi et al 1998, Nishimura and Yoshikawa 1936, reviewed by Nishiyama 1985, Sasaki and Wada 1975). Nitrogen generally promotes shoot growth more than root growth, and consequently reduces root/shoot ratio. In addition, many field observations and a few reports (Amano 1984, Yamamoto and Nishimura 1986) have suggested that root amount or activity plays an important role in reducing sterility caused by cool temperature at the critical stage in the rice plant. On the other hand, the relationship between sterility caused by cool temperature at the critical stage and the number of ripened pollen grains was negative (Nishiyama 1982 1983, Hayashi et al 1998). Thus, previous research suggests that roots played an important role in reducing sterility caused by cool temperature at the critical stage by preventing a reduction in the number of ripened pollen grains. Our objective in this study was to elucidate the relationship among root amount and sterility caused by cool temperature and the number of ripened pollen grains.

## Materials and methods

Cultivar Sasanishiki (moderately susceptible to cool temperature) and cultivar Hitomebore (very tolerant of cool temperature) were used. Germinated seeds were transferred to a nylon net floating on tap water that had been adjusted to pH 5.5 and were grown in a greenhouse in the summer of 1998. At the 3rd-leaf stage, one seedling was transplanted to each 500-mL plastic bottle, which was filled with nutrient solution. The basal nutrient solution, except nitrogen, was the same as described in Mae and Ohira (1981) and its strength was increased stepwise as plants developed: one-quarter, one-half, three-quarters, and full strength every 2 wk. In the low-N treatment, nitrogen was increased stepwise: 1.5, 3.0, 4.5, and 6.0 mg $L^{-1}$ every 2 wk. In the medium-N treatment, nitrogen was increased stepwise: 4.5, 9.0, 13.5, and 18.0 mg $L^{-1}$ every 2 wk. In the high-N treatment, nitrogen was increased stepwise: 6.8, 13.5, 27.0, and 54.0 mg $L^{-1}$ every 2 wk. After heading, the strength of all elements was decreased to half of the maximum strength. The nutrient solution was renewed every week. All the tillers were removed when they appeared. The cooling treatment was made at the young microspore stage at 17–12 °C day-night (12–12 h) under natural light for 5 d. The plants for the treatment were selected with the distance between the auricles of the flag leaf and penultimate leaves when the spikelets at the restricted positions within the panicle (that is, the 3rd, 4th, and 5th spikelets on the 1st, 2nd, and 3rd primary branches) were at the young microspore stage when observed by a microscope. When most of the grains within each panicle matured, the panicle was detached from the plant. The number of spikelets per panicle was counted and sterility of the restricted positions was estimated. The number of plants used for this estimation was 8–11 per treatment. At 1 d after heading, the spikelets at restricted positions were fixed with 50% ethanol solution. The longest anther was excised from each spikelet and dissected under iodine-potassium iodide solution. The number of ripened pollen grains stained dark was counted. The number of spikelets used for this estimation was 14–19 for each of the control plots and 24–39 for each of the cool treatments plots. At the onset of cooling, five plants for each treatment were sampled and divided into shoots and roots, dried at 80 °C, and then weighed.

## Results and discussion

Shoot dry matter, root dry matter, and spikelet number were higher at the higher levels of nitrogen (Table 1). Nitrogen generally promotes shoot growth more than root growth, and consequently reduces root/shoot ratio. Similar results were obtained in this experiment, and root/shoot ratio and root/spikelet ratio were lower at the higher levels of nitrogen (Table 1).

Sterility caused by cool temperature at the critical stage was higher at the higher levels of nitrogen (Fig. 1B). There was a negative correlation between sterility and root/shoot ratio and root/spikelet ratio in a single cultivar (Fig. 2). It was reported that sterility caused by cool temperature at the critical stage was lower in plants having a large amount of roots than in plants having a small amount of roots (Amano 1984), and cool temperature tolerance of varieties is closely correlated with the amount of root exudate (bleeding sap), which corresponds to root activity (Yamamoto and Nishimura 1986). These facts and our results strongly suggest that the relative amount

**Table 1. Shoot dry matter, root dry matter, root/shoot ratio, spikelet number, and root/spikelet ratio as affected by nitrogen treatment.**

| Cultivar | N treatment | Shoot dry matter[a] (g plant$^{-1}$) | Root dry matter (g plant$^{-1}$) | Root/shoot ratio (g g$^{-1}$) | Spikelet number (no. panicle$^{-1}$) | Root/spikelet ratio (mg no.$^{-1}$) |
|---|---|---|---|---|---|---|
| Sasanishiki | Low | 1.16 a | 0.28 a | 0.24 c | 65.4 b | 4.22 |
| | Medium | 2.34 c | 0.48 b | 0.21 b | 127.0 d | 3.80 |
| | High | 3.21 d | 0.50 b | 0.15 a | 154.9 f | 3.20 |
| Hitomebore | Low | 1.42 b | 0.36 a | 0.25 c | 55.9 a | 6.41 |
| | Medium | 2.45 c | 0.50 b | 0.20 b | 99.6 c | 5.00 |
| | High | 3.39 d | 0.48 b | 0.14 a | 144.1 e | 3.33 |

[a] Means followed by the same letter within a column are not significantly different at the 5% level according to Duncan's new multiple range test.

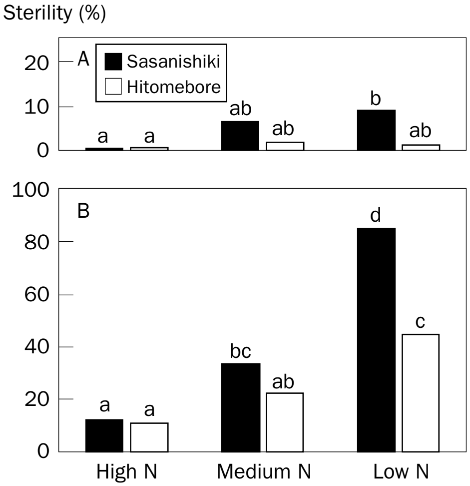

**Fig. 1. Percentage of sterility for cooled (B) or uncooled plant (A) as affected by nitrogen level and cultivar. Columns with the same letter are not significantly different at the 5% level according to Duncan's new multiple range test.**

or activity of root to shoot or spikelet is an important factor for sterility caused by cool temperature at the critical stage in rice plants.

It was observed as long as 300 years ago that a smaller amount of compost application resulted in better fertility under cool weather (Nishimura and Yoshikawa 1936). In addition, it has been known that increasing nitrogen fertilizer causes a higher percentage of sterility both in plants exposed to cold temperatures at the booting (critical) stage (Hayashi et al 1998, reviewed by Nishiyama 1985, Sasaki and Wada 1975) and in unexposed plants (Tatsuta et al 1996). These results coincided with our results in this study (Fig. 1) and it is possible that nitrogen affects sterility directly. But, it was reported that plants with applied compost showed lower sterility and a larger amount of roots than plants not receiving compost, even though the leaf N content was the same (Amano 1984). Therefore, it is considered that nitrogen affects sterility by increasing root/shoot or root/spikelet ratio.

The more resistant cultivars have a smaller change in sterility in response to changes in root/shoot ratio or root/spikelet ratio than the more susceptible cultivars (Fig. 2). Therefore, some physiological factors in roots might be involved in determining sterility in rice plants, and these factors might differ within cultivars.

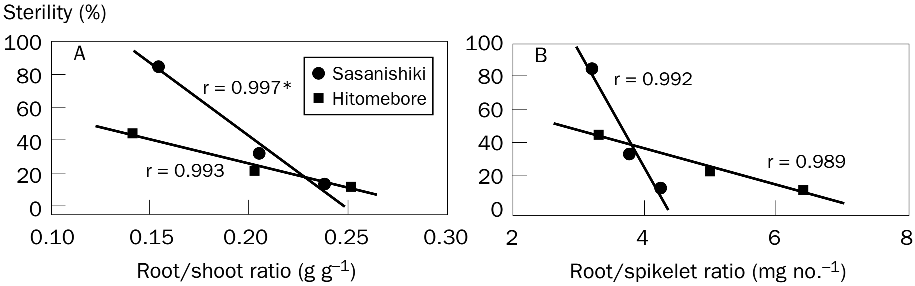

**Fig. 2. Relationship between percentage of sterility for cooled plant and root/shoot ratio (A) or root/spikelet ratio (B). * = significant at the 5% level according to Duncan's new multiple range test.**

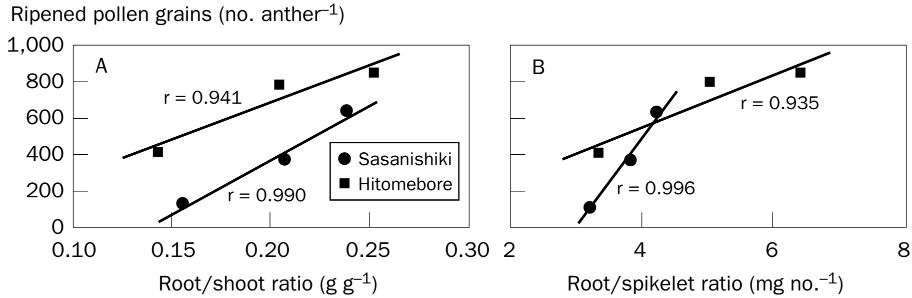

**Fig. 3. Relationship between number of ripened pollen grains for cooled plant and root/shoot ratio (A) or root/spikelet ratio (B).**

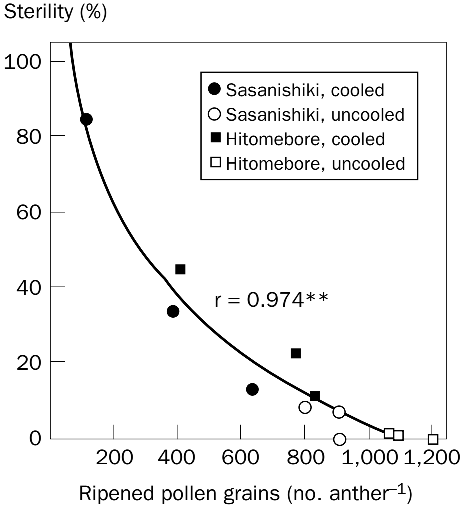

**Fig. 4. Relationship between number of ripened pollen grains and percentage of sterility. ** = significant at the 0.01 probability level.**

High nitrogen promotes sterility induced by cool temperature at the critical stage by reducing the number of ripened pollen grains (Hayashi et al 1998). In this study, there was a high negative correlation between root/shoot or root/spikelet ratio and sterility (Fig. 3), and between the number of ripened pollen grains and sterility (Fig. 4). These suggest strongly that the relative amount of root to shoot or spikelet affects sterility in part by regulating the development of pollen grains. But the mechanism by which roots affect this development and sterility under cool temperature is not known. Some physiological approaches to root activities and the development of pollen grains will expedite research in this field.

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# Sustainable rice production in temperate and subtropical climate areas: issues and opportunities

Van Nguu Nguyen

In 1997, rice was the main source of food calories for about 3 billion people. Rice was produced in 38 countries with temperate and subtropical climate areas. Temperate rice contributed about 9.4% to the world rice output and subtropical rice about 21.8% with average yields at about 64% and 52% higher, respectively, than the world rice yield. These high yields could be attributed to the favorable conditions and the advanced states of rice research and development. However, sustainable rice production in temperate and subtropical areas still requires technological breakthroughs to raise the yield potential of rice varieties, close yield gaps, and improve grain quality and water-use efficiency. It also needs innovations to reduce production costs and minimize environmental degradation caused by rice production. Collaboration among rice institutions and the exchange of information could speed up the development and use of technologies as well as prevent possible irreversible damage from the misuse of technologies. FAO promotes such collaboration.

In 1997, rice was the main source of food calories for about 3 billion people. Based on the 1997 world production of 573 million tons, at least an additional 190 million tons of paddy will be required in 2025 to meet the demand of the growing population. Therefore, deceleration in the growth of rice production after 1990 is a cause for concern for world food security.

In 1997, rice production in temperate and subtropical areas took place in 38 countries (Tables 1 and 2) and the crop was grown mostly under irrigated conditions. The rice varieties planted in temperate areas were mostly japonica (Table 1), whereas japonica and indica varieties were planted in subtropical areas. The aromatic indica varieties (e.g., Basmati and similar types) were dominant in several Asian countries (Table 2). In 1997, the harvested temperate rice area was 8.5 million ha, whereas it was about 21.48 million ha in the subtropics. Temperate rice contributed about 9.4% to the world rice output in 1997 and subtropical rice about 21.8%. The average yields

**Table 1. Climate type, dominant rice type, harvested area, yield, and production of rice in temperate zones in different countries in 1997.**

| Type of climate[a] | Country | Dominant rice type | Harvested area[b] (000 ha) | Yield[b] (t ha$^{-1}$) | Production[b] (000 t) |
|---|---|---|---|---|---|
| Steppe and semiarid | China[c] | Japonica | 822 | 5.4 | 4,448 |
| | Turkmenistan | Japonica | 38 | 1.4 | 55 |
| | Ukraine | Japonica | 24 | 3.6 | 86 |
| Desert and arid | Azerbaijan | Japonica | 2 | 3.5 | 7 |
| | Kazakhstan | Japonica | 85 | 3.0 | 255 |
| | Kyrgyzstan | Japonica | 5 | 2.6 | 13 |
| | Russian Fed. | Japonica | 164 | 2.0 | 328 |
| | Uzbekistan | Japonica | 174 | 1.7 | 294 |
| Continental | China[c] | Japonica | 899 | 7.0 | 6,271 |
| | Japan[c] | Japonica | 1,207 | 6.4 | 7,744 |
| | Korea (Rep. of) | Japonica | 151 | 6.8 | 1,206 |
| | Korea (DPR) | Japonica | 611 | 3.8 | 2,347 |
| | Bulgaria | Japonica | 5 | 1.8 | 9 |
| | Hungary | Japonica | 3 | 2.2 | 7 |
| | Italy[c] | Japonica | 30 | 6.0 | 179 |
| | Romania | Japonica | 4 | 2.7 | 11 |
| Highland | China[c] | Japonica | 4,285 | 6.9 | 29,376 |
| Temperate | | | 8,509 | 6.3 | 53,636 |
| World | | | 149,811 | 3.8 | 573,263 |
| Contribution from temperate to world | | | 5.7% | – | 9.4% |

[a]FAO (1996). [b]FAO (1997). [c]Estimates.

of temperate and subtropical rice were higher than the world rice yield in 1997 by about 64% and 52%, respectively (Tables 1 and 2).

Rice is the staple food in Korea, China, India, and Peru and it is an important food in Japan, Egypt, Iran, southern Brazil, and Iraq. Rice consumption in Australia, North America, and Europe has increased because of diversification in food consumption and immigration. In terms of trade balance, the United States, China, and India are major rice-exporting countries, whereas Brazil, Japan, and Korea are major rice importers.

## Major issues of sustainable rice production

Except in Africa and Latin America, there is little opportunity to expand the area under rice cultivation. Future increases in rice production must therefore come from higher yield per unit area, especially in irrigated rice. Rice yields in Australia, Egypt, and Greece in 1997 were the world's highest. These high yields could be attributed to the favorable growing conditions and the advanced states of rice research and development in these countries. Sustainable rice production, however, still faces several technical and socioeconomic constraints.

**Table 2. Climate type, dominant rice type, harvested area, yield, and production of rice in subtropical zones in different countries in 1997.**

| Climate type[a] | Country/region | Dominant rice type | Harvested area[b] (000 ha) | Yield[b] (t ha$^{-1}$)[b] | Production[b] (000 t) |
|---|---|---|---|---|---|
| Steppe and semiarid | Afghanistan | Aromatic | 170 | 1.8 | 300 |
| | Argentina[c] | Indica | 14 | 5.4 | 75 |
| | India[c] | Aromatic | 2,775 | 4.0 | 11 |
| | Iran | Aromatic | 550 | 4.7 | 2,600 |
| | Iraq | Aromatic | 121 | 2.0 | 244 |
| | Pakistan | Aromatic | 2,316 | 2.8 | 6,546 |
| | South Africa | Japonica | 1 | 2.3 | 3 |
| Desert and arid | Egypt | Japonica | 652 | 8.6 | 5,585 |
| | Morocco | Japonica | 10 | 5.0 | 50 |
| | Peru[c] | Japonica | 14 | 10.4 | 145 |
| Humid | Argentina[c] | Indica | 200 | 5.4 | 1,073 |
| | Brazil[c] | Indica | 1,000 | 5.0 | 5,000 |
| | China[c] | Indica | 12,700 | 6.2 | 78,740 |
| | Japan[c] | Japonica | 754 | 6.4 | 4,837 |
| | Korea (Rep. of)[c] | Japonica | 895 | 6.8 | 6,076 |
| | Paraguay | Indica | 29 | 5.9 | 171 |
| | Uruguay | Indica | 186 | 5.5 | 1,206 |
| | USA (other states)[c] | Japonica | 1,021 | 6.0 | 6,216 |
| Dry summer | Australia | Japonica | 164 | 8.2 | 1,352 |
| | Chile | Japonica | 26 | 4.1 | 107 |
| | France | Japonica | 21 | 5.7 | 120 |
| | Greece | Japonica | 30 | 7.7 | 230 |
| | Italy[c] | Japonica | 203 | 6.0 | 1215 |
| | Macedonia | Japonica | 5 | 4.5 | 22 |
| | Portugal | Japonica | 27 | 5.2 | 140 |
| | Spain | Japonica | 112 | 6.6 | 735 |
| | Turkey | Japonica | 55 | 5.2 | 285 |
| | USA (California)[c] | Japonica | 207 | 9.5 | 1,966 |
| Subtropical | | | 21,485 | 5.8 | 125,050 |
| World | | | 149,811 | 3.8 | 573,263 |
| Contribution from subtropical to world (%) | | | 14.3 | – | 21.8 |

[a]FAO (1996). [b]FAO (1997). [c]Estimates.

## Abiotic constraints

Rice production in temperate and subtropical areas is limited by low temperature. Rice yields in Aomori Prefecture in Japan dropped from about 6 t ha$^{-1}$ to about 3 t ha$^{-1}$ in 1980 because of low summer temperatures (Kushibuchi 1997). In the First Temperate Rice Conference held in February 1994 in Yanco, Australia, low temperature was also reported to be a major constraint to rice production in places such as California (USA), Australia, Italy, and southern Brazil. Inadequate water supply limits the rice area in Australia, Egypt, and Spain. Salinity is also a major constraint to rice production in those three countries. The delta of the Kuban River in Krasnodar, Russian Federation, has about 110,000 ha of saline soils, mostly grown to rice (Zelensky, personal communication). Increased salinity resulting from long-term rice production in irrigated areas has been increasingly observed.

### Biotic constraints

Blast (caused by *Pyricularia oryzae* Cav.), seedling rot caused by *Pythium* spp. and *Achyla klebsiana,* stem rot caused by *Sclerotium oryzae,* and sheath blight caused by *Rhizoctonia oryzae* are important diseases of rice in temperate and subtropical areas. Barnyardgrass (*Echinochloa crus-galli*), the dominant weed of lowland rice, has the capacity to develop resistance to herbicides after their repeated application (Baltazar and Smith 1994). Red rice is a major constraint to rice production in most European countries, the United States, and southern Brazil (Ferrero and Vidotto 1997).

### Socioeconomic constraints

Rice production costs in Europe, the United States, and Japan are high. If subsidies were reduced substantially, rice production in these areas could decline rapidly, unless significant progress is made in developing cost-reducing technologies. The demand for long-grain indica-type rice has increased in Europe and North America, whereas the demand for japonica is minimal outside of temperate and subtropical areas. Concern has increased about the accumulation of pesticides in rice cultivated under a high level of crop intensification. The increased use of pesticides associated with crop intensification in rice production has negative effects on nontargeted flora and fauna. Government environmental and chemical regulations and urban growth also may restrict farmers' use of optimum management practices. In California, the phaseout of straw burning creates management problems for rice growers. Moreover, wetland rice fields and irrigation schemes usually create ecological conditions favorable to the propagation of vectors of human and animal diseases.

### Yield plateau and yield gaps

Rice yields in Australia, California, and Egypt ranged from 8 to 9 t $ha^{-1}$, approaching the yield potentials of most current rice varieties. Varieties with higher yield potentials will be required if yields in these areas are to be increased substantially. On the other hand, Tables 1 and 2 indicate that large yield gaps still exist in some temperate and subtropical rice areas. Rice yields in France, Italy, Portugal, and Spain have stagnated at about 5–6 t $ha^{-1}$ since the 1970s.

### Decline in investments in rice production

Irrigation was one of the main factors of the increased rice production in Asia during the Green Revolution, but investments in irrigation infrastructure in Asia have declined around 60% since the 1960s. Similarly, public expenditures on rice research have been declining in many countries.

## Opportunities for and challenges of sustainable rice production

Apart from low temperatures, other climatic factors in temperate and subtropical areas, especially high solar radiation and long daylength, favor high rice yields. The challenges for rice research in temperate and subtropical areas are to harness these advantages toward increasing productivity and reducing costs. Promising research achievements have been made in this direction.

### Achievements in varietal improvement

In the First Temperate Rice Conference, breeding for cold tolerance was reported as a major objective in many rice programs. Using gamma rays, national agricultural research systems (in collaboration with the breeding program of the FAO/International Atomic Energy Agency) have released 13 cold-tolerant rice varieties for cultivation (Maluszynski et al 1999). Early maturing varieties can be used to avoid cold periods. For example, in China, variety Yuanfengzao has a yield potential of 10 t $ha^{-1}$ but matures about 45 d earlier than its parental variety IR8 (Maluszynski et al 1999). Early maturing varieties can also be planted to reduce the amount of water used in rice production. Rice breeders have not given much priority to breeding for efficient water use in rice. The different responses of rice varieties to sprinkler irrigation obtained from studies carried out in northern Italy suggest the potential of genetic improvement for more efficient water use in temperate rice (Russo 1996). Sprinkler irrigation could reduce water consumption in rice production by about 50% (Stone et al 1994). Recently, hybrid rice has shown an ability to increase the yield potential of rice. Hybrid rice has a 15–20% yield advantage over conventionally bred rice varieties. It has been commercially cultivated in China since 1976 and recently in Vietnam and India. The recently developed two-line hybrid Pei'ai 64S/Teqing yields 13 t $ha^{-1}$ or more. Its highest yield of 17.1 t $ha^{-1}$ was obtained from a 0.1-ha plot in Yongshen, Yunan, China, in 1992 (Yuan 1998). The adoption of hybrid rice technology outside China is limited by high seed prices. However, much improvement has been obtained and the average yield of $F_1$ seed production in China increased from less than 1 t $ha^{-1}$ in the early 1980s to about 2.5 t $ha^{-1}$ now (Tran and Nguyen 1998).

### Biotechnology

A comprehensive review of achievements in rice biotechnology was recently made by Khush et al (1999). Transgenic rice plants with better resistance to insects (*Bt, CryIIA*, and cowpea trypsin inhibitor) and herbicide (especially for resistance to glyphosate) have been developed. Quantitative trait loci (QTL) such as *Yld-1* and *Ydl-2* for yield improvement and genes for starch biosynthesis, for salinity and water stress resistance, and for resistance to *Rhizoctonia solani* have also been identified. There is, however, a need to evaluate the effects of transgenic rice on rice germplasm resources and biodiversity. Also, the effects of private-sector participation in biotechnology on the exchange of rice information and germplasm need to be closely examined to avoid irreversible damage. The free exchange of germplasm in the past has greatly contributed to advances in rice varietal improvement.

### Crop management

Much of the water in basin-flooded irrigated rice production is lost through seepage and percolation. About 40–70% of water use in rice can be reduced without significant yield losses by maintaining a saturated soil or alternate wetting and drying after the flowering stage. In Japan, an automatic water management system has been introduced to reduce labor for on-farm water management and to minimize water losses (Klemn 1999). However, there is a need for a greater understanding of the costs and benefits of these techniques, in terms of both monetary costs and environmental degradation. Much of the applied nitrogen fertilizer is lost to the environment, thus causing high production costs and environmental pollution. With long-term and more intensive cropping, other nutrients may become yield-limiting factors. Advanced technologies using computers and global positioning have made it possible to apply fertilizers in a precise manner according to crop need and soil conditions. These technologies may be too costly, however, for farmers with low incomes in developing countries; they could instead use chlorophyll meters or even the leaf color chart. Integrated management approaches have been developed for pest and nutrient management in rice production. *Ricecheck*, developed by the Australian program, has proved to be effective in increasing rice yields (Lacy 1994).

## FAO's activities in temperate and subtropical rice

A sustainable increase in rice production is a major concern of FAO, which hosts the Secretariat of the International Rice Commission (IRC). The IRC now has 61 member countries. Every four years, the IRC organizes a session to provide an opportunity for member countries to review the emerging issues and recent achievements relating to sustainable rice production and rice-based farming systems as well as orientation to national rice development programs for an adequate response to new challenges. FAO, recognizing the need for coordination of rice research and development in temperate and subtropical areas, established the Interregional Network on Collaborative Rice Research in Mediterranean Climate Areas in 1990 with the active participation of 12 countries. Since 1994, FAO extended support to the Working Group of Hybrid Rice in Latin America and the Caribbean. In 1995, FAO, in collaboration with the International Rice Research Institute and national rice programs, established an International Task Force for Hybrid Rice to promote efforts in developing and using hybrid rice technology.

## Conclusions

Rice research and development in temperate and subtropical areas are more advanced than in tropical areas. However, sustainable rice production in these areas still needs technological breakthroughs to raise the yield potential of rice varieties, close yield gaps, and improve grain quality and water-use efficiency. It also needs innovations to reduce production costs and minimize environmental degradation caused by rice production. Collaboration among rice institutions and the exchange of information could speed up the development and use of technologies for sustainable rice production in these areas as well as prevent possible irreversible damage from the misuse of technologies. FAO promotes such collaboration in view of the prevailing reduction in investment in and support to rice research and development activities.

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# Site-specific nutrient management in intensive rice systems in Asia

C. Witt and A. Dobermann

On average, irrigated rice farmers in Asia achieve only 60% of potential yield. High N losses into the environment occur because fertilizer N application is not fine-tuned to synchronize supply and plant demand or because unbalanced nutrition renders a better use efficiency of N. The supply of nutrients from indigenous sources is highly variable among rice farms. We therefore developed a new site-specific nutrient management (SSNM) technology for intensive irrigated rice systems. The major components are (1) modeling of crop nutrient requirements based on physiological requirements and an economically efficient yield target, (2) field-specific estimation of the indigenous supply of N, P, and K, (3) modeling of recovery efficiencies of N, P, and K, (4) estimation of the P and K balance to sustain soil P and K reserves without depletion, and (5) dynamic adjustment of N application based on monitoring of the plant N status during critical periods of rice growth by using a chlorophyll meter or a leaf color chart as diagnostic tools. In this chapter, we describe the steps involved in calculating fertilizer recommendations and report on initial results from field testing. The SSNM concept is currently being tested on more than 200 rice farms in China, India, Indonesia, Thailand, Vietnam, and the Philippines.

Farm- or field-specific management strategies that include site- or season-specific knowledge of crop nutrient requirements and indigenous nutrient supply will probably be required to achieve average yields of 8.0 t ha$^{-1}$ in irrigated systems of South and Southeast Asia in the near future (Hossain and Fischer 1995). Thus, more information-intensive fertilization strategies are needed to better fit fertilizer inputs to the seasonal pattern of crop nutrient demand and soil nutrient supply. The site-specific nutrient management (SSNM) approach discussed in this chapter allows us to work out fertilizer recommendations on a field-specific basis to account for the variability in indigenous nutrient supply. The general approach has been described by Dobermann and White (1999). This chapter therefore aims at summarizing (1) recent developments in the SSNM approach including more specific information on the calculation of fertilizer requirements and (2) initial results one year after introducing SSNM in 206 farmers' fields in Asia.

## Components of the site-specific nutrient management approach

The core of the site-specific nutrient management approach is formed by the model QUEFTS (QUantitative Evaluation of the Fertility of Tropical Soils) developed by Janssen et al (1990). In short, the model describes the relationship between (1) macronutrients in aboveground plant dry matter (DM) at physiological maturity and potential nutrient supplies from indigenous and fertilizer sources and (2) grain yield and plant nutrient accumulation acknowledging interactions among the three macronutrients. The model has been used to calculate grain yield as a function of N, P, and K availability, to evaluate crop response to fertilizers, and to estimate fertilizer input requirements for achieving a yield target (Janssen et al 1990, Janssen and Guiking 1990, Smaling and Janssen 1993). Although this work has been done in tropical maize, the model is also applicable to other crops once the basic relations between grain yield and nutrient supply are known.

### Indigenous nutrient supplies

A crucial step toward the calculation of site-specific fertilizer requirements is the estimation of the indigenous nutrient supplies, which we define as the cumulative amount of a nutrient originating from all indigenous sources (i.e., soil, water, and atmosphere) that circulates through the soil solution surrounding the entire root system throughout one entire crop cycle. Several approaches to predict indigenous nutrient supplies are currently being tested in our on-farm research, among which the estimation from nutrient uptake or grain yield measured in on-farm nutrient omission plots (i.e., a plot without fertilizer application of the respective macronutrient) appears to be most promising (Dobermann and White 1999). Plant-based measurements have the advantage of integrating all sources of variation such as crop establishment method, seasonal differences (climate), and cultivar differences (rooting patterns, nutrient uptake, nutrient-use efficiency). Measurements are ideally taken in a season with favorable climatic conditions and low pest pressure to minimize yield-limiting factors other than nutrient supply.

### Crop nutrient requirements

The QUEFTS model provides a generic approach for estimating crop nutrient requirements for a specified yield target taking the climate-adjusted, season-specific yield potential into account. This approach is likely to be more accurate and flexible than basing nutrient requirements on average values of internal nutrient efficiencies (IE, kg grain per kg nutrient in aboveground plant dry matter) obtained from on-station or on-farm measurements. Internal efficiencies vary greatly depending on variety, nutrient supply, crop management, and climatic conditions so that average IEs as "rules of thumb" for calculating fertilizer recommendations are of limited value.

The calibration of the QUEFTS model for irrigated rice required the estimation of two borderlines describing the possible minimum and maximum IEs (Fig. 1A-C). These were estimated at 42 and 96 kg grain kg$^{-1}$ N, 206 and 622 kg grain kg$^{-1}$ P, and 36 and 115 kg grain kg$^{-1}$ K, respectively. These IEs or slopes of the borderlines were derived by exploiting a large database on grain yield and plant nutrient accumulation in aboveground plant dry matter (DM) at the physiological maturity of rice. Full details are given by Witt et al (1999). The model predicted a linear increase in grain yield if nutrients are taken up in balanced amounts of 14.7 kg N, 2.6 kg P, and 14.5 kg K per 1,000 kg of grain until yield targets reached about 70–80% of the climate-

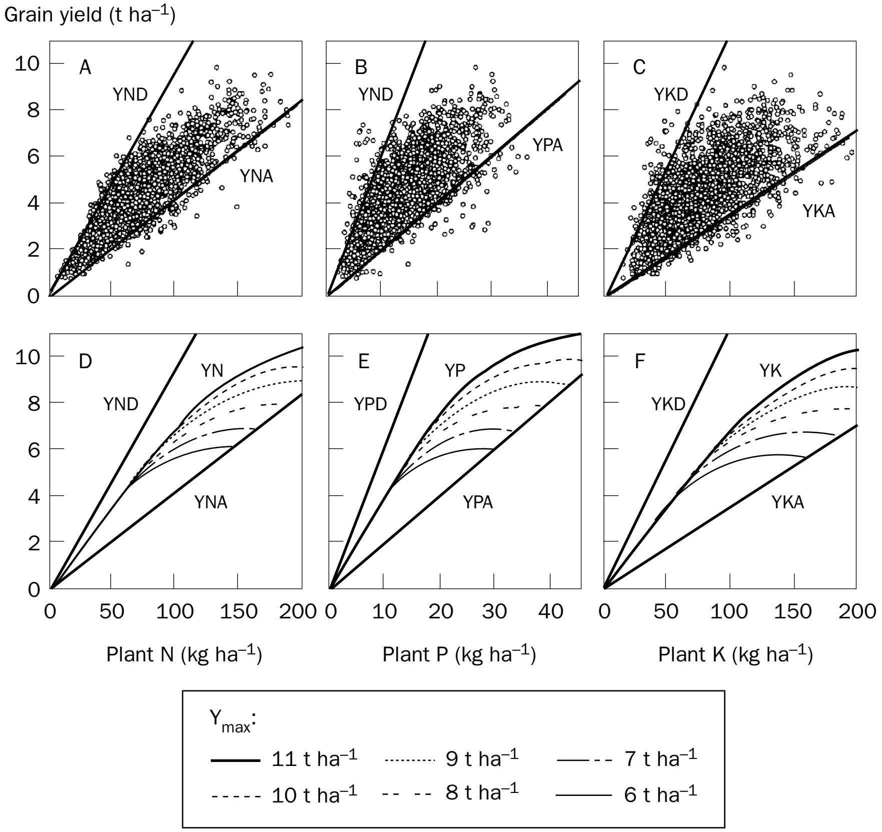

**Fig. 1. Relationship between grain yield and accumulation of N, P, and K in total aboveground dry matter at maturity of irrigated rice. Data in Figure 1A-C were obtained at experimental stations and from farmers' fields in six tropical Asian countries from 1992 to 1997 (n ≈ 2,200). The regression lines to the left of each figure represent the boundary of maximum nutrient dilution (YND, YPD, and YKD), whereas the lines to the right indicate the boundary of maximum accumulation (YNA, YPA, and YKA). Slopes of the boundary lines were calculated by excluding the upper and lower 2.5 percentiles of all internal nutrient efficiency data. For constants, see text. In Figure 1D-F, YN, YP, and YK represent the balanced uptake requirements of N, P, and K to achieve a certain rice grain yield target for the given boundaries as predicted by QUEFTS for yield potentials ranging from 6 to 11 t ha$^{-1}$ (Y$_{max}$).**

adjusted potential yield ($Y_{max}$, Fig. 1). The corresponding optimal IEs were 68 kg grain $kg^{-1}$ N, 385 kg grain $kg^{-1}$ P, and 69 kg grain $kg^{-1}$ K for a balanced nutrition. The optimal N:P:K ratio in plant DM as recommended by QUEFTS was about 5.7:1:5.6. The model predicted a decrease in IEs when yield targets approached $Y_{max}$. The optimal IEs as predicted by QUEFTS were greater than those estimates reported in the literature (e.g., van Duivenbooden et al 1996) or measured in many farmers' fields (Witt et al 1999). Low IEs in the farmers' practice were related to nutritional imbalances, inadequate irrigation, or problems with pests.

Treating the relationship of grain yield versus nutrient uptake in a linear to nonlinear fashion, the presented approach using QUEFTS also provides a useful tool for identifying nutritionally optimal yield targets. The derived borderlines are valid for current modern high-yielding indica cultivars with a harvest index of ca. 0.50 kg $kg^{-1}$ and can be used for all methods of crop establishment.

### Recovery fractions of applied fertilizer nutrients

In the current SSNM approach, field-specific fertilizer N, P, and K recommendations are calculated based on (1) the plant nutrient demand to support a grain yield target, (2) an estimate of the indigenous nutrient supplies, and (3) the expected fertilizer recovery efficiency (RE, kg fertilizer nutrient taken up per kg applied) by the plant. Recovery efficiencies are estimated in farmers' fields by the difference method: the increase in nutrient uptake due to fertilizer application comparing a fertilized plot and an unfertilized omission plot of the respective nutrient. For practical decision making, we currently use standard average REs of 0.45–0.55 kg $kg^{-1}$ for N, 0.20–0.25 kg $kg^{-1}$ for P, and 0.40–0.50 kg $kg^{-1}$ for K.

The RE of a nutrient varies depending on the indigenous supply, the amount of fertilizer nutrient applied, and the plant uptake or sink potential, which in turn depends on the availability of other nutrients and the climate-adjusted yield potential of a particular cultivar. Therefore, REs vary widely among farmers' fields and in the same field with time depending on differences in general soil properties, cropping history, current crop management, and climatic conditions. Additional variation may be introduced by problems with water supply, weeds, and pests. Fertilizer REs in irrigated lowland rice in Asia currently range from 0.31 to 0.56 kg $kg^{-1}$ for N, from 0.09 to 0.34 kg $kg^{-1}$ for P, and from 0.14 to 0.67 kg $kg^{-1}$ for K (interquartile ranges, i.e., 50% of $n \approx 300$ farms × seasons, data not shown).

QUEFTS indirectly offers the possibility to model REs of applied fertilizer nutrients. The modeling concept follows the reasonable assumption that the relationship between nutrient uptake and potential nutrient supply (see Fig. 2) can be expected to follow a curved line with a transition from source to sink limitation of nutrient uptake. The current model, however, uses the less flexible concept of maximum fertilizer RE (Janssen and Guiking 1990). This is due to a parabolic function that was originally chosen to describe the relationship between nutrient uptake and indigenous nutrient supply under the assumption that the indigenous supply equals the nutrient uptake in the respective nutrient omission plot ("original QUEFTS," Fig. 2). Extending the same concept to a system with fertilizer application, however, re-

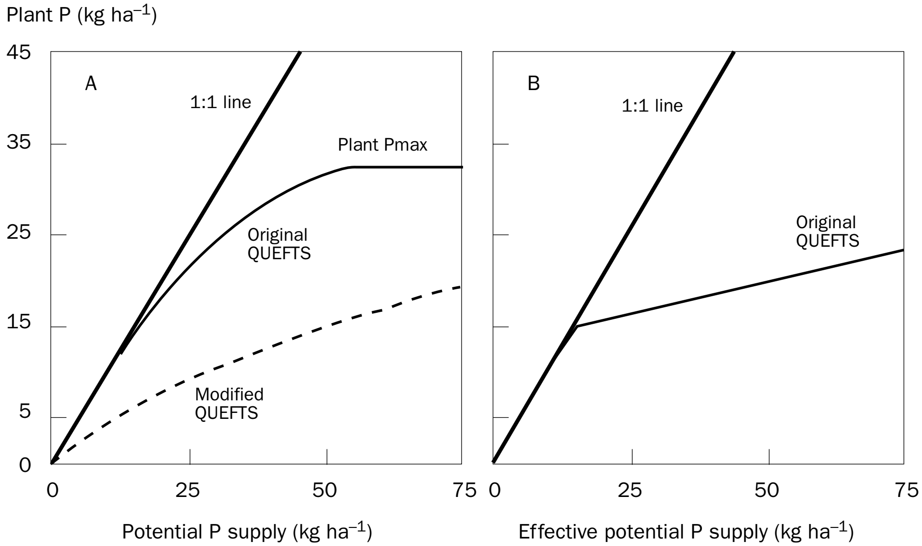

**Fig. 2. The relationship between plant P accumulation in aboveground plant dry matter at physiological maturity of rice and the potential P supply, that is, the sum of supplies from indigenous and fertilizer sources. "Effective" potential supply refers to indigenous supplies and the estimated fraction of applied fertilizer-P recovered by the plant. "Original QUEFTS" is based on a parabolic function suggested by Janssen et al (1990) for all three macronutrients, whereas the shape of the function "modified QUEFTS" can be adjusted and calibrated for each nutrient (J. Arah, IRRI, personal communication).**

quired the introduction of "effective" potential supplies (see Fig. 2B). As a consequence, the model suggests an effective uptake from indigenous nutrient sources and a more or less constant uptake from applied fertilizer sources (Fig. 2B). The actual difference in calculating fertilizer requirements between this modeling approach and the calculation described in the beginning of this chapter is negligible. We currently test a function that will allow a more realistic approach to model the relationship between nutrient uptake and nutrient supplies ("modified QUEFTS," Fig. 2A). The advantage of the "modified" QUEFTS is that the shape of the function depicted in Figure 2A is determined not only by the maximum possible nutrient uptake depending on the maximum yield potential and the availability of other macronutrients but also by a constant that could be calibrated for each macronutrient. Hence, REs could take on a much wider range of values than in the original model.

## P and K balance

Residual effects of fertilizer N in rice are small at constant crop management, but maintaining an adequate P and K balance appears to be crucial not only for sustaining present yield levels but also for increasing rice production. In our SSNM approach, we currently adjust the estimate of indigenous nutrient supplies from season to season according to a simple P and K balance taking into account (1) previous fertilizer rates, (2) nutrient removal with grain and straw, and (3) the amount of nutrients in residues remaining in the field after harvest. Although we currently assume the same constant recovery fractions for applied and residual fertilizer nutrients, recovery efficiencies of the latter would become a by-product if the relationship of nutrient uptake and potential indigenous supplies was based on the more flexible function of the modified QUEFTS model.

### Dynamic adjustment of N using diagnostic tools

As the plant demand for P and K is largely driven by yield potential and the availability of N, recovery efficiencies of P and K may be improved through improved N management and a more balanced nutrition of the rice crop. We recommend several management options to fine-tune N management and improve N efficiency in our SSNM approach including improved split application or tools for real-time N management such as the leaf color chart or chlorophyll (SPAD) meter (Peng et al 1996, Balasubramanian et al 1999).

## Initial results from farmers' fields

In 1997, the SSNM approach was implemented in 206 farmers' fields at key sites in South and Southeast Asia in addition to a farmers' fertilizer practice (FFP). On average, grain yields increased in the SSNM treatments compared with the FFP by about 10% (Fig. 3), but yield advantages of up to 20% were recorded on farms with good crop management. The yield increase was mainly related to an increase in nutrient uptake due to a more balanced nutrition (Fig. 4). Average uptake of N, P, and K increased by 20–31% compared with the baseline FFP data or 7–10% compared with the FFP plots sampled in the same year. Although the total amount of applied fertilizer N and P was similar in FFP and SSNM, negative K balances of more than 20 kg K $ha^{-1}$ $crop^{-1}$ in farmers' fields were reversed by SSNM. The more balanced nutrition

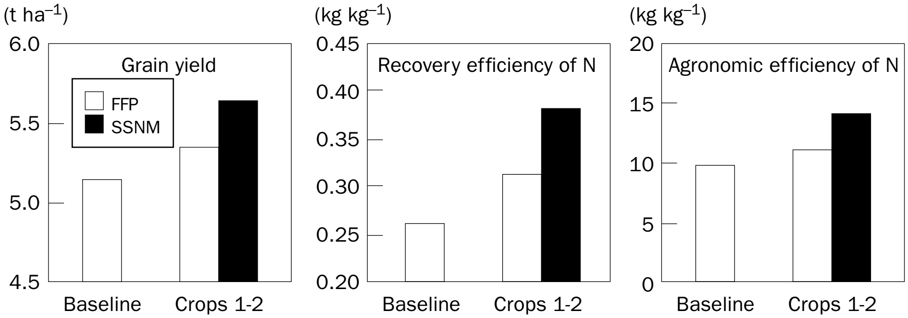

**Fig. 3. Medians of grain yield, attainable NPK-limited yield, and N-use efficiency in the farmers' fertilizer practice (FFP) and the site-specific nutrient management (SSNM) treatments on 206 rice farms in India, Indonesia, China, Vietnam, Thailand, and the Philippines during 1994-97. Data shown include baseline data collected before interventions (average values for two rice crops sampled in 1995-96 or 1997, n = 398 farms × seasons), and the average values for rice crops 1 and 2 after an SSNM treatment was established (1997-98, n = 338 farms × seasons).**

including greater fertilizer-K rates and improved splitting and timing of N applications increased N efficiency of irrigated rice in SSNM compared with FFP (Fig. 3). The recovery efficiency of applied N increased from an initial 0.26 kg kg$^{-1}$ to 0.31 kg kg$^{-1}$ in FFP and 0.38 kg kg$^{-1}$ in SSNM. A similar trend was observed for the agronomic N efficiency, that is, the increase in grain yield per kg fertilizer-N applied (Fig. 3).

A preliminary economic analysis showed that the slight increase in total fertilizer costs with the SSNM is offset by the larger increase in the value of grain produced, and that the increase in grain yield is accompanied by an increase in profit of similar magnitude.

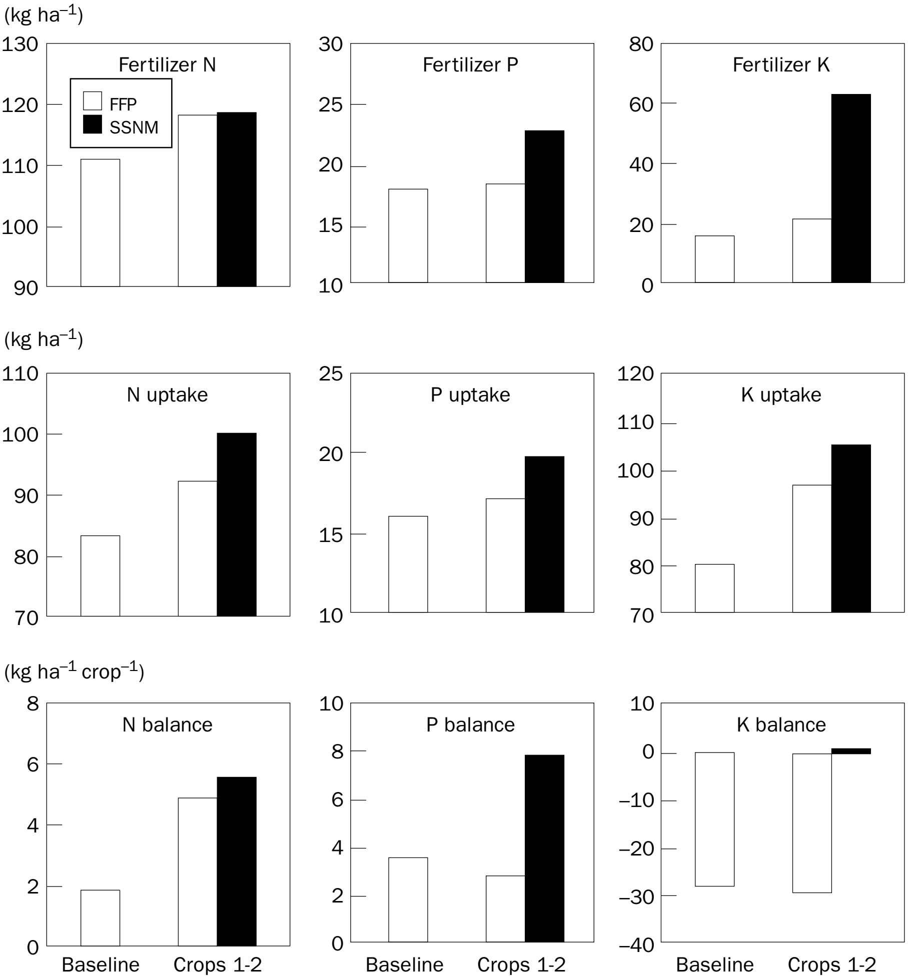

**Fig. 4. Medians of fertilizer use, nutrient uptake, and the estimated input-output balance in the farmers' fertilizer practice (FFP) and the site-specific nutrient management (SSNM) treatments on 206 rice farms in six Asian countries. For further information, see Figure 3.**

## Summary and outlook

Initial results suggested that yield increases of 0.5–1 t ha$^{-1}$ (10–20%) are feasible in Asian irrigated rice systems when following the presented SSNM approach with existing varieties and crop management technologies. The NPK modeling concept is simple, accurate, and flexible enough to be used at the farm level as well as for answering strategic questions on resource management. On the basis of these encouraging results, we are currently developing a nutrient decision support system that will integrate the various data inputs and provide users with cost-effective fertilizer recommendations. After completing a four-year performance evaluation (1997-2000), future work will include determining the optimum size of management domains, developing concepts and tools for extension, and implementing pilot studies for extension at the village scale. The integration of pest management will be a major challenge on the way toward integrated site-specific crop management.

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AGRONOMY

# Rice production in Uruguay: farmer-industry-research integration

G. Zorrilla de San Martín

Rice production has been growing steadily in Uruguay for more than 30 years. A continuous increase in area and yield allowed the country to reach 1 million tons of paddy rice in 1997, and forecasts estimated 1.1 million t for 1999. Rice is now the country's most important crop and it ranks third among export goods, behind beef and wool. Rice has become a key product in the Uruguayan economy.

With more than 90% of its production exported, the Uruguayan rice sector has had to develop in a mostly closed world rice market, with many commercial barriers and highly subsidized surpluses entering international commerce, which accounts for less than 5% of the world harvest.

In this environment, Uruguayan rice production has thrived without any kind of subsidy or direct government intervention in commercialization. The development of strong farmers' and industry associations with close relationships with research institutions and the government made this possible.

This chapter describes the structure and organization of the rice sector in Uruguay, and the key factors that explain its success.

Uruguay is situated between 30° and 35° S latitude and is bordered by Brazil and Argentina. Its rice production has been growing steadily for more than 30 years. A continuous increase in area and yield allowed the country to reach 1 million tons of paddy rice in 1997, and forecasts estimated 1.1 million t for 1999 (Fig. 1).

Rice production started in 1930 in the Merin Lagoon Basin in the eastern part of the country, and area has been growing continuously since then. In the past 20 years, it expanded to the north and crossed the Brazilian border.

Rice farmers are characterized by large commercial and high-investment operations, with modern machinery and equipment (Table 1). Industry is concentrated in a few big milling companies having the latest milling technologies (Fig. 2).

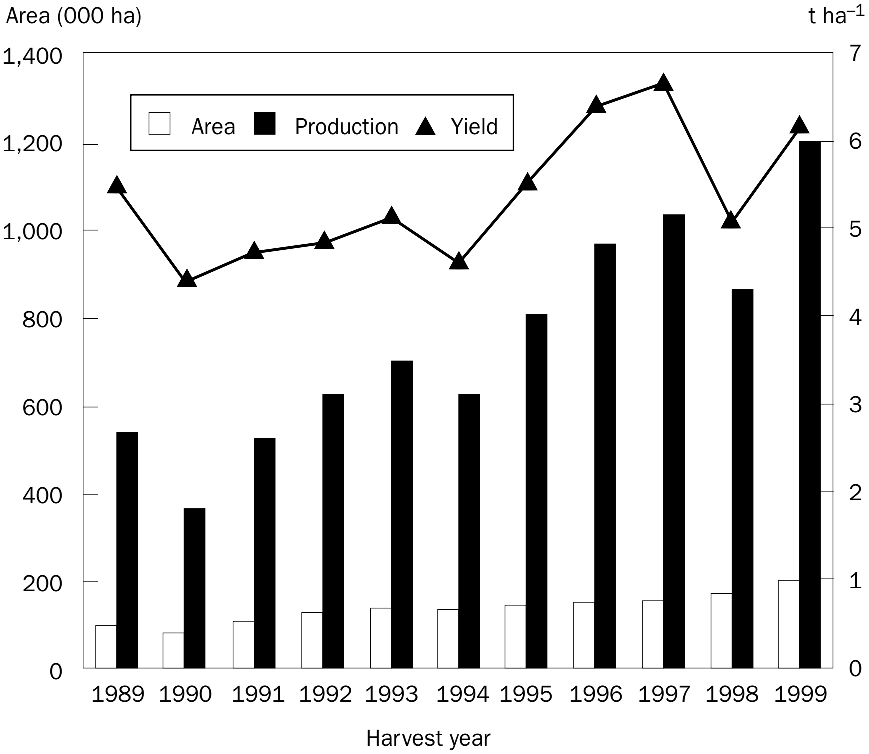

**Fig. 1. Uruguayan rice production statistics.**

**Table 1. Evolution of the number of rice farmers and area per farmer in Uruguay.**

| Crop year | Farmers (no.) | ha per farmer |
|---|---|---|
| 1985-86 | 429 | 200 |
| 1986-87 | 430 | 194 |
| 1987-88 | 424 | 192 |
| 1988-89 | 514 | 189 |
| 1989-90 | 528 | 156 |
| 1990-91 | 632 | 174 |
| 1991-92 | 783 | 162 |
| 1992-93 | 745 | 182 |
| 1993-94 | 722 | 186 |
| 1994-95 | 729 | 201 |
| 1995-96 | 750 | 200 |
| 1996-97 | 669 | 232 |

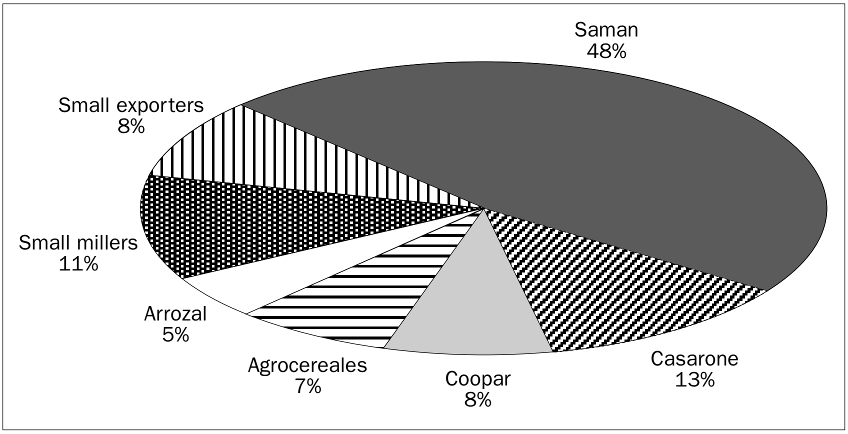

**Fig. 2. Market shares of millers and exporters in Uruguay, 1997.**

More than 90% of annual production is for export, Brazil being the main destination, followed by Peru, Senegal, Iran, and many other smaller markets. In 1997, Uruguay ranked sixth worldwide as a rice exporter, and rice became one of the most important products of Uruguay's economy in the past 10 years.

This development occurred in a mostly closed world rice market, with commercial barriers on the part of many potential buyers (Europe, Japan, etc.) and highly subsidized surpluses entering international commerce, which accounts for less than 5% of the world harvest.

In this environment, Uruguayan rice production has thrived without any kind of subsidy, or direct government intervention in commercialization. The development of strong farmers' and industry associations with close relationships with research institutions and the government is considered to be an important factor explaining this success.

Rice growers have a national association (Asociación de Cultivadores de Arroz), founded in 1948, and industry has been organized similarly for more than 50 years (Gremial de Molinos Arroceros). Both groups have developed strong relationships, allowing them to have common objectives. Almost all growers produce rice under contract with the milling company, and there are standards for premiums or discounts depending on the quality accepted by farmers and millers.

Both entities operate under an agreement to calculate an average price for rice, taking into account the real value obtained from total exports and the internal market.

This good relationship has had a large impact on local rice research and the application of new technologies. Most agricultural research in Uruguay is concentrated in the different research programs of the Instituto Nacional de Investigación Agropecuaria (INIA). One of them is the Rice Research Program, which has 13 scientists and conducts experiments all over the country. Since local research started more than 30 years ago, growers and industry have been tightly linked in discussing and defining short- and long-term goals for technology development.

They have also been active in transferring new technologies such as varieties and management strategies coming from the Rice Research Program. A good example is the evolution of cultivar area in the past 10 years (Fig. 3). Until 1990, more the 80% of the total rice area in Uruguay was planted with the old American variety Bluebelle. Now, it has less than 3% of the area, being replaced first by El Paso 144, an indica cultivar released by the local breeding project in 1987, and then by INIA Tacuarí, an American variety released by INIA in 1993. Both cultivars yield 20% more than Bluebelle.

Overall, this farmer-industry-research integration is one of the key factors that has supported the steady trend of increasing area and yield shown by Uruguay since 1970 (Fig. 4).

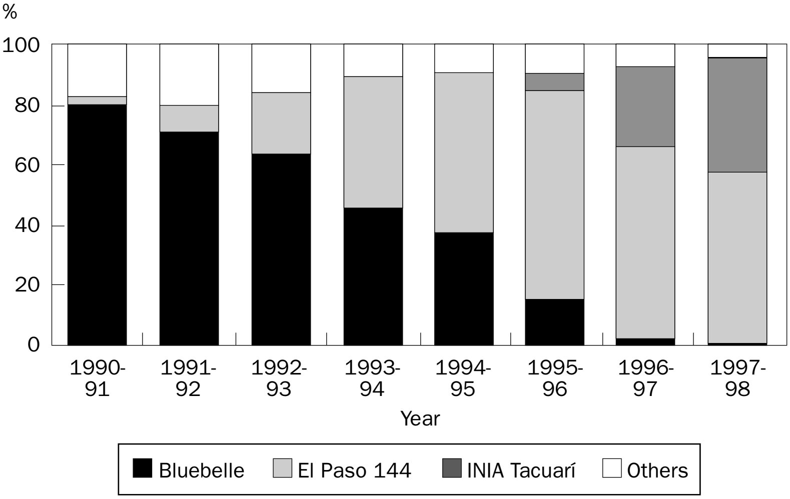

Fig. 3. Evolution of cultivar changes in Uruguay.

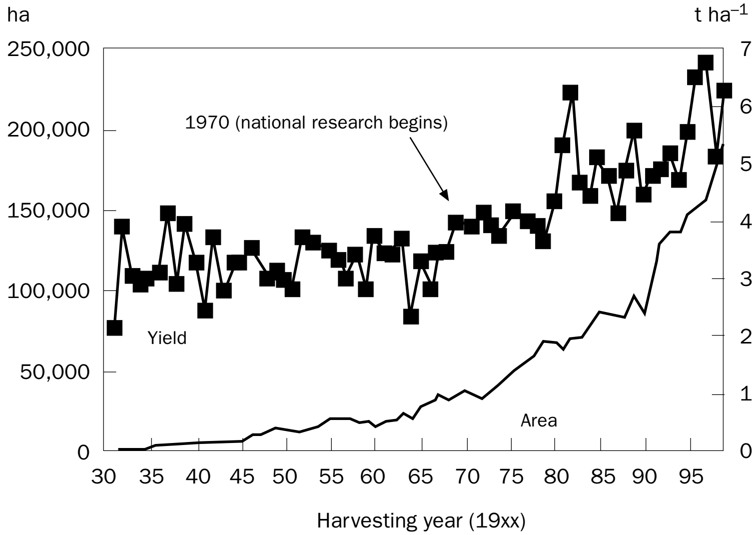

**Fig. 4. Impact of farmer-industry-research integration in Uruguay.**

## Notes

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AGRONOMY

# Seed longevity in the soil of japonica and indica rice cultivars and red rice

G. Zorrilla de San Martín, A. Acevedo, and M. Oxley

A long-term experiment was installed at the Paso de la Laguna research station of INIA Treinta y Tres to assess longevity of seeds from five commercial varieties and two red rice biotypes, buried in the soil at two depths (5 and 15 cm). Four varieties were japonica and one was an indica long-grain cultivar. Two red rice biotypes collected from rice fields were used: straw hull and black hull. The experiment was installed in June 1993 and, each September since then, seeds were removed from the soil and analyzed in the laboratory. Results from the first five extractions (1993-97) are discussed. Depth of burial in all cases favored longevity. Seeds from all japonica-type varieties lost almost all viability before the next spring (3 mo), whereas the indica cultivar maintained some viable seeds until the second spring (15 mo). All viable seeds from this cultivar were quiescent, with no dormancy at all. Both red rice biotypes showed a high capacity of seed survival. After the fifth extraction in September 1997, from 10% to 35% viability was still observed. Red rice seeds maintained fluctuating equilibrium between quiescent and dormant seeds among years.

Length of survival of seeds remaining in the soil from the last harvest is an important management factor in rice production. It permits us to plan changes in cultivars to avoid mixtures with volunteer plants, which may reduce grain or seed quality. Additionally, knowing the seed longevity in the soil of common Uruguayan biotypes of red rice is necessary for applying effective control strategies.

In the past decade, a new indica-type cultivar released in Uruguay and widely used across the country, El Paso 144, produced high levels of volunteer plants in the next crop. This problem was not usually observed with the japonica-type varieties used until then. Red rice infection in Uruguayan rice fields has been growing recently, and there are no studies on red rice seed survival in Uruguay.

The objective of this experiment was to assess seed longevity in the soil of commercial varieties and red rice biotypes under Uruguayan climatic and soil conditions.

## Materials and methods

A long-term experiment was installed at the Paso de la Laguna research station at INIA Treinta y Tres in a Solod clay soil typical of rice production fields in Uruguay. Depth of burial, year, and cultivar were the main factors combined in this trial. A split-split-plot design was used with three replications. Large plots were depths of burial (5 and 15 cm), subplots were years (5 y for rice cultivars and 20 y for red rice biotypes), and sub-subplots were cultivars.

Seed samples of five varieties were collected from a breeding experiment at maturity in May 1993, then sun-dried and cleaned in the laboratory. Four varieties were japonica types: Bluebelle, El Paso 48, INIA Tacuarí (American long-grain cultivars), and EEA-404 (medium grain). The fifth variety, El Paso 144, was an indica long-grain cultivar. At the same time, seed samples of two biotypes of red rice—straw hull and black hull—were collected in farmers' rice fields and treated similarly.

Two hundred seed samples were buried in June 1993, 1 mo after harvest. Seeds of rice cultivars presented very low or no dormancy, and both red rice biotypes had 100% dormancy at that time. PVC open cylinders (10 cm diameter, 4 cm height) were used to identify seed sample sites, but did not disturb the soil environment. Soil was removed to the treatment depth in each replication, cylinders were half-buried, and seed samples were distributed in a thin layer into them. Cylinders were then covered with the removed soil and all experiment surfaces leveled.

Each September since then (early spring in the southern hemisphere), a row of seven cylinders corresponding to each replication was cut from below and seeds carefully removed from the soil in the laboratory. Seeds with no visible deterioration were immediately placed in paper towels to germinate. Normal seedlings were counted and recorded as viable quiescent (nondormant) seeds. Seeds that did not rot at the end of the germination test were further analyzed by a tetrazolium test, and the number of viable seeds was recorded as viable dormant seeds. Total viability was obtained by adding quiescent and dormant seed counts, and results were expressed as percentage of total viable seeds buried in the soil. Results from the first five extractions are discussed (1993-97). Statistical analysis was conducted with SAS and mean separations were based on least significant difference when appropriate.

## Results

Main treatment effects were very significant, as can be observed in Table 1. Seeds placed 15 cm into the soil survived much better than the ones at 5 cm in all cases. Time was obviously a factor expected to affect seed longevity, and cultivars showed very different trends in the loss of seed viability, making all interactions highly significant.

Figure 1 summarizes results of seed longevity by depth of burial. At 5-cm depth, three types of responses could be defined. Both red rice biotypes showed a high rate of survival, maintaining some viability in spring 1997, 51 mo after burial. On the other hand, seeds from the four japonica varieties (Bluebelle, El Paso 48, INIA Tacuarí,

**Table 1. Analysis of variance. $R^2 = 0.966633$, CV = 36.78.**

| Source | DF[a] | SS | F Value | *P* > F |
|---|---|---|---|---|
| Depth of burial[b] | 1 | 2,026.17 | 48.33 | 0.0201 |
| Block[b] | 2 | 50.32 | 0.60 | 0.6249 |
| Depth of burial × block | 2 | 83.84 | 1.03 | 0.3592 |
| Year[c] | 4 | 25,516.47 | 145.94 | 0.0001 |
| Year × depth of burial[c] | 4 | 405.50 | 2.32 | 0.1015 |
| Year × block (depth of burial) | 16 | 699.38 | 1.08 | 0.3846 |
| Cultivar | 6 | 89,058.16 | 365.64 | 0.0001 |
| Depth of burial × cultivar | 6 | 1,472.79 | 6.05 | 0.0001 |
| Year × cultivar | 24 | 18,995.39 | 19.50 | 0.0001 |
| Year × depth × cultivar | 24 | 2,816.56 | 2.89 | 0.0001 |
| Error | 120 | 4,871.42 | | |
| Total | 209 | 145,995.98 | | |

[a]DF = degrees of freedom, CV = coefficient of variation. [b]Depth of burial × block used as an error term. [c]Year × block (depth of burial) used as an error term.

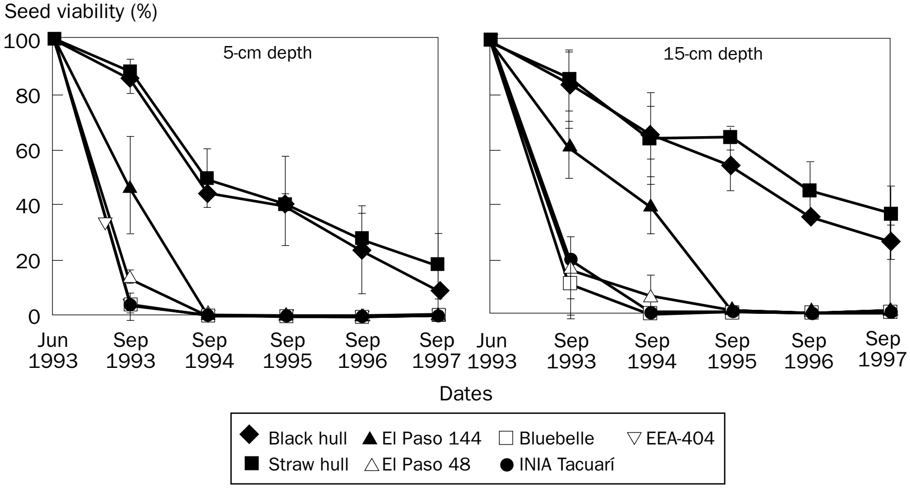

**Fig. 1. Evolution of seed viability of Uruguayan cultivars and red rice biotypes buried in the soil at two depths. Japonica cultivars El Paso 48, Bluebelle, INIA Tacuarí, and EEA-404; indica cultivar El Paso 144. Red rice biotypes are straw hull and black hull. Bars indicate standard error.**

and EEA-404) lost viability very soon, having few seeds alive in the first spring (September 1993), only 3 mo after burial. El Paso 144, the only indica cultivar, maintained 50% viability in September 1993, but all seeds were dead in the second spring (September 1994).

Results at 15-cm depth show similar trends, but with higher percentages of survival. Red rice biotypes maintained from 20% to 40% viability after 51 mo in the soil, and El Paso 144 had 60% viability in the first spring, 40% in the second spring, and only in the third spring were all seeds dead. Japonica cultivars presented some viable seeds in the first extraction, but had lost almost all viability in the second (September 1994).

Seeds from red rice biotypes and El Paso 144 showed different survival strategies. Red rice seeds were 100% dormant when buried, and after that they maintained a fluctuating equilibrium between quiescent and dormant seeds among years (Fig. 2).

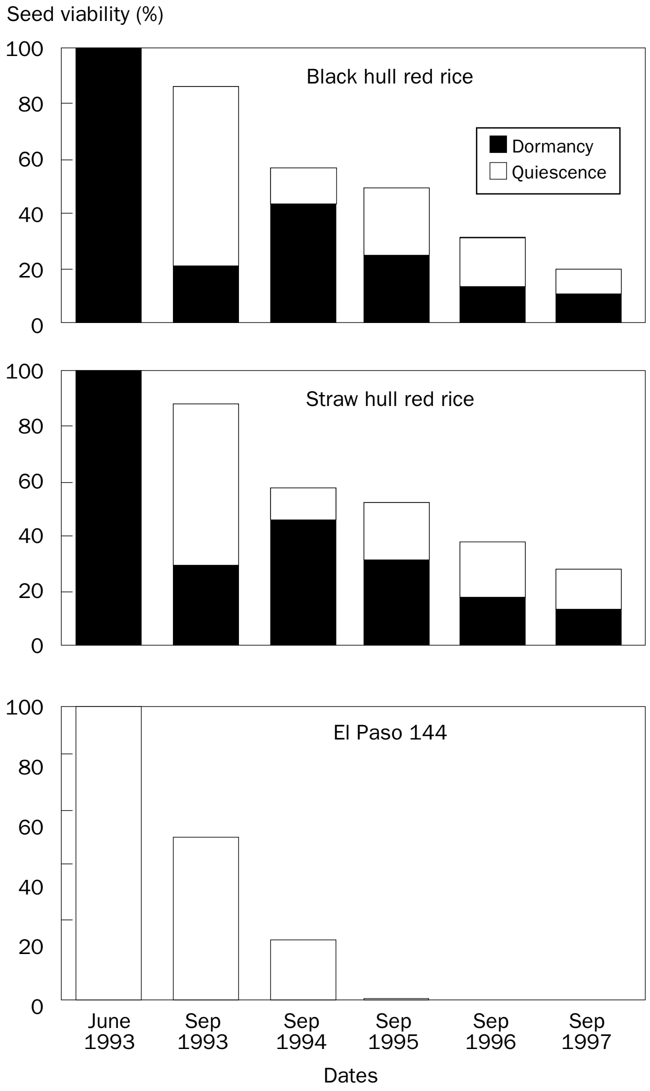

**Fig. 2. Quiescence/dormancy fluctuations in rice seeds of straw hull and black hull red rice biotypes and cultivar El Paso 144 (indica type) buried in the soil. Average of 5 and 15 cm depth of burial.**

Seeds apparently moved in and out of a secondary dormancy, probably induced by environmental changes.

On the other hand, El Paso 144 seeds were not dormant at all when buried, and no dormancy was detected after that, in any of the following years (Fig. 2). The capacity of survival of these seeds was probably sustained only by their physical structure.

## Discussion

Depth of burial benefits seed storage in the soil, not only in rice (Miller et al 1988, Noldin et al 1995), probably by diminishing temperature and moisture fluctuations, and by avoiding germination under low oxygen availability.

Previous studies have found similar longevity of red rice seeds in the soil. A frequently cited study by Goss and Brown (1939) determined that all red rice cultivars in their experiment were germinative after 3 y, whereas none of the white rice cultivars survived. Some of the red rice seeds retained germinability even after 10 y. A new study by Noldin et al (1995), however, showed that current red rice biotypes of the southern United States had a much shorter life span, having lost almost all viability after 17 mo of burial. A continuation of the present study will characterize the potential of survival of Uruguayan red rice biotypes. Results obtained up to now suggest a very strong capacity of survival because more than 20% viability was obtained after 51 mo of burial, and the rate of decay was slowing down in the last two years.

The difference in performance of japonica and indica cultivars cannot be easily described by the subspecies origin. Takahashi (1984), studying the relationship between dormancy and viability, found that japonica cultivars with weak dormancy had a short life span, whereas indica cultivars with strong dormancy had a long life span. The results of the present study coincide with Takahashi's in relation to seed longevity, although the only indica variety (El Paso 144) showed no dormancy.

In another paper discussing longevity and dormancy, Takahashi (1995) cites contradictory results on this relationship by Roberts (1963) and Ikehashi (1974). He concludes that dormancy has an important role in seed longevity of wild plants, but that there is no correlation between dormancy and the length of seed longevity in cultivated plants.

Our results support Takahashi's conclusion. Red rice biotypes, as wild species, relied on strong dormancy to maintain seed viability in the soil. The only cultivated variety that showed some capacity of survival (El Paso 144) did not have dormancy at all, but had some other physical or physiological characteristics protecting the seeds.

From these results, it is possible to give some recommendations to Uruguayan farmers for managing the rice seed bank in the soil. Changing varieties from one year to another may be possible, if a japonica type was used last in the cycle, but at least two years without rice should be allowed with El Paso 144. In this case, repeated superficial soil movement may effectively reduce the seed bank because of the lack of dormancy of this cultivar.

The long survival of red rice seeds in the soil in Uruguay has been confirmed in this study. Control strategies in red rice–infected fields won't be able to rely on short rotations with pastures, and they will need to include other management factors. Plowing immediately after harvest is not recommended if red rice is present because deeply buried seeds will last longer.

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DISEASES

# Economic consequences of rice blast disease in California

Jung-Sup Choi, D.A. Sumner, F.H. Buck, Jr., R.K. Webster, and C.A. Greer

Rice blast disease was first reported in California during 1996 and has already caused considerable losses to the rice industry. The disease seems to be well established and eradication does not appear to be economically feasible. The costs of the disease are manifest in higher production costs, lower yields, and higher prices for consumers. Industry-supported research for control began immediately and other public responses included relatively modest regulation to avoid further spread and efforts to restrict entry of new blast strains.

After many years in which it was thought to be unsuited to California conditions, rice blast disease was detected for the first time in the state during 1996 and it subsequently spread throughout a larger area in 1997 (Webster 1998). Even though severity of the disease was limited in 1998, it continues to have the potential to become the most serious disease affecting the California rice industry. As a consequence, individual growers and the industry have begun various activities to control the disease.

This chapter focuses on measuring the impacts of rice blast and its control on the California rice industry. More specifically, we attempted to assess the economic impact of rice blast on the price and quantity of rice production and related economic variables. We also analyzed the economic benefits and costs of alternative control measures, including the potential for eradication. The results of this analysis may provide a basis for better understanding the economic prospects of the industry and for public policy or industry-wide actions that could mitigate some of the negative consequences of rice blast disease.

## Rice blast disease in California

Blast was first detected in California during the fall of 1996 on about 12,000 acres of rice in Colusa and Glenn counties (Webster 1997). In 1997, the disease was reported on more than 55,000 acres, with about 30,000 acres in Colusa, 24,000 acres in Glenn, and 1,200 acres in Sutter County. In 1998, the disease was less severe than in 1996 or 1997 but the infected area expanded further east and south.

Blast can infect rice from the seedling stage through maturity (Scardaci et al 1997). Infection may result in lesions on most of the plant including leaves, the leaf collar, stems, nodes, panicles, and grain. Consequently, the disease has also been called leaf blast, collar rot, node and panicle blast, or rotten neck blast depending on the part of the plant infected. Once the pathogen is introduced and established, most of its dispersion is by airborne spores. Thus, fields without leaf blast may develop neck blast later in the season because of airborne conidia. The neck blast phase is the most damaging, resulting in yield losses that may reach 50%, with corresponding reductions in grain quality. Blast infects all of the cultivars presently grown in California with the relatively early maturing M-201 being the most susceptible. M-201 was planted on about 10% of the rice area in 1996, but its share fell to 3% by 1998.

Environmental conditions that favor the occurrence of blast include extended periods of free moisture on plant surfaces, night temperatures between 63 and 73 °F with little or no wind, and high relative humidity (RH). Conidia are produced and released under high relative humidity with no spore production below 89% RH. Optimum temperatures for germination, infection, lesion formation, and sporulation are 77–82 °F.

Management of rice blast disease requires an integrated approach including the development of cultivars with improved resistance, manipulation of cultural practices, and the judicious use of fungicides. Examples include

1. Destruction of infested crop residue limits initial inoculum but will not protect fields from airborne conidia from other fields.
2. Planting pathogen-free seed aids in preventing spread of the pathogen into uninfected areas but will not protect fields from other inoculum sources.
3. Water seeding reduces disease transmission from seed to seedlings.
4. Continuous flooding from planting to maturity minimizes blast.
5. Nitrogen fertilizer rates should not exceed that needed for maximum yield.
6. Protectant fungicides can be used to control neck blast and minimize reductions in yield and quality in years when conditions favor blast.
7. Resistant cultivars will be the most important control option, but it will take several years to incorporate resistance into California cultivars.
8. Border control measures for seed imports that reduce the chance that additional races of blast enter the state will continue to be necessary.

Occurrence and severity of blast have been sporadic in the infected areas since its introduction into California. Some individual fields have lost up to 35–40% of yield, whereas others have only a trace of disease. For an economic analysis, we needed aggregate, industry-wide effects of blast on rice yield and quality. Yield loss depends on the severity of individual infected fields and the portion of infected plants and area. Estimates indicated that, in California, the average percentage reduction in yield for an infected field attributable to blast ranged from 15% to 30% (California Rice Industry Association 1997). The geographic spread depended heavily on the proportion of area planted to cultivars differing in susceptibility. Agricultural specialists believe that the total infected area may have reached 100,000 acres or 20% of the total area and that the disease will continue to spread throughout the rice-producing area of the Sacramento Valley.

Rice blast also causes quality deterioration by uneven kernel moisture at harvest and smaller grain on infected plants. For the economic analysis, quality deterioration, measured by the milling percentage of head rice and total rice, was converted to the equivalent yield loss. In terms of total milling rate, a 1% loss reduced rice value by 4 to 6 cents per cwt and a 1% loss in head rice reduced rice value by 4.5 cents per cwt (98 Crop USDA Loan Values of Medium/Short Grain). Monitoring data showed that blast reduced the head/total milling yield from 48/68 to as low as 29/59. Considering that the normal milling ratio for short/medium grain in California is 58/68, blast appears to reduce the value of rice produced in the infected field by at least 10% to 20%. The average statewide additional yield loss attributable to milling quality loss may reach 2–4%. Considering that the statewide direct yield reduction may reach 3–6%, the industry-wide quality-adjusted yield loss could range from 5% to 10%. These figures apply to the potential effects of blast with no effort to control the disease.

Control of rice blast using fungicides involves costs and benefits. These costs to individual growers may reach 10% to 20% of cultural costs when both material and application costs are considered. As for benefits, field experiments show that chemical control may reduce potential yield loss from blast by 80–90%.

## Economic analysis

### Model

The contribution of the economic analysis is to consider the quantitative effects of blast and blast control in terms of prices, revenues, and resulting economic aggregates. Supply-side effects of rice blast appear as reduced production because of declines in yield and quality. Yield reduction also results in a corresponding increase in marginal production costs per cwt. Quality deterioration decreases revenue through lower milling quality. The benefits of blast control methods appear as reduced yield and quality losses from blast, whereas production costs increase by the cost of the control.

The spread, severity, and control of the disease vary with environmental conditions favorable for the disease so that information on the impact of blast is not known with certainty. But our model does not elaborate on the dynamics or the role of blast disease in risk to growers. The modeling approach provides a way to build in plausible ranges of estimates using a simple approach. The equilibrium displacement model, which is a simulation-based modeling approach, is used to project changes in the economic impacts of blast on the rice industry based on ranges of variables for the biological variables and economic parameters. Thus, the equilibrium displacement model projects how economic changes affect industry, including the introduction and occurrence of rice blast disease and its control methods. The model requires specification of the underlying equations that represent supply, demand, and market equilibrium.

Consider the following equilibrium displacement model system that is specified in log-linear differential form:

$EY = \delta$ (quality-adjusted yield) (1)
$EL = \varepsilon_l EP + \varepsilon_{lc} EC$ (acreage supply) (2)
$ES = \varepsilon_l EP + \varepsilon_{lc} EC + \delta$ (total supply) (3)
$ED = \eta EP$ (demand) (4)
$ES = ED$ (market clearing condition) (5)

The E operator denotes percentage changes. Quality-adjusted yield per acre, Y, is represented by equation 1, where $\delta$ denotes the percentage change in the quality-adjusted yield. Equation 2 denotes planted area, L, as a function of price, P, and production costs, C, where $\varepsilon_l$ is price elasticity of area (percentage change of area divided by percentage change of price). The elasticity of change in per hundredweight cost (percentage change of cost divided by percentage change of price) is given by $\varepsilon_{lc}$. (For simplicity we assume constant returns to scale.) Market supply, S, is given by equation 3 as the addition of percentage change in acreage and percentage change in yield from equations 1 and 2. Equation 4 represents the market demand, D, as a function of price, where $\eta$ is price elasticity of demand, that is, percentage change in demand divided by percentage change in price. Finally, equation 5 is the market clearing condition.

The approach begins by noting that blast has an exogenous shock shown in equation 1. The model is solved by using equation 5 to set equation 3 equal to equation 4 and by solving for EP. Then we substitute EP back into 1, 3, and 4 to get equilibrium values. For the second simulation, we note that blast control measures undertaken by growers affect production costs and yield in equations 1, 2, and 3. With these changes, by solving the equation system, we get the equilibrium values after incorporating control measures.

In the simulation, quality-adjusted yield losses of 5%, 10%, and 15%, the parameter $\delta$ in equation 1, are considered as plausible cases. The increase in per cwt production costs from control methods for blast, parameter EC in equation 2, is postulated as 10% and 20% of the cultural costs. The benefit of blast control is the reduction in yield loss. We examine the cases for which yield loss is decreased by 80% with a 10% per cwt cost increase, and by 90% with a 20% per cwt cost increase. The range of values of acreage supply elasticity is 0.2, 0.5, and 1.0 (Song and Carter 1996). To represent marginal cost elasticity with respect to acreage, the negative of these values equals the elasticity of production cost change on acreage, –0.2, –0.5, and –1.0. The final parameter needed is demand elasticity, for which we use values of –2.0, –4.0, and –6.0.

## Simulation results

*Impacts of introduction of blast with no effort to control.* Consider the case under which blast reduces yield by 10% when supply elasticity is 0.5 and demand elasticity is –4.0 (see the middle block of values in Table 1). In this case, the price of rice increases by 3.5% and the equilibrium quantity falls by 13.8%. As a result, the industry gross revenue falls by 10.8%. With a higher supply elasticity, price increases more and quantity falls more. With a higher demand elasticity, price falls less and quantity falls more. The ratio of producer surplus change to the total value produced is 3.1%. The equivalent ratio of the consumer surplus change is 0.4% (refer to Table 2).

*Impacts of the recommended control strategy.* We also consider the case where yield loss is 10%, supply elasticity is 0.5, and demand elasticity is –4.0, and blast control adds 10% to production costs and has 80% efficacy. In this case, industry total revenue falls by 5.4% and revenue net of blast control costs falls by 9.2% compared with no blast. But note that this is higher revenue than the loss of 10.8% when there is no attempt at control. Blast control that increases production costs by 20% and has 90% efficacy will result in industry revenue declining by 7.9% (Table 3). In this case, the net revenue falls by 15.6% compared with a decline of 10.8% with no control. Obviously, this level of control is not economical given increased production costs of 20%. It is shown that the reduction in revenue loss by blast control does not make up for the control costs if blast is less severe and control costs are high. For example, when the yield loss is 10%, a 10% control cost reduces the net revenue loss by 1.6 percentage points but a 20% control cost increases the net revenue loss by 4.8 percentage points. When disease severity increases, the reduction in net revenues exceeds the control costs.

**Table 1. Changes (%) in economic indicators by rice blast control measures.**[a]

| Yield reduction | Control costs | Change in area | Change in price | Change in quantity | Change in revenue | Change in net revenue |
|---|---|---|---|---|---|---|
| –5 | 0 | –1.8 | 1.7 | –6.8 | –5.2 | –5.2 |
| | 10 | –4.8 | 1.4 | –5.8 | –4.5 | –8.3 |
| | 20 | –9.1 | 2.4 | –9.6 | –7.4 | –15.1 |
| –10 | 0 | –3.8 | 3.5 | –13.8 | –10.8 | –10.8 |
| | 10 | –5.1 | 1.8 | –7.1 | –5.4 | –9.2 |
| | 20 | –9.2 | 2.6 | –10.2 | –7.9 | –15.6 |
| –15 | 0 | –6.2 | 5.3 | –21.2 | –17.0 | –17.0 |
| | 10 | –5.5 | 2.1 | –8.5 | –6.6 | –10.4 |
| | 20 | –9.4 | 2.7 | –10.9 | –7.7 | –15.4 |

[a] Supply elasticity is 0.5 and demand elasticity is –4.0. Control costs are additional costs of blast control as the percentage of cultural costs, that is, $4.04 (38%) of the total production costs per hundredweight, $10.53. The net revenue is revenue net of control costs.

**Table 2. Simulation result (in %): measuring costs from rice blast in California.**

| Yield reduction | Acreage elasticity | Demand elasticity | Acreage change | Price change | Quantity change | Revenue change | PS change[a] | CS change |
|---|---|---|---|---|---|---|---|---|
| –5% | 0.2 | –2.0 | –0.5 | 2.8 | –5.5 | –2.9 | –2.6 | –0.3 |
| | | –4.0 | –0.8 | 1.4 | –5.8 | –4.5 | –1.3 | –0.1 |
| | | –6.0 | –0.9 | 1.0 | –5.9 | –5.0 | –1.0 | –0.0 |
| | 0.5 | –2.0 | –1.1 | 3.1 | –6.1 | –3.2 | –2.5 | –0.6 |
| | | –4.0 | –1.8 | 1.7 | –6.8 | –5.2 | –1.5 | –0.2 |
| | | –6.0 | –2.0 | 1.2 | –7.0 | –5.9 | –1.1 | –0.0 |
| | 1.0 | –2.0 | –1.8 | 3.4 | –6.8 | –3.6 | –2.3 | –1.2 |
| | | –4.0 | –3.2 | 2.1 | –8.2 | –6.3 | –1.7 | –0.4 |
| | | –6.0 | –3.8 | 1.5 | –8.8 | –7.4 | –1.3 | –0.2 |
| –10% | 0.2 | –2.0 | –1.1 | 5.6 | –11.1 | –6.1 | –5.1 | –0.5 |
| | | –4.0 | –1.6 | 2.9 | –11.6 | –9.0 | –2.8 | –0.1 |
| | | –6.0 | –1.8 | 2.0 | –11.8 | –10.0 | –1.9 | –0.1 |
| | 0.5 | –2.0 | –2.4 | 6.2 | –12.4 | –7.0 | –5.0 | –1.3 |
| | | –4.0 | –3.8 | 3.5 | –13.8 | –10.8 | –3.1 | –0.4 |
| | | –6.0 | –4.4 | 2.4 | –14.4 | –12.3 | –2.2 | –0.2 |
| | 1.0 | –2.0 | –4.1 | 7.0 | –14.1 | –8.1 | –4.8 | –2.4 |
| | | –4.0 | –6.9 | 4.2 | –16.9 | –13.4 | –3.4 | –0.9 |
| | | –6.0 | –8.1 | 3.0 | –18.1 | –15.6 | –2.6 | –0.4 |
| –15% | 0.2 | –2.0 | –1.8 | 8.4 | –16.8 | –9.8 | –7.7 | –0.8 |
| | | –4.0 | –2.6 | 4.4 | –17.6 | –14.0 | –4.2 | –0.2 |
| | | –6.0 | –2.9 | 3.0 | –17.9 | –15.4 | –2.9 | –0.1 |
| | 0.5 | –2.0 | –4.1 | 9.5 | –19.1 | –11.4 | –7.7 | –1.9 |
| | | –4.0 | –6.2 | 5.3 | –21.2 | –17.0 | –4.8 | –0.6 |
| | | –6.0 | –7.0 | 3.7 | –22.0 | –19.1 | –3.4 | –0.3 |
| | 1.0 | –2.0 | –6.8 | 10.9 | –21.8 | –13.3 | –7.5 | –3.8 |
| | | –4.0 | –11.1 | 6.5 | –26.1 | –21.3 | –5.3 | –1.3 |
| | | –6.0 | –13.0 | 4.7 | –28.0 | –24.6 | –4.1 | –0.7 |

[a] The changes in producer (or consumer) surplus (PS or CS) are calculated as the ratio of PS (or CS) to the total industry revenue.

## Public policy

Since rice blast disease was only recently introduced to California in 1996, it is natural to consider the possibility of eradication. However, a brief review indicates that the economic costs of eradication appear to far exceed the potential benefits, even if eradication could be achieved. Blast is now known to be distributed over at least 70,000 acres and occurs sporadically at differing severities depending on environmental conditions. The only potential method of eradication would be to eliminate rice production in the whole region where blast has occurred and to burn all rice residue. Such a ban on production would need to last for at least three seasons. The region affected would be approximately one-third of the entire rice-growing area, so the cost of this operation would equal roughly the whole net revenue from rice production with collateral effects on workers and allied industries. Based on the costs

**Table 3. Simulation result (in %): measuring impacts of blast control costs in California.**

| Change in yield | Control costs[a] | Yield loss with control[b] | Total increase in control costs | Change in acreage | Change in price | Change in quantity | Change in revenue[c] |
|---|---|---|---|---|---|---|---|
| –5 | 10 | 1.0 | 11.0 | –4.8 | 1.4 | –5.8 | –4.5 (–8.3) |
| | 20 | 0.5 | 20.5 | –9.1 | 2.4 | –9.6 | –7.4 (–15.1) |
| –10 | 10 | 2.0 | 12.0 | –5.1 | 1.8 | –7.1 | –5.4 (–9.2) |
| | 20 | 1.0 | 21.0 | –9.2 | 2.6 | –10.2 | –7.9 (–15.6) |
| –15 | 10 | 3.0 | 13.1 | –5.5 | 2.1 | –8.5 | –6.6 (–10.4) |
| | 20 | 1.5 | 21.5 | –9.4 | 2.7 | –10.9 | –7.7 (–15.4) |

Note: The acreage supply elasticity is set at 0.5 and demand elasticity is set at –4.0.
[a]Control costs are denoted as the percentage of production costs per hundredweight. [b]Control costs of 10% and 20% are assumed to reduce yield loss by 80% and 90%, respectively. [c]Net revenue in parentheses.

shown in Table 1, even if eradication were biologically or politically feasible, its costs seem obviously larger than the costs of accepting the costs of blast disease.

At least three major public policies related to rice blast disease are important. The first relates to regulations on pesticides used to control the disease. The only chemical presently approved for use in California is effective for control, but is also relatively expensive. Thus, additional attention to balancing environmental concerns with the economic health of the industry is needed. A careful but expeditious regulatory review of chemicals and other control methods is particularly appropriate. Second, research on blast is also important and may demand public or industry support. Research should focus on developing resistant cultivars but might also include other control methods. Efforts to develop resistant cultivars are considered the most important and profitable industry-wide response to blast. The industry has already begun funding such research. Although blast seems to be established in California, thus far only one pathogenic strain of the fungus is known to be present. This should facilitate the development of resistant cultivars and further reductions in losses. The net annual payoff of a resistant cultivar is indicated by the net revenue column in Table 1. Third, research to find resistant cultivars emphasizes the need for measures to assure that additional pathogenic strains of the blast fungus are not introduced into California. This means that it is necessary to continue the policy measures to monitor and limit seed imports or other possible means of introduction.

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## Notes

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# Quadris: a novel fungicide for disease control in rice

S. Harrison and E. Tedford

Quadris (azoxystrobin) is a new fungicide from Zeneca Ag Products with a novel mode of action. This fungicide represents the first registered product from a new class of fungicides called "strobilurins." Azoxystrobin has a broad spectrum of activity against several important plant pathogenic fungi and therefore has the potential to be labeled for disease control in many crops. Quadris (SC formulation) has been evaluated for efficacy against several plant pathogenic fungi that attack rice and for potential to protect rice yield and milling quality. Quadris has demonstrated excellent control of major economic rice diseases such as sheath blight and panicle blast, caused by *Rhizoctonia solani* and *Pyricularia grisea*, respectively. Quadris also has good to excellent activity against stem rot and black sheath rot of rice caused by *Magnaporthe salvinii* and *Gaeumannomyces graminis*, respectively. As a result of disease control, Quadris has produced significant savings in rice yields and milling quality.

Azoxystrobin, the active ingredient in Quadris, originated from a family of natural products that have fungicidal activity. These natural products include strobilurins, oudemansins, and myxothiazols that are produced by the wood-rotting fungi *Strobilurus tenacellus* (Anke et al 1977) and *Oudemansiella mucida* (Musilek et al 1969) and the bacterium *Myxococcus fulvus* (Clough 1993), respectively. All three of these natural compounds are derivatives of β-methoxyacrylic acid. Of the many different β-methoxyacrylate derivatives characterized, strobilurin A and oudemansin A are two of the simplest compounds. These compounds are produced by *S. tenacellus* and *O. mucida* on decaying logs in the forest and are active against competitor fungi. However, when evaluated on agricultural plants under glasshouse conditions, their fungicidal activity was low. This was primarily due to rapid photolytic degradation that occurred in the glasshouse but not in the dense forest. To overcome this rapid degradation, Zeneca scientists evaluated a cascade of chemical analogues that varied slightly from the original compounds strobilurin A and oudemansin A. From these efforts,

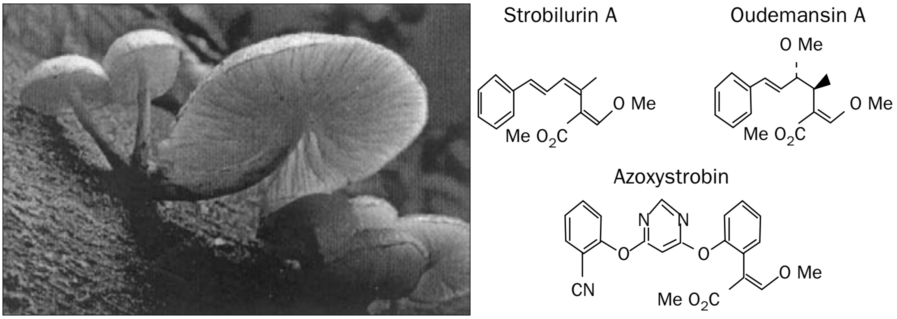

**Fig. 1. Photograph of *Oudemansiella mucida,* a Basidiomycete that produces the natural strobilurin oudemansin A, and chemical structures of strobilurin A, oudemansin A, and azoxystrobin (the active ingredient in Quadris™).**

they developed azoxystrobin, which contained the β-methoxyacrylate functional group similar to strobilurin A and oudemansin A (Fig. 1) yet maintained fungicidal activity in the presence of light.

## Spectrum of activity

Azoxystrobin has a broad spectrum of activity against plant pathogenic fungi in all four major fungal classes (Ascomycetes, Basidiomycetes, Deuteromycetes, and Oomycetes). This means that rice growers can use Quadris to provide protection against many of the key fungal pathogens that attack rice (Table 1). Azoxystrobin is an excellent protectant fungicide that also has systemic activity within the plant. This fungicide moves both translaminarly from one leaf surface to another and systemically upward within the xylem (Clough et al 1996). Therefore, with uniform coverage, azoxystrobin can provide systemic control of foliar diseases. However, this does not mean that it should be used as a curative fungicide. Azoxystrobin does have curative activity against sheath blight but is best used in a protectant fungicide program for multiple disease control.

## Mode of action

The fungicidal activity of azoxystrobin comes from its ability to inhibit electron transfer in fungal mitochondria by binding at a specific site on cytochrome b. The result of this activity is the cessation of normal energy production (ATP production) within the cell, which results in cell death. Evidence of this effect on fungi can be observed in spore mortality, mycelial collapse, inhibition of sporulation, or disruption of other vital stages of fungal development.

The ultimate goal of a preventive application of azoxystrobin is to provide disease control and maximize yield and quality. We will illustrate the advantage that azoxystrobin can provide in yield and quality by using examples from field studies where sheath blight and rice blast disease pressure were high.

**Table 1. Example of the broad spectrum of activity that azoxystrobin has against important rice diseases[a].**

| Target diseases | Pathogen |
|---|---|
| Sheath/stem diseases | |
| Aggregate sheath spot | *Rhizoctonia oryzae-sativae* |
| Black sheath rot | *Gaeumannomyces graminis* var. *graminis* |
| Sheath blight | *Rhizoctonia solani* AG1-IA |
| Bordered sheath spot | *Rhizoctonia oryzae* |
| Stem rot | *Sclerotium oryzae* |
| Foliar diseases | |
| Brown leaf spot | *Bipolaris oryzae* |
| Leaf smut | *Entyloma oryzae* |
| Narrow brown leaf spot | *Cercospora oryzae* |
| Panicle diseases | |
| Kernel smut | *Neovossia horrida* |
| Panicle blast | *Pyricularia grisea* |

[a]Azoxystrobin is the active ingredient in the fungicide Quadris.

## Efficacy and benefits of Quadris

### Sheath blight

Sheath blight, caused by *Rhizoctonia solani* AG1-IA, is an important disease of rice in both tropical and temperate climates. In the United States, this disease is particularly prevalent in the southern rice-growing regions. The pathogen survives between crops as sclerotia in the soil or as mycelium within infected plant debris. Sclerotia can survive for up to two years in temperate rice-growing areas and many natural or cultivated grass and leguminous hosts are associated with rice production (Webster and Gunnell 1992). For various reasons, it is not considered economically feasible to control this disease by crop rotation alone. Some rice cultivars have moderate resistance to this disease, but the major control measure in many areas is to apply fungicides. Left uncontrolled, sheath blight can reduce yields up to 50% under heavy disease pressure.

In the southern U.S., only one fungicide application is usually recommended for control of sheath blight—primarily for economic reasons. Currently registered fungicides for sheath blight are benomyl (Benlate$^{TM}$), propiconazole (Tilt$^{TM}$), flutolanil (Moncut$^{TM}$), and azoxystrobin (Quadris$^{TM}$). Over the past several years, many university cooperators have evaluated Quadris for efficacy against sheath blight and the results indicate that Quadris provides excellent control. As a result of disease control, rice yields and quality have been higher where Quadris was applied when compared with other treatments. For example, in an Arkansas trial, rice yields were measured in a field with heavy sheath blight disease pressure where plots were either not treated or treated with a single aerial application of benomyl, propiconazole, flutolanil, or azoxystrobin. Azoxystrobin (Quadris) was the only treatment in this trial that resulted

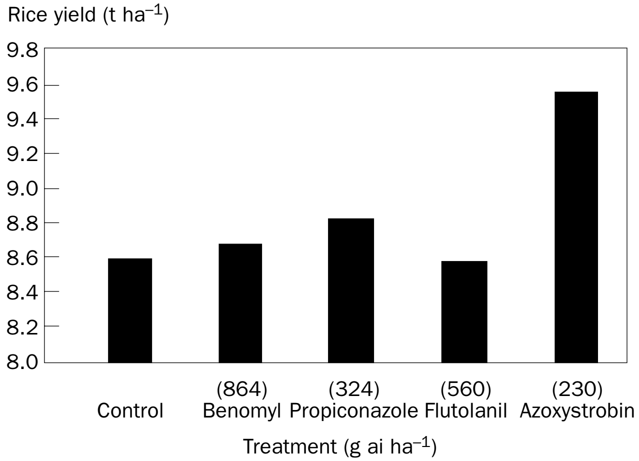

**Fig. 2. Effect of one aerial application of various fungicide treatments on yield of rice in a field under heavy sheath blight disease pressure in Arkansas (USA). Benomyl, propiconazole, flutolanil, and azoxystrobin are the active ingredients in Benlate™, Tilt™, Moncut™, and Quadris™, respectively.**

in substantially higher yields than the untreated control (Fig. 2). Similar results occurred in a Louisiana trial where a single aerial application of azoxystrobin (230 g ai ha$^{-1}$), flutolanil (392 g ai ha$^{-1}$), or benomyl (576 g ai ha$^{-1}$) was made in a field with extremely high disease pressure. In fact, the disease pressure was so high that the entire field was sprayed with propiconazole (324 g ai ha$^{-1}$) 2 wk before the fungicide treatment program. Quadris was the only fungicide in this study that provided significant disease control; the levels of disease in the other fungicide-treated plots were the same as in the untreated control (Table 2). The rice yield and milling quality in the Quadris-treated plots were higher than were those for the other treatments and this resulted in a much greater increase in value than for the other treatments (Table 2).

## Rice blast

Rice blast caused by *Pyricularia grisea* is one of the most important diseases of rice (Webster and Gunnell 1992). In the U.S., blast was known only in its southern rice-growing regions until 1996 when it was also reported in California. The rice blast pathogen survives between crops in infested residue or seed. Unlike for sheath blight, some rice cultivars have complete resistance to rice blast, although resistance may break down over time. Fertilizer management is critical with this disease as the susceptibility of rice increases with nitrogen and phosphorus fertilizers. Maintaining proper flood depth is also essential in minimizing blast in the southern U.S. Fungicides remain a major control option for this disease.

Two foliar fungicide applications are recommended to control rice blast on susceptible cultivars under favorable conditions for disease. While fungicides for blast control have been a long-standing practice in the southern rice-growing regions of the

U.S., this has not been the case in California, where the disease was not a factor in the past. Many of the rice cultivars grown in California are highly susceptible to this disease and under favorable conditions for disease development can be significantly damaged without fungicide protection.

Aerial fungicide field trials in the south have demonstrated that Quadris provides excellent control of rice blast compared with standard fungicide treatments. As a result of disease control, the use of Quadris resulted in higher yield, milling quality, and return on value in contrast to the other standard fungicide treatments tested (Table 3). Again, Quadris provides excellent to good control of several major diseases of rice (Table 4).

**Table 2. Effect of a single aerial application of various fungicide treatments on sheath blight disease ratings, rice yields, milling quality, and value of each treatment.[a]**

| Treatment[b] | Rate ($g$ ai $ha^{-1}$) | Disease rating (0–9)[c] | Yield ($t$ $ha^{-1}$) | Milling quality[d] | Value increase (\$ $ha^{-1}$) |
|---|---|---|---|---|---|
| Azoxystrobin | 231 | 2 | 8.2 | 65/72 | 246.31 |
| Flutolanil | 392 | 9 | 8.1 | 59/70 | 67.75 |
| Benomyl | 576 | 8 | 7.9 | 61/70 | 58.19 |
| Control | – | 9 | 7.8 | 58/70 | 0 |

[a]These data were generated by Ronnie Levy, Clayton Hollier, and Don Groth, Louisiana State University. [b]Azoxystrobin, flutolanil, and benomyl are the active ingredients in Quadris™, Moncut™, and Benlate™, respectively. [c]Because of heavy disease pressure, the entire field was sprayed with propiconazole (Tilt™) at 324 g ai $ha^{-1}$ 2 wk before the treatments listed above. [d]Numerator = percentage of whole kernels, denominator = total percentage of milled rice whole plus broken kernels. Values above 55 or 70 receive a premium and values below 55 or 70 are discounted.

**Table 3. Effect of two aerial applications of azoxystrobin or benomyl on rotten neck blast infection, rice yields, and milling quality[a].**

| Treatment[b] | Rate ($g$ ai $ha^{-1}$) | Rotten neck (%) | Yield ($bu$ $ha^{-1}$) | Milling quality[c] | Price (\$ $bu^{-1}$)[d] | Gross (\$ $ha^{-1}$) |
|---|---|---|---|---|---|---|
| Azoxystrobin<br>fb Azoxystrobin | 231<br>231 | 19 | 373 | 62/70 | 4.97 | 1,853.66 |
| Benomyl<br>fb Benomyl | 576<br>576 | 53 | 284 | 58/69 | 4.67 | 1,326.51 |
| Control | – | 81 | 207 | 56/70 | 4.57 | 948.18 |

[a]These data were provided by Steve Harrison from a Zeneca in-house study conducted in Arkansas. [b]Azoxystrobin and benomyl are the active ingredients in Quadris™ and Benlate™, respectively. [c]Numerator = percentage of whole kernels, denominator = total percentage of milled rice whole plus broken kernels. Values above 55 or 70 receive a premium and values below 55 or 70 are discounted. [d]Based on \$4.50 $bu^{-1}$ for rice with a standard milling quality of 55/70.

**Table 4. Relative ranking[a] of rice fungicides for control of major rice diseases.**

| Fungicide | Active ingredient | Sheath blight | Blast | Stem rot | Black sheath rot | Brown leaf spot |
|---|---|---|---|---|---|---|
| Quadris™ | Azoxystrobin | ++++ | ++++ | +++ | +++ | +++ |
| Benlate™ | Benomyl | ++ | +++ | ++ | | |
| Tilt™ | Propiconazole | ++ | | + | ++ | +++ |
| Moncut™ | Flutolanil | +++ | | | | |
| Rovral™ | Iprodione | ++ | | | | ++ |

[a] ++++ = excellent control, +++ = good control, ++ = some control, + = poor control. Information developed by Zeneca Ag Products.

## Ecological profile

Azoxystrobin has an excellent ecological profile and is therefore ideal for use in integrated pest management programs. This was the first fungicide to receive U.S. registration under the new FQPA guidelines of the Environmental Protection Agency as a "reduced risk" fungicide. It degrades in the soil, does not persist in the environment, has low soil mobility, and therefore does not pose a risk of groundwater contamination. Azoxystrobin has a low toxicity to mammals, birds, honeybees, earthworms, and a variety of beneficial arthropods, and presents only a low risk to fish and aquatic invertebrates.

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DISEASES

# Molecular characterization of a Latin American *Pyricularia grisea* population

A.B. Livore, C. Dezar, M.I. Plata, S. Avila, and M. Levy

Irrigated rice in the Southern Cone of Latin America is grown in southern Brazil, Argentina, and Uruguay. Although rice blast has been present in that region for a long time, it became a major constraint to rice production starting in 1996.

The MGR586 probe was used to generate DNA fingerprints (Eco*RI*-RFLPs, restricted fragment length polymorphism) from monoconidial isolates of *Pyricularia grisea* collected in southern Brazil, Argentina, and Uruguay from cultivars and breeding lines during the rice seasons of 1994, 1997, and 1998. A total of five different MGR-fingerprint lineages have been found in this region, but only one has been isolated on the tropical cultivars that are the most extensively grown. Even in breeding nurseries where other different isolates have been identified, the DNA fingerprints of isolates on tropical cultivars consistently belong to the same lineage. This suggests a specific susceptibility of those cultivars to that particular fungal lineage but, at the same time, also suggests resistance to the other lineages found in the region.

Comparisons made with other isolate fingerprints from Latin America showed a high similarity between the El Paso 144-associated lineage in the Southern Cone and a lineage from Colombia, for which a source of incompatibility (resistance gene) is known. It is possible that the addition of that gene to El Paso 144 would complete the spectrum of resistance (i.e., exclude all regional pathogen lineages) necessary to provide a more durable protection.

Irrigated rice in the Southern Cone of Latin America is grown in southern Brazil, Argentina, and Uruguay. Until approximately 1980, medium-grain types, with Italian (japonica) background, were cultivated in a large area. During the next decade, an indica long-grain cultivar (IRGA409) was released in southern Brazil, whereas a Texas variety, Bluebelle, was mostly grown in Uruguay and Argentina. Following the increasing demand from Brazil of indica-type rice, several cultivars closely related to the Brazilian cultivar IRGA409 were grown commercially in Uruguay and Argentina. Although rice blast has been present in that region for a long time, it became a major constraint to rice production in 1996. The spread of this disease has followed the diffusion of closely related germplasm. Consequently, the national breeding programs have started a novel approach for durable resistance based on the hypothesis of lineage exclusion, in which resistances are combined based on preventing infection by families of the blast fungus that exist in the region.

The first step in breeding for blast resistance is to identify the genetic components and structure of the pathogen population. Inoculation of differential cultivars helps to describe pathotype variation, but differences in assay conditions, intermediate readings on the differentials, and different scoring procedures make this method hard to standardize. On the other hand, the molecular characterization of *Pyricularia oryzae* monoconidial isolates yields an objective description of the individuals and their relatedness.

A family of *Magnaporthe grisea* repeated sequences (MGR sequences) was found in a high copy number (approximately 40 to 50 copies per genome) in isolates that infect rice (Hamer et al 1989). One of these repeated sequences, MGR586, has been used for phylogenetic analyses, pathotype variation and stability, estimates of strain relatedness (Levy et al 1991b), and genetic mapping of important genes in this fungus (Hamer 1991). A representative set of blast fungus isolates on rice collected in the southeastern region of the United States over a 30-year period was used to study pathotype variation and evolution (Levy et al 1991b). Another study was conducted in Colombia, where a large collection of isolates from a "blast hot spot" was analyzed (Levy et al 1991a). These and other studies in Asia (Borromeo et al 1993, Chen et al 1995, Zeigler et al 1995) support the hypothesis that isolates from a defined region form several discrete lineages, each of which has a specific and limited virulence spectrum. Such a relationship between lineages and virulence coupled with the identification of the resistant hosts for each lineage should help design a strategy for durable resistance.

## Materials and methods

Isolates were taken from lesions present on field-collected infected plants of different rice cultivars grown commercially and in experimental nurseries in Argentina, southern Brazil, and Uruguay. Samples were collected during the summers of 1994, 1997, and 1998 and most of the lesions collected were from neck-infected panicles after flowering. Plant tissue was thoroughly washed and placed in a high-moisture chamber to facilitate sporulation of *P. oryzae*. A monoconidial isolate was obtained using light microscopy and grown on rice polish agar plates. Samples of each isolate were stored on dried filter papers and a small piece was cultured in 250-mL flasks with 125 mL of complete liquid media to obtain enough fungus tissue for DNA isolation.

DNA was isolated following the CTAB protocol of Zeller and Levy (1994). Three micrograms of chromosomal DNA were digested with Eco*RI* and fractionated by electrophoresis on 0.8% agarose gels. A nonpathogenic strain of DNA digested with the same restriction enzyme was used as a reference standard that exhibited an

MGR-DNA profile of 30 RFLPs (restriction fragment length polymorphisms) of known length ranging from 0.9 to 38.2 kb (Levy et al 1993). The electrophoresed DNA was blotted to Hybond$^+$ membranes (Amersham Corp.). Electrophoresis and Southern blotting were carried out according to Sambrook et al (1989). A nonradioactive-labeled probe, MGR586, using the ECL (Amersham Corp.) direct nucleic acid labeling and detection system was hybridized to DNA blots to obtain the MGR-DNA fingerprints on blue-light-sensitive film.

The MGR-DNA RFLPs were visually scored as present or absent to build up the RFLP profile of each isolate. Groups on the basis of obvious similarities were made and variation within a profile group was evaluated from pairwise comparisons of the proportion of shared RFLPs between fingerprints. Nei and Li's index of genetic similarity (*Sxy*) for RFLP comparisons was calculated. The small range of RFLP variation within groups and the large distance between groups allowed the use of a consensus MGR-DNA profile fingerprint for each group in order to describe the structure of the population sampled.

## Results

A total of five MGR fingerprint groups have been found on the luminographs from the rice-infecting isolates collected in southern Brazil, Argentina, and Uruguay during the 1994, 1997, and 1998 rice seasons (Table 1). Two groups occurred more frequently than the others did throughout years, localities, and cultivars. The isolates from tropical cultivars expressed a common profile of about 66 bands (Fig. 1) and an *Sxy* ranging from 0.992 to 0.86 (Table 2), indicating that they belonged to one lin-

**Table 1. Cultivars and breeding lines collected by year and country.**

| Cultivar | Year | Country | Cultivar | Year | Country |
|---|---|---|---|---|---|
| Maybelle | 1994 | Argentina | El Paso144 | 1994 | Argentina |
| Tacuarí | 1997 | Uruguay | El Paso144 | 1997 | Uruguay |
| L1070 | 1997 | Uruguay | Chui | 1997 | Brazil |
| Lacasine | 1994 | Argentina | Bluebelle | 1996 | Argentina |
| Tacuarí | 1998 | Argentina | El Paso144 | 1998 | Argentina |
| Jackson | 1997 | Argentina | Yerbal | 1997 | Brazil |
| H121 | 1994 | Argentina | Don Juan | 1997 | Argentina |
| Epagri107 | 1998 | Argentina | El Paso144 | 1997 | Argentina, Brazil |
| Chui | 1996 | Argentina | IRGA416 | 1997 | Brazil |
| H205 | 1994 | Argentina | Bluebelle | 1998 | Uruguay |
| IRGA409 | 1998 | Argentina | El Paso144 | 1994 | – |
| 5CT | 1996 | Argentina | IRGA417 | 1997 | Brazil |
| Tacuarí | 1994 | Argentina | Caraguatá | 1998 | Uruguay |
| RP2 | 1998 | Argentina | L1119 | 1997 | Uruguay |
| IRGA409 | 1997 | Brazil | IRGA417 | 1996 | Brazil |
| El Paso227 | 1994 | Argentina | Tacuarí | 1998 | Uruguay |
| El Paso144 | 1998 | Uruguay | Fany | 1997 | Uruguay |
| El Paso144 | 1997 | Brazil | | | |

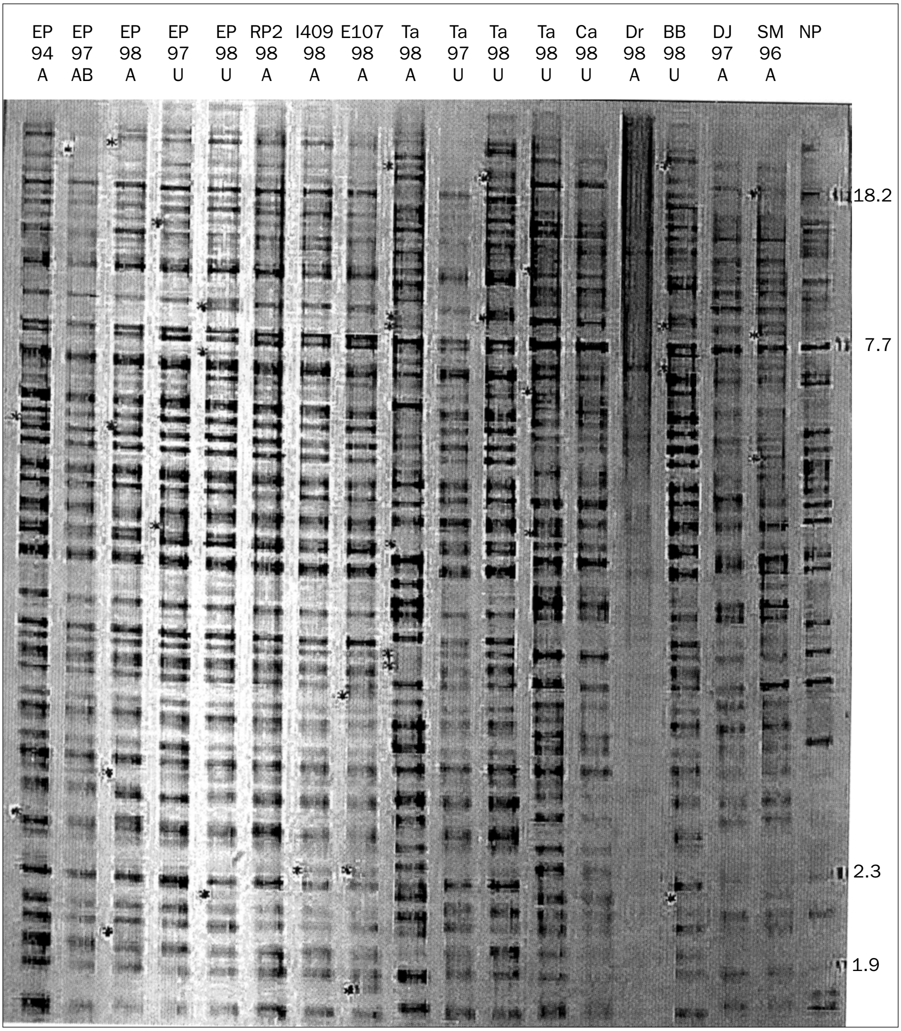

**Fig. 1. MGR fingerprints of *Pyricularia grisea* isolates from Brazil, Argentina, and Uruguay.**

eage. The second group was formed by three related lineages commonly found on American cultivars (Fig. 2), each 95% similar to each other but all >70% different from the lineage on tropical cultivars. The isolates from the Argentinean cultivar Don Juan showed only the profile belonging to a lineage common to all American varieties, whereas the isolates from the old Texas cultivar Bluebelle showed both profiles—but in different years.

The other three MGR fingerprints have been found on isolates from a Brazilian cultivar, Chui (not shown), and two Uruguayan cultivars, INIA Tacuarí and INIA

**Table 2. Index of genetic similarity (*Sxy*) in % (Nei and Li) of representative isolates of *Pyricularia grisea* from southern Brazil, Argentina, and Uruguay.**

| Cultivar | Year | EP | EP | EP | EP | EP | RP2 | I409 | E107 | Ta | Ta | BB | Ta | Ca | DJ | SM | Ta |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EP144 | 1994 | 1 | | | | | | | | | | | | | | | |
| EP144 | 1997 | 98 | 1 | | | | | | | | | | | | | | |
| EP144 | 1998 | 96 | 96 | 1 | | | | | | | | | | | | | |
| EP144 | 1997 | 97 | 98 | 96 | 1 | | | | | | | | | | | | |
| EP144 | 1998 | 96 | 97 | 95 | 96 | 1 | | | | | | | | | | | |
| RP2 | 1998 | 98 | 99 | 97 | 99 | 98 | 1 | | | | | | | | | | |
| IRGA409 | 1998 | 98 | 99 | 96 | 98 | 97 | 99 | 1 | | | | | | | | | |
| Epagri107 | 1998 | 96 | 97 | 95 | 96 | 96 | 98 | 99 | 1 | | | | | | | | |
| Tacuarí | 1997 | 98 | 99 | 97 | 99 | 98 | 99 | 99 | 98 | 1 | | | | | | | |
| Tacuarí | 1998 | 97 | 99 | 96 | 97 | 98 | 99 | 98 | 96 | 99 | 1 | | | | | | |
| Bluebelle | 1998 | 96 | 96 | 97 | 96 | 99 | 97 | 96 | 95 | 97 | 97 | 1 | | | | | |
| Tacuarí | 1998 | 26 | 26 | 24 | 24 | 29 | 26 | 27 | 27 | 26 | 27 | 30 | 1 | | | | |
| Caraguatá | 1998 | 26 | 26 | 25 | 25 | 29 | 27 | 28 | 28 | 28 | 28 | 31 | 98 | 1 | | | |
| Don Juan | 1997 | 12 | 10 | 10 | 10 | 13 | 10 | 12 | 12 | 10 | 12 | 13 | 70 | 71 | 1 | | |
| SM | 1996 | 12 | 10 | 10 | 10 | 15 | 10 | 11 | 11 | 10 | 13 | 15 | 68 | 69 | 97 | 1 | |
| Tacuarí | 1998 | 25 | 23 | 23 | 23 | 26 | 24 | 23 | 23 | 24 | 23 | 26 | 74 | 75 | 75 | 74 | 1 |

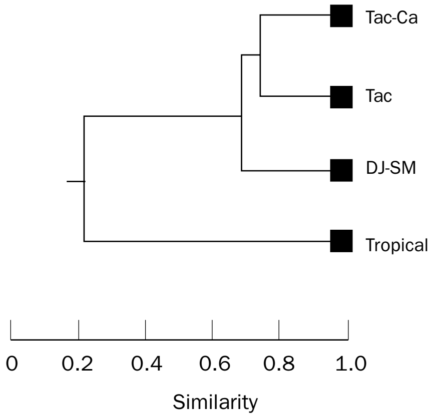

**Fig. 2. Dendrogram of the rice blast isolates from the Southern Cone population (Tac = Tacuarí, Ca = Caraguatá, DJ = Don Juan, SM = San Miguel isolates).**

Caraguatá. Only one MGR fingerprint was found on isolates from the most commercially grown cultivars, El Paso 144 and IRGA409. These cultivars have a common origin from a CIAT (Centro Internacional de Agricultura Tropical) population. Also, isolates from other tropical cultivars such as IRGA417, IRGA416, and Chui showed the same profile (not shown).

The five MGR fingerprint groups allowed the identification of discrete groups of *P. oryzae* isolates and defined lineages on the basis of a low index of genetic similarity (Table 2, Fig. 2) between members of different lineages and the high values of the members in each lineage.

## Conclusions

MGR-RFLP has been used successfully in understanding the population structure of the rice blast fungus in different regions. In this study, five discrete lineages were detected in southern Brazil, Argentina, and Uruguay. However, only one was isolated from tropical cultivars, which are the most extensively grown. The presence of other isolates belonging to different lineages at the same sites and times but on other hosts suggests a specific susceptibility of those tropical cultivars to that particular fungal lineage. At the same time, this association also suggests resistance to the other lineages found in the region. Only one isolate from the tropical cultivar Chui (not shown) showed a profile completely different from that of the others.

Comparisons made with other *P. oryzae* isolate fingerprints from Latin America showed a high similarity between the El Paso 144 associated lineage in the Southern Cone and a lineage (SRL-2) from Colombia, for which a source of incompatibility (resistance gene *Pi-ta* from Yashiro-mochi) is known. We are testing the possibility that the addition of that gene to El Paso 144 will complete the spectrum of resistance (i.e., exclude all regional pathogen lineages) necessary to provide a more durable protection.

Since El Paso 144 and IRGA409 are the cultivars that have the largest area planted in the region, a backcross program began in 1999 to introduce the *Pi-ta* gene from Yashiro-mochi into El Paso 144.

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# White tip disease in rice culture under temperate climate

I. Simon-Kiss and A. Szitó

The ectoparasite *Aphelenchoides besseyi* or rice leaf nematode sucks plant cell plasma and thus damages plants, opening the way for other pathogens. Infected plants are initially symptom-free; however, in the case of high worm density, a mosaic pattern of yellow patches may be observed on rice leaves from the plants' 4–6-leaf stage on. Later, the tips of the upper leaves become white and dry, hence the name of the disease. The spikelet of the plant remains entirely enclosed in the leaf sheath, the whole structure is enlarged into a spindle-shaped swelling, and it becomes distorted. A group of spikelets remains sterile and a high proportion of fertile seeds is infected with nematodes. Spikelet sterility, distorted and small panicles, and deformed kernels lead to a decrease in yield. These nematodes can be carried in the seed. Different seed treatment methods will be summarized and evaluated. Seeds can be treated with hot water at 56–57 °C for 10–15 min or at 54 °C for 10 min. An acid seed dressing followed by alkaline treatment can also be applied for neutralization. Fumigation of seeds with methyl bromide ($CH_3Br$) at 30 g $m^{-3}$ for 18 h at 16–18 °C was 96–97% effective in field experiments against *A. besseyi* nematodes. Chemical controls with nematicides have also been used worldwide. The results of our trials are presented for the nematicides $PH_3$ (phosphine) and methylbromide.

The species *Aphelenchoides besseyi* or rice leaf nematode causes "white tip" disease. It is a cosmopolitan species and causes significant damage to crops everywhere. It mainly spreads via seeds collected for sowing, but it also survives on infected plant tissues and grains left in the rice field. In the temperate zone, it overwinters in anabiosis and it keeps its biotic potential for years. It activates itself when rice germinates, subsists on the youngest parts of the rice plant, and reproduces there, too. It lays its eggs between the leaf sheath and the stem. In the vegetation period, 10 or even more generations may develop. One generation needs 2 or 3 wk for development, depending on the temperature. It needs a minimum temperature of 13 °C and the optimum is from 23 to 30 °C.

The nematodes move about and may transfer across the wet leaves that touch each other. Their spread is also aided by water motion. Some weeds serve as host plants.

Being an ectoparasite, *A. besseyi* sucks the plant cell plasma and thus damages the plants, opening the way for other pathogens. Infected plants are initially symptom-free; however, in the case of high nematode density, a mosaic pattern of yellow patches may be observed on rice leaves from the plants' 4–6-leaf stage on. Later, the tips of the upper leaves become white and dry, hence the name of the disease. The panicles of severely diseased tillers remain partly or entirely enclosed in the leaf sheath, the whole structure is enlarged into a spindle-shaped swelling as it develops, and it becomes distorted. A group of spikelets remains sterile and a high proportion of fertile seeds is infected with threadworms. Spikelet sterility, distorted and small panicles, and deformed grains lead to a significant decrease in yield (Butler 1919, Fernando 1993, Fortuner 1970, Fortuner and Merny 1973, Fortuner and Jacq 1976, Goto and Fukatsu 1952, Grist 1965, Huang and Chiang 1975, Ou 1985). The occurrence of rice nematode was reported first by Javor (1970) in Hungary.

For control, the following methods can be used: burning the stubble (prevention), plowing, varying monoculture with crop rotation, sowing noninfected seeds, and treating seeds. Seeds can be treated with hot water at 56–57 °C for 10–15 min (Ichinoche 1972, Yoshii and Yamamoto 1951) or at 54 °C for 10 min (Tichonowa et al 1975), or they can be given hot aeration at 55–60 °C (Budai and Elekes 1978). The treatment involved some risk of injury to seed germination unless the temperature was precisely maintained. Acid seed dressing followed by alkaline treatment for neutralization may be used (Simon-Kiss 1983, Simon-Kiss and Szepessy 1987). Gergon and Prot (1993) used benomyl and carbofuran treatments. Fumigation of seeds with methyl bromide ($CH_3Br$) at 30 g $m^{-3}$ for 18 h at 16–18 °C was 96–97% effective in field experiments against *A. besseyi* nematodes (Javor 1973).

## Materials and methods

Cultivars Dunghan Shali, Nucleoryza, Ringola, Oryzella, Cristalava, Ringola1, Ringola2, Dama1, Dama2, Sandora, and Risabell were investigated with two kinds of fumigation treatments to eliminate rice nematode (*A. besseyi*) infection. The infection level was determined by the Tichonowa scale (Tichonowa et al 1975). A 50-50% mixture of rice and maize kernels was placed in 26 petri dishes. Ten replications were treated with phosphine ($PH_3$) at 1 g $m^{-3}$ and the other 10 samples with phosphine at 3 g $m^{-3}$ for 96 h.

The air temperature fluctuated from 20 to 25 °C and the relative humidity from 80% to 87%, and six samples were used as controls during the experiment.

After the treatments were finished, the samples were aerated for 24 h. The samples were filled with hot water at 56–57 °C for nematode incubation for 24 h. Dead and live nematodes were counted and the seed samples cultured for 7 d to determine their germination ability after treatment, along with the controls (Tables 1–4).

**Table 1. Effect of treatments against the rice nematode on different rice cultivars.**

| Cultivar | Year harvested | Infection scale[a] |  |  |
|---|---|---|---|---|
|  |  | Before treatment | Treated with (worms 50 grains$^{-1}$) |  |
|  |  |  | Methyl bromide | Phosphine |
| Dunghan Shali | 1993 | Very heavy | 4 | 7 |
| Nucleoryza | 1993 | Heavy | 1 | 3 |
| Ringola | 1994 | Heavy | 0 | 5 |
| Oryzella | 1995 | Heavy | 0 | 3 |
| Cristalava | 1995 | Moderate | 2 | 8 |

[a]Source: Tichonowa et al (1975).

Seeds were fumigated with methyl bromide ($CH_3Br$) at 30 g m$^{-3}$ for 96 h for cultivars Dunghan Shali, Nucleoryza, Ringola, Oryzella, Crystalava, Dama, and Sandora. After treatment, we aerated the samples for 24 h and performed the nematode and germination ability test, detailed above (Tables 1–4).

## Results

The methyl bromide treatment was more effective than the phosphine treatment. All the nematodes died in the samples treated with methyl bromide in cultivars Ringola and Oryzella, but some survived the treatment in Nucleoryza, which was as much infected as the previously mentioned cultivars. The effect of the treatment was less in Dunghan Shali (heavily infested) and Cristalava (lightly infested). Nematodes stayed alive for 5 years in seeds (Table 1).

Cultivars Dama1 and Risabell were not resistant to the nematodes because the deformed seeds were slightly or intermediately infected. The weight of the deformed grains was 25–59% lower than that of the normal ones (Table 2). The average data showed that cultivars produced in 1997 were intermediately and heavily infected by *A. besseyi* in Hungary, excluding Risabell, which had a low infection. The germination ability fluctuated from 86% to 99% and it was independent of the infection level (Table 3). The loss of weight in deformed grains varied from 25% to 53% (Table 2).

## Conclusions

The nematode *Aphelenchoides besseyi* remained alive for 5 years in infected seeds. Methyl bromide was most effective in decreasing the infection levels although phosphine also decreased nematode infection. This nematode infected all rice cultivars. The infection level depended on floodwater depth and plant density.

**Table 2. Rice nematode infection of different cultivars in Hungary, 1997 (mean of the repetitions).**

| Cultivar | Rate of deformed grains in culture (%) | Nematodes 100 g$^{-1}$ grain |  | Rate of deformed grains (%) | Infection (Tichonowa scale) |  | 1,000-grain wt. (g) |  | Loss of weight in deformed grains (%) |
|---|---|---|---|---|---|---|---|---|---|
|  |  | GSL[a] | GD |  | GSL | GD | GSL | GD |  |
| Ringola1 | 2.8 | 580 | 7,000 | 91.7 | Moderate | Heavy | 34.5 | 20.0 | 42.0 |
| Ringola2 | 2.2 | 4,824 | 31,500 | 84.7 | Heavy | Very heavy | 34.0 | 16.0 | 52.9 |
| Dama1 | 9.4 | 0 | 2,353 | 100.0 | None | Moderate | 34.0 | 25.5 | 25.0 |
| Dama2 | 2.5 | 1,894 | 13,000 | 85.4 | Moderate | Heavy | 33.9 | 14.0 | 58.7 |
| Sandora | 5.0 | 600 | 24,666 | 37.6 | Moderate | Very heavy | 31.2 | 15.0 | 51.9 |
| Risabell | 6.2 | 0 | 70 | 100.0 | None | Weak | 32.0 | 19.0 | 40.6 |

[a] GSL = grain symptomless, GD = grain deformed.

**Table 3. Mean nematode infection and germination ability of rice seeds produced in 1997.**

| Cultivar | Grain infection (nematodes 100 g$^{-1}$ grain) | Infection (Tichonowa scale) | Germination ability (%) | 1,000-grain wt. (g) |
|---|---|---|---|---|
| Ringola1 | 2,355 | Moderate | 96.2 | 34.1 |
| Ringola2 | 9,958 | Heavy | 98.0 | 33.6 |
| Dama1 | 892 | Moderate | 86.4 | 33.2 |
| Dama2 | 3,683 | Heavy | 98.1 | 33.4 |
| Sandora | 6,081 | Heavy | 94.3 | 32.0 |
| Risabell | 20 | Initial | 99.0 | 31.2 |

**Table 4. Treatment of nematode infections on rice cultivars produced in 1998.**

| Cultivar | Germination (%) | | | Nematode infection (nematodes 50 grains$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Methyl bromide | Phosphine | Control | | Methyl bromide | | Phosphine | |
| | | | | Dead | Live | Dead | Live | Dead | Live |
| Ringola1 | 91 | 94 | 96 | 30 | 15 | 0 | 0 | 11 | 2 |
| Ringola2 | 93 | 96 | 97 | 15 | 1 | 17 | 9 | 2 | 8 |
| Ringola3 | 97 | 95 | 94 | 10 | 2 | 2 | 1 | 0 | 10 |
| Mean | 93.6 | 95 | 95.6 | 18.3 | 6.0 | 6.3 | 3.3 | 4.3 | 6.6 |
| Dama1 | 89 | 91 | 90 | 30 | 24 | 16 | 8 | 12 | 4 |
| Dama2 | 96 | 95 | 93 | 35 | 28 | 21 | 16 | 45 | 28 |
| Dama3 | 91 | 93 | 91 | 35 | 9 | 37 | 22 | 81 | 40 |
| Mean | 92 | 93 | 91.3 | 33.3 | 20.3 | 24.6 | 15.3 | 46.0 | 24.0 |
| Sandora1 | 93 | 96 | 94 | 15 | 0 | 9 | 3 | 23 | 16 |
| Sandora2 | 91 | 93 | 92 | 0 | 12 | 1 | 10 | 3 | 10 |
| Sandora3 | 95 | 93 | 91 | 10 | 3 | 4 | 2 | 5 | 13 |
| Mean | 93 | 94 | 92.3 | 8.3 | 5.0 | 4.6 | 5.0 | 10.3 | 13.0 |

Laboratory experiments showed that the germination ability of rice seeds was independent of nematode density. The infection in deformed seeds, which originated from panicles with typical infection symptoms, was markedly higher than in normal ones (Tables 2 and 3). We concluded that infection and crop loss might be decreased considerably with suitable cleaning and/or sorting of the paddy grain.

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# A recurrent selection strategy for durable resistance to rice blast (*Magnaporthe grisea*) disease

M.J. Vales, M.-H. Chatel, J. Borrero, D. Delgado, J. Dossman, Y. Ospina,
E. Tulande, M. Triana, R. Meneses, V. Kuri, M.C. Duque, and J. Silva

An original scheme allows recurrent selection for complete and partial rice blast (caused by *Magnaporthe grisea*) resistances, and for agronomic traits. Complete resistance is selected in greenhouse S1 lines and in the field during genetic recombination. Partial resistance and agronomic traits are evaluated in the field in S2 lines without complete resistance. Grain quality and resistance to *Tagosodes orizicolus* are evaluated in S3 seeds and S3 lines, respectively.

At least two nonexclusive strategies against rice blast are possible and used in rice breeding programs. The first employs the strategy of lineage exclusion using monogenic, complete, and specific resistances. The International Center for Tropical Agriculture (CIAT) developed the methods needed for this strategy (Levy et al 1993, Gibbons et al 1998). Complete resistance is considered most effective, but some rice lines escape low-frequency virulent *Magnaporthe oryzae* strains during the selection process (Vales et al, this volume) and this type of resistance may be overcome (break down) in time through shifts in the pathogen population.

The second strategy was developed by the Centre de Cooperation Internationale en Recherche Agronomique pour le Développement (CIRAD) and is used to limit the consequences of complete resistance breakdown. To increase the likelihood of durability, this second type of resistance against rice blast must be general, partial, and polygenic (Vales 1983, 1987). Polygenism will require the use of recurrent selection methods (Vales 1983, 1984, 1998a).

## Materials and methods

We used 13 strains of *Pyricularia oryzae* (*Magnaporthe grisea*) from seven lineages isolated from the Experimental Station of Santa Rosa (EESR), Villavicencio, Meta, Colombia (Correa-Victoria et al 1997, Flor Payan 1998).

The indica recurrent population PCT-6\HB has a recessive male-sterility gene and was also selected for resistance to the hoja blanca virus (RHBV) (Borrero Correa et al 1998). It has complete resistance against the 13 *P. oryzae* strains previously mentioned. This original recurrent selection scheme (Vales 1998a,b) employs this population and has three parts with parallel execution and common genetic recombinations (Fig. 1).

In greenhouse selection, rice plants with complete resistance against the seven lineages of *P. oryzae* continue to be indentified under controlled conditions. S1 lines with broad complete resistance are then used for the next genetic recombination.

During this recombination, a massal field selection is made for complete resistance against a natural *P. oryzae* population in a rice blast "hot-spot" field located near Santa Rosa.

S1 plants showing no complete resistance against a given *P. oryzae* strain are multiplied. The S2 lines are then selected in the field for partial leaf and neck blast resistance using this pathogenic strain as inoculum. Different *P. oryzae* strains are used for subsequent recurrent cycles to avoid selection of highly specific partial resistance. Agronomic traits are simultaneously selected in the field as well. S3 seeds derived from the best S2 plants are used for grain quality analysis and in greenhouse trials for resistance to *Tagosodes orizicolus*. Afterward, the best S3 seeds are used for the next genetic recombination.

To avoid risks of genetic drift and breakdown of polymorphism, a population sample must be maintained by successive harvest of male sterile plants. The S0 seeds are used for genetic recombinations.

## Results and discussion

Significant genetic progress for selection of rice plants with complete resistance to *P. oryzae* was made after the first cycle of selection and recombination (Fig. 2).

One and a half recurrent selection cycles have been completed thus far. When the concentration of complete resistance genes is considered sufficient, it will then be possible to fix the population for this type of resistance. Successive samples of this fixed population will be further defined by drastic recurrent selection. Finally, it should be possible to rapidly develop elite breeding lines from this enhanced population through conventional pedigree selection.

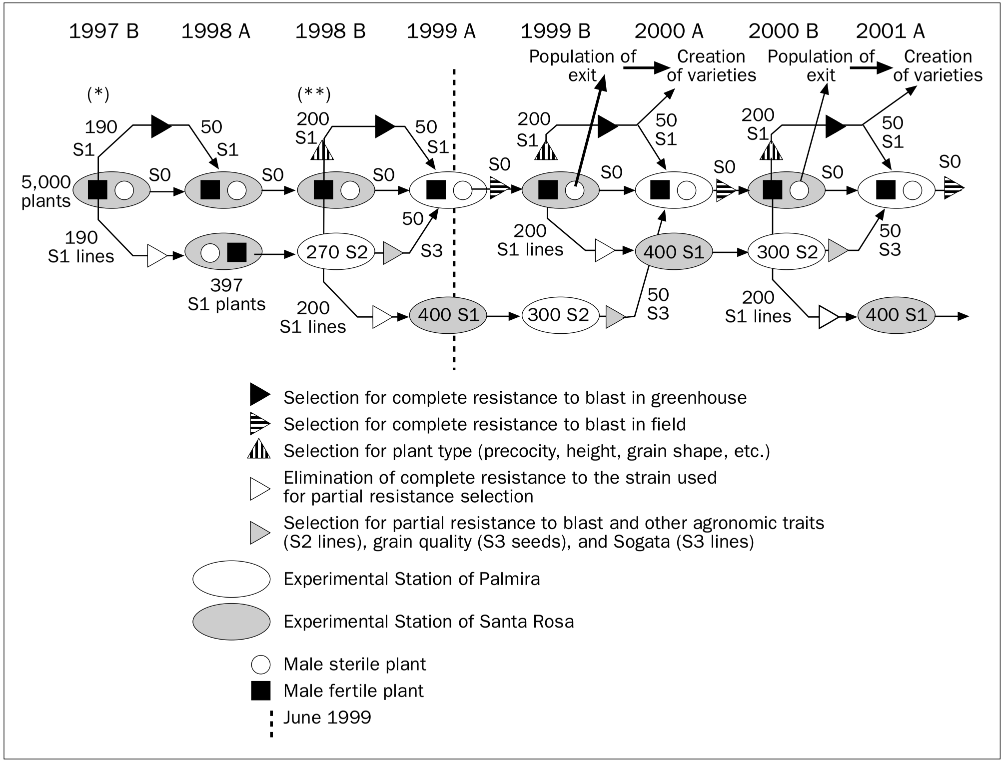

**Fig. 1. Recurrent selection in the population PCT-6\HB for durable resistance to blast and other agronomic traits.**

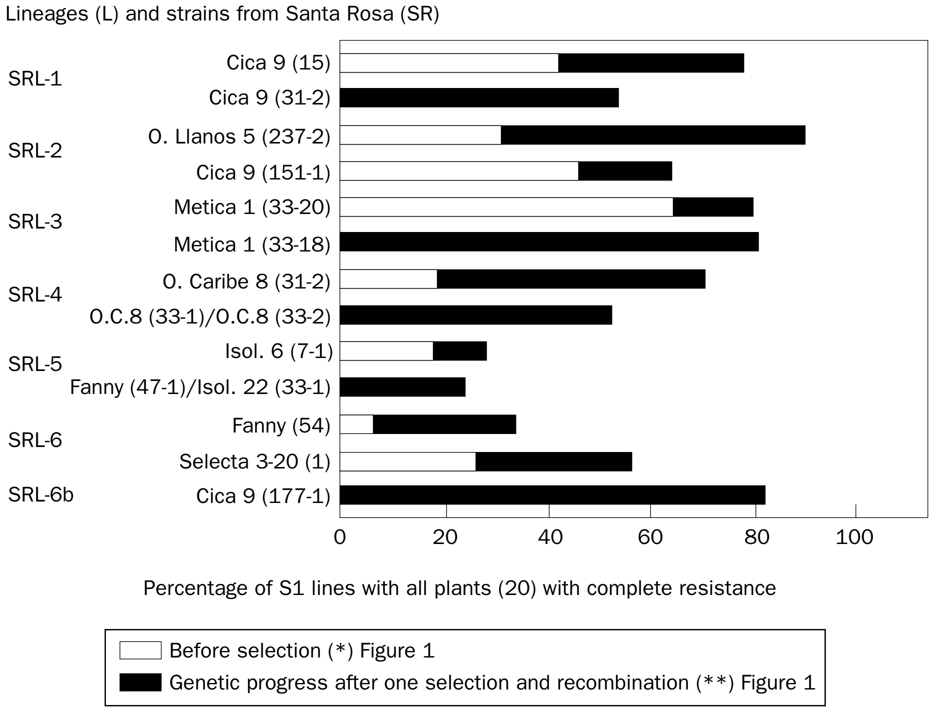

**Fig. 2. Genetic progress for complete resistance to blast in the population PCT-6\HB.**

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# A pedigree selection strategy for durable resistance to rice blast disease

M.J. Vales, J. Borrero, D. Delgado, Y. Ospina, E. Tulande, J. Gibbons, and D. González

An original scheme uses conventional pedigree selection for complete and partial resistance to rice blast (caused by *Magnaporthe grisea*). Complete resistance is selected in both the field and greenhouse since some plants escape low-frequency virulent strains of *M. grisea* in field trials. Partial resistance is evaluated in the field using progenies of plants without complete resistance.

At least two nonexclusive genetic strategies against rice blast (caused by *Magnaporthe grisea*) are possible and used in rice breeding programs. The first is the strategy of lineage exclusion using monogenic, complete, and specific resistances (Levy et al 1993, Correa-Victoria et al 1997, Gibbons et al 1998). The second limits the consequences of a breakdown in complete resistance by shifts in the pathogen population. To increase the likelihood of long-term resistance durability, it should be general, polygenic, and partial (Vales 1983, 1987).

It is not possible to observe partial resistance if all plants express complete resistance. It is also not possible to maintain genes with complete resistance if all plants in a population have lost those genes. Only the descents of heterozygous plants for complete resistance show plants with and without complete resistance. So, only heterozygous plants allow pedigree selection for both partial and complete resistance (Vales 1984).

## Materials and methods

The best 95 S2 lines from the Fondo Latinoamericano para el Arroz de Riego (FLAR) were selected for complete resistance in field trials against a natural *Pyricularia oryzae* (*M. grisea*) population as well as for desirable agronomic traits and grain quality.

Thirteen strains of *P. oryzae* from the seven lineages isolated from the Experimental Station of Santa Rosa (EESR), Villavicencio, Meta, Colombia, were used. The virulence spectrum for each fungus lineage was accounted for by using two complementary strains.

This original pedigree selection scheme (Fig. 1) allows efficient detection of lines with complete and/or partial resistance to rice blast disease.

For greenhouse selection, the S2 lines were inoculated with 13 *P. oryzae* strains of the seven lineages previously mentioned. S2 lines were selected for segregating response against one of the strains and for complete resistance. Plants with and without complete resistance against this strain were transplanted and the seeds harvested.

Field selection was done for complete resistance against a natural *P. oryzae* population in the EESR "hot-spot" field using S3 descents of the plants with complete resistance against the strain of *P. oryzae* used in the greenhouse studies. Agronomic traits and grain quality were also selected for and seeds of the best plants were harvested.

In a field trial, the S3 descents of plants without complete resistance against *P. oryzae* were selected for partial leaf and neck blast resistance using one strain of the fungus for inoculum.

The S4 lines used for the next selection cycle will come from the best S3 plants that showed complete resistance, desirable agronomic traits, and grain quality in the field trial, and from the best S3 families found to have partial resistance.

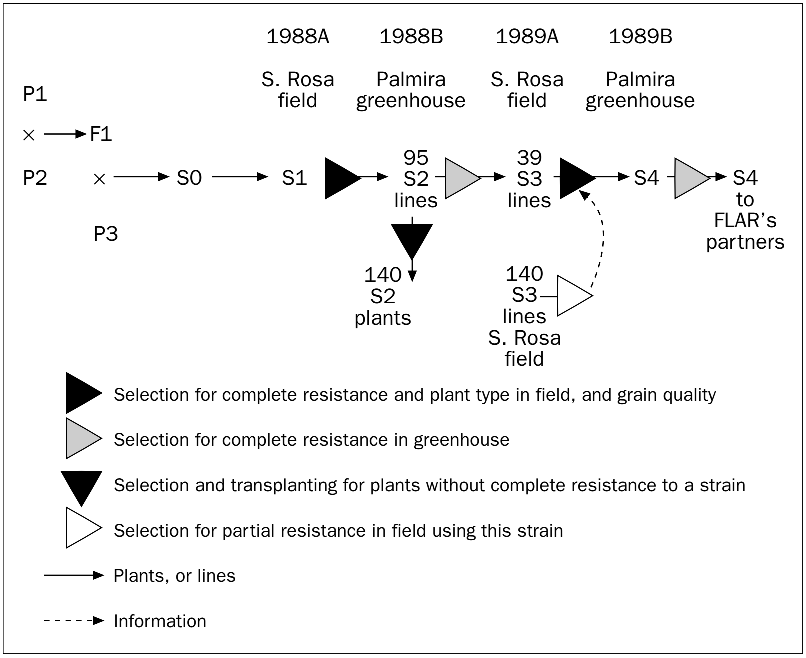

**Fig. 1. Pedigree selection for complete and/or partial rice blast resistance.**

## Results and discussion

It was observed that rice plants often escaped infection in field trials even though they were susceptible to *P. oryzae* strains present in the study area. Approximately 22% of the S1 plants escaped existing virulent strains (Table 1). The percentage of escape was greater for *P. oryzae* strains occurring at low frequency (Table 2). A strain had a low frequency in the field if its lineage also had a low frequency and/or a broad

**Table 1. Escape of S1 plants based on evaluation of S2 progenies.**

| Lineage | Strain | % of escape[a] | |
|---|---|---|---|
| SRL-6 | Fanny 54 | 0.00 | 4.21 |
| | Selecta 3-20(1) | 4.21 | |
| SRL-5 | Isolinea 6(7-1) | 10.53 | 12.36 |
| | Isolinea 22(3-1) | 8.42 | |
| SRL-4 | O. Caribe 3(31-2) | 3.16 | 5.26 |
| | O. Caribe 8(33-2) | 4.21 | |
| SRL-3 | Metica 1(33-18) | 0.00 | 0.00 |
| | Metica 1(33-18) | 0.00 | |
| SRL-2 | O. Llano 5(237-2) | 1.05 | 0.05 |
| | Cica 9(151-1) | 0.00 | |
| SRL-1 | Cica 9(15) | 0.00 | 0.00 |
| | Cica 9(31-2) | 0.00 | |
| SRL-6b | Cica 9(177-1) | 4.21 | 4.21 |

[a]From 95 progenies of S1 plants, 20 plants were used for each S2.

**Table 2. Relationship between the escape of S1 plants and frequency and virulence spectrum of lineages.**

| Lineage[a] | % of escape in S1 |
|---|---|
| Lineages with low frequency and broad virulence spectrum | |
| SRL-5 | 12.63 |
| SRL-6b | 4.21[b] |
| Lineages with high frequency and broad virulence spectrum | |
| SRL-4 | 5.26 |
| SRL-6 | 4.21 |
| Lineages with narrow virulence spectrum | |
| SRL-2 | 1.05 |
| SRL-3 | 0.00 |
| SRL-1 | 0.00 |

[a]Correa-Victoria et al (1997). [b]With one strain.

spectrum of virulence. In the greenhouse, we used strains with the broadest possible spectrum of virulence. It was hypothesized that *P. oryzae* strains with broad virulence occurred at low frequency in the field because of the genetic costs of the virulence. With only 13 *P. oryzae* strains, it was not possible to represent all the pathogen population variability, so, even in inoculated greenhouse trials, plants believed to be immune will likely be susceptible to other strains of the fungus present in the field. Regardless, both inoculated greenhouse and natural field selections are considered necessary for developing breeding lines with the greatest degree of durable blast resistance.

Thirty-nine of the original 95 S2 rice lines were segregating for complete resistance against the *P. oryzae* strain Isol 22 (3-1) of the lineage SRL-5. From these 39 S2 lines, 691 plants were transplanted with 451 showing complete resistance and 140 without complete resistance to the strain Isol 22 (3-1) in greenhouse studies. Selections showing complete resistance in the greenhouse are now in a field selection trial for complete resistance against a natural *P. oryzae* population. Those without complete resistance in the greenhouse tests are in a field selection trial for partial resistance against the strain Isol 22 (3-1).

S4 lines with complete and/or partial resistance to blast and having desirable agronomic traits will be provided to FLAR's partners.

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# Responses of blackbirds to aerial application of Flight Control bird repellent to ratoon rice in Cameron Parish, Louisiana

M.L. Avery, J.S. Humphrey, and E.A. Tillman

Blackbird damage to ripening rice is an economically important problem for many producers in Louisiana and elsewhere. Currently, management options for dealing with this problem are limited and generally ineffective. One possible option is the application of a chemical feeding deterrent. In October 1998, we tested the commercial bird repellent Flight Control, which has anthraquinone as its active repellent ingredient. Blackbird use of a 4-ha plot of ratoon rice treated with Flight Control at a rate of 18.7 L $ha^{-1}$ declined dramatically and birds stayed off the plot for 7 d postspray. These results corroborate those obtained in a similar trial in 1997 and suggest that Flight Control can be an effective component of blackbird damage reduction strategies in ripening rice.

Red-winged blackbirds (*Agelaius phoeniceus*) and related species cause millions of dollars of damage to rice annually in southeastern United States (Wilson et al 1989, Decker et al 1990). While recent research efforts to alleviate this problem have focused on the newly planted crop (e.g., Holler et al 1982, Wilson et al 1989, Decker et al 1990), losses to the ripening crop are considerable. Although the level of damage has not been measured, estimated losses in Texas alone exceed $8 million annually (M.O. Way, Texas A&M University, Beaumont, Texas, personal communication). Furthermore, blackbirds also damage wild rice, a valuable and rapidly growing crop in northern California (Gorenzel et al 1986).

When confronted with blackbird damage to ripening rice, rice producers have few options. Recommended practices include harassment with various scare devices and noisemakers and shooting (Dolbeer 1994). Chemical feeding deterrents constitute another set of potential blackbird management options, but as yet no compound is registered for use in ripening rice.

For blackbird control in ripening rice, a chemical repellent must be cost-effective and environmentally benign. The latter is particularly important because rice is grown in water and toxicity to aquatic organisms is often a major concern. One candidate compound that appears to meet these criteria is Flight Control$^{TM}$, a product developed by Environmental Biocontrol International (EBI), Wilmington, Delaware. This product contains 50% anthraquinone as the active ingredient. Anthraquinone is an effective blackbird repellent when applied to rice seed (Avery et al 1997, 1998), and Flight Control is registered for use as a bird repellent on turf.

In a preliminary trial conducted during October 1997, Flight Control applied at 18.7 L ha$^{-1}$ discouraged bird use of a 4-ha plot of ratoon rice for several days (M.L. Avery, unpublished data). On the basis of that result, we felt that further evaluation of Flight Control was warranted to determine blackbird responses to treatment on ripening ratoon rice, to document residues on rice grains, and to establish relationships between application rate and residue of the seed.

## Methods

The test site in Cameron Parish, Louisiana, was selected because of consistent bird pressure (approximately 500–1,500 blackbirds) on the site. The 28-ha field that we used had been harvested using a stripper-header combine and was approximately 8 km from the nearest field of ripening rice. In the part of the field where we observed the most bird activity, we delineated two 201 × 201-m plots separated by a 31 × 201-m buffer. We selected the west plot for treatment because of its higher use by birds. There appeared to be a roost established beyond the field to the west as birds usually arrived from and departed in that direction. From 1 d before treatment to 7 d posttreatment, we observed the plots twice daily (between 0700–1000 and 1500–1800) for 2 h. We monitored bird activity within the test site and recorded the total number of birds in each plot at 5-min intervals.

The treatment was applied late in the morning on 15 October 1998. The sky was clear with a light wind from the east. The application consisted of 76 L of EBI Flight Control, 3.8 L of Latron CS-7 (Rohm and Haas, Philadelphia, Pennsylvania) sticker, and 299 L of water. The treatment was applied aerially at 93 L ha$^{-1}$. The aircraft used 33 number 12 Lund nozzles, which covered a 15.3-m swath.

We established four north-south transects within the treated plot and randomly located two sampling points on each transect. At each sampling point, we clipped five rice panicles 1 d before treatment and 1 h and 1, 3, 7, and 14 d posttreatment. The clipped panicles were stored in an air-conditioned room and seeds from these panicles were subsequently sent to EBI for determination of anthraquinone residues. Differences among sampling dates were assessed in a one-way analysis of variance.

## Results

On the morning just before treatment, we recorded an average of 177 blackbirds per 5-min interval in the west plot, more than three times the number we recorded in the east plot (50 interval$^{-1}$). Bird pressure increased in the afternoon following treatment of the west plot, with 223 and 123 red-winged blackbirds recorded per interval in the treated and control plots, respectively. Thereafter, bird use of the treated plot steadily declined and no birds used the plot after day 2 posttreatment (Fig. 1). Bird numbers in the control plot also declined after the second posttreatment day, and for the next 2 d almost all bird activity shifted to the east end of the rice field beyond the boundary of the control plot. Activity in the control plot resumed in the final 3 d of observation. Boat-tailed grackles (*Quiscalus major*), which had previously occurred in negligible numbers, became a larger constituent of the feeding flocks (up to 50% at times).

Although birds were recorded in the treated plot for 2 d following treatment, their behavior during those sporadic visits was noticeably different from that during pretreatment. The birds spent less time feeding and more time restlessly moving about. The rate at which the flock drifted and "leapfrogged" through the plot seemed to increase and birds departed the plot relatively quickly (after approximately 20 min) for no apparent reason. In the untreated plot and beyond to the east, flocks fed for extended periods of time, moved more slowly, and often relocated only when persistently harassed by raptors.

Anthraquinone residues averaged 337 ppm (SE = 43 ppm) immediately after application of Flight Control and declined to 209 ppm (SE = 39 ppm) 14 d posttreatment (Fig. 2). Only the 1-h and 14-d residues were statistically distinct ($F_{1,14} = 1.46$, $P = 0.044$). Residues varied considerably among the eight sample locations; samples 1 and 2 were consistently much greater than samples 7 and 8 (Fig. 3).

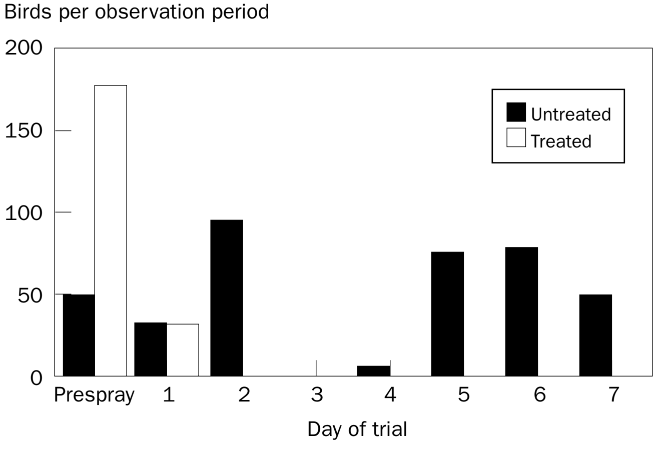

**Fig. 1. Blackbird activity during 700–1000 at two 4-ha study plots in ratoon rice, Cameron Parish, Louisiana. The treated plot received one application of Flight Control bird repellent (18.7 L ha$^{-1}$).**

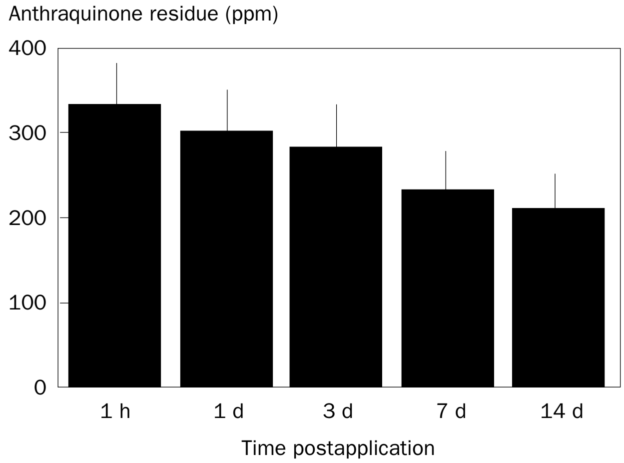

**Fig. 2. Anthraquinone residues on rice seed collected from a 4-ha plot treated with Flight Control bird repellent (18.7 L ha$^{-1}$), Cameron Parish, Louisiana. Vertical bars denote 1 standard error.**

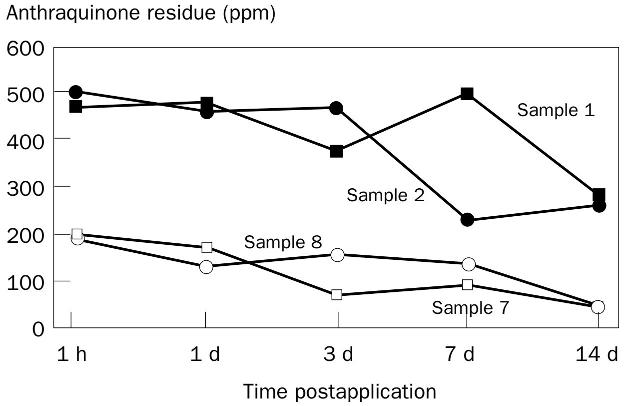

**Fig. 3. Variation in anthraquinone residues on ripe rice seed obtained from sampling points throughout a 4-ha study plot treated with Flight Control bird repellent (18.7 L ha$^{-1}$), Cameron Parish, Louisiana.**

We recorded 2.5–5.0 mm of precipitation on four of the seven days following Flight Control application. Total rainfall during this period was 14 mm.

## Discussion

Application of Flight Control resulted in an 80% decline in use of the treated plot within the first 24 h and abandonment of the plot after 2 d. Interestingly, a corresponding decline in the use of the control plot also occurred as the birds shifted from the test plots at the west end of the 28-ha study site to the eastern end. When blackbird flocks did return to the control plot, they stayed almost exclusively on the eastern end of the plot, several hundred feet from the treated plot. Possibly, this behavior was due to the birds' inability to distinguish reliably the boundary between the treated and untreated rice.

Conceivably, the observed response was a consequence of the birds' feeding method. Flocks of foraging blackbirds advance in a leapfrog-type movement, often at a pace of 3–6 m $\text{min}^{-1}$. It follows then that a strip pattern of application may be just as effective as complete field coverage. For example, perhaps the same 18.7 L $\text{ha}^{-1}$ application rate could be used, but with 5–6 m between swaths, without a loss of effectiveness. If so, then the economic viability of Flight Control would be enhanced.

When we applied Flight Control to a 4-ha study plot in October 1997, we obtained a result similar to that in this trial. Although the 1997 trial was not monitored as closely as in 1998, postapplication visits to the study site revealed little or no bird activity where there had been thousands of blackbirds and grackles present before treatment with Flight Control. Anthraquinone residues from the 1997 study were greater than those obtained in 1998, however (Fig. 4). Although the application rate and general procedures did not change between years, this discrepancy may be due to differences in weather conditions or aircraft spraying systems. Given that both years produced effective blackbird control, the consistent results suggest that aerial application of Flight Control is robust over different sets of conditions.

To date, testing of Flight Control in ripening rice has been limited to two 4-ha ratoon crop sites. The results have been encouraging but considerable additional information is needed. Trials need to be conducted in a variety of rice-growing areas. Further testing should be conducted at different rates and patterns of application to determine cost-effectiveness and to establish a relationship between application rate

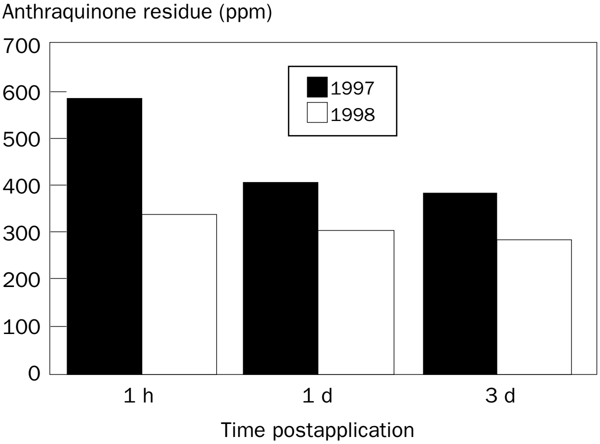

**Fig. 4. Anthraquinone residues on ripe rice seeds from test plots in 1997 and 1998 that received applications of Flight Control bird repellent (18.7 L $\text{ha}^{-1}$).**

and residue on the rice panicle and on the processed grain. In addition, it will be important to know the duration of adequate repellency. The trials to date have shown effective protection from depredation for at least 7 d postapplication, but such assessments need to be extended to 2–3 wk at least.

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# Integrated management of rice water weevil—California style

L.D. Godfrey and T.D. Cuneo

The rice water weevil is the most serious insect pest for rice production in California. Several other arthropod pests can sporadically reach damaging levels, but rice water weevil infestations consistently have the potential to reduce rice yields, especially in the areas adjacent to field levees. Cultural control measures provide partial rice water weevil control and fit as part of an integrated management program. Insecticidal control, however, is still a critical part of management schemes for this pest. Granular carbofuran has been used to manage this pest for more than 20 years. The registration and availability of this product to growers are threatened annually. New insecticides for rice water weevil control have been evaluated each year since 1992. Two materials with activity as postflood treatments and one preplant incorporated product have shown considerable promise for rice water weevil management. The two postflood materials, lambdacyhalothrin and diflubenzuron, received California registration in early 1999. These products control rice water weevil by affecting the adults and thereby reducing the number of larvae, the damaging stage. Because these products are short-lived in water, application timing is critical to achieve control. Several studies have been conducted to evaluate and refine treatment timing.

Rice production in California occurs annually on about 200,000 ha, primarily in the Sacramento Valley, where a water-seeded, continuously flooded system is used to cultivate the crop. Several arthropod pests hinder rice production, including insects and crustaceans. A group of arthropods that includes tadpole shrimp, *Triops longicaudatus;* crayfish, *Procambarus clarki* and *Orconectes virilis;* and seed midges, *Cricotopus sylvestris*, *Paralauterborniella subcincta*, and *Paratanytarsus* spp., inhibits rice stand establishment. These pests occur sporadically in time and space, but can be important in limited areas. They threaten rice yields by feeding on germinating seeds, uprooting seedlings, and reducing light penetration by affecting water clarity. The rice leafminer, *Hydrellia griseola*, damages rice seedlings near the time of emergence through the water. During years characterized by cool spring conditions, populations of this pest and the resulting damage reach a peak. The rice water weevil, *Lissorhoptrus oryzophilus*, is the most important insect pest of rice in California and this insect will be the emphasis of this chapter. Rice water weevils damage rice from about 4 to 8 wk after rice emergence.

The rice water weevil (RWW) was first found in California in the late 1950s (Lange and Grigarick 1959) from an apparent introduction from its native range in the eastern United States. All RWWs in California are females (the insect reproduces parthenogenically) and there is one generation per year (a partial second generation occurs during some years). This biology differs from that in this pest's native range where both sexes are present and the pest has multiple generations per year.

In California, RWWs overwinter on levees, roadsides, basins of harvested fields, native riparian areas, etc. The adults break diapause in the spring and disperse, that is, fly, during warm, calm evenings from April to June. The timing and magnitude of this dispersal flight are monitored annually with light-trap sampling. This flight period generally coincides with the time of rice seeding. Adults readily infest flooded fields but typically do not move into dry fields (before flooding or temporarily drained fields). The adults feed on rice leaves; however, the characteristic longitudinal feeding scars do not negatively affect plant productivity. After feeding for a few days, the adults deposit eggs in the leaf sheaths just below the water line. The eggs eclose in 3–5 d and the first instars briefly mine in the sheath tissue. The larvae then drop through the water to the soil surface and complete development by feeding on plant roots. This feeding constitutes the primary damage to the rice plant. Root pruning reduces plant growth, tillering, and ultimately grain yield. Yield losses of up to 35% can occur, but RWW infestation and yield losses in California are most severe in the areas about 15 m adjacent to levees (Godfrey and Palrang 1996). Pupation also occurs in the soil adjacent to the roots. The new-generation adults emerge from the pupae after 10–14 d.

RWWs in California have historically been controlled with preventive preflood applications of a soil insecticide. The only registered insecticide for more than 20 years has been a granular formulation of carbofuran. A single application at 0.56 kg (ai) ha$^{-1}$, incorporated into the soil before flooding, provides excellent control. Since the early 1990s, the registration of granular carbofuran has been under scrutiny by federal regulatory officials. The initial point of contention regarding the product was avian toxicity. Carbofuran has been available to California rice growers yearly through 1998, although the approval process has been an annual challenge and registration has been issued by federal and/or state agencies depending on the year. Given this uncertain status of carbofuran, considerable research has been conducted to find alternative chemicals to manage RWW and to develop and refine cultural control measures into a more complete integrated approach. This chapter briefly summarizes highlights of this research.

## Procedures

Studies designed to better understand the biology and behavior of RWW are conducted annually by the University of California-Davis Rice Entomology Project. Through improved knowledge of this pest's habits, we hope to identify weak links in its life history and design appropriate cultural management techniques. During the past 10 years, we have examined the role of levee vegetation removal, delayed seeding, drill seeding, and winter flooding in supplementing RWW management. Each of these techniques has a fit as part of an integrated approach, but each has some drawbacks that limit its utility.

Insecticides for RWW management have also been evaluated in California since 1992. The initial screening trials are conducted in 0.8-m$^2$ ring plots at the Rice Experiment Station near Biggs, California. These plots allow for maximum control over the studies, for example, with each ring having the same number of rice plants, infested with the same number of RWW adults, etc. The efficacy of the candidate insecticides can be evaluated impartially and compared. RWW adult feeding scars (% of plants with feeding scars on either of the two newest leaves), larval populations (RWW immatures per 1,200-cm$^3$ soil core containing at least one rice plant with individuals recovered with a soil-washing–flotation technique), grain yield, and plant growth are quantified. The second "phase" of the testing process takes place in small basin plots (3 × 6.1 m). This allows for more "natural" conditions with more commercial application, seeding, and harvest procedures. In addition, RWW infestation depends on the movement of native adults into the plots. In some years, a noneconomic infestation occurs and limits the usefulness of this approach. The final and most important test of the efficacy of new insecticidal tools for RWW management is in grower fields. Plots of 2+ ha located in grower fields are treated using the treatment parameters derived from the previous small-plot studies. Quantitative data, similar to data obtained from the ring plots, are collected from these plots.

The characteristics of several of the compounds available for evaluation against RWW dictated that the materials be applied postflood to optimize efficacy. This application timing, although more appropriate in terms of pest management, required additional research on application timing. A short persistence of the insecticide in water favored product registration; however, this attribute also meant that the application had to be timed appropriately. Carefully designed research efforts were used to define this timing.

## Results

Several cultural control measures are combined with chemical controls to form the integrated approach used in California to manage RWW. Details on these methods and the associated research can be found in Palrang (1994) and Palrang et al (1994). The strategy that has shown the greatest utility in California is the removal of weedy vegetation on the levees in the spring or early summer. This weedy vegetation provides a habitat for RWW adults between the spring flight and the invasion of flooded rice fields. Removing weeds by cultivation in the spring results in a lower RWW infestation in the adjacent rice field basin compared with fields with weedy levees. During the three years of research, the percentage of rice plants with RWW adult feeding scars (indicative of infestation severity) was less at the study sites with weed-free levees versus those with weedy levees. The effectiveness of this technique depends on the specific area, that is, environment of neighboring areas, riparian areas, etc. The drawbacks of the destruction of levee vegetation include the costs for tillage and the loss of potential wildlife nesting habitat.

Chemical insecticides represent an important component of the RWW integrated management scheme. Up-to-date research data under California conditions and with currently used rice practices are needed to fully evaluate the applicability of new insecticides. Ring plots offer a means to screen many treatments and to quickly identify ineffective products. Performance in grower fields, however, is obviously the criterion used to judge new product applicability. Figure 1 shows results from ring plots in 1998. Lambdacyhalothrin, deltamethrin, diflubenzuron, zeta-cypermethrin, and fipronil provided excellent control of RWW larvae, whereas control with carbofuran was less efficacious.

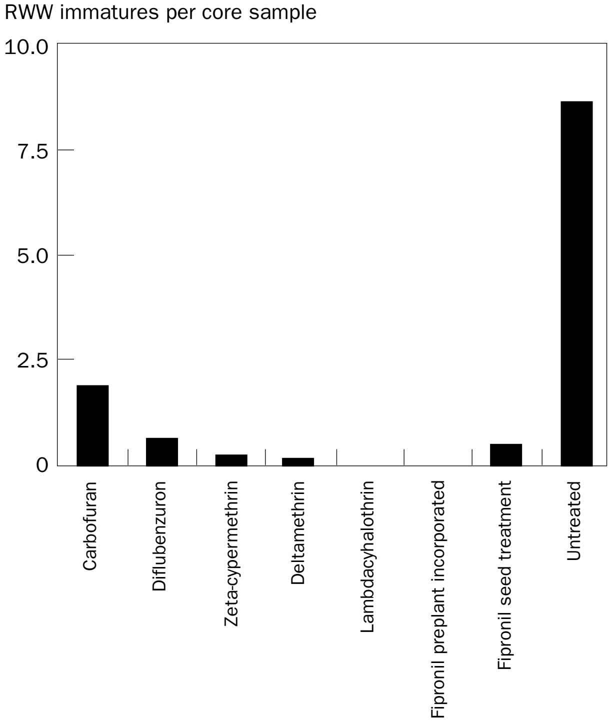

**Fig. 1. Efficacy of candidate insecticides against rice water weevil in ring-plot tests conducted at the Rice Experiment Station in 1998.**

Studies conducted in 1998 in grower fields under commercial conditions showed that lambdacyhalothrin and fipronil provided slightly better RWW larval control than the standard carbofuran treatment and diflubenzuron performed slightly less efficaciously than carbofuran (Fig. 2). We also compared the use of postflood treatments for the border areas (areas about 15 m adjacent to levees) with application to entire basins. Based on this limited sample size, there were no differences in RWW control between these two treatment approaches. Granular carbofuran is often applied only to the border areas since this coincides with the areas of highest RWW infestation. In terms of protecting yield, plots treated with lambdacyhalothrin yielded about 1,100 kg ha$^{-1}$ more grain than untreated areas but less than the carbofuran-treated plots. In the diflubenzuron test fields, diflubenzuron-treated plots yielded about 350 kg ha$^{-1}$ more grain than the untreated plots and were comparable to the carbofuran-treated

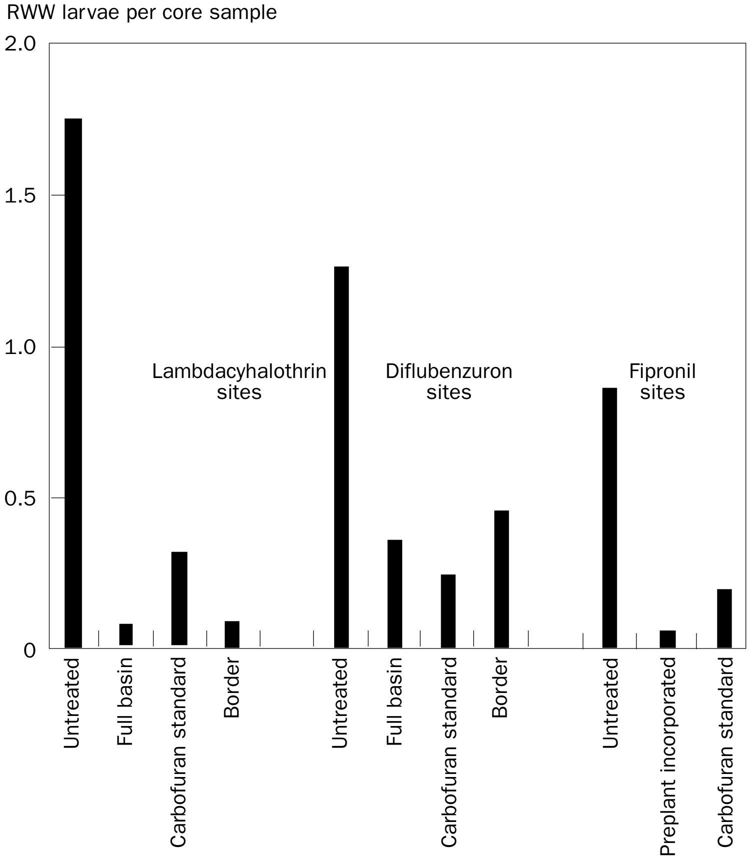

**Fig. 2. Efficacy of candidate insecticides against rice water weevil in tests conducted in grower fields (1998). Treatments applied to blocks of 2+ ha each. Lambdacyhalothrin, diflubenzuron, and fipronil evaluated at 4, 6, and 2 locations, respectively.**

areas. The fipronil-treated areas yielded similarly to the carbofuran areas and both yielded about 800 kg ha$^{-1}$ more than the untreated plots. Lambdacyhalothrin and diflubenzuron were registered for RWW control in California rice in early 1999.

In these tests, fipronil was applied to the soil surface before flooding; this insecticide controls RWW by larval mortality. Lambdacyhalothrin and diflubenzuron are applied postflood and the modes of action are toxicity to RWW adults and sterilization of adults, respectively. Because these two materials have a short residual in the water, the challenge is to time applications before RWW oviposition, but not so early that the RWW adults have not yet infested the rice fields. Two studies were conducted to attempt to quantify the best timing for these products. In 1997 and 1998, ring plots were treated with diflubenzuron at several time intervals. These plots were infested with RWW adults 1 d before the initial application and again from 5 to 10 d after 50% emergence applications. These infestation timings simulated a natural infestation pattern. In 1997, diflubenzuron provided excellent RWW control with 5 and 10 d after 50% emergence applications and moderate control with the application immediately preceding and following these two timings (Fig. 3). In 1998, the first three application timings (first emergence through water to 5 d after 50% emergence) provided 90%+ RWW control.

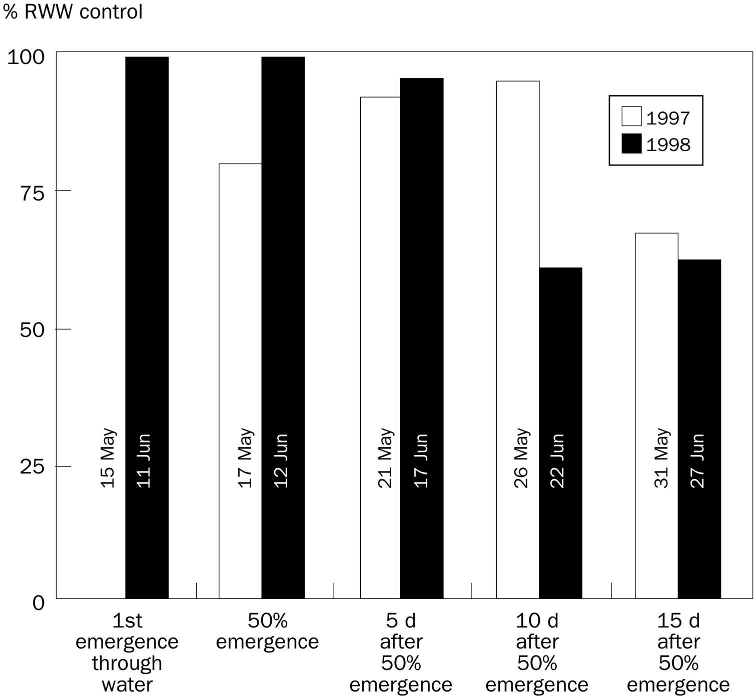

**Fig. 3. Evaluation of residual control with diflubenzuron in ring plots against rice water weevil in 1997 and 1998. Applications made at several intervals after rice plant emergence through the water (application dates indicated).**

The initiation of and pattern of RWW oviposition are the second aspect of determining the timing of application for postflood materials. RWW is not a new insect pest in California, but control relied on preplant-incorporated materials for more than 20 years, so the exact timing of egg-laying was not important. This obviously has changed with the current atmosphere of postflood applications. Based on observations and dogma, it was thought that RWWs typically infest rice fields at the time of seedling emergence through the water, that is, about the 3-leaf stage, and that the adults begin egg deposition after a 2–4-d preovipositional feeding period. Studies conducted in 1998 showed that some eggs were deposited as early as the 1- to 2-leaf stage and oviposition peaked at the 2- to 4-leaf stage (Fig. 4). In terms of days after seeding, eggs were found at the first sample period (8 to12 d) and new eggs were deposited at 33 to 37 d after seeding. A 28-d ovipositional period was found in some fields, but others had as short as a 14-d period. Reasons for these differences are unknown, but will certainly be an important factor in product performance.

Management schemes in place for RWW, and the lack of other significant arthropod pests of California rice, have facilitated the high level of productivity in this

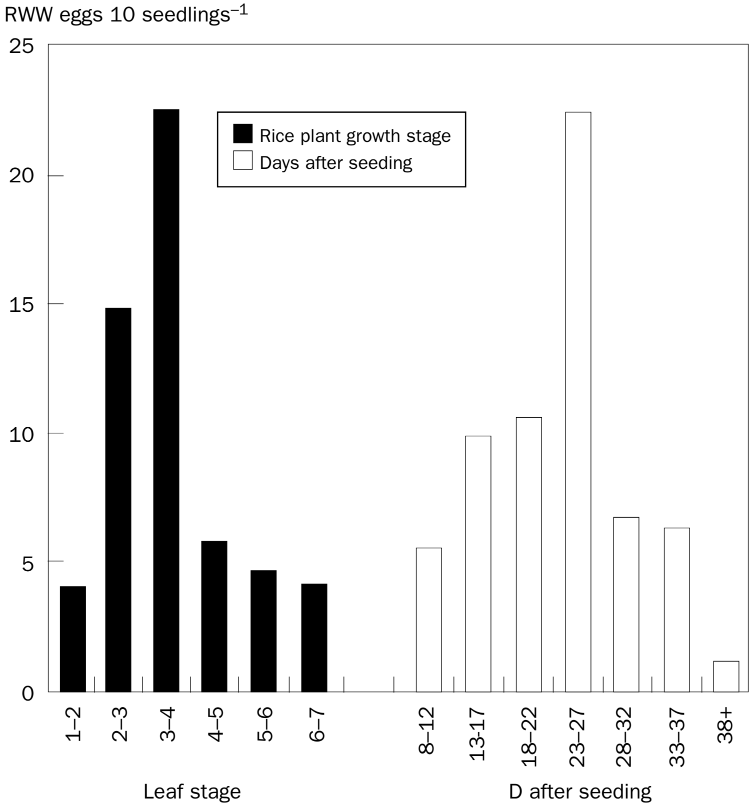

**Fig. 4. Rice water weevil oviposition timing and pattern in grower fields in the Sacramento Valley, California, in 1998.**

system. Insecticides, facilitated by cultural control measures, form the backbone of the RWW management system. Research will continue to attempt to refine this system and to maintain excellent control of this significant pest.

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# Efficacy and environmental fate of alphacypermethrin applied to rice fields for the control of chironomid midge larvae (Diptera: Chironomidae)

S. Helliwell and M.M. Stevens

The pyrethroid insecticide alphacypermethrin was evaluated for the control of chironomid midge larvae in small-plot field trials at Yanco Agricultural Institute during the 1997-98 and 1998-99 rice seasons. Treatments were applied at sowing in both trials. In the 1997-98 trial, alphacypermethrin provided up to 73% control of target Chironominae in the first 19 d after application (DAA), while a chlorpyrifos standard treatment provided 73% control. Populations of Chironominae were very low during the 1997-98 season and control was not reflected in improved plant establishment.

In the 1998-99 season, target Chironominae were far more abundant. Alphacypermethrin provided >99% control of Chironominae for 19 DAA at all rates evaluated, while the chlorpyrifos standard treatment gave 97% control over the same period. There were significant differences in plant establishment between control basins and those containing either alphacypermethrin or chlorpyrifos.

Our results indicate that alphacypermethrin applied at 8 g ai ha$^{-1}$ will be equal or superior to chlorpyrifos (75 g ai ha$^{-1}$) for the control of chironomid larvae during rice crop establishment. Concentrations of alphacypermethrin in the water column and sediment were monitored during each trial. During the 1998-99 season, residues were 0.008 μg L$^{-1}$ at 18 DAA and 18 μg kg$^{-1}$ at 38 DAA for the overlying water and sediment, respectively. No alphacypermethrin residues were detected in grain or forage samples at harvest.

Chironomid midges have been recorded as pests of rice in many temperate rice-growing countries (Surakarn and Yano 1995). The larvae either attack the seed itself or feed on the roots or shoots or both of young seedlings. When high larval densities are present in rice fields, secondary damage can arise through their tunneling activity in the sediment, which can destabilize the root systems of young plants and increase water turbidity, which reduces photosynthesis and slows the growth of submerged seedlings.

In southwestern New South Wales (NSW), more than 90% of the rice crop is sown by fixed-wing aircraft. Although aerial sowing has several advantages for rice growers, it leaves the germinated seed sitting at the soil/water interface, where it is vulnerable to attack by several invertebrate pests, particularly chironomid larvae. Chironomid communities during the first 20 d after basin flooding are often dominated by a single, highly synchronized generation of the rice bloodworm, *Chironomus tepperi* Skuse, a specialized colonist species (Stevens 1998) that often reaches densities of more than 1,000 larvae m$^{-2}$. *C. tepperi* larvae attack the seed embryo and endosperm, and the roots of developing seedlings, but do not attack the shoots. More than 90% of seedlings can be lost if effective chemical control is not implemented (Stevens et al 1998). A range of other chironomid species also colonize NSW rice fields during the crop establishment period, and, although some of these taxa clearly contribute to crop damage, the significance of individual species has not yet been determined.

Although chlorpyrifos is the mainstay of chironomid midge control programs in NSW rice fields, its future availability to rice growers is uncertain and its use is currently under review by the National Registration Authority for Agricultural and Veterinary Chemicals. Doubts about the ongoing availability of chlorpyrifos have prompted a research program aimed at developing alternative compounds.

Synthetic pyrethroid insecticides have been reported as highly toxic to chironomids (Ali and Mulla 1978, 1980, Ali 1981), and alphacypermethrin, like many pyrethroids, is substantially more toxic to *C. tepperi* larvae than chlorpyrifos in laboratory bioassays (Stevens 1993). Comparatively little work has been done on the efficacy of pyrethroids against chironomids under field conditions, and this study was conducted to assess the efficacy and environmental fate of alphacypermethrin when applied to rice fields, with a view to developing this product as a possible alternative to chlorpyrifos for use in NSW.

## Materials and methods

Alphacypermethrin was evaluated in two trials conducted during the 1997-98 (trial 1) and 1998-99 (trial 2) rice seasons at Yanco Agricultural Institute (34°37′S, 146°26′E) in southwestern NSW. The two trials were conducted on adjacent sites on a Birganbigil clay-loam soil (van Dijk 1961).

Two rows of nine rectangular basins with earthen banks (each approximately 30 m$^2$) were used in each trial. Each basin was supplied with water from a central channel. Alternate basins in each row were used as treatment basins, with intervening basins being used as buffer zones. In each trial, two basins were designated as untreated controls, two were treated with a standard chlorpyrifos treatment (Lorsban® 500 EC [DowElanco Australia Ltd.], 500 g L$^{-1}$ applied at 75 g active ingredient [ai] ha$^{-1}$), and six (two at each of three application rates) were treated with alphacypermethrin (Dominex® 100 EC, FMC International AG, 100 g L$^{-1}$).

In trial 1, alphacypermethrin was applied at 10, 20, and 30 g ai ha$^{-1}$, whereas, in trial 2, application rates of 6, 10, and 20 g ai ha$^{-1}$ were evaluated. All chemical treatments were applied 6 d after flooding to the water surface in approximately 5 L of water using a single-nozzle hand sprayer. All treatment and control basins were sown with pregerminated rice (cultivar Namaga, 120 kg dry weight $ha^{-1}$) by hand broadcasting within 2 h of chemical treatments being applied. Following severe damage to trial 1 by ducks, cages (2.5 m × 1.5 m × 0.45 m [height]) covered with wire mesh were constructed and randomly placed (one per basin) in all control and treatment basins immediately after trial 2 was sown to protect at least part of each basin from duck damage.

Chironomid populations were assessed using soil core sampling as previously described (Stevens and Warren 1992). In trial 1, three samples were taken from each basin 4, 9, 14, 19, 24, and 29 d after chemical application (DAA), whereas, in trial 2, four samples were taken from each basin 4, 9, 14, 19, and 24 DAA. Extracted larvae were divided into two groups, Chironominae and "other chironomids" (predominantly Tanypodinae). Data were transformed to $y' = \log_e (y + 1)$ and analyzed using ANOVA and Tukey's honestly significant difference (HSD) test to separate means.

Plant establishment counts were made in both trials using a 35-cm internal diameter sampling ring. In trial 1, 30 plant counts were made at 30 DAA; however, severe duck damage was evident across the trial. In trial 2, severe duck damage also occurred, and plant establishment counts were restricted to the areas under the duck-proof cages. Eight plant counts were made per basin at 30 DAA. Results from both trials were analyzed using ANOVA, with Tukey's HSD test used to separate means.

Single 1-L composite water samples were taken from the control and alphacypermethrin-treated basins at set intervals. In trial 1, samples were collected at 1, 2, 5, 9, 14, 19, and 25 DAA. Each sample was acidified with 10 mL of 6 M HCl and then chilled to 5 °C until extraction and analysis. All extractions were conducted within 1 wk of sample collection. In trial 2, samples were collected at 1, 2, 3, 4, 7, 10, 14, and 18 DAA and chilled to 5 °C. Although samples were not acidified, extractions were completed within 10 h of sample collection.

Core sediment samples were collected for chemical analysis from control and alphacypermethrin-treated basins in both trials. In trial 1, two samples were collected from each basin at 5, 14, and 25 DAA, whereas, in trial 2, two samples were collected from each basin at 15 and 38 DAA. Cores were frozen at –17 °C until analyzed.

Plant samples were collected for residue analysis from trial 1 only. Twenty complete plants were removed at random from each control and alphacypermethrin-treated basin at 42 DAA, whereas grain samples (20 random panicles per basin) and aboveground forage samples (six random plants per basin) were collected at grain maturity (136 DAA).

Samples from trial 1 were analyzed using solvent extraction (2 × 25 mL hexane, dried over $Na_2SO_4$, evaporated to dryness under nitrogen, and taken up in 0.2-mL acetonitrile), whereas solid-phase microextraction (SPME) (100-µm thick polydimethylsiloxane fiber [Supelco] exposed for 10 to 30 min at 25 °C) was used for trial 2. Quantification was carried out via gas chromatography with an electron capture detector on a DB5 (J&W) capillary column (30 m × 0.25 mm i.d.) with a film thickness of 0.25 µm.

Cores were thawed and the top 2 cm from duplicate cores were combined and air-dried. Ten-g subsamples were sonicated and analyzed according to Clark et al (1989) using solvent extraction followed by gas chromatography.

Samples of chopped rice forage, stems, or grain forage (40 g) were extracted with 150 mL of 1:1 acetone:petroleum (150 mL) in the presence of $Na_2SO_4$. Extracts were cleaned with florisil and evaporated to dryness before being taken up in 10 mL of petroleum ether. Quantification was as described for water samples.

## Results

In trial 1, populations of target Chironominae remained low in the control basins throughout the trial, reaching a density of approximately 485 larvae $m^{-2}$ at 14 DAA. Control was relatively poor during the first 14 DAA, with the standard chlorpyrifos treatment providing only 81.8% control of the main target group. Although the 10 g ai $ha^{-1}$ alphacypermethrin treatment provided 97% control over this period (Table 1), activity of alphacypermethrin was not dose-dependent.

Target Chironominae populations were far higher in trial 2 (estimated density in control basins at 14 DAA approximately 13,100 larvae $m^{-2}$), and much higher levels of control were achieved. Both the chlorpyrifos standard and all rates of alphacypermethrin provided >99% control of the target group for 14 DAA, and only the chlorpyrifos standard had dropped below 99% control (to 97.1%) at 19 DAA (Table 1). All treatments significantly ($P < 0.05$) reduced populations of Chironominae at each sampling period up to and including 19 DAA (Fig. 1).

No significant differences in plant densities were recorded between treatments at 30 DAA in trial 1. This contrasts with trial 2, in which substantial differences were

**Table 1. Percentage reductions in target Chironominae larvae (relative to untreated controls) resulting from applications of chlorpyrifos and alphacypermethrin, cumulative data.**

| Treatment[a] | First 14 DAA[b] | First 19 DAA |
|---|---|---|
| *Trial 1* | | |
| Chlorpyrifos 75 | 81.8 | 73.2 |
| Alphacypermethrin 10 | 97.0 | 73.2 |
| Alphacypermethrin 20 | 70.0 | 53.7 |
| Alphacypermethrin 30 | 84.8 | 65.6 |
| *Trial 2* | | |
| Chlorpyrifos 75 | 99.6 | 97.1 |
| Alphacypermethrin 6 | 99.8 | 99.1 |
| Alphacypermethrin 10 | 99.9 | 99.3 |
| Alphacypermethrin 20 | 100.0 | 99.1 |

[a]Values represent application rate in g ai $ha^{-1}$. [b]DAA = days after chemical application.

recorded (Fig. 2). All chemical treatments significantly (*P* <0.05) improved plant establishment from the control level (23 plants $m^{-2}$) to between 169 and 204 plants $m^{-2}$. There were no significant differences among the chemical treatments.

No alphacypermethrin was detected in any of the plant samples taken at 42 DAA or in grain or forage samples collected at harvest (detection limit 20 μg $kg^{-1}$).

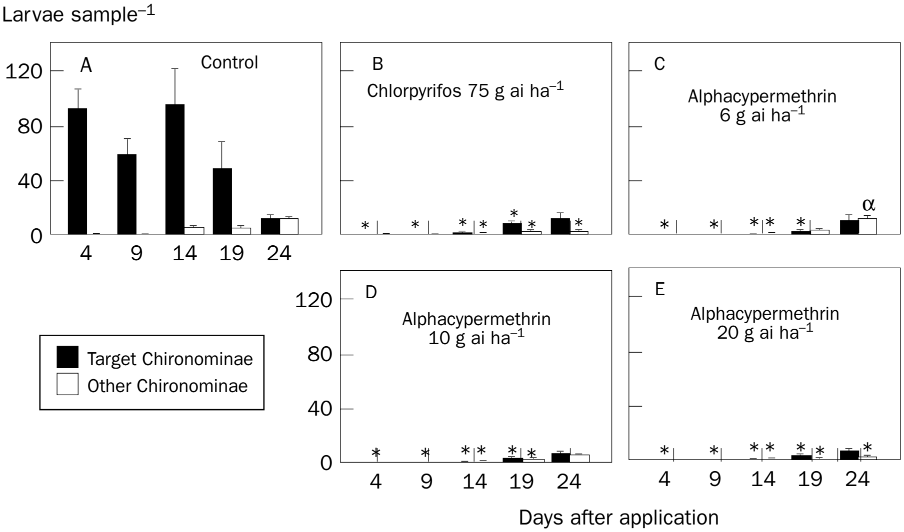

**Fig. 1. Larval chironomid populations in trial 2, 1998-99 season. * = significantly (*P* <0.05) lower than equivalent control population. α = significantly (*P* <0.05) higher than equivalent chlorpyrifos-treated population. No other significant differences were detected.**

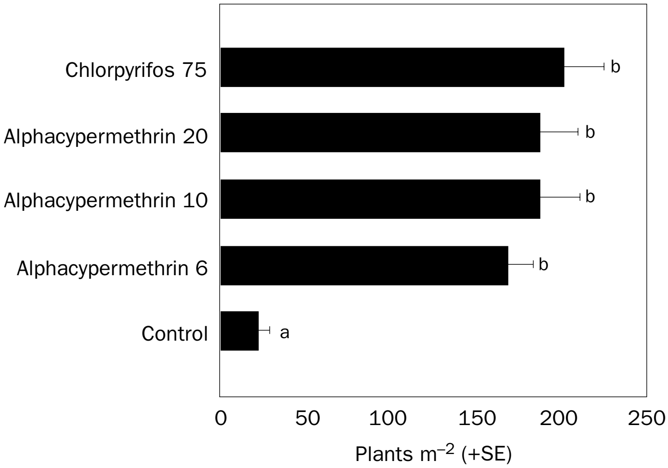

**Fig. 2. Plant establishment in trial 2, 1998-99 season, at 30 d after chemical application. Values next to treatment types represent application rates in g ai $ha^{-1}$. Columns followed by different letters are significantly different (*P* <0.05).**

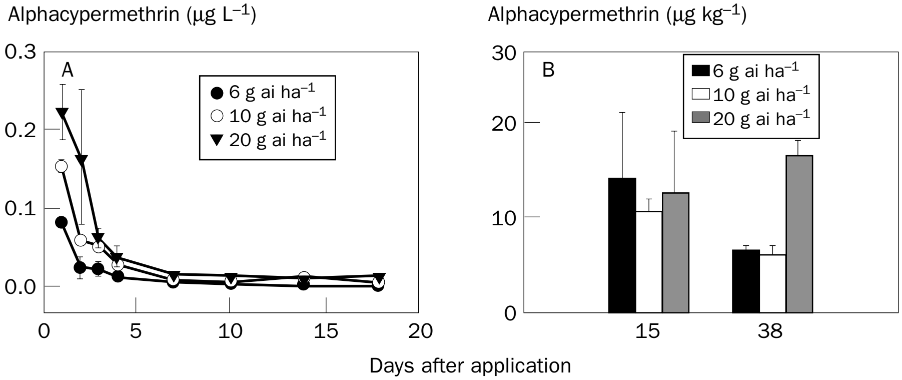

**Fig. 3. Alphacypermethrin residues in water column (A) and sediment (B) in trial 2, 1998-99 season.**

Alphacypermethrin concentrations in both trials decreased in an exponential-like manner as the alphacypermethrin became incorporated into the sediment. In trial 1, levels for the 10- and 20-g ai ha$^{-1}$ treatments were below 0.01 µg L$^{-1}$ at 5 DAA. During trial 2, alphacypermethrin concentrations for the highest treatment application (20 g ai ha$^{-1}$) diminished to 0.008 µg L$^{-1}$ at 18 DAA (Fig. 3A).

Alphacypermethrin residues were found in the sediments on all sampling occasions for both trials (detection limit 2 µg kg$^{-1}$). Figure 3B shows the alphacypermethrin sediment levels for trial 2; however, unlike the levels in the water, it is difficult to ascertain any decay trends over the time period sampled.

## Discussion

The two trials provided substantially different results, primarily as a consequence of the different levels of colonization by target Chironominae (particularly *Chironomus tepperi)* in the two seasons. In percentage terms (Table 1), the control provided by all chemical treatments in trial 1 appears relatively poor. This can be explained by the fact that the main target species, *C. tepperi*, was almost totally absent during trial 1, and those Chironominae that were present belonged to different taxa that may be more tolerant of the pesticides being evaluated. The response of chironomid populations to increasing rates of alphacypermethrin in trial 1 was not clearly dose-related, suggesting that only limited emphasis can be placed on the result of this trial because of the very low pest pressure.

In contrast, *C. tepperi* was the dominant component of the Chironominae found in trial 2 control basins, and its high susceptibility to both alphacypermethrin and chlorpyrifos resulted in >97% control of Chironominae being achieved in the first 19 DAA. In both trials, alphacypermethrin at the lowest rates evaluated provided control of the main target group at least equivalent to that provided by chlorpyrifos at 75 g ai ha$^{-1}$. Our results indicate that alphacypermethrin at an application rate of approximately 8 g ai ha$^{-1}$ will be an effective alternative to chlorpyrifos for chironomid control in rice crops. This result is in accordance with the findings of Pasini et al (1997), who found that alphacypermethrin applied at a higher rate (23.7 g ai ha$^{-1}$) provided effective control of chironomids in Italian rice fields.

## Conclusions

Alphacypermethrin has been field-evaluated for control of chironomid midge larvae in rice crops over two growing seasons. Because of low target Chironominae numbers during the first trial, alphacypermethrin provided only up to 73% control at 19 DAA, as did the standard chlorpyrifos treatment. Significantly greater Chironominae numbers during the second trial increased control to >99% at 19 DAA for all alphacypermethrin treatments compared with 97% control for the standard chlorpyrifos treatment. Its efficacy and low environmental residuals suggest that alphacypermethrin at an application rate of 8 g ai ha$^{-1}$ would be an effective alternative to chlorpyrifos in the establishment of water-seeded rice crops.

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# Oxygen demand and behavior of the rice midge *Cricotopus bicinctus* Meigen 1818 (Diptera: Chironomidae)

A. Szitó

*Cricotopus bicinctus* Meigen is one of the most important rice pests of flooded rice culture in Hungary. It damages young rice leaves on the water surface. An experiment was conducted to test the assumption that the behavior of the larvae is influenced by their oxygen demand, which in turn explains why they become common rice pests.

In laboratory experiments, mature larvae started to move toward the water surface after the optimum of about 10.9 mg $dm^{-3}$ of dissolved oxygen was reached in the water. The same behavior was observed with younger larvae at 3.7 mg $dm^{-3}$. The $LC_{50}$ value was 2.7 mg $dm^{-3}$ and the $LC_{100}$ value was 1.8 for mature larvae and 1.2 mg $dm^{-3}$ for young larvae. The mature larvae had a high oxygen demand, whereas the younger larvae were more tolerant of oxygen deficiency. The oxygen content of the water is considerably lower near the mud surface, especially at night or at daybreak, which makes *C. bicinctus* larvae move toward the water surface. Rice leaves present both a substrate and food for the larvae, together with the overgrowth of algae and bacteria. In this way, the originally polyphagous *C. bicinctus* larvae become rice pests.

The sown area of rice in Hungary increased quickly in the 1960s. It reached 12,000–15,000 hectares and stayed at that amount for an extended time. Rice was grown in monoculture for 4–6 years on soils unsuitable for other plants. Damage by larvae of the rice midge *Cricotopus bicinctus* was first observed by Bognar, and it was first described as a rice pest by Berczik (1957) in Hungary. Further investigations revealed that the larvae caused serious damage in flooded rice fields exclusively when the plants had no leaves above water and all leaves were under or on the surface of the water. Damage decreased when the first leaves appeared above the water surface (Bognar 1957).

Szilvassy (1961, 1964) considered the rice midge larva a major rice pest, causing significant (90–100%) damage in crops in Hungary. Darby (1962) in California and Berczik (1970, 1971, 1974) reported the same. According to these observations, until the end of the first instar, the larvae lived on the surface of the flooded soil and fed on the detritus. At the beginning of the second instar, the larvae migrated to the leaves on the water surface and caused serious damage by feeding. Berczik saw food deficiency as the cause of the upward migration, since he found as many as 10,000–15,000 larvae $m^{-2}$ (Berczik 1977).

My own examinations showed that the larvae fed on algae and bacterial matter covering the soil. Larvae continued to migrate up to the rice leaves on the water surface even when their individual density $m^{-2}$ was low; thus, no food deficiency could develop. I assumed that a decrease in oxygen concentration forced the larvae toward the water surface. The experiments confirmed my hypothesis.

## Materials and methods

In the rice field, we filled 12 test tubes of 25 mL each with water in a way so that the inflowing water did not bubble. In three test tubes, we fixed the oxygen content of the water and sealed them from air. Water temperature was 21 °C. Each of the remaining nine test tubes received 10 larvae and larval tubes (with the rice leaf piece containing them) and the test tubes were also sealed from air. We placed them vertically on a stand in the laboratory and observed the behavior of the larvae, which were in their second to fourth instars. The temperature of the laboratory was 21 °C. In three of these test tubes, we recorded the dissolved oxygen content of the water when the first larvae left their larval tubes for the water surface. In another three test tubes, the dissolved oxygen content was measured when the first larvae perished, and the same was done in the remaining three test tubes when all of the larvae perished.

During the experiment, from 19 to 20 May 1981, the dissolved oxygen content of the floodwater in the rice field from where the larvae were collected was measured every other hour. We took water samples from the water/sediment line with a glass tube that had a rubber ball attached to one end, and placed the samples into test tubes. The dissolved oxygen content of all samples was established by a semimicro method modified from Winkler (1888).

## Results

On the surface of the flooded soil in the rice field, the lowest oxygen content was 1.5 mg $L^{-1}$, measured at 0400, whereas on the surface of the floodwater it was 19.2 mg $L^{-1}$ (Fig. 1).

The larvae started to swim up to the surface when the oxygen content of the water decreased to 19.5 mg $L^{-1}$. The first larvae perished at 3.5 mg $L^{-1}$ oxygen content, 50% of the larvae perished at 2.7 mg $L^{-1}$, and all perished at 1.2–1.8 mg $L^{-1}$ (Fig. 2).

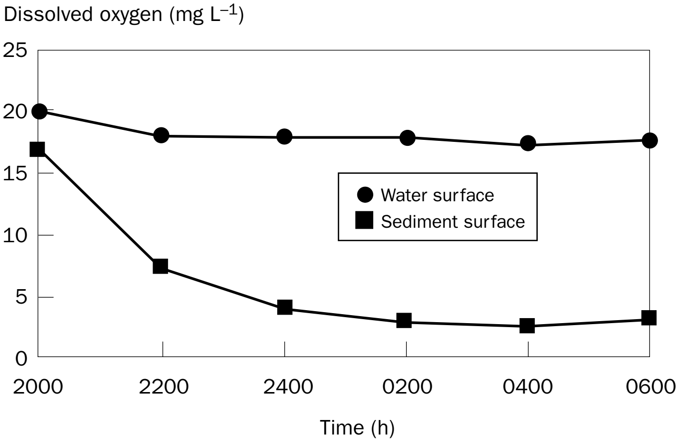

**Fig. 1. Changes in dissolved oxygen content in floodwater on the water surface and on the sediment surface (water depth = 200 mm).**

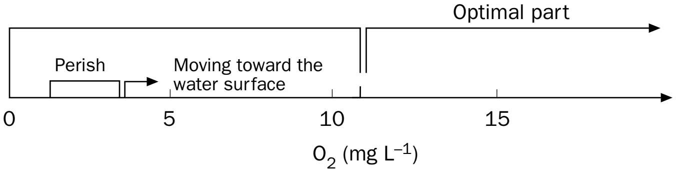

**Fig. 2. Oxygen demand of the rice midge larvae.**

## Conclusions

Older larvae were more sensitive to and younger larvae were more tolerant of the oxygen deficiency. The lowest value of the optimal domain for larvae in their third and fourth stage was 10.9 mg $L^{-1}$ oxygen content, whereas younger larvae started to move toward the surface at the 3.6 mg $L^{-1}$ value. This explains why younger larvae can stay on the soil surface where oxygen concentration is lower, but must move toward the water surface when they get older and need more oxygen.

From the various oxygen content figures measured in the floodwater, it becomes clear that the dissolved oxygen content taken at the water/sediment line is high (18 mg $L^{-1}$) during the day and low at night (1.5 mg $L^{-1}$). The minimum value at dawn causes the larvae to perish. Thus, swimming toward the surface is nothing other than trying to avoid death, that is, drowning.

At dawn, masses of larvae in the rice field floodwater moved toward the water surface. In search of a refuge and food, they settled on the rice leaves, where they could find bacteria and algal masses on the epidermis. We observed that the larvae damaged the epidermis of the leaves while feeding. The injured parts undergo bacterial decomposition and a new layer of algae and bacteria develops, which continuously offers food for the larvae. While feeding, the larvae continue to damage the leaves, thus becoming secondary pests of rice.

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# The pests of sprinkler-irrigated upland rice in Hungary

A. Szitó and I. Simon-Kiss

Sprinkler-irrigated rice yields the same as irrigated lowland rice but requires less water. The goal of this study was to describe the pests of this crop. The results of examinations made on the stems, leaves, and panicles of rice plants, and of the soil by soil traps, are as follows: root pests were the black cricket *(Melanogryllus desertus* Pallas*)*, bulb mite *(Rhyzoglyphus echinopus* Fumouse-Robin*)*, and elm leaf aphid *(Tetraneura ulmi* Linnaeus*)*. Stem and leaf pests were springtail *(Tomocerus plumbeus* Linnaeus*)*, yellow springtail *(Bourletiola lutea* Lubbock*)*, leaf beetle *(Lema melanopus* Linnaeus*)*, corn gound beetle *(Zabrus tenebroides* Goeze*)*, red spider mite *(Tetranychus urticae* Koch*)*, cereal spider mite *(Bryobia graminum* Schrank*)*, green grain aphid *(Schizaphis graminum* Rodani*)*, grain aphid *(Macrosiphum avenae* Fabricius*)*, and barley flea beetle *(Phyllotreta vittula* Redtenbacher*)*. Stem and panicle pests were the meadow mouse *(Microtus arvalis* Pallas*)* and hare *(Lepus cuniculus* Linnaeus*)*. *Zabrus tenebroides* Goeze was detected as a panicle pest as well.

The abundance and species composition of insect pests depended on crop rotation. *Tetraneura ulmi* and *Rhyzoglyphus echinopus* were present during all seasons when rice followed maize, but these pests were absent with systematically repeated irrigation. Insect damage situations could occur in all cases but the individual density of pests decreased with periodic irrigation, although irrigation was not always effective.

In tropical areas, rice is known to have many pests, the most significant of which are the flea beetle species (Alam 1967, Cendana and Colora 1967, Fernando 1967, Nasu 1967, Paik 1967, Soenardi 1967, Yunus 1967, Wongsiri and Kovitvadhi 1967, Pathak and Dyck 1975, Pathak and Pawar 1982). Kisimoto and Dyck (1976) observed that meteorological and climatic factors play an important role in damage formation. These factors are temperature, relative humidity, precipitation, wind, and the frequency of the latter two factors.

The cultivation of sprinkler-irrigated rice is a familiar practice in Hungary and was first discussed in relation to *Oryza montana* L. (Bogdanffy 1920). The fundamental questions of production have been discussed continually ever since (Mathe 1947, Simon-Kiss and Ipsits 1992). The production of varieties was already an issue in Hungary in the 1950s (Mathe 1950a,b). However, research did not extend to pests. With the development of agricultural technology, the production of sprinkler-irrigated rice became possible. We studied the potential pests of sprinkler-irrigated rice since a need for control could emerge.

## Materials and methods

We studied the composition of pest species in the cropping season recording changes in the number of species and in the seasonal individual density. Our field experiments were conducted on 7-ha and 15-ha fields in Tiszafoldvar and on the experimental plots of the Irrigation Research Institute, Szarvas, in 1987, 1988, 1989, 1993, and 1994.

After rice was sown but before it emerged, we placed plastic cups into the soil for soil traps, at 2-m distance from one another, with a total of 18 for 1 ha. The traps were placed from one edge of the field to the other, with their rims 1–2 cm beneath the soil surface. The sampling surface of one cup was 38.5 $cm^2$. Data were recalculated for a per-square-meter value. The observations and experiments were conducted from May to September.

To decrease surface tension, we added two drops of Nonite into the 3% formaldehyde solution in the traps. The traps were emptied every 2 or 3 days and contents were continually determined by species. When foliage developed, we randomly sampled the stems, leaves, and panicles of rice simultaneously with sampling the soil traps. Fields were irrigated weekly with 37–45 mm $m^{-2}$ of water from the beginning of June to the end of August.

Ten stems, 10 leaves, and 10 panicles were randomly sampled and the species collected were conserved in 80% alcohol. Before rice was sown, we determined the number of species and individual density of the insects living in the soil. On the 10 1-$m^2$ areas designated for examination, we removed the leaves damaged by corn ground beetles (*Zabrus tenebroides*) after sampling. To determine the individual density of springtail species, we used sticky sheets whose size was equivalent to the leaf surface. The sheets were at a 30–40° angle with the soil surface, and their end closest to the soil was at 5 cm. We also cut off the leaves damaged by leaf beetles (*Lema melanopus*) after each sampling.

To identify the insects, we followed the work of the Hungarian researchers Benedek (1989), Deseo (1990), Loksa (1988), Manninger (1990), Nagy (1989), Saringer (1988, 1990), and Szalay-Marzso (1988).

## Results

### Potential root pests

*Black cricket* (Melanogryllus desertus *Pallas*). This pest was present in Tiszafoldvar following a crop of pepper, after the end of May. Its average individual density was 0–2 $m^{-2}$, a medium value in May. In 1987, the individual density decreased and led to disappearance by the end of June. In 1988, individual density was similar to the 1987 value in rice sown after maize (Fig. 1A).

In Szarvas, individual density was four times greater than in Tiszafoldvar at the beginning of the cropping season. We found black crickets at 20 $m^{-2}$ density, and this value increased to 25 at the beginning of June. It dropped to 7–13 individuals in July but peaked again in August. After a strong decrease, the value stayed at 7 $m^{-2}$ until the end of the season. After sprinkler-irrigated upland rice was sown in 1988, individual density was low, but, on 17 May, we found double the amount measured at the same time in the previous year. In the middle of June, the top value was 20 $m^{-2}$, but it decreased quickly to 7 $m^{-2}$. It stayed the same until 22 July, increased at the end of July and at the beginning of August, but dropped back to 7 $m^{-2}$ after 10 August and stayed at this level until the end of the cropping period (Fig. 1B).

*Bulb mite* (Rhyzoglyphus echinopus *Fumouse-Robin*). At the start of the examinations in April, we found 65 $m^{-2}$ of this species, but this value decreased drastically later. In May, individual density was from 32 to 45 $m^{-2}$ and, after large fluctuations, the bulb mite disappeared in June and July (Fig. 1C).

*Elm leaf aphid* (Tetraneura ulmi *L.*). In the soil of rice sown after maize, the individual density of this species was 91 $m^{-2}$ at the end of April, but only 28 in the middle of May. In May, June, and July, it decreased after fluctuations and was as low as 12 $m^{-2}$ at the beginning of August. It finally disappeared from the area (Fig. 1D).

### Stem and leaf pests

*Springtail* (Tomocerus plumbeus *L.*). After maize, we found 400 springtail individuals $m^{-2}$ in the soil even before rice was sown. From 1987, we found individuals in varying numbers on the leaves and shoots of young plants, but in great numbers in the soil traps. There were 1 to 8 insects continuously on the leaves and shoots, and exuvia as well. We found these insects, together with yellow springtails (*Bourletiola lutea*), on the leaves and shoots only to the end of August (Fig. 1E, 2B). The regulatory effect of irrigation on individual density could not be proved.

*Leaf beetle* (Lema melanopus *L.*). In 1987, in the experimental plots in Szarvas, leaf beetle damage was visible on the first leaves of the plants from the end of May. The damage decreased from the end of July. In 1988, less damage was experienced and no burn damage developed. We considered the damage of 14 leaves $m^{-2}$ $wk^{-1}$ as burn damage. On the experimental area, individual density varied within wide boundaries year by year (Fig. 2C,D). The regulatory effect of irrigation on population was not apparent.

*Corn ground beetle* (Zabrus tenebroides *Goeze*). In 1987, damage affected 2 to 8 leaves per week and the damage ceased in August. The maximum value observed

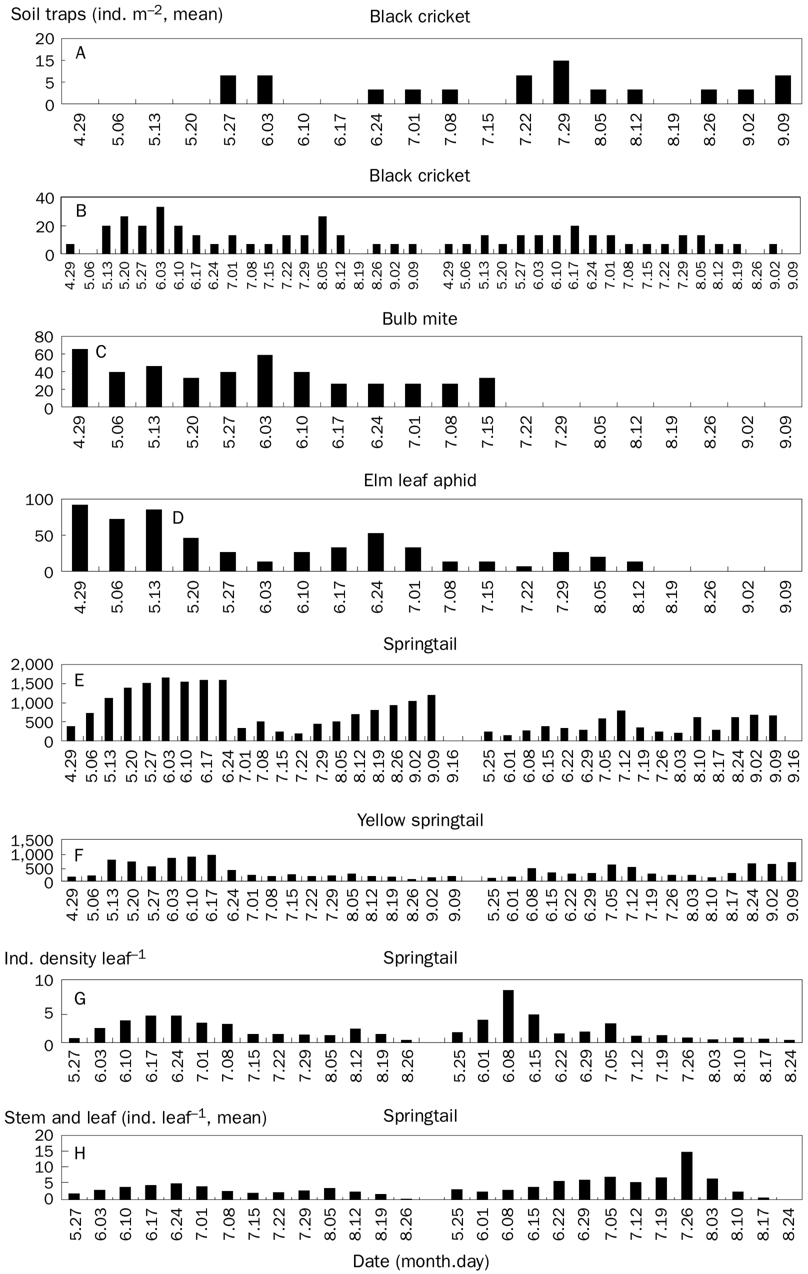

**Fig. 1. (A) On-farm experiment, 1987, after red pepper; (B) in plots, 1987, after maize and in 1988 after sprinkler-irrigated rice (SIR); (C) in plots, 1987, after maize; (D) in plots, 1987, after maize; (E) in plots, 1987, after maize, and in 1988, after SIR; (F) in plots, 1987, after maize, and in 1988 after SIR; (G) in plots, 1987, and in 1988, after SIR; (H) on-farm experiment, 1987, after red pepper, and in 1988, after maize.**

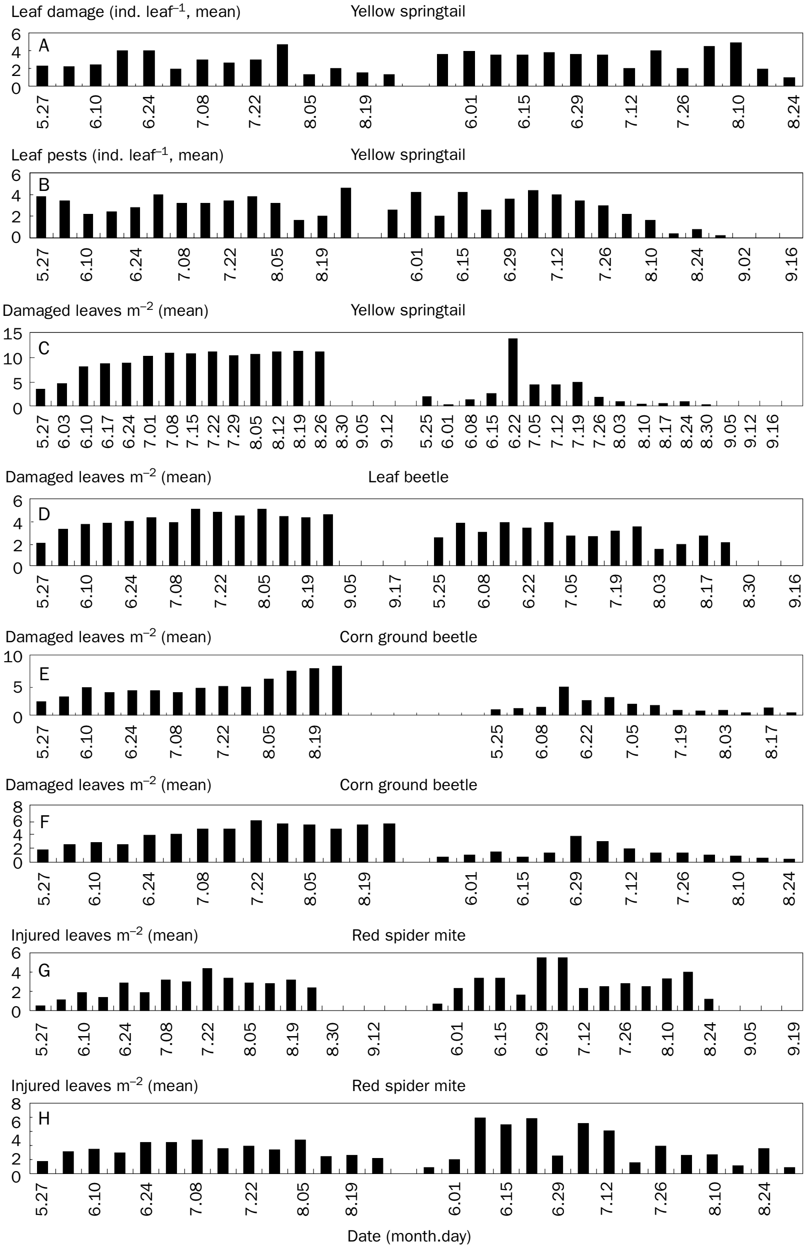

**Fig. 2. (A) Plots, 1987, after maize, and in 1988 after sprinkler-irrigated rice (SIR); (B) on-farm experiment, 1987, after red pepper, and after maize in 1988; (C) plots after maize in 1987 and in 1988 after SIR; (D) on-farm experiment, 1988, after maize, and after winter wheat in 1989; (E) plots, 1987, after maize, and in 1988 after SIR; (F) on-farm experiment, 1987, after red pepper, and after maize in 1988; (G) plots, 1988 and 1989, after SIR; (H) on-farm experiment after maize in 1988 and after winter wheat in 1989.**

was considered burn damage. No such burn damage occurred in 1988 (Fig. 2E,F). The regulatory effect of irrigation on individual density was significant (*P* = 1%) only once, in 1987, during farm experiments.

*Red spider mite* (Tetranychus urticae *Koch*). Red spider mite density per leaf was from 2 to 7 from the beginning of May until the end of August. More than 4 individuals per leaf caused burn damage (Fig. 2). In 1987, the regulatory effect of irrigation was significant (*P* = 0.1%); however, this could not be repeated in 1988. Together with the gradual desiccation of leaves, individual density decreased until the end of August and the species disappeared. In farm experiments, burn damage occurred for a short while, in 1988 at the beginning of July and in 1989 in June (Fig. 2G,H).

*Cereal spider mite* (Bryobia graminum *Schrank*). In Szarvas, the average density varied from 0.3 to 2.4 per leaf in 1988 and 1989. In Tiszafoldvar in farm experiments, it was from 0.5 to 1.8 per leaf in 1988. In 1989, the seasonal variation was 0.8–1.8. The individual density of the cereal spider mite varied from 0.5 to 2.4 individuals in August (Fig. 3A,B). In none of these years did irrigation affect population control.

*Green grain aphid* (Schizaphis graminum *Rodani*). In 1987 in Szarvas, we found this pest at the end of May. The average individual density was 0.4 leaf$^{-1}$. The maximum was 4.9 leaf$^{-1}$ in July, followed by a sudden drop in one week, after which it stayed low until the end of August. The seasonal dynamics of this species was similar to that of the previous year. The farm experiments produced the same results in 1987 and 1988 (Fig. 3C,D). The regulatory effect of irrigation on the population numbers was not detectable on the experimental plots, but was shown at *P* = 5% in the farm experiments near Tiszafoldvar in both years.

*Grain aphid* (Macrosiphum avenae *Fabricius*). In 1987 in Szarvas, the damage of the individuals of this species was present immediately after the appearance of the first leaves, at the end of May. At the beginning of the cropping period, individuals averaged 0.8 leaf$^{-1}$. Their individual density grew until 10 June and peaked at 4.5 after 20 June. The fluctuation in individual density was great in July and August. Our experience is that 3–4 grain aphids per leaf cause burn damage (Fig. 3E,F). Irrigation did not significantly change individual density.

*Barley flea beetle* (Phyllotreta vittula *Redtenbacher*). In many years, summer drought was favorable for the massive appearance of the barley flea beetle. We first observed its damage on the leaves and panicles of rice on both sprinkler-irrigated and flooded fields (Fig. 3I). At blossoming, the fully developed beetles went into the hollows of the open glumellas and damaged the capsule. When the glumellas closed, quite a few individuals were trapped in the hollows.

During our investigation, we wanted to assess the damage caused. Therefore, we set up experimental plots at the sides of sprinkler-irrigated and flooded rice fields, one set at the edges, one set at 10-m distance toward the center of the field, and one set in the middle. Because of prolonged flowering, the investigation was in progress for 3 weeks starting from heading of rice. On five 1-m$^2$ surfaces in the areas, we counted all healthy and damaged rice seeds in all panicles for 3 days.

Panicle damage was assessed as 6–15% in 1993 and 12–15% in 1994.

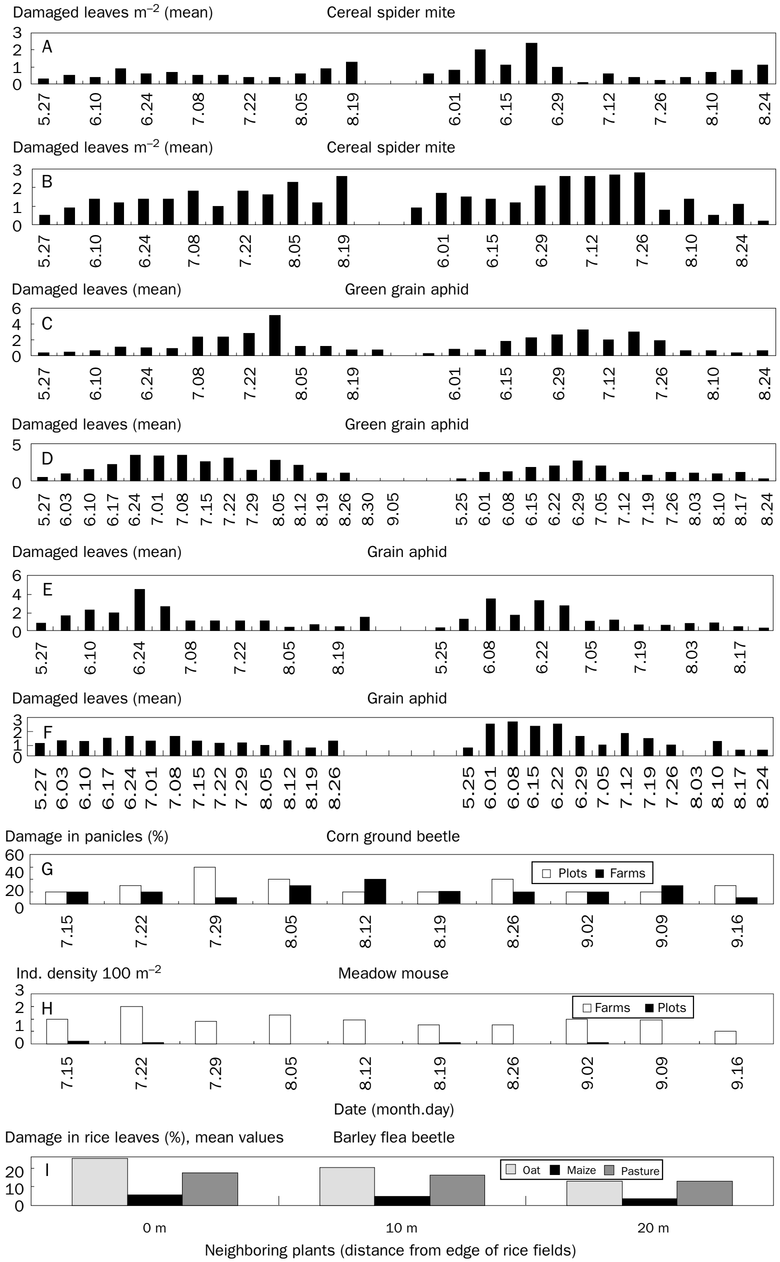

**Fig. 3. (A) Plots, after maize in 1988 and after sprinkler-irrigated rice (SIR) in 1989; (B) on-farm experiment, after maize in 1988 and after wheat in 1989; (C) plots, after maize in 1987 and after SIR in 1988; (D) on-farm experiment, after red pepper in 1987 and after SIR in 1988; (E) plots, after maize and after SIR in 1988; (F) on-farm experiment, after red pepper in 1987 and after SIR in 1988; (G) plots and experiment farms in 1989; (H) farms and plot in 1989; (I) the importance of the neighboring crop for barley flea beetle damage in SIR.**

We did not find a significant difference in the extent of the damage close to the levees or farther away from them in the middle range of the fields. The damage was larger, however, on rice fields bordering grazing land/pasture or cereal crop (especially oat) fields. With oat fields nearby, rice fields regularly exhibited damage of nearly double. This shows pest mobility on the one hand and that the rice field became an important source of food for the fully developed insects on the other.

Larvae have been found only in the soil of sprinkler-irrigated rice fields. In flooded rice fields, the primary habitats of these pests are the levees, which are the source of infestation. The weed foliage is the most damaged. Among the host plants, there were all kinds of dicotyledonous weeds as well.

### Stem and panicle pests

*Corn ground beetle* (Zabrus tenebroides *Goeze*). The degree of this beetle's damage was similar both in the experimental plots and the farm experiments. In 1989 at the beginning of the vegetative stage, corn ground beetle damaged 20% of the panicles at both sites, but ranged up to 40–60% in specific areas (Fig. 3G).

*Meadow mouse* (Microtus arvalis *Pallas*). In 1989 on the Szarvas experimental plots, damage was minimal and was observed from the beginning of August. Typically, the mice cut the stem and chewed the panicles.

In farm fields, however, damage reached as much as 50% in 1989 and was even greater in several smaller-sized fields. After harvest of neighboring grain fields, the pests moved toward rice fields. Green rice meant a rich food source for the mice. As many as two mice $m^{-2}$ were observed at some sites.

Regular irrigation constantly decreased the individual density of these pests, but even so the number stayed at 0.5 $m^{-2}$ by mid-September (Fig. 3H). Irrigation decreased individual density only when the amount of water was equivalent to 10 mm precipitation per week, as measured in fields at $P = 2\%$.

*Hare* (Lepus europapaeus *L.*). We observed hare damage in each of the three years of the study (1987-89), on both the experimental plots and farms. The animals ate the plants from as early as their one- or two-leaf stage, and usually cut off the whole plant above the ground. These plants perished or were seriously retarded in their growth. The animals were feeding along regular paths and their trails grew wider each day from rice plant feeding. The degree of damage varied from 5% to 20% in different parts of the field.

## Discussion

### Root pests

Regular irrigation did not significantly reduce the individual density of black cricket. We assume that no damage will occur in rice crops, as the damage of this species is not significant in other grain crops.

Bulb mite was observed in rice sown after maize. Sprinkler-irrigated rice was not a sufficient food source and regular irrigation was unfavorable for this species. It disappeared after July and did not reappear. The situation is similar with elm leaf aphid also.

### Stem and leaf pests

The individual density of springtail was 5–8 times greater after maize than after sprinkler-irrigated rice. Rice reduced individual density although it is possible that other factors not examined here may have played a role in the seasonal dynamics.

Sprinkler-irrigated rice as the previous plant was not favorable for the massive reproduction of yellow springtail, whereas maize or pepper as the previous crop increased its individual density. Burn damage from this species is not likely to occur or may occur only very infrequently.

When 6 leaves $m^{-2}$ are damaged by the leaf beetle, burn damage should be expected, especially when maize is grown before rice in that area. If rice is grown after rice, no burn damage occurs since the population of these insect pests is low throughout the whole vegetative period, and also because regular irrigation has a regulatory effect on the population.

We observed regular damage by the corn ground beetle in 1987 on the experimental plots after maize as the previous crop, from the beginning of the cropping season. Its individual density was relatively stable. Maize is not favorable for this species. We were surprised to find a high individual density after pepper on farms.

The polyphagous red spider mite found favorable living conditions on sprinkler-irrigated rice fields. In our opinion, two mites per leaf fall into the category of burn damage. The literature does not contain relevant data. Irrigation reduced the population, but in other cases this effect could not be shown.

The cereal spider mite caused damage but no burn damage. Its individual density was greater on farms than on experimental plots. Regular irrigation reduced individual density. Its natural predators, such as *Coccinella septempunctata* adults and larvae and *Chrisopa perla*, were frequent in fields. If sprinkler-irrigated rice was sown in two consecutive years, the population of this species was greater than in the previous year. Other previous crops probably had no positive effect on the individual density of this species.

The previous crop plays a minor role in the case of the green grain aphid since wind is known to be the most effective aid in spreading leaf aphids. They travel to great distances even under windless conditions (Szalay-Marzso 1988).

On both plots and farms, the increase in individual density was simultaneous with the period of time when the winged forms set out to leave the ripening grain fields where quantity and quality of food were decreasing. This phenomenon was first noticed in Szarvas on the cereal crop fields near the experimental plots. In the evening hours, when there was no wind, millions of aphids left the grain field to settle on the rice field a few hundred meters away. Migration was also observed from a grain field to a farm rice field, although it was several hundred meters away.

The grain aphid's migration to rice fields took place at the same time as that of the green grain aphid. Sprinkler-irrigated rice aided the birth of new generations of grain aphids. Population figures varied excessively, possibly because of several factors yet unknown to us.

### Panicle pests

The proportion of panicles damaged by the corn ground beetle normally varied from 20% to 40% and on a single occasion was more than 60% on the experimental plots. On one panicle, the number of damaged grains was 1 at the minimum, 13 at the maximum, and 7 on average. Burn damage did not develop, but irrigation did not play a role in this.

For the meadow mouse, 2–5 $m^{-2}$ means burn damage (Bognar-Huzian 1979). On the experimental plots, burn damage occurred only occasionally and was stopped by regular irrigation.

Irrigation reduced the number of pests on the farms, but local groups survived where the soil did not become saturated with water and thus the microclimatic conditions were favorable for them. The pests moved around and fed during the day, unlike they normally do. They felt safe from pedators. Their damage should be expected in dry summers, especially after grain crops are harvested.

Hare damage on sprinkler-irrigated rice fields was general and eye-catching. Immediately after rice sprouted and continued to grow, fresh droppings left behind signaled the animals' regular feeding.

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# Simultaneous phenologies between Chironomidae larvae (Diptera) and rice germination in rice fields in the Camargue (South of France)

J.-C. Vala, V. Pons, S. Arnoux, and M. Roux-Cuvelier

In the rice fields of the Camargue, in the South of France, during two cropping periods in 1997 and 1998, Chironomidae populations (number of species, densities, and time of appearance) in fields showed major differences with phenological stages. On the whole, a shift in chironomid emergence time was clearly observed.

Camargue rice fields located at 42°N are at the northern limit of European rice culture. This geographical distribution allows only one harvest at the end of summer—August and September. Rice is sowed at the end of April or in early May when temperature is optimal and above 12 °C. Including the annual crop rotations with wheat, Camargue rice fields cover 20,000 to 25,000 ha. In this area of the South of France, the major stresses are water salinity, strong cold wind (called the Mistral), temperature variations occurring early in germination, and the action of *Chiro suppresalis* and Chironomidae larvae. This study presents information on the relationship between temperature and Chironomidae larvae.

Some *Chironomidae* spp. are among the most injurious pests and damage can be observed early, starting during seed germination. Plantlet development is reduced because the larvae feed on roots, leaves, and stems. Attacks are quite variable: in some years, attacks can first be detected as early as the first week following field flooding; in other years, they may occur later and can be observed only during the third or fourth week. The economic consequences of this timing may be of major importance to production.

## Materials and methods

Chironomidae are classically captured by hand with a PVC tube (inner diameter 5.6 cm) in the mud. For the species that damage rice yield, larvae are trapped directly from rice roots and stems by using a net case (20 × 10 cm), the mesh of which (2 × 2 mm) is big enough to let roots and stems go through. Each case contains 100 rice seeds. Five to eight groups of five traps are laid out a few hours before sowing. Each group constitutes five traps placed at a distance of 0.50 m. To capture adults, we used classical emergence traps. Moreover, at various points in the rice fields, we placed yellow sticking label pegs, with 30–40 cm extending above the water.

Physical parameters such as temperature, diluted oxygen, pH, and conductivity were also recorded in the course of samplings every 5 days. For the present results, only temperature was taken into account.

## Results

In Camargue, sowing usually takes place from 20 April to 5 May. In the rice fields studied here, sowing took place from 22 to 24 April.

### Chironomidae species collected

A total of 69 species were identified from 22 April to 30 May in the rice fields (Vala and Moubayed n.d., Vala et al n.d.). Taxonomically, the larvae are distributed in 3 subfamilies: 4 Tanypodinae species, 25 Orthocladiinae, and 40 Chironominae, including 26 Chironomini and 14 Tanytarsini. In flooding, the Orthocladiinae are more numerous because of their dispersal in draining water, where they are abundant. Among these are *Cricotopus sylvestris*, *C. bicinctus*, *C. tricinctus*, *Limnophyes minimus*, *Orthocladius rubicundus*, *Tvetenia calvescens,* and *T. verralli*. Of the Chironomini, those mainly present in incoming waters were *Chironomus annularius*, *C. aprilinus*, *C. pseudothummi*, *C. riparius*, and *Dicrotendipes notatus*. Among Tanytarsini we identified *Tanytarsus eminulus*, *T. ejuncidus*, *T. fimbriatus*, *T. brundini*, *T. heusdensis*, *Rheotarnytarsus ringei*, and *Micropsectra* cf. *contracta*. The imported Tanypodinae were mainly *Ablabesmya monilis*, *Rheopelopia ornata,* and *Macropelopia nebulosa*; the fourth Tanypodinae species, *Procladius (Holotanypus) choreus*, appeared later in the fields.

### Annual observations

During rice production of 1997, temperature was optimal on the sowing date (22 April, Fig. 1). Then, average values varied from 16 to 18 °C. However, under the influence of the strong wind, a decrease in temperature was noted on 25 April (7 °C) and on 6 May (10 °C). Sixty-nine species were counted in rice fields as early as 15 d after flooding. Some species were carried by draining water.

The highest density was reached 20 d after flooding. Phytophagous larvae, mainly *Cricotopus sylvestris* and *C. trifasciatus,* were the most numerous (30% to 60% according to the rice field studied) and consumed the roots of germinating seeds or of young growing plants that had not reached the first leaf stage. Also, in some rice fields, one *Tanytarsus* sp. and one *Paratanytarsus* sp. were found feeding inside the seeds near the young buds. Consequently, rice plants could not take root and these seedlings were transported by the wind to the edges of the field. Some farmers had to resow. Worldwide damage observed on rice seeds and seedlings was also reported from genera *Tanytarsus, Cricotopus,* and *Chironomus* (Way and Wallace 1989, Ferrarese 1992, Pasini and Dalla Montà 1997).

The populations of the two main pest species showed similar population dynamics (Fig. 2) in both Mas d'Yvan located in the north of Camargue and Mas du Vedeau in the south. In Mas du Vedeau, the Chironomidae population was not so abundant, probably because of the effect of salt (conductivity rising to 1,300–1,400 µS cm$^{-1}$ but only 350–400 at Mas d'Yvan). Point 0 on the 10th day of observation at Mas d'Yvan is correlated to the drying of the field.

In 1998, during the first fortnight following flooding, temperature varied from 12 to 14 °C (Fig. 1). Only a few chironomid larvae could be observed. Although smaller, rice plantlets developed a good set of roots strongly implanted in the mud. Fewer than 10 species were counted at water flooding. The presence of Chironomidae larvae was low or nil when germination started, but they appeared in large numbers 25–28 days after flooding, at the third-leaf stage of the rice plants. At this time, there were 8–10 roots, 3 to 4 cm long. Thus, partial destruction of a few roots affected the growth of rice plants only slightly. This delay in Chironomidae phenology limited damage to rice growth and rice production.

The differences observed from 1997 to 1998 were induced by climatic factors, temperature, and precipitation. The low rainfall that prevailed in 1996 continued during 1997. Floodwater for rice fields was still pumped out of the Rhone River. In 1997, the Rhone water level was very low and temperature was 13–26 °C. With pumping into the fields, many Chironomidae were transported at the second or third instars and

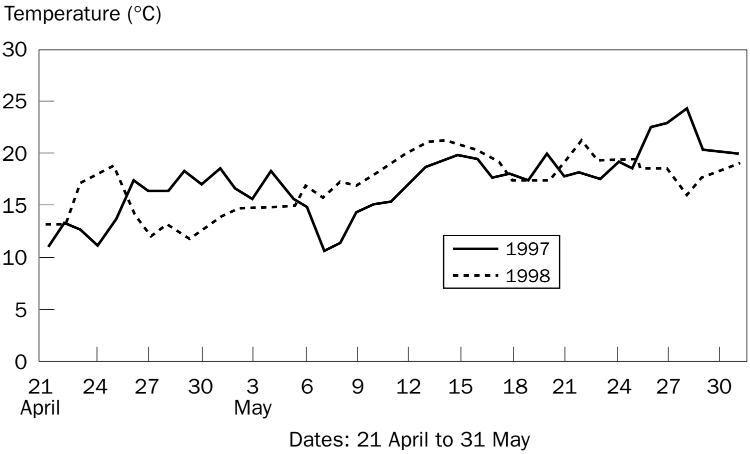

**Fig. 1. Average temperature for the 1997 and 1998 planting seasons.**

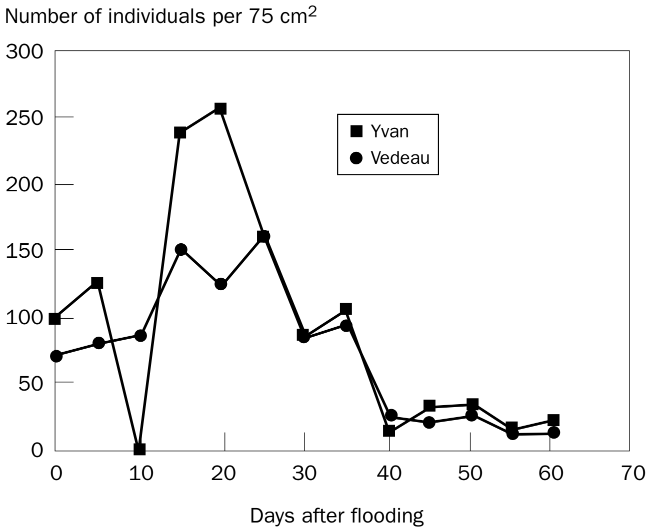

**Fig. 2. *Cricotopus sylvestris* and *C. trifasciatus* found in two rice fields in 1997.**

pupae. They immediately attacked rice roots (Fig. 3). In contrast, in 1998, the river water level was higher, the temperature lower, and the density of Chironomidae larvae transported into the rice field lower and composed of more juvenile larval instars. When chironomid populations exploded, rice plants were close to the three-leaf or tillage developmental stage and were vigorous enough not to be weakened by the larvae.

In Camargue, the relationship between temperature and Chironomidae larvae is well established. The observations for 1999 are apparently similar to the data obtained in 1997. The first generation of *Cricotopus* spp. is also very precocious with a heavy attack on the young plantlets. A study is now being carried out to determine the importance of larvae attacks for seed germination at intervals from 20 April to 25 May, very late dates for rice sowing in Camargue, where harvesting after the end of August is extremely difficult.

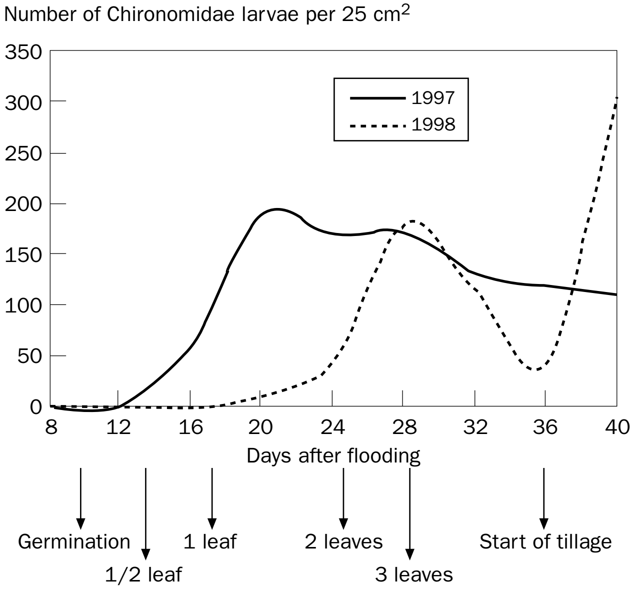

**Fig. 3. Comparative phenologies between Chironomidae larvae and rice seeds in germination.**

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## Notes

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ENTOMOLOGY

# Update on new management tools for the rice water weevil

M.O. Way and R.G. Wallace

In 1997 and 1998, granular carbofuran use for rice water weevil (RWW) management in Texas was increasingly restricted and was completely banned in 1999. Lambda-cyhalothrin (Karate) received a United States Environmental Protection Agency (USEPA) registration in the summer of 1997 and was used on a significant area in Texas in 1998 for RWW, chinch bug [*Blissus leucopterus* leucopterus (Say)], and fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] control. Fipronil (Icon 6.2FS) received a USEPA registration as a seed treatment for RWW control in the late summer of 1998. Diflubenzuron (Dimilin 2L) received a USEPA registration in the spring of 1999.

Results from several years showed that Karate 1EC or Karate Z applied at 0.034 kg ai ha$^{-1}$ early postpermanent flood provided RWW control similar to granular carbofuran. Results in 1997 and 1998 showed that Karate 1EC or Karate Z applied prepermanent flood provided control of RWW comparable to postpermanent flood applications. Results from several years also showed that Icon 6.2FS applied at 0.035 to 0.056 kg ai ha$^{-1}$ as a seed treatment control led to RWW comparable to granular carbofuran.

The United States Environmental Protection Agency (USEPA) deregistered the use of granular carbofuran for rice water weevil (RWW), *Lissorhoptrus oryzophilus* Kuschel, control after the 1999 growing season. Since 1989, we have evaluated other insecticides to replace granular carbofuran. For Texas, the USEPA approved the use of Karate 1EC/Z (active ingredient lambda-cyhalothrin) in 1997 and Icon 6.2FS (active ingredient fipronil) and Dimilin 2L (active ingredient diflubenzuron) in 1999.

Most rice in Texas is drill- or broadcast-planted in dry soil. Rice is usually flushed (temporary flood) as needed until application of the permanent flood (about 3 to 6 wk after rice emergence through the soil). This method of irrigation is important to understanding the proper timing of insecticide applications for RWW control. For this method of irrigation, our data show that most RWW oviposition occurs after application of the permanent flood (Way 1992).

## Materials and methods

Experiments were conducted in 1996-98 at the Texas A&M University Agricultural Research and Extension Center at Beaumont and Western Area Operations at Eagle Lake (Way et al 1997). The experimental design was a randomized complete block with four replications. Plot size was 4.6 m × 2.4 m with each plot surrounded by a metal barrier to prevent movement of insecticides. Rice was drill- or broadcast-planted in dry soil and flushed as needed. The permanent flood was applied 3 wk after emergence. All experiments relied on natural infestations of RWW. Standard Texas cultural practices (weed control and fertilization) were employed (Texas Agricultural Extension Service 1999). Liquid formulations of treatment insecticides were applied with a 4-nozzle (tip size 800067, 50-mesh screens) hand-held spray rig pressurized with carbon dioxide. Granular treatment insecticides were applied with a hand-held shaker jar. Seed treatments were applied by placing the proper amount of insecticide and seed in a plastic bag and mixing until the seed was thoroughly coated. About 3 and 5 wk after the permanent flood, five 10-cm-diameter by 10-cm-deep soil cores (each containing at least one rice plant) were removed from each plot. Rice plants were washed and immature RWW recovered from the roots. At maturity, plots were harvested with a small-plot combine. Yields were adjusted to 12% moisture. Insect counts were transformed using the square root of $x + 0.5$ and all data analyzed by two-way analysis of variance and Duncan's multiple range test.

## Results

Icon 6.2FS provided control of RWW comparable to granular carbofuran, regardless of seeding rate (Table 1). Yields were not significantly different from those of the untreated plots. Karate Z applied before the permanent flood provided control of RWW comparable to granular carbofuran (Table 2). Dimilin 2L provided control of RWW comparable to granular carbofuran. A single application of Dimilin 2L 3 d after the permanent flood at 0.21 kg ai ha$^{-1}$ yielded 1,500 kg ha$^{-1}$ more than the untreated plots (Table 3).

In Texas, Dimilin 2L, Icon 6.2FS, and Karate Z applied at the proper rates and times provided control of RWW comparable to granular carbofuran. All three insecticides must be applied earlier than recommended applications of granular carbofuran. Also, no economic thresholds are currently available to use in association with new replacement insecticides. Thus, efforts are in progress to educate Texas rice farmers regarding the use of the new products and to develop associated economic thresholds.

**Table 1. Icon 6.2FS seeding rates vs rice water weevil (RWW), Beaumont, Texas, 1998.**

| Treatment | Application rate (kg ai ha$^{-1}$) | Seeding rate (kg ha$^{-1}$) | Timing | Av no. of immature RWW per 5 cores[a] |  | Av yield (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|
|  |  |  |  | 2 Jun | 13 Jun |  |
| Untreated | – | 95 | – | 50.8 a | 57.0 a | 5,310 |
| Icon 6.2FS (fipronil) | 0.028 | 67 | st[b] | 3.0 c | 4.8 c | 5,580 |
| Icon 6.2FS (fipronil) | 0.039 | 95 | st | 3.8 c | 2.5 c | 5,910 |
| Icon 6.2FS (fipronil) | 0.05 | 123 | st | 2.8 c | 1.8 c | 5,870 |
| Furadan 3G (carbofuran) | 0.67 | 95 | 14 dapf[c] | 2.8 c | 2.8 c | 5,890 |

[a]Means in a column followed by the same or no letter are not significantly different at the 5% level (analysis of variance, Duncan's multiple range test). [b]st = seed treatment; seed was treated with Icon 6.2FS at 0.041 kg ai ha$^{-1}$ given a seeding rate of 100 kg ha$^{-1}$. [c]dapf = days after application of the permanent flood.

**Table 2. Efficacy of selected insecticides for rice water weevil (RWW) control, Beaumont, Texas, 1998.**

| Treatment | Application rate (kg ai ha$^{-1}$) | Timing | Av no. immature RWW per 5 cores[a] |  | Av yield (kg ha$^{-1}$) |
|---|---|---|---|---|---|
|  |  |  | 12 Jun | 22 Jun |  |
| Icon 6.2FS (fipronil) | 0.041 | st[b] | 0.8 b | 2.3 b | 6,646 |
| Karate Z (lambda-cyhalothrin) | 0.034 | 3 dapf[c] | 0.3 b | 0.3 b | 6,026 |
| Karate Z (lambda-cyhalothrin) | 0.034 | bpf[d] | 0 b | 0.3 b | 6,162 |
| Karate Z (lambda-cyhalothrin) | 0.034 | 3 dbpf[e] | 0.5 b | 0 b | 6,377 |
| Karate Z (lambda-cyhalothrin) | 0.034 | 6 dbpf | 0.3 b | 0 b | 6,271 |
| Karate Z (lambda-cyhalothrin) | 0.034 | 12 dbpf | 0.8 b | 0.5 b | 6,168 |
| Dimilin 2L (diflubenzuron) | 0.28 | 3 dapf | 2.0 b | 1.5 b | 6,629 |
| Furadan 3G (carbofuran) | 0.67 | 14 dapf | 0.8 b | 1.0 b | 6,260 |
| Untreated | – | – | 34.0 a | 16.5 a | 5,775 |

[a]Means in a column followed by the same letter are not significantly different at the 5% level (analysis of variance, Duncan's multiple range test). [b]st = seed treatment (0.041 kg ai ha$^{-1}$) based on seeding rate of 100 kg ha$^{-1}$. [c]dapf = days after application of the permanent flood. [d]bpf = immediately before application of the permanent flood. [e]dbpf = days before application of the permanent flood.

**Table 3. Dimilin 2L vs rice water weevil (RWW), Eagle Lake, Texas, 1996.**

| Treatment | Application rate (kg ai $ha^{-1}$) | Timing | Av no. immature RWW per 5 cores[a] | | Av yield (kg $ha^{-1}$) |
|---|---|---|---|---|---|
| | | | 29 May | 18 Jun | |
| Untreated | – | – | 83.3 a | 67.3 a | 8,160 b |
| Dimilin 2L (diflubenzuron) | 0.21 | 3 dapf[b] | 0.5 b | 14.8 b | 9,734 a |
| Dimilin 2L (diflubenzuron) | 0.28 | 3 dapf | 0 b | 6.5 bc | 9,457 ab |
| Dimilin 2L (diflubenzuron) | 0.11 + 0.11 | 3 + 10 dapf | 1.0 b | 7.5 bc | 10,069 a |
| Dimilin 2L (diflubenzuron) | 0.14 + 0.14 | 3 + 10 dapf | 0 b | 2.0 c | 9,800 a |
| Furadan 3G (carbofuran) | 0.67 | 13 dapf | 0 b | 11.3 bc | 9,556 ab |

[a] Means followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test). [b] dapf = days after application of the permanent flood.

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GENETICS

# Early maturing rice cultivars have small root systems

J.F. Angus, R.L. Williams, T.C. Farrell, and R.F. Reinke

Rice cultivars differing in maturity were grown in two field experiments at Yanco, Australia, and sampled for aboveground and root biomass. In the first experiment, nine cultivars were sampled on the same day, when their stages of development differed from early stem elongation to early grain filling. The aboveground weights of the cultivars on that day were similar and unrelated to maturity. However, root weight increased with later maturity, with an additional 11 g $m^{-2}$ for each day's delay in anthesis. The ratio of aboveground to root weight (the shoot:root ratio) decreased from 11.2 to 2.7 between the earliest and latest flowering cultivars. In another set of observations in the first experiment, six cultivars were sampled on their date of anthesis. For each day's delay in anthesis, shoot weight increased by 12 g $m^{-2}$ and root weight increased by 21 g $m^{-2}$. The shoot:root ratio decreased from 8.0 to 2.6 between the earliest and latest flowering cultivars.

In the second experiment, shoot and root weights of four cultivars were measured every 2 wk throughout the growing season. The results confirmed that later maturing cultivars produced more root dry matter, but there were two patterns of root growth. For three early maturing cultivars, roots grew at a similar rate (2.6 g $m^{-2}$ $d^{-1}$) until the panicle initiation stage, after which they stopped growing, leading to root weights proportional to the duration of vegetative growth. However, the root growth rate of a later maturing cultivar, Amaroo, was 8.2 g $m^{-2}$ $d^{-1}$ at the same stage, and continued at that rate until panicle initiation. The combination of faster growth and longer duration led to a root weight of Amaroo at maturity of 5 t $ha^{-1}$ compared with 2–3 t $ha^{-1}$ for the other cultivars. However, shoot growth rate was unrelated to cultivar maturity, so the additional roots were not produced at the expense of shoots.

These results suggest that breeding for earliness may lead to cultivars with small root systems. It is not clear that small size is necessarily a disadvantage since Australian early maturing cultivars have yield potential similar to that of cultivars that mature later.

The increased cereal yield potential brought about by plant breeding has been mostly due to increased harvest index, the ratio of grain to aboveground dry matter (Evans 1993). There is little evidence of a change in total biomass productivity and the increased yield has been offset by reduced stem weight associated with genes for dwarfing and earlier maturity. Much of the evaluation of the physiological basis of yield improvement has not considered root growth. It is possible that more rapid shoot growth comes at the expense of root growth, as suggested for wheat by Davidson et al (1990). Their hypothesis is supported by the results of Gómez-Macpherson et al (1998), who compared seedlings of near-isogenic lines of wheat growing in artificial canopies and found that early lines grew more shoot and less root than late lines.

Confirmation of this result in the field is difficult for upland crops because of the difficulty of recovering complete root systems. Flooded rice is more easily studied because its roots are shallow and it grows in soft soil. The objective of this study was to evaluate and understand the relationship between maturity and dry matter production of shoots and roots in a range of rice cultivars.

The development of early maturing rice cultivars is important in many environments. In the tropics, the advantage is being able to grow more crops in one year. In temperate environments, earliness is required to adapt rice to cooler regions; in arid environments, it is needed to reduce the requirements for irrigation water.

## Materials and methods

Cultivars and breeding lines of rice were grown in two field experiments at the Yanco Agricultural Institute (lat. 34°37′S, long. 146°25′E, 150 m elevation). All plots were 20 m long and 1.4 m wide with 8 rows, 18 cm apart. They were drill-sown with 120 kg $ha^{-1}$ of seed into dry soil. The fields were irrigated intermittently until mid-November, when permanent floodwater was applied. Local best-practice management of fertilizer and weed control, as described in Reinke et al (1994), was maintained in all experiments. Nine cultivars were selected on the basis of differing flowering dates for the measurements of shoot and root growth (Table 1). All were japonica with the exception of IR20897, which was indica. The progress of anthesis was recorded by counting the percentage of panicles with exserted anthers on every second day. Anthesis was defined as the first day when anthers had dehisced on at least 50% of the panicles. For measurements of aboveground dry matter production, a quadrat of 0.5 $m^2$ from the inner six drilled rows was harvested at the level of the soil surface from each of the three replicates. Roots were then sampled from the soil within each quadrat. In 1996-97, six cores were collected between the rows with a circular stainless-steel tube 10 cm in diameter and 10 cm deep, with a 1.5-mm wall thickness and a sharpened cutting edge. In 1997-98, a larger sampler (15 cm in diameter, 15 cm deep) was used to collect two cores, one within and one between the rows. The top of the sampling tube was attached to a vertical shaft with a horizontal handle, which was rotated to cut the soil and roots to the sampling depth. The corer was then tilted slightly to break the suction and a hand inserted into the soil to prevent the core from falling out of the corer as it was removed. The cores were frozen until ready for processing,

**Table 1. Cultivars and details of the two experiments.**

| Cultivar | Origin | Experiment 1 (sown 10 Oct 1996) |  |  | Experiment 2 (sown 2 Oct 1997) |
|---|---|---|---|---|---|
|  |  | Anthesis date in 1997 | Measurements[a] on 22 Jan 1997 | Measurements at anthesis 1997 | Anthesis date in 1997-98 |
| H473 | Hungary | 13 Jan | ● |  | 27 Dec |
| HSC55 | Hungary | 15 Jan | ● |  |  |
| Plovdiv 22 | Russia | 20 Jan | ● | ● |  |
| Barkat | India | 20 Jan |  | ● | 3 Jan |
| Jarrah | Australia | 25 Jan | ● | ● | 4 Jan |
| Illabong | Australia | 30 Jan | ● | ● |  |
| YRL39 | Australia | 6 Feb | ● |  |  |
| Amaroo | Australia | 8 Feb | ● | ● | 22 Jan |
| IR20897 | IRRI | 12 Feb |  | ● |  |

[a]● indicates cultivar was sampled on this date.

when they were thawed and the soil washed from the roots. The process of root washing consisted of placing the core in a tray with 2-mm holes and hosing gently with a water jet. After most of the soil was washed away, the mat of roots was placed in a 20-cm-diameter soil sieve with a 1-mm mesh. There the roots were repeatedly hosed vigorously, submerged in water, and agitated while still in the dish. To ensure that no roots were lost in the process, a second dish with a 0.5-mm mesh was placed below the first dish. The quantity of roots reaching this dish was negligible. The shoots and roots were dried at 80 °C for 48 h and then weighed.

In experiment 1, measurements of shoot and root biomass were made on nine cultivars. One set of measurements was made on a set of seven cultivars on 22 January 1997 when four cultivars were past anthesis. A second set of measurements was made as close as possible to the date of anthesis, in all cases within 3 d of anthesis. Measurements of four of the cultivars were included in both data sets (Table 1). Experiment 2, in 1997-98, involved sampling shoots and roots of four cultivars at 2-wk intervals. The number of samplings varied from seven for the earliest cultivar, H473, to 11 for the latest, Amaroo.

## Results

### Experiment 1

Figure 1 shows that root weight was greater in later flowering cultivars when all cultivars were sampled on the same day. Root weight varied from 100 g $m^{-2}$ for the earliest flowering cultivar to 406 g $m^{-2}$ for the latest. There was a linear increase of $11.0 \pm 1.4$ g $m^{-2}$ for each day's delay in anthesis. The biomass of aboveground tissue was unrelated to the time of flowering and, consequently, the shoot:root ratio was

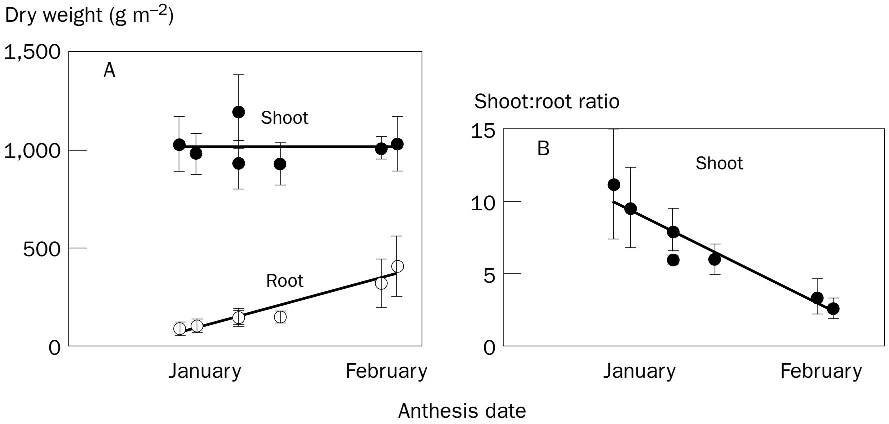

**Fig. 1. Aboveground and root dry weights of seven rice cultivars growing in field plots harvested on 22 January 1997 in relation to date of anthesis. (A) Aboveground and root weights, (B) the same data expressed as the shoot:root ratio. Bars represent standard errors of the means.**

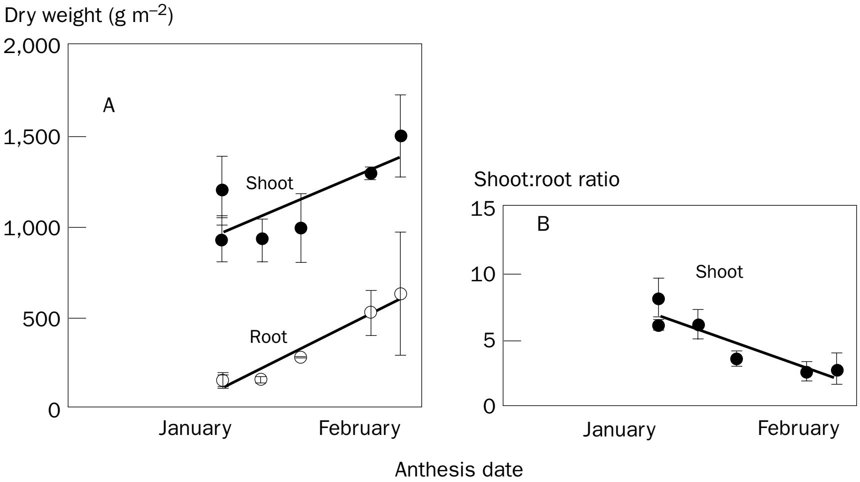

**Fig. 2. (A) Aboveground and root dry weights of six rice cultivars and lines harvested at anthesis (±3 d) in relation to date of anthesis. (B) The same data expressed as the shoot:root ratio. Bars represent standard errors of the means.**

strongly related to flowering time, decreasing from 11.2 for the earliest flowering cultivar to 2.7 for the latest flowering one.

Figure 2 shows the equivalent data when all crops were sampled close to their flowering dates. In this case, the weights of both roots and aboveground organs increased with later flowering, with each day's delay in flowering associated with an additional 21.1 ± 2.3 g $m^{-2}$ of roots and 12.5 ± 6.6 g $m^{-2}$ of aboveground organs. The shoot:root ratio measured in this way varied from 8.0 for the earliest flowering cultivar to 2.6 for the latest flowering. The outlying point in Figure 2 refers to cultivar Barkat, which had a relatively large shoot weight in relation to its maturity and a consequently large shoot:root ratio. This outlier is also responsible for the large standard errors in the estimate of shoot growth in relation to flowering date.

### Experiment 2

Four cultivars were selected for serial harvests of shoots and roots in the following season because of their different maturities. Figure 3 shows that root growth of the three earlier cultivars was similar until soon after panicle initiation, after which root growth slowed or stopped. Over the period of the first four harvests, their growth rate was 2.6 ± 0.8 g $m^{-2}$ $d^{-1}$, with no significant differences between cultivars. Root growth of the later cultivar Amaroo also stopped at about panicle initiation, but before that time its growth rate was significantly greater (8.2 ± 0.5 g $m^{-2}$ $d^{-1}$) than for the earlier cultivars. The mean shoot growth rate at the same time was 19.3 ± 1.0 g $m^{-2}$ $d^{-1}$, with no significant differences between cultivars.

Figure 4 shows that the shoot:root ratios of the four cultivars reflected root growth, with the rankings the reverse of root weight. The values for root weight and shoot:root ratio at anthesis in experiment 2 were similar to those measured in experiment 1 (Fig. 2).

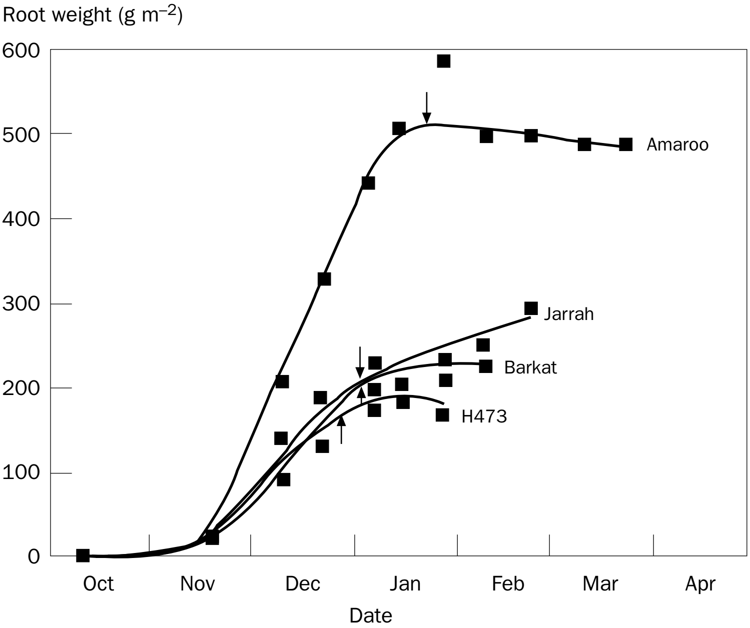

**Fig. 3. Root weight of rice cultivars growing in experiment 2. The arrows indicate anthesis dates.**

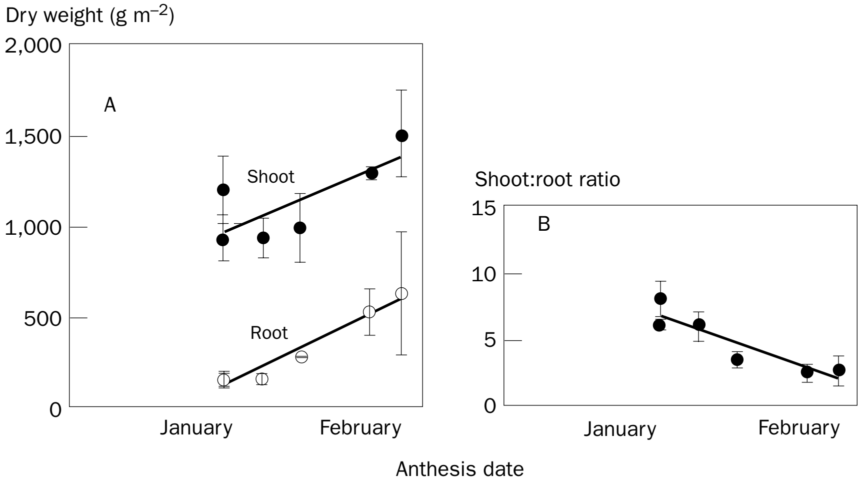

**Fig. 4. Shoot:root ratios of the rice cultivars shown in Figure 3.**

## Discussion

The results show that early flowering cultivars produced less root biomass than later flowering cultivars, suggesting that breeding for earliness may lead to reduced root growth. Gómez-Macpherson et al (1998) reported a similar result with wheat growing in simulated canopies in controlled environments, but the differences in root growth in relation to flowering date in this study are much greater. Their explanation was that plants allocated less assimilates to roots because of competition by stems when they elongated. This explanation is consistent with the data for root growth of the three early cultivars shown in Figure 3, where growth of roots slowed or stopped at about the time of panicle initiation. However, the explanation of Gómez-Macpherson et al (1998) is not consistent with the rapid root growth of the late cultivar Amaroo. The root growth of this cultivar was clearly greater than that of the other cultivars for three harvests before it initiated panicles. The exception provided by Amaroo leads to the conclusion that it is possible to breed for a different size of root system, other than by altering maturity, and that earliness does not necessarily lead to small roots.

The differences in root weight raise a question whether a large root system is essential for high productivity. For example, at the time of flowering, the early maturing cultivars had root weights that were 40% of those of the later maturing cultivars. Despite this difference, early and late maturing cultivars have produced similar yields (about 13 t ha$^{-1}$) when grown under optimal conditions (Reinke et al 1994, Williams and Angus 1994). In less favorable conditions, indicated by average farm yields of about 9 t ha$^{-1}$ in the Riverina, the average yields of early flowering cultivars are generally less than those of later flowering cultivars. The lower efficiency of early flowering cultivars with suboptimal management suggests that earliness may come at the expense of resilience. Reinke et al (1994) suggested that the smaller biomass of early cultivars remobilized less reserves to grain, so that yields were reduced if assimilation after anthesis was inadequate for grain filling. Roots may also contain reserves available for redistribution, and research is needed on whether the root reserves complement the retranslocated stem reserves.

Apart from their possible role in redistribution, the functions of rice roots are water and nutrient uptake, providing aeration of the rhizosphere via the aerenchyma, and providing mechanical support for the shoot. Modest root densities are sufficient for uptake of water, and, provided fertilizer is adequate, for uptake of nutrients. We are unaware of any studies on the root density needed to supply oxygen for respiration of roots and soil in the rhizosphere. However, even if a particular genotype has inadequate capacity to transport oxygen, there is a management option of midseason drainage, as widely practiced in Japan, enabling the rhizosphere to briefly become aerobic for a period of hours or days before reflooding (Matsushima 1980). Poor mechanical strength of the stems and crowns may be a more significant limitation of early maturing cultivars, and large roots may reduce the risk of lodging. Other possible advantages of large root systems are competition with weeds and resistance to some soil pests and diseases. Overall, there may be situations in which a large root system is an advantage but there is no evidence that it is essential, and good crop management may be an adequate substitute.

The results are puzzling when interpreted in terms of assimilate sources and sinks. The greater root growth of late-flowering cultivars suggests that shoot growth was not limited by the amount of assimilates available, that is, growth was not source-limited. If growth had been source-limited, one would expect greater root growth to be offset by reduced shoot growth. The results are more consistent with growth of roots being limited by the size of the sink, perhaps because branching of the roots as they become older leads to an exponentially increasing demand for assimilates. As such, the total sink activity at panicle initiation is greater for longer duration rice types, resulting in greater total biomass accumulation. However, there was no evidence in our data of increased shoot growth as seen during stem elongation of early maturing wheat cultivars (Davidson et al 1990).

These results suggest that roots represent an important and flexible sink for vegetative assimilates and a potential reserve of assimilates to buffer against stress during grain filling. Further research is justified on the significance of crop maturity for root growth, for example, to clarify when additional roots compete with aboveground tissue for assimilates, and when additional sinks in the root system stimulate assimilation. It is important to quantify how much root biomass consists of nonstructural material that is available for retranslocation to grain and whether root reserves contribute to yield and whether these reserves contribute to the apparent resilience of later flowering cultivars. The wider role of roots in farming systems should also be considered in the light of these results, since the smaller roots of early maturing cultivars will leave less residue to replenish soil organic matter.

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## Notes

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# Improving rice through somaculture and induced mutation

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Rice (*Oryza sativa* L.) is one of the most important crops in São Paulo State, Brazil, for both production and consumption. It is cultivated predominantly in an upland system in which warm temperatures and humidity provide an excellent environment for the rapid spread of blast disease (caused by *Pyricularia grisea*). The objective of this research was to induce mutation in conjunction with somaclonal variation for enhanced blast resistance and increased yield potential in the commercial upland rice variety IAC 201.

Seeds of IAC 201 were irradiated with a source of $^{60}$Co gamma rays at 30, 35, and 40 krad. The germination rate for all irradiation treatments was less than 50%; only the 30 krad treatment resulted in an acceptable germination rate. This finding is consistent with previous results in rice and in barley. Immature panicles and seeds were harvested from $M_1$ plants irradiated at 30 krad to provide material for in vitro and in vivo mutation. Selection was not done in the $M_2$ generation. Panicles harvested were evaluated in the $M_3$ generation for blast reaction. From 10,340 panicles screened, 267 lines were selected with a possible blast tolerance. In the $M_4$ generation, these populations were again evaluated for blast reaction and for agronomic traits. Fifty-three possible mutant lines were selected with tolerance for blast, and more than 15% had better yield potential.

Results from the $M_3$ generation in selection for blast resistance indicated that, in vivo, 37 lines were moderately resistant to blast, and these results were confirmed.

The results described in the fourth phase showed that the population selected in the $M_3$ generation had normal distribution for all other characters and genetic variability was both positive and negative, indicating that selection for the traits studied can improve the possibility of finding an appropriate mutant.

From the 52 lines selected, we expect that at least one will be released in the future as a new upland rice cultivar for São Paulo State.

Rice (*Oryza sativa L.*) is grown on every continent except Antarctica. It is an important food staple for almost two-thirds of the world's population. In São Paulo State, Brazil, rice is a very important crop for both production and consumption. Most of the rice in São Paulo is cultivated in an upland system and depends on rainfall for moisture, but some areas are irrigated. The rice-growing areas of São Paulo are characterized by warm temperature and humidity, providing an environment conducive to the rapid spread of the blast disease pathogen (*Pyricularia grisea*). Rice blast is considered the most important disease in this state. It causes severe losses in yield and rice quality. Unfortunately, blast resistance in rice varieties can be lost soon after large-scale cultivation because of the development of new races of the pathogen.

At the Instituto Agronômico de Campinas (IAC), the main objectives of the rice breeding program, besides yield, are grain quality and blast resistance. We released an upland variety, IAC 201, with excellent grain quality and agronomic traits. However, this variety is susceptible to blast. The objective of this project was to induce genetic variability in cultivar IAC 201 by irradiating seeds with $^{60}$Co gamma rays and developing somaclonal populations to select plants that combine blast tolerance with the good agronomic characteristics of the original cultivar.

## Materials and methods

### First phase

Seeds of IAC 201, an upland rice variety developed by IAC, were irradiated at 30, 35, and 40 krad using a $^{60}$Co source of gamma ray. About 4,500 seeds receiving each dose were planted in field plots to obtain the $M_1$ generation used for developing subsequent in vivo and in vitro populations. Only plants from the 30-krad irradiation treatment were used for population development because the higher irradiation levels resulted in very low survival percentages (Table 1). At the booting stage, 450 immature panicles (about 1.5 cm long) were harvested from $M_1$ plants as well as 300 panicles from nonirradiated IAC 201 plants and used as explant material for somaculture. All regenerated plants were grown in the greenhouse to maturity and harvested individually. Seeds were also harvested from field-grown $M_1$ plants and nonirradiated IAC 201 control plants. After harvest, plants were thrashed by hand and seeds were dried to 13% moisture. The $M_1$ population was divided into three groups according to sterility rate (Table 2); a total of five populations were advanced to the $M_2$ (Table 2).

**Table 1. Percentage of surviving plants after seeds irradiated at three $^{60}$Co gamma ray doses.**

| Material | Doses at | | | Nonirradiated |
|---|---|---|---|---|
| | 30 krad | 35 krad | 40 krad | |
| Seeds planted | 4,500 | 4,500 | 4,500 | 4,500 |
| Plant survival (no.) | 1,576 | 756 | 125 | 4,155 |
| % | 35.8 | 16.8 | 2.7 | 92.3 |

**Table 2. $M_2$ populations according to sterility rate in the $M_2$ generation.**

| Population | | Sterility rate |
|---|---|---|
| $M_2$ 1 | (A) | Plants with 50–80 seeds (mutant in vivo) |
| $M_2$ 2 | (B) | Plants with more than 100 seeds (mutant in vivo) |
| $M_2$ 3 | (C) | Plants with less than 50 seeds (mutant in vivo) |
| $SM_2$ | (D) | Somaclones from 30 krad (mutant in vitro) |
| $R_2$ | (E) | Somaclones from normal IAC 201 |

**Table 3. $M_2$ generation with five populations planted in upland conditions.**

| Population | Rows planted (no.) | Seeds sown (no.) | Plant survival (no.) | Albino plants (no.) | Albinism (%) |
|---|---|---|---|---|---|
| A | 62 | 3,100 | 2,577 | 61 | 2.31 |
| B | 113 | 11,300 | 8,868 | 207 | 2.28 |
| C | 65 | 6,500 | 5,754 | 101 | 1.73 |
| D | 31 | 3,100 | 2,352 | 60 | 2.49 |
| E | 126 | 12,600 | 11,066 | – | – |
| IAC 201 (control) | 34 | 3,400 | 3,089 | – | – |
| Total | 431 | 36,600 | 30,617 | 429 | |

## Second phase

The $M_2$ generation composed of populations A, B, C, D, and E was sown in a pathogen-free field in 431 single-plant rows (Table 3). These yielded 30,617 plants. IAC 201 was planted in check rows at a rate of one row every 10 mutant rows, for a total of 34 rows yielding 3,089 plants. No selections were made in the $M_2$ generation. Albinism was observed and recorded (Table 3). At maturity, single panicles were harvested from each plant in each row, constituting the $M_3$ generation seeds. A total of 25,000 panicles were harvested. All these panicles were thrashed by hand and dried to 13% moisture. The best panicles were put in small paper bags and recorded by row number and panicle number within each row. A total of 10,340 panicles were advanced to the $M_3$ generation.

## Third phase

In the $M_3$ generation, 10,340 panicles were evaluated for blast tolerance, using the standard nurseries for homogeneous reaction to leaf blast.

In November 1995, two continuous rows of a blast-susceptible rice variety (IAC 165) were planted surrounding the area where the $M_3$ generation was to be established to build up inoculum for blast screening. In January 1996, seedbeds were prepared and planted with the $M_3$ generation. Each of the 10,340 panicles was sown in small rows 20 cm long, spaced 10 cm apart. After each 10 rows of $M_3$ panicles, one row was sown to the original variety (IAC 201). The first and the last five rows, as well as the two rows surrounding the experiment, were planted with a mixture of blast-susceptible varieties as spreaders. In total, there were 52 plots with 200 rows each. All plots were fertilized with 240 kg N $ha^{-1}$ applied one-third during sowing, one-third 15 d after germination, and the other one-third 25 d after germination. The blast evaluation was done 40 d after germination, according to visual symptoms and rated on a scale from 1 to 9 as adopted in the Symposium on Rice Blast Disease in 1963 at the International Rice Research Institute. The original parent material (IAC 201) was rated 6 (considered susceptible). Materials were selected from rows with ratings of 3 or lower. We selected 267 rows that appeared disease-free and all of these were transplanted to another field plot for neck blast evaluation. All lines were harvested and dried to 13% moisture; panicles from each row (line) were thrashed separately at maturity and stored in paper bags as the $M_3$ to $M_4$ generation.

### Fourth phase

This phase was conducted over two seasons (1996-97 and 1997-98). In each season, we conducted a yield trial and blast evaluation.

*Yield experiment.* The 267 lines selected for blast tolerance were planted in upland conditions. The experimental design was seven blocks with 40 plots each and one block with 16 plots for a total of 296 plots. Plots were 1.5 m long with 3 rows spaced 40 cm apart. After each 10 plots of mutant lines, one plot had IAC 201 as a control. These experiments were conducted using normal practices for upland rice cultivation and harvested by hand.

*Blast test.* One panicle from the 267 lines in the $M_4$ generation was planted as described in the third phase and replicated at two locations. The two locations represented approximately 95% of all blast races occurring in São Paulo State.

Data collected in the fourth phase included blast tolerance reaction, panicle length, number of filled grains per panicle, number of empty spikelets per panicle, 1,000-grain weight at 13% moisture, estimated yield potential, number of days to flowering, and grain shape. No panicle blast was observed. The field plots were harvested by hand and panicles dried to 13% moisture for data analysis.

## Results

### First phase

In all irradiated populations of the $M_1$ generation, except for the control of nonirradiated IAC 201, a low germination rate was observed, less than 50% survival for plants in all treatments (Table 1). These data suggest that using a low dose such as 20 krad will be better for establishing sensitivity for each specific rice cultivar before beginning to work with the mutation technique in a breeding program.

Although 30 krad was considered a slightly high dose, it was selected for this study. During the somaclonal work, no difference was found in the plant boots that came from a 30-krad population or from a normal population. Calli were induced very well for both populations. However, the regeneration rate was very poor. We had 33 plants from the normal population and 91 plants from the 30-krad population that gave us some indication that irradiation increased the regeneration rate. At maturity, a high level of sterility was observed for all somaclones, so we decided to harvest the plants according to the sterility rate and we divided them into five $M_2$ populations (Table 2).

### Second phase

Table 3 presents the data for all five $M_2$ populations that were planted in an upland field. The number of rows, number of seeds sown, number of surviving plants, and number and percentage of albinos were recorded. There was no difference in the percent of surviving plants among the populations for albinism, which came from the induced mutation. Only for the somaclonal population did no albino plants occur. From the 30,617 plants, 25,000 panicles were harvested and we chose the best 10,340 to constitute the $M_3$ generation. This choice was made by visual appearance only and not by selection.

### Third phase

Evaluation and selection for blast tolerance were carried out at 40 d after germination. Leaf blast infection was severe as the spreader lines were completely burned by blast. Disease-free rows were identified.

Table 4 presents the selected number of $M_3$ in each population. Population $M_3$ A gave a high number of selected mutants for blast tolerance and $M_3$ E was the worst one. Comparing all populations, 220 mutants selected were from mutation in vivo, 41 from mutation in vitro, and only 6 from somaclonal variation.

This study suggests that induced mutation in connection with somaculture increased the selection rate for blast tolerance. Data also showed that, in mutation in vivo compared with mutation in vitro, the rate was 5:1. All 267 potential mutants selected for improved tolerance for leaf blast also showed potential tolerance for panicle blast.

**Table 4. Number of panicles harvested from survival of $M_2$ generation and number of mutants selected for blast tolerance in the $M_3$ generation.**

| Population | $M_2$ plant survival | $M_2$ panicles harvested | $M_3$ mutants in vivo | Selected in vitro | Selection pressure |
|---|---|---|---|---|---|
| A | 2,577 | 1,013 | 120 | – | 0.12 |
| B | 8,868 | 3,820 | 75 | – | 0.19 |
| C | 5,754 | 1,682 | 25 | – | 0.02 |
| D | 2,352 | 611 | – | 41 | 0.07 |
| E | 11,066 | 3,182 | – | 6 | 0.002 |

## Fourth phase

Table 5 presents the frequency distribution for the blast reaction for all 267 possible mutant lines in the $M_4$ generation. The average of 28 readings for IAC 201, the original cultivar used as a control, was graded 6.3, which is considered susceptible. One hundred and ninety-four lines, 65.8% of the population, were graded 4.0, considered partially resistant; 60 lines, 20% of the population, were graded 5.0, which indicated moderately susceptible; 35 lines (11.9%) were like the original variety; and only 2% were considered to be highly susceptible. In the $M_3$ generation, blast was the only character selected, which was why the $M_4$ generation showed this type of distribution. This also gives us an idea of how efficient the methodology used was for attaining the project's objective. These results suggest that further selection in a further generation has to continue to guarantee improvement for blast tolerance to produce mutants that could become a new upland rice cultivar for São Paulo State.

Tables 6, 7, 8, and 9 show the frequency distribution for panicle length, 1,000-grain weight, percent sterility, and days to flowering. For the 267 mutants in the $M_4$ generation and the 267 mutant lines selected in the $M_3$ generation, all characters studied showed a normal distribution. For all characters measured, the original cultivar fit

**Table 5. Frequency distribution for tolerance for leaf blast (grade 1–9).**

| Value | Frequency | Percentage | Cumulative | |
|---|---|---|---|---|
| | | | Frequency | Percentage |
| 4 | 194 | 65.8 | 194 | 65.8 |
| 5 | 60 | 20.3 | 254 | 86.1 |
| 6 | 35 | 11.9 | 289 | 98.0 |
| 7 | 2 | 0.7 | 291 | 98.6 |
| 8 | 3 | 1.0 | 294 | 99.7 |
| 9 | 1 | 0.3 | 295 | 100.0 |
| Total | 295 | 100.0 | | |

**Table 6. Frequency distribution for panicle length in centimeters.**

| Value | Frequency | Percentage | Cumulative | |
|---|---|---|---|---|
| | | | Frequency | Percentage |
| 22 | 1 | 0.3 | 1 | 0.3 |
| 24 | 10 | 3.4 | 11 | 3.7 |
| 25 | 42 | 14.2 | 59 | 17.9 |
| 26 | 83 | 28.0 | 136 | 45.9 |
| 27 | 93 | 31.4 | 229 | 77.4 |
| 28 | 49 | 16.6 | 278 | 93.9 |
| 29 | 16 | 5.4 | 294 | 99.3 |
| 30 | 2 | 0.7 | 296 | 100.0 |
| Total | 296 | 100.0 | | |

Table 7. Frequency distribution for 1,000-grain weight in grams.

| Value | Frequency | Percentage | Cumulative | |
|---|---|---|---|---|
| | | | Frequency | Percentage |
| 17 | 3 | 1.0 | 3 | 1.0 |
| 18 | 4 | 1.4 | 7 | 2.4 |
| 19 | 13 | 4.4 | 20 | 6.8 |
| 20 | 23 | 7.8 | 43 | 14.5 |
| 21 | 35 | 11.8 | 78 | 26.4 |
| 22 | 45 | 15.2 | 123 | 41.6 |
| 23 | 49 | 16.6 | 172 | 58.1 |
| 24 | 48 | 16.2 | 220 | 74.3 |
| 25 | 45 | 15.2 | 265 | 89.5 |
| 26 | 21 | 7.1 | 286 | 96.6 |
| 27 | 7 | 2.4 | 293 | 99.0 |
| 28 | 2 | 0.7 | 295 | 99.7 |
| 29 | 1 | 0.3 | 296 | 100.0 |
| Total | 296 | 100.0 | | |

Table 8. Frequency distribution for sterility in percentage.

| Value | Frequency | Percentage | Cumulative | |
|---|---|---|---|---|
| | | | Frequency | Percentage |
| 8 | 1 | 0.3 | 1 | 0.3 |
| 11 | 6 | 2.0 | 7 | 2.4 |
| 12 | 4 | 1.4 | 11 | 3.7 |
| 13 | 9 | 3.0 | 20 | 6.8 |
| 14 | 12 | 4.1 | 32 | 10.8 |
| 15 | 13 | 4.4 | 45 | 15.2 |
| 16 | 18 | 6.1 | 63 | 21.3 |
| 17 | 17 | 5.7 | 80 | 27.0 |
| 18 | 18 | 6.1 | 98 | 33.1 |
| 19 | 21 | 7.1 | 119 | 40.2 |
| 20 | 39 | 13.2 | 158 | 53.4 |
| 21 | 28 | 9.5 | 186 | 62.8 |
| 22 | 13 | 4.4 | 199 | 67.2 |
| 23 | 19 | 6.4 | 218 | 73.6 |
| 24 | 13 | 4.4 | 231 | 78.0 |
| 25 | 15 | 5.1 | 246 | 83.1 |
| 26 | 13 | 4.4 | 259 | 87.5 |
| 27 | 5 | 1.7 | 264 | 89.2 |
| 28 | 5 | 1.7 | 269 | 90.9 |
| 29 | 4 | 1.4 | 273 | 92.2 |
| 30 | 7 | 2.4 | 280 | 94.6 |
| 31 | 5 | 1.7 | 285 | 96.3 |
| 32 | 5 | 1.7 | 290 | 98.0 |
| 33 | 2 | 0.7 | 292 | 98.6 |
| 34 | 3 | 1.0 | 295 | 99.7 |
| 40 | 1 | 0.3 | 296 | 100.0 |
| Total | 296 | 100.0 | | |

**Table 9. Frequency distribution for number of days to flowering.**

| Value | Frequency | Percentage | Cumulative | |
|---|---|---|---|---|
| | | | Frequency | Percentage |
| 70 | 12 | 4.1 | 12 | 4.1 |
| 75 | 48 | 16.3 | 60 | 20.3 |
| 80 | 100 | 33.9 | 160 | 54.2 |
| 85 | 63 | 21.4 | 223 | 75.6 |
| 86 | 3 | 1.0 | 226 | 76.6 |
| 88 | 9 | 3.1 | 235 | 79.7 |
| 90 | 28 | 9.5 | 263 | 89.2 |
| 92 | 3 | 1.0 | 266 | 90.2 |
| 93 | 12 | 4.1 | 278 | 94.2 |
| 95 | 16 | 5.4 | 294 | 99.7 |
| 96 | 1 | 0.3 | 295 | 100.0 |
| Total | 295 | 100.0 | | |

**Table 10. Descriptive statistics for all traits evaluated and average of the 28 controls (IAC 201).[a]**

| Trait | N | Mean | SD | Minimum | Maximum |
|---|---|---|---|---|---|
| Panicle length (PL) | 296 | 26.6 | 1.23 | 22.0 | 30.0 |
| 1,000-grain weight (1,000gW) | 296 | 22.9 | 2.19 | 17.0 | 29.0 |
| Sterility (STER) | 296 | 20.8 | 5.21 | 8.0 | 40.0 |
| Number of filled grains in 4 panicles (NFG) | 296 | 723.0 | – | 471 | 1,298 |
| Days to flowering (DF) | 295 | 82.6 | 6.32 | 70 | 96 |
| Leaf blast (LB) | 295 | 4.5 | 0.85 | 4.0 | 9.0 |
| Estimated yield potential (EYP) | 295 | 2,722 | 837.29 | 920 | 5,280 |

| IAC 201 | PL | 1,000gW | STER | NFG | DF | LB | EYP |
|---|---|---|---|---|---|---|---|
| N | 28 | 28 | 28 | 28 | 28 | 28 | 28 |
| Mean | 26.7 | 22.8 | 20.3 | 813 | 80 | 6.3 | 2,960 |

[a] SD = standard deviation.

exactly around the average, which gives us assurance that we are selecting mutant lines in this generation for other traits besides blast reaction.

Table 10 presents data on descriptive statistics for all traits evaluated. The estimated yield potential was normally distributed and the average of the data for the 28 original cultivars almost equaled the population average.

Table 11 shows 53 possible mutants selected for improved tolerance for blast and good agronomic performance. The original cultivar IAC 201 has a low yield potential, so we selected these mutants based on their being 15% better than the average of the original cultivars. For days to flowering, we selected early and late plants, but, in a further generation and selection, we will attempt to select early types, which

**Table 11. Mutants selected in the $M_5$ generation and their agronomic performance, blast tolerance, and grain shape.**

| Mutant no. | Population pedigree | Yield potential (kg ha$^{-1}$) | Days to flowering | Leaf blast[a] (1–9) | 1,000-grain weight (g) | Panicle length (cm) | Sterility (%) | Grain shape |  |  | Ratio L/W |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  |  | Length (mm) | Width (mm) | Thickness (mm) |  |
| M96020 | Pop. A L 9 - P13 | 3,080 | 75 | 4 | 25.7 | 28.0 | 18.6 | 6.72 | 2.07 | 1.73 | 3.25 |
| M96022 | Pop. A L 10 - P 1 | 4,320 | 85 | 5 | 25.4 | 25.2 | 29.2 | 6.77 | 1.99 | 1.70 | 3.40 |
| M96023 | Pop. A L 10 - P 3 | 4,080 | 75 | 4 | 24.2 | 26.6 | 20.2 | 6.52 | 1.95 | 1.66 | 3.34 |
| M96024 | Pop. A L 10 - P 4 | 3,220 | 75 | 5 | 24.8 | 24.9 | 23.2 | 6.69 | 2.06 | 1.74 | 3.25 |
| M96036 | Pop. A L 24 - P 2 | 3,440 | 85 | 4 | 25.9 | 24.4 | 16.1 | 6.77 | 1.99 | 1.70 | 3.40 |
| M96037 | Pop. A L 24 - P 3 | 3,360 | 85 | 4 | 23.7 | 26.4 | 15.9 | 6.61 | 2.02 | 1.72 | 3.27 |
| M96043 | Pop. A L 34 - P16 | 3,960 | 75 | 4 | 28.9 | 20.6 | 24.4 | 2.49 | 1.97 | 1.67 | 3.29 |
| M96076 | Pop. A L 56 - P 5 | 2,720 | 80 | 4 | 25.8 | 25.5 | 15.9 | 6.51 | 1.99 | 1.69 | 3.27 |
| M96077 | Pop. A L 56 - P 6 | 3,140 | 75 | 4 | 26.1 | 25.3 | 18.6 | 6.62 | 2.01 | 1.70 | 3.29 |
| M96078 | Pop. A L 56 - P 7 | 3,920 | 80 | 4 | 24.2 | 28.5 | 21.0 | 6.69 | 2.01 | 1.71 | 3.33 |
| M96079 | Pop. A L 56 - P 8 | 3,300 | 80 | 4 | 26.3 | 27.9 | 21.8 | 6.90 | 2.00 | 1.70 | 3.45 |
| M96080 | Pop. A L 56 - P 9 | 4,140 | 75 | 4 | 26.5 | 28.4 | 33.9 | 6.41 | 1.93 | 1.67 | 3.32 |
| M96081 | Pop. A L 56 - P10 | 2,940 | 93 | 4 | 24.7 | 28.0 | 20.8 | 6.47 | 1.97 | 1.66 | 3.28 |
| M96083 | Pop. A L 56 - P14 | 4,160 | 88 | 4 | 24.3 | 28.2 | 30.3 | 6.38 | 1.98 | 1.68 | 3.22 |
| M96084 | Pop. A L 56 - P15 | 3,640 | 80 | 4 | 21.5 | 25.9 | 23.2 | 6.63 | 1.95 | 1.67 | 3.40 |
| M96085 | Pop. A L 56 - P16 | 4,280 | 80 | 4 | 23.2 | 27.4 | 23.3 | 6.80 | 1.95 | 1.65 | 3.49 |
| M96086 | Pop. A L 57 - P 1 | 4,480 | 75 | 4 | 23.2 | 27.0 | 21.4 | 6.77 | 1.99 | 1.69 | 3.40 |
| M96093 | Pop. A L 57 - P 8 | 2,940 | 80 | 4 | 24.0 | 25.4 | 12.4 | 6.58 | 2.08 | 1.58 | 3.16 |
| M96094 | Pop. A L 57 - P 9 | 3,760 | 75 | 4 | 28.6 | 25.5 | 12.1 | 6.68 | 1.99 | 1.69 | 3.36 |
| M96096 | Pop. A L 57 - P11 | 3,340 | 75 | 4 | 27.1 | 25.3 | 11.1 | 6.90 | 2.03 | 1.73 | 3.40 |
| M96097 | Pop. A L 57 - P13 | 4,680 | 75 | 4 | 26.3 | 25.6 | 13.1 | 6.31 | 2.01 | 1.70 | 3.14 |
| M96098 | Pop. A L 57 - P14 | 3,540 | 80 | 4 | 24.5 | 25.7 | 20.8 | 6.49 | 1.95 | 1.68 | 3.33 |
| M96099 | Pop. A L 57 - P15 | 3,440 | 80 | 4 | 25.4 | 24.3 | 15.0 | 6.60 | 1.97 | 1.69 | 3.35 |
| M96100 | Pop. A L 57 - P16 | 2,980 | 80 | 4 | 21.8 | 27.2 | 15.8 | 6.66 | 2.04 | 1.72 | 3.26 |
| M96101 | Pop. A L 57 - P17 | 3,220 | 85 | 4 | 24.4 | 25.9 | 23.7 | 6.63 | 1.96 | 1.64 | 3.38 |

*continued on next page*

**Table 11. continued**

| Mutant no. | Population pedigree | Yield potential (kg ha$^{-1}$) | Days to flowering | Leaf blast[a] (1–9) | 1,000-grain weight (g) | Panicle length (cm) | Sterility (%) | Grain shape |  |  | Ratio L/W |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  |  | Length (mm) | Width (mm) | Thickness (mm) |  |
| M96102 | Pop. A L 57 - P18 | 3,260 | 75 | 4 | 25.1 | 25.8 | 19.5 | 6.55 | 1.97 | 1.69 | 3.32 |
| M96139 | Pop. B L117 - P27 | 3,760 | 80 | 4 | 23.0 | 25.3 | 26.1 | 6.43 | 1.93 | 1.64 | 3.33 |
| M96154 | Pop. B L159 - P16 | 3,900 | 80 | 4 | 19.3 | 27.3 | 15.4 | 6.08 | 2.00 | 1.70 | 3.04 |
| M96160 | Pop. B L162 - P 2 | 4,420 | 80 | 4 | 21.8 | 26.7 | 17.5 | 6.25 | 1.93 | 1.66 | 3.24 |
| M96161 | Pop. B L162- P 3 | 4,620 | 70 | 4 | 19.0 | 26.3 | 19.4 | 6.19 | 1.90 | 1.66 | 3.26 |
| M96165 | Pop. B L162 - P 7 | 3,640 | 80 | 8 | 21.6 | 26.6 | 12.3 | 6.21 | 1.93 | 1.66 | 3.21 |
| M96167 | Pop. B L162 - P 9 | 3,900 | 80 | 5 | 22.2 | 26.2 | 18.2 | 6.49 | 1.99 | 1.68 | 3.26 |
| M96178 | Pop. B L184 - P 8 | 3,840 | 75 | 4 | 20.7 | 27.1 | 26.4 | 6.19 | 1.97 | 1.67 | 3.14 |
| M96184 | Pop. B L184 - P14 | 3,880 | 80 | 4 | 21.7 | 26.1 | 18.8 | 6.32 | 2.00 | 1.71 | 3.16 |
| M96185 | Pop. B L184 - P15 | 4,200 | 85 | 5 | 21.2 | 25.8 | 18.5 | 6.56 | 1.99 | 1.69 | 3.30 |
| M96186 | Pop. B L184 - P16 | 3,400 | 90 | 4 | 20.5 | 27.1 | 18.7 | 6.47 | 1.99 | 1.69 | 3.25 |
| M96189 | Pop. B L188 - P 6 | 3,380 | 85 | 5 | 23.3 | 29.0 | 13.9 | 6.47 | 1.98 | 1.68 | 3.26 |
| M96190 | Pop. B L188 - P19 | 3,500 | 80 | 5 | 20.7 | 25.0 | 29.2 | 6.00 | 1.94 | 1.64 | 3.09 |
| M96191 | Pop. B L188 - P20 | 3,300 | 95 | 4 | 21.7 | 27.5 | 21.4 | 6.32 | 1.97 | 1.68 | 3.21 |
| M96192 | Pop. B L190 - P 1 | 3,320 | 80 | 4 | 23.3 | 26.1 | 7.8 | 6.28 | 1.99 | 1.68 | 3.16 |
| M96193 | Pop. B L190 - P 2 | 3,580 | 85 | 4 | 22.6 | 28.1 | 20.1 | 6.61 | 1.97 | 1.69 | 3.35 |
| M96198 | Pop. C L196 - P14 | 2,960 | 80 | 5 | 24.6 | 28.0 | 14.9 | 6.13 | 1.92 | 1.60 | 3.19 |
| M96202 | Pop. C L217 - P 4 | 3,880 | 92 | 4 | 19.7 | 27.0 | 31.5 | – | – | – | – |
| M96211 | Pop. C L248 - P25 | 3,400 | 93 | 6 | 22.6 | 27.0 | 14.6 | 6.52 | 1.95 | 1.70 | 3.34 |
| M96219 | Pop. D L274 - P19 | 3,820 | 75 | 4 | 21.1 | 27.9 | 23.8 | 6.02 | 1.92 | 1.63 | 3.13 |
| M96220 | Pop. D L274 - P22 | 4,700 | 70 | 5 | 23.3 | 26.0 | 19.9 | 3.64 | 2.01 | 1.69 | 3.30 |
| M96231 | Pop. D L276 - P10 | 4,000 | 80 | 5 | 22.0 | 25.6 | 13.4 | 6.16 | 1.98 | 1.67 | 3.11 |
| M96233 | Pop. D L276 - P12 | 5,280 | 80 | 4 | 23.9 | 27.0 | 14.1 | 6.31 | 2.01 | 1.70 | 3.13 |
| M96234 | Pop. D L276 - P13 | 4,220 | 80 | 5 | 18.9 | 27.4 | 29.7 | 6.19 | 1.97 | 1.68 | 3.14 |
| M96244 | Pop. D L276 - P27 | 3,900 | 95 | 4 | 21.3 | 24.5 | 19.9 | 6.72 | 2.00 | 1.70 | 3.36 |
| M96245 | Pop. D L277 - P 1 | 4,200 | 70 | 4 | 19.6 | 25.3 | 30.7 | 6.37 | 2.06 | 1.78 | 3.09 |
| M96257 | Pop. E L213 - P 2 | 3,440 | 80 | 5 | 22.3 | 27.5 | 26.1 | 6.51 | 2.00 | 1.71 | 3.26 |
| M96258 | Pop. E L213 - P 3 | 4,180 | 75 | 4 | 21.2 | 26.6 | 23.2 | 6.13 | 1.95 | 1.70 | 3.14 |
| IAC 201 | IAC 165//Labelle | 2,722 | 80 | 6.3 | 22.8 | 26.7 | 20.3 | 6.59 | 1.99 | 1.71 | 3.31 |

[a] On scale of 1–9, where 1 = tolerant and 9 = susceptible.

will be better for upland conditions. Table 11 also presents data for grain shape. The selected mutant lines had no difference compared with the original cultivar, which was one of our objectives in this project. Finally, Table 11 shows that 26 selected mutant lines came from population A in the $M_2$ generation (Table 2), 15 from population B, 3 from population C, 7 from population D, and 2 from population E. This means that 44 mutants selected were generated from mutation in vivo and only 9 were generated from mutation in vitro. This result suggests that it is not necessary to use somaculture in connection with mutation technology in a rice breeding program when the objective is to select for blast tolerance and agronomic traits.

## Discussion

The germination rate for all irradiated treatments was less than 50%, but 30 krad was an acceptable dose and our results confirmed previous findings in rice (Madris et al 1996, Montepeque et al 1996). But these results also suggest that it is important to establish the best dose for each specific rice cultivar. The data presented indicated that the application of mutagenic agents affects the rate of regeneration, with a stimulating effect of 30 krad using $^{60}$Co gamma ray irradiation. These results were compatible with data found in barley (Atanassov et al 1998).

Results from the $M_3$ generation with selection for blast resistance indicated that mutations in vivo were better than in vitro. The data showed that, from 52 rice mutants in vivo, 37 lines were moderately resistant to blast, and these results were confirmed by Madris et al (1996).

The results described in the fourth phase showed that the population selected in the $M_3$ generation was normally distributed for all other characters and that the genetic variability was both positive and negative (Yokoyama et al 1996), indicating that selection for this trait can improve our possibility of finding a mutant, such as for the main objective of this project.

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# Development of improved mutants from the traditional medium-grain variety EEA-404

P.H. Blanco and F.B. Pérez de Vida

Interest in short- and medium-grain varieties has increased in Uruguay, where only a reduced area of those cultivars was grown in the past, and the traditional variety EEA-404 has been the most popular of them, obtaining premium prices in special markets. However, EEA-404 has several undesirable agronomic traits and the purpose of this work was to use mutation techniques to reduce its plant height and growth duration.

Seeds were irradiated in 1994 with 250 and 350 Gy. In 1994-95, 1,000 $M_1$ plants were grown and high sterility was observed. One panicle per plant was collected and 1,000 $M_2$ headrows were grown in 1995-96. Selection was done based on agronomic traits and the $M_3$ and $M_4$ lines were grown in individual rows in 1996-97 and 1997-98. A total of 118 lines were selected and tested in replicated trials in 1998-99.

Radiation treatment induced important variability in plant height, growth duration, pubescence, and grain shape. The parental variety required 100 days to heading and the $M_3$ lines ranged from 80 to 110 d. Plant height of EEA-404 was 1.22 m and $M_3$ lines ranged from 0.64 to 1.36 m. Several mutants with improved plant type and good grain quality yielded higher than the parental variety and check cultivars.

Rice is a major crop in Uruguay, where 200,000 ha of irrigated long-grain rice are grown and 90% of the production is exported. Average yield is 6.2 t $ha^{-1}$ and the main long-grain varieties have an indica or japonica background. Only a reduced area of short- or medium-grain cultivars has been grown and local breeding work has focused on developing high-yielding long-grain cultivars with improved grain quality and cold tolerance during the reproductive phase. However, interest in short- and medium-grain varieties, to be exported to special markets, has increased in recent years and a crossing program to develop adapted cultivars with this grain type has begun.

Only an area of medium- or short-grain varieties was grown in the past, and the traditional medium-grain variety EEA-404 has been the most popular of them, obtaining premium prices in special markets. This old Brazilian variety has large and bold grains, with low amylose content and low gelatinization temperature. However, EEA-404 has several undesirable agronomic traits and lower yield potential than improved long-grain cultivars. It is lodging-susceptible (plant height 1.4 m), long-season, and cold-susceptible and its grain and leaves are pubescent. Introduced short-grain cultivars grown in Uruguay, such as Sasanishiki and Koshihikari, have received high scores in sensory tests in Japan, but they have also shown lodging, limited yield potential, and poor adaptation to mechanized harvest (Pérez and Blanco 1995).

Induced mutations in rice have been widely used for semidwarfness and earliness, in many cases without greatly modifying the desirable genetic background of the parental variety (Maluszynski 1998, Rutger 1992, Deus et al 1996, Tisseli Filho et al 1996). The purpose of this work was to use mutation techniques to develop improved mutants from the traditional variety EEA-404. This work is part of the activities conducted under a research contract with the International Atomic Energy Agency (IAEA).

## Materials and methods

Seeds were irradiated with Gamma rays at the Centro de Investigación Nuclear (CIN), Montevideo, in 1994, with 250 and 350 Gy. The populations were managed in the following way:

- $M_1$: 1,000 $M_1$ plants were grown in 1994-95 and high sterility was observed. One panicle per plant was collected.
- $M_2$: 1,000 headrows were grown in 1995-96. In general, the population showed severe lodging and 360 panicles from 66 rows were selected, based on agronomic traits.
- $M_3$: 360 $M_3$ lines were grown in individual rows in 1996-97 and heading date and plant height were recorded. At harvest, the selected lines were not bulked because some of them still showed some degree of variability. A total of 337 panicles were selected from 115 $M_3$ lines.
- $M_4$: 337 $M_4$ lines were grown in individual rows in 1997-98 and plant height, heading date, grain shape, lodging, leaf angle, and pubescence were recorded. A total of 118 lines with desirable traits were selected for yield and grain quality testing.
- $M_5$: 118 lines were evaluated in replicated trials in 1998-99.

The 118 $M_5$ lines were tested in two trials with two replications, with a randomized complete block design, to determine grain yield, milling and cooking quality, plant height, days to heading, lodging, and grain shape. Plots had 6 rows of 3.5 m and 0.20 m between rows. Parental variety EEA-404, short-grain varieties Koshihikari, Sasanishiki, and S-201, and high-yielding long-grain cultivar INIA Tacuarí were used as checks. Experiments 1 and 2 were seeded 28 October and 4 November 1998, respectively. Heavy rains after planting and low temperatures resulted in a nonuniform stand in experiment 1 and only results from experiment 2 are reported here.

## Results and discussion

### $M_3$ generation

$M_3$ lines from parental variety EEA-404 showed high variability in growth duration, plant height, pubescence, and grain shape. The parental variety required 100 d to heading and the $M_3$ lines ranged from 80 to 110 d. About 34.5% of the $M_3$ lines required less than 95 d to heading and 44% required from 96 to 100 d (Fig. 1).

Plant height of EEA-404 was 1.22 m and $M_3$ lines ranged from 0.64 to 1.36 m, 88% of them being shorter than 1.2 m and 28% shorter than 1 m (Fig. 2). Thirty semidwarf $M_4$ lines were backcrossed to the parental variety to recover the parental grain type and populations can be used for genetic studies of dwarf genes involved. The parental variety EEA-404 is pubescent but several $M_3$ lines were glabrous. In $M_4$, 62.5% of the selected lines were glabrous. Glabrous mutants were also obtained in previous work on indica variety BR(IRGA)409, one of them being used as a parent in the cross from which the commercial variety INIA Cuaró was selected (Blanco et al 1997). The $M_3$ lines also showed variability in grain shape, from medium to short grain type. The parental grain type was difficult to recover and it was probably related to the dose of mutagenic treatment applied (250–350 Gy), which, according to the sterility observed in $M_1$, was too high for a parental variety.

### Field testing of $M_5$ lines

The mean grain yield of experiment 2 was 6.3 t $ha^{-1}$ and significant differences among cultivars were found for all variables tested (Table 1). Maximum and minimum yields

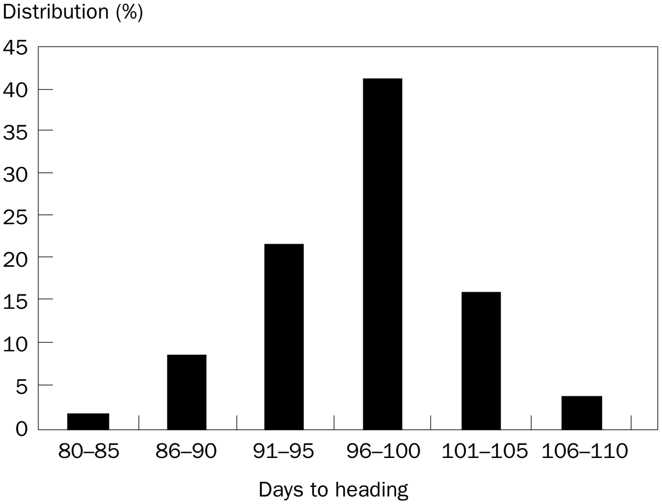

**Fig. 1. Days to heading for $M_3$ lines.**

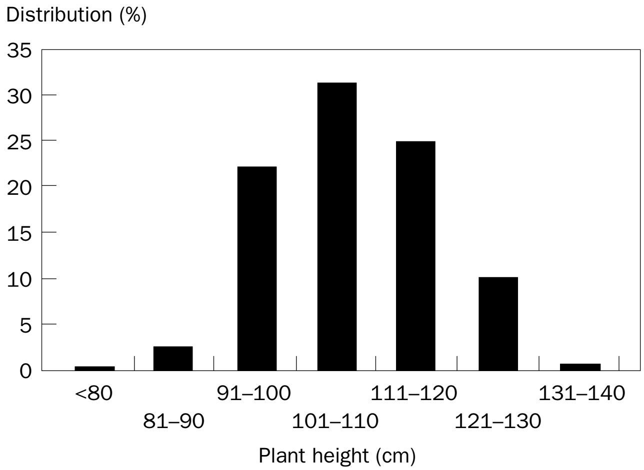

Fig. 2. Plant height of $M_3$ lines.

Table 1. Mean, range of variation, and statistical analysis of selected variables.

| Item[a] | Grain yield (t ha$^{-1}$) | Whole kernels (%) | Chalkiness (%) | Plant height (m) | Heading (d) |
|---|---|---|---|---|---|
| Mean | 6.3 | 52.2 | 8.2 | 0.98 | 106 |
| Maximum | 8.4 | 70.4 | 25.1 | 1.29 | 127 |
| Minimum | 2.7 | 23.1 | 0.5 | 0.81 | 92 |
| EEA-404 | 2.7 | 61.7 | 1.7 | 1.29 | 108 |
| CV% | 11.6 | 14.1 | 15.0 | 5.2 | 1.2 |
| LSD 0.05 | 1.46 | 14.76 | 4.627 | 0.109 | 2.638 |

[a]CV = coefficient of variation. LSD = least significant difference.

were 8.4 and 2.7 t ha$^{-1}$, the latter corresponding to the parental variety. This low yield was linked to the high sterility observed in the parental variety as a result of the unusually low temperatures registered during the reproductive phase in the 1998-99 crop season. The long-term yield of EEA-404 was 5.8 t ha$^{-1}$. The parental variety had a plant height of 1.29 m and 108 d to heading and the mutants ranged from 1.21 to 0.81 m and from 127 to 92 d, respectively, for both variables (Tables 1 and 2). All mutants except one were significantly shorter than the parental variety, but only 17 required less days to heading. There was a strong negative correlation between days to heading and yield ($r^2 = -0.451$, $P = 0.0001$), which may be related to the low temperatures registered in the season as was mentioned above. Some milling variables, such as whole kernel % and chalkiness %, also showed high variability, ranging from 70.4% to 23.1% and from 25.1% to 0.5%, respectively (Table 1).

The mean performance of the best-yielding mutants and check varieties is shown in Table 2. A group of eight mutants had grain yields significantly higher than that of Sasanishiki, which has been the best adapted introduced short-grain variety. The mean yield of those mutants was 34% higher than that of Sasanishiki. The mean plant height of the best-yielding mutants (0.99 m) was average for the experiment, but their mean growth duration (100 d to heading) was 6 d shorter than the overall mean. The amylose content of EEA-404 and Koshihikari was normal, but Sasanishiki and S-201 showed higher values than usual, along with the long-grain check INIA Tacuarí, which may be because of an interaction caused by temperature.

Some of the eight best-yielding mutants showed poor milling yields or high chalkiness, but others combined high yield potential with good milling and grain appearance. The amylose content of those mutants was similar to that of the parental variety, with the exception of one that showed very low amylose (Table 3). Six of them were pubescent and three had grain type similar to that of the parental variety. Weather conditions during maturity and harvest were good and the lodging-suscep-

**Table 2. Performance of check varieties and mean of best-yielding mutants.**

| Cultivar | Grain yield (t ha$^{-1}$) | | Whole kernels (%) | Chalkiness (%) | Plant height (m) | Heading (d) | Amylose (%) | Lodging[a] |
|---|---|---|---|---|---|---|---|---|
| 8 lines > yield[b] | 8.1 | 134 | 53.6 | 11.0 | 0.99 | 100 | 18.5 | 1 |
| EEA-404 | 2.7 | 45 | 61.7 | 1.7 | 1.29 | 108 | 21.2 | 3 |
| Koshihikari | 6.4 | 106 | 59.7 | 6.2 | 0.92 | 98 | 18.8 | 4 |
| Sasanishiki | 6.0 | 100 | 61.5 | 7.5 | 0.86 | 97 | 23.1 | 2 |
| S-201 | 5.7 | 95 | 63.2 | 1.6 | 0.92 | 107 | 23.9 | 1 |
| INIA Tacuarí | 7.1 | 117 | 58.1 | 5.6 | 0.81 | 93 | 28.6 | 4 |

[a] Lodging: 1 = no lodging, 9 = all plants lodged. [b] Significantly higher than Sasanishiki.

**Table 3. Performance of best-yielding mutants (significantly higher than Sasanishiki) and the parental variety.**

| No. | Grain yield[a] (t ha$^{-1}$) | Whole kernels (%) | Chalkiness (%) | Plant height (m) | Heading (d) | Amylose (%) | Lodging |
|---|---|---|---|---|---|---|---|
| 56 | 8.4 + | 44.3 – | 8.8 + | 0.92 – | 101 – | 21.5 | 1 |
| 42 | 8.3 + | 56.3 | 11.3 + | 1.02 – | 101 – | 17.6 | 1 |
| 21 | 8.1 + | 59.7 | 3.4 | 1.07 – | 107 | 19.2 | 1 |
| 48 | 8.1 + | 51.0 | 25.1 + | 0.94 – | 98 – | 20.0 | 1 |
| 55 | 8.1 + | 31.2 – | 21.2 + | 0.84 – | 97 – | 23.1 | 1 |
| 41 | 8.0 + | 56.4 | 7.4 + | 1.21 | 92 – | 17.6 | 1 |
| 22 | 7.9 + | 64.3 | 5.6 | 1.03 – | 103 – | 20.0 | 1 |
| 36 | 7.7 + | 66.0 | 5.4 | 0.94 – | 105 | 9.1 | 1 |
| EEA-404 | 2.7 + | 61.7 | 1.7 | 1.29 | 108 | 21.2 | 3 |

[a] + and – signs indicate significant differences with EEA-404.

tible check varieties showed only moderate lodging (3–4 on the 1 to 9 scale, INGER-IRRI 1996), whereas the best mutants showed no lodging at all (1 on the 1 to 9 scale, Tables 2 and 3).

## Conclusions

The mutagenic treatment was highly effective in reducing plant height in the parental variety EEA-404, but important variation was also observed in growth duration, pubescence of grain and leaves, milling and cooking quality, and grain shape. Several high-yielding mutants with desirable agronomic traits were selected and will be tested next season in more advanced trials.

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# Rice anther culture expedites variety improvement for Louisiana

Q.R. Chu, S.D. Linscombe, and H.X. Cao

Rice anther culture is an effective and time-saving technology for obtaining homozygous lines in varietal improvement. An efficient anther culture breeding system has been established for southern U.S. rice. It includes an annual inoculation of a half million anthers from more than 300 populations of $F_1$ and $F_2$ breeding materials. Ninety percent of the crosses showed a response to the culture with a mean callus induction rate of 20%. Annual production of approximately 8,000 doubled-haploid plants (DH1) resulted in field evaluation of several thousand DH2 panicle rows and selection of anther culture lines for yield testing. We have optimized culture conditions to improve anther culturability of U.S. rice and designed a basal medium designated as Chu, which is threefold higher in callus induction rates than the widely used N6 medium. A novel regeneration medium shows a twofold increase in regeneration rates when compared with the commonly used MS medium. Using "bridging parents" with high regeneration rates and superior agronomic traits is an important approach that improves culturability and breeding value. A total of 14,000 homozygous lines (DH2) were planted in 1999 for field evaluation, which resulted in the selection of 162 long-grain and 58 medium-grain elite lines. Current research emphasizes those crosses having high yield, good grain quality, disease resistance, and high culturability. Continued research activities in anther culture will focus on transferring genes of blast and sheath blight resistance, photoperiod-sensitive genetic male sterility, and herbicide-tolerant transgenes into elite rice lines.

Rice has been an important crop in Louisiana, both historically and economically. Research on rice varietal improvement including genetics, biotechnology, disease resistance, insect resistance, weed control, fertilization, and cultural management practices has been of tremendous positive benefit to United States rice production. Anther culture is an important technique for immediate fixation of homozygosity and expediting varietal improvement. The early work on the origin of doubled haploids (DH) derived from rice anther culture revealed that the great majority of regenerated plants from $F_1$ anthers were homozygous with microspore origin. Progeny tests of doubled haploids showed that the plants were uniform for a wide range of morphological characteristics. No segregation was observed in most selfed progenies of the DH plants, indicating that more than 90% of diploid plants from anther culture were homozygous arising from haploid microspores through spontaneous chromosome doubling during in vitro development. Segregation observed in a few progenies derived from anther culture may be due to variations in chromosome number and structure, nonhaploid microspores, or clonal variations (Chu et al 1985). The selection efficiency with DH lines is higher, especially when dominance variation is significant. This genetic feature of DH lines makes the anther culture technique a useful tool in rice breeding.

The capacity to regenerate a sufficient number of DH plants to produce adequate population sizes from which to select is an important component in the use of anther culture for varietal improvement. Typical laboratory materials such as Taipei 309 and Nipponbare are of japonica type, which showed high response in anther culture; however, the commercial value of using these genotypes in breeding long-grain rice is quite low. U.S. medium-grain rice averages 3–4% in culturability, which is acceptable in breeding; however, medium-grain rice varieties occupy limited area in the southern rice-growing area. Most U.S. long-grain varieties respond similarly in anther culturability to indica rice, producing less callus, which in turn results in fewer regenerated DH plants. The $F_1$ or $F_2$ breeding materials involving long-grain parents generally respond less in culturability, which hinders the use of anther culture technology. Furthermore, enlarging the genetic diversity in long-grain crossing parents by introducing indica types results in problems such as loss of grain quality, unstable segregation of agronomic characteristics in a quantitative nature, and lower probability of selection of desirable genotypes. Therefore, a unique anther culture system must be established to develop long-grain varieties with high yield, superior quality, and disease resistance, as well as for increasing diversity.

This paper reports the establishment of an anther culture breeding system for southern U.S. rice, which includes determining parental materials with high yield, superior quality, and disease resistance; improving anther culturability of $F_1$ and $F_2$ breeding materials; development of novel culture media; and using "bridging parents."

## Anther culturability of $F_1$ and $F_2$ breeding materials

Our preliminary work on rice anther culture breeding at the LSU Rice Research Station has focused on $F_1$ and $F_2$ populations of southern U.S. crosses involving Cocodrie, Jodon, Jackson, Alan, Maybelle, Tebonnet, Millie, L-202, Cypress, Katy, Kaybonnet, Drew, Lafitte, Lemont, Gulfmont, Newbonnet, Rico 1, Bengal, Orion, Mars, Toro 2, Teqing, Tetep, Taducan, Gui Chow, Jasmine-85, LSBR-5, and LSBR-33, and their derivatives (Chu et al 1997a). Among those genotypes, only the long-grain variety Cocodrie and the medium-grain Bengal are acceptable in anther culture response for breeding purposes.

In 1996, 8,926 plants were derived from the anther culture of 321 $F_1$ and $F_2$ populations (Table 1). The crosses with medium-grain type in general produced high regeneration rates compared with long-grain crosses. In $F_1$ populations, medium-grain types from conventional breeding averaged 3.24% in anther culturability, whereas long-grain types produced 0.22%. The $F_2$ populations showed the same response in anther culturability. The medium-grain types averaged 3.26% in anther culturability, which is higher than that of long-grain types (0.51%). The high anther culturability of medium-grain crosses leads to the production of adequate population sizes for the selection of DH2 lines, whereas low culturability (less than 1%) in most long-grain populations needs to be improved.

In 1997, a total of 505,320 anthers were plated for 294 $F_1$ and $F_2$ breeding materials (Table 1). Ninety percent of the crosses showed anther culture response, with a mean callus induction rate of 16.3%. Some 7,764 plants were regenerated from these crosses, with a mean of 1.6% in anther culturability. $F_2$ populations showed a mean callus induction rate of 20.9%, among which CR118, 129, 176, 205, 7085, 7479, 7565, and 7639 showed high culturability, with rates of 6.3%, 9.2%, 8.3%, 11.8%, 12.3%, 7.6%, 9.5%, and 8.7%, respectively.

In 1998, 527,760 anthers of 553 $F_1$ and $F_2$ populations were plated and 10,838 plants were regenerated (Table 1). The $F_1$ populations with high anther culture responses were T21, T24, T55, T135, T149, T159, T160, T161, T163, T172, T244, T245, T266, T279, and T299. $F_2$ populations showed a mean callus induction rate of 15.1% and a mean anther culturability of 1.8%. The $F_2$ populations with high culturability were 7075, 7083, 7119, 7131, 7135, 7143, 7147, 7209, 7211, 7223, 7267, 7277, 7301, 7335, 7377, 7411, 7413, and 7417.

The success of anther culture techniques assisting in and integrating into rice breeding programs is commonly judged at three levels. The first level aims to regenerate adequate numbers of doubled haploids (for example, 2,000 DH1 annually). The second level optimizes regenerants from the number of crosses covering desirable genetic diversity (40–50 crosses with 30 different parents). The third level focuses on the breeding and commercial value of the regenerated lines (50 homozygous lines for yield testing). Anther culture systems vary among laboratories because of the breeding objectives and the genotypes used; however, we have established an anther culture system that includes (1) annual production of 8,000 DH plants, (2) annual inoculation of a half million anthers from 300 populations of $F_1$ and $F_2$ material, (3) callus

**Table 1. Plant regeneration in anther culture (AC) breeding of U.S. rice (Rice Research Station, Crowley, Louisiana).**

| Year | Number of crosses used for AC | Number of anthers plated | Number of plants regenerated | Number of lines selected |
|---|---|---|---|---|
| 1996 | 321 | 540,230 | 8,926 | 127 |
| 1997 | 294 | 505,320 | 7,764 | 220 |
| 1998 | 553 | 527,760 | 10,838 | 321 |

induction rates averaging 20%, (4) mean anther culturability of 2%, (5) field evaluation of several thousand DH2 lines, and (6) annual selection of more than 100 anther culture lines (DH3) for yield testing. We believe that these standards are satisfactory for using anther culture technology in rice breeding.

## Optimizing culture media to improve anther culturability of U.S. rice

Although rice anthers respond to many basic media such as N6, MS, LS, R4, SK, and He5, the medium compositions required by japonica and indica are different. Japonica crosses respond well to N6 medium with mean culturability of 4–8%, whereas indica crosses respond to He5 medium with anther culturability of 1–2%. Southern U.S. long-grain crosses are composed of mostly javanica or tropical japonica germplasm, whereas medium-grain crosses possess more japonica types. Based on the comparison of medium compositions of MS, N6, LS, and R4, we have specifically designed a basal medium, designated as Chu, for callus induction of southern U.S. crosses. A stepwise test of Chu basal with various combinations of carbon sources, auxins, and cytokinins was made. The induction rate of embryogenic callus of nine $F_1$ populations on Chu medium averages 23.5% versus 10.9% on N6 medium. The function of Chu basal to increase callus induction was further confirmed by anther culture in summer 1997. The mean callus induction rate of 54 long-grain $F_1$ populations on Chu medium was 13.5%, which is a threefold increase over that on N6 medium (Chu et al 1997b).

The production of DH plants in rice anther culture usually involves three developmental processes, which are dedifferentiation of microspore (callus induction), differentiation of callus (plant regeneration), and maturation of plantlets. The nutritional and hormonal requirements for these steps are different. After comparing Chu basal and MS, we have further developed a regeneration medium for U.S. rice crosses. Results obtained from 1997 and 1998 indicated that plant regeneration rates of 16 $F_1$ populations on Chu1 medium averaged 43.4%, which is twofold higher than that on MS medium. By transferring regenerated plantlets onto hormone-free Chu1 medium, 98% of the plantlets initiated shoots and roots (Chu et al 1998a).

Carbon source in a medium is important in callus induction in rice anther culture. Sucrose is added into the culture medium as a carbon source and an adjuster of osmotic pressure. Most southern U.S. long-grain crosses show less response to a single carbon source medium than the medium-grain crosses. Various carbon sources and concentrations on N6 and Chu basal media were tested in anther culture to reveal the effects of a compound carbon source on callus induction. All crosses cultured on a compound carbon source medium produced more calli than a single-source carbon medium. The callus induction rate averaged 22.1% in a compound carbon source and 11.5% in a single carbon source. When callus induction rates of 521 crosses were pooled, supplementation with a compound carbon source (2% sucrose + 2% sorbitol + 2% maltose) improved the callus induction rate to 18.0% when compared with that of using sucrose alone of 11.1%. The two-year results suggest that using a compound carbon source in the induction medium is better than using sucrose alone when dealing with the anther culture of U.S. long-grain and medium-grain materials. The compound carbon source also improved callus emergence by 7 to 10 days when compared with single sucrose (Chu et al 1998b).

## Using bridging parents with high culturability to improve regeneration and breeding value

Two approaches are considered to be important for improving anther culturability and obtaining regenerants with breeding value. The first approach deals with optimizing culture conditions, such as medium components, hormone combinations, sugar resources, and cold pretreatment. The second approach, focused on genotypic effects (Chu and Croughan 1989), is to use bridging parents with high regeneration ability. Based on three-year data of more than 500,000 anthers annually and about 300 $F_1$ and $F_2$ populations, the mean anther culturability of long-grain types ranged from 0.5% to 1.6%, whereas that of medium-grain types varied from 2% to 4.2%. It is obvious that the anther culture system of long-grain rice needs to be improved for breeding purposes. Several bridging parents, such as AC101DH2, AC127DH2, AC157DH2, AC176DH2, AC179DH1, AC255DH1, AC268DH1, AC272DH1, AC273DH1, AC274DH1, and AC303DH1, were selected because of their high culturability in anther culture and their morphological traits (panicle size, plant type, heading date, grain quality, disease resistance, and yield potential) and were incorporated into crossing nurseries. The single or double crosses have one or two DH lines as recurrent parents. The mean callus induction rate for 18 $F_1$ crosses carrying the bridging parent was 46.7% and anther culturability averaged 9.4%, which is four times as high as that of the crosses without the bridging parents. We noticed that crosses with two bridging parents (DH2/DH2) had anther culturability that ranged from 10.1% to 44.5%. Therefore, using bridging parents in anther culture breeding can significantly improve the culturability and breeding value of southern U.S. long-grain crosses (Chu et al 1999).

## Current rice anther culture breeding strategy

The most important agronomic traits in rice breeding are high yield, good grain quality, and disease resistance. A large number of anther culture–derived pure lines have been developed and tested in the last several years. This germplasm, AC101DH2, AC108DH2, AC110DH2, AC116DH2, AC117DH2, AC127DH2, AC134DH2, AC139DH2, AC156DH2, AC157DH2, AC176DH2, AC179DH2, AC255DH1, AC268DH1, AC272DH1, AC273DH1, AC274DH1, and AC303DH1, carries desirable high yield, superior grain quality, and disease resistance traits. Some superior lines developed through anther culture are listed in Table 2 and are available for research upon request. Further crosses will be made among these parents, and anther culture of breeding material with DH2/DH2 will, we hope, increase the breeding value of long-grain rice.

The photoperiod genetic male sterile (PGMS) lines were obtained from somaclonal variation or natural mutation. We have used anther culture to develop

**Table 2. Anther culture–derived pure lines (Rice Research Station, Crowley, Louisiana).**

| 99 DH2 | 98AC | Pedigree | Agronomic characters |
|---|---|---|---|
| 9952002 | 101DH1 | CCDR/9771333DH2 | Long grain, high yield |
| 9952578 | 102DH1 | CCDR/97HB51 | Long grain, high yield, disease-resistant |
| 9952704 | 103DH1 | CCDR/9771186DH2 | Long grain, high yield, semidwarf, multitillers |
| 9952759 | 105DH1 | CCDR/9770532DH2 | Long grain, high yield |
| 9952805 | 106DH1 | CCDR/9771357DH2 | Long grain, high yield, disease-resistant |
| 9953013 | 109DH1 | CCDR/9771394DH2 | Long grain, high yield |
| 9953359 | 110DH1 | CCDR/9770532DH2 | Long grain, high yield, high culturability |
| 9954529 | 116DH1 | CPRS/9771190DH2 | Long grain, high yield |
| 9954948 | 121DH1 | BNGL/9770558DH2 | Long grain, high yield |
| 9955449 | 124DH1 | BNGL/97HB52 | Long grain, high yield, disease-resistant |
| 9955652 | 127DH1 | BNGL/97HB53 | Long grain, high yield, disease-resistant |
| 9956323 | 139DH1 | 9771186H2/CCDR | Long grain, high yield |
| 9956947 | 151DH1 | 97ACF65$F_1$/96CR02DH1 | Long grain, high yield |
| 9956966 | 152DH1 | DREW/96CR90DH1 | Long grain, high yield, disease-resistant |
| 9957278 | 157DH1 | BNGL/96CR90DH1 | Long grain, high yield, disease-resistant |
| 9963879 | 176DH1 | 97T1080DH1/97T1307DH1 | Long grain, high yield, high culturability |
| 9965705 | 179DH1 | 97T1130DH1/97T1080DH1 | Long grain, high yield, high culturability |
| 9965722 | 234DH1 | 97AC105DH1/97AC140DH1 | Long grain, high yield, high culturability |
| 9965775 | 248DH1 | 97AC152$F_1$/97AC174$F_1$ | Long grain, high yield, high culturability |
| 9965790 | 252DH1 | 97AC152$F_1$/97AC156$F_1$ | Long grain, high yield, high culturability |

suitable PGMS lines adapted to the rice environments of Louisiana. Sixty-three crosses were made to transfer PGMS gene(s) into southern U.S. commercial cultivars. In these crosses, we used microspore-derived lines, which carry PGMS genes as female parents, whereas the U.S. varieties were used as male parents. Anthers from the $F_1$ plants of 25 crosses were inoculated. The callus induction rate averaged 45.9%, ranging from 12.8% to 129.7%, which is much higher than that derived from $F_1$ and $F_2$ populations of long-grain and medium-grain types. High callus induction rates may be attributed to the female parents in these crosses as bridging parents, which underwent one cycle of anther culture. Some 2,526 plants were regenerated, with a mean of 6.1%, ranging from 0.2% to 22.5% in anther culturability. Field evaluation of pollen and spikelet sterility and agronomic characteristics of these DH lines was going to be done in 1999 to distinguish stable sterility traits (Chu et al 1998c).

Rice anther culture is a fast and highly efficient technology in varietal improvement. Recent release of a specialty rice variety derived from anther culture by the LSU Rice Research Station is one example. Current research emphasizes those crosses having high yield, good grain quality, disease resistance, and high culturability. Continued research activities in anther culture will focus on transferring genes of blast and sheath blight resistance, photoperiod-sensitive genetic male sterility, and herbicide-tolerant transgenes into elite rice lines.

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# Temperate rice varieties with wide $F_1$ hybrid compatibility and incompatibility

G. Clement, B. Courtois, and J.L. Seguy

We observed the $F_1$ hybrid compatibility of temperate rice varieties grown under Mediterranean France conditions.

When used as a parent in crosses, Estrela, a Portuguese japonica-indica extra-long-grain variety, gives $F_1$ hybrids with good fertility whatever the genetic nature (japonica, indica, or japonica-indica) of the other parent. In spite of its wide compatibility, Estrela did not produce better progenies than incompatible japonica varieties when crossed with the indica varieties of our working collection. The beneficial role of wide compatibility in improving the outcome of japonica × indica crosses is therefore questionable.

Based on $F_1$ fertility, the only variety that does not combine well with Estrela is Carinam, an extra-long-grain variety derived through anther culture from the $F_1$ of a japonica × japonica cross. Evaluation of the fertility in crosses of Carinam shows that this variety induces semisterility of the $F_1$ hybrid whatever the genetic nature (japonica, indica, japonica-indica, or "basmati") of the other parent. This wide incompatibility can be partially explained by the intervention of a single major gene. Spikelet sterility is gradually disappearing during the first generation of inbreeding. Therefore, the wide $F_1$ incompatibility of Carinam does not hamper its use as a progenitor.

The term "$F_1$ hybrid sterility" is used to describe spikelet sterility coming from a lack of fitness between two parents. The rate of sterility is considered to be a function of the genetic distance between the parents. We observed two types of $F_1$ hybrid compatibility in genetically close and distant crosses made in the framework of our rice breeding program conducted in Mediterranean France conditions.

## Estrela: a wide $F_1$ hybrid-compatible variety

Estrela is a japonica-indica extra-long-grain variety introduced from Portugal. Estrela produces fertile $F_1$ hybrids regardless of the genetic nature (japonica, indica, japonica-indica) of the other parent (Table 1).

**Table 1. Sterility (%) measured on 10 $F_1$ involving Estrela as a parent. Average of 4 panicles (about 500 spikelets).**

| Cross: P1 × P2 | Genetic nature | | % sterility | | |
|---|---|---|---|---|---|
| | P1 | P2 | P1 | P2 | $F_1$ |
| Estrela × Cristal | Japonica-indica | Japonica | 7.3 | 7.7 | 10.8 |
| Estrela × Bonni | Japonica-indica | Japonica | 7.3 | 4.9 | 6.1 |
| Estrela × Miara | Japonica-indica | Japonica | 8.2 | 11.9 | 13.7 |
| Estrela × L 202 | Japonica-indica | Japonica | 27.3 | 19.0 | 7.8 |
| Estrela × Alan | Japonica-indica | Japonica | 27.3 | 21.0 | 13.5 |
| Estrela × CNA 6159 | Japonica-indica | Japonica-indica | 17.8 | 16.4 | 25.0 |
| Estrela × IR58-3102 | Japonica-indica | Indica | 17.8 | 4.0 | 10.1 |
| Estrela × IRCTN 141 | Japonica-indica | Indica | 17.8 | 9.7 | 14.0 |
| Estrela × Artiglio | Japonica-indica | Indica | 24.6 | 14.8 | 18.9 |
| Estrela × Carinam | Japonica-indica | Japonica | 24.6 | 7.2 | 34.1 |

Sterility observed in Estrela varies from 7.3% to 27.3% depending on stem rot incidence (to which Estrela is very susceptible) during the cropping season. Despite this problem, the rate of sterility is clearly low or quite low in almost all the $F_1$ progenies. One of the highest rates of sterility is observed in the cross between Estrela and CNA 6159, another japonica-indica variety.

Despite its wide $F_1$ hybrid compatibility, Estrela did not produce any useful progenies in crosses of indica or indica-japonica varieties from our working collection such as IR58-3102, IRCTN 141, Artiglio, or CNA 6159. Even if japonica varieties with $F_1$ incompatibility (rate of sterility above 40%) when crossed with the same indica parents did not give the best results, this observation put in doubt the beneficial role of $F_1$ fertility in generating progenies out of genetically distant crosses. This result cannot be explained by the poor general combining ability of Estrela; indeed, in spite of the risk of transferring stem rot susceptibility to its progenies, Estrela remains a good progenitor in crosses with japonica varieties. Two of its progenies have even been included in the 1998 cropping season preregistration trials.

Among the crosses studied, the only variety that does not combine well with Estrela is Carinam (with an $F_1$ sterility of 34.1%). This result does not put in doubt the wide $F_1$ hybrid compatibility, but only indicates that Carinam does not combine well with any other rice varieties.

## Carinam: a wide $F_1$ hybrid-incompatible variety

Carinam is a japonica extra-long-grain variety derived through anther culture from the $F_1$ of a japonica × japonica cross. It was registered in 1997 in the E.U. Catalog. $F_1$ hybrids involving Carinam display semisterility regardless of the genetic nature (japonica, indica, japonica-indica, or "basmati") of the other parent (Table 2). Wide $F_1$ hybrid incompatibility can be attributed to Carinam.

We can see that sterility is relatively constant (26.1% to 34.1%). On the other hand, the two japonica parents of Carinam (Delta Luxe B and Miara) do not display $F_1$ semisterility when crossed with japonica varieties.

To understand the genetic origin of this $F_1$ semisterility, intra-japonica backcrosses were made with Carinam as the recurrent parent. Sterility was measured on the parents, the $F_1$, and each $BC_1$ plant (Table 3). With a qualitative analysis of the data, the distributions of the sterility of the $BC_1$ plants is bimodal, with 50% of the plants as fertile as Carinam and 50% of the $BC_1$ plants with semisterility. This distribution is characteristic of a Mendelian single-gene segregation. With a quantitative analysis of the data, the genetic determinism appears more complex because of the distribution continuity with the presence of transgressive plants in the two directions. So, we can conclude that the semisterility is controlled by a major gene that is modified by minor genes.

As in genetically distant crosses, spikelet semisterility is gradually disappearing during the first generation of inbreeding and the wide $F_1$ hybrid incompatibility does not hamper the use of Carinam as a progenitor. Crosses involving Carinam as a parent are ongoing. In intra-japonica crosses, we have obtained interesting results depending on the other parent. On the other hand, the genetically distant cross Artiglio × Carinam did not produce any interesting progenies.

**Table 2. Sterility (%) measured on 9 $F_1$ involving Carinam as a parent. Average of 4 panicles (about 500 spikelets). Carinam was always used as a male parent to avoid possible cytoplasmic effects because of its tissue culture origin.**

| Cross: P1 × P2 | Genetic nature | | % sterility | | |
|---|---|---|---|---|---|
| | P1 | P2 | P1 | P2 | $F_1$ |
| Panda × Carinam | Japonica | Japonica | 14.4 | 9.1 | 33.3 |
| (T × M-AA) × Carinam | Japonica | Japonica | 14.0 | 9.1 | 29.3 |
| (Q × A-D) × Carinam | Japonica | Japonica | 16.2 | 7.6 | 26.1 |
| Idra × Carinam | Japonica | Japonica | 20.4 | 8.0 | 33.3 |
| 66/88 × Carinam | Japonica | Japonica | 4.6 | 8.0 | 31.2 |
| Helene × Carinam | Japonica | Japonica | 5.6 | 8.0 | 29.0 |
| Artiglio × Carinam | Indica | Japonica | 14.8 | 7.2 | 31.5 |
| Basmati C621 × Carinam | "Basmati" | Japonica | 7.9 | 7.2 | 28.5 |
| Estrela × Carinam | Japonica-indica | Japonica | 24.6 | 7.2 | 34.1 |

**Table 3. Sterility distribution of 36 $BC_1$ plants in two backcrosses involving Carinam as the recurrent parent. Average of 10 panicles for parents and $F_1$, of 4 panicles for $BC_1$.**

((T.A. × M.-A.) × Carinam) × Carinam (1): distribution of $BC_1$ plant sterility

| Classes of sterility | 0–5 | 5–10 | 10–15 | 15–20 | 20–25 | 25–30 | 30–40 | 40–50 |
|---|---|---|---|---|---|---|---|---|
| Number of $BC_1$ plants | 3 | 9 | 6 | 4 | 7 | 5 | 1 | 1 |
| Frequencies | 50 | | | 50 | | | | |
| Range | 2.3% | | 13.7% | | 17.0% | | 40.4% | |

(Preto × Carinam) × Carinam: distribution of $BC_1$ plant sterility

| Classes of sterility | 0–5 | 5–10 | 10–15 | 15–20 | 20–25 | 25–30 | 30–40 | 40–50 |
|---|---|---|---|---|---|---|---|---|
| Number of $BC_1$ plants | 4 | 7 | 7 | 2 | 7 | 5 | 4 | – |
| Frequencies | 55.6 | | | | 44.4 | | | |
| Range | 2.7% | | 15.8% | | 20.9% | | 35.9% | |

| | % sterility | | | % sterility | |
|---|---|---|---|---|---|
| Parents-$F_1$ | Average | Range | Parents-$F_1$ | Average | Range |
| Carinam | 7.6 | 4.5–11.6 | Carinam | 9.1 | 6.8–12.9 |
| (T.A. × M.-A.) | 13.1 | 9.9–16.5 | Preto | 11.4 | 9.5–15.2 |
| $F_1$ | 25.4 | 15.9–38.0 | $F_1$ | 28.9 | 18.9–32.3 |

## Notes

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# Breeding japonica × indica crosses to improve rice under Mediterranean France conditions

D. Tharreau, G. Clement, D. Louvel, and J.L. Seguy

Under Mediterranean France conditions, all the cultivated rice varieties and most of the working collection are japonica types. To obtain new plant phenotypes and/or transfer some genes of agronomic interest, japonica × indica crosses have been bred.

To choose the indica parent, two methods were tested using either indica varieties from the working collection or unadapted tropical indica varieties.

Despite the well-known problems in genetically distant crosses ($F_1$ semisterility, poor character recombination), we obtained a few good progenies with blast resistance and/or improvements in grain quality. Moreover, one of the japonica × indica progenies included in the preliminary trials of the 1998 cropping season outyielded the cultivated japonica reference, which indicates promise for the future of this breeding method.

The use of tropical indica varieties as parents produces more interesting progenies (including for earliness) than the use of indica varieties suited to local conditions.

Molecular characterization of French Mediterranean cultivated rice varieties and the working collection shows that most of them are japonica types, although a few varieties that come from the Philippines, northern China, South Korea, or Brazil are indica types. These results show the considerable possibilities for creating a new type of variety by breeding distant japonica × indica crosses and keeping problems of progeny lateness to a minimum.

## Materials and methods

To plan distant crosses, japonica progenitors representative of the different morphological types determined in our working collection were chosen. To choose an indica parent, it was important to give priority to adapted indica varieties because of the expected progeny earliness. But tropical indicas were considered also because of a wide range of varietal choice and the possibility of increasing genetic variability with genes of agronomic interest not now present or scarce in our working collection. According to their well-known blast resistance or grain quality, varieties such as Eloni (Suriname), ITA 212 (IITA), Tolima (CIAT), Couachi (French Guyana), or the Brazilian IRGA varieties were used as parents.

Progenies were bred by the conventional pedigree method adapted for distant crosses. According to the large variability produced, the more or less late appearance of useful recombinants, and the slow fixation of progenies, screening was undertaken on plant value till the $F_4$-$F_5$ generation.

## Results

Despite the problems related to genetically distant crosses ($F_1$ semisterility, poor character recombination), the first results point to the possibility of obtaining some interesting original plant phenotype progenies with improvement for blast resistance and/or grain quality.

### Grain quality

Breeding distant crosses allowed us to screen some useful progenies with extra-long grain and suitable-quality grain. Grain shape and quality can be similar to those of the indica variety Pygmalion × Eloni or positively transgressive according to the two parents (IRGA 409 × Miara); in this last case, extra-long grain is borne by an early dwarf plant type.

### Blast tolerance

Analysis of the blast population in north Mediterranean Europe by molecular markers allowed us to determine four main races. To test the blast susceptibility of japonica-indica progenies, inoculations were made in the greenhouse on plants at the 4th-5th-leaf stage with representative strains of these four races. Disease scoring was done 7 d after inoculation. Table 1 reports the observed results. These data show no particular recombination constraint for blast resistance in japonica × indica crosses. Pygmalion × Eloni and Indio × ITA 212 progenies were as resistant as Eloni or ITA 212 when others were susceptible. However, we can point out the loss of supposed progenitor-specific genes in their progenies (R × R = S), particularly from Koral × ITA 212, Miara × ITA 212, and Miara × IRGA 409 (we do not have any results on IRGA 409 behavior, but all adapted or tropical indica varieties are resistant to all the European blast races). The results could be explained by polygenic control of resistance in parents and the loss, in the absence of selection pressure for blast resistance, of these gene combinations in the tested progenies. Resistance genes might also be lost in chromosomal rearrangements, which can be frequent in distant crosses. Nevertheless, in most progenies, the level of partial resistance remains sufficient to control blast in France. A blast resistance evaluation in the field (unusually strong blast inci-

**Table 1. Blast scoring (in % of susceptible reference Maratelli lines according to affected leaf area, average of two replications) recorded on japonica-indica progenies and their parents 7 d after inoculation at the 4th-5th-leaf stage.**

| Genotype | Blast race | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Eloni | 0 | 0 | 0 | 0 |
| ITA 212 | 0 | 0 | 0 | 0 |
| Indio | 0 | 100 | 75 | 0 |
| Pygmalion | 50 | 75 | 25 | 25 |
| Koral | 0 | 100 | 0 | 0 |
| Miara | 0 | 25 | 25 | 0 |
| Pygmalion × Eloni-C | 0 | 0 | 0 | 0 |
| Indio × ITA 212-B | 0 | 0 | 3 | 0 |
| Indio × ITA 212-D | 0 | 0 | 0 | 0 |
| Koral × Eloni-A1 | 0 | 80 | 20 | 0 |
| Koral × ITA 212-B2 | 15 | 2 | 10 | 4 |
| Koral × ITA 212-B4 | 25 | 5 | 3 | 2 |
| Miara × ITA 212-B1 | 60 | 12 | 4 | 15 |
| IRGA 409 × Miara-D4 | 70 | 45 | 60 | 90 |
| Maratelli | 100 | 100 | 100 | 100 |

**Table 2. Yields for three japonica-indica progenies and the local reference variety from the preliminary trial in the 1998 cropping season (6-$m^2$ plots, 3 replications).**

| Line | Yield (t $ha^{-1}$, 14% moisture content) | Yield (% of reference) |
|---|---|---|
| Koral × Eloni-A1 | 6.69 | 97 |
| Koral × ITA 212-B4 | 7.26 | 105 |
| Miara × ITA 212-B1 | 6.80 | 98 |
| Ariete (reference) | 6.93 | |

dence) in the 1997 cropping season confirmed the susceptibility of IRGA 409 + Miara-D4 when other progenies were free of symptoms.

## Yield

With these distant crosses, we first expected some well-recombined progenies expressing some of the useful indica characters for use as a progenitor-relay. To evaluate their yield, three japonica-indica progenies were included in a preliminary trial in the 1998 cropping season. Table 2 shows yields for the three progenies and the local reference variety Ariete. Two progenies yielded almost the same as the reference variety and one of them, Koral × ITA 212-B4, even outyielded Ariete. These results are promising for a direct release, under Mediterranean France conditions, of japonica-indica varieties and to extend the prospects for this type of cross.

## Discussion

Breeding japonica-indica crosses is a promising way to obtain genetic improvement under Mediterranean France conditions by increasing the use of readily available genetic variability and even cultivar release.

Until now, the use of distant crosses for breeding has made it impossible to characterize a particular morphological japonica group that has a better combining ability with indica varieties. Practical results depend on specific combining ability even if some parents, such as Koral, seem to fit quite well with indica varieties. On the other hand, the use of indica varieties adapted to Mediterranean France temperate conditions is paradoxically of less interest than the use of tropical indica varieties. The adaptation of indica varieties to Mediterranean France seems to be related to a certain genetic uniformization with local japonica varieties. As with the use of tropical indica varieties, distant crosses using adapted indica varieties display $F_1$ semisterility and poor recombination, but with a weak $F_1$ heterosis and an effective homogeneity in the $F_3$ generation.

## Notes

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GENETICS

# Using rice germplasm collections to develop breeding strategies

R.H. Dilday, K.A. Moldenhauer, J.W. Gibbons, and W.G. Yan

The USDA-ARS rice germplasm working collection contains 17,279 accessions from 110 countries or regions. Data for more than 30 different descriptors have been collected and are part of the germplasm resources information network (GRIN). The descriptors include allelopathy, amylose content, days from emergence to flowering, disease resistance, grain type, herbicide tolerance, kernel length, kernel weight, lodging, plant height, salt tolerance, yield, etc. A broad range of genetic variability is present in GRIN for each descriptor. For example, amylose content ranges from 0% to 53%, days from emergence to flowering from 37 to 219, kernel length from 3.0 to 9.9 mm, kernel length/width ratio from 1.0 to 8.0, plant height from 41 to 208 cm, protein content from 1.7% to 13.6%, salt tolerance rating from 0.0 to 5.6, straighthead rating from 3.0 to 9.0, and 1,000-kernel weight from 6.9 to 46.0 g. Some accessions are tolerant of nonselective herbicides such as glyphosate and sulfosate whereas other accessions apparently are allelopathic to weed species such as ducksalad (*Heteranthera limosa*) and barnyardgrass (*Echinochloa crus-galli*).

The USDA-ARS rice germplasm working collection maintained at Aberdeen, Idaho, contains 17,279 accessions from 110 countries. A comparison of the USDA-ARS rice germplasm collection and the International Rice Research Institute (IRRI) rice collection reveals that the U.S. collection contains more accessions from several Central and South American countries (Argentina, Bolivia, Chile, Colombia, Costa Rica, El Salvador, Honduras, Panama, Paraguay, Uruguay, and Venezuela) than the IRRI collection. Therefore, the USDA-ARS rice germplasm collection is an important germplasm base for rice varietal development programs in both North and South America. Data for more than 30 different descriptors have been collected for many of the accessions in the USDA-ARS collection and the data are included in the germplasm resources information network (GRIN).

Before 1988, the USDA-ARS collection had not been systematically evaluated; consequently, it had not been efficiently used in varietal development programs (Dilday 1990). Since 1988, a systematic evaluation of the collection has been coordinated by the USDA-ARS rice germplasm project at Stuttgart, Arkansas. Germplasm accessions with important characters such as allelopathic weed suppression, herbicide tolerance, quality traits, earliness, and stress tolerance have been identified. A thorough and systematic evaluation and characterization of the collection is ongoing and promising germplasm is being enhanced so it can be used in varietal development programs.

From their inception, two primary objectives of rice varietal development programs in the U.S. were to increase field yields and improve grain quality through conventional breeding. Selecting parental material with tolerance of or resistance to diseases has also been an important component of each breeding program. For example, during the early years of the rice breeding program at Beaumont, Texas, germplasm was selected that was tolerant of straighthead, a physiological disorder in rice. Blast has always been an important disease in Arkansas and the breeding program at Stuttgart initially selected germplasm that was tolerant of blast. The influence of these early germplasm selections as parents for specific characteristics is still present in the respective breeding programs.

## Procedures

It has been estimated that a total of 100,000 rice cultivars exist in Asia alone (Chang 1985). Four major areas must be addressed before rice germplasm collections can be effectively and efficiently used in varietal development programs. The germplasm must be introduced, characterized, and evaluated and must undergo genetic analysis. IRRI holds about 81,000 accessions of rice. China has several rice germplasm collections, but the collection at Beijing has more than 50,000 accessions. The national rice collection in India has more than 35,000 accessions and is located at several storage sites. The Japanese national rice collection contains about 26,000 accessions. The USDA-ARS rice collection has more than 17,000 accessions from 110 countries. The IRRI collection is probably the most genetically diverse rice collection in the world because acquisition and field collection efforts were implemented in the appropriate places and at opportune times before advanced genetic erosion occurred (Chang et al 1989).

Phenotypic plant and seed characteristics of each accession will be described and catalogued in GRIN for use by national and international scientists. In addition, genotypic differences and similarities will be characterized and catalogued. These data, along with the evaluation and passport data for each accession, will serve as the database in developing strategies for a core collection of rice germplasm.

Field and laboratory evaluation experiments for useful characters are conducted or coordinated through the germplasm project at the Dale Bumpers National Rice Research Center (DB NRRC). Emphasis will be on evaluating for (a) allelopathy (Dilday et al 1994), (b) herbicide, salt, and drought tolerance or increased water-use efficiency, (c) disease and insect resistance (Bernhart 1994, Lee 1992), (d) milling quality, (e) nitrogen efficiency (Wells et al 1973, Norman et al 1995), (f) seedling vigor, (g) superior yield, (h) ratooning ability, and (i) plant and seed characteristics.

Most of the new and exotic rice germplasm that is now being identified has agronomic or quality deficits that limit its direct adaptation. This germplasm must be combined with locally adapted cultivars or otherwise improved before it can be used in U.S. rice breeding programs.

## Results and discussion

### Collection and preservation

Although the USDA-ARS rice germplasm collection has grown to 17,279 accessions from 110 countries, the collection has obvious gaps. For example, five countries make up 60% of the collection, 10 countries make up 81% of the collection, and 78% of the collection was introduced before 1980. Central and South America, Africa, Russia, and Asia are areas where rice germplasm must be acquired to fill obvious gaps. Furthermore, 97% of the rice germplasm from Bangladesh, 96% from China, 94% from Hungary, 92% from Indonesia, and 94% from Japan was introduced into the collection before 1980. There have been 1,843 accessions from Indonesia, 1,543 from Colombia (CIAT), 1,041 from Bangladesh, 451 from Japan, and 204 from China introduced into the National Plant Germplasm System (NPGS) since 1993. Rice germplasm that is in the NPGS is preserved at two locations in the U.S. The base collection is maintained at Fort Collins, Colorado, and the working collection is maintained at Aberdeen, Idaho.

### Rejuvenation and evaluation

When the grain weight of stored accessions in the USDA-ARS rice working collection drops below 50 g, the accession is increased (rejuvenated) at DB NRRC and returned to the National Small Grains Collection (NSGC). Also, after a rice accession has cleared quarantine initially and before it is introduced into the NPGS, the accession is increased at DB NRRC and sent to the NSGC at Aberdeen, Idaho, and assigned a plant introduction (PI) number. From 1993 to 1998, 5,217 low-inventory accessions and 2,057 accessions had cleared quarantine and been rejuvenated at DB NRRC. The following quality and agronomic characteristics have been evaluated and entered into GRIN, with the range of diversity in parentheses: alkali/spreading value (2.00 to 7.04); allelopathy (387 accessions); apparent amylose % (9 to 54), aromatic rice (130 accessions); awning—absent (7,710), short and partly awned (3,307), short and fully awned (68), long and partly awned (596), long and fully awned (826); blast[1]—IB1 (145), IB33 (25), IB49 (62), IC17 (155), IE1K (122), IG1 (163), and IH1 (153);

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[1] Blast, listed here by race and with the number of accessions with a rating of zero susceptibility on a scale of 0 to 5.

sheath blight (3 to 9); bran color—white (750), light brown (14,033), speckled brown (44), brown (465), red (2,942), variable purple (3), and purple (49); days to anthesis (coded value); days from emergence to flowering: 37 to 59 (90), 60 to 81 (1,072), 82 to 104 (5,965), 105 to 126 (4,101), 127 to 148 (1,075), 149 to 171 (391), 172 to 193 (87), 194 to 216 (1), and 217 to 238 (1); endosperm type—common or nonwaxy (6,897), glutinous or waxy (300); gelatinization temperature—high (103), intermediate (3,339), low (3,804); grain type—short (2,585), medium (6,562), long (4,497), extra long (196), other (8), and mixed grain length (242); hull color white (75), straw (10,422), gold, light, or deep (1,217), tawny or russet (281), furrowed-gold, dark, or purple (613), mottled, speckled, or piebald (594), purple (248), black (105), and mixed or intermediate (519); hull cover—glabrous (1,752), hairs on lemma keel only (121), hairs on upper portion only (185), short hairs throughout (11,570), long hairs throughout (415), hairs on both lemma and palea keels (3), and mixed types (41); kernel length (3 to 10 mm); kernel weight in milligrams of milled rice (7.70 to 29.57); length/width ratio for brown dehulled rice (1.00 to 5.76); kernel width in millimeters for brown dehulled rice (1.00 to 4.01); weight of 1,000 kernels (3.37 to 46.93 g); lodging 0–10% (1,764), 11–20% (1,260), 21–30% (3,609), 31–40% (1,384), 41–50% (2,553), 51–60% (586), 61–70% (1,554), 71–80% (944), and 81–100% (1,581); panicle type—compact (401), intermediate (13,496), and open (1,220); parboil loss based on the percentage of solids lost when parboiled (7.00 to 58.03); plant height (coded values) in cm—less than 70 (349), 80–89 (1,098), 90–99 (1,565), 100–109 (1,606), 110–119 (1,654), 120–129 (1,915), 130–139 (1,839), 140–149 (1,274), 150–159 (674), and 160 or more (446); plant height measured in actual values (20 to 209 cm); plant type—erect (2,043), intermediate (4,787), open (7,561), spreading (798), and procumbent (8); protein % (1.70 to 14.57); ratooning—no growth (2,286), vegetative growth (3,521), head formed but no mature seed (1,544), and mature seed (726); tolerance for 1.0 lb ai of glyphosate and sulfosate (16); salt-tolerant—scale of 0 = sensitive to 10 = tolerant, 0.00–0.80 (12,636), 0.81–1.62 (1,051), 1.63–2.43 (242), 2.44–3.24 (103), 3.25–4.05 (26), 4.06–4.86 (8), and 4.87–5.67 (4); sterile lemma color—straw (9,671), gold (236), red (91), and purple (217); straighthead—no symptoms (5), less than 1.0% malformed panicles (14), 5% malformed panicles (58), 10% malformed panicles (239), 30% malformed panicles (725), 50% malformed panicles (1,089), 70% malformed panicles (1,329), 100% malformed panicles (929), and severely damaged with plants not heading (252); and upland type (331). Additional data can be obtained from the following Internet address: www.ars-grin.gov.

## Enhancement

Enhancement activities include allelopathy or weed suppression to both ducksalad and barnyardgrass; disease resistance for blast, sheath blight, and straighthead; stress tolerance for drought and herbicide resistance; salinity; earliness; quality; and high yield.

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# Rice wild relatives as a source of disease resistance for U.S. rice cultivars

G.C. Eizenga, J.N. Rutger, and F.N. Lee

Wild relatives of rice (*Oryza sativa* L.) are a useful source of disease resistance genes. The International Rice Research Institute reported 77 accessions of rice wild relatives (*Oryza* spp.) as resistant to sheath blight (caused by *Rhizoctonia solani* Kühn). The objectives were to (1) cross southern U.S. rice cultivars with resistant *Oryza* spp. to introduce additional sources of sheath blight resistance, (2) determine the amount of sheath blight resistance in the *Oryza* spp. and their $F_1$ progenies, and (3) ascertain whether any *Oryza* spp. and their $F_2$ progenies were resistant to U.S. blast (caused by *Pyricularia grisea* Cav.) races. Twenty-eight accessions, representing eight wild *Oryza* spp. and *O. rufipogon* (PI 590420), were crossed with the long-grain experimental line RU9401188 and the medium-grain cultivar Bengal. $F_1$ progenies were obtained from crosses with 23 of the 28 accessions. $BC_1$ and $BC_2$ progenies were derived from crosses with A genome species. Parents and $F_1$ hybrids were screened for sheath blight resistance using ratoon tillers. The *Oryza* spp. rated resistant or moderately resistant (MR), RU9401188 was moderately susceptible, Bengal susceptible (S), and $F_1$ hybrids MR to S. *Oryza* spp. and $F_2$ progenies were inoculated with U.S. blast races. Blast screening indicates that individual *O. nivara* accessions and *O. rufipogon* have resistance to common races.

Wild relatives of *Oryza* are an important source of useful genes for improvement of cultivated rice (*O. sativa* L.). Efforts to introgress genes from these *Oryza* species have been limited. In U.S. cultivars, only stem rot resistance has been introgressed from *O. rufipogon* (Rutger et al 1987, Tseng and Oster 1994). Reports from the International Rice Research Institute (IRRI) include transfers from the following species for resistance: grassy stunt virus resistance from *O. nivara* (Khush 1989), brown planthopper and whitebacked planthopper resistance from *O. officinalis* (Jena and Khush 1990), bacterial blight and blast resistance from *O. minuta* (Amante-Bordeos et al 1992), and brown planthopper resistance from *O. australiensis* (Ishii et al 1994). At the West Africa Rice Development Association (WARDA), Jones et al (1997) have made crosses with the cultivated African rice *O. glaberrima* in an attempt to identify additional genes for rice improvement. Sitch (1990) summarized the *Oryza* spp. and related genera that contain traits of economic importance for rice improvement.

Sheath blight, caused by *Rhizoctonia solani* Kühn (Lee and Rush 1983, Rush and Lee 1992), and blast, caused by *Pyricularia grisea* Cav. (Bonman 1992), are the major fungal diseases affecting rice in the U.S. IRRI reported 77 accessions of rice wild relatives (*Oryza* spp.) as having resistance to sheath blight (IRRI 1992). The objectives of this study were to (1) cross southern U.S. rice cultivars with resistant *Oryza* spp. to introduce additional sources of sheath blight resistance, (2) determine the amount of sheath blight resistance in the *Oryza* spp. and their $F_1$ progenies, and (3) ascertain whether any *Oryza* spp. and their $F_2$ progenies were resistant to U.S. races of blast.

## Materials and methods

Twenty-seven accessions, representing eight wild *Oryza* species, reported by IRRI as having a sheath blight resistance rating of >1 on a 0–9 scale in field tests (IRRI 1992), and *O. rufipogon* (IRRI no. 100912/PI 590420), the source of stem rot resistance (Tseng and Oster 1994), were included in this study. The long-grain experimental line RU9401188 and the medium-grain cultivar Bengal (Linsombe et al 1993) were the cultivated parents.

These accessions (*Oryza* spp.) were male parents in crosses with emasculated RU9401188 and Bengal panicles. The $F_1$ hybrids were verified by the presence of pubescent leaves and awns. Percentage of stainable pollen was determined for the parents, $F_1$, and $BC_1$ plants using Lugol solution ($I_2$/KI).

Embryo rescue was used in attempts to acquire $F_1$ hybrids that were not obtained using traditional crossing methods. For embryo rescue, embryos were dissected out of the developing ovary approximately 14 d after pollination, placed on Knudson C modified orchid medium (Sigma Chem. Co., St. Louis, MO), and placed in soil when the plants had three to four leaves.

For sheath blight inoculations (Eizenga et al 1998), toothpicks were infiltrated with potato dextrose (PD) broth, then cultured with *R. solani* on PD agar until well colonized. Colonized toothpicks were placed in the leaf sheath at the collar in up to five leaves of a particular ratoon tiller, and placed in a growth chamber at 100% humidity, 28 °C, and 14 h light/10 h dark. Approximately 1 wk after inoculation, plants were given an overall rating on a scale of 0 = no infection to 9 = severely infected. The individual ratings were summarized using the scale susceptible (S), moderately susceptible (MS), moderately resistant (MR), and resistant (R).

The *Oryza* spp. and $F_2$ progenies were inoculated with U.S. blast races IB-1, IB-33, IB-49, IC-17, IE-1K, IG-1, and IH-1 (Xia et al 1993, Correll and Lee 1996) at the four-leaf growth stage. One week after inoculation, plants were rated for blast susceptibility using a scale of 0 = no lesions to 9 = large susceptible lesions and/or leaves dying. Plants were inoculated again and rated using the same procedure about

2–3 wk after the first inoculation. Individual ratings were summarized using the scale very susceptible (VS), susceptible (S), moderately susceptible (MS), moderately resistant (MR), and resistant (R).

## Results and discussion

$F_1$ progenies were obtained from crosses with 21 of the 28 accessions without embryo rescue (Table 1). All but one of the successful crosses were with an A genome species, *O. barthii*, *O. glumaepatula*, *O. meridionalis*, *O. nivara,* or *O. rufipogon.* Backcross ($BC_1$) progenies were derived from crosses with 15 accessions and $BC_2$ progenies from crosses with 12 accessions. Pollen stainability was 50% or higher in the $F_1$ and $BC_1$ plants that produced $BC_1$ and $BC_2$ progenies, respectively. $F_1$ plants from crosses with some accessions of *O. barthii*, *O. meridionalis*, *O. nivara,* and *O. rufipogon* produced $F_2$ seed that was used to screen for blast resistance.

One C genome species, an *O. officinalis* accession (IRRI no. 101399), hybridized with Bengal. *O.australiensis* (IRRI no. 103318), an E genome species, and *O.latifolia* (IRRI no. 100169), a CD genome species, hybridized with Bengal using embryo rescue. No $BC_1$ or $F_2$ seed was obtained from the three non-A genome hybrids with Bengal. Embryo rescue will probably be needed to obtain backcross progenies from crosses with the non-A genome species.

Introgression of blast and bacterial blight resistance from *O. minuta* with a BBCC genome into *O. sativa* required embryo rescue to obtain $F_1$, $BC_1$, and $BC_2$ seeds (Amante-Bordeos et al 1992). Jena and Khush (1990) reported only 1.3% seed set when backcrossing *O. sativa* to completely sterile $F_1$ hybrids with *O.officinalis* , a C genome species. These examples suggest that, in most cases, embryo rescue will be required to obtain progenies from the first generations of backcrossing with non-A genome species.

Screening parents and $F_1$ hybrids (Table 2) for sheath blight resistance indicated that the *Oryza* spp. rated resistant or moderately resistant, RU9401188 rated moderately susceptible, and Bengal susceptible. The $F_1$ progenies tested rated moderately resistant to susceptible. In some cases, it appears that partial resistance is being transferred into the cultivated rice parent. Ratings from the growth chamber screening were higher than ratings from field screening for both the *Oryza* spp., which rated very resistant in IRRI tests, and the cultivated parents, RU9401188, which rated moderately resistant, and Bengal, which rated moderately susceptible in U.S. field tests. Even though several accessions have been reported to be resistant to sheath blight, transfer of the sheath blight resistance into cultivated rice at IRRI has been unsuccessful (D.S. Brar, IRRI, personal communication 1998). In addition, attempts to transfer the resistance from four different *O. rufipogon* accessions into three southern U.S. cultivars have proven unsuccessful (Oard and Groth 1994; J.H. Oard, Louisiana State University, personal communication 1997). Our research confirms these reports that incorporation of sheath blight resistance from *Oryza* spp. into cultivated rice is a difficult task requiring innovative methods of transferring the resistance.

**Table 1. List of the *Oryza* species accessions included in this study and the $F_1$, $BC_1$, and $BC_2$ plants obtained to date in crosses with the long-grain experimental line RU9401188 (1188) and the medium-grain cultivar Bengal (BNGL).**

| Species | Genome | Accessions (no.) | Accessions crossed (no.) | Female parent | No. of accessions that gave progenies | | |
|---|---|---|---|---|---|---|---|
| | | | | | $F_1$ plants | $BC_1$ plants | $BC_2$ plants |
| *O. alta* Swallen | CCDD | 1 | – | – | – | – | – |
| *O. australiensis* Domin | EE | 1 | 1 | BNGL | 1[a] | – | – |
| *O. barthii* A. Chev. | AA | 6 | 5 | 1188 | 5 | 3 | 2 |
| | | | | BNGL | 3 | 1 | 1 |
| *O. glumaepatula* Steud. | AA | 3 | 2 | 1188 | 2 | 1 | 1 |
| | | | | BNGL | 2 | – | – |
| *O. latifolia* Desv. | CCDD | 2 | 1 | BNGL | 1[a] | – | – |
| *O. meridionalis* N. Q. Ng | AA | 3 | 3 | 1188 | 3 | 2 | – |
| | | | | BNGL | 3 | 2 | 1 |
| *O. nivara* Sharma & Shastry | AA | 5 | 5 | 1188 | 5 | 5 | 4 |
| | | | | BNGL | 5 | 3 | 1 |
| *O. nivara/O. sativa* L. | AA | 2 | 2 | 1188 | 2 | 1 | 1 |
| | | | | BNGL | 2 | 1 | 1 |
| *O. sativa/O. nivara* | AA | 1 | 1 | 1188 | 1 | 1 | 1 |
| | | | | BNGL | 1 | 1 | – |
| *O. officinalis* Wall. ex Watt. | CC | 3 | 2 | 1188 | 2[b,c] | 1[c] | 1[c] |
| | | | | BNGL | 1 | – | – |
| *O. rufipogon* W. Griffith | AA | 1 | 1 | 1188 | 1 | 1 | 1 |
| | | | | BNGL | 1 | 1 | – |

[a] Plants obtained through embryo rescue. [b] All plants of one accession died in the seedling stage. [c] One accession is probably not *O. officinalis;* it looks like *O. meridionalis*.

**Table 2. Sheath blight ratings for *Oryza* spp., long-grain experimental line RU9401188, medium-grain cultivar Bengal, and $F_1$ hybrids (cultivated rice/*Oryza* sp.). Plants had ratoon tillers and were inoculated using a growth chamber method.**

| Species/ cultivar | Accessions (no.) | Sheath blight rating[a] | |
|---|---|---|---|
| | | Parent | $F_1$ |
| *O. alta* | 1 | MR | – |
| *O. australiensis* | 1 | MS | – |
| *O. barthii* | 6 | MR | MR-S |
| *O. glumaepatula* | 3 | MR | MR |
| *O. latifolia* | 2 | MR | – |
| *O. meridionalis* | 3 | MR | MR-MS |
| *O. nivara* | 5 | R-MR | MS |
| *O. nivara/O. sativa* | 2 | R | MR |
| *O. sativa/O. nivara* | 1 | MR | MR |
| *O. officinalis* | 3 | MR | MR-MS |
| *O. rufipogon* | 1 | MR | MR |
| Bengal | | S | S-MR |
| RU9401188 | | MS | S-MR |

[a] Ratings were summarized as susceptible (S), moderately susceptible (MS), moderately resistant (MR), and resistant (R).

Blast screening (Table 3) indicated that individual *O. nivara* accessions and the *O. rufipogon* accession (PI 590420) have resistance to common blast races found in the U.S. Also, *O.nivara* accessions and *O. rufipogon* appear to have resistance to race IC-17 (data not presented). Preliminary data from hybrids with the A genome accessions indicate that resistance to U.S. blast races is still present in $F_2$ progenies.

IRRI obtained rice lines with blast resistance from *O. nivara* (Khush 1989) and *O. minuta* (Amante-Bordeos et al 1992). In addition, blast resistance was reported in *O. officinalis* (D.S. Brar, IRRI, personal communication 1999). This implies that the *Oryza* spp. will provide additional sources of blast resistance that could be incorporated into cultivated rice.

Results to date suggest that additional sources of resistance to blast will probably be transferred from the wild relatives into U.S. cultivars. On the other hand, to transfer sheath blight resistance from the *Oryza* spp. into U.S. cultivars may require more than just backcrossing. Future studies will focus on transferring the identified resistance into adapted cultivars and tracking the introgression of the selected *Oryza* spp. genome into cultivated rice using molecular markers.

**Table 3. *Oryza* species rated for blast tolerance in the seedling stage using blast races found in the U.S.**

| Species | Accessions (no.) | Blast race[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | IB-33 | IB-49 | IB-1 | IE-1K | IG-1 | IH-1 |
| *O. alta* | 1 | MS | S | S | – | – | – |
| *O. australiensis* | 1 | VS | S | VS | S | VS | VS |
| *O. barthii* | 6 | S | S | MS-S | S | S | MR-S |
| *O. glumaepatula* | 3 | S | S | S | MS-S | S | – |
| *O. latifolia* | 2 | S | S | MS | S | MS | S |
| *O. meridionalis* | 3 | S | S | MS-S | S | S | MR |
| *O. nivara* | 5 | MR-S | MR-S | MR-S | R-MS-S | R-S | MR-S |
| *O. nivara/O. sativa* | 2 | S | S | MS | S | R | S |
| *O. sativa/O. nivara* | 1 | S | S | MS | S | S | S |
| *O. officinalis* | 3 | S | S | S | MR | S | S |
| *O. rufipogon* | 1 | S | MR | MS | MS | MR | MR |

[a]Ratings were summarized as very susceptible (VS), susceptible (S), moderately susceptible (MS), moderately resistant (MR), and resistant (R).

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GENETICS

# Anticipating obstacles to achieving transgenic promises and performances

C.W. Johnson

A large share of the United States area of soybeans, cotton, and maize is seeded to transgenic insect- and/or herbicide-tolerant varieties and/or hybrids. Herbicide transformants in rice have been produced and the race is on to get these products to the market place.

Significant changes will occur in all phases of research, development, and marketing. Important lessons already learned in other crops can be helpful in identifying the best agronomic and most regulatory acceptable varieties. Insertion methods are improving to reduce yield drag. Other important issues include, but are not limited to, germplasm exchange, limited yield testing before release, negative pleiotropic effects, outcrossing to related species, gene stacking, little genetic improvement for yield, protected germplasm and varieties, development of resistant weeds, the narrowing germplasm base, licensing agreements, and consumer acceptance of transgenic products.

The bottom line is that transgenic herbicide-tolerant varieties provide a different tool for weed control. This has application in particular locations, but is not a cure-all for every production field.

Varieties/hybrids with direct transgenic inserts conferring insect, disease, and herbicide resistance need further selection in breeding programs. In 1999, these rice transgenic lines were principally for herbicide tolerance to be used as new weed management tools for crop production. Information from other crops would suggest that a multitude of genetic traits could be incorporated into rice that would expand the product line. Many of these innovative end products are currently on the drawing board for rice. The timetable involved for release of these products and the amount of research effort are unknown because of the confidentiality contracts of commercial companies. This paper focuses on rice as the raw product used by consumers.

The function of a breeding program is to have a well-defined set of objectives and at the same time anticipate future needs. Consumer acceptance of transgenic rice for the next five years is now unknown. Sampling of various end-point consuming countries indicates that the range of acceptance varies from complete acceptance to a total refusal to purchase any transgenic material. Recent events in Europe have questioned the use of transgenic crops in manufactured products. Some consumer groups are demanding only nontransgenic products; they do not want even a trace of transgenic material in the products being marketed in their particular country.

Marketing strategies have to be well defined for the specific country of consumption. Cooking and quality tests are currently being done on limited transgenic samples. It is not anticipated that any significant changes will occur when comparing a transformed variety to the original variety. Probability is very low that changes could have occurred if the insertion methods caused point mutations and/or somoclonal variations that were selected during generation testing. It is therefore important to evaluate several transformants to detect any changes and to select for the original genotype.

In the case of herbicide-tolerant rice, the physical properties of the various general-purpose herbicides vary. These properties influence what spray method is used. The potential for drift on nontargeted fields, crops, and other vegetation may place limitations on spray methods. Among all factors, the success of transgenics is going to be based on economic costs and benefits for producers and consumers.

The following summarizes some of the advantages and disadvantages of transgenics that will be applicable to rice and that have already been experienced in maize, soybean, cotton, and canola. In this paper, direct transformation refers to the progeny, after several generations of selection, for the normal phenotype of the original variety whose origin was a single event. Most events were obtained by using "gene guns" to insert the gene with appropriate promoters into callus tissue. This process can cause substantial chromosome damage. Somoclonal variation can also be expressed in the progeny obtained from the callus. Promoters and methods of insertion are evolving that cause less chromosome damage.

Transgenics now have two advantages:

- They provide a different tool for weed and/or insect control.
- They have application in particular environments or locations, but are not a cure-all for every production field.

Transgenics have many disadvantages:

- Most direct transformations reduce yield potential. It can take three to four breeding cycles to get back to the original variety performance.
- In general, there has been less than adequate yield testing over years and locations and more concern about producing a large amount of seed for an expedited variety release time line. Lines derived from direct insertions are often less adapted and could produce less high-quality rice than the original variety.

- Most transformants are progeny from initial direct events. These varieties (99%) have been less than adequate in one or more important characters than the original variety.
- It is necessary to clean up a variety every time a new gene insertion is made. The cost involved with registration almost makes it a prohibitive process to insert genes into several varieties.
- Negative pleiotropic effects can be associated with the transformants. Some insertions are in chromosome locations that can adversely affect other important characters such as lodging resistance.
- It takes time to eliminate all the negative characters being expressed because of the insertion procedure. Examples include taller and/or shorter stature, more lodging, lower head rice, and higher sterility in the transgenic selections when compared with the original variety.
- Transgenic varieties are being protected in various countries by such methods as utility patents and plant variety protection acts (PVPA). Patent holders of the gene and variety have the right to sign licensing agreements for use of their variety with the transformed gene and to ensure that the producer buys new seed every year. It is important for various institutions developing varieties to protect genes and germplasm when it is released. Greater protection will be required for varieties than the PVPA of the United States currently allows.
- It costs at least US$8 million to register a new transformation. Once a transformed variety is registered, it can be used without reregistering. Any variety containing the inserted gene will be controlled by the gene patent holder.
- Outcrossing to related species and wild relatives could create super weed problems. It has been reported that canola has outcrossed to mustards in Canada.
- Because successful herbicides are often used continually, natural selection will create resistant and or harder-to-kill weeds.
- Gene stacking (i.e., herbicide and *Bacillus thuringiensis*) will further reduce yield. The gene-stacking sequence can be developed in several ways. The easiest would be to put genes together in one insert.
- In the long term, yield will have little genetic improvement. The short-term goal of immediately making sequential backcrosses to place the transgenic trait into another variety without genetic recombination contributes little to accumulating quantitatively inherited traits. Emphasis is now on obtaining a financial return on investment required for registration.
- Licensing transgenics agreements and all the assorted details have to be worked out before insertions can be used in a breeding program and in germplasm exchanges (material transfer agreements) with other programs. A paperwork nightmare is being created.
- Confidentiality agreements will become a part of plant breeding programs. Restricting the distribution of information about developing germplasm will decrease breeding progress and hide weaknesses/flaws of new cultivars.

Lawyers are going to be members of the plant breeding team to protect germplasm and varieties.

- Isolation requirements for transgenic and production fields and for commercial paddy fields will be necessary. Can you imagine the lawsuits from spray drift and spraying the wrong field?
- Every grower may need to sign licensing agreements that permit company inspections of fields and seed storage records for a certain time period after the production year. Often the contract license spells out financial liabilities for producers who keep or sell seed for replanting.
- It is important to consider information from all people involved in the herbicide transgenic variety development program. The popular magazines, press, and certain chemical sales personnel have definitely oversold the value of varietal tolerance of herbicide. Transgenic herbicide tolerance is an important tool for weed control, but the final commercial product and its intended use are equally important economic factors to consider.
- Gene silencing is a term referring to shutting off the inserted gene after a period of time. Even though this occurs at a low frequency, it is becoming a significant problem. No information is now available on the exact cause(s).
- Genetic diversity has long been discussed as being necessary for plant improvement. Backcrossing the registered transgenic varieties into other leading varieties will further narrow the germplasm base. Depending on the chromosome insertion site, the stage could be set for another crop disaster like the Southern corn leaf blight episode that occurred in 1970 in the U.S. It has been stated more than once that uniformity is stupidity.
- Considering the present grain-handling systems and methods, it is going to be extremely difficult to keep traces of transgenic rice out of nontransgenic rice being shipped into markets with zero tolerance for any transgenic. The potential for losing these markets is significant and a real threat.

The challenge is to register the best agronomic transformants that have regulatory acceptability and combine well with other varieties. Some long-time leading varieties are poor combiners. The inclusion of each transgenic type of herbicide tolerance, or other characteristic, in effect creates another breeding program that requires additional land, isolation, and economic resources. For large programs, this eventually will lead to diminished efforts in other plant breeding objectives.

## Notes

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# Early vigor: an important foundation for rapid biomass accumulation

R.F. Reinke, R.A. Richards, J.F. Angus, and L.G. Lewin

The development of rice varieties with shorter growth duration is an important objective of the New South Wales (NSW) rice improvement program. Such varieties use less water, allow greater time for alternative enterprises, and can be sown later, thus allowing faster establishment under warmer conditions. The challenge facing breeders is to maintain yield potential as duration is reduced, thus increasing the efficiency of rice production. Attaining adequate biomass production during the shorter vegetative phase is likely to be an important factor in obtaining high yields while reducing crop duration.

Three field experiments compared the biomass accumulation patterns of a range of 37 cultivars to determine whether useful genetic variability exists for this characteristic. All experiments had significant differences between cultivars in biomass accumulation throughout the vegetative stage and, for most cultivars, the patterns of biomass accumulation were similar. In one experiment, three cultivars exhibited a markedly different pattern of biomass accumulation.

Interestingly, there was a significant relationship between seedling biomass and midseason biomass in both trials. Increasing seedling vigor was associated with an increase in midseason biomass of 100–200 g $m^{-2}$, or 1–2 t $ha^{-1}$. Evidence of an association of seedling vigor with improved midseason biomass accumulation is a key finding of this research. Future research needs to examine the factors influencing early vigor and other traits that influence biomass production for future improvement of NSW cultivars.

The development of rice cultivars with shorter growth duration is an important objective for rice production in southwest New South Wales (NSW). Such cultivars use less water, allow greater time for alternative enterprises, and can be sown later, thus allowing faster establishment under warmer conditions. If current high yields can be maintained, the efficiency of rice production would also increase. Reinke et al (1994) have suggested that an important issue in obtaining high yields while reducing crop duration is attaining adequate biomass production during the shorter vegetative phase. This experiment was conducted to determine whether useful genetic variability exists for faster biomass accumulation. This was done by comparing the biomass accumulation patterns of several diverse cultivars with those of NSW cultivars.

## Materials and methods

A total of 37 rice cultivars were selected from the 1991 and 1992 International Rice Cold Tolerance Nurseries (IRCTN), which are organized by the International Rice Research Institute. Cultivars originating from 11 countries were selected on the basis of tolerance for low temperatures during the germination, vegetative, reproductive (including microspore development and anthesis), and/or maturation stages. Two groups of cultivars were differentiated on the basis of maturity (Table 1). The "short-season" group had 22 cultivars of shorter duration, similar to the NSW cultivar Jarrah, which takes around 100 d from sowing to anthesis and 150–160 d to maturity. The "long-season" group consisted of 15 cultivars of maturity similar to that of the NSW cultivar Amaroo, which takes around 120 d from sowing to anthesis and 170–180 d from sowing to maturity.

Three field experiments were conducted during the 1995-96 rice season. Each experiment used four NSW cultivars: the long-season cultivar Amaroo and the short-season cultivars Illabong, Millin, and Jarrah. Experiments involving short-season cultivars were conducted at the Yanco Agricultural Institute (146.5°E, 34.5°S) and Deniliquin Field Station (145.0°E, 35.5°S). Only one experiment, located at the Yanco Agricultural Institute, was conducted for long-season cultivars. All three experiments were designed as randomized complete blocks with three replicates. Plots were 12.0 m long and 1.43 m wide and consisted of eight rows spaced at 175 mm. Because of seed restrictions, three cultivars in the long-season experiment were included in one replicate only. All three experiments were drill-sown at 130 kg ha$^{-1}$ into a dry seedbed.

At Yanco, the long-season experiment was sown on 13 October and intermittent flooding began on 16 October. Intermittent flooding consisted of inundating the field with water to a depth of approximately 10 cm, allowing the floodwater to remain for 24 h, and then draining the field. The field underwent three cycles of intermittent flooding until 16 November, when seedlings had reached the 3-leaf stage. On that day, plots were fertilized with urea at 100 kg N ha$^{-1}$, herbicide was applied with a tractor and boom spray to control *Echinochloa crus-galli*, and the field was subsequently flooded to a depth of 10 cm.

The short-season experiments were sown at Deniliquin on 28 October and at Yanco on 13 November. Intermittent flooding began immediately. After four cycles of intermittent flooding, plots were fertilized, sprayed, and flooded as described above. Thereafter, all experiments remained flooded until all cultivars had reached physiological maturity.

An initial sample to determine height and biomass per seedling was taken from both the Yanco experiments 1 wk before permanent flood. For the long-season ex-

**Table 1. Cultivars included in field experiments at the Yanco Agricultural Institute (34.5°S) and Deniliquin Field Station (35.5°S).**

| Cultivar name | Origin | Type | Heading (d) | Height (cm) |
|---|---|---|---|---|
| *NSW commercial cultivars* | | | | |
| Amaroo | Australia | Japonica | 113 | 85 |
| Illabong | Australia | Japonica | 107 | 85 |
| Jarrah | Australia | Japonica | 97 | 89 |
| Millin | Australia | Japonica | 108 | 86 |
| *Short-season cultivars* | | | | |
| M-103 | USA (Calif.) | Japonica | 98 | 85 |
| Dubovsky 129 | Russia | Indica | 83 | 105 |
| Kalin | Russia | Indica | 81 | 95 |
| 80007 TR210 | Turkey | Japonica | 92 | 110 |
| 80023 TR166 | Turkey | Japonica | 96 | 135 |
| Barkat | India | Japonica | 86 | 98 |
| H433 | Hungary | Japonica | 79 | 102 |
| H448 | Hungary | Japonica | 84 | 110 |
| H467 | Hungary | Japonica | 79 | 102 |
| H473 | Hungary | Japonica | 79 | 85 |
| HR 4856-1-1-1-1-2 | Korea | Japonica | 97 | 100 |
| HSC 14 | Hungary | Japonica | 82 | 85 |
| HSC 236 | Hungary | Japonica | 81 | 85 |
| HSC 55 | Hungary | Japonica | 82 | 100 |
| HSC 6 | Hungary | Japonica | 80 | 75 |
| HSL 447 | Hungary | Japonica | 84 | 111 |
| Jinbu 8 | Korea | Japonica | 97 | 95 |
| Suweon 235 | Korea | Japonica | 92 | 92 |
| Unbong 3 | Korea | Japonica | 97 | 95 |
| Plovdiv 22 | Bulgaria | Javanica | 88 | 110 |
| *Long-season cultivars* | | | | |
| L-202 | USA (Calif.) | Indica | 114 | 87 |
| California Belle | USA (Calif.) | Indica | 105 | 105 |
| China 1039 | India | Indica | 105 | 106 |
| CT6744 F2 CA 10 | Chile | Indica | 105 | 97 |
| IR20897-B-8-1-4 | IRRI | Indica | 130 | 120 |
| IR36 | IRRI | Indica | 140 | 95 |
| IR72 | IRRI | Indica | 141 | 89 |
| RAU 4045-2A | India | Indica | 106 | 93 |
| Milyang 93 | Korea | Japonica | 108 | 95 |
| SR11327-22-3-2 | Korea | Japonica | 102 | 115 |
| SR11451-T204 | Korea | Japonica | 113 | 95 |
| SR13925-13-1 | Korea | Japonica | 104 | 102 |
| Stejaree 45 | Russia | Japonica | 103 | 101 |

periment, this was taken on 8 November, 23 d after the initial watering, when the third leaf of most cultivars had fully expanded. The short-season experiment was sampled on 13 December, 30 d after the initial watering. This initial sample consisted of 20 seedlings that were cut at ground level and dried for 4 d at 80 °C before weighing.

Plots in all three experiments were then sampled every fortnight, with samples being dried for 4 d at 80 °C before weighing. Two rows of 1 m length were cut at soil level from each plot. Since the row spacing was 175 mm, the sampled area was 0.35 $m^2$. The sample weight (in g) was then multiplied by 2.857 to convert it to g $m^{-2}$.

## Results and discussion

### Seasonal conditions

The season was characterized by variable temperatures. During mid-October, when the long-season experiment was sown, maximum temperatures averaged 21 °C, which is 5 °C above average. Both maximum and minimum temperatures were below average throughout December, and for the last 10-d period in January and the first two periods in February. The low minimum temperatures during the latter period resulted in damage to the developing pollen microspores, leading to reduced seed set. This was reflected in a 25% drop in the industry-wide average yield of the commercial rice crop compared to the previous season.

### Seedling vigor

There were significant differences in seedling vigor between cultivars in both experiments. There was a twofold difference in seedling biomass in the long-season experiment, from 8 to 15 mg. The tropical cultivars IR72 and IR36 had the lowest biomass, whereas the NSW cultivars Amaroo, Jarrah, Illabong, and Millin averaged around 12 mg per seedling. The Chilean cultivar CT6744 had the largest biomass.

In the short-season experiment, the difference in biomass between cultivars was more than twofold, from 28 to 68 mg. Seedlings were larger at the time of sampling as 30 d had elapsed since initial watering (versus 23 d for the long-season experiment), and temperatures during the period from sowing to the time of the first sample were higher, resulting in greater growth. Several cultivars had biomasses around 65 mg, significantly greater than the NSW cultivars, which had biomasses of around 45 mg. It was interesting that four of the cultivars with the greatest biomass came from Hungary (HSC55, HSC6, H433, and H467), whereas 80023 TR166 came from Turkey. Given their origin, these cultivars would be expected to be adapted to temperate conditions.

### Biomass accumulation patterns

There was significant variation in midseason biomass. In both experiments at Yanco, and for 21 of the 24 cultivars grown at Deniliquin, the patterns of biomass accumulation were similar. In particular, only small differences occurred in the rate of growth of cultivars, but differences during early growth were maintained throughout much of the growing season. This is indicated by the parallel logistic curves for representative cultivars in Figures 1 and 2. The long-season experiment had greater differences in biomass accumulation than the Yanco short-season experiment. In the long-season experiment (Fig. 1), the tropical cultivars IR36 (shown) and IR72 (not shown) had the smallest biomass at each of the sample dates, whereas SR11327 consistently had the greatest biomass, averaging 150 g $m^{-2}$ more than Amaroo. In the short-season experiment at Yanco (Fig. 2), many cultivars had a biomass similar to that of Amaroo at each of the sample dates. However, two cultivars (H473 and 80023 TR166) consistently performed better than Amaroo, averaging 100 g $m^{-2}$ greater biomass across all sample dates.

In the short-season experiment at Deniliquin, 21 cultivars followed the pattern observed in Yanco. However, three cultivars had a very different pattern. Among these, differences in biomass were not apparent until about 70 d after sowing. This was followed by a period, from about 80 to 110 d after sowing, during which all three accumulated biomass more rapidly than the NSW cultivar Amaroo. During this period, marked differences occurred in the rate of biomass accumulation among the three varieties. Each then reached a stage at which it stopped growing, but those with the fastest rates of midseason biomass accumulation reached this stage earlier than others (Fig. 3). It was thought that these differences might relate to differences in maturity, with early flowering cultivars ceasing biomass production earlier, but it was found that any relationship between maturity and biomass production 147 d after sowing for all 24 cultivars in the trial was not significant (r = 0.36 ns).

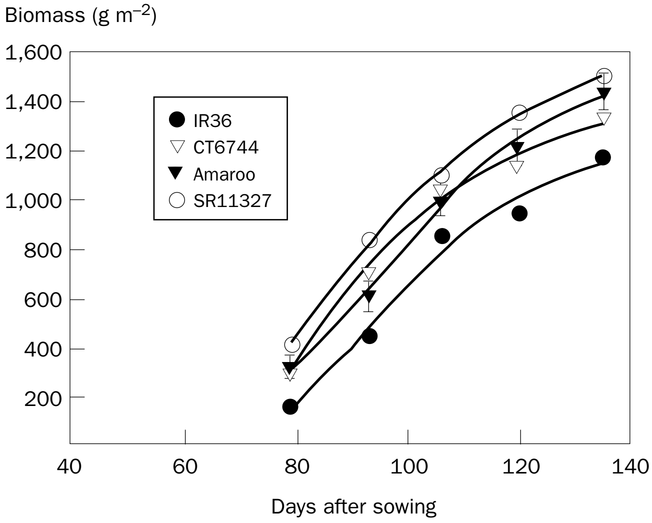

**Fig. 1. Biomass accumulation patterns of selected long-season cultivars. Symbols indicate biomass accumulation (g $m^{-2}$) and lines indicate a fitted four-parameter logistic function. Vertical bars indicate standard error.**

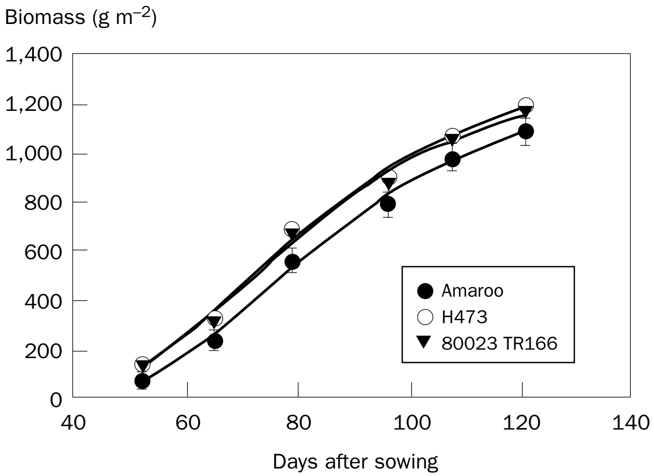

**Fig. 2.** **Biomass accumulation patterns of selected short-season cultivars. Symbols indicate biomass accumulation (g $m^{-2}$) and lines indicate a fitted four-parameter logistic function. Vertical bars indicate standard error.**

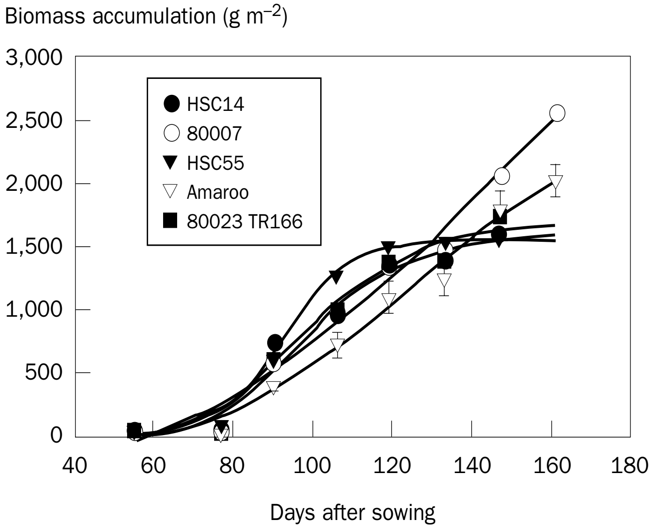

**Fig. 3.** **Biomass accumulation patterns of selected short-season cultivars from the experiment at the Deniliquin Field Station. Symbols indicate biomass accumulation (g $m^{-2}$) and lines indicate a fitted four-parameter logistic function. Vertical bars indicate standard error.**

### Midseason biomass and maturity

Maturity was associated with midseason biomass in all experiments, with early flowering being associated with increased biomass accumulation. The correlation was strongest for the short-season cultivars at Deniliquin (r = 0.69, *P* <0.01), with biomass production declining by 9 g $m^{-2}$ for each day's delay in flowering. It was weakest for the long-season cultivars (r = 0.54, *P* <0.05).

One possibility is that differences in phenology may have contributed to the variation in midseason biomass. For example, if there is an increase in the rate of aboveground biomass accumulation associated with the change from vegetative to reproductive growth, those cultivars that initiate growth earlier would be expected to have greater biomass. This phenomenon has been reported, among others, by Kemp (1985) for temperate pasture grasses, by Kemp et al (1989) for perennial ryegrass, and by Davidson et al (1990) for wheat.

Nevertheless, biomass production patterns at both experiments at Yanco demonstrate that differences in biomass were evident during the midvegetative stage, well before the commencement of stem elongation. Only three cultivars in the short-season experiment at Deniliquin fit the abovementioned pattern of increased aboveground growth commencing around the time of panicle initiation and stem elongation. It was interesting to note that there was no such rapid change in the rate of biomass accumulation associated with the commencement of reproductive growth in any of the remaining cultivars.

Earliness thus does not guarantee greater midseason biomass production. The results of these experiments are supported by Reinke et al (1994), who reported that rice cultivars with differences of 20 d from sowing to flowering had similar patterns of biomass production throughout the growing season.

### Seedling vigor and midseason biomass production

To assess how long the effects of early seedling vigor persisted throughout the growth cycle, the biomass of individual seedlings was plotted against midseason biomass production in both experiments at Yanco.

There was a significant and positive relationship between seedling biomass and midseason biomass in both experiments (Figs. 4 and 5). The slope of the regression line was greater in the long-season experiment, in which an increase in seedling biomass from 10 to 14 mg was associated with an increase in midseason biomass of approximately 200 g $m^{-2}$, or 2 t $ha^{-1}$. In the short-season experiment, an increase in seedling biomass from 40 to 70 mg was associated with an increase in midseason biomass of 100 g $m^{-2}$, or 1 t $ha^{-1}$.

The correlation coefficient between seedling biomass and midseason biomass was 0.52 in both experiments; however, the differing slopes of the linear regressions indicate that the effect of increased vigor was greater in the long-season experiment. Temperature conditions during early growth may have had some influence on this, as the 10-d period immediately after the long-season experiment was sown had an average minimum temperature of 7.7 °C and a maximum of 21 °C. In the period immediately after sowing the short-season experiment, conditions were more conducive for

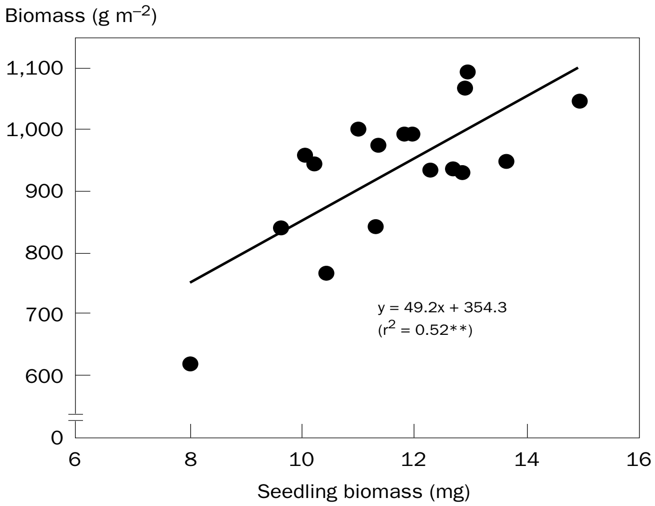

**Fig. 4. Relationship between seedling biomass and biomass accumulation 106 d after sowing among 17 long-season cultivars at the Yanco Agricultural Institute.**

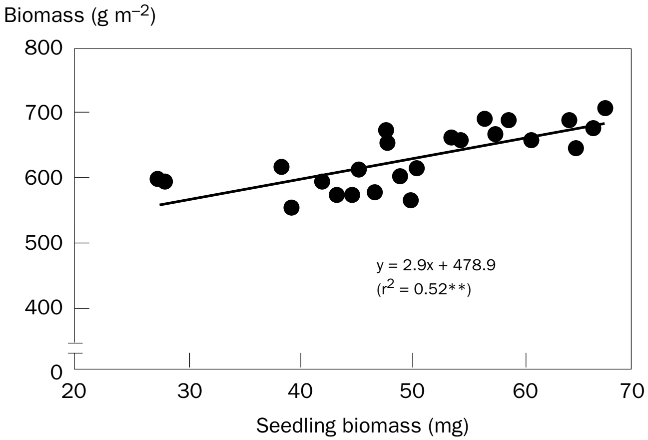

**Fig. 5. Relationship between individual seedling biomass and biomass accumulation 79 d after sowing among 24 short-season cultivars at the Yanco Agricultural Institute.**

growth, with an average minimum temperature of 13.5 °C and a maximum of 25 °C. The relationship between seedling biomass and midseason biomass accumulation indicated that cultivars with vigorous seedlings were more likely to have greater midseason biomass. It was a surprising result that greater seedling biomass (measured on an individual plant basis, not as biomass per unit area) was associated with greater midseason biomass accumulation. That vigorous seedlings should result in greater midseason biomass is not self-evident. Many factors influence the rate of biomass accumulation, including the speed of emergence and establishment of seedlings and their relative growth rate. Further, when seedlings interact to form canopies, the canopy structure influences the efficiency of use of incident radiation and thus the rate at which the crop grows. If increased early vigor results from the production of longer, less erect leaves, as is generally the case with tall cultivars (Yoshida 1977), the early advantage may be diminished through the development of an inefficient crop canopy with reduced crop growth rate. This is due to canopy structure, in which the upper leaves intercept most of the incident radiation and the lower leaves are shaded. On this basis, early vigor seems unlikely to be associated with greater midseason biomass.

The relative length of the periods during which seedlings are independent and when they form a crop canopy will also influence the accumulation of biomass. In these experiments, crop canopies were established at or shortly after the time of the second sample; thus, for 20–25% of the period from sowing to the midseason sample date (106 and 79 d after sowing for the long-season and short-season experiments, respectively), biomass accumulation was influenced by canopy characteristics.

## Conclusions

This study showed that there is genetic variation for seedling vigor and for biomass accumulation during the vegetative stage of rice growth. Maximizing biomass production during the reduced period of vegetative growth available to short-season cultivars should lessen the yield penalty associated with such cultivars and contribute to the maintenance of current high yields.

At issue is the determination of the physiological factors that underpin rapid biomass accumulation. Results from the experiments at Yanco suggest that early seedling vigor is important, as early advantages in biomass production were maintained throughout the vegetative stage. Conversely, the results from three of the cultivars at Deniliquin suggest that differences in the linear growth phase are important. Further research should address the relative importance of seedling vigor and subsequent growth to identify promising traits for improving midseason biomass production. Cultivars with increased early vigor and high growth rate combined with an efficient canopy structure will allow NSW rice growers to capitalize on the benefits of reduced growth duration while minimizing negative effects on yield.

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# Current directions at the Dale Bumpers National Rice Research Center

J.N. Rutger

The Dale Bumpers National Rice Research Center (DB NRRC) opened on 24 August 1998. An International Symposium on Rice Germplasm Evaluation and Enhancement was held a week later, and the DB NRRC was dedicated on 22 October 1998. The mission of the DB NRRC is to conduct research to help keep the U.S. rice industry competitive in the global marketplace by assuring high yields, superior grain quality, pest resistance, and stress tolerance.

The DB NRRC builds upon 70 years of cooperative research between the U.S. Department of Agriculture (USDA) and the University of Arkansas. From 1931 to 1993, there were only one or two USDA scientists at Stuttgart, and then growth began. There are now eight USDA scientist positions at the DB NRRC. Research groups include genetics, germplasm evaluation and enhancement, physiology of weed control, cereal chemistry, molecular genetics, cytogenetics, molecular plant pathology, and molecular biology.

The Dale Bumpers National Rice Research Center (DB NRRC), then known as the National Rice Germplasm Evaluation and Enhancement Center, opened on 24 August 1998. A week later, the DB NRRC and the University of Arkansas Rice Research and Extension Center (UA RREC) co-sponsored an International Symposium on Rice Germplasm Evaluation and Enhancement. On 22 October 1998, the new facility was dedicated and the name was officially changed to the DB NRRC, in honor of U.S. Senator Dale Bumpers of Arkansas, who was instrumental in securing Congressional funding for the facility. Extensive support for the concept of a national center has been provided by rice growers and industry leaders in Arkansas and the entire U.S. rice community.

The mission of the DB NRRC is to conduct research to help keep the U.S. rice industry competitive in the global marketplace by assuring high yields, superior grain quality, pest resistance, and stress tolerance. Major emphasis is on genetic improvement, ranging from conventional genetics and germplasm to rice genomics. Intensive studies are also conducted on the physiology of weeds and their control and molecular biology of stress tolerance. Although located in Arkansas, the nation's principal rice-growing state (49% of the total national rice area), the DB NRRC serves the research needs of all U.S. rice-producing areas.

The $12.4-million DB NRRC is a state-of-the-art facility with laboratories, offices, seed storage, and greenhouse space. Located on land leased from the University of Arkansas, the DB NRRC expands upon the long-time cooperative federal-state rice research program at Stuttgart. The Arkansas rice experiment station was established in 1927 and the first USDA scientist at Stuttgart, Dr. C. Roy Adair of the Division of Cereal Crops and Diseases, a predecessor agency of the Agricultural Research Service (ARS), was employed in 1931. The staffing level remained at one to two USDA-ARS scientists until 1993, when current expansion began. From the beginning, the University of Arkansas provided office and field facilities for the ARS scientists. Thus, it is appropriate that laboratory space in the DB NRRC be shared with UA RREC scientists.

The DB NRRC is a research center and it has considerable low-temperature storage, but it is not the germplasm repository for rice. That function is ably handled in the "working collection" of rice at the National Small Grains Collection in Aberdeen, Idaho, which curates, preserves, and distributes the U.S. rice germplasm collection, as it does the other small grains. In addition to the working collection at Aberdeen, the National Seed Storage Laboratory in Ft. Collins, Colorado, provides additional security by preserving a "base collection," which intentionally duplicates materials in the working collection. Since rice cannot be grown outdoors at Aberdeen, the DB NRRC serves as the grow-out location for rice germplasm regeneration and increase, and then transfers these materials to Aberdeen.

The mission of the DB NRRC is being addressed by eight research groups:

- *Rice Genetics*—Determines inheritance of high yield, superior grain quality, and pest resistance; develops and releases improved germplasm and genetic stocks; and conducts base-broadening research with indica semidwarfs (J. Neil Rutger, director and supervisory research geneticist).
- *Evaluation and Enhancement of Rice Germplasm*—Evaluates U.S. rice collection; expands collection of rice species; determines inheritance of allelopathy, pest resistance, and stress tolerance; and develops and releases diverse gene pools, genetic populations, and breeding stocks (vacancy, research geneticist, and Wengui Yan, geneticist).
- *Biology, Physiology, and Control of Weeds in Rice*—Identifies and exploits natural biological properties and mechanisms and discovers biological principles of economically important weeds, such as red rice and barnyardgrass, which will facilitate improved weed control (David R. Gealy, plant physiologist).
- *Cereal Chemistry*—Conducts research in rice grain chemistry, using the newest analytical techniques for identifying factors affecting grain quality in conventional and specialty rice varieties, including aroma and taste. Under-

takes a trait discovery program to identify added-value grain traits for rice (Rolfe J. Bryant, research chemist).

- *Molecular Genetics*—Uses molecular marker technology to accelerate rice germplasm enhancement, collaborates with other laboratories to develop map-based cloning strategies, and uses genetic transformation to discover and introduce valuable genes for yield, grain quality, pest resistance, and stress tolerance (Thomas H. Tai, research geneticist).
- *Cytogenetics*—Transfers useful characters such as disease resistance and genes for high yield from other rice species, and transfers apomixis through wide hybridization (Georgia C. Eizenga, research geneticist).
- *Molecular Plant Pathology*—Identifies sources of disease resistance, especially for sheath blight and blast, and accelerates incorporation of resistance genes into improved germplasm (Yulin Jia, research plant pathologist).
- *Molecular Biology*—Uses molecular techniques to study biology and genetics of stress tolerance and stress avoidance, for abiotic stresses such as micronutrient deficiency or excess and temperature extremes (Helen B. Miller, research biologist).

A new thrust at the DB NRRC is the establishment of a Rice Genomic Resources Program. In this program, scientists will examine opportunities to use genome sequencing technologies emerging from the International Rice Genome Sequencing Project to study functional genomics—how genes work—and applied genomics—the solving of important agricultural problems such as those described in the mission.

## Notes

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# Short-duration rice: implications for water-use efficiency in the New South Wales rice industry

R.L. Williams, T. Farrell, M. Hope, R. Reinke, and P. Snell

A reduction in growth duration is an ongoing objective of the New South Wales (NSW) rice breeding program. One of its benefits is a reduction in the period of ponding, and hence in water use. This is important in light of current water restrictions and the increasing cost of irrigation water. The effect of growth duration on water-use efficiency (WUE), here defined as yield divided by total water use (t $ML^{-1}$), had until now not been quantified.

This effect has now been estimated using the rice crop model TRYM, which was used to simulate rice yield and total water use for three crop durations and three sowing dates over 42 seasons of weather data.

As duration from sowing to flowering decreased, yield declined by 0.12 t $ha^{-1}$ $d^{-1}$ and water use declined by 0.078 ML $ha^{-1}$ $d^{-1}$. Thus, in response to a 20-d reduction in time to anthesis, WUE declined from 0.80 to 0.71 t $ML^{-1}$. A similar reduction in yield in response to growth duration was observed in two replicated trials of advanced breeding lines in the 1997-98 season (0.11 t $ha^{-1}$ $d^{-1}$). To ensure that WUE does not decline, the yield of new short-season cultivars should not decline by more than 0.062 t $ha^{-1}$ for each day's reduction in growth duration.

Rice is a major user of irrigation water in southern New South Wales (NSW) irrigation schemes, accounting for 60% of total water diversion. Increased competition for the limited water supply is focusing attention on the water-use efficiency of the NSW rice production system.

In response to this, the rice improvement program at the Yanco Agricultural Institute has aimed to produce short-duration rice varieties that would reduce total water use. Unfortunately, these rice varieties also have a lower yield.

Another reason for developing short-duration rice (which takes 10 to 20 d less to flower than the full-season standard variety Amaroo) is that it increases on-farm flexibility of planting or harvesting time. Short-duration rice can either be planted at the same time as full-duration rice and harvested earlier, or be planted later and harvested at the traditional time. Late planting allows previous pastures to have an extra cut, and earlier harvest allows a greater chance of harvesting in dry and favorable conditions, before winter rains. Rice variety Jarrah was released in 1993 and has a 20-d shorter growth duration than the standard variety Amaroo. It is grown for the benefits of its short season, especially to resow medium-grain crops where the first planting failed. Millin (which takes 10 d less to flower than Amaroo) was released in 1995, and its shorter duration, when combined with an early planting date, allows it to be sold to niche markets.

There is general agreement that these short-duration varieties have a lower water-use requirement and a lower yield than full-season varieties, but little has been done to investigate the possible implications for water-use efficiency in using such varieties. This chapter describes a rice model used in the NSW rice industry to investigate the water-use efficiency of shorter-season rice types and concludes that it is lower than that of standard full-season types. This conclusion has been supported by results of field trials.

## Materials and methods

The rice crop model TRYM (Williams et al 1994) was used to simulate crop development, growth, and yield for three sowing dates (5, 15, and 25 October) and a range of preflood N application rates for each of the 42 growing seasons from 1955 to 1996. The N rates used were in increments of 25 kg N ha$^{-1}$ for the range from 0 up to 150 kg N ha$^{-1}$. The simulations used daily solar radiation and maximum and minimum temperatures for each of these seasons. Deep water at early pollen microspore was assumed, as this is the recommended practice to reduce damage from low temperature.

Parameters for the model were based on the full-season variety Amaroo, except that the simulations were run not only for the full duration of Amaroo but also for two reduced durations. Duration was reduced only by reducing the number of days to panicle initiation (PI). PI was set to occur 90, 80, and 70 d after planting for what are defined as the full-, medium-, and short-duration rice types. This range in days to PI is similar to that observed in currently available NSW rice cultivars. PI dates were input into the model for all simulations, whereas flowering and maturity were estimated by TRYM.

The simulation set the initial soil fertility so that 75 kg N ha$^{-1}$ was taken into the full-season crop during the 90 d from planting to PI. Yield was simulated with preflood N rates ranging from 0 to 150 kg N ha$^{-1}$. The optimal N application rate for each simulation was defined as the highest rate for which the addition of the last 25 kg N ha$^{-1}$ increased yield by 0.5 t ha$^{-1}$. The optimal rate for most simulations was 100 or 125 kg N ha$^{-1}$.

Water use was estimated for each of the three sowing dates for each crop by adding estimates of the amount of water required to fill the profile (a total of 1 ML ha$^{-1}$), deep percolation (1 mm d$^{-1}$), and daily evapotranspiration (ET). Daily ET was estimated by multiplying a crop factor of 1.0 by the estimated evaporation from a class A pan at the meteorological station at Griffith, NSW.

## Results

### Grain yield

Grain yield declined with growth duration for all sowing dates. As there was no interaction between crop duration and planting date, the average yields of the three planting dates are presented (Table 1). Average simulated yields of almost 12 t ha$^{-1}$ were achieved with the full-season variety when 125 kg N ha$^{-1}$ was applied preflood. The full-season variety had a yield advantage of 1.2 and 2.5 t ha$^{-1}$ over the medium- and short-duration types, respectively. The reduction in yield was entirely due to a reduction in total biomass accumulation and not due to changes in harvest index. The simulation took no account of the possible increase in harvest index with a reduced growing season, and may thus overestimate the yield loss with a reduction in growing season.

The simulated yield potential gap of more than 2 t ha$^{-1}$ between the full- and short-duration types is similar to that observed by Reinke et al (1994). They found that, in the absence of cold damage, short-duration cultivar M101 yielded 1.8 t ha$^{-1}$ less than full-season cultivar M7, which has a phenology similar to that of Amaroo. The field trial also showed, as in the simulation, that the lower yield of the short-duration variety could not be increased by increasing the application of N.

### Water use

Average water use decreased with crop duration. The reduction in crop duration by 10 or 20 d reduced water use by 0.78 ML ha$^{-1}$ 10 d$^{-1}$. This is a combination of deep drainage (1 mm d$^{-1}$) and the extra reference evaporation (6.6 mm d$^{-1}$) for the additional period during which a full-season rice crop still requires water.

This study compares crops that were sown on the same date. If we instead compared crops harvested on the same date, the savings in water use would be similar to or less than those estimated here, as ET is at least as high in March (at the end of the season) as it is in October.

The savings of water is therefore not commensurate with the decline in grain yield. Consequently, water-use efficiency declines from 0.80 to 0.71 t ML$^{-1}$ as duration decreases by 20 d (Table 1).

**Table 1. Average grain yield, water use, and water-use efficiency for full-, medium-, and short-duration rice simulated over 42 years. Results are averaged over three sowing dates (5, 15, and 25 October) with 125 kg N ha$^{-1}$ applied preflood.**

| | Full | Medium | Short |
|---|---|---|---|
| Days to panicle initiation | 90 | 80 | 70 |
| Grain yield (t ha$^{-1}$) | 11.9 | 10.7 | 9.4 |
| Water use (ML ha$^{-1}$) | 14.7 | 13.9 | 13.2 |
| Water-use efficiency (t ML$^{-1}$) | 0.80 | 0.76 | 0.71 |
| Yield loss due to 10-d reduction (t ha$^{-1}$) | | 1.2 | 1.3 |
| Water saved by 10-d reduction (ML ha$^{-1}$) | | 0.78 | 0.78 |

### Target yield loss with reducing duration

Based on the numbers in Table 1, a target yield decline for shorter-duration varieties can be estimated. The minimum target yield decline is the product of water-use efficiency of the full-duration crop and the water savings of the short-season variety, that is,

$$\text{Target yield decline (t ha}^{-1}) = \text{water-use efficiency (t ML}^{-1}) \\ \times \text{ water saved (ML ha}^{-1})$$

The minimum target yield decline estimated by this study is 0.062 t $\text{ha}^{-1}$ $\text{d}^{-1}$.

*Field validation.* The effect of growth duration on yield was further investigated within the high-yielding 1998 rice breeding population. This population was a set of advanced rice lines at the $F_5$ stage that was grown for yield and quality testing. The trial was sown on 27 October and had two rates of applied N at preflood. As there was no effect of applied N, the results were pooled for the following analysis.

Yield of the lines ranged from 10.3 to 16.1 t $\text{ha}^{-1}$ and the days to flowering ranged from 87 to 111 d after flooding. The linear effect of crop duration on yield accounted for 26% of the variation in yield. The slope of the regression shows that yield was reduced by 0.11 t $\text{ha}^{-1}$ per day reduction in duration (Fig. 1). This field estimate of yield loss with shorter-season rice varieties confirms the modeled value in the absence of cold damage. Unfortunately, this value is almost twice the target yield reduction required to maintain water-use efficiency in the shorter-duration types.

*Physiological basis.* During March, when the full-season crops are still growing but the short-season types have finished, the full-season crops are each day intercepting 0.9% (21.1 MJ $\text{m}^{-2}$) of the total radiation intercepted during the season. However, they are using only 0.56% (7.8 mm $\text{d}^{-1}$) of the total water used during the season. It is this that accounts for the fact that grain yield (which is a function of radiation interception) declines more rapidly than does water use.

## Conclusions

Short-season rice is a valuable asset to the NSW rice industry in providing on-farm flexibility for planting and harvest times. These varieties make sowing possible as late as November, thus allowing extra pasture to be grown. Alternatively, they allow an earlier harvest, thus increasing the chances of a winter crop being established.

Nevertheless, this chapter shows that this flexibility comes at a cost. Short-season rice in the NSW environment cannot maintain, let alone increase, the water-use efficiency of the rice component of the overall cropping system because the yield advantage of full-season varieties outweighs the water savings of the shorter-duration types.

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GENETICS

# A retrotransposon *Tos 17* in a mutated rice population and cloning of rice genes by transposon tagging with *Tos 17*

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*Tos 17*, a rice retrotransposon, is transcribed and makes inserts of its copies at other sites in the rice genome when rice cells are cultured in vitro. When this insertion occurs inside a working gene, the gene can be identified from a change in plant phenotype and the gene can be cloned by using the *Tos 17* sequence as a probe against the genomic DNA library in a way similar to transposon tagging with transposons.

In our research on gene cloning with *Tos 17*, we are making a database of mutations found in a regenerated rice population. A part of these mutations is expected to be *Tos 17* mutants. Based on this database, the relationship between the mutant phenotype and the *Tos 17* insertion was investigated to identify the *Tos 17* inserted gene, which affected the mutant phenotype. In this research, to this point, we have investigated 1,880 strains and identified nine *Tos 17* mutations.

Transposon tagging is a widely used method to clone genes in a variety of plant species. In this method, a gene is recognized when it loses its function after the insertion of a transposon in its coding region and is cloned using the DNA fragment of the transposon as a DNA probe.

In rice, however, the tagging method had not been adapted because transposons available for this purpose had not been found. In their search for a transposon in the rice genome, Hirochika et al (1996) found a series of putative retrotransposons based on their sequence similarity to a retrovirus. They also proved that one of the retrotransposons, *Tos 17*, makes its copies at other sites in the genome when rice cells are cultured in vitro. They suggested that the retrotransposon could be used as a probe in a transposon-tagging method to clone genes (Hirochika et al 1996, Hirochika 1999). Because *Tos 17* is a retrotransposon of rice, cloning with *Tos 17* has an advantage in our work over cloning with other alien transposons or retrotransposons, which must be introduced into rice cells by the transformation procedure (Izawa et al 1997, Izawa and Shimamoto 1999, Nakajima et al 1996, Noma et al 1997).

Most Japanese rice cultivars have two copies of *Tos 17* in their genome, except for some Japanese cultivars that have one. During in vitro cell culture, *Tos 17* is transcribed to mRNA and then, possibly in the cytoplasm, the mRNA is transcribed to complementary DNAs. Some of these DNAs, in turn, are inserted into the rice genome again. When this insertion occurs inside a gene, the gene might lose its function and this might lead to the expression of a mutant phenotype.

In this research, we investigated two rice populations that were regenerated from in vitro cell culture. As the first step, we made a database of mutations in the populations. Then we detected *Tos 17* mutations among the observed mutations by the analysis of genetic segregation with genomic Southern hybridization analysis.

## Materials and methods

Calli from seeds of Japanese japonica rice cultivar Akitakomachi were cultured in vitro and regenerated plants from the calli in the $R_1$ generation were obtained by Tsugawa et al (personal communication) at the National Agricultural Research Center. After the cultivation of two generations in a greenhouse at the National Institute of Agrobiological Resources (NIAR), the plants of the $R_3$ generation in $R_1$ generation families were cultivated in the field of the Hokuriku National Agricultural Experiment Station (HNAES). Regenerated plants from seed calli of Japanese japonica rice cultivar Nipponbare were obtained at NIAR and the plants of the $R_2$ generation were cultivated in the field of HNAES as $R_1$ families. In 1997 and 1998, 380 $R_1$ families of Akitakomachi and 1,500 $R_1$ families of Nipponbare were observed.

In the nursery, seedlings were observed for leaf color, plant shape, and lethality, among other traits. For the observation of phenotype at growing, heading, and maturing stages, plants were transplanted in the paddy field of HNAES. Observed mutants were harvested individually along with wild-type plants. The phenotype of the embryo and endosperm was observed in dehulled seeds.

*Tos 17* insertion was investigated by genomic Southern hybridization analysis. The 1.0-kb *Xba*I and *Bam*HI fragment of *Tos 17* was used as a probe against *Xba*I digestion of genomic DNA. The probe and restriction enzymes were chosen to make a signal in the genomic Southern hybridization analysis to represent a copy of *Tos 17* in the genome.

## Results and discussion

### Observation of mutations

In the seedling stage, dwarf mutants, leaf morphology mutants, and lethal mutants were found besides many chlorophyll mutants. In the observation in the paddy field, mutants of dwarf, long culm, leaf morphology, leaf color, tillering traits, leaf withering, panicle morphology, heading date, weak growth, and lethality were found. In the observation of seeds, mutants of seed shape, seed coat color, endosperm character, viviparous embryo, and shoot-less embryo were found.

In the observation of 1,880 family lines, more than 600 mutations were detected, though the observation of seed traits of the Nipponbare population has not been completed at this point. This indicated that more than 32% of the regenerated plants had mutations.

Among the mutations of the Akitakomachi population found in the first year, 103 mutations were observed in the second year to confirm their mutant phenotype and to find single-gene mutations. For this purpose, progeny plants from the mutant phenotype plants and wild-type phenotype plants were cultivated. From this observation, 67 of the 103 mutations were confirmed to be single-gene mutations, which account for 65% of the mutations found in the first year. Some of the mutations observed in the first year were accompanied by too many other mutations to analyze. Some of the mutations found in the first year might not be transferred to the next generation for any reason. Taking this into account, we consider that the actual number of single-gene mutations might be higher in our populations than the number shown above if a more precise genetic analysis could be carried out.

The mutations were documented for each individual plant. The observed plants were denoted to make a pedigree record for the genetic analysis in the following years. For this document, a database was constructed in a computer server at NIAR. The document, along with pictures of the mutants and the results of genomic Southern analysis, which will be mentioned below, was registered in the database. The name of the mutants, the description of the mutant phenotype, and their pictures can be retrieved by Internet Web browsers.

## Detection of the *Tos 17* mutation

Among the mutations confirmed above as single-gene mutations, 25 were investigated by genomic Southern hybridization analysis. The average copy number in a genome in the Akitakomachi population increased to more than 12. Because of this increase in number of the copies, the genetic segregation analysis of the *Tos 17* insertion was difficult to make among the progenies of hybrids between mutants and the plants of the original cultivar. Therefore, we made our analysis in plants in each segregating family and the genetic constitution of *Tos 17* was compared between mutant phenotype plants and wild-type plants in each family.

*Tos 17* mutations might appear as recessive mutations, possibly complete recessive mutations, in which the transcription of a gene is blocked by the insertion of *Tos 17*. Therefore, a mutant should be homozygous for the inserted gene. Figure 1 shows one of our analyses on mutant A158. The left four lanes represent *Tos 17* genotypes of mutant-type plants. The mutant-type plants shared six common copies of *Tos 17* besides the original two copies. Among 12 wild-type plants presented here, three plants did not have one of the six copies indicated by the arrows, while the other nine wild-type plants have faint signals for the copy. The faint signal might represent a heterozygous genotype of the inserted gene. From this segregation along with our other analyses, we considered that the *Tos 17* copy indicated with the arrows might be the one inserted in a gene, which controls the mutant phenotype of A158.

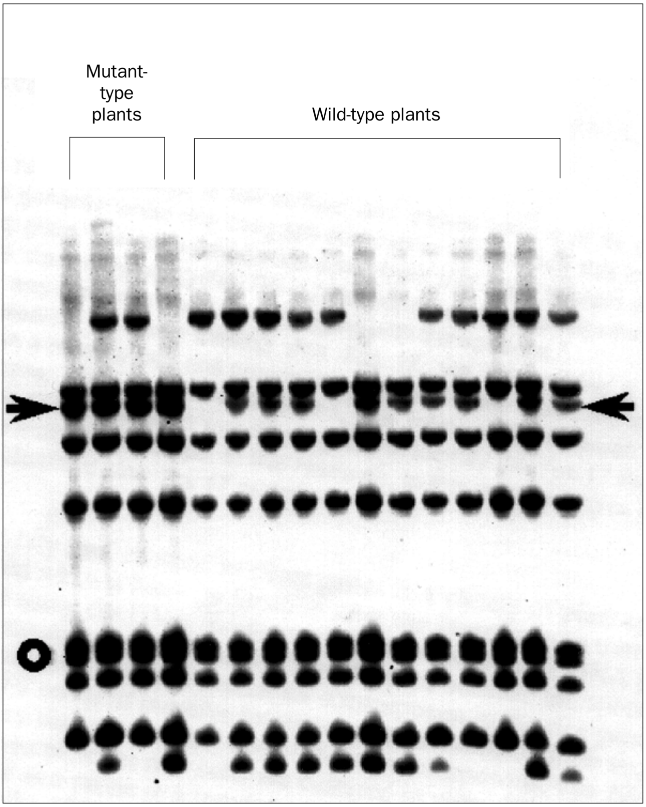

**Fig. 1. Genomic Southern hybridization analysis of a *Tos 17* mutant, A128. The arrows indicate the signal of the *Tos 17* inserted gene. The circle indicates the original two copies of *Tos 17*.**

## *Tos 17* inserted mutations

We have investigated 25 mutations by genomic Southern hybridization analysis as mentioned above. At this point, we believe that nine of the 25 mutations, with a high possibility, might be *Tos 17* mutations (Table 1).

A53 is a dark green dwarf mutation with short panicles and round grain. Leaves of A109 begin to wither at the early stage of each leaf, while the other traits of this mutant are almost the same as those of the original cultivar. In A158, the outermost layer of endosperm shrinks and this leads to fine wrinkles on the grain surface. The endosperm of A250 shows an appearance similar to that of a floury mutant. The analysis of the branching structure of amylopectin of this mutant indicated that the longer branches increased relatively. A269 is a semidwarf mutation with moderately small grain. Necrotic fine spots appear on the leaves of A276 plants. Also, the heading of A276 plants is delayed a few days. A278 is a small-grain mutation. A361 is a sterile mutation. Plants of A369 have faded green leaves.

**Table 1. Mutations controlled by *Tos 17* inserted genes.**

| A lines | Mutation | Number of *Tos 17* copies |  | Signal of *Tos 17* insertion (kb) |
|---|---|---|---|---|
|  |  | Minimum | Maximum |  |
| 53 | Dwarf | 8 | 11 | 6.9 |
| 69 | Semidwarf | 9 | 11 | None |
| 87 | Narrow leaf | 11 | 15 | None |
| 93 | Dwarf/dark green | 5 | 8 | None |
| 99 | White core grain | 11 | 14 | None |
| 109 | Leaf tip withering | 8 | 13 | 5.8 |
| 110 | Small grain | 5 | 10 | None |
| 129 | Floury | 11 | 16 | None |
| 158 | Fine wrinkled grain | 8 | 10 | 8.7 |
| 215 | Narrow leaf | 12 | 13 | None |
| 222 | Dwarf/zebra | 2 | 9 | None |
| 250 | Floury | 14 | 16 | 3.8 |
| 257 | Semidwarf | 11 | 15 | None |
| 259 | Semidwarf | 11 | 13 | None |
| 269 | Semidwarf/small grain | 10 | 11 | 3.3 |
| 276 | Necrosis/leaf withering | 6 | 10 | 7.5 |
| 278 | Small grain | 17 | 20 | 6.8 |
| 281 | Late heading | 2 | 2 | None |
| 288 | Waxy | 9 | 13 | None |
| 340 | Semidwarf | 14 | 15 | None |
| 355 | Yellow green | 12 | 13 | None |
| 361 | Sterile | 5 | 6 | 6.4 |
| 369 | Faded green | 14 | 19 | 4.4 |
| 407 | Small grain | 13 | 14 | None |
| 425 | Dwarf | 10 | 14 | None |

## Efficiency of detecting *Tos 17* mutants

In our cloning work with *Tos 17*, an incident of *Tos 17* insertion was found only by intensive genomic Southern hybridization analysis. Genomic Southern hybridization analysis is among the most laborious procedures and its sensitivity is not high. Therefore, the efficiency to detect a gene in our work had been a major concern of ours since the beginning of our research.

The efficiency of our cloning work was calculated as follows and is shown in Table 2. During two years, we observed 1,880 families of regenerated plants and found more than 600 mutations. In the following observation of 103 mutations of the 600 mutations, 67 were confirmed as single-gene mutations, though there might be more single-gene mutations among them. In the analysis of the *Tos 17* insertions, 9 of 25 single-gene mutations were found to be *Tos 17* mutations. Based on this, we could expect that 36% of the single-gene mutations or 23% of the mutations found in the first observation could be *Tos 17* mutations.

**Table 2. Estimated efficiency to detect *Tos 17* inserted genes.**

| Observation | Number in 1997 and 1998 | Estimated efficiencies |
|---|---|---|
| Observed plants | 1,800 lines × 25 plants | |
| Mutation | About 600 in 1,800 lines | 33% of observed lines |
| Gene mutation confirmed | 67 of 103 mutations | 65% of mutations<br>21% of observed lines |
| *Tos 17* insertion | 9 of 25 gene mutations | 36% of gene mutations<br>23% of mutations<br>8% of observed lines |

It is well known that somatic mutations inevitably occur in a plant population regenerated from cell culture. The mutations in our populations in this research could also be considered as somatic mutations. The calculation above indicated that more than 23% of the somatic mutations of a rice population, or of our rice populations, might be induced by *Tos 17* insertion in genes.

## Molecular cloning of rice genes

Based on the investigation mentioned above, we are now cloning the nine *Tos 17* mutant genes. As the first step, DNA fragments of the flanking region of each inserted *Tos 17* are sequenced and used as cloning probes against cDNA and genomic DNA libraries to clone wild-type genes that correspond to the *Tos 17* inserted mutant genes. In our method, (1) any gene could be cloned as long as it affects plant phenotype, (2) there is no essential information on the transcription product of genes before the cloning work, and (3) the information on the molecular mechanism of phenotypic expression is not required before the cloning work. Therefore, our method has an advantage in the cloning of genes, for which molecular mechanisms of the phenotype have not been elucidated though the phenotype is important. Many genes that control agronomically important traits, such as heading behavior, plant stature, and disease tolerance, might belong to these genes.

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GENETICS

# Rice panicle response to in vitro and in vivo systems

X. Zhao and N. Darvey

This chapter describes a systematic approach to in vitro culture of rice for both young panicles and controlled-environment experimentation on tolerance for various stresses from early microsporogenesis through to pollen development. This experimentation evaluates current progress with panicle culture technology, with special reference to microsporocytes and uninucleate microspores. In this procedure, immature detached rice panicles were cultured in medium, using different combinations of growth regulators, and young panicle development in vitro resulted in well-developed panicles with normal glumes; however, the process was much slower than in vivo. The anthers failed to undergo further development and did not show physical growth in most of the cultures. In addition, further development was not observed in cultures in which panicle size was less than 2 cm in length. Individual florets were also cultured, and they gave a similar response as in panicle culture.

Different basic media did not have a significant effect on panicle culture response. Growth regulators such as indole acetic acid (IAA) and benzylaminourine (BAP) enhanced panicle development in vitro, whereas gibberellic acid ($GA_3$) showed negative effects. Liquid medium gave better results than solid medium.

Other experimentation using a hydroponic system was discussed. This system aimed at producing large numbers of panicles for controlled-environment experiments.

Culture of excised immature floral organs in vitro allows us to study the factors regulating floral development without the mediating influence of the vegetative portion of the plant. It is a useful tool to study and manipulate the developmental program of flowering. The development of male and female fertile florets in vitro by cereal crops such as maize (Pareddy et al 1987, Bommineni and Greyson 1987), wheat (Trione and Stockwell 1989, Barnabas and Kovacs 1992), and barley (Astwood and Hill 1995) has been achieved. Immature tassel meristem explants of maize (isolated several days before meiosis) have been cultured in defined medium, and these explants underwent further growth and completed microsporogenesis and microgametogenesis in vitro (Polowick and Greyson 1982, 1984). In wheat and barley, in vitro culture of young spikes gave rise to trinucleate pollen only in spikelets that contained anthers with microspores beyond the uninucleate stage (Trione and Stockwell 1989, Barnabas and Kovacs 1992, Astwood and Hill 1995). In vitro development of rice florets has not been documented. Another approach to studying the factors regulating floral development is to carry out experiments in vivo under controlled conditions. This would require an adequate degree of replication within experiments to precisely delineate the outcome from each experimental treatment. Hydroponic systems in particular can be used for such controlled experiments as plant growth conditions and nutrient supply can be precisely defined and large numbers of panicles can be produced on a single plant or genotype, which can be easily cloned and replicated over a range of experimental conditions.

## Materials and methods

The rice cultivars Doongara, Kyeema, Illabong, and Jarra were grown in hydroponics in a greenhouse at 28 °C day and 24 °C night with 13.5 h photoperiod. Panicles were collected at different stages of pollen development (from premeiosis to the uninucleate stage) and surface-sterilized with 70% ethanol. Whole panicles, spikelets, and florets were cultured in solid or liquid medium. The culture was incubated at 26 °C for 3 d in the dark before moving to 26 °C with light. Murashige and Skoog's (MS) and $N_6$ media complemented with 500 mg $L^{-1}$ of casein enzymatic hydrolysate, 1,000 mg $L^{-1}$ of malt extract, 500 mg $L^{-1}$ of glutamine, 100 mg $L^{-1}$ of myoinositol, and 30,000 mg $L^{-1}$ of sucrose at pH 5.8 were used. Different combinations of plant growth regulators were tested.

The hydroponic units were locally constructed and contain four parallel 2-m-long channels connected to the nutrition tank with a hose, and the nutrient solution is recirculated by a water pump. The composition of the nutrient solution is essentially Hoagland's solution with reduced nitrogen (40 ppm) just before booting.

## Results and discussion

### Panicle culture in vitro

Different modifications of media were used in this work. There was no significant difference in culture response between the MS and $N_6$ basic media. The media solidified with different gelling agents such as agarose, Phytagel, and Gelrite and did not show any difference in culture response. Young detached panicles and spikelets at several stages of microspore development were cultured. On solid media, panicles at the mid-uninucleate stage grew physically to almost full length in size but the anthers failed to undergo further development and did not give rise to trinucleate pollen. Calli and roots often grew from panicles collected before the early uninucleate stage, but these panicles did not undergo further growth. However, growth of the panicles and

**Table 1. Comparison of spikelet development in vitro in liquid media containing different combinations of plant growth regulators.**[a]

| Constituent of medium[b] (mg L$^{-1}$) | | | Development of spikelets (%) | Development of glumes (%) | Development of anthers (%) |
|---|---|---|---|---|---|
| IAA | BAP | GA$_3$ | | | |
| 0 | 0 | 0 | 80, NN | 70, AS | OS |
| 10 | | | 100, N | 100, N | OS |
| 10 | 5 | | 100, N | NN | OS |
| 10 | | 2 | 100, AS | OS | OS |
| 10 | | 4 | 100, AS | OS | OS |

[a]Incubated 10 days at 26 °C with light. Development of spikelets and glumes in vitro was compared with that in plants under normal growing conditions. The panicles were cultured for 10 d. Percentage indicates the size compared to in vivo. N = normal in shape, NN = near normal in shape, AS = abnormal in shape, OS = retained in original size and shape. [b]IAA = indole acetic acid, BAP = benzylaminopurine, GA$_3$ = gibberellic acid.

glumes occurred when the panicles and spikelets were cultured in liquid media. On the other hand, panicles at the microsporocyte stage or earlier did not grow in any of these media.

Different plant growth regulators were used (Table 1). The panicles grew slowly in the media without plant regulators and did not produce fully developed spikelets or glumes. In the media with indole acetic acid and benzylaminopurine (BAP), the spikelets and glumes showed normal development. It has been reported that cytokinins are associated with the successful completion of meiosis in cultured anthers of *Allium cepa* (Vasil 1957). Kinetin and BAP enhance the development of spikelets in vitro in maize tassel culture (Polowick and Greyson 1984). In detached spike culture of wheat and barley, plant growth regulators were not required (Trione and Stockwell 1989, Barnabas and Kovacs 1992, Astwood and Hill 1995). In this study, several plant growth regulators were tested in various combinations. Some of them showed some improvement in panicle and glume development, but none of them resulted in normally developed anthers.

Panicles at different developmental stages demonstrated different responses in culture. The spikeletes or glumes did not undergo further development when panicles were cultured at the microsporocyte stage or earlier. Similar results have been reported in detached spike culture of wheat and barley (Trione and Stockwell 1989, Barnabas and Kovacs 1992, Astwood and Hill 1995). In detached tassel culture in maize, microsporogenesis, gametogenesis, and pollen maturation were completed in vitro (Pareddy et al 1987). Thus, the culture response in vitro varied among the different plant species for the developmental stage at which microspores can result in normal functional pollen grains.

### Floret organ culture in vitro

We failed to obtain normal functional anthers from detached panicle culture. To examine the physiological parameters affecting anther development in vitro, research on in vitro culture of florets began. The male and female organs or single anthers were separated from the glumes and cultured in liquid medium with $N_6$ salts, an organic mixture of wheat spike culture medium (Trione and Stockwell 1989), and different combinations of plant growth regulators.

This work is still under way. Physical growth of anthers was observed. After 10 days in culture, the anthers cultured at the microsporocyte stage grew to 70–80% of their normal size, but failed in pollen physiological development. Anthers cultured at the tetrad and early uninucleate stage underwent pollen physiological development, but did not fully mature. Thus, we are still unable to obtain functional pollen grains from these cultures.

### Clonal propagation in vivo

Large numbers of panicles were successfully generated in hydroponics. These are being used both for studies on cold tolerance during flowering and for anther and isolated microspore culture. Arzani and Darvey (1993) obtained much higher levels of anther culture response with hydroponically grown triticales than with plants grown in pots. The hydroponic system can be considered as an optimal system for plant growth. Minihydroponic units with individual sodium lights are being constructed for controlled experiments under various temperature regimes to determine the critical stage of damage by cold on pollen development.

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QUALITY

# Quality traits that influence rice nutrition

A.B. Blakeney and P.J. Griffiths

Rice is one of the world's most important cereals for human consumption, providing up to 80% of the daily energy intake in some Asian countries. Nutritional quality of rice typically does not attract a market premium, but is important collectively with milling quality and cooking/eating/processing quality. Correlations exist between nutritional value and quality traits that determine the essential cooking and processing characteristics of rice, which may represent a potential opportunity in some markets.

Characteristics such as protein content, amylose content, and gelatinization temperature, which are important determinants of rice appearance, cooking, and eating quality, may also be nutritionally significant. Amylose content and gelatinization temperature appear to be related to varietal differences in the glycaemic index (GI) of rice. Amylose that escapes digestion in the small intestine accounts largely for the resistant starch content of rice.

With increasing interest in nutrition and health in many markets around the world, consideration of nutritional qualities of rice in conjunction with other quality attributes may represent a significant opportunity for the industry.

We have reviewed the world quality data with the aim of establishing the nutritional differences of the world's major rice classes and from this examine the possibility for developing rice varieties for nutritional niche markets.

Rice is one of the world's most important cereal crops for human consumption, being the staple for more than half the world's population. It provides as much as 80% of the daily energy intake in some Asian countries and is the single most important source of protein in the world because of the quantity consumed.

In Western countries, where consumption is typically much lower than in Asia, higher consumption of rice and other grain-based foods is encouraged as a means of solving escalating health problems resulting largely from excess consumption of high-fat foods. In Australia, rice consumption has increased steadily over recent years in response to the introduction of new varieties, effective marketing strategies, and an increasing proportion of the population being of Asian extraction.

In view of the vast consumption of rice throughout the world, identifying quality characteristics in rice that may have nutritional implications—especially those that can be manipulated by breeding or processing—could benefit millions of people, both in lower-value commodity markets and in higher-value health-driven markets around the world.

This chapter will discuss correlations between quality attributes that determine essential cooking and processing qualities of rice—primarily amylose content—and the nutritional characteristics of rice that may represent future opportunities for the industry.

## Nutrition and consumer food choice

In many developed markets around the world, interest in nutrition and health is one of the major factors influencing consumer food choice. Markets that may once have been considered purely commodity markets now focus on health and are driven, at least in part, by interest in nutrition.

In Asia, particularly in the more developed countries such as Singapore, Japan, Hong Kong, Malaysia, China, and Republic of Korea, food habits have changed dramatically in recent years as a result of high levels of urbanization and rapidly increasing disposable incomes. The trend toward healthier eating that began during the 1980s in the West rapidly emerged in the 1990s in the East. In 1980, China had fewer than 100 health-food shops; in 1992, there were more than 3,000!

## Nutritional characteristics of rice

As already noted, rice is an important source of nutrients for much of the world's population. The nutritional value of rice is usually determined by its protein content—generally considered a secondary quality factor, although it has been correlated with cooking and eating quality.

Among cereals, rice's protein quality is high (rice is unique among cereals, being higher in lysine because of its higher content of glutelins and lower content of prolamins), although the total protein content is lower (av 9.5%). There is some evidence that not all the glutelin is digested and that improving its digestibility would improve the overall nutritional quality of rice.

As with other grains, the milling of rice, which involves removal of practically all the germ and the pericarp and most of the aleurone layer, has a significant influence on its nutritional value. Protein, fat, vitamins, minerals, and fiber are all present in higher amounts in the bran, which is removed, than in the remaining endosperm. Milled rice does, however, have lower levels of phytate and fiber, both of which can reduce the availability of some nutrients.

Contemporary nutrition interest is moving from a focus on the presence or absence of macro- and micronutrients (protein, fat, carbohydrate, dietary fiber, vitamins, and minerals) in foods toward a focus on the health benefits of foods *over and above* that attributed to traditional nutrients ("functional foods"). In Asia, the functional food market is a billion-dollar market segment that is expanding rapidly and driving product development and market growth.

Rice has potential opportunities in this lucrative nutrition-related market, largely related to amylose content—the major determinant of rice appearance and cooking and eating quality. The amylose content of rice correlates with the nutritional characteristics of *glycaemic index* and *resistant starch,* both of which offer health benefits. Because amylose content can be maximized by breeding programs and processing techniques, correlations between these characteristics may be of potential significance in marketing rice to health-conscious consumers.

## Quality traits that influence rice nutrition

The term "grain quality" is perceived differently depending on the intended end use, but generally encompasses milling quality; cooking, eating, and processing quality; nutritional quality; cleanliness; soundness; and purity.

Nutritional quality is usually determined by protein content, but could be considered in terms of amylose content, which is correlated with two important areas of nutrition interest: glycaemic index (GI) and resistant starch content (RS). Varietal differences are observed for both GI and RS, correlating with differences in amylose content (also with gelatinization temperature and degree of starch branching). GI correlates negatively with amylose content.

Glycaemic index is a means of classifying carbohydrate-containing foods according to the rate at which they raise the blood-sugar level. The GI is measured on human subjects. It compares foods on an equal carbohydrate basis (50 g) and ranks them (from 1 to 100) relative to a standard food (usually glucose, but sometimes white bread) based on their effect on blood-sugar levels.

Low GI will provide a slow, steady release of carbohydrate into the bloodstream, whereas high GI foods will result in a shorter, sharper effect on blood sugar. The health benefits of low GI foods include:

1. Control of blood sugar and lipid levels—regular consumption of slowly digested carbohydrates leads to improvement in glucose and lipid metabolism beyond that produced by typical high-carbohydrate diets.
2. Weight control—slow digestion of carbohydrate, that is, as would result from a diet containing a proportion of low GI foods, is associated with higher satiety, with obvious implications for weight control. Such diets have also been shown to promote weight control by promoting fat oxidation (a result of lower insulin levels).
3. "Sustained energy"—low GI foods, that is, slowly absorbed carbohydrates, are important in providing sustained energy for endurance in sports. Con-

versely, high GI foods, as quickly absorbed carbohydrates, have a role in replenishing energy reserves postcompetition.

The relationship between amylose and GI may be exponential rather than linear—reducing the amylose content from 20% to 0% appears to make little difference to GI. Increasing the amylose content above 25%, however, has a pronounced effect on GI.

Table 1 demonstrates varietal differences and the effect of processing on the glycaemic index of rice. Clearly, many rice varieties and products do not currently qualify as low GI. This, however, could be examined in rice breeding and gene technology programs and by attention to processing—as discussed later for resistant starch. Resistant starch refers to that portion of ingested starch which reaches the large bowel undigested, that is, related to the extent of starch digestion.

Contrary to traditional beliefs, it is now known that not all starch consumed is fully digested but that a proportion—estimated to be about 10% in Western diets—reaches the large bowel undigested. This portion, termed "resistant starch," is mainly amylose.

**Table 1. Glycaemic index (GI) of different rice types and products.**

| Type of use | | GI |
|---|---|---|
| *Type* | | |
| Waxy (0–2% amylose) | | 88 |
| Low amylose (12–20%) | | |
| Calrose (mid-grain): | white | 83 |
| | brown | 87 |
| Pelde (long grain): | white | 93 |
| High amylose (25–30%) | | |
| Doongara (long grain): | white | 64 |
| | brown | 66 |
| | instant | 94 |
| Basmati | | 59 |
| *Use* | | |
| Parboiled rice | | 47 |
| Calrose rice bread | | 92 |
| Doongara rice bread | | 61 |
| Rice bran | | 19 |
| Rice Bubbles/Chex/Krispies | | 88 |
| Rice cakes | | 82 |
| Brown rice pasta | | 92 |
| Wheat pasta | | 58 |
| Rolled barley | | 66 |
| Rolled oats | | 58 |

Source: Brand Miller et al (1992).

Starch may be resistant to digestion for several reasons: (1) physically indigestible ("trapped") starch, e.g., grains, legumes; (2) resistant starch granules, e.g., high-amylose starches; (3) retrograded starch, e.g., cooled, cooked starch; and (4) chemically modified, e.g., commercially manufactured starches.

RS content correlates positively with amylose content, but the effect of varietal differences and processing has been observed to be less for resistant starch than for glycaemic index (Juliano 1992), suggesting that differences in starch properties are expressed better in the rate than in the extent of digestion of cooked starch.

In the large bowel, resistant starch undergoes fermentation, acting in a similar fashion to dietary fiber. Resistant starch promotes bowel health, reducing the risk of bowel cancer and other such diseases via several mechanisms, including lowering the pH in the bowel, increasing the production of short-chain fatty acids, particularly butyrate, increasing the bowel's beneficial microflora, and reducing the level of secondary bile acids in the feces.

The potential to optimize the RS in rice lies in the ability to

1. Increase the amylose:amylopectin ratio to produce high-amylose rice varieties through breeding programs (some already exist, such as Doongara and Basmati).
2. Increase the resistant starch content formed during processing by repeated cooking and cooling. The resistant starch content generally increases with parboiling and noodle extrusion.
3. Reduce the degree of gelatinization in processed rice products by lowering pressure and temperature during processing.
4. Increase viscosity—aging rice leads to interactions between fatty acids and amylose, resulting in increased viscosity during cooking and cooling.

Storage of rice results in changes in the physical and chemical characteristics of the grain. As milled rice ages, cooking, processing, eating, and nutritional qualities are modified—cooked kernels have a firmer texture, drier surface, larger volume, and reduced cohesiveness. These changes may be considered desirable or undesirable depending on consumer preferences in particular markets.

Storage of milled rice also results in a higher paste viscosity—shown to be a result of the increased levels of free fatty acids (FFAs) that occur during storage. The amount, rather than the type, of FFAs is the dominant factor in determining the shape of the amylogram (Barber 1972). This effect does not occur with waxy rice varieties, but there is a dramatic effect with high-amylose varieties (Reece and Blakeney 1994), suggesting that interactions between fatty acid content and amylose are important in obtaining increased viscosity during cooking and cooling. These FFA/amylose complexes are indigestible, representing a further source of resistant starch.

## Nutritional significance of varietal preference and cuisine

As the staple food for more than half the world's population, rice is consumed by a huge diversity of people in an equally diverse range of cuisines. The preferred texture, flavor, and other cooking characteristics vary with ethnic group and geographical region. This diversity could be expected to have significant nutritional implications, particularly for the intake of resistant starch (and hence dietary fiber), which may be influenced by

1. Regional varietal preferences. Rice could be an important source of dietary fiber (resistant starch) in countries preferring high-amylose varieties. High-amylose varieties predominate mainly in tropical countries such as India, China, Malaysia, Myanmar, the Philippines, and Taiwan (China) (high-amylose rice noodles). Intermediate-amylose (20–25%) rice is preferred over high-amylose varieties in some areas of China, Indonesia, Malaysia, the Philippines, Thailand, and Vietnam. Low-amylose rice varieties predominate in temperate countries such as Japan and northern China. Waxy (glutinous) rice is the staple food in China, Lao PDR, and north and northeast Thailand. Even though regional varietal preferences are clearly evident, specific rice varieties are usually consumed within ethnic groups, for different culinary uses.
2. Method of preparation. Cuisines in which rice is cooked and then cooled will contribute to higher resistant starch intakes than those in which the rice is boiled and eaten straight away.
3. Freshness of rice. The practice of aging rice before it is consumed influences resistant starch content through its effect on viscosity.

## Overview

Rice is a nutritionally important crop in terms of the vast numbers of people that rely on it as their staple diet. It does, however, have greater potential to be positioned to address consumer interest in health and nutrition.

The extent to which this potential has been developed, particularly in Asia, largely reflects the socioeconomic status of the population rather than the nutritional potential of the crop. In many countries, rice is the staple food crop and continues to be marketed purely as a commodity. In comparison, in developed countries such as Japan, nutrition research programs are more advanced in exploring the potential niche marketing opportunities for rice products.

In conclusion, it is evident from this discussion that rice is not “just rice” and that, in deciding which rice to use for nutritional applications, the type of rice is important.

Improving rice nutritional properties through conventional processing techniques and genetic engineering remains a continuing challenge for the industry.

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# The effect of weather on whole-kernel milling yield of selected cultivars of *Oryza sativa* L. grown in the Po Valley of Italy

A. Finassi, P. Noris, and F. Vidotto

A survey to determine the influence of grain moisture (Gm) of spikelets, time of harvest, and climatic conditions, with particular attention to rainfall, on head rice yield (HRY) at the milling of paddy rice was carried out over six years. The survey was conducted on seven japonica varieties characterized by different dimensions of caryopsis (ratio of length/width) and the presence or absence of seed pearl. The humidity of the spikelets situated in the apical, central, and basal zones of the panicle was determined weekly for about two months to monitor the process of ripening and demonstrate the extent of difference. During the six years of observation, notable differences were seen in the frequency and intensity of rainfall, whereas variations in temperature and relative humidity of the air were slight. Even in the most favorable cases, the average humidity of the paddy never fell below 15%. It was also noticed that, for varieties with a round caryopsis and with the presence of a large pearl, a reduction of about 6–8% in HRY takes place only when the harvest and subsequent drying follow a rehumidification of the paddy rice after repeated rainfall of at least 2 d or that is above 15 mm. This reduction is greatly decreased or not observed if the harvest is delayed at least 24 h after the rainfall and the drying air is at 30 °C. Varieties with a caryopsis without pearl and with a ratio of length/width near or greater than 3 are less sensitive to the phenomenon. On the strength of these results, it was concluded that the climatic conditions that exist in the Po Valley do not induce a notable reduction in HRY following rehumidification by rainfall because drying in the field never reaches the critical values reported in the literature.

Rice growing in Italy occupies an area of 220,000 hectares (1998), with a production of $1.4 \times 10^6$ t of paddy. More than 93% is grown in the western Po Valley at a latitude above 45°N. The harvest begins in the second week of September and may last until the end of October, in a period of lower temperatures and higher rainfall. Harvesting is frequently suspended and the grain moisture (Gm) of the paddy rice changes considerably. Rainy days alternating with sunny days cause a rapid change in the Gm of the paddy rice, as the water absorbed from the rain or from night moisture is not bound to the cellular structures and is therefore rapidly released into the environment. These wetting-drying cycles subject the kernel to physical stresses that, under certain conditions, can cause cracking of the endosperm, resulting in an increase in broken kernels during milling.

As the commercial value of broken rice is about half that of whole kernel, the result is that the commercial value of the milled rice is closely linked to the percentage of whole kernels obtained from milling. Therefore, it is very important economically to adopt measures to keep the potential whole-kernel yield of the paddy rice at the highest level. In Italy, more than 40 cultivars of rice are grown, all belonging to japonica types. Commercially, rice is subdivided into types according to kernel length and length-width ratio (round, medium, long A, and long B). Farmers grow several different varieties each season to use the characteristics of the land and market demands. However, this strategy requires different cultural practices, harvest times, and drying techniques for each type of rice.

The recent introduction of new cultivars with a very slender kernel (length/width ratio >3) and thin and smooth glumes has made it necessary to change the harvesting and drying techniques. These new cultivars dry more quickly than traditional types and can reach a relatively low Gm in the field.

Cracking of the endosperm is caused by strains following the gradient of the Gm content between the surface and the inner part of the kernel (Kunze and Hall 1965). The hypotheses on the factors that determine cracking have been the subject of several studies (Kondo and Okamura 1930, Nagato et al 1964, Wasserman and Calderwood 1972).

The latest studies agree in attributing cracking to the reabsorption of moisture rather than to quick drying. Thus, the hypothesis of sun cracking would give way to the reabsorption of water after rain or cyclic daily variation (day-night) in relative humidity. Numerous studies also reveal that cracking occurs only when the Gm of the paddy rice goes below 15% (Kunze 1964, 1977).

In Italy, the harvesting period coincides with the end of summer and is often characterized by sharp drops in temperature and repeated rainfall. This weather trend prevents the Gm of the paddy rice in the field from dropping below 16%. Nevertheless, appreciable reductions in the yield in milled whole kernels are noted, especially in cultivars with round pearled grain harvested immediately after a rainy period.

The study aimed to identify the factors that affect whole-kernel milling yield of paddy rice and offer strategies to growers on harvesting time in relation to the mean Gm of the paddy rice of different types of varieties, the possibility of postponing the harvest to reduce drying time, the correlated energy expense, and the adjustment of drying facilities when harvesting is done after rain.

## Materials and methods

The research was carried out for 6 years, from 1988 to 1993. Seven japonica cultivars of *Oryza sativa* that are characterized by different kernel sizes (length/width ratio) and by the presence or absence of pearl in the caryopsis were compared (Table 1). Starting from the beginning of ripening, corresponding to a spikelet moisture content of about 35%, the Gm of the spikelets in the apical (A), central (C), and basal (B) area of the panicle was measured on a weekly basis for about 2 mo. Fifty panicles were dehulled by hand, keeping the three sections of the spikelet separate. From the samples obtained, two subsamples of 15 g each were taken to assess Gm by drying in the oven (ISO 712). At the same time, a sample of panicles sufficient to supply 0.5 kg of paddy rice was gathered and dehulled. The paddy rice was kept in a thin layer at a temperature ranging from 20 to 22 °C and relative humidity of 40–60% until reaching 14% Gm. Then, the sample was kept in closed polythene bags until milling. Milling was carried out with a standard Amburgo Universal polishing machine (with abrasive cone), officially used in Italy to determine yield in whole kernel. Polishing was carried out under standard operating conditions, up to removal of the embryo from the kernel. Each operation was repeated twice.

## Results

Ripening of the rice panicle is progressive, but this characteristic is expressed differently depending on the cultivar. This character has a biological basis typical of each cultivar and is not affected by the weather. In certain conditions, the Gm in the A position of the panicle is 6–7 percentage points lower than that of the grains in the B position, even when agronomic ripeness is reached. The panicles in the A position often reach a moisture content of 15–16%, whereas those in the B position rarely drop to 20%. During harvesting, the presence at the same time of spikelets with large differences in Gm may cause cracking in the kernel (Kunze and Prasad 1976), but, in most cases, the phenomenon does not occur in Italy because the moisture content of the A area is above 15%.

**Table 1. Dimensions and characteristics of the caryopsis of selected Italian rice varieties.**

| Cultivar | Length (mm) | Width (mm) | Thickness (mm) | Ratio (l/w) | Weight (g) | Pearl |
|---|---|---|---|---|---|---|
| Cripto | 5.0 | 3.1 | 2.2 | 1.6 | 25 | Present |
| Lido | 6.0 | 2.5 | 1.7 | 2.3 | 22 | Absent |
| Drago | 6.8 | 2.8 | 1.8 | 2.4 | 28 | Absent |
| Ariete | 6.8 | 2.7 | 1.9 | 2.4 | 25 | Absent |
| Ringo | 6.5 | 2.8 | 2.0 | 2.3 | 27 | Present |
| Roma | 6.9 | 3.1 | 2.2 | 2.2 | 34 | Present |
| Thaïbonnet | 8.1 | 2.1 | 1.7 | 3.8 | 25 | Absent |

In Italy, harvesting is done with combines and the paddy is dried in heated air dryers on the farm. Harvesting starts when the dew has dried and ends at sunset, when the dew forms again. Drying facilities operate at night, taking the paddy rice to a Gm of 14% for japonica types with round grain and to 13% for cv. Thaïbonnet with slender kernels.

The HRY obtained with drying in a thin-layer environment reveals a typical trend for all cultivars. As ripening progresses, the whole-kernel yield increases and, after reaching a maximum value (which is around 22–24% Gm for all the varieties tested), undergoes reductions corresponding with harvesting after heavy rain. These reductions are greater when drying takes place in hot-air dryers working at standard temperatures of 35 to 40 °C.

During the observation period, the amount and distribution of rainfall differed. In 1989, rainfall was scarce and practically nil for about 50 d from 10 September to the end of October. In 1993, it rained heavily from the second week of September until the first week of November, involving the entire harvesting period.

In 1989, a long period without rain allowed the paddy rice, which was left in the field after ripening, to reach a Gm of around 15%. Rain for three consecutive days thereafter caused the Gm of the paddy rice to increase over 27%. In this case, the HRY dropped considerably. This behavior was more obvious in the cultivars with pearl (Figs. 1 and 2) than in those without pearl (Fig. 3). In 1993, the continuous alternation of rain with sunny days caused repeated cycles of Gm reabsorption and drying, but these cycles always occurred with a Gm of the paddy rice of around 20%.

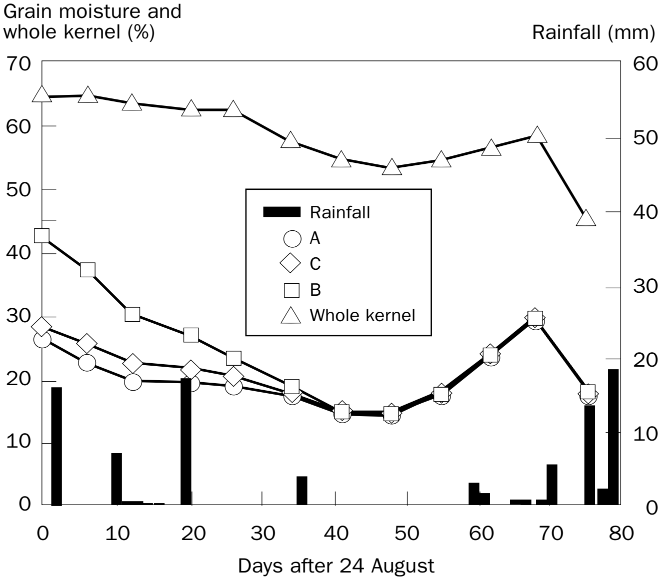

**Fig. 1. Evolution of paddy moisture content and whole-kernel percentage during 1989 experiment, cultivar Cripto. Kernel position on panicle: A = apical, B = basal, C = central.**

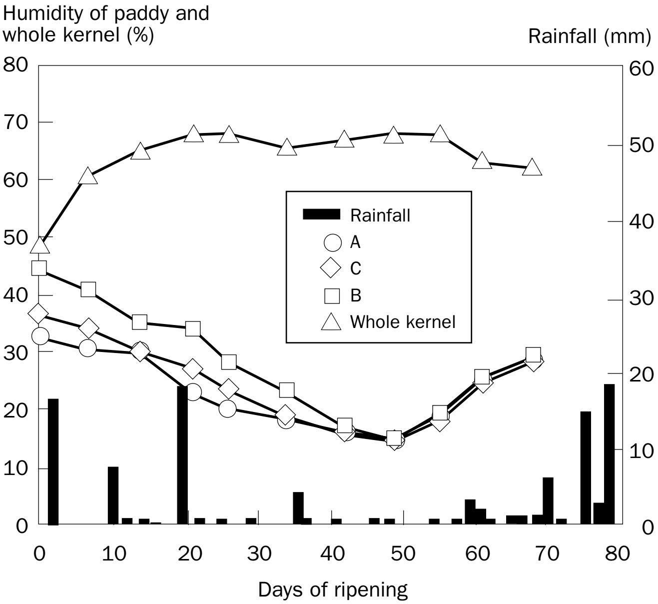

**Fig. 2. Evolution of paddy moisture content and whole-kernel percentage during 1989 experiment, cultivar Roma. A = apical, B = basal, C = central.**

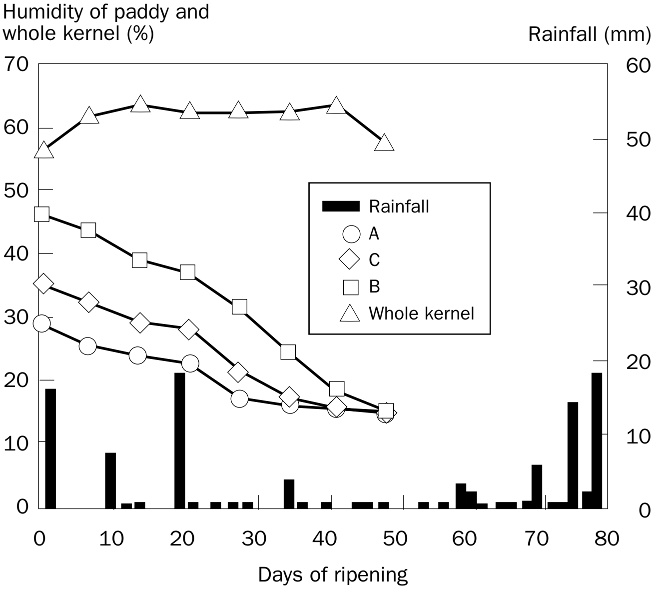

**Fig. 3. Evolution of paddy moisture content and whole-kernel percentage during 1989 experiment, cultivar Lido. A = apical, B = basal, C = central.**

Under these conditions, the cultivars with both pearl and translucent kernel did not undergo any significant change in HRY even after remaining in the field for more than 2 mo after normal harvest time (Figs. 4 to 8).

## Discussion

A considerable amount of data for six harvest seasons has made it possible to evaluate the behavior of seven cultivars in relation to the effect of the weather on HRY at milling. Under particularly dry and sunny years at harvest in the Po Valley, the mean Gm of the paddy rice never fell below 15%. For the panicle, the spikelets in the A position often reach a Gm of 15%, but earlier in the harvest season their Gm is almost 8 percentage points lower than in the B position. At the time of harvest, this difference is usually reduced by 5–6 points; therefore, no problems of resorption occur from harvest to drying. In any case, this period is very short as drying begins 2–3 h after harvesting. From these data, drying the paddy rice in ambient air on a thin layer did not reduce HRY even when harvesting was carried out more than 1 mo after the paddy rice Gm content reached 24% to 18% (physiological maturity). This behavior is more evident in cultivars with translucent kernels, whereas the cultivars with central-side pearl tended to be more sensitive. Drying tests on the same samples and in the same operating conditions, carried out in a controlled environment with air heated to a temperature of 30, 35, 40, 45, and 50 °C at a relative humidity of 20% revealed that, when harvesting is carried out after a period of rain, pearl kernels must be dried in air with a temperature of 30 °C and relative humidity of 20% to avoid lower HRY.

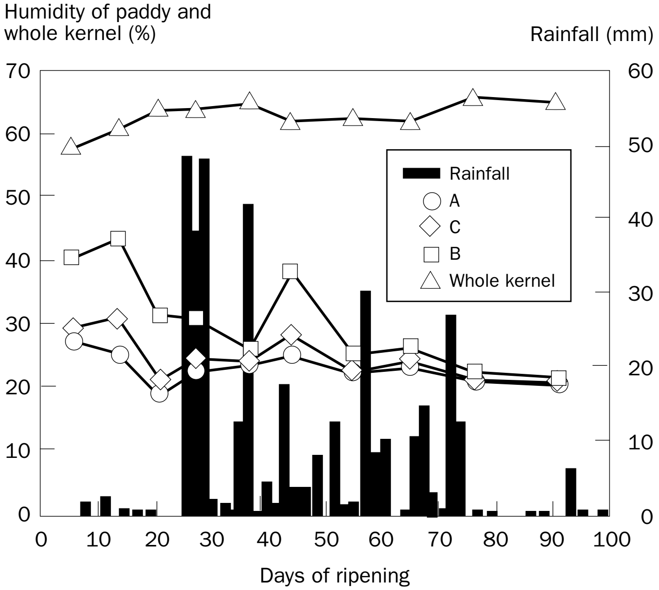

**Fig. 4. Evolution of paddy moisture content and whole-kernel percentage during 1993 experiment, cultivar Cripto. A = apical, B = basal, C = central.**

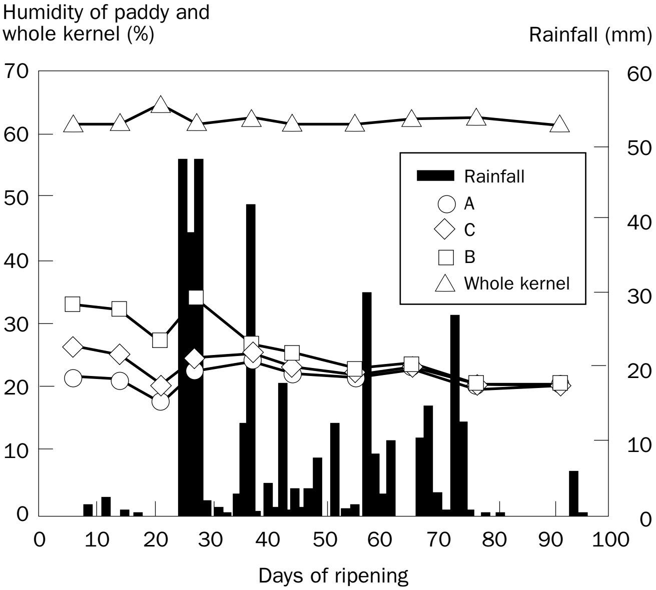

**Fig. 5. Evolution of paddy moisture content and whole-kernel percentage during 1993 experiment, cultivar Drago. A = apical, B = basal, C = central.**

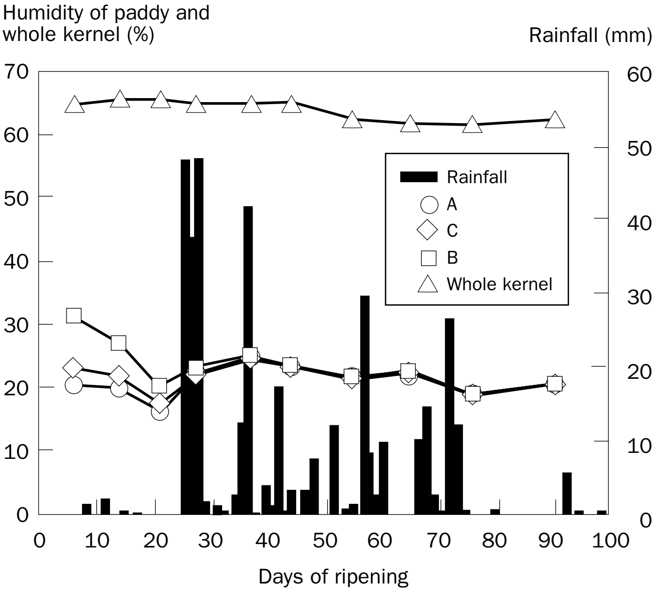

**Fig. 6. Evolution of paddy moisture content and whole-kernel percentage during 1993 experiment, cultivar Lido. A = apical, B = basal, C = central.**

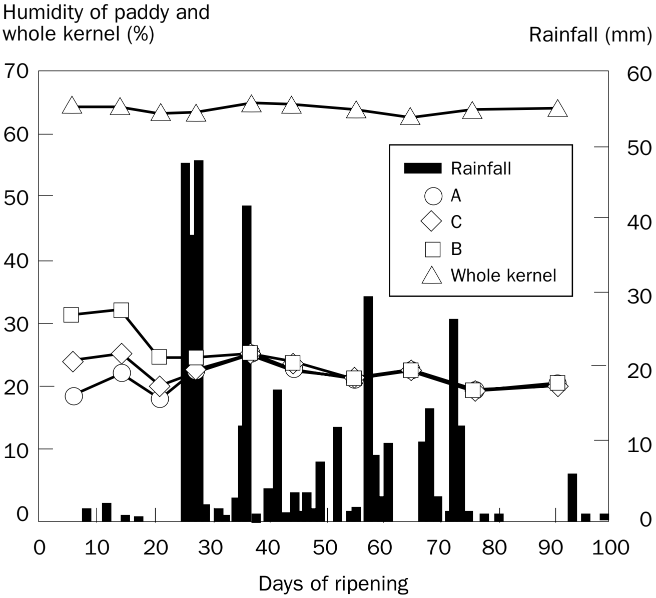

**Fig. 7. Evolution of paddy moisture content and whole-kernel percentage during 1993 experiment cultivar Ariete. A = apical, B = basal, C = central.**

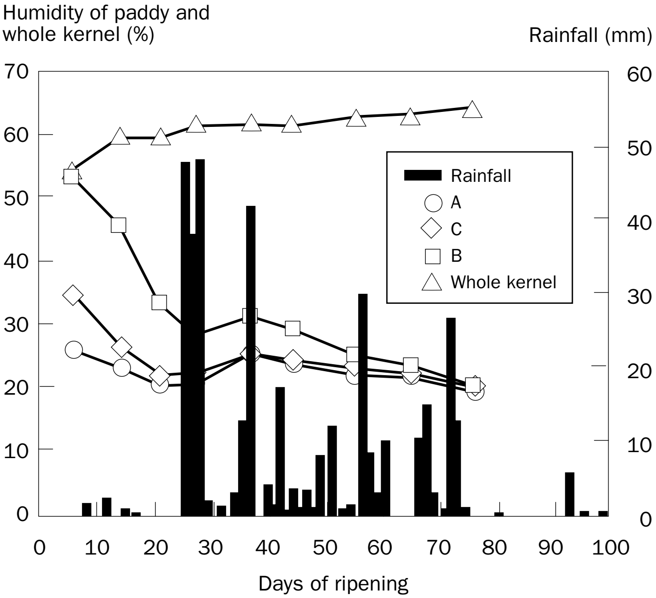

**Fig. 8. Evolution of paddy moisture content and whole-kernel percentage during 1993 experiment, cultivar Thaïbonnet. A = apical, B = basal, C = central.**

Alternatively, harvest can be postponed by 24–36 h and the drying temperature raised to 35 °C. Conversely, for cultivars without pearl and of a slender type, these precautions are not necessary.

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# Sequence polymorphism in granule-bound starch synthase is strongly correlated with rice cooking quality

P.D. Larkin, A.M. McClung, and W.D. Park

In the United States, rice has traditionally been classified on the basis of apparent amylose content. Although apparent amylose content is clearly a key indicator of rice quality, the relationship between amylose content and cooking and processing quality is not absolute. Thus, secondary analyses of grain quality, such as starch paste viscosity tests, have been established to help distinguish rice with different cooking qualities. To identify the genes that control rice pasting characteristics, we examined the segregation of the genes for the primary enzymes involved in starch biosynthesis in a cross between the high-amylose, strong-pasting-curve variety Rexmont and the low-amylose, weak-pasting-curve variety Toro-2. Somewhat surprisingly, we did not see a significant relationship between pasting characteristics and the genes for branching enzyme, debranching enzyme, or soluble starch synthase. Granule-bound starch synthase (GBSS), however, was highly correlated with several parameters of the starch pasting curve, including starch paste breakdown and setback viscosity. In agreement with GBSS playing a key role in determining pasting characteristics, we also found differences in the structural gene for GBSS between rice varieties with similar apparent amylose contents but very different pasting properties.

The apparent amylose content of milled rice, as measured by iodine binding, is a key index of cooking and processing quality (Takeda et al 1987, Reddy et al 1993). In an extended pedigree of current and historically important rice cultivars, we were able to identify eight different alleles of the enzyme that makes amylose, granule-bound starch synthase (GBSS) (Ayres et al 1997). These alleles explained more than 85% of the observed variation in apparent amylose content among the nonglutinous cultivars examined. They efficiently differentiate United States rice market classes and have been used for marker-assisted selection to produce two commercial rice varieties (McClung 1999). Most strikingly, a single G/T polymorphism at the 5′ leader intron splice site differentiated all varieties with less than 18% amylose from those with higher amounts (Ayres et al 1997). Overall, this single nucleotide polymorphism could explain 79.7% of the variation in apparent amylose content in the 89 nonglutinous varieties tested. As shown by work in several laboratories, this G/T polymorphism interferes with processing of GBSS mRNA (Bligh et al 1998, Cai et al 1998, Ishiki et al 1998, Wang et al 1995). This results in the accumulation of partially processed GBSS mRNA that retains the first intron and in the use of several alternate splice sites in low-amylose varieties.

Although apparent amylose content is clearly a key indicator of rice quality, the relationship between apparent amylose content and cooking and processing quality is not absolute. The "apparent amylose" measured by iodine binding or near infrared spectroscopy is a complex mixture of amylose and long chain amylopectin whose proportions vary substantially between varieties (Reddy et al 1993, Ramesh et al 1999). Thus, it is not surprising that some varieties with the same apparent amylose content have markedly different cooking and processing properties. For such cases, secondary analyses of grain quality, such as starch paste viscosity tests, have been established to help distinguish rice with different cooking qualities.

To identify the genes that control rice pasting characteristics, we examined the segregation of the genes for the primary enzymes involved in starch biosynthesis in a cross between the high-amylose, strong-pasting-curve variety Rexmont and a low-amylose, weak-pasting-curve variety, Toro-2. We did not see a significant relationship between pasting characteristics and the genes for branching enzyme or debranching enzyme. The allelic forms of soluble starch synthase and GBSS, however, were highly correlated with several parameters of the starch pasting curve, including starch paste breakdown and setback viscosity. Since these genes are located 5–10 cM apart on chromosome 6, we used the method of path coefficient analysis (Dewey and Lu 1959) to examine their individual contributions to pasting characteristics. Somewhat surprisingly, GBSS was 2- to 3-fold more important even for starch paste breakdown—a parameter traditionally associated with amylopectin structure. However, GBSS playing a key role in a wide range of starch functional properties is consistent with recent work on *Chlamydomonas* demonstrating that GBSS is involved in the synthesis of both amylose and long chain amylopectin (Delrue et al 1992, Van de Wal et al 1998). In agreement with the importance of GBSS in determining pasting characteristics, we also found differences in the structural gene for GBSS between rice varieties with similar apparent amylose contents, but very different pasting properties.

In understanding the relationship of GBSS gene structure and cooking quality, it is necessary to account for genotype × environment interactions. In particular, it is well known that the quality of low-amylose japonica varieties is particularly sensitive to temperature during grain development (Asaoka et al 1984, 1985, Sano et al 1985). While several different mechanisms are involved in G × E interactions, we recently found that the same G/T polymorphism in GBSS that is responsible for differences in amylose content between varieties also plays a key role in differential temperature sensitivity (Larkin and Park 1999). Varieties with the GBSS 5′ leader intron sequence AGGTATA splice pre-mRNA efficiently and accumulate high levels of mature GBSS mRNA over a wide range of temperatures. In contrast, varieties in which the corresponding sequence is AGTTATA show a temperature dependence in RNA processing. At 25 or 35 °C, there is a striking reduction in the steady-state level of mature GBSS mRNA in these varieties, thus leading to their typical "low amylose content." At 18 °C, however, the steady-state level of mature GBSS mRNA and amylose in AGTTATA varieties is similar to that in varieties with the sequence AGGTATA. The biochemical mechanism responsible for this differential temperature sensitivity is complex, involving the differential use of multiple alternate splice sites in AGTTATA varieties as a function of temperature (Larkin and Park 1999). In some cases, this results in the formation of short premature open reading frames and in other cases it leads to the use of a nonconsensus TT/AG splice site rather than the canonical GT/AG (Bligh et al 1998, Ishiki et al 1998, Larkin and Park 1999).

In summary, although other genes also clearly play key roles, we have found that differences in both the amount and structure of GBSS profoundly influence rice cooking and processing quality. Most strikingly, a single G/T polymorphism in GBSS often appears to be the most important factor controlling the G × E interactions that affect cooking quality.

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# Finding the link between chalkiness and starch synthesis

A.J. Lisle, M. Martin, and M.A. Fitzgerald

The propensity of rice grains to form chalk is related to the temperature during the development of the grain. Both temperature and chalk affect pasting temperature and setback (parameters used in assessing cooking quality) in lines that are tolerant for and susceptible to forming chalk. In susceptible lines, amylose content decreases as temperature increases, and starch structures differ in chalky and translucent areas. The supply of sucrose to the endosperm does not limit starch synthesis at high temperatures, but less sugars are incorporated into chalky areas than translucent areas regardless of temperature.

Because Australia is such a small producer of rice by world standards, it is difficult for the rice industry to compete with others in terms of quantity. The Australian rice industry is, however, proportionally a large exporter in the market, exporting around 85% of the rice produced. The marketing strategy of the Australian rice industry has been to target the premium end of niche markets by breeding varieties with quality parameters that meet requirements of specific markets. Chalk (opaque areas in the grain) is one important quality character that is evaluated in the breeding program. Most of our export markets will tolerate only around 2% chalk. The presence of chalk in Australian varieties alters cooking quality (Fitzgerald et al 1999). We have also found that high temperatures during grain development increase the propensity of Australian rice grains to form chalk, and this has also been shown in other varieties (Tashiro and Ebata 1975). Since we used high temperatures to induce chalk, however, it was necessary to separate effects caused by chalk from effects caused by high temperature. The aims of this chapter are to (1) confirm that the presence of chalk induced by high temperatures in a glasshouse system alters cooking quality and (2) investigate the effects of chalk and high temperature on starch structure and deposition.

## Materials and methods

Three rice lines of varying tolerance for chalk were used in this study. Opus is a Japanese-style rice newly released in Australia, and can only be induced to form chalk at very extreme temperatures (i.e., above 45 °C daytime temperature). Millin, another Japanese variety, is considered tolerant for forming chalk, but is less tolerant than Opus. Illabong is an arborio-style rice that has been bred to produce a chalky center. Because a high percentage of chalk is desirable and normal, we have considered this a susceptible line. However, we have been able to eliminate chalk from Illabong grains in very cool conditions (28/15 °C day/night).

Cooking quality was measured by a Rapid Visco Analyser (RVA). A paddle was inserted into a slurry of rice flour and water and the sample was stirred while a heating procedure was carried out. The temperature regime simulated cooking and the viscosity of the mixture was measured throughout. The parameters used when assessing cooking quality from the RVA curve were the pasting temperature, peak viscosity, final viscosity, and setback (difference between final and peak viscosities).

## Results and discussion

Our results show that, in both tolerant and susceptible lines, pasting temperature increases as temperature increases and as the amount of chalk increases, while setback becomes more negative as temperature and amount of chalk increase.

In the susceptible line, the amount of amylose decreases as temperature increases; this supports the results of work by Asaoka et al (1984). In Millin, amylose decreased above 30 °C, but did not change at higher temperatures. Opus showed only a very slight, probably insignificant, decrease in amylose content as temperature increased. The structure of amylose differs between chalky and translucent grains.

We have previously shown that amylopectin structure differs between chalky and translucent grains, indicating that amylopectin molecules are wider and denser in translucent grains than in chalky grains (Lisle et al 2000). Together, these results show that both the amylopectin and amylose fractions differ between chalky and translucent grains, which could explain differences in cooking quality. In both tolerant and susceptible lines grown at high temperatures, the supply of sucrose to the endosperm does not limit starch synthesis. Regardless of temperature, however, endosperms are less able to incorporate sugars into chalky areas than into translucent areas. Therefore, although high temperatures induce chalk, some factor(s), other than simple heat stress, is involved in the formation of chalk.

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# Red rice control in Southern Europe with pre- and postemergence applied herbicides

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A. Ferrero, K. Dhima, G. Feougier, and J.C. Mouret

Within the framework of an EU (European Union) project, field experiments were carried out in Italy, Spain, France, Greece, and Portugal during 1997 and 1998 to evaluate the efficacy of various pre- and postemergence herbicides for red rice control and their selectivity on water-seeded cultivated rice. Preemergence herbicides were applied under water-saturated soil conditions and rice was sown 15 to 20 days after treatment (DAT). Postemergence herbicides were applied at the 2–4-leaf stage of red rice on water-seeded rice sown 5 to 8 DAT. These trials indicated that the preemergence herbicides alachlor, acetochlor, dimethenamid, and metolachlor could be safely applied 15 days before rice sowing on emerged red rice seedlings grown under soil-saturated conditions. These herbicides generally controlled red rice with little effect on cultivated rice sown 15 d later. The postemergence herbicides quizalofop, haloxyfop, fluazifop, cycloxydim, clethodim, sethoxydim, dalapon, glufosinate, paraquat, and glyphosate controlled red rice from 58% to 100%, and these herbicides can contribute substantially to red rice management.

Red rice (*Oryza sativa* L.) is one of the most important weeds in almost all regions where cultivated rice is grown. The name comes from the red color of the pericarp, caused by anthocyanins. Its appearance in table rice lowers commercial quality, while the removal of the red pericarp increases the proportion of broken white grains and consequently reduces milling yield (Smith 1971).

Red rice is very competitive with cultivated rice since it is generally taller and produces more tillers (Diarra et al 1985a,b, Kwon et al 1991, Smith 1988). Competition studies carried out by Pantone and Baker (1991) showed that 4, 16, and 25 red rice plants $m^{-2}$ reduced grain yield by 13%, 37%, and 48%, respectively.

Control of this weed in cultivated rice is very difficult because both belong to the same genus and species and share many morphological and physiological characteristics (Pantone and Baker 1991). Mechanical control is seldom successful unless the land can be fallowed and disked two to six times for at least two years (Klosterboer

1978). Crop rotation, involving soybeans and sorghum for two or three seasons, and water management may also reduce red rice infestations (Smith 1976, Sonnier 1977), but rotation is not always applicable or commercially acceptable.

Chemical control is also very difficult because of the close genetic relationship between the crop and weed, thus reducing herbicide selectivity. Smith (1971) reported that seed treatment with the antidote 1, 8-naphthalic anhydrite increased the tolerance of rice for molinate, but only 60–70% control was obtained in field studies (Baker and Henry 1971). Preplant-incorporated molinate in water-seeded rice partially controlled red rice, but this method was weather-dependent, while the risk for the spread of molinate-tolerant red rice was possible (Baker and Sonnier 1983). The objective of this study was to examine the effectiveness of various pre- or postemergence herbicides against red rice as well as their selectivity on commercial rice.

## Materials and methods

### Preemergence herbicides

Experiments were carried out by all Southern European countries (Italy, France, Spain, Greece, Portugal) where cultivated rice is grown and red rice is one of the main weed problems. Red rice was evenly sown in all plots during early to mid-May in 1997 and 1998 and afterward all plots were irrigated. Eight days after irrigation, the plots were treated with selected preemergence herbicides when the soil was at water saturation. Five days later, all plots were flooded and cultivated rice (cv. Ariette or Thaibonnet) was planted 15 days after treatment (DAT). At 8 d after red rice sowing (herbicide application time) and 15 to 20 DAT, red rice seedlings were counted. In all experiments, an untreated control was included. The efficacy of the treatments was evaluated at the emergence of cultivated rice and 7, 35, 55, and 100 d after cultivated rice emergence (DAE), while the phototoxicity of the treatments was evaluated at 7, 55, and 100 DAE. The evaluation was performed by counting the number of surviving plants of both cultivated and red rice. Phytotoxicity was also evaluated by observing crop growth, density, and production.

A randomized complete block design was used with four replications. The plot size was 4 × 5 m. Although the herbicide treatments differed somewhat among the countries, the results are discussed, where possible, as an average over the countries.

### Postemergence herbicides

Experiments were again carried out by all Southern European countries (Italy, France, Spain, Greece, Portugal). In these trials, red rice was evenly sown in all plots during early to mid-May in both 1997 and 1998 and three days later all plots were irrigated with 1,500 $m^3$ $ha^{-1}$ of water. Postemergence herbicide treatments were applied when the red rice seedlings were at the 2–4-leaf stage. Two days after treatment, the experimental field was flooded and 3–6 d later cultivated rice (cv. Ariette or Thaibonnet) was planted. Red rice seedlings were counted at herbicide application and 12 DAT.

Surviving plants were also counted at 36 and 80 DAT. Herbicide phytotoxicity was evaluated by recording crop growth, density, and production.

A randomized complete block design was used with three replications. The plot size was 5 × 10 m. Although the herbicide treatments differed somewhat among the countries, the results are discussed, where possible, as an average over the countries.

## Results

The trials carried out in Italy in 1997 and 1998 showed that red rice control with pretilachlor (1.5 kg ai $ha^{-1}$), dimethenamid (0.96 kg ai $ha^{-1}$), dithiopyr (0.24 kg ai $ha^{-1}$), pretilachlor + dimethenamid (1.25 + 0.64 kg ai $ha^{-1}$), and fluthiamide (0.30 or 0.36 kg ai $ha^{-1}$) ranged from 49% to 95%. None of the herbicide treatments were phytotoxic on cultivated rice sown 15 DAT. The postemergence-applied cycloxydim (0.95 kg ai $ha^{-1}$), dalapon (12.75 kg ai $ha^{-1}$), quizalofop (0.16 kg ai $ha^{-1}$), and clethodim (0.19 or 0.28 kg ai $ha^{-1}$) reduced red rice panicles at harvest by 85% to 100% compared with the untreated control. Cycloxydim was found to be the most effective. None of the herbicides caused any phytotoxicity on cultivated rice sown 5 DAT.

The trials carried out in France in 1998 showed that red rice control with the preemergence applied dimethenamid (1.08 kg ai $ha^{-1}$) and dimethenamid + oxadiazon (0.72 + 0.375 kg ai $ha^{-1}$) was excellent, while that with pretilachlor (1.001 = 1.01 kg ai $ha^{-1}$) and chlortoluron was 33% and 45%, respectively. None of the herbicide treatments caused any phytotoxic effect on cultivated rice sown 15 DAT. Oxadiazon + cycloxydim (0.25 + 0.20 kg ai $ha^{-1}$), oxadiazon + fluazifop (0.25 + 0.18 kg ai $ha^{-1}$), oxadiazon + quizalofop (0.25 + 0.06 kg ai $ha^{-1}$), oxadiazon + glyphosate (0.25 + 0.72 kg ai $ha^{-1}$), and oxadiazon + dalapon (0.25 + 10.2 kg ai $ha^{-1}$) applied postemergence controlled red rice from 38% to 98%. None of the treatments caused any phytotoxicity on commercial rice sown 5 or 8 DAT.

The trials carried out in Greece in 1997 controlled red rice with the preemergence-applied alachlor (2.4 kg ai $ha^{-1}$), acetochlor (1.54 kg ai $ha^{-1}$), dimethenamid (1.44 kg ai $ha^{-1}$), and metolachlor (2.50 kg ai $ha^{-1}$) from 58% to 80%, while in 1998 control was very good to excellent. None of the herbicide treatments caused any phytotoxicity to cultivated rice sown 15 DAT. The postemergence herbicides glyphosate (1.8 kg ai $ha^{-1}$), glufosinate (1.0 kg ai $ha^{-1}$), quizalofop (0.1 kg ai $ha^{-1}$), and paraquat (0.8 kg ai $ha^{-1}$) controlled red rice from 85% to 99%. None of the postemergence herbicide treatments had any adverse effect on cultivated rice sown 5 DAT.

The trials carried out in Portugal in 1997-98 controlled red rice with cycloxydim (0.20 kg ai $ha^{-1}$), glufosinate (0.45 kg ai $ha^{-1}$), glyphosate (1.80 kg ai $ha^{-1}$), and oxadiazon (0.40 kg ai $ha^{-1}$) from 58% to 95%.

## Conclusions

These trials indicated that the preemergence herbicides alachlor, acetochlor, dimethenamid, and metolachlor could be safely applied 15 days before rice sowing on emerged red rice seedlings grown under soil-saturated conditions. These herbicides generally controlled red rice with little effect on cultivated rice sown 15 d later.

The application of preemergence herbicides 15 days before cultivated rice sowing can contribute adequately to red rice control.

The postemergence herbicides quizalofop, haloxyfop, fluazifop, cycloxydim, clethodim, sethoxydim, dalapon, glufosinate, paraquat, and glyphosate controlled red rice from 58% to 100% and these herbicides can contribute substantially to red rice management.

These results across all countries showed clearly that the postemergence herbicides cycloxydim and quizalofop, as well as the soil-applied dimethenamid, were the most promising ones for the management of red rice.

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# Dry mass by height distributions of rice, watergrass, and sedge species in water-seeded rice

B.P. Caton, J.E. Hill, A.M. Mortimer, and T.C. Foin

Vertical leaf distributions strongly affect plant growth and competition, but have not been investigated in detail for direct-seeded rice and weeds. Rice (cv. M103) and weeds were harvested at 13, 28, 55, and 104 days after seeding (DAS) in a field experiment examining the role of water management in rice-weed competition. Treatments contrasted continuous flooding with drainage and reflooding. Weeds were early watergrass (*Echinochloa oryzoides*), smallflower umbrella sedge (*Cyperus difformis*), and ricefield bulrush (*Scirpus mucronatus*). Leaves and stems were stratified, and normalized data fit by regression. After 6 DAS, species heights differed significantly ($P < 0.05$). Vertical leaf dry mass distributions of rice and watergrass were always skewed toward the base, and both usually had 70% or more leaf dry mass in the top half of the plant. Grass stem distributions were conical, as were sedge shoot dry mass distributions. Only 46% of sedge foliage was found in the top half of the plant. It was concluded that (1) for grasses and sedges, vertical leaf or shoot distributions seemed uniform among morphological types; (2) at equal heights, these sedges will be less competitive for light than the grasses; and (3) competitive differences between rice and watergrass were not influenced by vertical leaf distributions.

Canopy growth traits of crops and weeds are important determinants of growth, competition for light, and crop yield or weed seed production. Examples of these traits are height growth rates, specific leaf and stem areas (kg $m^{-2}$), leaf angles, and leaf and stem area distributions over plant heights. Most detailed studies of rice canopy growth traits have been done in transplanted (Yamasue et al 1977) and upland (Johnson et al 1998) systems. Moreover, weed growth traits have rarely been studied, except for barnyardgrass (*Echinochloa crus-galli*).

Vertical leaf or stem distributions are the shape of area or dry mass (DM) over plant height (see Fig. 1). These distributions are important to study in both direct-seeded rice and weeds for three reasons. First, they have not been measured for rice and weeds in this system. Second, empirical and simulation studies indicate that they

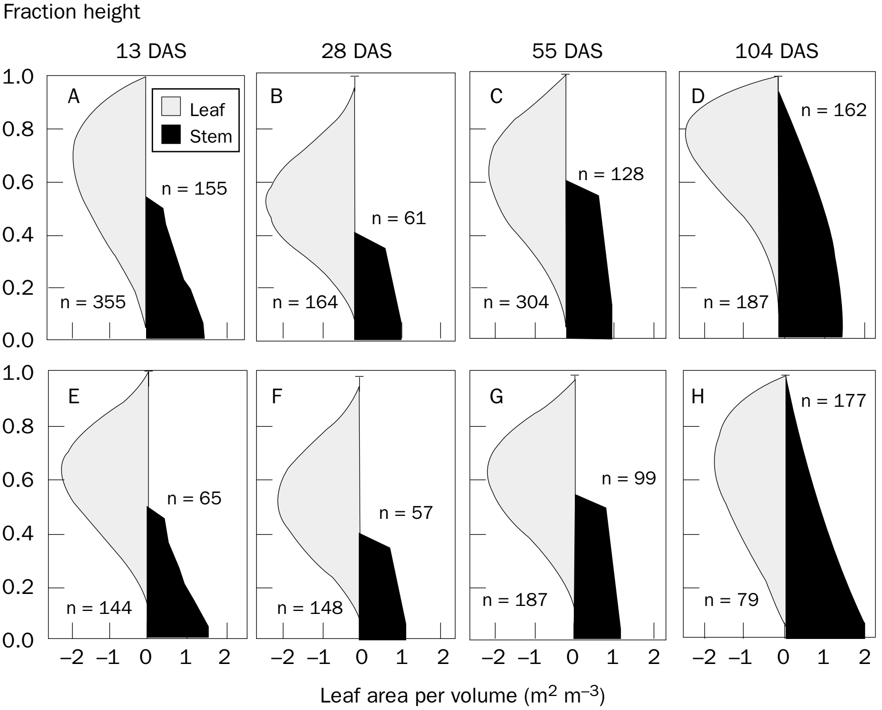

**Fig. 1. Summary of rice (A to D) and watergrass (E to H) leaf and stem dry mass distributions at 13, 28, 55, and 104 days after seeding (DAS). Curves were standardized so that the area under the curves equals 2.0 for leaves and 1.0 for stems.**

strongly affect growth potential, interception of light, and competitiveness (Dwyer et al 1992, Caton et al 1999). Third, reports about vertical leaf distributions of transplanted rice are contradictory. A parabolic vertical leaf distribution for rice was described by Noda et al (1968, cited in Lindquist and Kropff 1996), but Hoshikawa (1975, cited in Graf et al 1990) described it as bottom-skewed. Recently, however, Yamasue et al (1997) demonstrated in Japan that vertical leaf dry mass (kg $m^{-2}$) distributions of transplanted rice and *Echinochloa phyllopogon* (late watergrass, *E. oryzicola* in Japan) in competition with each other were both bottom-skewed.

Our objective was to measure vertical leaf and stem distributions of rice and important weeds in response to water management in direct-seeded rice. Water management affects height growth rates (Scardaci et al 1987), which in turn may affect vertical distributions. In a field experiment in 1998, vertical leaf and stem distributions were measured for rice and three naturally occurring weed species.

## Materials and methods

The experiment was done at the Rice Experiment Station near Biggs, California, as a completely randomized design because of water management requirements. Six 90-

$m^2$ basins had independent filling and draining capacity. Treatments (hereafter T1 to T6) were as follows: (T1) 0.15 to 0.20 m depth, continuously flooded (CF); (T2) 0.15 to 0.20 m, drained at the 3-leaf stage of rice (LSR); (T3) 0.15 to 0.20 m, drained at 6 LSR; (T4) 0.20 to 0.25 m, CF; (T5) 0.08 to 0.15 m, CF; and (T6) 0.0 to 0.05 m, drained at both 3 and 6 LSR. T6 was used to test how much rice and weeds responded to both shallow depths and multiple drainage periods. It was the least limiting environment, yet is consistent with occasional grower practices. Basins were flooded on 24 June 1998 and rice cv. M103 was aerially seeded on 25 June at 100 kg $ha^{-1}$. Rice seeds were soaked for 24 h before broadcasting. Naturally occurring weed species in the plots were *E. oryzoides* (early watergrass), late watergrass, *Scirpus mucronatus* (ricefield bulrush), and *Cyperus difformis* (smallflower umbrella sedge). *Echinochloa* spp. were not separable until late in the season, when it was determined at flowering that most were early watergrass. The two sedges were inseparable before 55 days after seeding (DAS).

Water depths were measured weekly until about 40 DAS, then only at harvests. Plants were harvested from 0.25-$m^2$ quadrats at 13, 28, 55, and 104 DAS. At 13 DAS, only rice and watergrass were large enough to harvest. Samples were kept at 4 °C before and after processing, dried (forced draft) to constant mass at 60 °C, and weighed to a precision of 0.0001 g. Four randomly selected plants were stratified. Increments were chosen to give at least seven partitions over species heights. Because leaves of sedge species are basal, total shoot DM was stratified. Four plants per species from two to four plots for each treatment were stratified, but later only four plants from one plot could be processed efficiently for each treatment.

Data were normalized for analyses. Relative height was calculated as the midpoint of the segment divided by total plant height. Relative leaf DM was calculated as the leaf DM in the segment over total leaf DM. Linear curves were fitted using least-squares regression. Skewed distributions were fitted with the following equation:

$$\mathrm{Y} = A \times (1 - \mathrm{h/H})^{B} \times (\mathrm{h/H})^{C} \tag{1}$$

where Y is DM fraction, h is the midpoint of the segment (m), H is total plant height (m), and $A$, $B$, and $C$ are parameters. Nonlinear regressions were done with NLREG (Phillip H. Sherrod, 6430 Annandale Cove, Brentwood, Tennessee 37027-6313). To compare parameters, we used Bonferroni family confidence intervals with $\alpha = 0.05$. Other nonlinear equation forms were fit by linear transformation, or with the simplest possible polynomial:

$$\mathrm{Y} = A \times (\mathrm{h/H})^{p} + B \times (\mathrm{h/H})^{p-1} + C \times (\mathrm{h/H})^{p-2} + \ldots \tag{2}$$

where $p$ was the highest power of (h/H). Leaf DM fractions in the top half of plants were found by integrating curves in Graphmatica (kSoft Inc., 345 Montecillo Drive, Walnut Creek, California 94595-2654).

## Results

Treatment effects on both rice and watergrass heights were significant at every sample date after 6 DAS (Table 1) but differences were small. Rice and watergrass in drained treatments had slower height growth at 13 and 19 DAS. No clear responses were apparent for sedge species. Species heights differed significantly at every sample date. Rice or watergrass was always tallest.

From confidence intervals, no treatment effects were detected on vertical leaf or shoot DM distributions for any harvest or species (not shown). Thus, normalized distributions were best described by means over all treatments (Table 1). Parabolic (quadratic) distributions did not fit the data well at any harvest (not shown).

Rice and watergrass leaf DM distributions were bottom-skewed over the whole season (Fig. 1). Distributions were less skewed at 28 DAS (Figs. 1B,F), giving a more parabolic shape, but skewness increased after that (Fig. 1). That was near the stage of maximum tillering, so the change in shape may have been related to tiller number. Changes in parameter $B$ were significant for rice, but equations at 13 and 55 DAS were not significantly different (Table 2). Significant differences between rice and watergrass distributions were not apparent until 104 DAS (Table 2). Fractions of rice leaf DM in the top half of plants were 0.70, 0.54, 0.70, and 0.87 at 13, 28, 55, and 104 DAS, respectively. These fractions for watergrass were 0.71 at both 13 and 55 DAS, but 0.55 and 0.68 at 28 and 104 DAS, respectively.

Vertical shoot DM distributions for the sedges at 28 DAS and ricefield bulrush at 55 and 104 DAS were cubic polynomials (Figs. 2A,C,D). Nonlinearity seemed to be caused by large basal DM portions. Bulrush shoot DM distributions at 55 and 104 DAS differed significantly from that at 28 DAS (Table 2). The fraction of leaf DM in the top half of the plant increased from 0.24 at 28 DAS to 0.35 and 0.36 at 55 and 104 DAS, respectively.

**Table 1. ANOVA results for water depth and drainage treatment effects on plant heights of rice, watergrass, ricefield bulrush, and smallflower umbrella sedge, and between species. Numbers in parentheses show total degrees of freedom.**

| | Plant heights[a] | | | | |
|---|---|---|---|---|---|
| Sample date (DAS) | Rice | Watergrass | Bulrush | Umbrella sedge | Species |
| 13 | *** (250) | *** (190) | – | – | *** (430) |
| 19 | ** (59) | * (59) | ns[b] (59) | | *** (179) |
| 28 | *** (239) | *** (192) | ns[b] (209) | | *** (642) |
| 55 | ** (239) | *** (211) | *** (233) | ** (94) | *** (780) |

[a] *, **, and *** denote significant treatment effects at the 0.05, 0.01, and 0.001 levels, respectively; ns denotes no significant treatment effects detected. DAS = days after seeding. [b] Smallflower umbrella sedge and ricefield bulrush were combined in samples at this date.

**Table 2. Estimates (*A* to *D*) and 95% family (Bonferroni) confidence intervals (CI) of parameters from curves describing vertical dry mass distributions for leaves (rice and watergrass) or shoots (ricefield bulrush). Rice and watergrass curves were skewed (3 parameters), whereas those for ricefield bulrush were cubic polynomials (4 parameters). See text for equations.**

| Species | DAS[a] | *A* | CI(*A*) | *B* | CI(*B*) | *C* | CI(*C*) | *D* | CI(*D*) |
|---|---|---|---|---|---|---|---|---|---|
| Rice | 13 | 2.11 | 0.71–3.51 | 1.05 | 0.72–1.38 | 2.22 | 1.59–2.85 | – | – |
| | 28 | 20.96 | 4.50–37.42 | 3.11 | 2.58–3.64 | 3.32 | 2.76–3.88 | – | – |
| | 55 | 2.51 | 1.56–3.46 | 1.54 | 1.33–1.75 | 2.69 | 2.36–3.02 | – | – |
| | 104 | 3.98 | 1.35–6.61 | 1.10 | 0.82–1.38 | 3.76 | 2.94–4.58 | – | – |
| Watergrass | 13 | 10.88 | –8.38–30.19 | 2.07 | 1.08–3.06 | 3.46 | 1.91–5.01 | – | – |
| | 28 | 10.46 | 2.57–18.35 | 2.64 | 2.15–3.13 | 2.98 | 2.43–3.53 | – | – |
| | 55 | 11.07 | 2.73–19.41 | 2.12 | 1.70–2.54 | 3.56 | 2.90–4.22 | – | – |
| | 104 | 1.25 | –0.32–2.82 | 0.85 | 0.22–1.48 | 1.79 | 0.63–2.95 | – | – |
| Bulrush[b] | 28 | –1.94 | –3.0–0.84 | 3.55 | 1.66–5.21 | –2.16 | –2.87–1.45 | 0.53 | 0.45–0.61 |
| | 55 | –0.94 | –1.36–0.52 | 1.56 | 0.93–2.19 | –0.90 | –1.17–0.63 | 0.30 | 0.27–0.33 |
| | 104 | –1.19 | –1.59–0.79 | 2.11 | 1.48–2.74 | –1.23 | –0.95–1.51 | 0.35 | 0.32–0.38 |

[a] Sampling dates as days after seeding. [b] Note from equation 2 that polynomial parameters are listed from highest to lowest order, i.e., $Ax^3 + Bx^2 + ....$

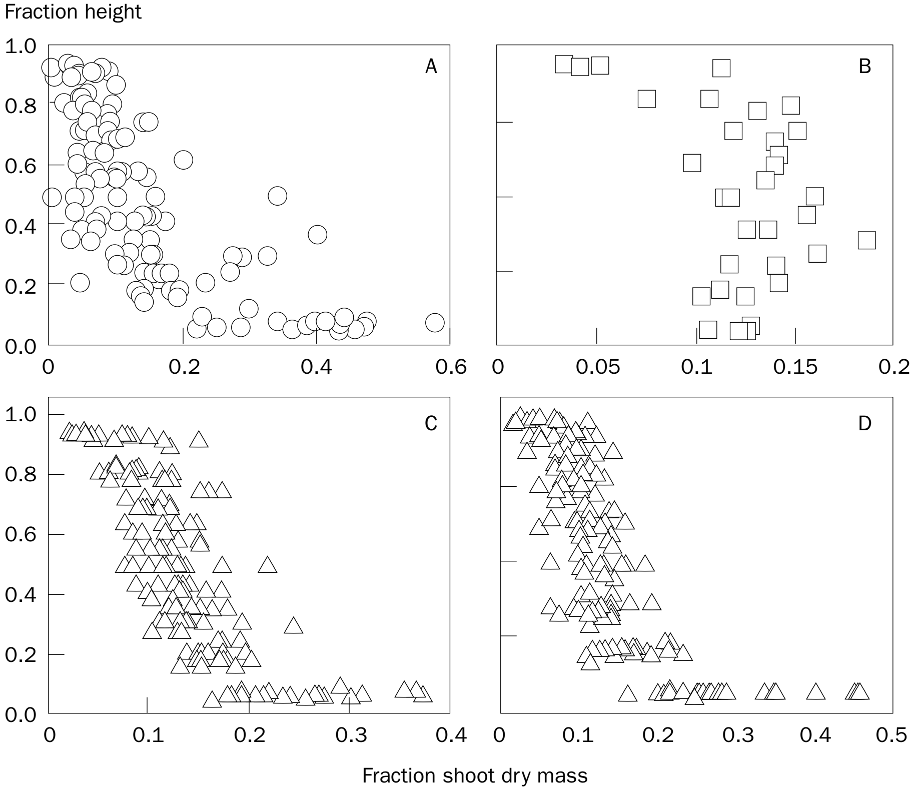

**Fig. 2. Normalized shoot dry mass distributions over plant heights for sedge species at 28, 55, and 104 days after seeding (DAS). (A) Combined species at 28 DAS, (B) smallflower umbrella sedge at 55 DAS, and ricefield bulrush at (C) 55 DAS and (D) 104 DAS.**

Vertical shoot DM distribution of smallflower umbrella sedge was constant at 55 DAS (Fig. 2B). Its fraction of shoot DM in the top half of the plant was approximately 0.5.

Rice and watergrass stem DM distributions were similar (Fig. 1). Distributions were conical and best fit by either cubic (13, 104 DAS) or linear (28, 55 DAS) equations (not shown).

## Discussion

These results and those of Yamasue et al (1997) indicate that vertical leaf distributions of rice and *Echinochloa* spp. are almost always bottom-skewed. Thus, parabolic distributions only seem realistic for monoculture transplanted rice at midseason (Yamasue et al 1997). Similar leaf distributions of rice and watergrass indicate that their competitive differences are probably more affected by other traits, such as rates of photosynthesis or height growth. Differences at 104 DAS probably occurred because early watergrass flowers and senesces before rice.

The height growth responses of the two grasses to depths and drainage were also similar. Height growth rates slowed during drainage, whereas submerged plants elongated more rapidly. Bulrush heights were generally unresponsive to draining.

Morphology determined gross patterns of leaf and stem or shoot DM distributions in this study. The architecture of the two grasses, with tillers and variably aged overlapping leaves that start well up the stem, led to bottom-skewed leaf distributions. In contrast, the morphology of the two sedges, with very erect basal leaves on tillers, gave conical shoot DM distributions.

Shoot DM distributions for the sedges indicated that they probably have a severe competitive disadvantage against rice and watergrass. Relative heights largely determine whether species shade each other, but at equal heights the two grass species would have about 1.5 and 2 times more leaf DM in the top half of plants than smallflower umbrella sedge and ricefield bulrush, respectively.

These results have implications for modeling interspecific competition for light in direct-seeded rice. Given that vertical distributions strongly affect simulated growth (Caton et al 1999), our results indicate that using parabolic vertical leaf area distributions for simulations of rice-*Echinochloa* spp. competition (e.g., Lindquist and Kropff 1996) in direct-seeded systems was unrealistic and inaccurate. These results also indicate, but do not prove, that skewed distributions for sedges (Graf et al 1990) in transplanted rice were unrealistic. The effects of temporal changes in vertical leaf area distributions on model predictions remain to be tested.

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# Water management affects competitive processes in water-seeded rice systems

B. Caton, J. Hill, M. Mortimer, and T. Foin

The effects of water depths and drainage on the growth and competition of rice (cv. M103) and weeds in water-seeded culture were investigated at Biggs, California, to determine whether drainage increased *Echinochloa oryzoides* (early watergrass) interference. Six treatments combined three depths (0.05, 0.10, and 0.15 m) and continuous flooding (CF) or early season drainage (3- or 6-leaf stage of rice). Plants were harvested at 13, 28, 55, and 104 d after seeding (DAS). Shoot dry mass (DM) and leaf nitrogen fractions were measured for rice and watergrass. Rice total shoot DM ($W_{RICE}$, g m$^{-2}$) at 104 DAS was best explained by watergrass shoot DM ($W_{WG}$) but varied with drainage:

$$W_{RICE} = 1{,}075 - 1.02 \times W_{WG} \qquad \text{Drained}$$
$$W_{RICE} = 1{,}075 - 0.61 \times W_{WG} \qquad \text{Continuously flooded}$$

(adjusted $R^2 = 67.1\%$, $P < 0.05$, $n = 18$). We hypothesized that this difference was due to increased root partitioning in response to root zone aeration, a known response of both species. At 28 DAS, leaf nitrogen fractions of watergrass and rice varied significantly between treatments ($P < 0.005$), and for watergrass was highest in drained treatments. This supported the hypothesis. These results have important implications for optimizing water management with applications of foliar-active herbicides.

Water management is a critical weed control method in direct-seeded rice systems (Hill et al 1994). Scardaci et al (1987) and Williams et al (1990) established that water depths and early season drainage in California rice affected weed densities and compositions, rice and weed height gain rates and growth, and rice yields. Despite this, little is known about the mechanistic effects of water management on rice and weed growth or corresponding plant responses.

Recently, Caton et al (1999) found that, in validation tests of DSRICE1, a rice-weed competition and management model for direct-seeded systems, the model underpredicted *Echinochloa oryzoides* (early watergrass) interference with rice in drained and reflooded plots. In contrast, DSRICE1 correctly predicted rice-watergrass competition in continuously flooded (CF) plots. This discrepancy suggested that drainage affected rice-watergrass competitive interactions by some process not yet identified and understood.

Therefore, the objective of this research was to investigate in greater detail rice and weed growth responses to early season water depths and drainage in the water-seeded system. This was done in field experiments in 1998 for rice and naturally present weeds in California. This study focused on the questions raised by the modeling results (above): Does early watergrass interference with rice growth increase as a result of drainage, and, if so, why?

## Materials and methods

### Experiment design and materials

The experiment was done at the Rice Experiment Station, Biggs, California, in a completely random design because of irrigation requirements. Six 90-m$^2$ basins were constructed with independent filling and draining capability. Treatments (hereafter T1 to T6) were designed as follows: (T1) 0.15 to 0.20 m depth, CF; (T2) 0.15 to 0.20 m, drained at the 3-leaf stage of rice (LSR); (T3) 0.15 to 0.20 m, drained at 6 LSR; (T4) 0.20 to 0.25 m, CF; (T5) 0.08 to 0.15 m, CF; and (T6) 0.0 to 0.05 m, drained at both 3 and 6 LSR. The last, T6, was included to test rice and weed responses to both shallow depths and multiple drainage periods. It represents the least limiting environment, yet is consistent with occasional grower practices.

Basins were flooded on 24 June 1998 and rice cv. M103 was aerially seeded on 25 June at 100 kg ha$^{-1}$. Rice seeds were soaked before broadcasting. Treatments were established by 4 d after seeding (DAS). At 0 DAS, depths varied by as much as 0.05 m from intended levels but no plots had exposed soil. Because of rainfall just before flooding, some watergrass plants germinated and established in the plots before the plots were flooded and seeded. Because this was atypical and would have compromised the experiment, these weeds were hand-weeded at 7 DAS.

Important weeds in the plots were early watergrass, late watergrass (*E. phyllopogon*), ricefield bulrush (*Scirpus mucronatus*), and smallflower umbrella sedge (*Cyperus difformis*). *Echinochloa* spp. were inseparable until late in the season when it was determined at flowering that most were early watergrass. At the harvest at 28 DAS, the two sedges were also inseparable.

### Sampling and analysis

Water depths were measured weekly until 40 DAS, then at each harvest. Plant heights were measured at 6 and 19 DAS and at each harvest. Plants were harvested from 0.25-m$^2$ quadrats at 13, 28, 55, and 104 DAS. Only rice and watergrass were large enough for harvest at 13 DAS. Samples were kept at 4 °C before and after processing. Plant densities of all species and tiller densities of rice and watergrass were counted. Heights of ten randomly selected plants were measured per species and shoots of these subsamples were separated into leaves, stems, and seed. Then samples were dried (forced draft) to constant mass at 60 °C and weighed.

Data were analyzed by ANOVA with treatment as the main factor. Significant test results were compared with Tukey's test using $\alpha = 0.05$. Multiple linear regression was used to test for drainage effects.

## Results and discussion

### Growth and competition

Depths and drainage significantly affected rice and weed dry mass (DM) accumulation at most harvests (data not shown). By 104 DAS, increased depths led to more rice growth and lower weed growth (Fig. 1). Moreover, rice growth was much better in CF than in drained treatments, although drainage at 6 LSR (T3) had little effect on either rice or weeds compared with CF plots. Growth of watergrass and ricefield bulrush was highest in the two treatments with early drainage (T2 and T6). Watergrass growth was also high in the shallow CF treatment. Bulrush growth was also high in the deep CF treatment (T4), which may indicate competitive release from watergrass.

For watergrass competition, rice shoot DM ($W_{RICE}$, g m$^{-2}$) at 104 DAS was best explained by the following equation:

$$W_{RICE} = 1{,}075 - 1.02 \times W_{WG} + 0.413 \times W_{WG} \times C$$

where $W_{WG}$ is watergrass shoot DM (g m$^{-2}$) and $C$ is a dummy variable equal to 0 if the plot was drained and 1 if the plot was CF (adjusted $R^2 = 67.1\%$, $P < 0.05$ for full vs reduced model, n = 18). This gave the following equations (Fig. 2):

$$W_{RICE} = 1{,}075 - 1.02 \times W_{WG} \quad \text{Drained}$$
$$W_{RICE} = 1{,}075 - 0.61 \times W_{WG} \quad \text{Continuously flooded}$$

Thus, watergrass interference in drained plots was 67% greater than in CF plots (Fig. 2), which supported the DSRICE1 model validation results (Caton et al 1999).

These results help explain why the two water-active herbicides, molinate and bensulfuron-ethyl, were a successful combination in water-seeded rice (before resistance to the latter developed). Flooding reduced weed establishment (Williams et al 1990), the high efficacy of the two chemicals gave excellent control, and continuous flooding further reduced interference by any escaped weeds. Thus, for water-seeded rice growers, water-active herbicides have ecological as well as practical advantages over foliar-active chemicals.

Most current or new herbicides in California, however, are foliar-active. For them, a trade-off exists between maintaining a continuous flood and facilitating chemical contact with weed foliage, especially for very early applications (i.e., by or before the 2-leaf stage). It may be possible to maintain flooding and still achieve good control with foliar-active chemicals. For example, lowering water without completely

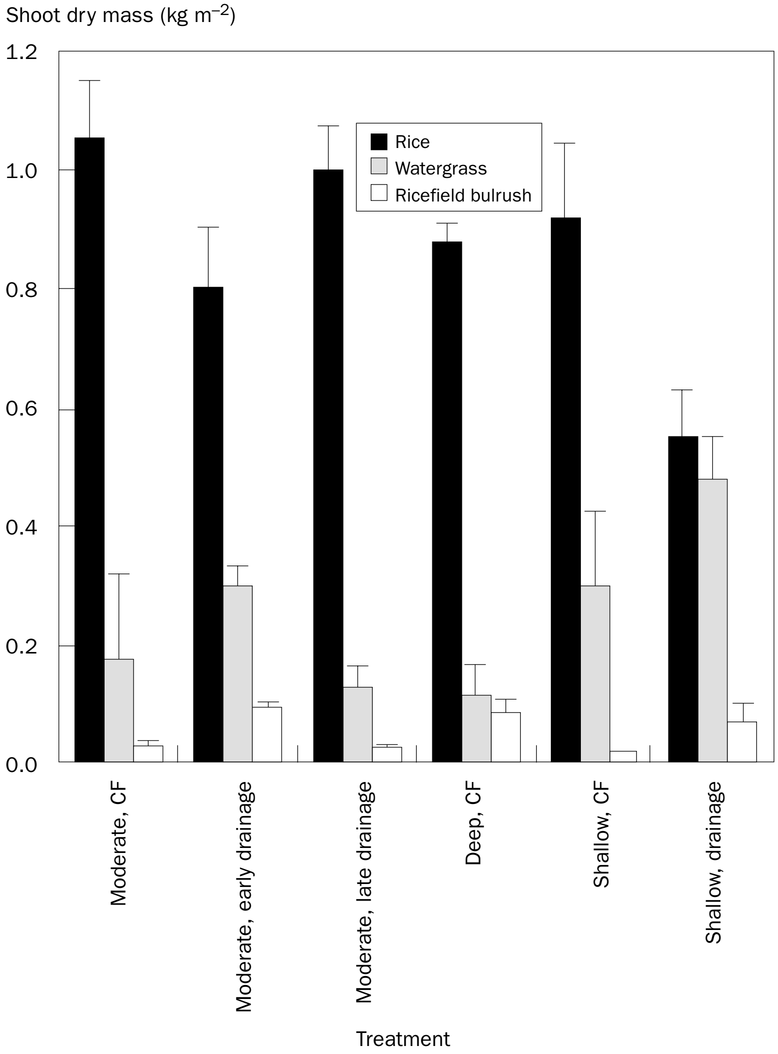

**Fig. 1. Water management effects on rice, watergrass, and ricefield bulrush shoot dry masses (± SD) at 104 d after seeding for shallow, moderate, and deep water depths, and continuous flooding (CF) or early and late drainage timings. See text for detailed descriptions.**

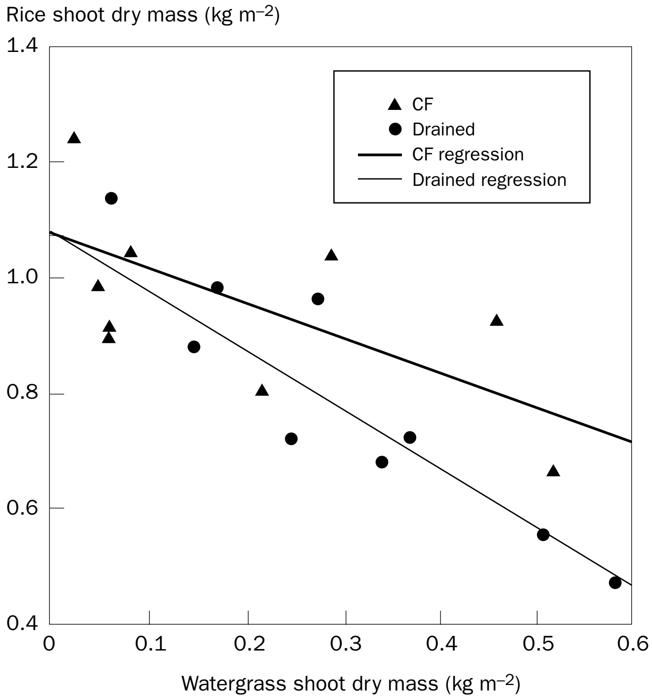

**Fig. 2. Relationship between rice and watergrass shoot dry masses, showing simultaneous multiple regression lines for continuously flooded (CF) and drained plots (see text).**

draining fields may allow enough chemical contact with weed foliage for successful control. Such tactics are increasingly being tested in California herbicide trials. Another option may be to delay herbicide applications until "target" weeds are no longer submerged, but chemical efficacy often decreases with weed age. Finally, reevaluating the need for early control of weeds that grow and emerge more slowly, such as redstem (*Ammannia* spp.), may be useful. Early season control of such weeds typically requires complete drainage, but lowering water may be sufficient to control taller and more important weeds such as watergrass. If necessary, slow-growing weeds could be controlled later and without draining the field.

The mechanism by which watergrass interference increased in response to drainage may be hypothesized to be related to shoot:root partitioning in response to soil aeration. Both rice and watergrass increase root partitioning after aeration (Kennedy et al 1980, Pearce and Jackson 1991). Increased root DM would probably allow earlier or increased uptake of nitrogen or other nutrients. This could increase plant competitiveness in several ways: by making plants larger and more competitive for light, by reducing available nutrients, or both. As a first test of this hypothesis, leaf nitrogen fractions of rice and watergrass were analyzed.

**Table 1. Water management effects on leaf nitrogen of rice and watergrass at two harvests, for mean leaf %N and mean total leaf N (g m$^{-2}$).**

| | | | Leaf nitrogen content | | | |
|---|---|---|---|---|---|---|
| DAS[a] | Depth[b] | Drain[c] | Rice | Watergrass | Rice | Watergrass |
| | | | (± SE)[d] | | g m$^{-2}$ (± SE) | |
| 28 | Mod. | – | 4.77 (0.19) | 3.83 (0.29) | 2.16 (0.18) | 0.60 (0.09) |
| | Mod. | 3 LSR | 5.42 (0.11) | 4.59 (0.13) | 2.21 (0.13) | 0.21 (0.05) |
| | Mod. | 6 LSR | 5.31 (0.08) | 4.22 (0.22) | 1.99 (0.10) | 0.30 (0.16) |
| | Deep | – | 4.42 (0.20) | 3.50 (0.00) | 2.24 (0.04) | 0.15 (0.00) |
| | Shall. | – | 4.81 (0.13) | 3.89 (0.00) | 2.59 (0.11) | 0.49 (0.00) |
| | Shall. | 3/6 LSR | 4.53 (0.08) | 3.07 (0.07) | 2.15 (0.38) | 0.59 (0.10) |
| 55 | Mod. | – | 2.59 (0.10) | 1.68 (0.08) | 3.30 (0.22) | 0.64 (0.19) |
| | Mod. | 3 LSR | 2.61 (0.14) | 1.76 (0.16) | 2.85 (0.23) | 0.62 (0.17) |
| | Mod. | 6 LSR | 2.62 (0.11) | 1.74 (0.29) | 4.55 (0.74) | 0.18 (0.10) |
| | Deep | – | 2.31 (0.06) | 1.74 (0.08) | 3.99 (0.07) | 0.42 (0.08) |
| | Shall. | – | 2.50 (0.19) | 1.70 (0.17) | 4.01 (0.58) | 1.14 (0.41) |
| | Shall. | 3/6 LSR | 2.41 (0.07) | 1.57 (0.10) | 2.95 (0.38) | 1.66 (0.22) |

[a]DAS = days after seeding. [b]Mod. = moderate, 10 cm; deep = 15 cm; shall. = shallow, 5 cm. [c]– indicates no drainage (continuous flooding); LSR = leaf stage of rice. [d]SE = standard error.

## Leaf nitrogen content

Significant treatment effects on both rice and watergrass leaf nitrogen fractions ($P$ <0.005) were found at 28 DAS but not at 55 DAS (Table 1). The latter results indicated that leaf N fraction had probably equilibrated by that date. Total leaf N content (g m$^{-2}$, the product of leaf N fraction and leaf DM m$^{-2}$) of watergrass at 28 and 55 DAS was significantly affected by treatments ($P$ <0.05). For rice, treatment effects on total leaf N were only marginally significant at 55 DAS ($P$ <0.08) (Table 1).

At 28 DAS, leaf N percentages of rice and watergrass were highest in the two single drainage treatments (Table 1). For watergrass, this percentage was lowest in the deep treatment. At 55 DAS, total leaf N was highest (but most variable) for rice and lowest for watergrass in the late drainage treatment (Table 1). In contrast, at this date total leaf N was lowest for rice in the two treatments with early drainage (T2 and T6) and highest for watergrass in the two shallow treatments (T5 and T6). This indicated that rice and watergrass competed for N in the study, but, more importantly, that drainage and perhaps water depths affected that competition.

Using only the empirical data, it is difficult to fully understand the processes that produced these results. Several feedback loops link water depths and drainage, root:shoot partitioning, competition for N and light, and plant growth in this system. Current understanding of this complex system is poor. Model analyses should give more insight into relevant physical and growth processes, competitive mechanisms, and interactions. Since DSRICE1 only simulates competition for light (Caton et al 1999), this will require further model development.

## Conclusions

The effect of drainage on rice-watergrass competition had not been shown previously. Leaf nitrogen analyses supported the hypothesis that changes in root partitioning were one possible mechanism. Moreover, these results have important practical implications for integrating water management with herbicide applications. Draining should be avoided if possible to reduce the competitive effect of watergrass. This, in turn, means using water-active chemicals when possible and ensuring that foliar-active chemicals are applied as efficaciously as possible to reduce weed escapes. Further study is needed, particularly on trade-offs between flooding suppression of weeds versus drainage to facilitate herbicide contact with weed foliage.

Interactions between management practices and crop-weed establishment and growth occur routinely in agroecosystems, with crop rotation effects on weed populations being one widely known example. These interactions are not often a focus of weed research and modeling, however, especially for effects on competitive dynamics. As shown here, studying these interactions may provide practical weed management information as well as increase our understanding of agroecosystem function.

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WEED SCIENCE

# Development of the mycoherbistat fungus *Rhynchosporium alismatis* for control of Alismataceae weeds in rice

E.J. Cother, F.G. Jahromi, W. Pitt, G.J. Ash, and V. Lanoiselet

The fungus *Rhynchosporium alismatis* is being investigated as a bioherbistat for management of the weeds *Alisma lanceolatum* and *Damasonium minus* in temperate Australian rice crops. The fungus sporulated on a range of solid and liquid media and the medium in which an isolate was grown had a significant effect on the virulence of the resulting conidia. Sporulation, germination, germ-tube length, and resulting lesion development on leaf tissue were greatest at 25 to 30 °C. Inoculation of juvenile plants caused growth suppression and there was a synergistic response between the fungus and low rates of bensulfuron herbicide. The pathogen produces chlamydospores in liquid and solid media and these are being investigated as an alternative, and more robust, form of inoculum. Pathogenicity to host and related non-host species is being studied at the cellular and molecular level. *Rhynchosporium alismatis* has a limited host range and is not considered to pose any threat to crops grown adjacent to, or in rotation with, rice.

Among the aquatic weed flora in temperate Australian rice crops are the Alismataceae plants *Alisma lanceolatum* L. (alisma), *A. plantago-aquatica* L. (water plantain), *Damasonium minus* (R.Br.) Buch. (starfruit), *Sagittaria montevidensis* Cham. et Schlecht. (arrowhead), and *S. graminea* Michx. The fungus *Rhynchosporium alismatis* (Oudem.) J.J. Davis is pathogenic to several species in the Alismataceae and is being investigated as a bioherbistat for use in alternative control strategies for management of alisma and starfruit.

## Biology

*Rhynchosporium alismatis* sporulates on a range of solid and liquid media. The nutrient composition of liquid-shake cultures significantly influenced conidial production after 6 d at 25 °C. Lima bean broth at pH 7.5 produced the largest number of viable and infective conidia ($4.99 \times 10^7$ $mL^{-1}$). Media pH per se did not appear to affect yield directly. The medium in which an isolate was grown had a significant effect on the virulence of the resulting conidia as measured by disease severity scores in leaf discs of *A. lanceolatum* after 3 and 13 d. There was a significant difference between isolates, produced in the same medium, in the subsequent rate of disease development.

Sporulation, germination, and germ-tube length were greatest at 25 and 30 °C. Lesion development on leaf tissue was greatest at 25 °C but reduced at temperatures >30 °C. If the water level is kept constant before and after inoculating plants that possess floating leaves, a dew period is not critical as long as the relative humidity is above 60%. The effects of the disease vary with plant age; in adult plants, it causes mostly necrosis and chlorosis on aerial parts, while in juveniles it significantly reduces plant growth. Growth suppression in juvenile plants does not occur if they are submerged during inoculation. For disease development and subsequent growth suppression of juvenile plants, they must be inoculated after lowering the water to the soil level. Early results suggest that a dew period may not be needed for infection of juvenile plants. Fungal isolate DAR 73158 and lima bean broth are considered to be the combination of choice for further studies to explore the fitness of conidia produced in small-scale biofermentors.

In addition to conidia, *R. alismatis* produces chlamydospores in culture. Large numbers ($3.6 \times 10^6$ $cm^{-2}$) were produced on potato dextrose agar within 8–15 d. Sixty percent of chlamydospores, 8 d to 3 mo old, germinated within 24 h at 30 °C, producing up to four germ-tubes (Table 1). One-mo-old chlamydospores were pathogenic to excised leaf discs of *A. lanceolatum* and *D. minus* and produced conidia within 4 d of application. The role of this secondary inoculum in increasing disease incidence and severity needs elucidation. Although chlamydospores are readily produced in broth,

**Table 1. Proportion, with standard errors, of 3- and 6-mo-old chlamydospores of isolate DAR 73158 producing multiple germ-tubes after 24 h at 30 °C.**

| Age | Number of germ-tubes[a] | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 3 mo | 0.54 (0.02) | 0.39 (0.02) | 0.06 (0.01) | 0.01 (0.01) |
| 6 mo | 0.46 (0.02) | 0.44 (0.02) | 0.08 (0.01) | 0.02 (0.01) |
| | (*P*<0.05) | ns | ns | ns |

[a] ns = nonsignificant.

**Table 2. Influence of *Rhynchosporium alismatis* infection of the scape on seed weight and germination in *Damasonium minus*.**

| Disease severity (% of scape infected) | Seed weight (mg 250 seeds$^{-1}$) | Seed germination[a] (%) | % of seeds infected with *R. alismatis*: Not surface-sterilized | % of seeds infected with *R. alismatis*: Surface-sterilized |
|---|---|---|---|---|
| 0 | 46 | 25.8 c | 14 | 6 |
| 1 | 49 | 21.3 c | 10 | 2 |
| 5 | 41 | 10 ab | 12 | 0 |
| 20 | 38.5 | 10 ab | 8 | 4 |
| 50 | 30.5 | 3.3 a | 16 | 10 |
| 80 | 23.5 | 15 bc | 10 | 2 |
| LSD ($P$ = 0.05) | 3.8 | | ns | ns |

[a] Mean of six replicates, each of 40 seeds. Values followed by the same letter are not significantly different at the 5% level. ns = nonsignificant.

attempts to produce them in liquid shake-culture, free from large numbers of conidia, have so far been unsuccessful.

As well as seedling death or significant growth suppression, *R. alismatis* infection of inflorescence stalks of *D. minus* caused significant reductions in seed weight and viability (Fox et al 1999). A 49% reduction in seed weight was recorded when 80% of the stalk was infected (Table 2). The numbers of nodes and florets per plant were not affected. The fungus was seed-borne at low levels in starfruit.

## Herbicide-fungus interaction

The effect of Londax®, MCPA, propanil, and Roundup® on conidial germination was studied. It was found that Londax and MCPA have the least effect on spore germination, whereas propanil and Roundup both inhibit germination, even with doses as low as 1% of recommended rates. Preliminary tests have indicated synergy between the fungus and sublethal doses of Londax (bensulfuron) at 1% of the recommended dose, which could enhance the suppressive effects on juvenile plants. Current research focuses on herbicide-fungus interactions to reduce the competitiveness of these weeds.

## Molecular pathogenicity studies

Using the leaf disc bioassay method of Jahromi et al (1998), 40 isolates of *R. alismatis* were assayed for pathogenicity against two host (*D. minus*, *A. plantago-aquatica*) and two nonhost (*Sagittaria graminea* and *S. montevidensis*) Alismataceae weed species. Pathogenicity was rated visually on a 0 to 10 scale (Cother and van de Ven 1999). The objectives of this study were to determine the most pathogenic isolate on each plant variety. A few isolates were pathogenic to all three species, *D. minus*, *A.*

*plantago-aquatica*, and *S. montevidensis*. A subset of the original 40 isolates will be reassessed for their effectiveness on juvenile plants. Juvenile *D. minus* plants suffer a stunting effect after inoculation with the pathogen. This stunting effect on juvenile plants was not consistent with those isolates causing the greatest disease on adult plants. As such, the subset of isolates chosen will be assessed for their pathogenicity on juvenile plants of *A. plantago-aquatica* and both species of *Sagittaria*. Using one or two isolates, studies will be undertaken to map the infection process of the pathogen. These studies have been performed on the adult *D. minus* plants but thus far have not been conducted on the other species mentioned above. Understanding the infection process could be critical to expanding the potential host range of the pathogen. In addition, differential display studies may determine the molecular mode of pathogenesis of *R. alismatis* against host-plant species *D. minus* and *A. plantago-aquatica* and nonhost *Sagittaria* species.

Repetitive DNA elements will be used to differentiate isolates to the subspecies level. Fungal isolates have been grown in liquid culture and DNA extracted. Preliminary polymerase chain reaction using REP (repetitive extragenic palindromic), ERIC (enterobacterial repetitive intergenic consensus), ITS (internal transcribed spacers), IGS (intergenic spacers), and SSR (single sequence repeat) primers is being assessed to determine genetic variation within the isolate population and to relate this to host affiliation and geographic origin. REP sequences so far have shown only minimal variation among isolates from the same genus and species. Those isolates that attack one plant species do not appear to be genetically distinct from those that attack another species. Moreover, the data suggest that the isolates collected from different geographic locations are not distinct populations and that they all arise from one central population from which they have been dispersed over time.

## Host-range studies

Twenty-eight species of aquatic plants in the Alismataceae and related families and 39 cultivars of 25 species of agriculturally important plants were tested for their reaction to inoculation with conidial suspensions of *R. alismatis* under glasshouse conditions. The pathogen produced lesions in species of *Vallisneria, Triglochin,* and *Marsilea* but the fungus was only reisolated from *Vallisneria*. Scattered infrequent lesions developed on leaves of barley, oats, triticale, lupin, soybean, lettuce, and tomato, but the pathogen was only reisolated from lesions on soybean cv. Bowyer. Emphasis on sampling any areas of discolored tissue resulted in a much higher rate of reisolation of *R. alismatis*. Cucurbits and tomato were the most susceptible plants, based on the frequency of reisolation of the pathogen. There was no progression of disease in any of the infected plants and infection did not appear to influence plant growth and development.

The use of this pathogen as a mycoherbistat for Alismataceae weeds is considered to pose a negligible risk to crops grown adjacent to, or in rotation with, rice crops in southern Australia.

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WEED SCIENCE

# Structure-activity relationships of heteroaryl carbinol analogs in transplanted rice

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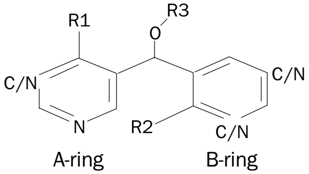
Heteroaryl carbinol analogs are selective herbicides in maize (*Zea mays*), soybean (*Glycine max*), wheat (*Triticum aestivum*), and rice (*Oryza sativa*). In rice, these analogs control *Echinochloa crus-galli* and *Cyperus difformis* at low rates. The chemistry of heteroaryl carbinol analogs has three distinct fragments: an A-ring, a B-ring, and a capping group. Analogs with a t-butylcarbonyl capping group were selective to rice, whereas analogs containing an N-alkyl carbamyl capping group were injurious to rice. Ring type (phenyl, pyridyl, or pyrimidinyl) or substitution on either ring did not affect rice selectivity but rather capping group determined selectivity. Specific analogs with a pyrimidinyl A-ring and phenyl B-ring were an exception to this rule. Pyrimidinyl phenyl analogs containing a 4-ethyl thio or 4-isopropyl thio pyrimidinyl substitution were selective to rice regardless of capping group. However, analogs with the t-butylcarbonyl capping group were always more selective than the corresponding analog with the N-alkyl carbamyl capping group. Based on selective control of *E. crus-galli*, the chloropyridyl trifluoromethylphenyl analog with the t-butylcarbonyl capping group was the best analog for field testing. In field trials in Japan, this analog selectively controlled *E. oryzicola* and other annual weeds in transplanted rice.

Heteroaryl carbinols are preemergence herbicides that selectively control grass weeds and selected broadleaf weeds in maize (*Zea mays*), soybean (*Glycine max*), and wheat (*Triticum aestivum*). Additionally, many heteroaryl carbinols also control problem annual weeds in rice (*Oryza sativa*) when applied postflood, postemergence.

Zeneca scientists discovered heteroaryl carbinol analogs from a chemical synthesis program aimed at making HPPD (p-hydroxyphenylpyruvate dioxygenase) inhibitors (Chin et al 1994). Unlike HPPD inhibitors, heteroaryl carbinol analogs do not bleach plant tissue, but cause leaf crinkling, malformed leaves, reduced root growth, stunting, chlorosis, and eventual necrosis by inhibiting obtusifoliol 14-↑-methyl demethylase during sterol biosynthesis (Howard et al 1998, 1999). Heteroaryl carbinol analogs contain three distinct fragments, which have been referred to as the A-ring, the B-ring, and the capping group on the carbinol moiety:



The objectives of this project were to (1) identify heteroaryl analogs that controlled *Echinochloa crus-galli* (ECHCG) while being selective to rice and (2) develop structure-activity relationships (SAR) that could be used to design analogs that would be selective rice herbicides.

## Materials and methods

Analogs were synthesized at the Western Research Center in Richmond, California. Initial glasshouse studies were conducted in the primary herbicide screen. Seeds of rice variety Koshihikari, ECHCG, and *Cyperus difformis* (CYPDI) were sown in individual pots containing a clay soil and placed into 28 cm × 18 cm × 12 cm tubs. One day before application, the tubs were flooded 1.5 cm above the soil surface and the following day the analogs were injected into the flood. At application, ECHCG and CYPDI were in the two-leaf stage. Weed control and rice injury were assessed at 19 to 21 d after application (DAA).

Analogs of interest advanced to a secondary rice screen in which additional weeds (*Monochoria vaginalis* [MOOVA], *Sagittaria pygmaea* [SAGPY], *Scirpus juncoides* [SCPJU], *Eleocharis acicularis* [ELOAC], and *Cyperus serotinus* [CYPSE]) were tested. The methods for the secondary screen were similar to those of the primary screen, but the secondary screen used transplanted rice, additional weeds, larger tubs, and two growth stages of the weeds and rice, and was assessed at 28 DAA.

## Results and discussion

### Selectivity ratio

We have used selectivity ratios to indicate selective analogs. The selectivity ratio was defined as the $ED_{10}$ for rice injury divided by the $ED_{80}$ for the ECHCG control. A selectivity ratio equal to 1.0 indicated that the associated analog gave 80% control of ECHCG and 10% rice injury. Ratios greater than 1.0 indicate more selective analogs whereas ratios less than 1.0 indicate less selective analogs. We used ECHCG in generating the selectivity ratios rather than CYPDI because ECHCG was determined to be a more important target weed and ECHCG is one of the most competitive weeds with rice (Smith 1968).

## Basis for SAR work

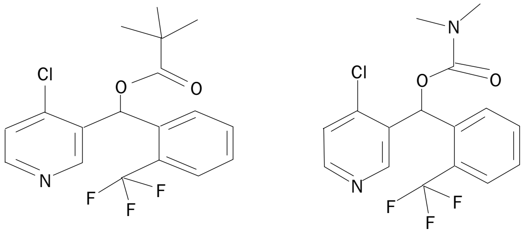
Early in the analog synthesis program, two lead analogs were compared in the secondary rice screen. The 4-chloropyridyl 2-trifluoromethylphenyl analog with the t-butylcarbonyl capping group (compound I) and the corresponding analog with the N,N dimethyl carbamyl capping group (compound II) were tested to determine the breadth of weed control (see Figure 1 for chemical structures). In the secondary rice screen, both analogs controlled ECHCG, CYPDI, and SCPJU at rates of 125 to 250 g $ha^{-1}$. Compound II was the more active analog; it also controlled ELOAC, SAGPY, and MOOVA but did not control CYPSE. There were distinct selectivity differences between the two analogs. Compound I was selective to rice and it did not injure rice at rates up to 1,000 g $ha^{-1}$. Compound II was not selective to rice and caused unacceptable rice injury at 250 g $ha^{-1}$.

Because compound II was more active but unfortunately less selective than compound I, we began the SAR project to determine whether analogs with N-alkyl carbamyl capping groups could be selective rice herbicides and maintain their breadth of weed control. With this goal in mind, we embarked on the SAR project.

## SAR of the heteroaryl carbinol analogs

Heteroaryl carbinol analogs with a pyridyl A-ring and a phenyl, pyridyl, or pyrimidinyl B-ring (t-butylcarbonyl capping group) were selective to rice. Analogs with an N-alkyl carbamyl capping group were not selective herbicides (Tables 1 and 2). For example, compound I (t-butylcarbonyl capping group) had a selectivity ratio of 14.13, but the corresponding analog with the N,N dimethyl carbamyl capping group, compound II, had a selectivity ratio of 0.06 (Table 1). Analogs with chloro, di-chloro, methyl, or ethyl substitution on the B-ring followed the SAR of compound I or compound II, depending on the capping group. The capping group rather than the substitutions on the A- or B-ring influenced selectivity.

Heteroaryl carbinol analogs with a pyrimidinyl A-ring and a phenyl B-ring followed the same SAR pattern as previously described, provided the substitution on the pyrimidinyl ring was alkyl (Table 3). However, when 4-alkyl thio analogs were synthesized, we observed a deviation from the SAR pattern. Analogs with the A-ring substitution of 4-methyl thio pyrimidinyl followed the expected SAR with only the t-

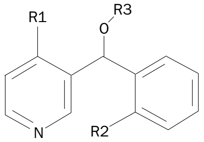


Compound I
t-butylcarbonyl analog

Compound II
N,N-dimethyl carbamyl analog

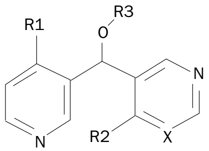
**Fig. 1. 4-chloropyridyl 2-trifluoromethyl phenyl analogs with t-butylcarbonyl and N,N-dimethyl capping groups.**

**Table 1. Selectivity ratio of heteroaryl carbinol analogs with A-ring = pyridyl and B-ring = phenyl.**



| R1 | R2 | R3 | | Selectivity ratio |
|---|---|---|---|---|
| 4-Cl | 2-$CF_3$ | $COC(CH_3)_3$ | (compound I) | 14.13 |
| 4-Cl | 2-$CF_3$ | $CON(CH_3)_2$ | (compound II) | 0.06 |
| 4-Cl | 2-Cl | $COC(CH_3)_3$ | | >1.0 |
| 4-Cl | 2-Cl | $CON(CH_3)_2$ | | <0.3 |
| 4-Cl | 2,3-$Cl_2$ | $COC(CH_3)_3$ | | >1.0 |
| 4-Cl | 2,3-$Cl_2$ | $CON(CH_3)_2$ | | 0.13 |
| 4-Cl | 2-$CH_3$ | $COC(CH_3)_3$ | | >2.0 |
| 4-Cl | 2-$CH_3$ | $CON(CH_3)_2$ | | 0.14 |
| 4-Cl | 2-$CH_2CH_3$ | $COC(CH_3)_3$ | | >6.5 |
| 4-Cl | 2-$CH_2CH_3$ | $CON(CH_3)_2$ | | 0.22 |

**Table 2. Selectivity ratio of heteroaryl carbinol analogs with A-ring = pyridyl and B-ring = pyridyl or pyrimidinyl.**



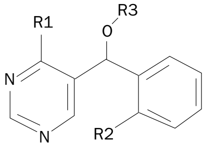
| R1 | R2 | R3 | X | Selectivity ratio |
|---|---|---|---|---|
| 4-Cl | 2-$CF_3$ | $COC(CH_3)_3$ | CH | 1.02 |
| 4-Cl | 2-$CF_3$ | $CON(CH_3)_2$ | CH | 0.20 |
| 4-$CF_3$ | 2-$CF_3$ | $COC(CH_3)_2$ | CH | 1.58 |
| 4-$CF_3$ | 2-$CF_3$ | $CON(CH_3)_2$ | CH | 0.30 |
| 4-$CF_3$ | 2-$CF_3$ | $COC(CH_3)_3$ | N | 1.25 |
| 4-$CF_3$ | 2-$CF_3$ | $CON(CH_3)_2$ | N | 0.12 |

butylcarbonyl analogs being selective to rice. But with a 4-ethyl thio (see Fig. 2) or 4-isopropyl thio pyrimidinyl substitution on the A-ring, analogs with both a t-butylcarbonyl or an N,N dimethyl carbamyl capping group were selective to rice (selectivity ratio equal to or greater than 1.0; Table 4). Nevertheless, selectivity ratios were greater for analogs with the t-butylcarbonyl capping group. Alkyl thio groups with more than two carbon units likely also impart some selectivity to rice.

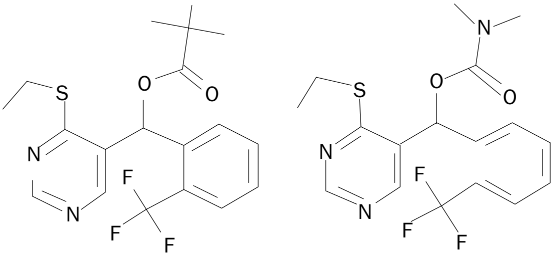
## Field testing

In follow-up glasshouse testing, we determined that compound I was the best analog for field testing, based on selective ECHCG control. Compound I was tested at Zeneca's Japanese field research station, where it selectively controlled *Echinochloa oryzicola* (ECHOR) when applied at 3 d before transplanting, at transplanting, at 5 d after transplanting, and at 2 1/2-leaf ECHOR (Table 5).

**Table 3. Selectivity ratio of heteroaryl carbinol analogs with A-ring = pyrimidinyl and B-ring = phenyl.**



| R1 | R2 | R3 | Selectivity ratio |
|---|---|---|---|
| 4-$CF_3$ | 2-$CH_2CH_3$ | $COC(CH_3)_3$ | >2.0 |
| 4-$CF_3$ | 2-$CH_2CH_3$ | $CON(CH_3)_2$ | <0.25 |
| 4-$CH_3$ | 2-$CF_3$ | $COC(CH_3)_3$ | 2.0 |
| 4-$CH_3$ | 2-$CF_3$ | $CON(CH_3)_2$ | 0.25 |
| 4-$CH_2CH_3$ | 2-$CF_3$ | $COC(CH_3)_3$ | 16.0 |
| 4-$CH_2CH_3$ | 2-$CF_3$ | $CON(CH_3)_2$ | <0.5 |

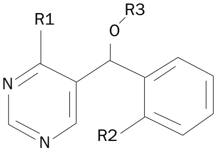


Compound III
t-butylcarbonyl analog

Compound IV
N,N-dimethyl carbamyl analog

**Fig. 2. 4-ethyl thio pyrimidinyl 2-trifluoromethyl phenyl analogs with t-butylcarbonyl and N,N-dimethyl capping groups.**

**Table 4. Selectivity ratio of heteroaryl carbinol analogs with A-ring = pyrimidinyl and B-ring = phenyl.**



| R1 | R2 | R3 | Selectivity ratio |
|---|---|---|---|
| 4-$SCH_3$ | 2-$CF_3$ | $COC(CH_3)_3$ | >1.0 |
| 4-$SCH_3$ | 2-$CF_3$ | $CON(CH_3)_2$ | 0.75 |
| 4-$SCH_3$ | 2-$CF_3$ | $CON(CH_2CH_3)_2$ | 0.48 |
| 4-$SCH_3$ | 2-$CH_2CH_3$ | $COC(CH_3)_3$ | 2.3 |
| 4-$SCH_3$ | 2-$CH_2CH_3$ | $CON(CH_3)_2$ | 0.52 |
| 4-$SCH(CH_3)_2$ | 2-$CF_3$ | $COC(CH_3)_3$ | >1.2 |
| 4-$SCH(CH_3)_2$ | 2-$CF_3$ | $CON(CH_3)_2$ | 1.00 |
| 4-$SCH_2CH_3$ | 2-$CF_3$ | $COC(CH_3)_3$ (compound III) | >2.23 |
| 4-$SCH_2CH_3$ | 2-$CF_3$ | $CON(CH_3)_2$ (compound IV) | 1.06 |

**Table 5. Rice injury and *Echinochloa oryzicola* (ECHOR) control by compound I at Zeneca's Japanese field station.**

| Rate (g ha$^{-1}$) | Treatment timing | Percent rice injury[a] | | | | Percent control of ECHOR | |
|---|---|---|---|---|---|---|---|
| | | 7 DAA | 14 DAA | 28 DAA | 49 DAA | 28 DAA | 49 DAA |
| 125 | 3 DBT | 0 | 5 | 5 | 2 | 98 | 98 |
| 250 | 3 DBT | 0 | 6 | 5 | 1 | 99 | 98 |
| 500 | 3 DBT | 0 | 12 | 12 | 9 | 100 | 100 |
| 125 | At transplant | 0 | 5 | 5 | 1 | 100 | 100 |
| 250 | At transplant | 0 | 3 | 3 | 0 | 99 | 100 |
| 500 | At transplant | 0 | 4 | 3 | 2 | 100 | 100 |
| 125 | 5 DAT | 0 | 1 | 0 | 0 | 98 | 98 |
| 250 | 5 DAT | 0 | 4 | 6 | 0 | 100 | 98 |
| 500 | 5 DAT | 0 | 2 | 3 | 0 | 100 | 100 |
| 125 | 2-1/2 leaf | 0 | 0 | 0 | 0 | 79 | 63 |
| 250 | 2-1/2 leaf | 0 | 0 | 0 | 0 | 97 | 90 |
| 500 | 2-1/2 leaf | 0 | 5 | 0 | 0 | 98 | 95 |

[a] DAA = days after application, DBT = days before transplanting, DAT = days after transplanting.

## Conclusions

Heteroaryl carbinol analogs controlled ECHCG and other annual weeds in rice. These analogs can have phenyl, pyridyl, or pyrimidinyl rings. When the analogs were capped with a t-butylcarbonyl capping group (i.e., compound I), they were useful as selective herbicides in rice. Corresponding analogs with N-alkyl carbamyl capping groups (i.e., compound II) were not sufficiently selective to rice. Substitutions on the phenyl, pyridyl, or pyrimidinyl rings generally did not affect rice selectivity but did affect overall activity. The capping group determined selectivity.

There was one main exception to this SAR pattern. Pyrimidinyl-phenyl analogs (i.e., compounds III and IV) with a 4-ethyl thio pyrimidinyl or with a 4-isopropyl thio pyrimidinyl substitution were selective to rice regardless of capping group. However, analogs with a t-butylcarbonyl capping group always had a higher selectivity ratio than analogs with N-alkyl carbamyl capping groups.

Compound I was determined to be the most active selective analog in the chemical series. It was tested at Zeneca's Japanese field station, where it selectively controlled ECHOR and other annual weeds in transplanted rice.

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## Notes

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# Biology and control of red rice *(Oryza sativa L. var. sylvatica)* infesting Italian rice fields

A. Ferrero and F. Vidotto

Red rice infestations were already reported in the 19th century, but started to cause severe yield losses in Italian rice fields in the 1960s after the shift from rice transplanting to direct seeding and the cultivation of weak, semidwarf indica-type varieties. This chapter reports the results of several experiments carried out in Italy starting in 1995 under the framework of a research project funded by the European Commission and aimed at studying the biology and control of the weed. The main studies regard seed bank evolution, seed longevity, emergence behavior, competitive and shattering ability, acquisition of viability after flowering, and control in rice pre- and postplanting and in rotational crops. The information acquired from these studies makes it possible to define integrated weed management programs based on a combination of prevention, rotation, and chemical practices.

Red rice is undoubtedly the main weed problem in European rice fields. Known in Italy since the beginning of the 19th century, the weed spread relatively slowly until the end of the 1960s (Tarditi and Vercesi 1993, Ferrero and Finassi 1995, Ferrero and Vidotto 1997a). After this period, the presence of this weed in rice fields increased dramatically because of the shift from transplanting to direct seeding and the increasing cultivation of poorly competitive, indica-type semidwarf rice varieties, which was encouraged by European Commission policies and the growing demand of European consumers (Yap 1994). The serious spread of red rice was also favored by the planting of commercial rice seed containing grains of weedy plants, and by the difficulties in controlling the weed with chemical and mechanical means in cultivated rice. In 1998, the weed was estimated to be significantly present in at least 65% of Italian rice crops.

Red rice is classified botanically as the same species as cultivated rice. It can be distinguished from the cultivated crop only after tillering. After this stage, the red rice plants are taller and have more slender culms than the cultivated varieties, with more tillers and greener and hispid leaves. The weedy plants disseminate more seeds during ripening and before crop harvesting, thus feeding the soil seed bank (Baker et al 1986, Coppo and Sarasso 1990, Kwon et al 1992). Shattered grains can persist in the soil in a dormant condition and remain viable up to 7 years (Goss and Brown 1939, Diarra et al 1985a).

The seeds of most weedy biotypes have a pigmented pericarp because of the presence of a variable content of different anthocyanins. Red rice seeds harvested with the crop reduce the value of a paddy. The pigmented layer can be removed with an extra milling, but this operation results in broken grains and grade reduction (Smith 1981, Diarra et al 1985a,b). Red rice is highly competitive toward crops and affects rice yield in relation to weed density and vigor of the variety. According to Diarra et al (1985a), a 22% yield loss occurs with an infestation of 5 plants $m^{-2}$. The main control techniques are based on integrated weed management systems. These systems typically are based on the planting of seeds that are free from red rice grains, mechanical or chemical destruction of the seedlings that emerge before crop planting, and, sometimes, rotation with other crops.

The seriousness of red rice infestation caused the European Commission to fund a joint project to study the ecobiological traits of the weed and the strategies used to control it in all European countries involved in rice cultivation. This chapter reports the main results of the experiments carried out in the framework of the European project under Italian environmental conditions to study the main biological traits and the possibilities of controlling this weed.

## Seed bank evolution

Different tillage conditions commonly adopted in set-aside management had different effects on red rice seed banks. The main soil tillages were plowing or harrowing or a combination of both, in spring and in summer. Sometimes mechanical interventions were replaced by nontillage followed by a glyphosate application in summer or by a cultivation of soybean or sunflower for nonfood (industrial) use.

In all tillage conditions, only the single application of glyphosate at the red rice flowering stage markedly reduced the red rice seed reserve in both the upper and deeper soil layers (Fig. 1). All the management techniques of the weed resulted in a good seed bank reduction in the upper layer (0–5 cm). Harrowings and soybean cultivation only partially reduced the seed reserve in the 5–10- and 15–20-cm layers. Harrowing, carried out at the seedling stage of red rice, favored subsequent flushes of the weed, allowing some plants to disseminate and feed the seed bank. Moreover, harrowings permitted the burial of some weed seeds, thus stimulating their dormancy (Ferrero et al 1998).

The experiments carried out in rice monoculture showed that, starting from a seed bank of about 2,500 and 680 seeds $m^{-2}$ in 0–10- and 10–20-cm soil layers, respectively, the seed bank varied markedly over 2 years of continuous rice cultivation, according to the weed management techniques that were adopted (Fig. 2). The spraying of dalapon on red rice seedlings before rice planting still allowed the seed bank to

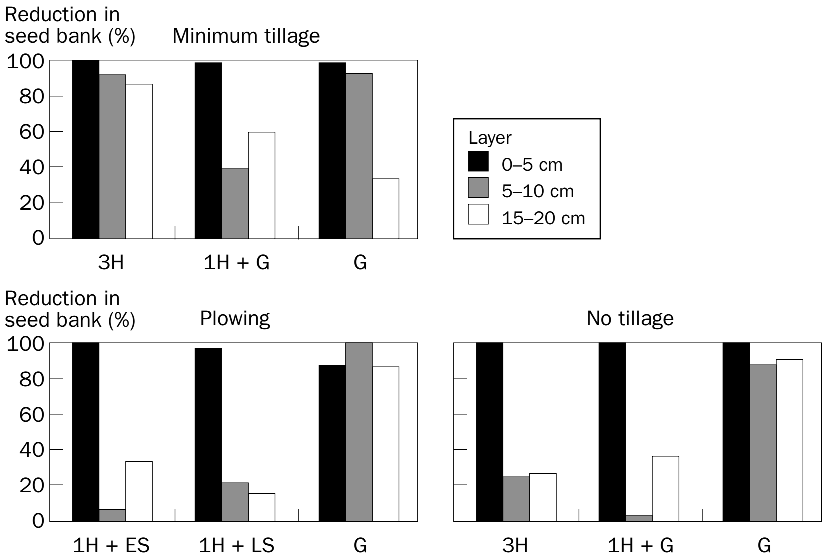

**Fig. 1. Reduction in red rice seed bank after 2 y of different set-aside management: 3H = 3 harrowings, 1H + G = 1 harrowing followed by glyphosate application, 1H + ES = 1 harrowing and early soybean planting, 1H + LS = 1 harrowing and late soybean planting, G = glyphosate application.**

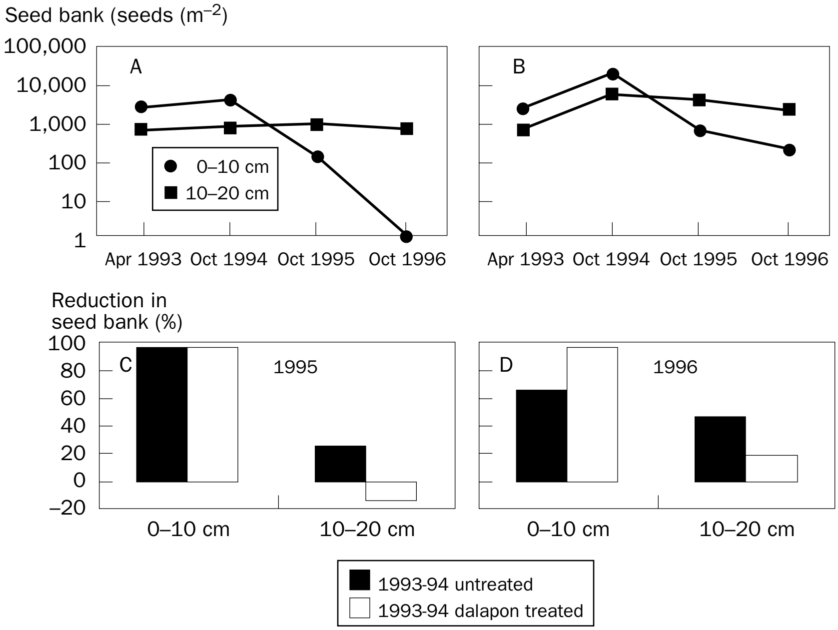

**Fig. 2. Red rice seed bank evolution for different management systems. (A) 1993-94 rice cultivation with dalapon control, 1995 soybean, 1996 rice, (B) 1993-94 rice cultivation without dalapon control, 1995 soybean, 1996 rice, (C) annual rate of decrease in the red rice seed bank in 1995 soybean cultivation, and (D) 1996 rice cultivation.**

grow by 65%, whereas the noncontrol had an increase of about tenfold (Ferrero and Vidotto 1997b).

Rotation greatly reduced the seed bank. One year of soybean cultivation allowed the control of all the emerged red rice plants with selective graminicides (cycloxydim and clethodim) and permitted a reduction of the seed bank in the 0–10-cm layer by about 97%. The reduction in the same layer was still higher (99%) when soybean was planted at the end of May after the spring flush of weed emergence (Ferrero and Vidotto 1997b).

### Seed longevity

The burial of red rice seeds at a depth caused by plowing can greatly reduce their viability. In the present experiments, the number of viable seeds in loam soil decreased to 6% after 1 year and to 5% after 2 years of burial. The nonviable seeds appeared empty, without embryos and reserve matter. It is presumed that most seeds can germinate under favorable environmental conditions (high temperature and oxygen content because of tillage operations), but cannot emerge from the soil (Ferrero and Vidotto 1998b). The germination percentage of the viable seeds varied in time, decreasing from 91% at the beginning of the experiment to 73% after 1 or 2 years of burial. This behavior can most likely be explained by the fact that many of the seeds that were dormant at the beginning of the experiment did not germinate and remained dormant over 2 years. The seeds dug up after 1 year required, on average, less time to germinate than those buried for 2 years.

### Emergence

The emergence of red rice was strongly influenced by the type of soil tillage adopted for seedbed preparation. The seedlings that emerged before rice planting were the main contributors to the total emergence from the seed bank. Almost all the plants freely grown in an undisturbed soil were able to emerge from mid-April to mid-May, after reaching an accumulation of 200 growing degree-days (Fig. 3; Ferrero et al 1996).

If we take harrowing and plowing into consideration, the emergence of red rice seedlings in relation to the 0–10-cm seed bank was influenced markedly by the type of tillage. On average, the emergence percentages in harrowed and plowed plots were 7.2% and 2.5%, respectively. Different values of emergence were probably caused by the movement of the red rice seeds in the soil and this was determined by plowing. Inversion of the top soil layer buries newly shed seeds and stimulates their dormancy. At the same time, plowing returns the seeds buried in the previous season to near the soil surface, but many of these seeds lose their ability to germinate (Ferrero and Vidotto 1997a).

Seed depth and flooding conditions have a negative influence on weed emergence. The experiments carried out in greenhouse and field conditions showed that abundant and contemporaneous emergence occurred with good soil moisture condi-

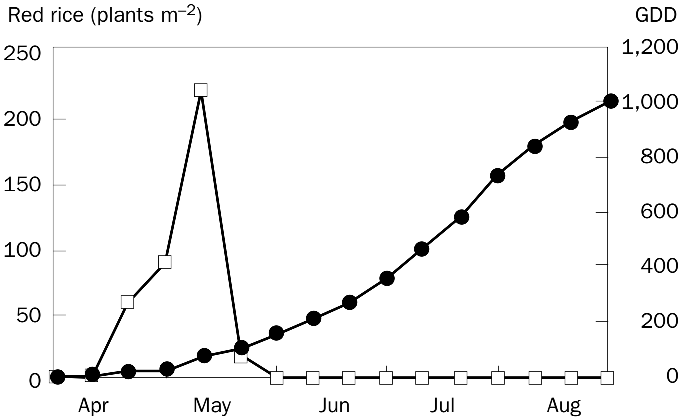

**Fig. 3. Red rice emergence as influenced by growing degree-days (GDD), determined in undisturbed soil (calculated with base temperature of 10 °C).**

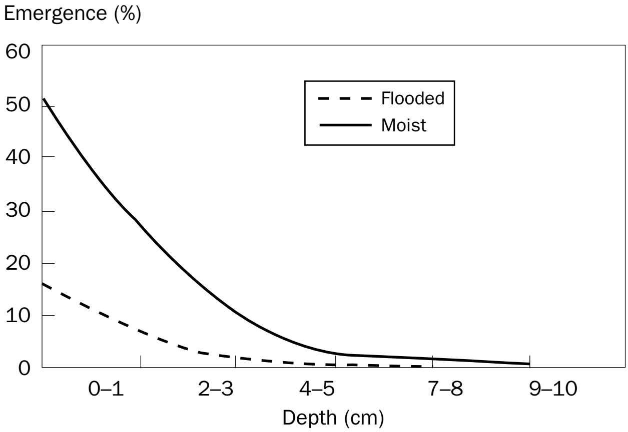

**Fig. 4. Red rice emergence at increasing depth in flooded and moist soils.**

tions for the seed placed at the soil surface. Seeds placed in the upper soil layer (0–1 cm) reached 50% germination when the soil was kept continuously moist, and 18% when submerged by 2–3 cm of water (Fig. 4). An emergence reduction occurred with an increase in depth for both moist and flooded soil. Germination at the 4–5-cm depth was only 8% in the moist soil and nil in the flooded soil. Seeds that did not emerge showed the formation of seedlings in the soil. In moist soils, the proportion of these seedlings to the total nonemerged seeds ranged from 45% at 4–5 cm to 2% at 9–10 cm. In flooded soils, this proportion ranged from 15% at 4–5 cm to 0% at 9–10 cm. Emergence from the 0–1-cm layer was completed in 14 d in the moist soil and in 18 d in the flooded soil. This behavior could be one of the reasons for the continuous emergence of the weed in rice fields (Ferrero and Finassi 1995).

## Dormancy

The germinability of red rice seeds in field conditions was negligible immediately after shattering but increased rapidly as time went by. In this study, the red rice seeds collected at crop harvest were left on the soil surface of the rice plots during autumn and winter. One month after the collection, the seeds required 2–3 mo in petri dishes to reach 50% germinability depending on the biotype. Six months after harvesting, all the seeds were able to germinate by about 90% in 1 mo (Fig. 5). This experiment clearly showed that seeds shattered in autumn are not able to germinate in the same season even with favorable temperature and water conditions (data not published).

## Acquisition of shattering ability and viability after flowering

Red rice seeds began to shatter within 9 d after flowering and increased gradually for 30 d until complete development of the panicle (Fig. 6). At this stage, 65% of the total grains had shattered and shattering did not appear to be influenced markedly by different N supplies (Ferrero and Vidotto 1998b).

Shattered and nonshattered seeds, considered on the whole, started to become viable at about 9 d from the beginning of flowering, with a germinability of about

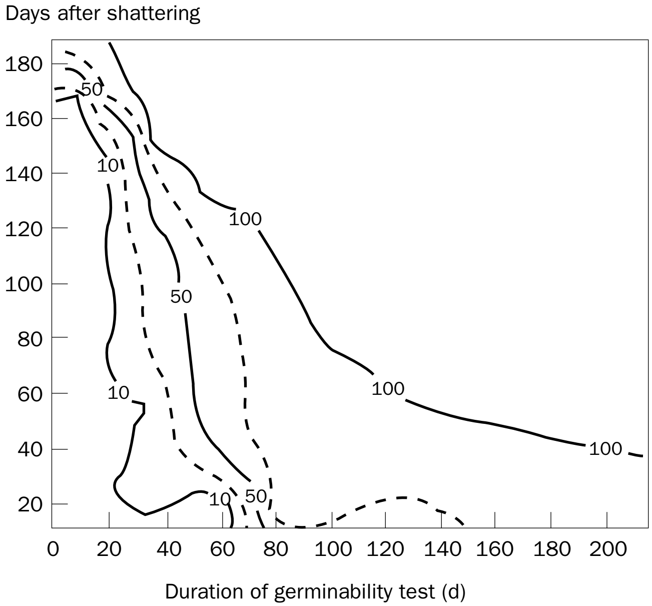

**Fig. 5. Evolution of red rice seed germinability as a function of days after shattering and duration of germinability test.**

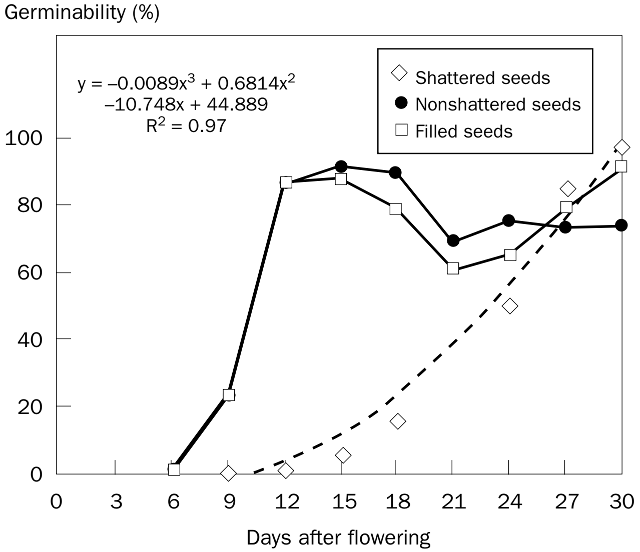

**Fig. 6. Germinability evolution of the shattered and nonshattered seeds and mean germinability of filled seeds.**

20%. This value increased quickly and had already reached about 85% at 12 d after flowering. In general, the shattered grains showed a lower germinability until 24 d after flowering, compared with that of nonshattered seeds (Ferrero and Vidotto 1998a). From this time on, the germinative capacity of the two groups of seeds was different. In particular, the germinability of the shattered seeds was very low during the first 15 d after flowering, with a maximum value of about 5%. This behavior can most likely be explained by the incomplete development of the early shattered grains, which broke off mainly because of wind. The seeds that shattered after 15 d from flowering contained nearly filled and physiologically mature grains.

## Competitive ability

The experiment carried out from 1996 to 1998 pointed out that yield losses caused by red rice infestation in rice fields vary as a function of weed density and, in tall varieties, of nitrogen rate. At 100 kg N ha$^{-1}$, densities ranging from 10 to 40 plants m$^{-2}$ gave rice yield reductions of 7% to 35% in both tall (e.g., Arborio) and semidwarf varieties (e.g., Thaïbonnet, Fig. 7). Yield losses increased by about 10% in tall varieties at an N rate of 150 kg N ha$^{-1}$. This behavior was mainly due to the high degree of lodging that occurred at high N rates in this variety (data not published).

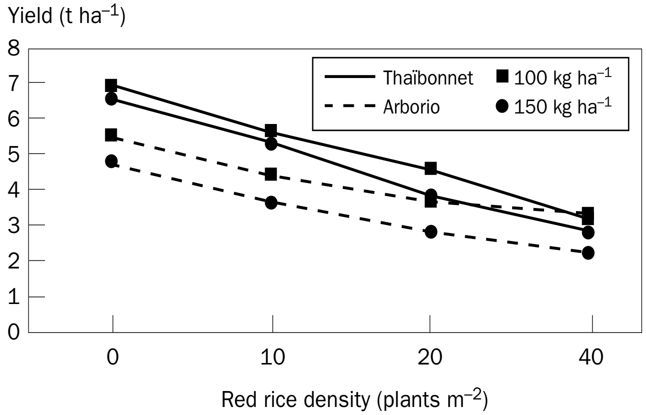

**Fig. 7. Yield of "Thaïbonnet" and "Arborio" rice varieties in different levels of red rice infestation (0, 10, 20, and 40 plants m$^{-2}$) and at two levels of nitrogen supply.**

## Control

### Preplanting in rice

At present, interventions before crop planting, both before and after the emergence of red rice, are the most successful means of controlling the weed. In preemergence applications, pretilachlor is the only herbicide authorized in Italy for red rice control, but this research has pointed out that dimethenamid is also valuable for this purpose. In several experiments, dimethenamid used alone at 0.48 to 0.96 kg ai ha$^{-1}$ provided 86–93% control of red rice. Pretilachlor at 1.5 kg ai ha$^{-1}$ had, on average, an efficacy of 84%. The mixture of dimethenamid and pretilachlor at 0.64 + 1.25 kg ai ha$^{-1}$ also proved to be very effective (90% of activity).

The main system adopted in Italy is that of controlling red rice postemergence on stale seedbeds. This practice, commonly known in Italy as "false seeding," is feasible only if weather conditions allow the weed to reach at least the 2–3-leaf stage in the first 10 d of May, in order to plant a still productive variety.

Cultural and chemical practices are primarily those of early seedbed preparation, followed by field irrigation to stimulate red rice germination and a treatment with systemic herbicides 2 d before rice planting or a pass of a blade or disk harrow to destroy red rice seedlings. Success of the stale seedbed technique mainly depends on the number of weed seedlings that can emerge before the treatment. The experiments conducted in the past few years have indicated that the type of tillage for seedbed preparation and soil humidity have a marked influence on seed germination. Harrowing approximately tripled the percentage emergence compared with plowing. Abundant and contemporaneous emergence occurred with good soil moisture conditions but it was strongly affected by continuous flooding. The most commonly used herbicide for the control of red rice seedlings with the stale seedbed technique is dalapon at 10–13 kg ai ha$^{-1}$, which is usually more effective than mechanical interventions. A comparison between chemical control and harrowing at the 2–3-leaf stage of red rice seedlings showed that dalapon treatment results in more than 95% weed control, whereas mechanical means rarely achieve 70% control (Ferrero et al 1999). Advancing the treatment to the 1–2-leaf stage reduces the effectiveness to that of harrowing. Other studies showed that dalapon could be successfully replaced by herbicides such as cycloxydim and clethodim at 0.28 and 0.95 kg ai ha$^{-1}$, respectively (Vidotto et al 1998).

### Postplanting in rice

Red rice control postplanting in rice relies mainly on the cultivation of short-statured varieties to allow panicle cutting or the localized application of systemic herbicides. Both systems need to be applied at maximum plant height but before shattering or the acquisition of seed germinability.

The machine used for cutting interventions was made up of a 5-m bar derived from the cutting device of a combine harvester, mounted onto the front of a tractor. Cutting equipment is usually fitted with a roll crusher made up of a couple of counter-rotating rollers. The experiment carried out with Thaïbonnet indicated that the double intervention of cutting equipment resulted in a decrease of 94% of panicles compared with the untreated plots (Table 1). The first treatment was performed at 10 d from the beginning of flowering, whereas the second was performed 15 d later to control panicle regrowth. The cut panicles that escaped passage through the crushing roller showed a germinability of 19%. The single cutting intervention controlled the panicles present in the field by 89%, but the panicles that escaped the crushing roller still had a residual germinability of about 52%. The localized application of systemic herbicides to the top of red rice plants is based on the use of wiping bar equipment mounted onto a self-propelled machine. Research conducted to assess the effectiveness of glyphosate and cycloxydim at different concentrations showed that both herbicides markedly reduced the germinability of red rice seeds.

The application of glyphosate and cycloxydim at 20% and 5% concentration, respectively, resulted in a germinability decrease of more than 90% with both herbicides. This percentage concerned only the seeds of the panicle that were in contact with the wiping equipment. About one-third of the panicles in the experimental field escaped treatment as they were equal to or lower in height than the crop. The seeds of the escaped panicles, on the one hand, feed the soil seed bank, whereas, on the other

**Table 1. Influence of panicle cutting on red rice control.**

| Treatment | % of cut panicles (referring to pretreatment number) | Germinability of red rice seeds (as % of total seeds) | | |
|---|---|---|---|---|
| | | Cut but not passed through crushing roller | Passed through crushing roller | Not cut |
| 1 cutting | 89 | 51.7 | 19.0 | 89.3 |
| 2 cuttings | 94 | 9.3 | 0.3 | 85.7 |

hand, they allow the selection of short biotypes for the following years that cannot be controlled either with cutting or wiping bar equipment.

## Conclusions

Red rice belongs to the same species as cultivated rice. For this reason, all control strategies are based on the application of nonselective chemical or mechanical means, avoiding contact with the cultivated varieties. The success of the main red rice managing systems mainly depends on the understanding of weed biology and growth habit.

The main flush of germination of the seeds buried in the soil occurs from the beginning of April and is more favored by good soil moisture conditions and minimum tillage for seedbed preparation than by continuous soil flooding and plowing. Knowledge of the germination behavior of red rice seeds in different environmental conditions allows farmers to regulate weed emergence in relation to the different management systems adopted. With low infestations and small seed reserves in the soil, it would be advisable to plow and plant as soon as possible late rice varieties that are more productive than early ones. This management approach results in an improved control of the weed if farmers combine plowing, in flooded soil, with the treatment of pretilachlor (the only antigerminative herbicide authorized in Italy). On the contrary, with high infestations and large seed banks, it would be better to harrow and irrigate the fields at the beginning of March to stimulate red rice emergence. This sequence of practices, which characterizes the stale seedbed technique, ends with the control of the weed with a graminicide, at the 2–3-leaf stage, a few days before crop planting. The only herbicide authorized for this application is dalapon, which shows good control of the weed at 10–13 kg ai ha$^{-1}$. In the experiments, cycloxydim and clethodim were as effective as dalapon and potentially may control red rice at dosages much lower than those used for dalapon.

Red rice seeds start to become viable and shatter at about 9 d after flowering. This is the period during which it is then necessary to carry out panicle cutting or localized treatment of systemic herbicides.

Red rice seeds buried in rice fields maintain viability for more than 2 years, allowing the weed to survive in the soil seed bank. The knowledge of seed bank dynamics allows farmers to assess the risks of the spread of the weed and offers the possibility of indirectly evaluating the efficacy of different control techniques. The highest reduction of red rice seed banks can be obtained either in fallow set-aside with weed control before seed set and dissemination by applying glyphosate or rotating rice with other crops.

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## Notes

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WEED SCIENCE

# Italian rice-field weeds and their control

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The composition of the weed communities of Italian rice fields has become quite degraded in the past decade. Five main floristic scenarios represent this situation: (1) *Echinochloa* spp., found in all rice fields; the main herbicides applied against these species are molinate, tiocarbazil, dimepiperate, thiobencarb, quinclorac, propanil, and azimsulfuron; (2) exotic *Heteranthera* species, present in 60–80% of the rice production area and controlled mainly with oxadiazon and pretilachlor; (3) *Alisma* spp. and Cyperaceae weeds, found in more that 50% of the fields and controlled with ALS inhibitor herbicides (sulfonylurea compounds and metosulam), MCPA, and propanil; (4) various weedy rice biotypes, controlled with a combination of cultural and chemical (dalapon and pretilachlor) means; and (5) other weeds of minor importance that are normally not subjected to specific interventions.

The Italian rice production area in 1998 was about 223,000 ha and this was almost all located in the northwest region. About 85% of this area is cultivated with japonica varieties under flooding conditions. Since the beginning of the 1960s, rice has been mechanically direct-seeded. Now, 85% of the production area is broadcast-planted in flooded fields. The remaining area is row-planted in dry soil and flooded, starting from the beginning of crop tillering. In these conditions, rice has no competitive growth advantage over weeds, which can compete with the crop from the beginning of stand establishment.

The rice-field ecosystem is notably complex. It has numerous weed species, characterized by particular morpho-physiological traits. These plants are associated differently according to the specific ecological conditions and anthropic pressure, which lead in time to the appearance and spread of some species and disappearance of others. According to Angelini (1936), about 30 weed species were present in Italian rice fields in 1870, and 133 were present in 1913. The diffusion of mechanization and introduction of selective herbicides, which began to take place from the end of the 1950s, had a large impact on rice cultivation, modifying cultural practices and then the traditional composition of the flora that infested this crop. The main changes were due to the abandoning of transplanting in favor of direct broadcast seeding in flooded fields, the spread of rice monoculture, and the introduction of selective herbicides, which was first effective against Cyperaceae and Alismataceae species (2,4-D, MCPP, MCPA, 2,4-DP) and afterward against *Echinochloa* spp. (molinate and propanil).

Recent surveys carried out at the end of the 1980s (Sparacino et al 1985, Sgattoni et al 1989) pointed out that there were only 22 exotic species or species of recent introduction infesting the main rice area. Weeds cause the greatest damage to rice in Italy. Without weed control, crop losses were estimated to be as high as 92% at a yield level of 7 to 8 t ha$^{-1}$ (Moletti et al 1987).

## Scenarios of Italian rice weeds and control techniques

According to the various observations made over the past few years, the composition of the weed communities of rice fields can be represented by five main floristic groups: (1) *Echinochloa* spp.; (2) *Heteranthera* spp.; (3) *Alisma* spp. and Cyperaceae weeds, associated with sulfonylurea-resistant species; (4) various weedy rice biotypes; and (5) other minor species.

### ***Echinochloa* spp.**

The plants of the genus *Echinochloa* are mainly represented by the following species: *E. crus-galli* (L.) Beauv., *E. crus-pavonis* (H.B.K.) Schultes, *E. hostii* (Bieb.) Boros, *E. colona* (L.) Link, and *E. phyllopogon* (Stapf) Koss. The results of a recent survey carried out in a large area of rice cultivation on the basis of the main morphological traits showed that only 44% of the examined plants were attributable with certainty to these species (Sparacino et al 1994), whereas the remaining 56% are represented by biotypes with intermediate characteristics.

This study indicated that *E. crus-galli* was found throughout the rice-growing area with a 53% degree of coverage (as an average of the values determined in the areas where the plants were present, Table 1). *E. crus-pavonis* was present in 95% of the rice fields with a 25% degree of coverage. *E. hostii*, *E. colona*, and *E. phyllopogon* showed a distribution (as percentage of the infested area in the total area cultivated with rice) ranging from 65% to 54% and a 7% to 3% degree of coverage.

Various residual and foliar herbicides are applied in Italy to control *Echinochloa* spp. in water- and dry-seeded rice at different application times and rice growth stages (Table 2). Molinate, dimepiperate, tiocarbazil, and thiobencarb are common residual herbicides used in water-seeded rice, with presowing or early postemergence applications (Moletti 1993). Quinclorac and recently azimsulfuron (with prevalent foliar activity but also partially absorbed by roots), propanil, and cyhalofop-butyl (foliar herbicides) are the other chemicals that are applied in early or late postemergence to control *Echinochloa* spp. in water- and dry-seeded rice (Rapparini 1998).

Molinate is the main residual herbicide applied against all *Echinochloa* spp. It controls these weeds at the 3- to 4-leaf stage and can be applied at preplanting (mixed

**Table 1. Average spread (as a percentage of the infested area on the total area cultivated with rice) and cover (as an average of the values determined in the areas where the plants were present) of the main weeds in Italian rice fields.**

| Species | Spread (%) | Cover (%) |
|---|---|---|
| *Echinochloa* spp. group | | |
| *E. crus-galli* | 100 | 53 |
| *E. crus-pavonis* | 95 | 15 |
| *E. hostii* | 65 | 7 |
| *E. colona* | 65 | 4 |
| *E. phyllopogon* | 54 | 3 |
| *Heteranthera* group | | |
| *H. reniformis* | 80 | 75 |
| *H. rotundifolia* | – | – |
| *H. limosa* | 60 | 45 |
| *Alisma* and Cyperaceae group | | |
| *Alisma plantago-aquatica* | 79 | 72 |
| *A. lanceolata* | 35 | 48 |
| *Schoenoplectus* (*Scirpus*) *mucronatus* | 61 | 57 |
| *Bolboschoenus* (*Scirpus*) *maritimus* | 48 | 50 |
| *Butomus umbellatus* | 12 | 8 |
| Weedy rice group | | |
| *Oryza sativa* | 75 | 30 |
| Others | | |
| *Cyperus serotinus* | 5 | 15 |
| *Eleocharis* spp. | 3 | 6 |
| *Sparganium erectum* | 3 | 4 |
| *Alopecurus geniculatus* | 2 | 5 |
| *Ammania coccinea* | 1 | 3 |
| *Bidens cernua* | 1 | 3 |

with oxadiazon) or at early postemergence. It has a relatively high water solubility and is most active in clay or silt-clay soils where deep water levels can be more easily maintained for several days.

Dimepiperate is a residual herbicide that is highly selective toward rice and is commonly applied at presowing or early postemergence in standing water to control *E. crus-galli* and late *E. phyllopogon* before their 2-leaf stage. To control *Echinochloa* spp. at the 2-leaf stage, dimepiperate is often mixed with molinate.

Tiocarbazil is similar to dimepiperate, but it can also be applied in sandy soils because of its lower solubility.

Thiobencarb is effective as a residual herbicide against *Echinochloa* spp. until the 2- to 3-leaf stage; it is also partially active against *Heteranthera reniformis* seeds. Thiobencarb is applied in presowing (mixed with oxadiazon) or in early postemergence alone or mixed with molinate. With high temperatures, this herbicide sometimes stunts the shoots of rice.

**Table 2. Main weeds in Italian rice fields and active ingredients used for their control.**

| Active ingredient | Rate (kg ai ha$^{-1}$) | Application timing |
|---|---|---|
| Target weed: *Echinochloa* spp. | | |
| Molinate | 3–4.5 | Presowing |
| | 3–4.5 | Postemergence |
| Thiobencarb | 3–4 | Presowing |
| | 3–4 | Early postemergence |
| Tiocarbazil | 4–5 | Early postemergence |
| Dimepiperate | 2.5–3.0 | Early postemergence |
| Quinclorac | 0.5–0.6 | Postemergence |
| Propanil | 3.5–4.2 + 3.5–4.2 | Late postemergence |
| Azimsulfuron | 0.02 | Early postemergence |
| Cyhalofop-butyl | 0.2–0.3 | Early postemergence |
| Target weeds: Alismataceae and Cyperaceae | | |
| Bensulfuron-methyl | 0.06 | Postemergence |
| Cinosulfuron | 0.06–0.08 | Postemergence |
| Ethoxysulfuron | 0.06 | Postemergence |
| Bensulfuron-methyl + metsulfuron-methyl | 0.05 + 0.002 | Late postemergence |
| Metosulam | 0.06–0.08 | Postemergence |
| Azimsulfuron | 0.02 | Postemergence |
| MCPA | 0.4–0.6 | Postemergence |
| Triclopyr | 0.3–0.4 | Late postemergence |
| Bentazone | 1.2–1.6 | Postemergence |
| Pretilachlor | 1.25 | Presowing (30 d before sowing) |
| | 1 | Postemergence |
| Target weed: *Heteranthera* spp. | | |
| Oxadiazon | 0.25–0.375 | Presowing |
| Pretilachlor | 1–1.1 | Early postemergence |
| Triclopyr | 0.3–0.4 | Late postemergence |
| Target weed: weedy rice | | |
| Dalapon | 12–15 | Presowing (after stale seedbed) |
| Glufosinate-ammonium | 1 | Presowing (after stale seedbed) |
| Glyphosate | 1.0–1.2 | Presowing (after stale seedbed) |
| Pretilachlor | 1.25 | Presowing (after stale seedbed) |

Quinclorac is commonly applied in postemergence of *Echinochloa* spp. on drained and saturated fields, which can be reflooded 4 to 5 d after treatment. Quinclorac controls *E. phyllopogon* well until the tillering stage, but its activity is weak against *E. crus-galli*. Rice growers frequently mix quinclorac with propanil to broaden the spectrum of controlled weeds.

Propanil is the most important herbicide applied in Italy to control *Echinochloa* spp. in rice, especially in sandy soils where it is easy to drain the soil or where rice is dry-seeded with delayed flooding. Propanil is normally sprayed with repeated postemergence applications on drained soil (usually twice, with 6–7 d interval). Propanil must be applied at the 2–3-leaf or larger stage of rice to avoid crop injury, and before the tillering stage of *Echinochloa* plants to obtain good weed control.

Azimsulfuron is a new sulfonylurea applicable during postemergence of rice weeds. This herbicide restrains the acetolactate synthase (ALS) action on susceptible weeds and therefore the amino acids valine, leucine, and isoleucine synthesis. Applied at 20 g ai ha$^{-1}$, with a nonionic surfactant at 0.1% v/v, it gives good control of *Echinochloa* spp. and also of the most common Italian rice weeds, particularly sedges and broadleafs (Massasso et al 1996). Application can be carried out about 20 to 25 d after sowing, on both the drained rice field and a soil with a shallow water layer. Watergrass and white ecotypes of *E. crus-galli* are less sensitive to azimsulfuron than red ecotypes.

Cyhalofop-butyl is a new graminicide that belongs to the aryloxy phenoxy propionate family, which is applied for postemergence control of *Echinochloa* spp. in rice (Barotti et al 1998). This herbicide inhibits acetyl-coenzyme A carboxylase in the biosynthesis of fatty acid. Cyhalofop-butyl is effective against *Echinochloa* spp. at 2–4 leaves of the weeds under drained paddy conditions. The product is always used in combination with a nonionic adjuvant. It is highly selective toward the crop, but its efficacy is greatly reduced when it is mixed with some broadleaf-weed herbicides, such as cinosulfuron.

### *Heteranthera* spp.

The *Heteranthera* spp. are exotic plants that were first reported in Italy in 1968 (Pirola 1968). From that time, these plants have shown an enormous diffusion. The main species now are *H. reniformis* Ruiz et Pavon and *H. rotundifolia* (Kunth) Griseb, which show a distribution of 80% with an average degree of cover of 75%. The third species, *H. limosa* (Sw.) Willd., has reached a distribution of 60% and a degree of cover of 45%. These plants may germinate throughout the rice growing period and can build up an abundant and persistent seed bank (Schiele 1988). Weed survival through vegetative organs occurs only if the soil is kept wet (Gabela and Doll 1974). In the first 2 mo after emergence, the weed develops deep roots and only 2–3 reniform leaves (Viggiani 1988). After this period, it forms a long rhizome. *H. reniformis* emerging together with rice at a density of 48 plants m$^{-2}$ can reduce yield by 65% (Ferrero 1996).

Preplant application of oxadiazon is the main weed control strategy that rice farmers employ to control *Heteranthera* species. Among these species is *H. reniformis*, which is considered difficult to control postemergence with the herbicides presently available in Italy. It is estimated that oxadiazon is now applied to about 80% of the Italian rice area. This herbicide is usually applied to flooded fields 5–6 d before sowing or to dry soil 3–4 d before flooding and sowing. *H. reniformis* seedlings that escape oxadiazon application are partially controlled with postemergence applications of pretilachlor, cinosulfuron, or bensulfuron-methyl + metsulfuron-methyl.

Pretilachlor (alone or mixed with the safener fenclorim) is an antigerminative herbicide that controls several weeds through preemergence or early postemergence applications in flooded rice fields. An early postemergence application of pretilachlor is effective against the seeds and young seedlings of *H. reniformis*. The full rate of cinosulfuron or bensulfuron-methyl plus metsulfuron-methyl (alone or with propanil) partially controls this weed, thus reducing its competitive ability and allowing the rice crop (especially tall varieties) to improve panicle density. Partial control of the weed can also be obtained in late postemergence with triclopyr used in combination with propanil.

### Alismataceae, Cyperaceae, and *Butomus umbellatus*

This group of weeds has been present in Italian rice fields since the beginning of the 19th century. These plants are considered together as they are normally subjected to the same control program and are often sensitive to the same herbicides. *Alisma plantago-aquatica* L. and *A. lanceolatum* With. are present in about 80% and 15% of the rice fields, respectively, averaging 45% and 30% degree of coverage, respectively. *Bolboschoenus maritimus* (L.) Palla and *Schoenoplectus mucronatus* (L.) Palla are spread over 55% and 60% of the rice fields, averaging 40% and 60% coverage, respectively. *Butomus umbellatus* L. is estimated to be present in 12% of the rice fields, averaging 8% coverage. The infestation of this plant has shown a remarkable reduction since 1989, when it was present on 23% of the rice area with 25% coverage (Sparacino and Sgattoni 1993).

A few years after the introduction of sulfonylurea herbicides, some of these species developed resistance to ALS inhibitors. This phenomenon was first noticed in 1995 in *A. plantago-aquatica* and *S. mucronatus* plants that were continuously treated for at least 3 years with ALS inhibitors (data not shown). Until now, resistance cases have been reported on rice areas of more than 10,000 ha. The studies of Sattin et al (1999) on *S. mucronatus* have shown that there is a generalized cross resistance among several sulfonylureas (azimsulfuron, bensulfuron-methyl, cinosulfuron, ethoxysulfuron). Some of these resistant populations were also insensitive to the triazolopyrimidine herbicide metosulam at three times the recommended field dose. Many herbicides are available in Italy to control *Alisma* spp., *B. maritimus*, and *S. mucronatus* in rice fields. The most common herbicides applied to control these weeds are bensulfuron-methyl (alone or in combination with metsulfuron-methyl), cinosulfuron, ethoxysulfuron, azimsulfuron, metosulam, MCPA, 2,4-DP, triclopyr, bentazon, and pretilachlor, alone or together with propanil.

Bensulfuron-methyl was the first sulfonylurea introduced in Italy at the end of the 1980s. Applied postemergence, it is very selective for rice and provides very good control of nearly all annual aquatic broadleaf weeds and sedges under different application timings and paddy conditions. It is frequently used together with propanil to improve activity against *B. maritimus*.

Ethoxysulfuron is a new sulfonylurea that has been authorized in Italy since 1998. The efficacy of this herbicide is, on the whole, similar to that of bensulfuron-methyl, but with a slightly better activity against *B. maritimus*.

Cinosulfuron and bensulfuron-methyl plus metsulfuron-methyl are frequently applied at reduced rates as they are reported to be less selective than other sulfonylureas. Metosulam is an ALS inhibitor that belongs to the triazolopyrimidine chemical group. Applied alone or in combination with propanil, this herbicide is effective against the same weeds that are controlled by sulfonylurea herbicides, but it is usually more selective toward the crop than sulfonylureas. Metosulam is less effective against *B. umbellatus* than sulfonylureas.

MCPA and 2-4 DP are phenoxy herbicides that commonly perform well against all broadleaf weeds, especially when they are applied together with propanil.

Bentazone provides good control of *B. umbellatus*, Cyperaceae, and Alismataceae. The use of this herbicide has at present been notably limited by many local restrictions because its residues have been found in well water.

Pretilachlor (alone or mixed with the safener fenclorim) is an antigerminative herbicide that controls the seeds of *Alisma* spp., *S. mucronatus*, *Heteranthera* spp., and *Echinochloa* spp. in preemergence or early postemergence applications. Pretilachlor is usually used when the crop has reached the 2-leaf stage to avoid injuries to the cultivated plants.

### Weedy rice

*Oryza sativa* L. var. *sylvatica* (weedy rice) has been reported in Italian rice fields since the beginning of the 19th century, but it started to spread significantly in the 1960s after the shift from rice transplanting to direct seeding. Infestations became severe several years ago because of the cultivation of poorly competitive, semidwarf indica-type rice varieties, the planting of commercial rice seeds containing grains of the weed, and the difficulty in controlling its infestations with mechanical and chemical means in cultivated rice. Weedy rice comprises a group of several biotypes characterized by a wide morphological variability (Coppo and Sarasso 1990) that have in common a great capacity to spontaneously disseminate the grains before crop harvesting. Some plants are awnless and some awned; some awns are dark colored and some straw colored. Most weedy biotypes have a red pericarp and show some morphological characteristics after the tillering stage, which make them different from the crop: more tillers, taller plants, and lighter green leaves. The high possibility of crossing that can occur in rice fields results in a great variability of biological and physiological features (Craigmiles 1978, Kwon et al 1992) that have important agronomic consequences. The high elasticity of the germination process may, for example, favor the competitiveness of weedy biotypes that can emerge earlier than the crop or allow those plants that emerge late to escape weed interventions.

Recent surveys have shown that weedy rice plants infest about 75% of the total rice field area and average a 30% degree of cover. Weedy red rice control strategies are mainly based on a combination of preventive, cultural, and chemical practices to avoid large infestations of weedy rice. The preventive means are mainly represented by the planting of certified seed and seed free of weedy rice or turning to rotational crops such as maize and soybean.

Cultural practices are mainly based on late seedbed preparation using disk harrows to destroy already emerged young seedlings. Precision land grading, obtained with laser-directed equipment, is another agronomic practice that has greatly contributed to weedy rice management in Italian rice production. Regular slopes within basins enable appropriate water management leading to both limiting weed growth and assuring a uniform emergence of weeds, which consequently results in better control thanks to the use of herbicides.

The precise management of water is essential for controlling weedy rice with the adoption of the stale seedbed practice. The stale seedbed, known in Italy as the "false seeding technique," is applied by preparing the seedbed early in the season (March) and then flooding the rice field to stimulate weedy rice germination. Weed seedlings are then treated with dalapon, glyphosate, or another total graminicide when they have reached at least the 2–3-leaf stage (normally in the first 10 d of May). After this treatment, the water level is immediately increased to 15–20 cm to further weaken the weed. A few days (2–3) after the treatment, the field is planted with an early but still productive variety. The success of the stale seedbed technique mainly depends on the number of weed seedlings that can emerge before the treatment.

Weedy rice can also be controlled with preemergence pretilachlor, an antigerminative herbicide recently authorized for this specific application. This product is applied to flooded fields at the end of March to early April, when most weed germination normally occurs (Ferrero et al 1996). Pretilachlor represents an interesting tool for weedy rice management, even though its control does not exceed 60–70%, as it allows the application of ordinary management techniques without any delay in planting time. The only condition that has to be satisfied is to apply the herbicide at least 25–30 d before planting to avoid risking injury to the crop.

### Other weeds

This group is mainly represented by *Cyperus serotinus* Rottb., *Eleocharis* spp., *Sparganium erectum* L., *Alopecurus geniculatus* L., *Ammania coccinea* Rottb., and *Bidens cernua* L. These species are usually less important as they are present on a limited rice area and are commonly well controlled by the treatments carried out against *Alisma* and the Cyperaceae species. *A. coccinea* frequently escapes early treatments with sulfonylureas because it germinates late, starting in June. This weed is normally well controlled by treatments with propanil or MCPA carried out to control *Echinochloa* spp., *Alisma* spp., and Cyperaceae species that have escaped previous treatments.

## Conclusions

The important changes that occurred in Italy during the 1960s in rice management such as the change from transplanting to direct seeding, the expansion of mechanization, and the introduction of chemical weed control have caused a significant modification of the composition of the weed flora in rice fields. Weeds such as *Echinochloa* spp., *Alisma* spp., Cyperaceae species, and weedy rice, which were previously controlled by hand picking or limited in their growth by transplanting, found an ecological environment that increasingly favored their spreading. The availability of new herbicides suited for every floristic situation led to a minimization of yield losses but did not limit weed spread and pressure. In the past 30 years, weed infestations have become more severe because of the spread of existing weeds and the introduction of exotic plants. The area of infestation of *Heteranthera* species, which were sporadically reported for the first time only at the end of the 1960s, increased by 80% in about 30 years, and, in spite of the good performance of oxadiazon, a further expansion of infestation is foreseen. A similar situation can be observed with weedy rice, a plant with negligible importance until rice was transplanted and which is now present in 75% of the rice fields. Experience acquired with this weed has shown that the best results in controlling weed infestations can be obtained by integrating agronomic and chemical means of management.

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# Effects of straw management practices on watergrass population dynamics

M.W. Hair, C. van Kessel, J.E. Hill, S.C. Scardaci, and W.H. Horwath

Because of state-mandated reductions in rice straw burning, a 30-ha field trial was established near Maxwell, California, in 1993 to study and evaluate alternative straw management practices. Objectives were to evaluate alternative straw management effects on watergrass (*Echinochloa oryzoides*) populations. Main plots were winter-flooded or nonflooded treatments (four replications) split by four postharvest straw treatment operations: (1) burned, (2) baled and removed, (3) chopped and incorporated by chiseling, discing, or both, and (4) rolled. All plots received the same standard herbicide applications. Weed levels were relatively high in most plots when the trial began.

In burned and baled plots, watergrass control was good and improved over the period 1994-98, but in rolled and incorporated plots watergrass control was poor and did not improve. In 1998, in burned plots, a density of about 0.01 watergrass panicle $m^{-2}$ was estimated after rice heading. In contrast, in incorporated plots, a density of about 10 watergrass panicles $m^{-2}$ was estimated, a 1,000-fold difference. Watergrass densities in flooded plots were approximately three times smaller than in nonflooded plots that received the same straw treatment.

California rice growers have historically burned the rice straw that remains after harvest. To control air pollution, state law now requires that the area burned be reduced to a maximum of 25% by 2001. In the fall of 1993, a 30-ha field trial was established near Maxwell, California, to study and evaluate alternative straw management practices. The agronomic effects of winter flooding, straw incorporation by tillage, rolling, and straw removal by baling were investigated and compared with the conventional practice of burning.

These alternative practices present a cost to growers that is significantly greater than the cost of the present practice of burning. Rolling, incorporating, or baling the straw entails operational costs of approximately \$32, \$50, and \$75 $ha^{-1}$ (Table 1). The cost of winter flooding varies widely but may be about \$20 $ha^{-1}$ for extra water from irrigation districts. In comparison, an additional herbicide application for control of

**Table 1. Estimated cost of field operations and 1998 watergrass panicle density for straw management practices (see Fig. 1 for explanation of practices).**

| Practice | | Cost | Watergrass panicle density |
|---|---|---|---|
| Winter | Fall | (\$ ha$^{-1}$) | (no. m$^{-2}$) |
| Flood | Burn | 22 | 0.0005 |
| Flood | Bale | 95 | 0.02 |
| Flood | Roll | 55 | 1.3 |
| Flood | Inc | 70 | 5.8 |
| Drain | Burn | 2 | 0.01 |
| Drain | Bale | 75 | 0.46 |
| Drain | Roll | 32 | 6.4 |
| Drain | Inc | 50 | 14.4 |

weeds that escape primary control measures may cost as much as \$175 ha$^{-1}$. Clearly, if certain alternative straw management practices are associated with increased weed control costs, economic considerations may slow or even prevent their adoption.

The objective of this part of the project was to evaluate the effect of these alternative straw management practices on watergrass (*Echinochloa oryzoides*) population dynamics and control.

## Materials and methods

Thirty-two individually irrigated 0.75-ha plots received one of eight treatments (four replications) applied each year. Main plots were winter-flooded or nonflooded treatments split by four postharvest straw treatment operations: (1) burned, (2) baled and removed, (3) chopped and incorporated by chiseling, discing, or both, and (4) rolled. Weed levels were relatively high in most plots when the trial began. All herbicide applications were uniformly applied by air across all plots. Each year, granular thiobencarb or molinate and bensulfuron methyl were applied at standard rates into a continuous flood at the 1.5- to 2.0-leaf stage of rice (about 10 d after seeding) according to conventional practices. Also, propanil, applied at 6.7 kg ai ha$^{-1}$ under drained conditions at the late-tillering rice stage (about 42 d after seeding), was used in 1995 through 1998 to suppress watergrass that escaped primary control.

Watergrass control was evaluated subjectively for each plot each year using a scale of 1 to 10, where 1 indicates no control and 10 indicates excellent control. Rice seedling vigor was also rated subjectively and in 1998 rice stand density was determined by counts in 0.5-m$^2$ quadrats. In 1998, the average density of watergrass panicles visible above the rice late in the season was estimated for each plot. Significant differences among treatment means within a year were determined by analysis of variance ($P = 0.05$) with means separation by the LSD test. Trends over years were examined by regression analysis.

## Results and discussion

Subjective ratings for degree of watergrass control during 1994-97 indicated that, in burned and baled plots, weed control was good and improved over the 4-y period (Fig. 1). In contrast, watergrass control was poor in rolled and incorporated plots and perhaps worsened over the 4-y period (Fig. 1). Watergrass control was significantly better in burned plots than in incorporated plots every year since 1994 (Scardaci et al 1996). In 1997 and 1998, watergrass control was almost as good in baled plots as in burned plots, both baled and burned plots had significantly better control than rolled plots, and rolled plots had significantly better control than incorporated plots (data not shown).

In 1995, rice seedling vigor ratings indicated significantly greater thiocarbamate phytotoxicity in the baled and burned plots than in the rolled and incorporated plots (Scardaci et al 1996). In 1998, rice stand density was significantly lower in burned plots (data not shown).

In 1998, in burned plots, a density of about 0.01 water grass panicle $m^{-2}$ was estimated after rice heading (Table 1). In incorporated plots, however, a density of about 10 watergrass panicles $m^{-2}$ was estimated, a 1,000-fold difference (Table 1). In rolled plots, about 4 panicles $m^{-2}$ were found. Mean watergrass panicle density in seven of eight baled plots was 0.02 $m^{-2}$, but the remaining plot was quite weedy at 1.8 $m^{-2}$. In incorporated and rolled plots that were flooded, watergrass densities were three times lower than in nonflooded plots (Table 1). Winter flooding also appeared to considerably reduce the watergrass density in the burned and baled plots: three of eight plots were completely weed-free.

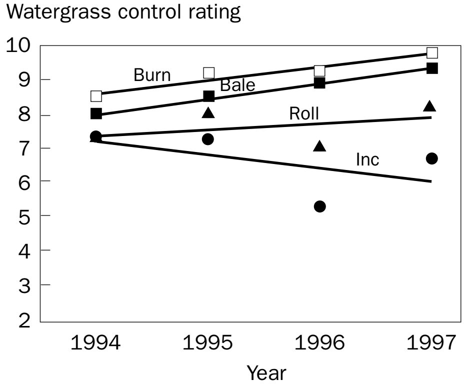

**Fig. 1. Watergrass control rating by year for four straw management treatments: burned (Burn), $R^2 = 0.73$, $P = 0.007$ for t-test of slope >0; baled (Bale), $R^2 = 0.50$, $P = 0.05$ for t-test of slope >0; rolled (Roll); and incorporated (Inc). Rating scale is 1 to 10, where 1 = no control and 10 = excellent control.**

The only other weed species to appear in 1998 was *Saggitaria longiloba*, which was also significantly more prevalent in the straw-incorporated and -rolled plots.

In plots where straw was rolled or incorporated by tillage after harvest, an aggressive herbicide program failed to provide acceptable watergrass control. The same herbicide program provided excellent control in plots where straw was burned or baled and removed after harvest, however. The practice of winter flooding improved watergrass control regardless of straw treatment. The two straw management alternatives to burning that leave the straw in the field, whether rolled or incorporated, winter-flooded or not, resulted in higher watergrass population densities, necessitating increased herbicide use at higher cost. From a weed control perspective, baling and removing the straw appeared to be an acceptable alternative to burning.

It is possible that survival of watergrass seed during the winter and early spring was enhanced by burial when the straw was incorporated. Other studies have found that watergrass seed buried by fall tillage remained 100% viable during the winter (Hair 1996). Seed survival in nontilled plots was 14% and dropped to only 6% when straw was burned (Hair 1996). Predation from the undisturbed soil surface by savanna sparrows caused these losses (Hair 1996). Waterfowl could cause similar or even greater seed losses in the winter-flooded plots at the Maxwell site. Also, germination in the early spring was reduced and survival enhanced by burial and by the presence of straw cover in the rolled treatments (Hair 1996). Perhaps seed burial and straw cover reduced natural seed losses by predation and germination, thus increasing viable preplant seed density until the herbicide treatment was simply overwhelmed by force of numbers.

It is also possible that the presence of straw residue may reduce herbicide efficacy by providing binding sites that reduce the effective concentration. Evidence that thiobencarb phytotoxicity on rice was more severe where straw was removed from the baled and burned plots (results reported above) gives indirect evidence that this may be the case.

The straw-incorporated and -rolled treatments differ from the baled and burned treatments in two important respects: straw remains in the field and weed seeds are buried. There is a need to determine the role played by these factors individually. A treatment that leaves the straw in the field but does not bury the weed seed should be tested. The principal goal of studies currently being conducted at the Maxwell site is to discover the mechanism(s) by which straw treatments applied in the fall could determine the success or failure of the weed control program in the subsequent summer. This information might then be used to devise an acceptable alternative to burning that leaves the straw in the field without increasing weed populations and weed control costs.

Periodic soil sampling to monitor changes in seed-bank numbers should indicate whether predation during the winter or early spring germination was a significant seed loss mechanism. Preliminary analysis of preplant soil samples taken in 1998 from the Maxwell plots confirms that viable seed numbers were as much as 50 times higher in the straw-incorporated plots than in the burned and baled plots. A more extensive sampling program began in the fall of 1998.

If the presence of straw itself reduces the efficacy of the granular thiocarbamate soil-active herbicides, then a major change in herbicides and weed control strategy may be required when straw is left in the field. It is also possible that this population of watergrass has developed some resistance to thiocarbamate herbicides and this should be investigated further. Further studies in small basins will be important in isolating the effects of straw and soil on herbicide efficacy.

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# Managing the potential for developing herbicide-resistant weeds in herbicide-tolerant rice

K. Johnson, M. Elhlardt, L. Smith, S. Linscombe, C. Johnson, A. Abreu, and D. Mitten

Herbicide-tolerant rice varieties IMI (tolerant of sulfonylureas and imidazilinones), Roundup Ready™ (glyphosate-tolerant), and Liberty Link™ (glufosinate-tolerant) represent three separate herbicidal modes of action. The commercial release of herbicide-tolerant rice cultivars is imminent as these herbicide/rice systems are currently in advanced stages in private and public breeding programs. Rice varieties tolerant of herbicides offer unique management tools that provide flexibility in the timing of herbicide application. Because of the economics in some regions, viable crop rotation systems are sometimes the only management tools to control problem weeds such as red rice (*Oryza sativa*). Commercial-scale development of herbicide-tolerant rice varieties requires both a biosafety assessment and management strategies for the potential development of herbicide-resistant weeds.

The first commercial sale of herbicide-tolerant rice varieties is planned within the next few years. Three herbicide/rice systems are currently in advanced stages in private and public breeding programs targeted for temperate rice production. Herbicide-tolerant rice varieties are IMI rice (tolerant of sulfonylureas and imidazilinones), Roundup Ready™ (glyphosate-tolerant) rice, and Liberty Link™ (glufosinate-tolerant) rice[1] and they represent three separate herbicidal modes of action (Schmidt 1997). Rice varieties tolerant of herbicides offer needed solutions for the control of serious weed problems. Coupled with unique management tools, they provide flexibility in the timing of herbicide application and rotation of herbicide modes of action. Genetic-based herbicide resistance in the crop will be especially important for the con-

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[1] IMI rice, developed by the Louisiana State University Agricultural Center and American Cyanamid, is derived from an EMS-mutant. Roundup Ready rice and Liberty Link rice are derived from recombinant DNA techniques and are being developed by Monsanto and AgrEvo, respectively.

trol of the conspecific weeds of cultivated rice, including rice mimic (*Echinochloa phyllopogon*) and red rice (*Oryza sativa*).

Product stewardship of herbicide-tolerant rice varieties requires both a biosafety assessment of the genetic modification and management strategies for the potential development of herbicide-resistant weeds in advance of commercial release. Regionally based agronomic research is necessary to define the best management practices for weed control and resistance management. Communication of the best agronomic practices and a commitment to evaluation and adjustment of recommended practices as the experience base grows (monitoring) are necessary elements for the stewardship of these new products.

## Potential for herbicide-resistant weeds

Herbicide resistance can be achieved by either mutation and selection within the weed populations or gene flow to sexually compatible species. The intensive use of herbicides could select for resistant individuals in weed populations. For example, propanil-resistant barnyardgrass and bensulfuron-resistant sedges have been reported (Heap 1998). Resistance was found where there was continuous cropping to rice and little rotation with different herbicide modes of action. Bensulfuron has residual activity and can offer prolonged selection pressure within a weed population.

The second path to the development of herbicide-resistant weeds requires the pollination of sexually compatible species and subsequent introgression of a herbicide-resistant trait into weed populations. Gene flow depends upon cross-pollination, growing environment, and selection pressure for the trait. Domestic rice, *Oryza sativa*, is predominantly self-pollinating. Wild rice and weedy rice that share the AA genome have varying degrees of sexual compatibility and thus would be potential recipients of gene flow (Langevin et al 1990).

In the temperate rice production regions, both the crop and weedy rice grow only in agricultural environments (Table 1). Neither has escaped cultivation and neither has naturalized or established a population outside of cultivation. Characteristics of temperate rice production are irrigation, a discrete growing season with high radiation, and extended daylength.

In tropical regions, cultivated rice may be naturalized in disturbed regions near cultivation or in wetland ecosystems. Hybrid populations have been documented in regions where wild and domestic rice cultivation overlap. Two intensely studied hybrid populations are located in the likely centers of origin for *O. sativa* in India and China and have not eliminated the presence of the parental landraces (Morishima 1984). Although geographic distributions may list the *Oryza* relatives in coincident regions, ecosystems must overlap for outcrossing to occur and this may reduce the potential risks.

It was the consensus of an international delegation assembled by the World Bank (Clegg et al 1993) that there is “a low but finite risk of outcrossing of transgenes to wild and weedy rice, especially where there is a close association between them.” The group concluded that “It is conservative to assume that any gene inserted into

**Table 1. Rice-growing regions of the world where sexually compatible relatives do exist and present opportunities for gene flow.**

| Rice production region | Sexually compatible relatives (naturalization status) |
|---|---|
| Temperate regions of USA | |
| Delta and Gulf Coast | Red rice, *Oryza sativa* (introduced weed) |
| California | None present |
| Temperate regions of Brazil, Argentina, Colombia, and Uruguay | Red rice, *O. sativa* (introduced weed) |
| Temperate regions of Italy and Spain | Red rice, *O. sativa* (introduced weed) |
| Temperate regions of Japan and Australia | None present |
| Tropical regions of Asia and the Americas | *O. perennis* complex, for example, *O. rufipogon* (wild species) |
| Tropical Africa | *O. breviligulata* (wild species)/*O. glaberrima* (cultivated) |

cultivated rice that is used on a widespread basis ultimately may be transferred into the AA wild relatives, if they are located within the cultivated region and especially if the gene increases the fitness of the related rice."

For temperate rice, the weed target of gene flow is red rice. Management techniques to control red rice before flowering can greatly reduce the opportunity for cross-pollination. However, beyond restricting cross-pollination, rotation of herbicides can reduce the selection pressure for herbicide-tolerant individuals within a population and thus slow the introgression of herbicide-tolerant genes into weed populations.

## Herbicide application practices can delay and may prevent resistance development

Herbicides with residual activity provide strong selection pressure. For example, weeds resistant to the rice herbicide bensulfuron (trade name Londax), even with only an approximately 30-day residual, identified in California include smallflower umbrellaplant (*Cyperus difformis*), arrowhead (*Sagittaria montevidensis*), and redstem (*Ammannia coccinea*). Nonselective herbicides do not distinguish between weed and crop plants, except in the case of crops genetically modified to resist a specific herbicide action. The option to use herbicides with alternative modes of action and, in the case of glufosinate and glyphosate, with little residual activity can greatly reduce selection pressure for naturally occurring mutations in the weed population. Rotation of herbicides with different modes of action minimizes opportunities for resistance. It is important that rice growers have different options for weed control. Rice varieties tolerant of herbicides offer a unique management tool providing flexibility in the timing of herbicide application. In regions where crop rotation systems are not an option, these herbicide-tolerant varieties may be the only management tool to control difficult weeds.

The use of rice varieties with genetic tolerance of nonselective herbicides will provide new control options for weeds such as rice mimic (*Echinochloa phyllopogon*) and red rice (*O. sativa*) that have life cycles similar to cultivated rice and are tolerant of currently registered rice herbicides.

Red rice was introduced with the initial cultivation of rice as an impurity in the planting seed and has become a weed problem of significance to temperate rice production systems. The same species as cultivated rice, red rice has adapted to the changes in rice production practices and genetics over time. Red rice is tolerant of the same rice herbicides as cultivated rice. Red rice can be highly competitive and, in cases of severe infestation, can result in a yield loss of such magnitude as to make the crop economically unfeasible. Reported yield loss from red rice has been as high as 80% (Hill et al 1994). In addition, contamination of milled rice with red rice grains will reduce the grade and the grain price.

Its early season plant habit and life cycle make rice mimic, *E. phyllopogon,* a highly competitive weed with rice. Biotypes known to be tolerant of rice herbicides have been identified. Populations of *E. phyllopogon* tolerant of molinate (trade name Ordram), thiobencarb (trade names Bolero and Abolish), and fenoxaprop (trade name Whip) have been reported in California (Fischer et al 2000).

## Herbicide resistance management recommendations

Research efforts to date have identified the following recommendations for minimizing weed resistance to herbicides (Anonymous 1995). We propose possible new rice weed control systems (Table 2) in temperate rice culture:

- *Minimize opportunity for gene flow and never allow red rice to set seed.* Techniques include timely selective herbicide applications, irrigation management to suppress red rice germination, the use of maleic hydrazide to suppress red rice seed head formation, and hand roguing of fields, as is commonly done for certified seed production.
- *Rotate crops and class of herbicides.* We can learn from experiences with the development of propanil-resistant barnyardgrass and bensulfuron-resistant sedges. In both cases, resistance was found where continuous cropping to rice and no rotation of alternative herbicides were practiced (Hill et al 1994). For information on herbicides and modes of action, see the Web site maintained by Dr. Ian Heap, International Survey of Herbicide-Resistant Weeds, at http://weedscience.com.
- *Use best management practices for the region.* Crop rotation patterns that promote spring germination of red rice followed by cultivation to destroy the seedlings before the next crop is planted will help to deplete the red rice seed bank. Several over-the-top grass herbicides labeled for use in cotton and soybean can be used to control rice (white or red) in the rotational crop.

**Table 2. New rice weed control systems for rice. New weed management tools that combine genetic technology with chemical herbicides will soon be available. Herbicide-tolerant rice varieties representing three separate herbicidal modes of action are currently in advanced breeding programs.**

| Genetics | | Chemistry | |
|---|---|---|---|
| Variety trade name | Genes and gene action | Active ingredient (trade name) | Herbicidal mode of action |
| LibertyLink Rice | *pat* or *bar* gene to inactivate herbicide | Glufosinate ammonium (Liberty®) | Inhibition of glutamine synthetase resulting in accumulation of ammonia in plants. |
| IMI Rice | *als* gene for a tolerant ALS[a] enzyme insensitive to the herbicide; EMS[a] and somaclonal mutations of the *als* gene are available | Imazethapyr (Pursuit®, Cadre®) | Inhibition of ALS, an important enzyme for the synthesis of branched amino acids. |
| Roundup Ready Rice | *aroA* or *EPSP* gene for alternative enzyme, *GOX* gene degrades herbicide | Glyphosate (Roundup®), sulfosate (Touchdown®) | Inhibition of EPSP[a] synthase blocking shikimate pathway and the formation of aromatic amino acids. |

[a] ALS = acetolactate synthase, EMS = ethylmethane sulfonate, EPSP = 5-enol pyruvylshikimate-3-phosphate.

In regions where crop rotation is not an option, rotate herbicide mode of action.

- *Monitor for signs of herbicide resistance.* Look for weed survivors following herbicide application, especially in rotational crops that may allow the application of the same herbicide mode of action.

## A resistance-monitoring and response plan

An effective resistance-monitoring and response plan should have the following elements:

- Communication
    - Develop a grower education program combining the best agronomic practices for the local region and monitoring for volunteers
    - Do training for field representatives, local pest control advisers, and local extension agents
    - Alternate herbicides with different modes of action
- Monitoring
    - Independent surveys by private and/or public-sector weed specialists
    - Company and grower responsibility
- Response
    - Have a management plan for unexpected results
    - Use an 800 phone number for access and follow-up
    - Apply the "weed resistance management action tree" recommendations when resistant red rice or rice mimic is reported
    - Use sampling procedures to confirm the presence of the gene for herbicide tolerance in field-grown rice varieties

## Conclusions

1. Rice varieties tolerant of herbicides offer unique management tools that provide flexibility in the timing of herbicide application, and in some regions that lack economically viable crop rotation systems may be the only management tools for controlling red rice or herbicide-resistant weed biotypes.
2. Three types of herbicide-tolerant rice varieties are under development. The use of more than a single mode of action is an important tool for herbicide resistance management.
3. Herbicide resistance in weed populations may be achieved by intensive use of a herbicide that can select for a naturally occurring resistant mutant in weed populations. In addition, outcrossing of genes from herbicide-tolerant rice to red rice can occur.
4. Knowledge of red rice biology and currently developed weed management practices can be applied to minimize the potential occurrence of herbicide-resistant red rice populations and to control populations that might develop.

5. If red rice populations become resistant as a result of gene flow, current red rice cultural practices will be effective in managing the populations. Regions that rely on reduced tillage "burn-down" management prior to planting should use caution in releasing a rice variety with genetic tolerance for the "burn-down" herbicide.
6. Herbicide resistance management, monitoring, and response plans have the same elements as those already in use by the agricultural community for managing weeds that are resistant to conventional herbicide technology.

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# Clomazone: a new herbicide for grass control in direct-seeded rice

I. Pegg, P.V. Grassick, and M.C. Taylor

Clomazone (Command® 480 EC), an inhibitor of both chlorophyll and carotenoid biosynthesis, is a new water-applied herbicide for control of grasses such as *Echinochloa crus-galli* and *Leptochloa fusca (*syn: *Diplacne fusca)* in direct-drilled and water-seeded rice in New South Wales (NSW), Australia.

Results from replicated field trials in NSW, conducted over five seasons (1993-97), demonstrated that pre- and early postemergence treatments of clomazone at 180 to 240 g ai $ha^{-1}$ consistently achieved control of *E. crus-galli* in water-seeded rice equal to or better than molinate at 2,400–3,600 g ai $ha^{-1}$ or thiobencarb at 3,000 g ai $ha^{-1}$. *Leptochloa fusca* is less susceptible to clomazone than *E. crus-galli.* Higher rates of clomazone at 240 to 300 g ai $ha^{-1}$ gave a level of control equal to or better than that of molinate at 2,400 g ai $ha^{-1}$ when applied early postemergence.

Crop tolerance in efficacy trials and varietal trials conducted under weed-free conditions shows that all commercial rice varieties are tolerant of clomazone at 240 g ai $ha^{-1}$. The most sensitive variety was an arborio type, Illabong, which can exhibit transient bleaching.

Results obtained in these small-plot trials were subsequently confirmed in six large-scale commercial trials in 1997-98–1998-99.

Crop residue trials demonstrated that applying clomazone to floodwater, at or shortly after inundation of rice bays, at rates up to 480 g ai $ha^{-1}$ did not result in detectable clomazone residues (LOD = 0.01 mg $kg^{-1}$) in straw or grain at harvest.

Field recropping studies show that conventional rotation crops of wheat, barley, canola, and subterranean clover can be planted safely 5 mo after treatment with clomazone at 240 to 480 g ai $ha^{-1}$ in direct-seeded rice.

Clomazone is used in the United States and South America for preemergence weed control in soybean, cotton, and sugarcane. Commercial development of clomazone for grass weed control in drill-sown rice in the early 1990s in South America prompted a global evaluation in a variety of rice-growing cultures. Clomazone is now registered in rice in South America, the Philippines, and Indonesia. This research focuses on evaluating clomazone in drill- and water-seeded rice culture of New South Wales (NSW), Australia.

*Echinochloa crus-galli* (L.) P. Beauv. (barnyardgrass, ECHCG) and *Leptochloa fusca* (Lam.) Gray (silvertop grass, LEFFA) are the predominant grass weeds infesting water-seeded rice in NSW. Molinate and thiobencarb are herbicides commonly applied in NSW to control grass weeds in rice.

The soluble nature of clomazone lends itself to application by dripping into the incoming floodwater (water-run) as well as the soluble chemical water injection in rice technique (SCWIIRT) (Taylor 1994). In the SCWIIRT, the product is diluted in water and applied at low pressure via a 4-wheel all-terrain vehicle (ATV) or helicopter directly into the flood for subsequent dispersal in the water.

## Materials and methods

### Weed control and crop tolerance

Eleven small-plot field experiments were conducted at dedicated laser-leveled sites in the Murray Valley irrigation area during the seasons 1992-93–1996-97. Randomized complete block (RCB) designs with four replications with 4.5 m by 8-m plots were used, with earthen bunding and independent irrigation. Normal agronomic practices were emulated, with pregerminated seed sown by aircraft. Herbicide was applied by administering dilute emulsions (delivering 7.5 to 13 L ha$^{-1}$ total spray volume) directly to floodwater or using a hand-held gas-powered small-plot boom sprayer delivering 90 to 115 L ha$^{-1}$.

Trials were assessed using visual ratings of rice injury and weed control, counts of rice plants, weed seedlings, and inflorescence density, and direct harvest of grain yields using small-plot harvesters.

To further confirm efficacy and evaluate application methods under practical commercial conditions, six large-scale, unreplicated product evaluation trials were undertaken in 1997-98 and 1998-99. Applications were compared by water-run versus SCWIIRT in direct-drilled, dry-sown, and water-seeded rice. For drip treatment, clomazone was diluted in water to a total volume of 4 L ha$^{-1}$. A constant head siphon with a single 4916 T-jet regulator fitted with a disk orifice plate no. 22 was used to apply the drip treatment into the floodwater. SCWIIRT treatments were applied using a 4-wheel ATV with two nozzles on a 2-m boom. The spray volume was typically 5 to 10 L ha$^{-1}$ at a swath width of about 20 m traveling at 10 to 12 kph.

### Rice varietal tolerance

A dedicated crop tolerance trial examined rice varietal tolerance under weed-free conditions. An RCB design was used with four replications, earthen bunding, and independent irrigation. Plot size was 10 m by 25 m (varieties 2.1 m by 10 m). Varieties tested were Millin, Amaroo, Jarrah, YRM43, Langi, YRL38, and Kyeema. Seed was broadcast on the dry soil surface using a 2.1-m linkage seeder. Following inundation, clomazone 480 EC was applied at 240, 360, and 480 g ai ha$^{-1}$ directly into floodwater when rice was at the 1- to 2-leaf growth stage.

Trials were assessed using visual ratings of rice injury with a 0 to 100% linear scale where 0 = no rice injury and 100 = complete death of all rice seedlings. Grain yields were obtained by direct harvest of plots using a modified small-plot harvester.

### Crop residues

Four residue trials were conducted to evaluate clomazone residues in rice treated with clomazone in NSW in 1995-96 and 1996-97. Clomazone was applied by SCWIIRT at or near sowing of water-seeded rice at 180, 240, 360, and 480 g ai ha$^{-1}$. Samples of rice forage were taken from 52 d after application and grain and straw samples were taken at harvest, ranging from 122 to 170 d after application. Samples were frozen and sent to Analchem Bioassay Pty. Ltd. for residue analysis.

### Recropping intervals for rotational crops

Two recroppings were conducted at Tongaboo (1996-97) and Jerilderie (1997-98) in an RCB design with six replications. Plots were typically 13.5 m by 20 m, with earthen bunding and independent irrigation. Normal agronomic practices were emulated, with pregerminated rice seed sown by aircraft.

Clomazone 480 EC was applied directly into standing water at 240 and 480 g ai ha$^{-1}$. The rotational crops of wheat, barley, canola, and subterranean clover were seeded following the rice crop, after burning and rotary hoeing of the site. Rotational crop seedling densities were recorded in ten 0.1-m$^2$ randomly selected quadrats from each plot. Grain yields were obtained by direct harvest of (plot-size) plots using a modified small-plot harvester.

## Results

Consistently high levels of *E. crus-galli* control were achieved across all experiments with clomazone at 180–300 g ai ha$^{-1}$ applied either presowing or at the rice 1-leaf stage (Table 1). Clomazone at 180 g ai ha$^{-1}$ was less effective when applied at the rice 2-leaf stage as larger *E. crus-galli* escaped control (Table 1). *Leptochloa fusca* was less susceptible to clomazone than *E. crus-galli*, especially when clomazone was applied presowing (Table 2).

Under commercial conditions (Table 3), clomazone (Command 480 EC) applied by water-run or by SCWIIRT, in direct drill-, dry-, and water-sown rice, achieved an overall high level of *E. crus-galli* control. Clomazone at 300 g ai ha$^{-1}$ applied by water-run achieved control less than or equal to that of 300 g ai ha$^{-1}$ applied by SCWIIRT. Rice injury ratings from all experiments showed rising rice phytotoxicity from 120 to 300 g ai ha$^{-1}$, surpassing that noted with molinate at 2,400 g ai ha$^{-1}$ but less than thiobencarb at 3,000 g ai ha$^{-1}$. Less rice injury occurred with presowing

**Table 1. Mean percent control ratings of *Echinochloa crus-galli* collated from nine replicated trials in water-seeded rice, 1993-96, New South Wales.**

| Treatment rate (g ai ha$^{-1}$) | Application timing[a] | | |
|---|---|---|---|
| | Presowing | 1 LSR | 2 LSR |
| Clomazone 120 | 84 | 46 | – |
| Clomazone 180 | 94 | 96 | 90 |
| Clomazone 240 | 97 | 98 | 94 |
| Clomazone 300 | 100 | 100 | 99 |
| Molinate 2400 | 61 | 89 | 73 |
| Thiobencarb 3000 | 69 | 93 | 100 |

[a] LSR = leaf stage of rice.

**Table 2. Mean percent control ratings of *Leptochloa fusca* collated from three replicated trials in water-seeded rice, 1994-95, New South Wales.**

| Treatment rate (g ai ha$^{-1}$) | Application timing[a] | | |
|---|---|---|---|
| | Presowing | 1 LSR | 2 LSR |
| Clomazone 180 | 45 | 70 | 77 |
| Clomazone 240 | 78 | 80 | 85 |
| Clomazone 300 | 74 | 89 | 90 |
| Molinate 2400 | 69 | | 58 |

[a] LSR = leaf stage of rice.

applications of clomazone than postsowing applications (Table 4). Injury was expressed as transient bleaching of leaves. Clomazone at 240–480 g ai ha$^{-1}$ did not significantly affect grain yields of any rice varieties grown under weed-free conditions (Table 5). High levels of experimental error in this trial were associated with varieties that suffered severe floret sterility because of cold weather at flowering. No clomazone residues were detected (limit of detection = 0.01 mg kg$^{-1}$) in any forage, grain, or straw samples at any of the rates tested (180–480 g ai ha$^{-1}$ ).

Seedling densities and yield data (Tables 6 and 7) show that rotational crops such as wheat, barley, canola, and clover can be planted safely as little as 5 mo after a clomazone application in rice, at up to 480 g ai ha$^{-1}$ (1.6 times the highest use rate).

**Table 3. Percent control rating of *Echinochloa crus-galli* collated from six unreplicated commercial-scale trials evaluating water-run and SCWIIRT[a] application methods for direct-seeded rice, 1997-98–1998-99, New South Wales.**

| Treatment (g ai ha$^{-1}$) | Trial | | | | | |
|---|---|---|---|---|---|---|
| | Duffty | Wiltshire | Mannes | Malcolm | Sampson | Taylor |
| *Drip* | | | | | | |
| Clomazone 240 | 85 | 95 | – | 90 | – | – |
| Clomazone 300 | 93 | 90 | 90 | – | 95 | 98 |
| Clomazone 360 | – | 95 | – | – | 95 | – |
| Molinate 3600 | 95 | 85 | 90 | 90 | 90 | 98 |
| *SCWIIRT* | | | | | | |
| Clomazone 300 | 95 | 95 | – | – | – | 98 |
| *Trial conditions* | | | | | | |
| Location | Coleambally | Coleambally | Coleambally | Leeton | Jerilderie | Tocamwal |
| Seeding technique | Sod | Sod | Sod | Sod | Dry | Water |
| Variety | Langi | Langi | Langi | Langi | Jarrah | Millin |
| Rice stage | 2–3 | 2–4 | 2 | 2–4 | 0–1 | 0–2 |
| BYG stage | 3–5 | 2–5 | 0–2 | 2–4 | 0–2 | 0–2 |
| Bay area (ha) | 1 | 4.5 | 4 × 3 | 5 | 2 × 3 | 4.5 |

[a]SCWIIRT = soluble chemical water injection in rice technique.

**Table 4. Mean percent injury ratings of water-seeded rice collated from nine replicated trials, 1993-96, New South Wales.**

| Treatment rate (g ai ha$^{-1}$) | Application timing[a] | | |
|---|---|---|---|
| | Presowing | 1 LSR | 2 LSR |
| Clomazone 120 | 3 | 7 | |
| Clomazone 180 | 7 | 12 | 13 |
| Clomazone 240 | 7 | 14 | 18 |
| Clomazone 300 | 5 | 16 | 24 |
| Molinate 2400 | 3 | 8 | 10 |
| Thiobencarb 3000 | 55 | 29 | 28 |

[a]LSR = leaf stage of rice.

## Discussion

At the rates evaluated, clomazone demonstrated a high level of *E. crus-galli* control and a moderate level of control of *L. fusca* when applied into floodwater. Clomazone can be easily applied by water-run or by SCWIIRT and offers a wide window of application in direct-seeded rice crops with adequate crop tolerance up to 300 g ai ha$^{-1}$. Applying clomazone to rice at up to 480 g ai ha$^{-1}$, which is 1.6 times the maximum recommended rate, did not result in detectable clomazone residues in grain or

**Table 5. Grain yields (t ha$^{-1}$) of seven weed-free dry-seeded broadcast rice varieties after treatment with clomazone, 1995-96.**

| Variety | Rate (g ai ha$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 0 | 240 | 360 | 480 | LSD (*P* >0.05) |
| Millin | 9 | 10 | 9 | 9 | 1 |
| Amaroo | 5 | 5 | 5 | 5 | 1 |
| Jarrah | 7 | 7 | 7 | 7 | 1 |
| YRM43 | 5 | 4 | 5 | 5 | 1 |
| Langi | 6 | 7 | 8 | 7 | 2 |
| YRL38 | 4 | 4 | 4 | 5 | 2 |
| Kyeema | 4 | 3 | 4 | 5 | 1 |
| Mean | 6 | 6 | 6 | 6 | 1 |

**Table 6. Effects of clomazone on seedling densities and yield in rotation crops planted 192 d (approx. 6 mo) after herbicide application in rice, 1996-97, Tongaboo, New South Wales.**

| Treatment rate (g ai ha$^{-1}$) | Seedling densities m$^{-2}$ (6 wk after sowing)[a] | | | | Yield (t ha$^{-1}$) (24 wk after sowing) | |
|---|---|---|---|---|---|---|
| | Wheat | Barley | Canola | Clover | Wheat | Barley |
| Clomazone 240 | 115.2 a | 121.7 a | 17.7 a | 112 a | 2.53 a | 3.30 a |
| Clomazone 480 | 122.5 a | 129.3 a | 10.2 a | 111 a | 2.50 a | 3.23 a |
| UTC[b] | 113.7 a | 116.8 a | 16.8 a | 102 a | 2.55 a | 3.48 a |
| LSD (*P* >0.05) | 22.7 | 17.1 | 12.5 | 22 | 0.37 | 0.43 |

[a]Numbers followed by different letters are significant at the 0.5 level of probability. [b]UTC = untreated check.

**Table 7. Effects of clomazone on seedling densities and yield in rotation crops planted 167 d (approx. 5 mo) after herbicide application in rice, 1997-98, Jerilderie, New South Wales.**

| Clomazone rate (g ai ha$^{-1}$) | Seedling densities m$^{-2}$ (6 wk after sowing)[a] | | | | Yield (t ha$^{-1}$) (30 wk after sowing) | | |
|---|---|---|---|---|---|---|---|
| | Wheat | Barley | Canola | Clover | Wheat | Barley | Canola |
| 240 | 64 a | 83 a | 102 a | 71 a | 1.74 ab | 2.04 a | 1.13 ab |
| 480 | 68 a | 75 a | 95 a | 61 a | 2.01 a | 2.17 a | 1.34 a |
| UTC[b] | 69 a | 84 a | 106 a | 98 a | 1.49 b | 2.39 a | 1.08 ab |
| LSD (*P* >0.05) | 16 | 13 | 17 | 26 | 0.32 | 0.47 | 0.24 |

[a]Numbers followed by different letters are significant at the 0.5 level of probability. [b]UTC = untreated check.

straw at harvest in rice forage 7 wk after application. Rotation crops such as wheat, barley, canola, and clover were safely recropped following clomazone applications in rice.

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## Notes

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WEED SCIENCE

# Benzofenap—a new herbicide for weed control in water-seeded rice

G.M. Skinner and M.C. Taylor

Benzofenap (MY-71) is a pyrazole herbicide first synthesized in 1981 by Mitsubishi Petrochemical Company. It was first registered in Japan in 1987 for pre- and early postemergent control of annual grass, sedge, and broadleaf weeds in transplanted rice. Rhone Poulenc Rural Australia Pty. Ltd. began evaluating benzofenap for water-seeded rice in New South Wales (NSW) in 1994. The commercial introduction of benzofenap (as Taipan® herbicide) in Australia occurred in the 1998-99 season.

Benzofenap exhibits low water solubility and volatility. It is primarily absorbed through roots, resulting in carotenoid biosynthesis inhibition. Toxicity to mammals, birds, and aquatic organisms is very low and no residues have been detected in grain and straw at harvest.

Forty-seven replicated field trials were conducted over four seasons to demonstrate the efficacy of benzofenap against seedlings of the following weed species in water-seeded rice: *Sagittaria montevidensis*, *Alisma plantago aquatica*, *A. lanceolatum*, *Damasonium minus*, and *Cyperus difformis*. Optimum results were obtained by applying benzofenap at 600 g ai $ha^{-1}$ to standing water within 10 d of flooding. The application of benzofenap to dry soil before inundation resulted in poorer weed control than when applied to floodwater. The addition of molinate at 2,400–3,600 g ai $ha^{-1}$ to benzofenap ensured the effective control of grass weeds (e.g., *Echinochloa crus-galli*).

Crop safety of benzofenap plus molinate combinations was acceptable and equivalent to or better than alternate commercial standards.

Monitoring of benzofenap performance under commercial conditions has demonstrated that prolonged floodwater retention after application is critical to attaining effective weed control. Where benzofenap-treated floodwater has been held static for a minimum of 5 d, excellent aquatic weed control was attained, resulting in high rice grain yields.

MCPA sodium has been recommended for application following benzofenap in NSW as a second mode-of-action herbicide to minimize selection for benzofenap-resistant weed biotypes.

In New South Wales (NSW), approximately 150,000 hectares of irrigated rice are grown annually, established principally by water seeding (pregerminated rice broadcast into the water).

Annual aquatic weeds are favored by constant ponding, so water-seeded rice tends to be infested with *Cyperus difformis* (dirty Dora, CYPDI), *Damasonium minus* (starfruit, DAMMI), *Sagittaria montevidensis* (arrowhead, SAGMO), *Alisma plantago aquatica* (water plantain, ALSPA), and *A. lanceolatum* (alisma, ALSLA). Cultural control methods such as clean seedbeds, clean seed, laser-guided land leveling, ridge rolling, farm hygiene, and floodwater management are all important components of weed control programs in NSW rice production; however, most growers cannot produce economic crops without herbicide inputs.

Bensulfuron methyl (Londax) has been applied to more than 90% of the NSW rice crop (most often in combination with molinate) for the past nine seasons, leading to the widespread development of bensulfuron-resistant populations of *C. difformis*, *D. minus*, and *S. montevidensis* (Davis and Fader 1994, Graham et al 1994). Herbicides with an alternate mode of action than that of bensulfuron are urgently required for water-seeded rice production.

Benzofenap was first synthesized in 1981 by the Mitsubishi Petrochemical Company. It has effective herbicidal activity applied as a pre- or early postemergent treatment against annual grass, sedge, and broadleaf weeds in transplanted rice. Current registrations in Japan include mixtures with pyributicarb and bromobutide.

As a pyrazole derivative, the compound exhibits nonhormonal herbicidal activity. Plant entry occurs through the roots and bases of target weeds. It is then thought that carotenoid biosynthesis inhibition occurs, resulting in morphological changes such as bleaching and yellowing before plant death (Ikeda and Goh 1991).

Benzofenap is not water-soluble (0.13 mg $L^{-1}$) but it is soluble in a range of organic solvents. The calculated Log $P_{ow}$ value (the partitioning coefficient of oil/water) is $4.69 \times 10^{-4}$. Benzofenap is odorless with a low vapor pressure and low propensity for evaporation in the field. It is stable in acid conditions, but readily degrades to the OH metabolite in alkaline conditions. Photolysis and microbial degradation are also key breakdown pathways. Benzofenap is heat-stable up to 250 °C.

The main metabolites of benzofenap are MY-71-OH and MY-71-Red. Both benzofenap and MY-71-Red convert to MY-71-OH through hydrolysis and microbial activity. MY-71-OH is subsequently degraded primarily by photolysis. Benzofenap is also easily reduced to MY-71-Red, which is subsequently hydrolyzed to MY-71-OH. Under certain field conditions, MY-71-Red may oxidize back to parent benzofenap although this is not clearly understood (Matano and Odanaka 1985, Uchiyama 1985).

Toxicity of benzofenap to mammals, birds, and aquatic organisms is very low and no residues have been detected in grain and straw at harvest (Anon. 1985, Savage 1998).

Benzofenap was identified as a potential herbicide for water-seeded rice culture in Australia in 1994 when field testing began. A local development program in subsequent seasons led to initial commercial sales of benzofenap to NSW rice growers in the 1998-99 season.

## Materials and methods

Forty-seven replicated field experiments were conducted at dedicated laser-leveled sites in the Murray and Murrumbidgee Valley irrigation areas of southern New South Wales during the seasons 1994-95 to 1998-99. Randomized complete block designs with four replications of plots typically 4.5 × 8 m were used, with earthen bunding and independent watering. Normal agronomic practices were emulated, with pregerminated seed sown by aircraft.

In the autumn after herbicide application, two recropping experiments used six replications of plots approximately 20 × 16 m, with plots burned and rotary-hoed before sowing winter crops into 2.1 × 16-m subplots with a cone seeder.

Herbicide applications were made using simulated SCWIIRT (soluble chemical water injection in rice technique) treatments (Taylor 1994), applying neat or concentrated working solutions directly to floodwater or using a hand-held gas-powered small-plot boom sprayer delivering 90 to 115 L ha$^{-1}$.

Experiments were assessed using visual ratings of rice injury and weed control, counts of rice and weed seedling and inflorescence density, and direct harvest of grain yields using modified Wintersteiger Seedmaster or Kincaid plot harvesters.

To determine what residues might remain after application, forage (fodder), straw, and unmilled rice grain samples from four NSW experiments were analyzed for benzofenap and metabolites.

Monitoring of benzofenap and metabolite concentrations in paddy water and sediment was conducted at five sites in the Murray Valley Irrigation Area during the 1997-98 season following commercial aerial applications of the proposed formulation (300SC) at the proposed rate (600 g ai ha$^{-1}$). Three sites were sampled weekly for up to 7 wk from the bottom bay in the field treated with benzofenap to identify any accumulation in the bottom bay and potential release of residue into drainage water. Two additional sites were sampled daily for 18 d and were located in the top bay to quantify the decline in paddy water and accumulation in sediment without potential contamination from upstream bays.

## Results

Figure 1 presents average percentage control ratings of five annual broadleaf and sedge aquatic weeds in a total of 47 replicated field trials conducted over four seasons. *Sagittaria montevidensis* exhibited a high susceptibility to benzofenap, followed closely by *A. plantago aquatica* and *A. lanceolatum*. *Damasonium minus* and *C. difformis* exhibited slightly less susceptibility to benzofenap than the above species.

Corm plants (i.e., arising from vegetative propagules) of *A. plantago aquatica* and *A. lanceolatum* were not controlled effectively by benzofenap. *Eleocharis* spp. (spikerushes) and *Typha* spp. (cumbungi) appeared not to be susceptible to benzofenap at 600 g ai ha$^{-1}$.

*Echinochloa crus-galli* (barnyardgrass, ECHCG) and *Leptochloa fusca* (silvertop grass, LEFFA) often escaped control with benzofenap alone. Tank-mix combinations

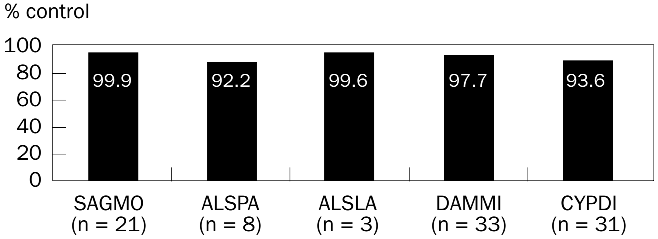

**Fig. 1. Mean percentage control ratings for annual aquatic weeds treated with benzofenap at 600 g ai ha$^{-1}$ in water-seeded rice, New South Wales, 1995-98 (47 trials over four seasons).**

**Table 1. Mean percentage control ratings and inflorescence densities of *Echinochloa crus-galli* and *Cyperus difformis* treated with benzofenap at 600 g ai ha$^{-1}$ (alone or tank-mixed with molinate) in water-seeded rice, New South Wales, 1996-97 and 1997-98 seasons. Compilation of 14 trials conducted over two seasons.**

| Treatment (g ai ha$^{-1}$) | ECHCG (percentage control rating) | ECHCG (panicles m$^{-2}$) | CYPDI (umbels m$^{-2}$) |
|---|---|---|---|
| Trials (no.) | 14 | 11 | 9 |
| Untreated control | 0 | 126 | 212 |
| Benzofenap 600 | 63.8 | 81 | 43 |
| Benzofenap 600 plus molinate 2,400–3,600 | 93.5 | 10 | 20 |

of benzofenap plus molinate at 2,400–3,600 g ai ha$^{-1}$ have been effective in controlling these two grass weeds. Molinate added to benzofenap also bolstered the control of *C. difformis* (see Table 1).

The application of benzofenap to dry soil before inundation of rice fields resulted in less effective control of annual aquatic weeds than if benzofenap was applied to standing floodwater at or around seeding (see Fig. 2).

The optimum efficacy of benzofenap occurred where applications were made within 10 d after the flooding of rice fields. Figure 3 shows the results from a benzofenap application timing experiment.

Table 2 presents average visual crop injury ratings for water-seeded rice from 27 ratings conducted in 12 experiments over three seasons. Benzofenap plus molinate combinations applied at or around sowing recorded less crop injury than the commercial standards in 24 of 27 ratings.

The following NSW rice cultivars were demonstrated to be tolerant of benzofenap at 600 g ai ha$^{-1}$ in two field trials and one glasshouse study (data not presented): Amaroo, Namaga, Millin, Jarrah, Langi, Doongarra, Koshihikari, Opus, Illabong, and Kyeema.

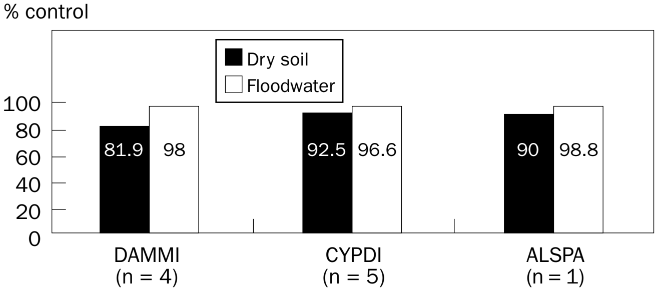

**Fig. 2. Mean percentage control ratings of annual aquatic weeds in water-seeded rice after dry soil and floodwater applications of benzofenap at 600 g ai ha$^{-1}$, New South Wales, 1997-98 and 1998-99 seasons. Compilation of five experiments conducted over two seasons (n = number of trials).**

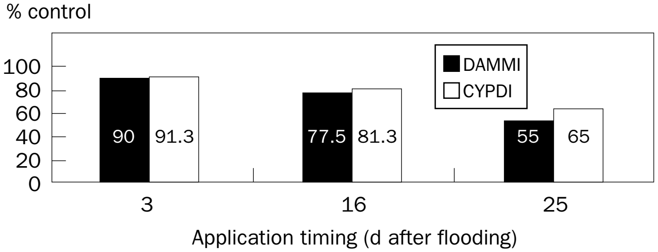

**Fig. 3. Effect of application timing of benzofenap at 600 g ai ha$^{-1}$ on percentage control ratings of annual aquatic weeds in water-seeded rice, New South Wales, 1996-97 season.**

**Table 2. Comparison of average crop injury ratings and grain yields of water-seeded rice treated with bensulfuron plus molinate or split thiobencarb plus bensulfuron treatments, New South Wales, 1996-97 to 1998-99.**

| Treatment (g ai ha$^{-1}$) | Percentage injury | Grain yield (t ha$^{-1}$ at 14% moisture) | Percentage increase (over untreated controls) |
|---|---|---|---|
| Trials (no.) | 12 | 10 | 10 |
| Benzofenap at 600 plus molinate at 3,600 | 14.6 | 8.54 | 210 |
| Thiobencarb at 800 followed by thiobencarb at 2,200 plus bensulfuron at 42–51 | 31.6 | 8.35 | 207 |

Table 2 presents average grain yields recorded from a total of 10 replicated experiments over two seasons. Benzofenap at 600 g ai $ha^{-1}$ plus molinate at 3,600 g ai $ha^{-1}$ produced grain yields slightly in excess of yields produced with the commercial standard of a split application of thiobencarb plus bensulfuron at 42 g ai $ha^{-1}$.

Wheat, barley, canola, and subterranean clover seeded at two sites 5–6 mo after the application of benzofenap at 600 g ai $ha^{-1}$ did not demonstrate any carryover phytotoxic effects (see Table 3).

Retreatment of benzofenap plots with MCPA sodium (to achieve two modes of herbicide action per weed species per season as a means of delaying herbicide resistance) did not significantly affect rice grain yield in four experiments (Table 4).

Studies conducted in Australian rice bays confirmed that the persistence of benzofenap in the rice bay system is short. Half-life values for floodwater were less than 7 d (total residue). As the sediment values varied over the first few weeks, it was difficult to estimate the half-life, but it was on the order of 30 d. Residue of benzofenap

**Table 3. Effect of benzofenap on average seedling densities and yield of rotation crops planted 167 and 192 d after herbicide application to water-seeded rice, New South Wales, 1996-97 and 1997-98 seasons.**

| Treatment (g ai $ha^{-1}$) | Seedling densities $m^{-2}$ (6 wk postsowing) | | | | Grain yield (t $ha^{-1}$) | | |
|---|---|---|---|---|---|---|---|
| | Wheat | Barley | Canola | Clover | Wheat | Barley | Canola |
| *1996-97 season* | | | | | | | |
| Untreated | 113.7 | 116.8 | 16.8 | 102 | 2.55 | 3.48 | |
| Benzofenap 600 | 126.3 | 121.5 | 19.2 | 117 | 2.46 | 3.26 | |
| LSD ($P<0.05$) | 22.7 | 17.1 | 12.5 | 22 | 0.37 | 0.43 | |
| *1997-98 season* | | | | | | | |
| Untreated | 69 | 84 | 106 | 58 | 1.49 | 2.39 | 1.08 |
| Benzofenap 600 | 70 | 80 | 94 | 76 | 1.56 | 2.00 | 1.07 |
| Benzofenap 1,200 | 73 | 86 | 97 | 72 | 1.64 | 2.19 | 0.98 |
| LSD ($P<0.05$) | 16 | 13 | 17 | 26 | 0.32 | 0.47 | 0.24 |

**Table 4. Effect of MCPA sodium on average grain yields of water-seeded rice after treatment with benzofenap plus molinate, New South Wales, 1997-98 season.**

| Treatment (g ai $ha^{-1}$) | Trial number | | | | Mean |
|---|---|---|---|---|---|
| | H52-97 | H54-97 | H58-97 | H63-98 | |
| Benzofenap 600 plus molinate 2,400 | 10.2 | 5.5 | 8.4 | 9.3 | 8.4 |
| Benzofenap 600 plus molinate 2,400 followed by MCPA sodium 700 | 9.8 | 5.5 | 9.2 | 9.1 | 8.4 |
| LSD ($P<0.05$) | 0.9 | 1.7 | 1.1 | 1.7 | |

and metabolites declined to about the limit of quantification (LOQ = 0.01 ppm) by day 35 for water and day 49 for sediment (Table 5 and Fig. 4).

Residue in bay sediment was generally low (max. 0.1 ppm) throughout the study period. At all three sites, residue levels increased from the time of application to day 7 and declined thereafter to just above the LOQ from week 5 onward (Fig. 5). At no site did the residue level consistently drop below the LOQ although individual

**Table 5. Results of MY-71-OH (expressed as benzofenap equivalents) residue in water and sediment from three sites following aerial application of benzofenap at 600 g ai ha$^{-1}$, New South Wales, 1997-98 season.**

| DAA[a] | "Ridgewell", Finley, NSW |  | "Bundilla" Deniliquin, NSW |  | "Kalawar" Deniliquin, NSW |  |
|---|---|---|---|---|---|---|
|  | Water | Sediment | Water | Sediment | Water | Sediment |
| 0 | 0.38–0.41 | 0.03–0.04 | 0.31–0.33 | 0.03–0.04 | 0.52–0.53 | 0.06 |
| 7 | 0.20–0.22 | 0.08–0.10 | 0.16–0.19 | 0.04–0.06 | 0.25–0.27 | 0.06–0.08 |
| 13 | 0.14–0.16 | 0.06–0.10 |  |  | 0.06–0.08 | 0.04–0.06 |
| 14 |  |  | 0.04–0.05 | 0.03–0.04 |  |  |
| 20 |  |  | <0.02–0.02 | 0.02–0.03 |  |  |
| 21 |  |  |  |  | 0.03–0.05 | 0.04–0.06 |
| 22 | 0.05–0.07 | 0.06–0.08 |  |  |  |  |
| 26 |  |  | <0.02 | 0.03–0.04 |  |  |
| 28 | 0.03 | 0.04 |  |  |  |  |
| 30 |  |  |  |  | <0.02–0.02 | 0.03–0.04 |
| 34 |  |  | <0.02 | 0.02–0.03 |  |  |
| 35 | <0.02–0.02 | 0.03–0.04 |  |  |  |  |
| 37 |  |  |  |  | <0.02 | 0.02–0.04 |
| 42 | <0.02 | 0.02–0.03 | <0.02 | <0.02–0.03 |  |  |
| 49 | <0.02 | 0.02–0.03 |  |  |  |  |

[a]DAA = days after application.

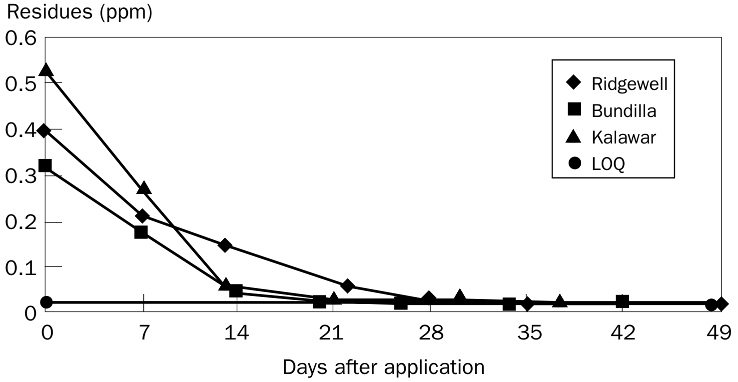

**Fig. 4. Mean residue of MY-71-OH (expressed as benzofenap equivalents) in water from three sites following aerial application of benzofenap at 600 g ai ha$^{-1}$, New South Wales, 1997-98 season. LOQ = limit of quantification.**

duplicate samples did so. The pattern of decline clearly indicates that this consistency would be achieved soon after week 7 and certainly well before the end of the season. For each site, the patterns of decline in water, accumulation, and subsequent decline in sediment were similar.

No residues of the parent compound or metabolite were found at any rate or application time, even at twice the proposed use rate (Table 6). Similarly, no residue was found in rice forage or fodder from these same crops (Table 7).

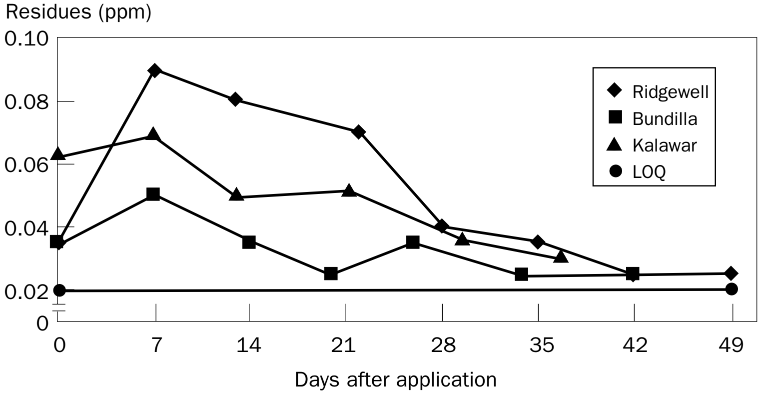

**Fig. 5. Mean residue of MY-71-OH (expressed as benzofenap equivalents) in sediment from three sites following aerial application of benzofenap at 600 g ai ha$^{-1}$, New South Wales, 1997-98 season. LOQ = limit of quantification.**

**Table 6. Residues in rice grain following application of benzofenap, New South Wales, 1996-97 season.**

| Year | Location | Variety | Treatment schedule | DALA[a] | Max. residues (ppm) at the specified rate (g ai ha$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 195 | 300 | 600 | 1,200 |
| 1996 | Tocumwal | Jarrah | 2 d presowing | 170 | <0.01 | <0.01 | <0.01 | <0.01 |
| | | | 8 d postsowing | 160 | | | | <0.01 |
| 1996 | Tongaboo | Millin | 15 d postsowing | 210 | | | <0.01 | <0.01 |

[a] DALA = days after last application.

**Table 7. Residues in rice forage/fodder following application of benzofenap, New South Wales, 1996-97 season.**

| Year | Location | Variety | Treatment schedule | DALA[a] | Max. residues (ppm) at the specified rate (g ai $ha^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 195 | 300 | 600 | 1,200 |
| 1996 | Tocumwal | Jarrah | 2 d presowing | 52 | <0.02 | <0.02 | <0.02 | <0.02 |
| | | | | 64 | <0.02 | <0.02 | <0.02 | <0.02 |
| | | | | 98 | <0.02 | <0.02 | <0.02 | <0.02 |
| | | | | 170 | <0.02 | <0.02 | <0.02 | <0.02 |
| | | | 8 d postsowing | 42 | | | | <0.02 |
| | | | | 54 | | | | <0.02 |
| | | | | 88 | | | | <0.02 |
| | | | | 160 | | | | <0.02 |
| 1996 | Tongaboo | Millin | 15 d postsowing | 52 | | | <0.02 | <0.02 |
| | | | | 80 | | | <0.02 | <0.02 |
| | | | | 210 | | | <0.02 | <0.02 |

## Discussion

Benzofenap has exhibited the most reliable results when applied within 10 d after flooding the field. In commercial practice, this has meant completion of inundation of the field within approximately 7 d, followed by application of benzofenap into standing water at or around sowing. This presents an opportunity to apply an insecticide for control of *Chironomus tepperi* (rice bloodworm) and an additional herbicide (e.g., molinate) for grass weed control. Considerable convenience is achieved by applying all treatments at one time with only a single water-holding period.

Benzofenap has been commercially formulated as a 300 g $L^{-1}$ suspension concentrate, enabling rapid and convenient commercial application directly into floodwater using the SCWIIRT on motorbikes or aerial application with fixed-wing or rotary-winged aircraft.

Data from the studies on the fate of benzofenap indicated a low potential for persistence in soil, floodwater, or the crop. Given the use pattern for this herbicide (a single application at sowing) and the length of the crop cycle (5–6 mo), there is unlikely to be a hazard to following crops or grazing livestock. Also, the local herbicide application techniques and current floodwater retention practices for rice limit the likely impact on off-target organisms through water movement.

The rapid metabolism of benzofenap in floodwater and sediment highlight the need to retain treated floodwater on the field for as long as possible, with no dilution or mass movement by entry of fresh (untreated) water. Such lock-up periods may induce exposure of the high sides of bays and risk establishment of a fresh cohort of *E. crus-galli* seedlings. In commercial practice, residual weed control from benzofenap in combination with molinate or clomazone has prevented reestablishment by grass weeds where mud has been exposed during prolonged lock-up periods.

Selection intensity for benzofenap-resistant biotypes is potentially high for highly susceptable species (e.g., *S. montevidensis*). To prevent or delay development of benzofenap-resistant aquatic weed populations, two strategies have been recommended to rice growers: avoidance of application to consecutive rice crops in the same field and retreatment of benzofenap-treated crops with MCPA sodium.

MCPA sodium is an attractive alternate mode-of-action herbicide to benzofenap or bensulfuron given its demonstrated activity against all aquatic weeds occurring in NSW rice. Additionally, it is relatively economical to apply. Previous studies in New South Wales have shown no reduction in rice grain yield where MCPA is applied to full-season varieties sown on time. Major grain yield penalties were, however, measured on short-season varieties, especially when sown late in the season (Taylor 1998). Off-target drift by MCPA sodium is a significant risk when applied by aircraft and this deters many growers from using this treatment.

Recommendations for benzofenap in water-seeded rice have integrated several agronomic practices aimed at producing dense and competitive rice stands (Anon. 1998). These include even field grades, rapid inundation of fields (in less than 7 d), application of rice bloodworm insecticides at or before sowing, and lowering of floodwater at the 1- to 2-leaf stage of rice development to encourage rice seedling root attachment.

Benzofenap at 600 g ai ha$^{-1}$ has exhibited good to excellent control of a range of annual aquatic weeds. Accordingly, it has now become a recommended alternative herbicide to bensulfuron for aquatic weed control in water-seeded rice in New South Wales, with full regulatory approval (trade name Taipan®) in the 1999-2000 rice season. Combination partner herbicides for benzofenap continue to be sought to broaden the spectrum of activity.

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# A first approach to the characterization of somatic red rice (*Oryza sativa* var. *sylvatica*) chromosomes

A.C. Sparacino, C. Halfer, F. Tano, V. Malvica, D. Ditto, and G. Fiore

Red rice has spread rapidly into the rice fields of Europe in recent years and has reached very high infestation levels. Thus, we began detailed studies to better understand the biology and propagation of this weed. The object of this work is to study the characteristics of the red rice chromosome somatic (*Oryza sativa* var. *sylvatica*) to determine possible differences from the karyotype of the cultivated rice (subsp. *japonica*). Studies carried out on chromosome samples obtained from the root apical meristem have shown that a 24-chromosome karyotype is also present in red rice. The identification of only one of the two homologues of the first pair was possible because it is easily recognizable as being longer than the others. Moreover, in rare cases, we observed cells containing 23 (2n – 1) and 25 (2n + 1) chromosomes. These preliminary results reveal the presence of mutations at both the chromosome and genomic levels.

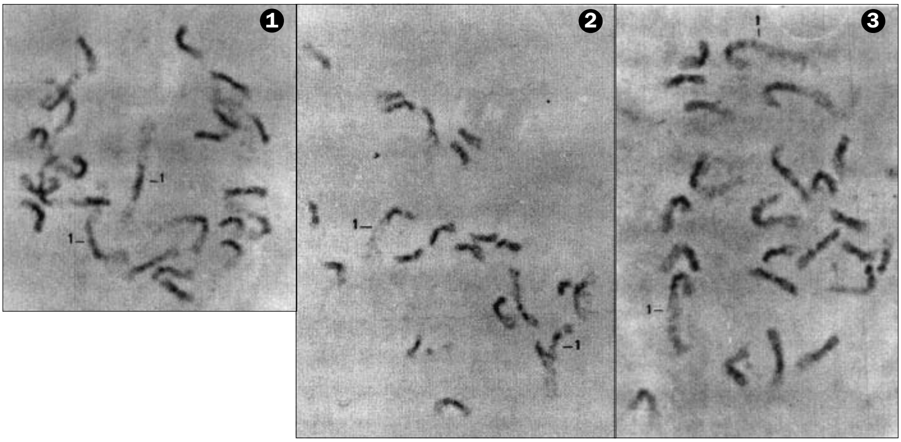
The infesting habit of red rice (*Oryza sativa* var. *sylvatica*) and its spread have become a serious problem in rice cultivation. In this work, we compare the karyotype of red rice to that of cultivated rice (*loto*, subsp. *japonica*) to discern differences between the two karyotypes.

## Materials and methods

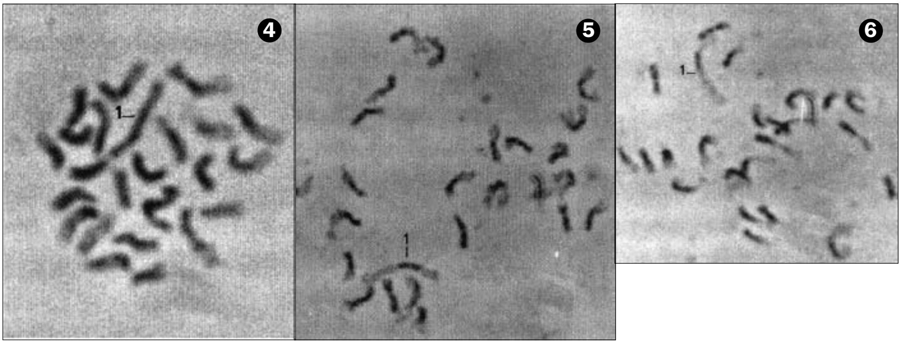
The cytological preparations were carried out following the procedure of Fukui and Iijima (1992) with minor modifications. Following fixation of root tips in methanol and acetic acid (1:1) for 1 h and enzymatic maceration in 4% cellulase Onozuka RS + 1% pectolyase Y-23, pH 4.2 at 37 °C for 50 min, the slides, prepared by the standard air-drying technique, were stained with a 4% Giemsa solution (pH 6.8) for 20 min.

## Results and discussion

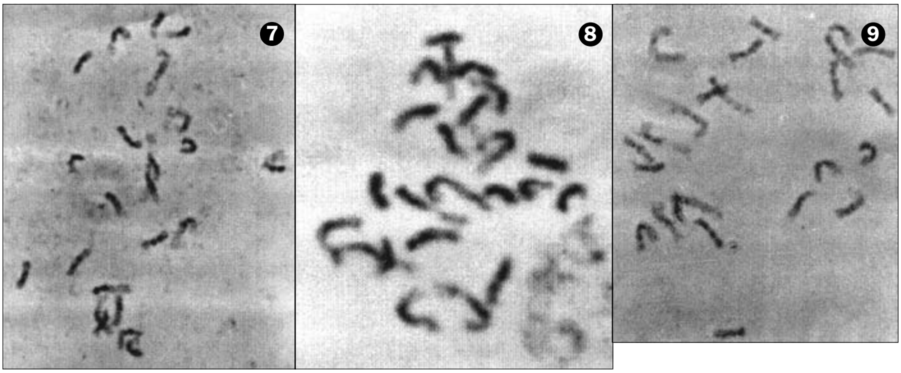
The karyotype was analyzed on chromosomes in the mitotic prometaphase stage since this stage is the best for chromosome identification. In this stage, in fact, the chromosomes are much longer, allowing the detection of differences in the length and distribution of the condensed chromatin (condensation pattern) along each chromosome. The analysis of cultivated rice (subsp. *japonica*) detected no chromosomal abnormalities (Figs. 1, 2, and 3). The red rice karyotype consists mainly of 24 chromosomes (2n=24). However, in the first pair of chromosomes, the longest of all the pairs, a single, very long component of the two homologues can be readily identified (Figs. 4, 5, and 6). In addition, occasional cells containing 23 (2n – 1) (Figs. 7, 8, and 9) or 25 (2n + 1) (Figs. 10 and 11) chromosomes are observed.



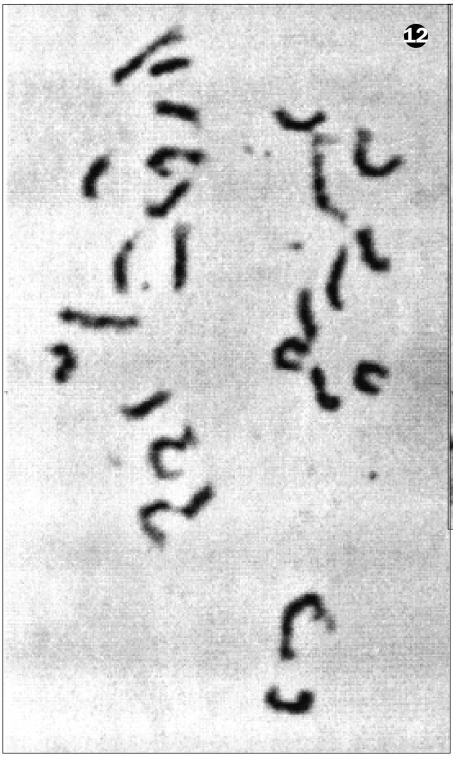
**Figs. 1, 2, and 3. Cultivated rice (*Oryza sativa* subsp. *japonica* var. *loto*). Mitotic prometaphase somatic cultivated rice chromosomes (2n=24). The two longest chromosomes of the first pair are clearly identifiable and distinguished by the number 1 within the figures.**



**Figs. 4, 5, and 6. Red rice (*Oryza sativa* var. *sylvatica*). Mitotic prometaphase somatic red rice chromosomes with 24 chromosome complements. A single chromosome of the first pair can be readily detected because it is the longest one and is labeled in the figures by the number 1.**



Figs. 7, 8, and 9. Metaphases of somatic red rice chromosomes with 23 chromosome complements (2n – 1).



Figs. 10 and 11. Metaphases of somatic red rice chromosomes with 25 chromosome complements (2n + 1).

The first observation seems to suggest the existence of a reciprocal translocation involving one chromosome of the first pair and another one not yet identified, whereas the occasional presence of aneuploids with 23 and 25 chromosomes might be the result of frequent nondisjunction at meiosis. The origin of the nondisjunction and the chromosomes involved in this process are now unknown.

Therefore, the question of suitable techniques and methods for characterizing the red rice karyotype calls for further investigation to establish how these abnormalities are related to red rice habit.

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WEED SCIENCE

# Preliminary study of the biology of *Echinochloa crus-galli* and *E. colona*

A.C. Sparacino, F. Tano, F.D. Vescovi, D. Sacchi, and N. Riva

This study was conducted in 1997 in Milan to determine the effects of three sowing depths (0, 5, and 10 cm) and two soil water conditions (saturated and submerged) on seedling emergence, third-leaf stage, number of tillered plants, and number of stems per hill of *Echinochloa crus-galli* and *E. colona.* The effects of sowing depth differed between soil water conditions for both species. Seedling emergence, rate of growth, and leaf and tiller development were negatively influenced by 5- and 10-cm sowing depths combined with submerged soil, and *E. colona* suffered more than *E. crus-galli* because of its lower 1,000-seed weight.

According to Pirola (1965) and Pignatti (1982), the *Echinochloa* genus consists of the following species: *Echinochloa phyllopogon* (Stapf.) Carv., *E. colona* (L.) Link, *E. crus-galli* (L.) Beauv., *E. crus-pavonis* (H.B.K.) Schultes, *E. erecta* (Pollacci) Pig., and *E. hostii* (Bieb.) Boros.

From the beginning of rice growth, these weeds compete with submerged rice, causing remarkable yield losses. Therefore, they have always been considered among the most troublesome weeds of rice fields. They were manually removed until the 1960s and have been controlled by chemical treatments thereafter.

The problem of *Echinochloa* spp. in rice can be dealt with by identifying the species, analyzing crop competition and control methods, and studying some biological aspects such as ecology, seed longevity and germinability, time of seedling emergence, time of dissemination, number and persistence of seeds in the soil, and reaction to herbicides.

This chapter focuses on some biological aspects of *E. crus-galli* (barnyardgrass) and *E. colona* (jungle rice). Morphological growth characteristics of *E. crus-galli* under various cropping patterns were reported by Chon et al (1994). The viability of *E. crus-galli* seed, its life cycle in the soil, and time and rate of germination were influenced by moisture and temperature (Roché and Muzik 1964). Moreover, *E. crus-galli* emerged from a depth of 15 cm but not from 20 cm. Chun and Moody (1984) found a significant reduction in germination of *E. colona* with increased sowing depth. Tolerance of flooding and poor soil aeration are quite variable among grass species (Jones 1985). Furthermore, caryopsis size is positively related to germination and seedling growth (Kneebone and Cremer 1955).

## Materials and methods

The trial was carried out in 1997 in Milan using plastic tanks (45 cm long by 37.5 cm wide by 19 cm deep). The tanks were filled to a depth of 15 cm with previously mixed sandy loam soil from cultivated land.

Two *Echinochloa* species (*E. crus-galli* and *E. colona*), three sowing depths (0, 5, and 10 cm), and two soil water conditions (saturated and submerged) were arranged in a factorial statistical design with four replications: the tanks were 48 in all. Ten seeds were placed at the appropriate sowing depth in each tank on 20 May. The seeds of the two species were 1 year old and germinability of both was 100%. The 1,000-seed weight was higher in *E. crus-galli* (4.99 g) than in *E. colona* (1.32 g). Before sowing, the soil water conditions were adjusted as required; after sowing, an electronically controlled drip system was used to maintain saturated or flooded (4 cm depth) soil conditions.

Observations were made (every 2–3 d from sowing date up to 2 mo later) to determine the number of emerged seedlings per tank, the number of plants at the three-leaf stage, and the number of tillered plants and stems per hill. Percentages of seedling emergence, plants at the three-leaf stage, and tillered plants were calculated. All the data were statistically evaluated (after transformations when necessary) by analysis of variance (ANOVA). Comparison of means was made using the t-test at 0.01 probability; least significant differences (LSD) are indicated in the Figures.

## Results and discussion

Seedling emergence, seedling growth, and tillering were, on average, more rapid in *E. crus-galli* than in *E. colona* (Tables 1, 2, and 3). The weight of 1,000 seeds and in particular the adverse environment (submerged soil and 5–10-cm sowing depths) influenced the results.

The percentage of seedling emergence (seedlings emerged per total seeds, Fig. 1) of *E. crus-galli* and *E. colona* was not affected by the soil water conditions when the seeds were put on the soil surface. In saturated soil, sowing depth did not influence the percentage of emergence, which was significantly greater in *E. crus-galli* than in *E. colona*. Therefore, we can exclude soil mechanical impedance as a cause of the differential emergence between the two species. In flooded soil, seedling emergence of *E. colona* did not occur at 5- and 10-cm sowing depths, whereas seedling emergence of *E. crus-galli* was reduced at 5 cm and was inhibited at 10 cm. That means that, in flooded soil, where oxygen is limited, germination of *E. crus-galli* and *E. colona* (as germination of rice) occurs normally if the seeds are put on the soil surface. If the seeds are covered with soil, however, the lack of oxygen causes poor

**Table 1. Percentage of seedling emergence of *E. crus-galli* and *E. colona*. Significantly different means are indicated for each species by different letters (*P* <0.01).**

| Days after sowing | *E. crus-galli* (%) | *E. colona* (%) |
|---|---|---|
| 10 | 26.6 b | 9.2 b |
| 17 | 49.4 a | 31.8 ab |
| 20 | 51.5 a | 17.9 ab |
| 22 | 52.7 a | 23.7 ab |
| 24 | 52.7 a | 26.2 a |
| 28 | 52.7 a | 26.2 a |
| 31 | 53.0 a | 26.2 a |
| 34 | 53.0 a | 26.2 a |
| 36 | 53.0 a | 26.2 a |
| 42 | 53.0 a | 26.2 a |

**Table 2. Percentage of plants at three-leaf stage in *E. crus-galli* and *E. colona*. Significantly different means are indicated for each species by different letters (*P* <0.01).**

| Days after sowing | *E. crus-galli* (%) | *E. colona* (%) |
|---|---|---|
| 17 | 8.3 c | 0.0 c |
| 20 | 20.3 bc | 1.2 c |
| 22 | 51.5 b | 7.4 c |
| 24 | 68.3 a | 34.3 b |
| 28 | 72.9 a | 49.4 ab |
| 31 | 73.3 a | 53.0 a |
| 34 | 74.3 a | 53.6 a |
| 36 | 74.3 a | 53.6 a |
| 42 | 74.3 a | 53.6 a |
| 51 | 74.3 a | 53.6 a |

seedling emergence. Differences between the two species were recorded and related to seed size; in fact, emergence of *E. colona* was reduced more at the deep sowing depth (and presumed insufficient supply of oxygen) than was that of *E. crus-galli*.

In saturated soil, the percentage of plants at the three-leaf stage (number of plants with three leaves per total plants) increased slowly until 20 d after sowing (17%), and linearly up to 13 June (90%); the peak (100%) was reached 2 mo after sowing (Fig. 2). In submerged soil, only 33% of the weed plants reached the three-leaf stage 50 d after sowing.

When the soil was saturated, these parameters were not affected by sowing depth in *E. crus-galli*; they were affected by the 10-cm sowing depth in *E. colona*.

**Table 3. Percentage of tillered plants per basin. Significantly different means are indicated for each species by different letters ($P$ <0.01).**

| Days after sowing | *E. crus-galli* (%) | *E. colona* (%) |
|---|---|---|
| 28 | 6.9 d | 4.4 c |
| 31 | 42.1 c | 15.0 c |
| 34 | 47.2 bc | 23.0 bc |
| 36 | 57.1 abc | 23.4 bc |
| 42 | 68.7 ab | 40.5 ab |
| 51 | 73.5 a | 51.6 a |
| 57 | 73.5 a | 53.7 a |
| 63 | 73.5 a | 55.7 a |

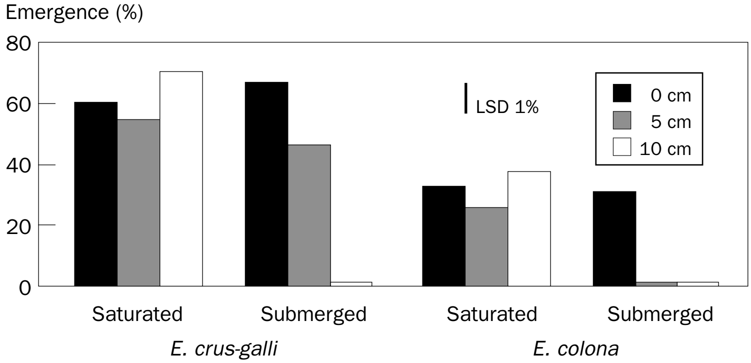

**Fig. 1. Percentage of seedling emergence of *E. crus-galli* and *E. colona* as affected by soil water conditions (saturated and submerged) and sowing depth.**

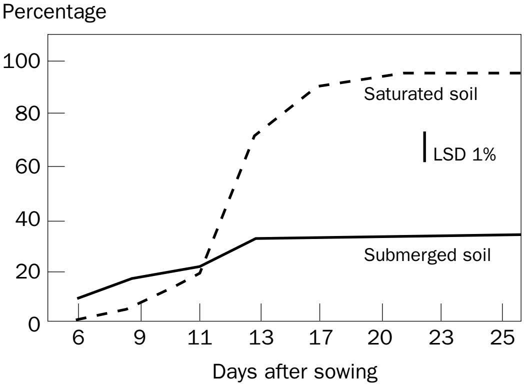

**Fig. 2. Percentage of plants at the three-leaf stage in saturated and submerged soil.**

The percentage of plants at the three-leaf stage (Fig. 3) and the percentage of tillered plants (number of tillered plants per total plants, Fig. 4) were strongly and negatively influenced when submerged soil was combined with 5-cm and especially with 10-cm sowing depths in *E. crus-galli*. The negative effect on *E. colona* was just visible with submersion in the case of surface sowing and became dramatic at the 5- and 10-cm sowing depth, as no plants reached the three-leaf stage or produced tillers.

The number of stems per plant of *E. crus-galli* and *E. colona* was greater in saturated soil than in flooded soil (Fig. 5); that number decreased at the 5- and 10-cm sowing depth (Fig. 6). In fact, in such adverse conditions, the buds in the axils of basal leaves failed to develop.

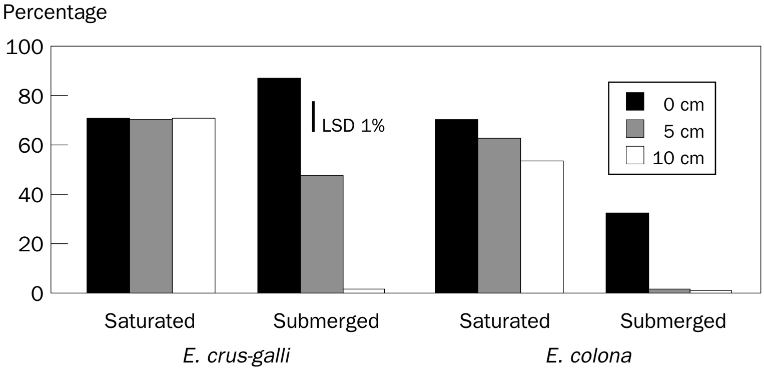

**Fig. 3. Percentage of plants at the three-leaf stage of *E. crus-galli* and *E. colona* as affected by soil water conditions (saturated and submerged) and sowing depth.**

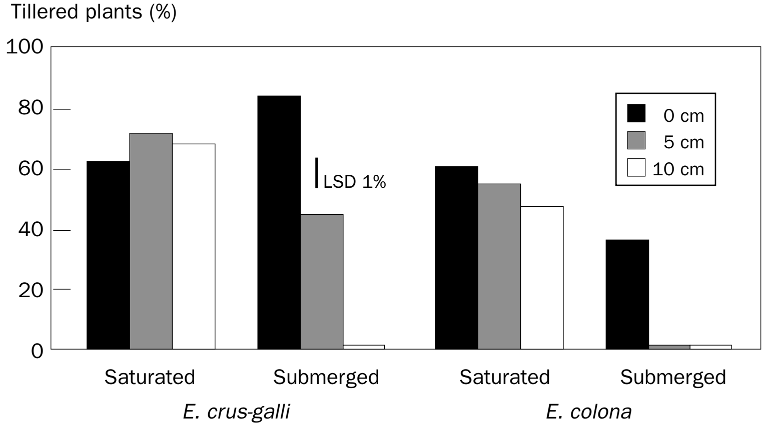

**Fig. 4. Percentage of tillered plants of *E. crus-galli* and *E. colona* as affected by soil water conditions (saturated and submerged) and sowing depth.**

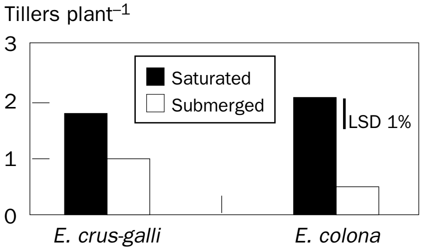

**Fig. 5. Number of tillers per plant as affected by soil water conditions.**

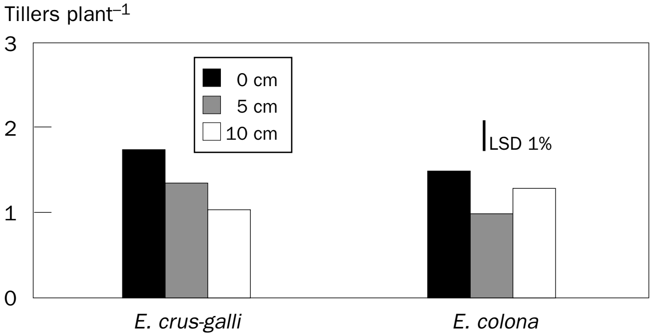

**Fig. 6. Number of tillers per plant as affected by sowing depth.**

## Conclusions

Adverse environment (submerged soil with 5–10-cm sowing depths) negatively influenced seedling emergence and leaf and tiller production of *E. crus-galli* and *E. colona*. In particular, the two species showed different morphological adaptation to poor aeration. *E. colona* suffered more than *E. crus-galli* probably because of the scarcity of its seminal reserves. In fact, the reserve of the endosperm constitutes most of the weight of the caryopsis, and the heterotrophic phase of seedling growth is responsible for early root and leaf area production.

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## Notes

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# *Echinochloa* spp. control with new herbicides in water- and dry-seeded rice in Italy

M. Tabacchi and M. Romani

Field experiments were conducted at the Rice Research Center near Castello d'Agogna (Pavia Province) and near Villanova Monferrato (Alessandria Province) in 1997 and 1998 to evaluate *Echinochloa* spp. control and rice yield with new herbicides (azimsulfuron, cyhalofop-butyl, and BAS 625 00H) applied as single or sequential postemergence treatments at different growth stages and rates compared with standard treatments of molinate, propanil, or propanil + quinclorac. Herbicide efficacy and rice injury were evaluated in five trials in both water- and dry-seeded rice. *Echinochloa* spp. control and selectivity with azimsulfuron were greater than or similar to the standard treatments, except in water-seeded rice in 1998. The efficacy of BAS 625 00H at all rates and growth stages generally exceeded sequential applications of propanil in water- and dry-seeded rice and rice injury increased at the higher rates, but without a significant yield reduction. Cyhalofop-butyl provided better control at higher rates when applied to *Echinochloa* spp. at the three- to four-leaf stage, and rice injury was lower than in all the other treatments in four of five trials.

*Echinochloa* (barnyardgrass) spp. represent one of the most common and troublesome weeds in both water- and dry-seeded Italian rice fields (Moletti 1989, 1993). Several species of the genus *Echinochloa* are present. In a survey carried out in 1991, seven species were found: *E. crus-galli*, *E. phyllopogon*, *E. crus-pavonis*, *E. colona*, *E. hostii*, *E. erecta*, and *E. frumentacea*, but only the first two were widespread in almost all rice fields (Sparacino and Sgattoni 1993).

Effective chemical control of these grasses in water-seeded rice is nowadays obtained with presowing and early postemergence application of molinate, dimepiperate, tiocarbazil, thiobencarb, or their mixtures; late postemergence (after 3–4 leaves) treatments with quinclorac and propanil are also usually used (Moletti 1993, Rapparini 1998). *Echinochloa* spp. control in dry-seeded rice is normally done with a preemergence application of pendimethalin alone or mixed with oxadiazon or thiobencarb and a double application of propanil (sometimes in combination with quinclorac) before permanent flood establishment (Moletti 1993, Moletti et al 1988). Rice weed control strategies in Italy differ depending on cultural system, soil type, water management, weeds, and herbicide restrictions in some areas (Moletti 1993, Rapparini 1998). Often, residual herbicides (such as molinate) are used to control *Echinochloa* spp. only where it is possible to maintain a flood depth of 5 to 15 cm after the application (Moletti 1989).

Furthermore, propanil is not as effective on larger barnyardgrass as it is on smaller plants and proper weather conditions at application time are needed to obtain satisfactory control. Repeated use has also resulted in the development of resistant biotypes of barnyardgrass in different countries (Baltazar and Smith 1994). Differential responses of *Echinochloa* species and biotypes to quinclorac were observed in rice fields in Spain (López-Martínez et al 1997).

This research was conducted to obtain further information on *Echinochloa* spp. control and rice yields by applying new herbicides with different modes of action in water- and dry-seeded rice. Azimsulfuron is a new sulfonylurea for postemergence application in paddy rice; it controls several common Italian broadleaf and sedge weeds, and *Echinochloa* spp. (Marquez et al 1995, Massasso et al 1996). Azimsulfuron obtained a registration permit for its use in rice in Italy in 1997.

Cyhalofop-butyl is a new graminicide belonging to the ariloxy phenoxy propionate family for the postemergence control of *Echinochloa* spp. in paddy rice. This herbicide inhibits acetyl coenzyme A carboxylase in the process of fatty acid biosynthesis (Barotti et al 1998). Cyhalofop butyl was labeled in late 1998 for paddy rice use in Italy.

BAS 625 00H (proposed name, clefoxydim) is an experimental herbicide belonging to the cyclohexanendione family (ACCase inhibitor), registered for use in rice in Latin America. Azimsulfuron, cyhalofop butyl, and BAS 625 00H were applied as single or sequential postemergence treatments at different growth stages and rates and were compared with standard treatments (molinate, propanil, or propanil + quinclorac) to control *Echinochloa* spp.

## Materials and methods

Five experiments were conducted in 1997 and 1998 at the Rice Research Center near Castello d'Agogna (Pavia Province) on a silty-loam soil with 2.0% organic matter and pH 6.0 and in the piedmont region near Villanova Monferrato (Alessandria Province) on a sandy-loam soil with 1.7% organic matter and pH 5.9. Location, sowing method, cultivar, date of planting, and herbicide applied for broadleaf weed control are provided in Table 1.

To avoid the influence of broadleaf weeds, bentazon was applied to all trials at the rate of 1.6 kg ai ha$^{-1}$ at 44 to 56 days after sowing (DAS). To control infestation of *Heteranthera reniformis* Ruiz & Pavon in water-seeded experiments, oxadiazon was applied in presowing at 0.3 kg ai ha$^{-1}$. All the trials were fertilized preplant with 250 kg ha$^{-1}$ of 18-8-30 fertilizer, 70 kg ha$^{-1}$ of prilled urea were applied at the tillering

**Table 1. Location, year, cultural system, variety, planting date, and herbicides used for broadleaf weed control.**

| Trial | Location | Year | Planting method[a] | Variety | Planting date | Broadleaf weed control Pre-emergence | Broadleaf weed control Post-emergence |
|---|---|---|---|---|---|---|---|
| 1 | Castello d'Agogna | 1997 | DS | Cripto | 3 May | – | Bentazon |
| 2 | Castello d'Agogna | 1998 | DS | Cripto | 5 May | – | Bentazon |
| 3 | Castello d'Agogna | 1997 | WS | Elio | 28 Apr | Oxadiazon | Bentazon |
| 4 | Castello d'Agogna | 1998 | WS | Elio | 6 May | Oxadiazon | Bentazon |
| 5 | Villanova Monferrato | 1998 | WS | Selenio | 30 Apr | Oxadiazon | Bentazon |

[a] WS = water-seeded, DS = dry-seeded.

**Table 2. Herbicide treatments in water-seeded trials (Castello d'Agogna).**

| Herbicide | Rate (kg ai ha$^{-1}$) | 1997 timing (DAS)[a] | 1998 timing (DAS) |
|---|---|---|---|
| Molinate | 0.36 | 24 | 26 |
| Azimsulfuron | 0.02 | 24 | 26 |
| Azimsulfuron | 0.02 | 32 | 33 |
| Cyhalofop-butyl | 0.2 | 28 | 28 |
| Cyhalofop-butyl | 0.3 | 28 | 28 |
| Cyhalofop followed by cyhalofop | 0.15 and 0.15 | 24 and 35 | 28 and 38 |
| Quinclorac | 0.6 | 30 | 33 |
| Propanil followed by propanil | 4.32 and 4.32 | 33 and 40 | 33 and 40 |
| Quinclorac + propanil | 0.5 + 2.8 | 30 | 33 |
| BAS 625 00H | 0.15 | 30 | 33 |
| BAS 625 00H | 0.2 | 30 | 33 |
| BAS 625 00H | 0.15 | 43 | 45 |
| BAS 625 00H | 0.2 | 43 | 45 |
| Untreated | – | – | – |

[a] DAS = days after seeding.

stage (1 to 3 d before permanent flood establishment in dry-seeded rice), and 50 kg ha$^{-1}$ of prilled urea were applied at the panicle initiation stage.

In Trials 1 and 2, dry-seeded rice (cv. Cripto) was planted at 170 kg ha$^{-1}$ in rows spaced 15 cm apart in a conventionally prepared seedbed. Plot size was 4.5 × 6 m. Permanent flood establishment was made 2 d after the last application of propanil followed by propanil. Trials 3, 4, and 5 were conducted in a water-seeded cultural system. In trials 3 (1997) and 4 (1998), rice (cv. Elio) was planted at 180 kg ha$^{-1}$ in flooded plots separated by plastic sheets and with independent water inlet and outlet gates. This type of plot was essential for following water management practices suggested by manufacturers to achieve good results with these herbicides. Trial 5 was

**Table 3. Herbicide treatments in water-seeded trial (1998, Villanova Monferrato).**

| Herbicide | Rate (kg ai ha$^{-1}$) | 1998 timing (DAS)[a] |
|---|---|---|
| Propanil followed by propanil | 4.32 and 4.32 | 38 and 45 |
| Cyhalofop-butyl | 0.3 | 30 |
| Cyhalofop-butyl | 0.3 | 43 |
| Quinclorac + propanil | 0.5 + 2.8 | 30 |
| BAS 625 00H | 0.1 | 30 |
| BAS 625 00H | 0.15 | 30 |
| BAS 625 00H | 0.2 | 30 |
| BAS 625 00H followed by BAS 625 00H | 0.075 and 0.075 | 30 and 38 |
| BAS 625 00H | 0.15 | 43 |
| BAS 625 00H | 0.20 | 43 |
| Untreated | – | – |

[a]DAS = days after seeding.

conducted without separated plots and rice (cv. Selenio) was water-seeded at 180 kg ha$^{-1}$. Plot size was 5.5 × 6 m.

For all the trials, the experimental design was a randomized complete block with treatments replicated four times. The herbicide treatments compared are reported in Table 2 (water-seeded treatments in Castello d'Agogna trials), Table 3 (Villanova Monferrato trial), and Tables 4 and 5 (for dry-seeded treatments).

Herbicides were applied at different weed and rice growth stages: 2nd-leaf stage to 2-tiller stage of *Echinochloa* spp. and 2nd- to 5th-leaf stage of rice in trial 1; 3rd-leaf stage to 5-tiller stage of *Echinochloa* spp. and 3rd-leaf stage to 1-tiller stage of rice in trial 2; 2nd-leaf stage to 3-tiller stage of *Echinochloa* spp. and 2nd-leaf stage to 4-tiller stage of rice in trials 3, 4, and 5.

To improve the efficacy of some treatments, the following adjuvants were added: Astrol® (nonil-phenol polioxyetilen 10%) at 1.5 L ha$^{-1}$ of formulated product in the case of cyhalofop-butyl, Trend® (nonil phenolpoliglicoletere 20%) at 0.1 % v/v together with azimsulfuron, and DASH HC (BAS 815, petroleum hydrocarbons 31.5%) at 1 L ha$^{-1}$ of formulated product with BAS 625 00H treatments.

Application of these new herbicides in water-seeded trials was made in moist or drained soil; a floodwater level (ranging from 5 to 15 cm) was established 1 to 3 d after treatments with cyhalofop-butyl and BAS 625 00H. Flooding of azimsulfuron-treated plots started 5 d after application. Herbicide treatments were carried out using a motorized backpack sprayer calibrated to deliver 300 L ha$^{-1}$ at 300 kPa.

Rice injury was visually estimated 1 to 2 wk after herbicide application by considering foliar chlorosis, plant stunting, and stand reduction (on a scale of 0 to 100 where 0 = no injury and 100 = plant death). *Echinochloa* spp. control in dry-seeded rice was determined from late July to early August by counting the number of plants

**Table 4. Rice injury, crop stand, grain yield, and *Echinochloa* spp. control in dry-seeded trial (1997, Castello d'Agogna).**

| Herbicide | Rate (kg ai ha$^{-1}$) | Timing (DAS)[a] | Injury (0–100 scale) | Rice stand (tillers m$^{-2}$) | Yield (t ha$^{-1}$) | *Echinochloa* spp. (plants m$^{-2}$) | *Echinochloa* spp. (% control) |
|---|---|---|---|---|---|---|---|
| Propanil followed by propanil | 4.32 and 4.32 | 30 and 36 | 8 | 505 | 7.1 | 8.6 | 83 |
| Quinclorac + propanil | 0.5 + 2.8 | 30 | 10 | 526 | 7.5 | 2.1 | 96 |
| Cyhalofop followed by cyhalofop | 0.15 and 0.15 | 26 and 36 | 0 | 512 | 7.3 | 5.7 | 89 |
| Cyhalofop-butyl | 0.3 | 30 | 1 | 499 | 7.7 | 0.7 | 99 |
| Azimsulfuron | 0.02 | 26 | 11 | 486 | 7.8 | 2.1 | 96 |
| BAS 625 00H | 0.1 | 30 | 11 | 472 | 7.6 | 0.7 | 99 |
| BAS 625 00H | 0.15 | 30 | 17 | 514 | 7.5 | 0.1 | 100 |
| Untreated |  | – | – | 243 | 2.1 | 51.3 | – |
| LSD (0.05) |  |  | 4.8 | 96 | 0.9 |  | 7.1 |

[a] DAS = days after seeding.

**Table 5. Rice injury, crop stand, grain yield, and *Echinochloa* spp. control in dry-seeded trial (1998, Castello d'Agogna).**

| Herbicide | Rate (kg ai ha$^{-1}$) | Timing (DAS)$^a$ | Injury (0–100 scale) | Rice stand (tillers m$^{-2}$) | Yield (t ha$^{-1}$) | *Echinochloa* spp. | |
|---|---|---|---|---|---|---|---|
| | | | | | | (plants m$^{-2}$) | (% control) |
| Propanil followed by propanil | 4.32 and 4.32 | 38 and 44 | 4 | 475 | 7.2 | 6.3 | 84 |
| Quinclorac + propanil | 0.5 + 2.8 | 40 | 4 | 465 | 7.8 | 4.3 | 89 |
| Cyhalofop followed by cyhalofop | 0.15 and 0.15 | 32 and 42 | 0 | 521 | 7.5 | 6.2 | 84 |
| Cyhalofop-butyl | 0.3 | 40 | 0 | 487 | 8.0 | 3.0 | 92 |
| Azimsulfuron | 0.02 | 40 | 5 | 464 | 7.5 | 3.3 | 91 |
| BAS 625 00H | 0.1 | 40 | 4 | 479 | 7.9 | 2.9 | 92 |
| BAS 625 00H | 0.15 | 40 | 9 | 441 | 8.1 | 0.1 | 100 |
| Untreated | | | | 280 | 4.3 | 38.2 | |
| LSD (0.05) | | | 3.1 | 124 | 1.0 | | 9.7 |

[a] DAS = days after seeding.

present in three 0.25-m$^2$ samples for each plot; in water-seeded trials, both barnyardgrass and late watergrass densities in the plots were determined.

Except for trial 5, the rice panicle density was assessed in three 0.25-m$^2$ areas for each plot. Rice was mechanically harvested with an Iseki small-plot combine when grain moisture was from 17% to 22%. Rice grain yield values were adjusted to 13% moisture.

Data obtained were subjected to analysis of variance. Means were separated using the least significant difference at $P = 0.05$. All percentage data were subjected to arcsin square root transformation before analysis of variance.

## Results and discussion

### Dry-seeded trials

*Weed control. Echinochloa* spp. control provided by BAS 625 00H at the higher rate of 0.15 kg ai ha$^{-1}$ was greater than propanil followed by propanil in both years (Tables 4 and 5). All the treatments were more effective than 83%; azimsulfuron and cyhalofop-butyl applications gave results similar to or better than standard treatments of propanil followed by propanil and quinclorac + propanil. The efficacy of chemicals was generally better in 1997 than in 1998; decreased activity was probably related to the delay in application because of heavy rainfall.

*Rice injury, crop stand, and grain yield.* All the treatments increased crop stand and grain yield when compared with the untreated control (Tables 4 and 5), but no significant differences were registered among herbicides. Cyhalofop-butyl caused less rice injury than all the other chemical treatments. BAS 625 00H at 0.15 kg ai ha$^{-1}$ caused more temporary rice injury (with a foliar chlorosis) than the other herbicides, but grain yield and stand density were unaffected.

### Water-seeded trials

*Weed control.* Azimsulfuron at 0.02 kg ai ha$^{-1}$ gave the best result (97% control) when applied at the 2–4-leaf growth stage of rice in water-seeded rice and with soil in drained conditions (Tables 6 and 7). In both years, azimsulfuron applied at the 4–5-leaf rice stage was more effective on barnyardgrass (89–94%) than on late watergrass (76–79%). Cyhalofop-butyl applied in 1997 and in 1998 at 0.3 kg ai ha$^{-1}$ on moist soil and at the 3–4-leaf stage of rice controlled *Echinochloa* spp. better than or similar to the standard treatments of molinate, propanil, or propanil + quinclorac. At the lower doses of 0.2 kg ai ha$^{-1}$, control of *Echinochloa* spp. with cyhalofop-butyl ranged from 62% in 1998 to 79% in 1997; when applied at 0.15 plus 0.15 kg ai ha$^{-1}$, barnyardgrass control was similar to that of the standard treatments, but late watergrass was controlled less than with the early application of molinate.

Over all the water-seeded experiments, barnyardgrass control with BAS 625 00H at all the rates ranged from 82% to 100%, whereas late watergrass was controlled 61% to 98% (Tables 6, 7, and 8). Greater control of *Echinochloa* spp. was obtained in 1998 with the early application of BAS 625 00H at 0.15 and 0.2 kg ai ha$^{-1}$; in 1997, late applications (1–4-tiller rice stage) at 0.15 and 0.2 kg ai ha$^{-1}$ gave

Table 6. Rice injury, crop stand, grain yield, and *Echinochloa* spp. control in water-seeded trial (1997, Castello d'Agogna).

| Herbicide | Rate (kg ai ha$^{-1}$) | Injury (0–100 scale) | Rice stand (tillers m$^{-2}$) | Yield (t ha$^{-1}$) | ECHCG[a] (% control) | ECHOR (% control) | *Echinochloa* spp. (% control) |
|---|---|---|---|---|---|---|---|
| Molinate | 0.36 | 8 | 456 | 7.6 | 99 | 95 | 97 |
| Azimsulfuron | 0.02 | 14 | 478 | 7.2 | 97 | 94 | 95 |
| Azimsulfuron | 0.02 | 11 | 487 | 7.8 | 94 | 79 | 85 |
| Cyhalofop-butyl | 0.2 | 0 | 470 | 6.7 | 87 | 73 | 79 |
| Cyhalofop-butyl | 0.3 | 0 | 483 | 7.9 | 99 | 92 | 94 |
| Cyhalofop followed by cyhalofop | 0.15 and 0.15 | 0 | 438 | 6.3 | 95 | 81 | 87 |
| Quinclorac | 0.6 | 9 | 501 | 6.9 | 81 | 98 | 92 |
| Propanil followed by propanil | 4.32 and 4.32 | 12 | 475 | 6.9 | 96 | 74 | 83 |
| Quinclorac + propanil | 0.5 + 2.8 | 10 | 490 | 7.7 | 96 | 94 | 94 |
| BAS 625 00H | 0.15 | 14 | 526 | 7.5 | 96 | 88 | 91 |
| BAS 625 00H | 0.2 | 23 | 472 | 7.6 | 100 | 97 | 98 |
| BAS 625 00H | 0.15 | 10 | 488 | 7.0 | 93 | 84 | 88 |
| BAS 625 00H | 0.2 | 13 | 480 | 7.6 | 100 | 94 | 96 |
| Untreated | | – | 243 | 3.3 | | | |
| LSD (0.05) | | 6.5 | 95 | 1.1 | 5.9 | 9.2 | 10.2 |

[a] ECHCG = *Echinochloa crus-galli*, ECHOR = *Echinochloa phyllopogon*.

**Table 7. Rice injury, crop stand, grain yield, and *Echinochloa* spp. control in water-seeded trial (1998, Castello d'Agogna).**

| Herbicide | Rate (kg ai ha$^{-1}$) | Injury (0–100 scale) | Rice stand (tillers m$^{-2}$) | Yield (t ha$^{-1}$) | ECHCG[a] (% control) | ECHOR (% control) | *Echinochloa* spp. (% control) |
|---|---|---|---|---|---|---|---|
| Molinate | 0.36 | 6 | 501 | 7.4 | 100 | 93 | 95 |
| Azimsulfuron | 0.02 | 7 | 468 | 7.4 | 95 | 90 | 91 |
| Azimsulfuron | 0.02 | 10 | 432 | 6.5 | 89 | 76 | 81 |
| Cyhalofop-butyl | 0.2 | 0 | 470 | 6.9 | 63 | 61 | 62 |
| Cyhalofop-butyl | 0.3 | 0 | 526 | 7.2 | 97 | 87 | 90 |
| Cyhalofop followed by cyhalofop | 0.15 and 0.15 | 0 | 438 | 6.3 | 91 | 70 | 78 |
| Quinclorac | 0.6 | 9 | 501 | 6.9 | 64 | 93 | 82 |
| Propanil followed by propanil | 4.32 and 4.32 | 7 | 444 | 6.7 | 92 | 40 | 59 |
| Quinclorac + propanil | 0.5 + 2.8 | 8 | 496 | 7.7 | 92 | 90 | 90 |
| BAS 625 00H | 0.15 | 9 | 532 | 7.3 | 100 | 91 | 94 |
| BAS 625 00H | 0.2 | 11 | 409 | 6.5 | 100 | 91 | 94 |
| BAS 625 00H | 0.15 | 10 | 488 | 7.0 | 97 | 69 | 79 |
| BAS 625 00H | 0.2 | 13 | 480 | 7.6 | 100 | 85 | 90 |
| Untreated |  |  | 169 | 3.6 |  |  |  |
| LSD (0.05) |  | 5.4 | 155 | 1.0 | 6.4 | 8.8 | 7.5 |

[a] ECHCG = *Echinochloa crus-galli*, ECHOR = *Echinochloa phyllopogon*.

**Table 8. Rice injury and *Echinochloa* spp. control in water-seeded trial (Villanova 1998).**

| Herbicide | Rate (kg ai ha$^{-1}$) | Rice injury (0–100 scale) | ECHCG[a] (% control) | ECHOR (% control) | *Echinochloa* spp. (% control) |
|---|---|---|---|---|---|
| Propanil followed by propanil | 4.32 and 4.32 | 4 | 56 | 71 | 67 |
| Cyhalofop-butyl | 0.3 | 0 | 94 | 80 | 84 |
| Cyhalofop-butyl | 0.3 | 0 | 86 | 63 | 70 |
| Quinclorac + propanil | 0.5 + 2.8 | 5 | 31 | 68 | 58 |
| BAS 625 00H | 0.1 | 4 | 100 | 90 | 93 |
| BAS 625 00H | 0.15 | 6 | 100 | 98 | 98 |
| BAS 625 00H | 0.2 | 11 | 100 | 98 | 98 |
| BAS 625 00H followed by BAS 625 00H | 0.075 and 0.075 | 0 | 100 | 83 | 88 |
| BAS 625 00H | 0.15 | 2 | 82 | 61 | 67 |
| BAS 625 00H | 0.2 | 3 | 87 | 71 | 75 |
| Untreated | | | | | |
| LSD (0.05) | | 3.8 | 11.4 | 15.1 | 13.2 |

[a]ECHCG = *Echinochloa crus-galli*, ECHOR = *Echinochloa phyllopogon*.

similar results (99–100% control) to the applications carried out at the 3rd- to 5th-leaf rice stage. When BAS 625 00H was applied late postemergence (1–4-tiller rice stage) at 0.15 kg ai ha$^{-1}$, late watergrass control was lower than that with all the rates applied early in the season.

*Rice injury.* Rice injury with azimsulfuron applications was similar to that with standard treatments and ranged from 7% to 14%, but rice crop stand and yields were unaffected (Tables 7 and 8). All the treatments increased crop stand density and grain yield when compared with the untreated control. No significant differences were registered for stand density; the lower doses of 0.2 kg ai ha$^{-1}$ and 0.15 plus 0.15 kg ai ha$^{-1}$ of cyhalofop-butyl resulted in lower grain yields than the standard treatments of molinate and propanil + quinclorac because of the poor *Echinochloa* spp. control. Minor rice injury was noted for applications of cyhalofop-butyl compared with all herbicide treatments except for BAS 625 00H applied at 0.15 and 0.2 kg ai ha$^{-1}$ when rice was at the 1- to 4-tiller stage. Temporary rice injury by BAS 625 00H increased at higher rates, but was greater than standard treatments only if applied at 0.2 kg ai ha$^{-1}$ when rice was at the 3–5-leaf stage.

In summary, this preliminary investigation showed that azimsulfuron, cyhalofop-butyl, and BAS 625 00H could be effective alternatives to standard herbicide treatments for controlling barnyardgrass and late watergrass in rice. In some cases, different responses against *E. crus-galli* and *E. phyllopogon* in water-seeded experiments (especially at the lower doses applied) were registered. Early postemergence applications of azimsulfuron and cyhalofop-butyl provided better results than late applications, whereas BAS 625 00H showed similar control of *Echinochloa* spp., also at the tillering stage. Soil conditions and rates and times of applications adequate to provide effective control in water- and dry-seeded cultural systems in Italy will be more precisely verified in the future.

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## Notes

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- Step 4. Identification of the main and subsidiary objectives of farmers at any time. A subsidiary objective is a checkpoint situated at a specific moment when the farmer evaluates the state of progress of his cultivation operations and compares it to the schedule planned for the achievement of the main objective. For example, the state of progress of primary tillage (plowing) is generally evaluated at the beginning of spring, but, if at a given time farmers believe that they are late (because they have not finished with this task), the scheduled program for the following month may be changed.

### Implementing the procedure for predicting sowing dates of rice

In the case we consider here, the date of sowing of rice has been chosen as the farmers' main objective. All the technical operations carried out from the harvesting of the preceding crop until the sowing of the next crop are performed to achieve this goal. Sowing dates are crucial for the success of rice productivity; thus, farmers have set deadlines they do not want to overshoot. Farmers for whom high yields are not an objective will set the completion of seeding on their farm for 20 May, whereas others, who aim at high yields, want to complete the sowing of the earliest varieties on 5 May and that of the latest on 30 April.

Throughout this clarification phase, the knowledge of agronomists (experts) is simply used for the inquiry and not to formulate opinions. At this stage, we assume that the expert knowledge is in concert with the farmers' circumstances and goals. A "model for action" or strategic work organization is developed. This means that farmers are asked to clarify management rules for normal conditions. Usually, the question is, "What kind of organization do you want to put into practice?" It is obvious that the farmers' personal "model for action" includes anticipation, which should lessen the effects of nonregular events.

So, the implementation of this methodology leads to the identification of farming resources and rules of time allocation to different blocks. These rules are based on "indicators" that are farmer-specific. All these rules, which reveal farm functioning, can be easily put in simple sentences of the following type:

if "checked indicator(s)," then "action"

At the end of this phase, a model has been built that represents the usual farmer's work organization. This is followed by a computerized application called OTELO (Attonaty et al 1990, Papy et al 1988), compatible with applying such rules. OTELO deals with work organization at the farm level for a given season, enabling an evaluation of the risks for various combinations of operations and the means of production used to implement them (labor force and equipment).

In this example, we assessed the risks of failure regarding the expected sowing dates of rice. The success or failure in reaching this objective was analyzed as a consequence of a specific organization and means of production at the farm level. An initial simulation used climatic data of the most recent year, then compared the results with the dates of sowing registered by the farmer, thus enabling validation of the

# Developing weed management strategies to achieve effective weed control in rice in New South Wales, Australia

M.C. Taylor and W.S. Clampett

Bensulfuron methyl, marketed as Londax®, was first used on Australian rice crops in the 1988-89 season. For a short period, in combination with molinate for grass weed control, it provided near perfect weed control for aerially sown rice crops. In the 1992-93 season, following confirmation of resistance to bensulfuron methyl in California, resistance was identified for the first time in the weeds *Cyperus difformis* (Dirty Dora), *Damasonium minus* (starfruit), and *Sagittaria montevidensis* (arrowhead).

In the face of developing resistance, growers confronted three choices: returning to the use of MCPA sodium, changing the establishment technique to drill seeding, or using the minor-use herbicide thiobencarb, marketed as Saturn®. None of these were attractive alternatives to rice growers and, at the onset of resistance, no promising new herbicides were known.

Initially, the problem of herbicide resistance was perceived as a poor herbicide product, a poor application, or a specific weed problem. We discuss the transition from this perception to a more holistic one that resistance is a problem of the rice industry in its broadest context—growers, agribusiness service providers, all agrochemical companies, industry organizations, and research and extension services.

As a result of these events, an "industry" Rice Weed Management Working Group to coordinate a holistic and cooperative approach to rice weed management has been developed. This has also facilitated the integration of new herbicide products into weed control programs and options and a broadening of weed management and resistance problems to include grass weeds.

In New South Wales (NSW), Australia, weed control is a major cost of growing rice, representing some 20% to 25% of the total costs of production of the 150,000-hectare rice crop. The development of herbicide resistance as a major problem during the last decade has provided the New South Wales rice industry with the twin challenges of maintaining both economic weed control and a range of effective herbicides from which to choose.

Before the 1988-89 rice season, the phenoxy herbicide MCPA sodium was used almost exclusively for the control of the aquatic weeds *Cyperus difformis* (Dirty Dora, CYPDI), *Damasonium minus* (starfruit, DAMMI), and *Sagittaria montevidensis* (arrowhead, SAGMO*),* the major weeds in aerial-sown rice crops. At that time, 60–65% of the 100,000-ha NSW rice crop was sown by this technique.

Control results with MCPA sodium were not always satisfactory, which, along with delayed application until the midtillering stage of rice, injury to the growth of the rice, and the risks of drift to susceptible crops, made it a less than satisfactory treatment. Nevertheless, along with the grass herbicide molinate, MCPA sodium was the standard for weed control in aerial-sown rice.

In the 1988-89 season, the sulfonylurea herbicide bensulfuron-methyl (Londax®) was first used on Australian rice crops. In the first season, it captured 50% of the aerial-seeded rice market and in the following season 95%. From 1988-89 to 1992-93, as aerial sowing increased to 93% of the 120,000-ha rice area, bensulfuron-methyl and molinate for grass weed control became the standard for weed control in aerial-sown rice, with MCPA sodium use declining to insignificant levels. The results were excellent, providing near perfect weed control in most cases. Rice growers became totally dependent on bensulfuron-methyl for aquatic weed control. In the 1992-93 season, after the identification of resistance to bensulfuron-methyl in California, resistance in four populations of *C. difformis* was confirmed in NSW rice fields after four to five treatments with bensulfuron-methyl (Davis and Fader 1994). The use of bensulfuron as the sole means of aquatic weed control coupled with the widespread movement of weed seed in soil and floodwater suggested that bensulfuron resistance would continue to develop in NSW rice fields and was a major threat to the industry. Soon afterward, resistance in the Alismataceae family weeds *D. minus* and *S. montevidenis* was also confirmed.

In early 1993, 30 weed seed samples from the 1992-93 rice crop were assessed for the presence of resistance under glasshouse conditions (Fowler and McCaffery 1994). Seventeen out of 19 samples of *C. difformis* showed signs of resistance; resistance was also apparent in *D. minus* (2 out of 5 samples) and *S. montevidensis* (2 out of 6 samples) populations. In the following 1993-94 season, 16 out of 24 samples of *C. difformis* tested positive for resistance as well as one sample of *D. minus*.

## The response

Initially, the rice industry's first question was, What about alternative herbicides? In the short term, the prospects for new herbicide registration were poor and many growers could not use them because of the increasing presence of susceptible crops within rice areas. Thiobencarb (Saturn® EC) was used on a small fraction of the rice area, mainly as a grass herbicide that also controlled the weed *C. difformis.* As a grass herbicide, it was less adaptable than molinate. Propanil was rarely applied because of its relatively poor reliability under cool conditions and high cost.

Because herbicide resistance was not a problem to 99% of the individual rice growers, obviously there was a need to educate growers about the realities of herbicide resistance and provide them with advice on how to avoid, delay, or react to the problem. Most growers chose not to respond to the threat until resistance was confirmed on their own farms.

Three basic needs were identified:

- Develop the best management strategies and practices to avoid, delay, and/or manage herbicide resistance using all currently available herbicides.
- Educate growers about herbicide resistance in rice fields.
- Encourage growers to adopt the best management practices developed.

Initially, extension messages focused on understanding resistance and recommendations based on existing knowledge and resources. At that time, the recommendations for management were to

- Monitor crops to identify resistance as soon as possible. To this end, a "bin test" was developed by DuPont Australia Ltd. This involved using a plastic garbage or trash can, with the bottom cut out as a simple means of isolating a plot. Once in place, a one, two, and/or three times rate of bensulfuron was applied to the weeds within the ring. Seed could be harvested from suspect surviving weeds assessed for resistance.
- Consider drill seeding where the aquatic weeds were not usually a problem. This was not particularly palatable to rice growers, who found aerial sowing (water seeding) logistically preferable.
- Use the alternative herbicides MCPA sodium and thiobencarb.

At the early stages, evidence of resistance was not readily visible to most growers. Thus, extension opportunities were used to promote the information. The problem of resistance was an abstract issue to most rice growers and understandably not their problem.

The interpretation of bin tests and resistance screening results was initially somewhat erratic. Furthermore, the extent of resistance was sometimes played down or it was suggested that higher rates of herbicide application would overcome the problem. As resistance became obvious, more growers returned to MCPA sodium and, as better application strategies developed, thiobencarb was increased.

## Developing the best management strategies for herbicide use

Bensulfuron resistance is reportedly caused by insensitive ALS, with the resistant biotypes exhibiting similar fitness to susceptible ones (Davis and Fader 1994); thus, rotating to herbicides of a different mode of action simply delays the onset of resistance development for a period directly proportional to the number of seasons away from bensulfuron (Cotterman 1994).

A computer model developed to predict herbicide resistance in annual ryegrass (*Lolium rigidum*) populations was adapted to predict outcomes from a variety of herbicide resistance management strategies in rice (C. Preston, personal communication). The model predicted rapid development of bensulfuron resistance (i.e., within six applications) in *C. difformis, S. montevidensis,* and *D. minus* populations if bensulfuron was the sole herbicide used for control of the species. When a second herbicide with an alternate mode of MCPA sodium was provided in the same season of use as bensulfuron, the frequency of resistance to bensulfuron did not increase markedly provided the efficacy of MCPA sodium exceeded 80% control. This work confirmed the necessity of an effective herbicide with a second mode of action to delay the development of herbicide-resistant populations.

Combination partners for bensulfuron must emulate the same pattern of susceptibility and residual control period to achieve an effective second mode of action (Gressel and Baltazar 1996). In practice, the options for New South Wales have been MCPA sodium (applied later than bensulfuron) to capture the same cohort and range of weed species and thiobencarb applied in a similar application window that targets only *C. difformis*.

## Research into alternate herbicides

Water-seeded rice culture in NSW is limited to a small number of herbicides (molinate, thiobencarb, bensulfuron, propanil, and MCPA sodium) that offer sufficient rice tolerance while possessing efficacy against what is often a synchronous germination of aquatic weeds and crop. Many herbicides that are tolerated by transplanted rice (e.g., chlor-acetamides, dinitroanilines, and s-triazines) are not suited to water-seeded culture, thus limiting opportunities to combine herbicides with an alternate mode of action to bensulfuron. Since 1994, a key objective of rice weed control research in NSW has been to search for new herbicides for water-seeded rice that offer alternate modes of action to bensulfuron against aquatic weeds. New ways to integrate existing herbicides in programs that achieved multiple modes of herbicide action were also evaluated.

### Thiobencarb

Thiobencarb is highly effective against *C. difformis* in addition to grass weeds and is active in floodwater. *Damasonium minus* may be suppressed by thiobencarb, while *S. montevidensis*, *Alisma plantago aquatica* (water plantain, ALSPA), and *Alisma lanceolatum* (alisma, ALSLA) are unaffected by this herbicide. It cannot be applied safely to water-seeded rice in the sowing to 1.5-leaf stage. By waiting until rice reaches the 1.5-leaf stage, grass weeds and *C. difformis* are often too advanced for control by thiobencarb. Thus, the timing of thiobencarb requires a very rapid (< 5 days) succession of field operations to prevent weed escape.

A presowing treatment of flooded fields with molinate or thiobencarb followed by treatment with a thiobencarb plus bensulfuron combination was identified as a means of ensuring effective control of grass weeds, as well as including thiobencarb into the program as a second mode-of-action herbicide against *C. difformis*. This program increased both the potential phytotoxicity to rice and the cost. Rate definition studies led to regulatory approval to use molinate at lower than standard rates of 960 to 1,440 g ai ha$^{-1}$ at or around the sowing of rice ("presow priming") before treatment with thiobencarb plus bensulfuron. A second derivation of this technique was the registration of split applications of thiobencarb at 800 to 1,200 g ai ha$^{-1}$ at sowing, followed by thiobencarb at 2,200 g ai ha$^{-1}$ at the 2-leaf stage of rice in a tank mixed with bensulfuron. These programs have seen widespread adoption over the past two seasons, achieving excellent control of grass weeds and of bensulfuron-resistant *C. difformis* populations as a result of the two modes of action. Bensulfuron-resistant broadleaf weeds were not challenged by a second mode-of-action herbicide with these programs unless MCPA sodium was subsequently applied.

### MCPA sodium

Optimum timing for MCPA sodium is at the early tillering stage of rice, whereas the agronomic advantage associated with bensulfuron from early weed removal arises from application at the 2-leaf stage of rice. Split applications of bensulfuron at the 2- to 3-leaf stage of rice development, followed by MCPA sodium at the 2–3 tiller stage, allow the objective of two modes of action per species per season to be achieved; however, this strategy is not favored by many rice growers.

Rice growers readily use MCPA sodium where there is a competitive weed problem and usually where scattered weeds indicate the likelihood of resistance. However, they generally will not spray visually clean fields to remove only a few weeds to prevent resistance for the following reasons:

- The additional expense associated with an extra herbicide and aerial application.
- Risk of phytotoxicity from MCPA sodium, thus reducing grain yield potential.
- Risk of aerial spray drift to off-target crops, gardens, and plantations.

Ground-based boom spraying in rice is uncommon in New South Wales; therefore, almost all growers rely on MCPA sodium applications via fixed-wing aircraft. Effective coverage of weeds with MCPA sodium is not always achieved by aerial application, thereby reducing the effectiveness of this strategy as a second mode-of-action herbicide. Limited studies of MCPA sodium tolerance under weed-free conditions concluded that rice injury caused by MCPA sodium phytotoxicity is unlikely to affect rice grain yield if full-season varieties are sown on time in average or warmer than average seasons. Severe grain yield penalties (averaging 12.8%) were measured where a short-season variety was sown late in November.

## Multiple modes of action versus herbicide rotation?

The adoption of a minimum of two modes of herbicidal action per species per season as the principal strategy to delay resistance was premised upon the assumption that fitness between resistant and susceptible biotypes was equivalent. This may not hold for resistance to classes of herbicides other than bensulfuron; thus, rotation of herbicide groups is also worthy of consideration and increasingly viable as new herbicides are introduced (e.g., clomazone or benzofenap). Label instructions for benzofenap specifically warn users not to apply the herbicide to consecutive rice crops in the same field.

Conscious adoption of herbicide-resistance management practices by NSW rice growers has tended to occur only when failures in weed control appear. A second (alternate) mode-of-action herbicide in most cases means using MCPA sodium. Its application to a seemingly clean crop (e.g., MCPA sodium after bensulfuron) is a particularly difficult decision for any crop manager, given the cost of the operation, the attendant risk of drift onto neighboring crops, and the absence of any obvious target for the herbicide. Yet, modeling of the development of herbicide-resistant weed populations in rice suggests that such retreatment is essential to prevent the rapid development of herbicide-resistant weed populations.

## Sequential delivery

Where the second mode-of-action herbicide requires sequential application, the survival of some weeds may provide a good reason for crop managers to apply the second herbicide. This suboptimal rate approach will necessitate choosing initial herbicide application rates that do not control all the weeds. Current herbicide recommendations in NSW are aimed at consistently controlling all target weeds with no appreciable survival.

A low-rate sequential strategy using all available herbicide options was devised and tested in New South Wales over three seasons (Taylor 1998). Lower-than-label rates of molinate, thiobencarb, and bensulfuron were sequenced with a full-label rate of MCPA sodium in water-seeded medium- and long-grain rice varieties. This achieved reliable crop safety and weed control while challenging all aquatic weeds against two or three modes of herbicide action. Only a suppressant rate of bensulfuron (12 g ai $ha^{-1}$) was applied, with the intention of allowing survival of a proportion of the susceptible weed population (albeit in a stunted and less competitive state) to trigger the crop manager to retreat with MCPA sodium. While reducing selection intensity for target-site resistance, this approach may increase selection intensity for metabolic resistance to bensulfuron and thiobencarb. Inclusion of MCPA sodium at a full rate guards against the latter occurring. A lower-rate strategy for the initial herbicide application poses a considerable dilemma to agronomists and rice growers as this may be contrary to commercial objectives or contravene legal and warranty requirements. Little commercial adoption of this strategy has occurred as few champions exist for this approach.

Simultaneous delivery of more than one mode-of-action herbicide can be achieved through tank mixtures of thiobencarb plus bensulfuron (against *C. difformis* only). Simultaneous delivery of multiple modes of action is clearly a desirable objective, yet is currently unattainable across a range of weed species because of differing windows of application (e.g., benzofenap at sowing versus bensulfuron at the 2-leaf stage of rice) and often inadequate crop tolerance by such combinations.

Rotation of herbicides may simply delay the development of resistance. However, this delay may be useful, providing it does not significantly increase the costs of weed control, as it can maintain a continuing choice of products and hence flexibility of weed control for the future. The time delay may also allow the development of new strategies or new herbicides.

## Rice Weed Management Working Group

Initially, the problem of herbicide resistance was perceived as a poor herbicide product, poor application, or a specific weed problem. The transition from this perception to a more holistic one that resistance is a problem of the rice industry in its broadest context—growers, agribusiness service providers, all agrochemical companies, industry organizations, and research and extension services—is seen as an important development in achieving better weed management in rice crops.

The consequent development of an "industry" Rice Weed Management Working Group to coordinate a holistic and cooperative approach to rice weed management occurred in 1996. The Working Group has representatives from the agrochemical companies supplying rice herbicides, the agribusinesses servicing and retailing herbicides to rice growers (pest control advisers), the Rice Research and Development Committee (the grower-run R & D funding body), rice herbicide R & D agencies, and NSW Agriculture. This Group develops and modifies weed management strategies and programs each year, which are then delivered to rice growers. The integration of new herbicide products into the weed control programs and options is also canvassed within the working group. The focus on weed management has also broadened to include grass weed control.

## Integration of new herbicide products

Two new herbicides (benzofenap and clomazone) entered commercial use in NSW water-seeded rice production during the 1998-99 season. Each represents a new and novel mode-of-action herbicide relative to currently used herbicides. Benzofenap (Taipan) is a group F2 herbicide (bleaching; inhibition of 4-HPPD) developed by Rhone-Poulenc Rural Australia Pty. Ltd. for aquatic broadleaf weed control in water-seeded rice (Skinner and Taylor, this volume). Clomazone (trade name Command) is a group F3 herbicide (bleaching induced by inhibition of carotenoid biosynthesis) developed by FMC International A. G. for *Echinochloa crus-galli* and *Leptochloa fusca* (silvertop grass, LEFFA) control in water-seeded rice (Pegg et al, this volume). Both clomazone and benzofenap enable rotation of multiple modes of herbicide action from season to season and within a single season. This is achieved by alternating seasonally between bensulfuron and benzofenap and always retreating with MCPA sodium. Approximately 40% of the crop was retreated in the 1999 season with MCPA sodium. These opportunities are now reflected in recommendations made to growers for commercial herbicide use (see Table 1).

**Table 1. List of the current six commercial herbicide programs and the number of modes of action on aquatic weeds.**

| Program<br>(rates as g ai ha$^{-1}$) | *Cyperus difformis* | *Damasonium minus* | *Sagittaria montevidensis* | *Alisma lanceolatum* and *Plantago aquatica* (seedlings) |
|---|---|---|---|---|
| *Standard molinate program* | | | | |
| Molinate 2.5–3.75 L at pre- to postsowing<br>Londax 50–85 g at 2-leaf stage of rice<br>MCPA sodium 2.7 L at 3-tiller rice stage | √√√ | √√ | √√ | ++ |
| *Standard Saturn program* | | | | |
| Saturn 3.75 L at secondary-root rice stage<br>Londax 50–85 g at 2-leaf stage of rice<br>MCPA sodium 2.7 L at 3-tiller rice stage | √√√ | √√ | √√ | ++ |
| *Molinate primer program* | | | | |
| Molinate 1–1.5 L at presow<br>Saturn 3.75 L at secondary-root rice stage<br>Londax 50–85 g at 2-leaf stage of rice<br>MCPA sodium 2.7 L at 3-tiller rice stage | √√√ | √√ | √√ | ++ |
| *Split Saturn program* | | | | |
| Saturn 1.0–1.5 L at presowing<br>Saturn 2.75 L at secondary-root rice stage plus<br>Londax 50–85 g at 2-leaf stage of rice<br>MCPA sodium 2.7 L at 3-tiller rice stage | √√√ | √√ | √√ | ++ |
| *Taipan program* | | | | |
| Taipan 2 L at presow to early postsowing<br>Molinate 2.5–3.75 L at pre- to postsowing<br>MCPA sodium 2.7 L at 3-tiller rice stage | +√ | √√ | √√ | √+ |
| *Command program* | | | | |
| Command 0.4–0.6 kg ai ha$^{-1}$ at presow to 2-leaf stage of rice<br>Londax 50–85 g at 2-leaf stage of rice<br>MCPA sodium 2.7 L at 3-tiller rice stage | √√ | √√ | √√ | ++ |

1. One check mark (√) for each herbicide action per species, minimum of two (√√) required for sound resistance management of aquatic weeds. 2. A plus sign (+) indicates a mode of action where the herbicide used may only suppress the weed or not carry a label claim for control of that weed species. 3. Number of modes of action assumes that no significant resistance to Londax is present.

Source: Clampett and Stevens (1998).

## Future opportunities

Several useful herbicide groups are not currently registered for use in NSW rice production, notably HRAC groups A (inhibition of ACCase), C (inhibition of photosynthesis), E (inhibition of protoporphyrinogen oxidase), G (inhibition of EPSP synthase), H (inhibition of glutamine synthetase), and K3 (inhibition of cell division). Some of these herbicides are currently being investigated for local registration. Additionally, a mycoherbicide exhibiting some potential control for *A. lanceolatum* and *D. minus* has been under evaluation in NSW for seven years (Cother and Gilbert 1994, Jahromi, this volume).

Rice varieties tolerant of glyphosate and glufosinate present opportunities to incorporate novel modes of herbicide action across a broader range of aquatic and terrestrial rice weed species. As both require foliar spraying in water-seeded rice, appreciable early weed competition is likely to occur until the weeds are large enough to spray. Irrigation water supply and management practices are not conducive to fully or partially draining fields to expose weeds at this stage. Aerial application of either herbicide will probably be problematic because of the attendant risks of spray drift. Ground-based boom spraying of these herbicides in drill-seeded culture appears to be the most practical means for commercial introduction in NSW.

Modeling has played only a peripheral role in determining what strategies could be adopted to manage herbicide resistance in rice weeds to date. We have applied estimates of selection intensity based on observations of optimal rates for the individual components (judged by agronomic performance). Yet, most of these strategies fail in the typical period of 3–15 seasons of application. A more helpful application of modeling would be to estimate what selection intensities are tolerable for each herbicide or cultural practice within the strategy (without jeopardizing their long-term viability) and how best to integrate these. Agronomic performance could then be measured against existing practices to determine whether such sustainable practices are economically viable. A simple example is to assume that one has two herbicides to target a single weed species and that one of these herbicides presents a high risk of resistance development and the other a moderate or low risk, and then answer the following question: What are the optimal percentage kills to be targeting if the two are used in succession or concurrently?

Opportunities to integrate nonchemical weed control methods with herbicides in water-seeded rice production in NSW appear limited. Options used in dryland crops such as rotation, delayed planting to germinate and kill weeds, in-crop tillage, mulching, and seed collection are of little practical benefit when producing a crop that is limited by water and infrastructure availability, growing season, and the logistics of working in wet fields. However, the best management practices that produce a seedbed free of germinated weeds before flooding and free of perennial weeds; a vigorous, competitive rice canopy; good water management to suppress weeds and optimize rice growth and herbicide activity; and effective herbicide rate and application techniques will remain essential to achieving total weed management.

## Current herbicide programs

Clampett and Stevens (1998) detailed the key strategies and programs for managing herbicide resistance and achieving effective aquatic weed control in rice for 1998-99:

- Maximizing the effectiveness of the herbicides used by good management before, during, and after application.
- Using two herbicides, that is, two different modes of action for each aquatic broadleaf weed.
- Rotation of herbicides, that is, where possible use a different herbicide with a different action in the subsequent rice crop.

### Two herbicides for each weed

Applying two herbicides with two different modes of action for each aquatic weed aims to ensure that weeds resistant to one herbicide are controlled by the other herbicide. In 1998, this meant applying MCPA sodium as the second herbicide in all the currently available herbicide programs.

The six basic herbicide programs for 1998 were

A. Standard molinate program—molinate followed by (fb)/plus Londax® fb MCPA sodium
B. Standard Saturn program—Saturn fb Londax fb MCPA sodium
C. Molinate primer program—molinate fb Saturn plus Londax fb MCPA sodium
D. Split Saturn program—Saturn fb Saturn plus Londax fb MCPA sodium
E. Taipan® program—Taipan plus or fb molinate fb MCPA sodium
F. Command® program—command fb Londax fb MCPA sodium

Table 1 provides details on the programs and the numbers of modes of action on aquatic weeds.

### Rotate herbicides

Rotating herbicides by using a different herbicide with a different mode of action when the next rice crop is grown will delay the development of resistance, that is, where two herbicides are available to control a weed or weed group, alternate from one herbicide one year to the other herbicide the next. The permit label for Taipan recommends the use of a different herbicide in the following rice crop.

### Good management for the effective use of all herbicides

Good weed control in rice involves *integrated weed management*—the combination of cultural methods, herbicide use, and water depth management before, during, and after herbicide application. Each weed species and each herbicide used have their own specific requirements that must be integrated into the rice management system.

Field layout—A land-formed well-graded field with 5-cm contour intervals will facilitate timely cultivation, rapid filling, and accurate water depth control. Land preparation should aim for no weeds being present or germinated before flooding. If weeds are present, a knockdown herbicide or further cultivation to control weeds should be considered.

Ridging rollers are ideal for reducing clod size, without further cultivation, thus enabling quicker water coverage and less water use at filling up.

Aim to fill up and sow within 7 days. Rapid filling and sowing bring weed and crop development closer together, a help to weed management. Water depth at and immediately after sowing should aim for full coverage of the field (for good weed management) but also shallow water of 10 cm or less (to encourage rice growth and vigor).

Use each herbicide properly as required by the directions for use. Particular attention must be given to the growth stages of the weeds and the rice, herbicide and water application rates, and water depth management before and after application. All herbicides used at establishment require water movement to cease before application, followed by a holding period of 3 to 5 days. Checks the label for details.

The rate of herbicide used should be effective within the recommended label range.

MCPA sodium is an effective herbicide—it requires careful use to maximize weed control, minimize injury to rice growth, and avoid drift to nontarget crops and other vegetation.

Encourage dense, and even vigorous, stands of rice to develop an early full canopy cover to compete with weeds and prevent late germination. Aim for 150–300 rice seedlings $m^{-2}$.

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## Notes

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# Molecular mapping of rice cold tolerance using an indica-japonica cross

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Low-temperature stress is commonly associated with rice cultivation in temperate climates. In these areas, rice cultivars with adequate tolerance of cold are needed at either the seedling or reproductive stage, or both. Progress in breeding for cold tolerance is particularly slow because simple and reliable methods for evaluation are lacking. Where controlled-environment facilities are not available, candidate tolerant lines are identified using natural low-temperature conditions. In these conditions, daily temperature fluctuations and differential development and maturity of test materials lead to less reliable selection. Thus, the identification of molecular markers associated with cold tolerance at critical plant development stages will be useful in breeding and elucidation of mechanisms of tolerance.

In this study, genetic loci controlling cold tolerance at the seedling stage were located using tolerance estimates from the $F_3$ generation of 198 $F_2$ plants derived from a cross between a cold-tolerant japonica cultivar (M-202) and a cold-susceptible indica cultivar (IR50). Rice seedlings at the 3-leaf stage (approx. 14 d after emergence) were exposed to 9 °C temperature for 12 d in a growth chamber. M-202 and IR50 were replicated 10 times and monitored closely for visible manifestation of cold injury. Withering because of leaf necrosis and wilting was observed for IR50 as early as 7 d of treatment compared with minimal damage observed for M-202 even at 12 d of treatment. The number of wilted plants per $F_3$ family was counted after 12 d of treatment as a measure of tolerance. Treated plants were allowed to recover in the greenhouse for 10 d and the number of surviving plants per family was counted.

A linkage map composed of 62 microsatellite markers and two RFLP markers was constructed using the $F_2$ population. QTL analyses were performed via analysis of variance (qGene) and interval analysis (MAPMAKER/QTL). A single major locus controlling cold tolerance at the seedling stage was detected on chromosome 12. The locus was flanked by microsatellite marker RM247 and RFLP marker RZ397 (9.2 cM apart) and accounted for more than 50% of the phenotypic variance.

# MANAGE RICE: a decision support system for nitrogen fertilizer management in relation to cold damage

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This poster describes a PC-based decision support system used by growers, advisers, and fertilizer resellers in the Australian rice industry. It is now in its fifth year of release and is used by about a quarter of the 2,000 rice growers, or half of those who own PCs.

MANAGE RICE addresses the problem of cold damage at the microspore stage, which is one of the key problems of rice growing in southeastern Australia. Cold damage is greatest in crops of high-N status but is reduced when the microspores are thermally insulated by increasing floodwater depth to about 20 cm. In the worst situation of a sensitive variety, with high-N status growing in shallow water when the minimum air temperature is less than 10 °C, total yield loss may occur. Rice growers need to balance the risk of cold damage in relation to crop N status and water depth.

The aim of the package is to give users information about the likely yield response and profitability of nitrogen fertilizer topdressing at the panicle initiation stage in relation to water depth and the risk of cold damage. The inputs required from users are sowing date, maturity of the selected variety, location, plant tests of shoot dry weight and N concentration at the panicle initiation stage, intended water depth at the microspore stage, and the expected rice price and fertilizer cost.

The package contains a simple model that simulates yield in response to different amounts of N and the weather for a specified year or years since 1953. It produces an estimated N-response curve and financial returns based on users' input data on costs and prices. As well as presenting average profitability, it shows the probability of achieving a specified financial return based on the responses simulated with the historical weather data.

The package includes a database so that growers can save and recall data for their own crops; it also contains records of regional experiments so that skeptics can check the accuracy of the model.

The package runs under Windows®, using a graphic user interface and database-handling routines written in DELPI, with a rice-growth simulation model written in FORTRAN. An Internet version is planned to provide on-line access to current weather and prices.

# Agar technique to confirm herbicide-resistant watergrass (*Echinochloa phyllopogon* and *E. oryzoides*)

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The unique water-seeded California rice production system in heavy soils resulted in great reliance on the few herbicides available in California. This consequently resulted in bensulfuron-resistant broadleaf weeds and sedges in 1993 and, most recently, thiobencarb-, molinate-, fenoxaprop-, propanil-, and bispyribac-sodium-resistant watergrasses—*Echinochloa phyllopogon* and *E. oryzoides.* The early identification and confirmation of herbicide-resistant weeds are important for implementing management techniques that would reduce the spread of these weeds. The whole-plant bioassay technique is presently the most used technique to detect and confirm herbicide resistance in weeds. With late watergrasses, this technique can take 35 to 45 days to implement, beginning with seed samples from suspected herbicide-resistant grass accessions. A more rapid and less costly technique than the whole-plant bioassay uses 3- to 4-wk-old young grass seedlings that are dissected to 0.5 to 1-cm length enclosing the meristem. The dissected shoots are grown for 7 days in petri dishes containing a solution of agar and herbicide at different doses to obtain a dose-response curve. A known susceptible (S) watergrass accession is grown in the same petri dish as the suspected resistant (R) accession. The number of dead shoots (R and S) per herbicide dose is determined and a probit-logit analysis is used to determine the dose at which 50% of the experimental plants are killed ($LD_{50}$). The fresh weight of the surviving shoots is also measured and the data plotted to get a dose-response curve that is used to determine the dose at which fresh weight is reduced by 50% ($GR_{50}$). The ratio of the $GR_{50}$ and $LD_{50}$ for resistant to susceptible accessions for each herbicide is determined to characterize the level of resistance. Unlike the whole-plant bioassay technique, which needs more materials, methods, and time, this technique is faster and more economical.

# Cultivar resistance to stem rot of rice in Uruguay

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Stem rot of rice, caused by the fungus *Sclerotium oryzae* Cattaneo, is of high importance in Uruguay. A group of lines in the final steps of selection by the breeding program and checks were included in field work that consisted of three experiments with a different degree of infection by *S. oryzae* in the 1996-97 crop season.

Infection severity by stem rot, yield, and grain quality lost were compared in experiments with natural infection, artificial inoculation, and protected with fungicide. The levels of infection reached did not establish differences between natural and artificial infection, and then comparisons were made between protected and nonprotected plots.

In the 1996-97 crop season, the levels of stem rot infection were unusually high: all the cultivars showed a high degree of severity because of an early infection and the yield in all the experimental fields was 18% lower than in anterior crop seasons.

The stem rot degree of severity % was 74.4% of the average in the naturally infected experiments and 55.2% in the protected ones. Grain yield was 4.8 and 6.4 t ha$^{-1}$, respectively. Sterility averaged 28.7% in the nonprotected plots and 14.6% in the protected ones. The disease also affected grain weight and industrial yield negatively and increased the chalky grains.

The cultivars that showed a lower degree of severity in the nonprotected plots were El Paso 144, L 1435 (actually INIA Cuaró), and PI574487, which registered as resistant to *Rhizoctonia solani* in the United States. Among the nine lines and five checks evaluated, we could detect significant differences in disease incidence and in the reaction to fungicide application.

# Factors affecting K absorption by rice

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Potassium deficiencies in lowland irrigated rice, although infrequent, appear to be increasing. However, visual deficiency symptoms do not appear among all cultivars grown in the same fields. This inconsistency in susceptibility to K deficiency suggests that cultivars may differ in the rate or quantity of K absorbed at critical times during the season. The objective of several of our studies is to identify factors that influence K absorption by rice roots under flooded conditions.

A mechanistic model was evaluated for its ability to predict K uptake by rice. Greenhouse and field studies were conducted with three cultivars—Lemont, Katy, and Mars—and associated root, soil, and K kinetic uptake model input parameters were measured. A satisfactory prediction of K uptake was obtained during vegetative growth but not during reproductive growth. The lack of prediction during reproductive growth may have resulted from rapid senescence of roots following booting, thus precluding an accurate measurement of root length development between measurements, a redistribution of K throughout the plant during reproductive growth, or changes in kinetic uptake parameters with plant age. Sensitivity analysis showed that the maximum rate of K uptake and the size of the root surface area most affected K uptake. Surprisingly, cultivars differed in the maximum rate of K uptake (Imax), suggesting that differences in Imax might account for differences in K uptake and susceptibility to K deficiencies.

On the basis of the information from the model, we began studies to measure the kinetic uptake parameters of Katy, Mars, Lemont, Bengal, Cypress, Kaybonnet, and Lagrue. Potassium depletions were conducted on each cultivar at maximum tillering in a growth chamber with a 16-h day temperature of 30 °C and an 8-h night temperature of 27 °C. Following the depletions, plants were harvested and shoots were separated from roots. The average root lengths for all cultivars except Bengal ranged from 774 to 1,657 m per plant. In contrast, the average root length for Bengal was 2,413 m per plant. Thus, it would appear that Bengal develops a much larger root system than the other commonly grown rice cultivars, thus having a greater root surface area in contact with a greater volume of soil to absorb more K. Often, root length development is in concert with shoot development, such that cultivars with larger root systems will also produce larger shoots. In our study, we found that the average shoot-to-root dry weight ratios for all cultivars except Bengal ranged from 8.2 to 9.8. The average shoot-to-root ratio for Bengal was 6.8. Thus, it would appear that Bengal allocates more photoassimilate to the development of a larger root system and less assimilate to the shoot than the other rice cultivars. These data suggest that, under similar concentrations of soil solution K, Bengal has the potential to absorb more K per unit of time than the other rice cultivars in this study.

Interestingly, Cypress also exhibits K deficiency in the field more frequently than many of the other cultivars. However, the size of the root system of Cypress was nearly half the size of that of Bengal and similar in size to that of the other cultivars in this study. Susceptibility of Cypress to K deficiency may result from a greater Imax for K. Values for Imax are being calculated from the depletion curves to allow us to evaluate the influence of K uptake rate on cultivar susceptibility to K deficiency.

The effect of salt type and concentration on K kinetic uptake parameters was also measured with depletion studies. Results showed that K uptake by Newbonnet, a salt-sensitive cultivar, was inhibited with the addition of Na and $CaCl_2$ at a high and low salt concentration. Uptake of K by Jasmine 85 was mostly affected by NaCl at the high salt concentration. Results suggest that K uptake is sensitive to salt concentration and salt cation.

# Dry-seeded delayed-flood rice in Italy: a historical review and the current situation

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Dry-seeded delayed-flood rice cultivation is not the common cultivation system in Italy, where wet broadcast seeding in flooded fields usually occurs. At the beginning of the 20th century, rice was commonly broadcast by hand and some studies were evaluating the dry-seeding method since it appeared advantageous in removing weeds by hand between rows for aquatic weed control, a better plant stand, and reduced labor. In 1913, transplanting in Spain and in Southeast Asia was reported. More and more studies were planned to verify the opportunity to transplant rice in Italy, which led to an increase in the use of this technique and a concomitant reduction in direct seeding in flooded fields and the abandonment of the dry-seeding method. The higher grain yield obtained by transplanting and the relatively low cost of human labor played an important role in making people forget the dry-seeding method for about half a century.

In 1973, Piacco was still assessing the dry-seeding method and indicated that it was not advantageous and that field trials clearly demonstrated this. In 1974, Baldi, a breeder at the Rice Research Centre in Mortara (Pavia Province), believed that dry seeding was not only possible but could lead to gains (such as rice cultivation in areas subject to a water shortage). He believed that the cause of low yield was in the genetic background of the rice varieties grown at that time. It was simply not possible to move varieties adapted to flooded fields to a system of drilling seed and with potential for early water stress. These varieties were not suited to those environmental conditions.

A breeding program started and, after the first unfortunate attempts, new varieties became available and a production gain became more evident. In 1983, the reported yield gain was 44% but still rice production was not satisfactory.

Agronomic constraints caused a further delay in the diffusion of the dry-seeding method, mainly because of the absence of herbicide for weed control in dry rice fields. Several experiments were conducted on weed control and crop management in dry fields and, at the end of the 1980s, everything was ready for rice cultivation with the "new" technique.

But what is the current situation?

Today, rice is dry-seeded on 25,000–30,000 ha (about 1/8 of the rice area) in Italy, mainly in Pavia and Milan provinces, where soils are characterized by a high sand content and low organic matter reserves.

Although in most cases wet-seeded rice is more productive (i.e., in Vercelli, Novara, and Ferrara provinces) in sandy soils, the dry-seeding method is the only one that guaranteed good seedling establishment with strong root anchoring, a higher and more uniform plant density, lower lodging risk, and a lower water shortage problem for water percolation in the first stages.

Nitrogen- and water-use efficiency have been analyzed. Water is flushed intermittently one or two times and fields are flooded at the 3–4-leaf stage until ripening. Water can be removed again for special purposes such as herbicide treatment or midseason N applications.

Later studies evidenced the effect of the N application method on several crop parameters, thus pinpointing the best fertilization strategy. Differences between wet- and dry-seeded rice management were highlighted. Because of the advantages recorded in some rice areas in Italy, studies will continue to increase the efficiency of production factors. Further work will focus on improving dry field management and studying the performance of new varieties to meet the yield target.

# The rice crop in Italy: a geographical analysis for spatial database construction

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Rice (*Oryza sativa* L.) probably reached the Mediterranean area after Alexander the Great's expedition to Asia. The Arabs and the Spanish introduced it to the Italian peninsula starting in the south, but the cultivated area remained limited until the 15th century, when the crop underwent experiments within flooded areas located in the Po Valley.

Po Valley rice production is located in a western area (in the provinces of Vercelli, Novara, Alessandria, Milano, and Pavia), a central area (in the provinces of Mantova and Verona), and an eastern area (in the province of Ferrara). Together, they comprise most of the total surface area given over to rice production in Italy (about 230,000 ha). Rice is also cultivated in central Italy (i.e., the province of Siena) and in Sardegna.

Rice crops are mostly planted on hydromorphic alluvial and fluvioglacial soils with a wide range of textures.

The climate of the Po Valley is classified as subcontinental temperate with an average annual temperature of 12–14 °C, the average temperature of the coldest month is –1/+1 °C, and 2–3 months have an average temperature higher than 20 °C. The average rainfall ranges from 600 to 1,400 mm per year and the average global solar radiation is about 85,000–100,000 mm per year. In Central Italy and Sardegna, the climatic conditions are much more heterogeneous because of the complex orography and the influence of the Mediterranean Sea. The climate of Italy's central plain is classified as warm temperate or sublitoraneum temperate. The global solar radiation reaches 100,000–120,000 mm per year. The Sardegna plain rice belt has warm temperate climatic conditions.

The variability in soil and climatic conditions corresponds to the variability of premium varieties and cooking traditions in the different areas of cultivation.

Since 1958, the law regulating the domestic rice market his forbidden the sale of mixed varieties and it is compulsory to specify the variety name on the packing. The variety name is viewed as a sort of brand name for the customer. As a consequence, some traditional varieties released more than 50 years ago are still grown even if their morphological and agronomic traits are inconsistent with modern rice-growing techniques.

About 130 rice varieties are now listed in the national register of cultivated species. They are classified as short, medium, long Arborio-type, and long indica-type grain according to European Union market classes. The traditional varieties belong to the third group. They are grown on about 20% of the total rice area.

This study aims at describing Italian rice environments and creating a database for rice with which it will be possible to collect and integrate the main soil, climatic, and agronomic variables that affect rice production and to characterize rice environments. The structure of the database is described, as well as the methods adopted in collecting and integrating data with different origin, quality, and type. This will be useful as an input for rice growth models or for a rice integrated geographic information system.

# Breeding for resistance to rice sheath blight

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Sheath blight (ShB), caused by *Rhizoctonia solani* Kühn, is an important fungal disease of rice. In this study, a total of 18 crosses in generations $F_3$ and $F_4$ in 1996 and $F_4$ in 1997 were screened for ShB reaction. Two susceptible rice cultivars (Lemont and Bond), two moderately susceptible cultivars (L-202 and RA73), and one tolerant cultivar (Katy) were used as parents in a set of reciprocal crosses made in 1993 and 1994. Crosses between Bond/L-202 and L-202/RA73 were not involved in the test. Tests were conducted at the Rice Research and Extension Center, Stuttgart, Arkansas, on a Crowley silt loam (fine montmorillonitic thermic Typic Albaqualfs). The $F_3$ and $F_4$ generations were space-planted at 20 to 25 seeds per hill with a spacing of 22 cm between hills within rows and 27 cm between rows.

Tests were field-inoculated with *R. solani* 50 to 55 days after emergence (i.e., at maximum tillering and panicle initiation) at 0.8 L $m^{-2}$ of ShB inoculum. Disease severity was visually evaluated, once, at maturity on a scale of 0 = no incidence of disease and 9 = severe infection on all leaves, with some plants killed. Plants were classified as being tolerant (rating 4–5), moderately susceptible (rating 6–7), or very susceptible (rating 8–9). Besides ShB reaction, heading date and plant height were also evaluated.

Katy showed the most tolerance of ShB, followed by L-202 and RA73. Katy crossed to either L-202 or RA73 and their reciprocal crosses produced populations with an overall lower ShB rating than when crossed to either Bond or Lemont. Bond and Lemont were the most susceptible cultivars tested and produced the populations with the highest overall mean ShB rating, indicating greater susceptibility to ShB.

Associations among ShB, plant height, and heading date showed negative correlations among generations and among years for most of the crosses. Cytoplasmic effects associated with ShB tolerance were not evident based on the detailed analysis of the frequency distributions and orthogonal contrast comparisons. Transgressive segregants, both high (for increased ShB tolerance) and low (for increased ShB susceptiblity), were repeatable over generations and years, and were obtained from all of the crosses involved in the test. Transgressive segregants for increased ShB tolerance, with a 3 rating, were obtained from several of the crosses. The transgressive effects for increased tolerance were more frequent when Katy was used as a parent.

Statistically significant ShB tolerance heritability estimates of 0.52* and 0.46* for L-202/Katy and Katy/L-202, respectively, were the highest of all crosses tested. RA73/Katy, L-202/Bond, and Lemont/Bond were also statistically significant for ShB tolerance with 0.40*, 0.35*, and 0.34* heritability estimates, respectively.

# Mechanisms of submergence tolerance associated with the rice *Sub1 gene*

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The rice *Sub1* gene may be useful in improving rice seedling vigor under flooded conditions. *Sub1* enhances survival under submergence but the physiological mechanisms that engender this response have not been completely characterized. Submergence tolerance, associated with *Sub1,* may derive from tolerance of submergence, tolerance of postsubmergence, or both. We have examined *Sub1*-associated physiology through comparisons of backcross lines that differ primarily in the *Sub1* trait. The backcross lines have been developed in an effort to clone *Sub1* using a map-based approach.

In our work on *Sub1*-associated physiology. we have studied fermentation end-product accumulation, antioxidant enzyme activity, and lipid peroxidation. Under anaerobiosis, a comprehensive profile of fermentation end-product accumulation was investigated in lines with the presence and absence of the *Sub1* trait. Lines containing *Sub1* had increased succinate, malate, and gamma amino butyrate accumulation and decreased ethanol production compared with *Sub1* minus lines. These data suggest that *Sub1* is associated with the diversion of metabolic carbon flux from ethanol to alternative fermentation end products. Ascorbate peroxidase and glutathione reductase activities and levels of lipid peroxidation were compared under submergence and during postsubmergence recovery. *Sub1* lines exhibit decreased lipid peroxidation during and immediately after submergence, suggesting that enhanced tolerance of oxidative stress is associated with *Sub1.*

Shoots of *Sub1* lines do not elongate under submergence. The submergence-induced shoot elongation response typical of most rice cultivars is completely inhibited in *Sub1* lines. We have used gibberellic acid and paclobutrazol, a GA biosynthesis inhibitor, to regulate elongation response under submergence. Results of these experiments suggest that reduced elongation is a major factor in *Sub1*-associated submergence tolerance. Results will be discussed with respect to the application of the *Sub1* trait to enhanced seedling vigor under submergence.

# Improved economics of weed control in weed-suppressive rice cultivars with reduced propanil rates

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A three-year field study was conducted to evaluate the economic benefit of reduced propanil rates in conjunction with weed-suppressive rice cultivars. The inherent suppressive abilities of four commercial U.S. rice cultivars (Lemont, Cypress, Kaybonnet, and Starbonnet) and three foreign cultivars (Teqing and Guichou from China and T65*2/TN 1 (PI312777) from the Philippines) were evaluated. In all cultivars (except PI312777), propanil application (regardless of rate) resulted in a marginal benefit-cost return (MBCR) of two or more. The naturally weed-suppressive rice cultivar PI312777 consistently reduced barnyardgrass growth and consequently produced higher grain yield with or without propanil application. The economic benefit of using propanil in PI312777 was marginal and probably unnecessary depending on the initial weed pressure early in the season. Propanil at 2.24 kg ai ha$^{-1}$ (1/2X) resulted in the highest marginal and actual returns for Kaybonnet and Teqing. Propanil at 1.12 kg ai ha$^{-1}$ (1/4X) resulted in the highest MBCR for Lemont, Starbonnet, and Guichou. Marginal returns decreased as the propanil rate increased for Lemont, Starbonnet, and Guichou. The foreign cultivars PI312777, Teqing, and Guichou produced higher yields resulting in higher actual returns than the local commercial cultivars Starbonnet, Kaybonnet, Lemont, and Cypress. Results indicate that the choice of cultivar is critical in achieving maximum returns. MBCR is a useful tool in evaluating the optimum rate of propanil to apply once the cultivar has been chosen. Results indicate that the use of weed-suppressive rice cultivars coupled with reduced herbicide rates can be an economically profitable and environmentally sound weed management strategy. Increased efforts to develop weed suppressiveness among local commercial cultivars are therefore needed.

# Signaling pathways in the rice defense response

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The rice *Xa21* gene, which confers resistance to *Xanthomonas oryzae* pv. *oryzae (Xoo),* was isolated by positional cloning. The sequence of the predicted protein encodes a receptor kinase with a leucine-rich repeat (LRR) motif in the presumed extracellular domain and a serine-threonine kinase in the intracellular domain. The structure of *Xa21* suggests a role in cell surface recognition of a pathogen ligand and subsequent activation of an intracellular kinase signaling pathway. Activation of this pathway is hypothesized to trigger plant defense responses such as the oxidative burst, hypersensitive cell death, and defense gene activation.

In support for this model, we have found that *Xa21* encodes an active kinase with serine/threonine specificity and that the LRR domain is responsible for race-specific pathogen recognition. We have also found that *Xa21* interacts specifically with rice catalase B (CATB) in the yeast two-hybrid system. We determined that catalytically deficient *Xa21* mutants fail to bind CATB. Catalase is a key component in regulating the levels of reactive oxygen species (ROS) in the cell. The accumulation of ROS has been shown to trigger the hypersensitive response (HR) and enhance disease resistance. HR is correlated with the onset of systemic acquired resistance (SAR), which requires salicylic acid (SA). SA accumulates following the oxidative burst around infection sites and is a known inhibitor of catalase. We therefore tested whether cell death and SAR play a role in the rice defense response.

Race-specific cell death was observed in cell lines expressing the *Xa21* gene when inoculated with an avirulent strain of *Xoo* but not with a virulent strain nor in the wild-type cell line. We then showed that BTH, an SA analog, could induce resistance to *Xoo* and we have isolated several BTH-induced genes, including pathogenesis-related (PR) genes. These results suggest that cell death and SAR contribute to the rice defense response.

It is known that NPRI is a key regulator of the SA-mediated SAR in *Arabidopsis*. Overexpression of NPRI in *Arabidopsis* enhances disease resistance. To test whether SA signaling components are conserved in rice, we used *Arabidopsis* NPR1 to screen a rice cDNA library by the yeast two-hybrid system. Two types of NPR1 interactors were isolated in this screen, a proline-rich NPR1 interactor and a class of bZIP transcription factors. These transcription factors have a high degree of similarity to transcription factors that target SA-responsive promoters in PR genes. The proline-rich NPR1 interactor and one of the bZIP transcription factors were used as baits to back-screen the rice cDNA library by the yeast two-hybrid system. Two NPR1 rice homologues and several other interacting proteins were isolated.

# Management of pesticide residues in rice irrigation systems in New South Wales, Australia

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Environmental problems are becoming of increasing concern to agriculture worldwide and the 150,000-ha New South Wales rice industry is no exception. Community concerns are changing and sustainability means developing a balance between maintaining the resource base and maintaining productivity and profitability to the benefit of the community as a whole.

In New South Wales rice, three environmental problems of concern to the rice industry, government agencies, and the community are rising water tables, the effect of pesticides, and burning of crop residues.

One pesticide problem is that of residues of agricultural pesticides in drainage water and the potential damage to the aquatic environment. The most widely used pesticide in the local irrigation areas, in terms of both area treated and rate of application, is molinate, the grass herbicide used on rice. In October 1993, residues of molinate exceeding 50 mg $L^{-1}$ (parts per billion) were found in a drain in the Murrumbidgee Irrigation Area, one of the main rice-growing areas. This followed changes in community attitudes, increased research activity, and increased monitoring by government agencies.

As a result, the rice industry and other irrigated agricultural industries involved, the local water authority, together with the government agencies involved with regulatory, research, and extension activities associated with agriculture, water, and the environment, joined together to establish realistic standards for drainage-water quality, review the best management practices, and implement programs to solve the problem. A spirit of cooperation developed an approach to solve the problem by improving the best management practices over time to meet short-term objectives and long-term aims. The strategies adopted focused on realistic changes in farmer practices to reach achievable standards in water quality, which were reviewed regularly in line with improvements, rather than an inflexible regulatory approach.

The changing patterns of increased pesticide use, rice-growing practices that are predisposed to residues in drainage waters, the effect of climate, particularly rainfall, and the institutional and regulatory arrangements that affect the situation are discussed. The short- and long-term water-quality objectives, involving notifiable and action levels for a range of pesticides used, the monitoring programs, the regulatory framework adopted, and the education and extension programs developed and implemented are outlined.

# Control of herbicide-resistant watergrass in northern California rice with bispyribac-sodium 80 WP herbicide

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Bispyribac-sodium is a postemergence selective herbicide for rice with excellent efficacy against certain grasses, sedges, and broadleaf weeds. It inhibits the plant enzyme acetolactate synthase (ALS), thus blocking branched-chain amino acid biosynthesis.

Bispyribac-sodium 80 WP has a wide application window for control of barnyardgrass (*Echinochloa crus-galli*) and watergrass (*E. phyllopogon)*. The herbicide can be applied to watergrass and barnyardgrass from the 1st leaf to the 2–3-tiller stage of growth at 10 to 18 g ai $ha^{-1}$. Optimum use rates are 4.1-4.9 g ai $ha^{-1}$ with the grass being at the 3- to 5-leaf stage. Higher use rates may be required for herbicide-resistant watergrass. A nonionic silicone-based surfactant is required at rates of 0.125 to 0.25 (% v/v).

Over the last 3 to 5 years, growers in the Sacramento Valley, California, have had difficulty controlling late watergrass, *E. phyllopogon*, in their rice fields. Grass control failures have been observed with maximum rates of thiobencarb, molinate, and fenoxaprop-ethyl applied alone and sequentially. In 1998, greenhouse tests confirmed that resistance to thiobencarb, molinate, and fenoxaprop-ethyl had developed in late-season watergrass biotypes and that populations varied in resistance levels from field to field.

Findings from greenhouse tests also indicated that certain watergrass biotypes exhibited resistance to bispyribac-sodium. To confirm greenhouse results, three field trials were established in the Sacramento Valley. All three fields, Argo, Glasgow, and Zoller, had histories of herbicide failures and confirmed resistance in the greenhouse.

Field tests were established to determine the application timing and rates for control of herbicide-resistant watergrass. Bispyribac-sodium 80 WP was used at 4.1, 7.3, and 9.7 g ai $ha^{-1}$ with Kinetic® at 0.125% v/v at all sites. The herbicide was applied at the 4- to 5-leaf and 1- to 2-tiller stage of watergrass at the Argo and Zoller sites, and at the 2- to 3-tiller stage of watergrass at the Glasgow site.

Consistently good grass control was observed at all three sites with the application timing at tillering. Earlier application at 4- to 5-leaf watergrass consistently controlled less than the watergrass at the later timing. Grass control of 85% to 99% was observed at rates of 7.3 and 9.7 g ai $ha^{-1}$ across all three trials. The 4.1 g ai $ha^{-1}$ rate did not provide adequate control regardless of application timing.

Bispyribac-sodium 80 WP appears to provide an additional tool for control of herbicide-resistant watergrass in Sacramento Valley rice.

# Molecular analysis of cold-induced pollen sterility

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Male reproductive development in rice is very sensitive to abiotic stresses such as cold. Low temperatures even for a short period from the young panicle differentiation stage to anthesis lead to pollen sterility and reductions in grain yield ranging from 20% to 40%. The molecular basis of this phenomenon remains poorly characterized. One of the earliest abnormalities observed after cold treatment is the increase in sugar levels, followed by a variety of physiological abnormalities and the absence of starch granules in the pollen grains. It is possible that cold induces a metabolic block, which in turn impairs normal pollen development. In wheat and rice, drought stress-induced sterility was shown to be associated with a reduction in invertase activity. We have therefore started to clone the rice invertase genes in order to use them as molecular markers to study the effect of cold treatment on sugar metabolism and pollen development. Three genes encoding apoplastic acid invertase and one gene encoding a vacuolar invertase gene were cloned and sequenced. Detailed expression studies are in progress to identify the invertase gene(s) expressed in the rice panicle during pollen development. By studying the expression (tissue specificity and response to cold stress, abscisic acid, and sucrose) of these genes and measuring invertase enzyme activity, we will be able to study how and at which level cold treatment disturbs normal pollen development.

# The value of rice fields as a habitat for waterbirds: a North American perspective

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Rice fields have become an indispensable component of waterbird habitat and a leading example of the potential for integrating agricultural and natural resource management in both temperate and tropical environments. We summarize the use and value of rice fields for waterbirds in North America and we consider some of the challenges that face farmers and wildlife managers in their efforts to promote rice land as a habitat for wildlife.

Residual rice, weed seeds and tubers, and invertebrates provide food resources for a host of avian species. Shallow water depths, typically less than 20 cm, provide preferred water depths for many species of ducks, geese, swans, shorebirds, and other wading birds. In North America, considerable information exists on the foraging ecology of wintering waterfowl in rice, on the role of rice fields as a breeding habitat for waterbirds in the southeastern states, on the effects of pesticides on birds that feed in rice fields, and on the occurrence of shorebirds and other nongame species in rice fields. This information suggests that rice fields can provide an important habitat for numerous species of waterbirds.

Several challenges exist for future waterbird habitat in rice. Expanding urban growth will greatly threaten rice culture in the Sacramento Valley, Texas prairie, and parts of Florida. This expanding human growth not only directly affects rice area but also affects remaining farms by increased demand for water, increased demand for pesticide controls and stubble burning, and increased periodicity of severe flooding. Irrigation district infrastructure may be affected as agricultural areas are converted to houses.

The fragmentation of large rice areas may result in reduced usage by migrant and wintering waterbirds. In a world market, increased high-quality rice area with expanding human populations and demand for rice will result in the desire to convert seasonal wetlands to rice. Human disturbance associated with growing population centers may reduce the quality of waterbird foraging and resting sites. The introduction of exotic species, such as the Chinese mitten crab, may have unknown consequences for the food chain balance.

Rice habitat can have both positive and negative consequences for migratory waterbirds. For example, several waterbird species that use rice have displayed declines in continental or regional populations, such as the northern pintail, king rail, black tern, and long-billed curlew. The provision of rice habitat during winter may be critical for these species. Other species, such as the white-faced ibis, white-fronted goose, and tundra swans, have displayed population increases. No species has shown greater increases than the mid-continent population of snow geese. The release from high winter/spring mortality with the expansion of rice, wheat, and other small-grain crops has, in part, allowed these birds to flourish. Current habitat conversions by these geese in key migration and breeding areas in the high arctic will have long-term effects on the fragile arctic environment.

# Herbicide-resistant watergrass in rice in California

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Farmers have repeatedly noted failures in controlling watergrass with the currently available herbicides. Watergrass seed samples were collected in the Sacramento Valley from selected fields where herbicide control had failed. Plants were grown in the greenhouse and treated with 0. 17 kg ai ha$^{-1}$ fenoxaprop-ethyl (4-leaf stage), 4.5 thiobencarb (1.5-1st), 4.5 molinate (1-1st), and 37 g ha$^{-1}$ bispyribac-sodium (3-1st). Fifteen days after treatment, the ranges of growth (fresh weight as % of untreated check) of surviving plants were fenoxaprop-ethyl 13–70%, thiobencarb 46–86%, molinate 19–80%, and bispyribac-sodium 13–63%. Several accessions survived (regrowth 20%) treatment with more than one herbicide, including chemicals with different modes of action. Dose-response experiments were conducted with two accessions that exhibited resistance to different herbicides. R/S (resistance/susceptibility) value ranges for each herbicide tested were thiobencarb 9.6–12.7, molinate 2–4.3, fenoxaprop-ethyl 18–29.5, bispyribac-sodium 2.6–12, bispyribac 4–12 (one accession), and propanil 1.4–1.6. One of these two accessions exhibited cross-resistance to bispyribac and bensulfuron. The overall most resistant accession behaved as fully susceptible when exposed to pendimethalin, clomazone, glufosinate, and glyphosate in another dose-response study. Thus, the repeated use of the few available grass herbicides in the predominantly monocultured rice of California may have selected for multiple resistance in late watergrass. The use of bensulfuron, which has already developed resistance in broadleaf and sedge species, may have selected indirectly for resistance to bispyribac-sodium, a herbicide not yet used in California. Both herbicides are ALS inhibitors. The introduction of transgenic rice cultivars with resistance to glufosinate and glyphosate may be a valuable tool for the management of multiple-resistance watergrass. However, to avoid further exacerbation of herbicide resistance, it is of paramount importance that herbicide action be complemented by other nonchemical, cultural means of weed control.

# Genotype, environment, and genotype × environment affect the cooking quality of rice

M. Fitzgerald

Our most useful tool for evaluating cooking and eating properties is Rapid Visco Analysis. RVA mimics the cooking process by heating a slurry of rice flour and water from 50 to 95 °C and then cooling back to 50 °C. Viscosity is measured as resistance to stirring as heating causes the starch granules to gelatinize and cooling causes a gel to form. Evaluating the cooking quality of rice and defining it in terms of current evaluation methods is difficult. An additional complication is that genotype, environment, and their interaction affect cooking properties.

Many of the eating qualities of rice have been statistically related to various parts of the viscosity curve. For example, setback has been correlated to firmness and to stickiness of the cooked rice. These correlations are useful, but, if we intend to use instrumental methods to measure or to predict what are basically sensory characters, we would be in a much more powerful position if we understood how starch structure, starch content, and protein affect the viscosity curve.

Viscosity curves were collected of

- five varieties of rice, all known to have about 18% amylose content and all grown in the same environmental conditions;
- one variety, again known to have about 18% amylose content and grown at either 38 °C or 28 °C by day;
- one variety, known to have about 18% amylose content and grown at four different levels of N nutrition; and
- a range of varieties known to have 0%, 16%, 19%, and 25% amylose content and grown in the same environmental conditions.

We examined the effect of protein content on viscosity characteristics by digesting away the proteins with a protease at 37 °C. We determined the structure of the starch by debranching the starch and injecting the solution into a gel permeation column on a high-performance liquid chromatography. Amylose elutes as a single peak and amylopectin elutes as three shouldering peaks.

None of the rice varieties had the same viscosity characteristics. There were small differences among the five varieties with the same amylose content, high temperature changes peak viscosity and setback, increased rates of N fertilizer decrease peak and final viscosities, and increased amylose content increases setback.

Different rice varieties with the same amylose content differed in amylopectin structure and in the effect of proteins on viscosity. Rice grown at high temperature has less amylose, different amylopectin structure, and different effects of proteins on viscosity than the same variety grown in cooler temperatures. N nutrition did not affect starch structure or amylose content, and removing proteins removed all differences in viscosity. Removing proteins in rice with different amylose content caused setback to move from negative toward zero or from positive toward zero. The results indicate that proteins are involved in the height of the peak and are involved in forming the viscosity of the cooled gel.

# QTL analysis for heading dates in rice

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Quantitative trait loci (QTL) analysis for heading dates in rice (*Oryza sativa* L.) was conducted using 191 recombinant inbred (RI) lines derived from a cross between indica rice variety Milyang 23 and japonica variety Akihikari. The segregation data and linkage map consisted of 165 restriction fragment length polymorphism (RFLP) markers. RI lines were cultivated under 10 different conditions: at the Kashimadai field of Tohoku University in 1996 and 1997 (two types of nitrogen conditions: 0 kg ha$^{-1}$ and 100 kg ha$^{-1}$); at Joetsu in 1995, 1996, and 1997 (two types of nitrogen conditions: 0 kg ha$^{-1}$ and 70 kg ha$^{-1}$); and at Tsukuba in 1997, Fukuyama in 1997, and Fukuoka in 1997. Cultivars were studied from the day of transplanting to paddies to panicle heading. From the results of QTL analyses, 12 QTLs were detected and tentatively named based on the trait, type of population, chromosome location, and number of QTLs: one QTL (*qDTH-MA1a*) on chromosome 1, two (*qDTH-MA2a, b*) on chromosome 2, two (*qDTH-MA3a, b*) on chromosome 3, one (*qDTH-MA6a*) on chromosome 6, one (*qDTH-MA7a*) on chromosome 7, two (*qDTH-MA9a, b*) on chromosome 9, one (*qDTH-MA10a*) on chromosome 10, one (*qDTH-MA11a*) on chromosome 11, and one (*qDTH-MA12a*) on chromosome 12. No QTL was detected in the region where a major photosensitive gene, *Se1*, was located on chromosome 6 in this analysis. These results indicate that Milyang 23 and Akihikari have the same allele, *se1*, on chromosome 6. The number of QTLs detected in this study is much greater than that in other reports. We assumed that functions of other genes were efficiently detectable when the effect of *Se1* was masked. Certain characteristic patterns in the two QTLs *qDTH-MA7a* and *qDTH-MA11a* indicate higher LOD scores than with other QTLs.

From this, we assumed that the segregation of heading dates in the RI population was controlled mainly by the two QTLs, while other QTLs modified the function of these two QTLs. The QTL reaction pattern showed no marked difference among nitrogen conditions, but certain patterns appeared characteristic of geography and year. The LOD of *qDTH-MA11a* indicated the same or a higher score than that of *qDTH-MA7a* at Joetsu, but the relationships of these two QTLs at other sites were the reverse. These differences in QTL function patterns are assumed to be caused by environmental factors, temperature, and length and degree of daylight during plant cultivation.

# Early watergrass and bearded sprangeltop control with cyhalofop-butyl in California water-seeded rice

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Cyhalofop-butyl is a selective postemergence graminicide for weed control in rice. It belongs to the aryloxyphenoxy proprionate class of chemistry and its mode of action is by the inhibition of the acetyl CoA carboxylase enzyme, which is essential for fatty acid synthesis. Cyhalofop-butyl is rapidly absorbed by the foliage and translocated freely throughout the plant. In grasses such as *Echinochloa* species, cyhalofop-butyl is rapidly metabolized (85% in less than 10 h) to the free acid form, which is an effective inhibitor of acetyl CoA carboxylase. Rice tolerance of cyhalofop-butyl is due to rice being able to rapidly metabolize cyhalofop acid to the "diacid" form, which is not an effective inhibitor of acetyl CoA carboxylase.

Six field trials were conducted in the Sacramento Valley during 1997-98 in water-seeded rice culture to investigate the relationship between application rate and stage of growth for control of natural populations of early watergrass and bearded sprangletop. Grasses were treated at either the 2–3-leaf or 4–6-leaf stage of growth with cyhalofop-butyl (285 g ai $L^{-1}$ EC) at rates of 70, 140, 210, 280, and 560 g ai $ha^{-1}$. Water was lowered or completely removed to expose foliage at the 2–3-leaf timing and maintained at a 7–10-cm level for the late timing. Commercial formulations of propanil (aqueous suspension) at 4,480 g ai $ha^{-1}$ and fenoxyprop-ethyl (resolved isomer) at 38 and 76 g ai $ha^{-1}$ were included as reference treatments. Cyhalofop-butyl at 140 g ai $ha^{-1}$ provided greater control (81%) of 2–3-leaf watergrass than propanil or the lower rate of fenoxyprop-ethyl and control equivalent to the higher rate of fenoxyprop-ethyl. Control of 4–6-leaf early watergrass with cyhalofop-butyl at 210 g ai $ha^{-1}$ averaged 87%, greater than propanil and both rates of fenoxyprop-ethyl. Similar trends were observed for the control of bearded sprangletop; however, control with propanil was significantly less than with early watergrass. All cyhalofop-butyl treatments caused little or no visual rice injury (0–5%) in all studies. Propanil caused only minor rice injury, averaging 9% and 8% injury 1–2 weeks after treatment for each timing, respectively. Fenoxyprop-ethyl at both rates caused significant injury to rice (18–53%) when applied at the 2–3-leaf grass timing and at the 76 g ai ha$^{-1}$ rate (21%) applied to 4–6-leaf grass at 1–2 weeks after application.

One trial was conducted in 1998 to determine the cyhalofop-butyl rate response on later-stage grasses. Cyhalofop was applied at 210, 280, 350, and 560 g ai ha$^{-1}$ and compared with propanil at 6,720 g ai ha$^{-1}$ and fenoxyprop-ethyl at 76 g ai ha$^{-1}$. Treatments were made 32 days after rice seeding to 2–3-tiller early watergrass and 10%-headed bearded sprangletop. All treatments provided excellent control of bearded sprangletop except propanil, which provided only 50% control. Both propanil and fenoxyprop-ethyl provided greater than 95% control of early watergrass. All rates of cyhalofop-butyl provided excellent initial control of early watergrass. No rice injury was observed with cyhalofop-butyl treatments; however, propanil and fenoxyprop-ethyl caused 8% and 26% injury 11 days after treatment, respectively.

Continued studies are planned to further elucidate the rate response of cyhalofop-butyl on these key grass weeds in California rice culture in anticipation of a United States registration. These initial studies indicate that cyhalofop-butyl, at rates ranging from 140 to 280 g ai ha$^{-1}$ depending on grass stage, will provide equal or better annual grass control than existing foliar products, with significantly less potential for rice injury.

# Growth, development, and biological characteristics of red rice accessions from the southern United States

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Red rice is an increasing weed problem in much of the drill-seeded rice produced in the southern United States and other temperate regions of the world. Seeds of red rice accessions (biotypes) were collected from Arkansas and other southern rice-producing states and planted in various field nurseries at Stuttgart, Arkansas, from 1995 through 1998. Presently, the red rice collection contains more than 150 entries. About one-third of the biotypes are "black-hull" types, two-thirds are "straw-hull" types, and there are more than ten crosses between rice and red rice. Generally, black-hull types are awned and straw-hull types are awnless.

Growth and development of these red rice biotypes were evaluated in a large nursery in 1997 and 1998 (1998 data not available). Maximum plant heights ranged from 87 to 158 cm, flag-leaf lengths and widths ranged from 22 to 42 cm and 0.7 to 1.6 cm, respectively, and time to heading ranged from 74 to 108 days after emergence. As a group, black-hull types headed later than straw-hull types. Most of the biotypes produced seeds that initially were moderately to highly dormant, but were no longer dormant after 6 months. Several biotypes remained highly dormant after 6 months. Generally, black-hull types were more dormant than straw-hull types. In separate field tests conducted from 1995 through 1998, tiller production and total biomass of the red rice biotypes ranged from two to three and one-half times those of commercial rice standards; relative chlorophyll content of seedlings averaged 15% less for red rice biotypes than commercial standards; and photosynthesis and transpiration of red rice flag leaves averaged 13% and 12% less, respectively, than the commercial standard. Most of the red rice biotypes were categorized as "medium-grain" types based on their seed dimensions. Amylose and alkali spreading reaction values for red rice seeds were not consistent with any one white rice standard tested. Amylose levels were generally similar to an indica medium-grain standard or a tropical japonica long-grain standard. Alkali reaction levels were similar to the tropical japonica long-grain standard, but not to the indica medium-grain standard.

Collectively, these results demonstrate the presence of diverse growth and development characteristics among a relatively small sampling of red rice populations from the southern U.S. Plant heights, tillering capacity, and flowering dates in particular may be important factors in predicting future effects and interactions of red rice with cultivated white rice cultivars.

# Differences in competitive ability among water-seeded rice cultivars

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The repeated use of a limited number of herbicides has led to the development of resistant weed species in California rice production. Competitive rice cultivars could help reduce herbicide dependency and decrease selective pressure for resistance. We conducted a field experiment in 1998 to identify rice traits related to competitive ability and to assess the relationship between competitive ability and rice yield. Five commercially available short-stature (1 m or less) cultivars, two tall (1.25 m) varieties no longer grown in California, three experimental short-stature lines, and an experimental line of intermediate (1.1 m) height were grown with and without competition from watergrass *(Echinochloa phyllopogon, E. oryzoides)*. A threefold difference in cultivar tolerance, measured as yield under weedy/weed-free conditions, among cultivars and a nearly fivefold difference in watergrass seed suppression were detected. While the most tolerant cultivars produced significantly less grain under monoculture conditions than the other varieties (although even these had yields in excess of 8.9 t $ha^{-1}$), the cultivars that interfered most with watergrass seed production had excellent yields in the absence of weed competition. We found a negative relationship (adjusted $r^2 = 44.1\%$, $P<0.001$) between watergrass seed production at 96 days after seeding (DAS) and the variables rice height and specific leaf area at 21 DAS. We found no significant relationship between watergrass seed production and rice height at maturity (the tall cultivars were not more competitive than the short cultivars), suggesting that superior height improved the ability to suppress watergrass only when the height advantage occurs early in the season. Our analysis suggests that the development of more competitive cultivars could improve weed control in water-seeded rice without reducing yields.

# Breeding iron- and zinc-dense rice for human nutrition

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Micronutrient deficiency—"hidden hunger"—affects more than 2 billion people worldwide, predominantly women and children because of their greater physiological need. Iron and zinc deficiencies have a wide range of adverse effects such as reduced immune competence, susceptibility to infection, complications in pregnancy and childbirth, poor child growth, delayed motor and cognitive development, and decreased work output. In 1992, IRRI began to examine the effect of certain soil characteristics on the iron content in rice grain. The work was expanded in 1995 to include zinc and collaboration with the University of Adelaide, Australia, for mineral analysis. Germplasm screening revealed the presence of large genetic variation for Fe and Zn in brown rice. Commonly grown cultivars contain about 12 mg $kg^{-1}$ of iron and 25 mg $kg^{-1}$ of Zn in brown rice. Some traditional varieties have double these amounts. Aromatic traditional rice seems to be associated with high iron and zinc in the grain.

Genotype by environment (G × E) interaction studies showed that high-iron and -zinc traits are expressed across most rice environments. The high grain iron trait can be combined with high-yielding agronomic traits. IRRI has developed two improved lines with high yielding ability and a high concentration of iron and zinc in the grain. Because of the high consumption of rice, the extra iron and zinc contained in improved rice lines, if it is bioavailable as in normal rice, would have a meaningful effect on human nutrition and health. Production of high-iron rice can also be an alternative to fortification.

# Rice with tolerance of adverse soils: bringing the green-green revolution to the tidal wetlands

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The rapidly growing population and the future production environment make tidal wetlands an important land resource for rice cultivation. Rice is often the only crop grown in these coastal wetlands during the wet season. But yields are low, usually less than 1 t ha$^{-1}$, because of adverse soil conditions and submergence.

The availability of genetic variability for the adverse soils and climatic stresses prevalent in tidal wetlands and the good possibility of combining desirable traits into a high-yielding background provide a challenging opportunity to bring a doubly green revolution to the tidal wetlands, that is, increasing productivity in a manner that protects the environment as well.

In developing rice with tolerance of adverse soils, the research strategy of the International Rice Research Institute (IRRI) is to concentrate on prebreeding activities that will enable the national agricultural research and extension systems to undertake varietal improvement. Research and experience on tidal wetlands in Asia are applicable to flood-prone rice lands in West Africa and Latin America.

Reliable and rapid greenhouse and field screening methods were developed to detect genotypic differences for tolerance of soil stresses. By 1998, IRRI had screened more than 200,000 rice varieties and breeding lines for tolerance of different soil stresses. Tolerance donors identified have been used successfully as parents in hybridization programs at IRRI and in national programs. Screening results were used in understanding the genetics and physiological mechanism of salt tolerance. The development of marker-aided selection techniques for salinity, phosphorus deficiency, and iron toxicity is in progress.

Screening results are stored and managed in the Problem Soils Germplasm Database, a component of IRRI's International Rice Information System (IRIS). It is envisioned to help examine key questions related to nutrient-use efficiency and germplasm improvement without costly experiments.

# Researching solutions to sustainability and environmental challenges for rice-based cropping systems in Australia

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The major threat to the sustainability of irrigated agriculture in the rice-growing regions of southern Australia is secondary salinization as a result of rising water tables. The main external adverse environmental effects of irrigated agriculture in general, including rice growing, are on natural flow patterns and consequently on ecosystems, and contamination of surface water with pesticide residues and salt. Stubble burning is also coming under increasing scrutiny because of air pollution, greenhouse gas emissions, nutrient loss, and effects on soil organic matter.

Rice growing contributes 40–50% of the accessions to the groundwater in the rice-growing areas. Diverse strategies for reducing the accessions from rice are applied, including restricting rice growing to soil assessed as being suitable for rice. In the past, this was based on soil texture, but increasingly surveys are being used to detect high-recharge or “leaky” areas, and the inclusion of soil sodicity constraints will further improve the ability to predict leaky soils. Recharge from leaky areas within a field can be greatly reduced by puddling or by compaction. After the rice crop, soil water content is high and recharge may continue. Research is under way to quantify the effect on accessions to the water table of growing a winter crop immediately after the rice harvest to use up the stored soil water and winter rains.

Diverse options for increasing rice water-use efficiency and reducing recharge have been or are being investigated, such as shorter-duration varieties and a range of water and soil management strategies. Intermittent and sprinkler irrigation can result in significant reductions in water use; however, yields decline markedly because of cold temperature damage associated with low night temperatures during early pollen microspore. For nonponded rice

culture to be viable, cold tolerance would need to be increased to cope with night temperatures as low as 10 °C during microspore development.

The model SWAGMAN Destiny (SWAGMAN = Salt Water And Groundwater MANagement) has been developed to simulate the interactions among management, irrigation water salinity, climate, water-table depth and salinity, soil type, and crop productivity. Models have also been developed to evaluate net recharge management options at the farm and regional scales. SWAGMAN Farm is an optimization model that predicts the most economical cropping mixes (for individual farms) that meet specified net recharge and change in root zone salinity objectives, taking into account farmers' preferences. Groundwater models include the model SWAGSIM to predict the effect of changes in rice-cropping intensity on the area of shallow water tables and a model based on MODFLOW to evaluate scenarios such as the effect of groundwater pumping from deep aquifers on pressure levels and the amount of net recharge allowable to maintain pressure levels at present levels.

A consequence of the development of shallow saline water tables is the need for methods to manage saline drainage waters. A pilot trial is under way to investigate the feasibility of serial farming systems, with the production of high-value crops in the first two stages, followed by salt-tolerant crops (stage 3), fish farming (4), evaporation basins, and (5) a solar pond to generate energy.

Research related to pesticide contamination has included monitoring the nature and concentrations of residues in the various components of the system from farms all the way to the river, ecotoxicological studies, and the development of rapid field assays. Future research on the development of area-specific environmental quality objectives, and on the fate and amelioration of pesticides, is proposed.

At present, most stubble is removed by burning, thus causing obvious atmospheric pollution. Perhaps even more important is the possible damage stubble removal and burning are doing to the soil's biological health. Stubble removal by burning causes a loss of active soil carbon and a depression in soil microbiota levels and activity. There is an associated decline in soil structure and, as the soil microbiota are also responsible for most nutrient cycling within the soil, this could have serious long-term consequences.

# Seedling inoculation with AMF and influence on growth and nutrient uptake of rice

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Paddy rice grows under water-saturated soil conditions and almost all mold cannot live actively under such anaerobic conditions. This might be why paddy rice has no appreciable continuous cropping injury. But, at the same time, this situation hinders the inoculation and use of any fungi even when a beneficial effect is expected.

In Japan, almost all rice plants are raised in small shallow seedling boxes under upland soil moisture conditions and are then transplanted into a water-saturated paddy field. In many cases, the seedling box soil is disinfected also to avoid any possible infection.

Pot experiments and field trials were conducted using rice plant seedlings inoculated with arbuscular mycorrhizal fungi (AMF) or symbiotic *Fusarium* spp. As an inoculum of AMF, “Serakinkon” (Central Glass Co.) was used. “Root Gain” (Eisai Co.) was inoculated as a symbiotic *Fusarium*.

AMF-inoculated seedlings showed yellowish leaf color and plant height was short or about the same when compared with seedlings that were not inoculated. AMF inoculation seemed to increase dry weight.

Seedlings inoculated with symbiotic *Fusarium* grew larger than those that received no inoculum, and yielded more. Symbiotic *Fusarium* was detected in rice roots at harvesting.

These findings demonstrate that the inoculated fungi are established in the rice seedlings raised under upland soil moisture conditions and that survived transplanting shock. Inoculated fungi show their activity even under an anaerobic water-saturated soil environment.

# Mycoherbicide approach for controlling starfruit, a major weed of Australian rice fields

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Starfruit is the major broadleaf aquatic weed in the Australian rice industry. The most important problem in controlling this weed is herbicide resistance, since the choice of chemical control is extremely limited. The endemic fungus *Rhynchosporium alismatis,* which causes disease in juvenile and adult starfruit plants, is being investigated as a potential mycoherbicide. This fungus has demonstrated effects on other important Alismataceae weeds of rice.

Several in vitro and in vivo experiments using detached leaf discs and plants in the glasshouse have been carried out. The fungus can produce large quantities of inoculum (conidia) in artificial culture, which are able to infect the plants within the first 24 h after inoculation. The effects of the disease vary with plant age; in adult plants, it causes mostly necrosis and chlorosis on aerial parts, whereas in juveniles it reduces plant growth significantly. Infection and disease development are reduced at temperatures below 20 °C, enhanced at around 25 °C, and reduced at 30 °C or higher. If the water level is kept constant before and after inoculating plants with floating leaves, a dew period is not critical as long as the relative humidity is above 60%. Growth suppression in juvenile plants does not occur if they are submerged during inoculation. For disease development and subsequent growth suppression of juvenile plants, these must be inoculated after lowering the water to the soil level and a dew period may be needed. Preliminary tests have indicated synergy between the fungus and sublethal doses of chemical herbicides, which could further enhance the suppressive effects on juvenile starfruit plants. The growth-reducing effects of the fungus on juvenile plants are likely to reduce significantly the competitiveness of the weed. This assumption is being tested in starfruit-rice competition experiments.

# Grain moisture exchange rate and fissuring resistance relationship in rice

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Rice milling yield can decrease drastically because of grain fissure developments that occur prior to harvest. Moisture absorption by relatively dry rice kernels is the primary cause of fissuring in preharvest and postharvest stages. Preliminary reports indicate that rice genotypes that have lower rates of moisture absorption or desorption by the rough rice may also be more resistant to fissuring. Two cultivars—Lemont and Cypress—were tested for the rate of moisture exchange near harvest maturity. These two cultivars are known to have distinctly different fissuring resistance characteristics, with Cypress being significantly more resistant to fissuring than Lemont.

Moisture absorption and desorption measurements were made at three different initial moisture contents (harvest moistures) of 260–270, 250–260, and 180–210 g kg$^{-1}$. Measurements were made twice daily on freshly harvested rough rice maintained under ambient conditions. Both varieties were planted at the same time. Cypress reached the 50% heading stage 1 day earlier than Lemont. Even though Cypress headed 1 day earlier, its drying rate was significantly lower than that of Lemont at all three initial moisture levels. Beginning with 271 g kg$^{-1}$ grain moisture content, Cypress maintained a 20 g kg$^{-1}$ (2%) higher moisture content than did Lemont. This differential lasted until both varieties dried to 80 g kg$^{-1}$ (8%) moisture. The moisture differential was slightly smaller at lower harvest moistures of 250–260 and 180–210 g kg$^{-1}$, possibly because of the higher dry matter accumulation by Cypress. At moisture contents below 120 g kg$^{-1}$, rough rice of both varieties regained moisture in the morning, with Lemont absorbing at a faster rate than Cypress. A comparison of moisture absorption by the hull and brown rice components when exposed as rough rice indicates that the additional moisture absorbed by Lemont grains was present in the brown rice and not in the hull. These results indicate that the lower moisture diffusivity observed by Cypress may be the primary cause of its fissuring resistance.

# Treatment of pregerminated rice seed: a new technique for commercial rice production

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A seed treatment application of the insecticide ICON® FS gives long-term control of rice water weevil in the southern United States. Standard seed treatment techniques cannot be used where rice culture requires the seed to be germinated before sowing because soaking treated seeds produces pesticide-contaminated water. A novel method of applying any seed treatment (e.g., insecticides, fungicides, plant growth regulators, micronutrients, biologicals) to pregerminated rice seed after the soaking process but before sowing was developed. Untreated rice seed is soaked in bulk bags for one day as usual. After the water has drained completely from the seed and the seed is free-flowing, it is treated with a slurry of ICON® FS and any other seed-applied products. A simple gravity-fed machine was developed specifically for treatment of pregerminated seed, with a high throughput (500 kg 1–1.5 min$^{-1}$), even coverage, and gentle, auger-free mixing to avoid damaging germinating seeds. A patent application has been filed in the U.S. and overseas for treatment of pregerminated seed. Commercial use of the method began in March 1998. In 1999, pregerminated seed had been successfully treated with ICON® FS at ten commercial locations in the southern U.S.

# Initial interference of barnyardgrass with the growth of rice variety INIA Tacuarí under nutritional restrictions

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The initial interference of the system *Echinochloa crus-galli–Oryza sativa* was evaluated by the treatments of *O. sativa* 100% (Or), *O. sativa* 65% and *E. crus-galli* 35% (Orl-Ecl), and *E. crus-galli* 100% (Ec) cultivated in complete nutritive solution of Kimura B or modified to have 1/4 nitrogen (Ns) or 1/4 phosphorus (Ps) or 1/4 nitrogen and 1/4 phosphorus (Ns, Ps). Measurements were made of the height variation of the plants, root length, dry matter weight of the aerial part and of the root system, and the absorption of N and P.

At the 19th day after transplanting, *E. crus-galli* produced meaningful interference with the height and dry matter production of the aerial part of *O. sativa*. In the treatments with lacking levels of N and P, it was observed that the height of *O.sativa* was more affected by the effect of N than by that of P in the interference of *E. crus-galli*. The deficiency of N affected the dry matter production of the aerial part and roots of *O. sativa* and *E. crus-galli*, with a proportionally greater effect in the latter. The deficiency of P did not affect the dry matter of the roots of *O. sativa*.

P deficiency significantly reduced the dry matter of the aerial part and roots of *E. crus-galli*.

The absorption of N and P by the two species revealed that, in the initial phase of cultivation, the requirements of N and P for *E. crus-galli* were greater than for *O. sativa*. In addition, the effects caused by the lower level of both N and P affected *E. crus-galli* in a greater proportion. The absorption of N and P, expressed in mg per g of dry matter, was more affected by the nutritional stress in *E. crus-galli* than in *O. sativa*. A greater power of recovery of P absorption when the two species received lower levels of N was observed in *O. sativa*.

It is important to note that the effects caused by the interference and by the nutritional stress began to be visualized at the 8th to 12th day.

# Flood depth role in "field" resistance to rice blast

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Blast-susceptible rice cultivars that develop symptoms during the growing season but do not often sustain significant damage are described as being "slow blasting" or "field"-resistant or as having "horizontal" resistance. Several variables, including the irrigation regime, are known to mediate the expression of field resistance. One long-established blast control recommendation is to maintain a proper flood depth during the growing season. In a unique test design, rice seeded in plots having a one inch per foot incline was maintained under a continuous variable-depth flood. This plot design provides rice plants that grow under varying flood depths but exposed to uniform inoculum levels and an aerial environment. Data collected from plants within the plots at desired locations along the incline provide cultivar response comparisons at different flood depths.

Data collected from these plots illustrate the effect of flood depth on blast development under field conditions. The data can be used to better estimate blast field resistance in cultivars or breeding lines. In general, as the flood mediation effect becomes evident, the incidence and severity of rice blast, although uniform at the beginning of flood, gradually decreases in the plots with increasing flood depth. In 1994 tests, leaf blast ratings, using the standard scale of 0 = no disease to 9 = maximum disease, of nine cultivars varied from 4.9 to 8.3 (average 6.1), 3.4 to 8.1 (average 5.5), and 0.4 to 4.1 (average 1.6) in plants growing under upland, a continuous 0–1 inch flood, or a continuous 4–6 inch flood, respectively. During 1995, as the flood depth varied from shallow flood conditions to increasingly deeper flood, the mean number of blast infections per exserted panicle (including neck infections) in 34 cultivars and breeding lines varied from 0.09 to 1.60 (average 0.50), from 0.08 to 1.04 (average 0.31), and from 0.08 to 0.74 (average 0.2) at the 0–1-inch, 4–5-inch, and 9–10-inch flood depths, respectively. In 1996, the mean number of leaf lesions on cultivar LaBelle decreased from 12.0 to 1.0 per plant. A corresponding decrease from 18.7 to 0.6 $mm^2$ also occurred in mean lesion area. A similar decline in the number of leaf lesions and leaf lesion area occurred in cultivars LaGrue and Rosemont.

# Potential for high-temperature tolerance in California rice

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High temperature (>35 °C) at the reproductive stage causes low fertility and can significantly decrease yield in rice. Continuous periods of up to 7 d with maximum daily temperatures over 39 °C have been recorded during the flowering period from July to August in the Sacramento Valley of California. Global temperature increases of 1 to 8 °C because of increasing greenhouse gases have been predicted for the 21st century. We have been studying the effect of high temperature on spikelet fertility of California rice cultivars and known tolerant and susceptible checks. In addition, we have been interested in the effect of high temperature on the fertility of putative thermo- or photosensitive genic male sterile (TGMS or PGMS) lines. Preliminary results showed that some California rice cultivars possess significantly higher heat tolerance than the standard check N22 in the growth chamber long-day and high-temperature treatment (14 h, 38/20 °C and 35/20 °C) for 5 d. The spikelet fertility percentages of S-102, M-202, and N22 were 56.7%, 35.2%, and 26.9% at 35/20 °C, respectively. These results are being further confirmed in additional tests. In a follow-up experiment, the simulated pattern of daily temperature and relative humidity for a maximum/minimum of 38/20 °C is being compared with 35/20 °C (constant for 14/8 h) for 7 d. The plants are being treated at the flowering stage. Pollen and spikelet fertility are being recorded. High temperature at the booting stage can also induce sterility in TGMS or PGMS cultivars. These cultivars possess genes that allow them to be fertile or sterile under the appropriate conditions. They are thus valuable for hybrid seed production.

# Rice biotechnology: a public plant breeder's perspective

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Biotechnology is a general term that describes a range of techniques including cell culture, molecular markers for simple and quantitative characters, isolation and manipulation of genetic control mechanisms, and transformation with alien DNA. This technology has developed rapidly in the past decade and it is seen as competing with traditional plant breeding. In reality, however, biotechnology techniques are supplementary to and an extension of plant breeding. They create variability, assist in selection, and aid the understanding of genetic control.

While not necessarily unique, biotechnology does provide important challenges to the plant breeding community. These are related to product acceptability, ethics, legislative regulation, variety ownership, freedom to operate and ownership of the techniques, use and control of the products in agriculture, and a shift in the balance between public and private plant breeding.

Ultimately, the technology will be used where it provides a real advantage in productivity, price, or cost of production. Most gains will be made via combinations of biotechnology and traditional breeding. The challenge for breeders is to keep abreast of the new techniques and to have sufficient imagination to capitalize on the technology and the new relationships that will develop with its use.

# The CRC for Sustainable Rice Production: an Australian investment in the rice industry

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The Cooperative Research Centre for Sustainable Rice Production (Rice CRC) is an unincorporated joint venture of seven core partners: two universities, two state departments, Commonwealth Scientific and Industrial Research Organisation, Ricegrowers' Cooperative Limited, and a research funding corporation. This seven-year program of research, education, and technology transfer was established under the Australian Government's Cooperative Research Centres Program. Total resources for the seven-year program are estimated to be $53 million (Australian), with $20 million in cash contributions.

Five interrelated programs cover the use of natural resources, sustainable production systems, genetic improvement for sustainable rice production, product and process control, and education. Each program focuses on those areas that will contribute to the sustainability of the rice industry.

There are 18 postgraduate studentships across all programs and supervision of these by university staff and those from other organizations serves to enhance cooperation. The objective is to add value to postgraduate training by promoting a balanced understanding of the rice industry.

Cold at the rice reproductive stage is the most important limit to production in New South Wales, Australia. Studies on this problem are aimed at both the physiology and genetics of cold response. They involve researchers from five research partners and several research disciplines. A solution to this problem will improve the reliability of production and allow the study of novel irrigation systems.

# Development of rice varieties for crawfish forage production

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Crawfish production on managed ponds is conducted on more than 100,000 acres in Louisiana each year. The majority of this production occurs in a double-crop system with rice grain production. In this system, after the grain crop is harvested, the field is reflooded in the fall and the rice stubble and ratoon growth serve as a forage source for crawfish production through the fall and spring.

However, some monoculture production of crawfish occurs. In this system, a forage crop (normally rice) is planted in mid- to late summer solely for the purpose of providing forage for crawfish throughout the season. Historically, the popular rice varieties for grain production have been used for this system. However, these varieties are somewhat lacking in characteristics to maximize crawfish production.

In an attempt to develop better rice varieties for this monoculture system, a study began in 1991. The USDA World Rice Collection was evaluated over a 4-year period (approximately 4,000 accessions per year). The accessions were planted in late summer under conditions that simulated commercial crawfish production. Lines were evaluated for characteristics such as seedling vigor, high biomass production, cold/freeze tolerance, spring regrowth, and persistence. Promising selections from each year were reevaluated throughout the study.

Twenty-two promising selections were planted in a replicated small-plot test in late summer of 1994 and 1995. This study measured biomass production on a periodic basis throughout the crawfish production season.

Three promising selections were entered into large-pond crawfish production studies in late summer of 1996 and each year since then. These studies evaluate crawfish production (both total and size grades) compared with a check variety. The three selections were also entered into the statewide rice commercial-advanced testing program to evaluate grain production characteristics. Though grain production is not directly important with this system, it will be necessary for seed production if one or more of these selections are released for crawfish forage production.

# Effects of rice attributes on the foraging behavior of *Cyrtorhinus lividipennis* Reuter

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A study was conducted in a laboratory to measure the effects of rice attributes on the foraging behavior of *Cyrtorhinus lividipennis,* a predator of the brown planthopper (BPH), *Nilaparvata lugens* (Stål). The results are as follows:

1. The predator females showed a different preference for BPH eggs on different rice genotypes, whereas they showed no preference for BPH eggs on different nitrogen-level rice plants.
2. The predator females were obviously attracted by the volatiles from healthy rice plants and preferred BPH nymph-damaged plants over healthy plants and BPH gravid female-damaged plants over BPH nymph-damaged plants. In a comparison of rice genotypes and nitrogen levels, obvious differences were found in the attractiveness of rice volatiles to the predators among rice genotypes, whereas no significant difference was found among nitrogen levels.
3. The morphological characters of rice varieties could influence the functional response of the predators to BPH eggs indirectly by affecting the vertical distribution of BPH eggs on the rice plants, which could be divided into three types: mainly on the upper and middle parts of the rice plant, mainly on the middle and lower parts of the rice plant, and uniform distribution. The functional response of the predators to BPH eggs mainly distributed on the upper and middle parts of the rice plant is obviously stronger than that mainly distributed on the middle and lower parts of the rice plant since the predators had an obvious preference for searching on the upper part of the rice plant.

The results show the important role of rice attributes, including rice volatiles and morphological characters, in the foraging behavior of *C. lividipennis.* Implications for enhancing the effectiveness of natural enemies by adjusting rice attributes and cultural practices were discussed.

# Microsatellites in rice: characterizing genetic resources for varieties adapted to European conditions

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A study of genetic diversity in rice (*Oryza sativa*) was carried out using 16 microsatellite biomolecular markers corresponding to 16 loci and two rice collections: (1) one rice collection involving 57 varieties that account for Asian rice variability and that was analyzed with enzymatic[1] and RFLP[2] (restriction fragment length polymorphism) markers and (2) the other involving 325 varieties from genetic resources supported by breeding programs of five Mediterranean European countries.

We can draw two main inferences from the analysis of the Asian collection:

1. The polymorphism shown by microsatellites (an average of 10.7 alleles with a range of 4–19) is more important than that of the isozymes (3.6 alleles with a range of 2–7) and the RFLP (2.7 alleles).
2. The bipolar structure shown by a factor analysis of correspondences with enzymatic and RFLP markers is confirmed: we get two major groups corresponding to indica and japonica subspecies (enzymatic groups I and VI) and three intermediate groups including the Indo-Pakistani basmati rice (group V). The subspecies japonica includes the morphological types japonica and javanica.

However, the important polymorphism of microsatellites makes the structuration more blurred and makes the differentiation confusing with some groups such as V and VI.

The study conducted with the Mediterranean collection shows that varieties are clustered in three groups: 85% belong to japonica, 10% to indica, and 5% to basmati (respectively, enzymatic groups VI, I, and V). This lesser genetic variability was expected, but the number of revealed alleles per locus is still important (an average of 8.7 alleles with a range of 4–17 for all groups and an average of 3 alleles with a range of 1–6 for the only japonica group).

The important polymorphism of microsatellites makes varietal identification possible; secondarily, we could check the homozygous state. Using a similarity index, the genetic distances between varieties were calculated and illustrated by a graphic representation, which gives a structuralization picture in a cluster tree. Three main clusters are well defined: the japonica, indica, and basmati ones. Inside the japonica cluster (about 270 varieties), we could differentiate six varietal subgroups with distinctive characters: typical japonica, typical javanica, North and South American varieties, Spanish varieties with javanica morphology, and varieties that suit special European cooking, such as for paella and risotto.

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[1] J.-C. Glaszmann, CIRAD (France), 1987.

[2] Second and Ghesquiere, IRD (France), 1994.

# Development of blast-differential near-isogenic lines for the southern United States

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Since the incorporation of several new blast-resistance (*Pi-*) genes into current southern U.S. rice cultivars, the international differentials have failed to discriminate among some pathotypes of *Pyricularia grisea* having different virulences on U.S. cultivars. For example, shortly after the release of the Arkansas cultivar Katy, isolates of *P. grisea* race IE-1, both virulent and avirulent to Katy, were discovered. In August 1995, we started developing a set of near-isogenic lines (NILs) to differentiate among races in the U.S. population of *P. grisea.* Our goal was to isolate all of the rice-specific *Pi*-genes found in the cultivars M-201 *(Pi-k*$^{s}$*),* Rexmont (*Pi-i*), Lemont *(Pi-k*$^{h}$*, pi-d),* Bengal (*Pi-z*), Kaybonnet *(Pi-ta2),* and Teqing *(Pi-*?*),* and introgress each *Pi*-gene into a Maybelle background by backcrossing. Maybelle is well adapted to the southern U.S. and has no known *Pi*-genes. Teqing was selected even though we have no clear understanding yet of its resistance genes. It possesses at least four *Pi*-genes effective against U.S. races of *P. grisea* and is being used in varietal improvement to increase the yield potential of U.S. cultivars. The five U.S. cultivars named above include all the *Pi*-genes presently identified in U.S. cultivars.

Some 524 $F_2$ plants were selected based upon their reaction to one of six key U.S. pathotypes—international races IB-1, IB-49, IB-54, IC-17, IG-1, mid IH-1—and were advanced to the $F_3$. The $F_3$ lines were challenged with all of the races. Among the $F_3$ lines from the six crosses, 25 combinations of resistant/susceptible responses or "signatures" to the six pathotypes were identified. There was evidence of multiple-resistance genes in Bengal, Lemont, Kaybonnet, and Teqing. Bengal appears to have the *Pi-k*$^{s}$ gene and another unrecognized resistance gene in addition to the *Pi-z* gene. Kaybonnet apparently carries the *Pi-k*$^{h}$ gene as well as the *Pi-ta*$^{2}$ gene. M-201 and Rexmont each have only one *Pi*-gene.

Plants from each of 31 $F_3$ lines, which included each of the 25 signatures, were backcrossed twice to Maybelle. The $BC_2$ plants were screened with races relevant to the signatures. Some 250 plants resistant to the chosen races have been grown to maturity for seed and the offspring were challenged with appropriate races. Plants with the appropriate resistances are being advanced and the resulting $F_3$ lines will be screened against all the races. From these, it is expected that we can select candidates for inclusion as blast differentials for the U.S. If further separation and purification of *Pi*-genes is needed, the above procedure can be repeated as necessary.

We anticipate that we will need to establish no more than 12 NILs as the new U.S. differentials. We propose to follow the current Japanese system of nomenclature modified to suit our needs. Each differential will be assigned a numerical value, half of them decimals: 32, 16, 8, 4, 2, 1, .01, .02, .04, .08, .16, and .32. The race number will be preceded by "NA" (North America) to avoid confusion with other systems. The sum of any combination of the above numbers is unique. For instance, an isolate of *P. grisea* that is virulent to the second, fourth, and ninth differential lines (reading from left to right) would be designated race NA20.04. No other combination adds up to 20.04. If we have fewer than 12 differential lines at the start, we will assign values beginning at the decimal point and working out in both directions, leaving the larger values for future additions. If we find it necessary to include more than 12 differentials, then race numbers will necessarily be expanded to six digits instead of four. As new *Pi*-genes are introduced through exotic germplasm into U.S. rice breeding populations, they should be isolated, characterized, and incorporated into the blast differentials. This may be accomplished more easily in the future through biotechnology. Pathotyping has played a key role in guiding breeding for blast resistance in the U.S. and should continue to do so in the future.

# Depth of emergence of red rice in flooded and moist soil conditions

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Several structural and technical factors determine rice production. Among others, we have to take into account weed management. Red rice (*Oryza sativa* L.) is undoubtedly one of the most troublesome weeds in rice production in some regions of Portugal. To be able to better control this weed, knowledge of red rice biological behavior in relation to different environmental conditions is considered essential to develop strategies of weed management for the integrated production of high-quality rice.

To partially accomplish this objective, an experiment was carried out in a Quinta do Marquês field in 1997 and 1998 to determine the effect of soil depth at sowing on awned and mutic red rice emergence in flooded and moist soil. In the third week of May, we planted 200 seeds of red rice (in pots) at a depth of 0–1, 2–3, 4–5, 7–8, and 9–10 cm in flooded (3 cm of water) and moist (field capacity) soil conditions. The experimental design was a randomized block with three replications.

Results indicated that, for awned and mutic red rice, in moist conditions red rice has emerged more than in flooded conditions, and, in the same conditions, awned red rice germinated better than mutic red rice.

# Is genomics relevant to plant breeding?

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Genomics is the study of how genes and genomes are structured (DNA sequence and chromosomal architecture), how genes function, when, where, and to what extent they are expressed, and how they evolved. Recent advances in automated, high-throughput sequencing have allowed scientists to describe the entire genetic repertoire of various organisms, but the ease of generating sequence data has brought with it new challenges in terms of biological interpretation. As we seek to understand how information about gene structure can be used to predict gene function, and ultimately to understand how genes interact to determine phenotype, biologists have begun to rely on a comparative approach. The degree to which comparative approaches to biology can provide insights into problems related to a specific species of interest depends very much on the interpretive ability of the researcher. A critical aspect of genomics-based inquiry is the use of computational approaches that are required to make sense of information in biological terms. Developing computational tools that allow plant breeders to find information of interest, to ask relevant questions, and to be able to view summaries of data presented in ways that are meaningful will be essential if we hope to make genomics relevant to plant breeding. Rice occupies an unusual position in the world of genomics today. The rice genome has been selected for complete genomic sequencing and will increasingly be looked to as a "model organism" in the world of plant biology. To take advantage of the intersection of interest from both basic and applied scientists around the world, the rice research community must carefully consider how this new level of interest in *Oryza sativa* is likely to affect its research and breeding activities. At a minimum, rice genomics will bring new information to the doorstep of those prepared to interpret it. If the rice research and breeding community takes a more active interest, it could help influence the direction of key activities in the rice genomics arena of the future.

# Textural differences among cooked Koshihikari harvested from different countries

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Koshihikari was obtained from several countries: the United States (California), Australia, and Uruguay. California Koshihikari was supplied from the California Rice Experiment Station. Koshihikari from Australia and Uruguay was obtained at the Foodex Japan'98. California medium grains, M-202 and M-401, were also supplied from the California Rice Experiment Station as references. Ten single rice kernels from each cooking-water ratio were tested by the uniaxial compression test using a Tensipresser. Hardness, stickiness, toughness, and adhesiveness were obtained from the test. Hardness and toughness were defined as the maximum force and work done to compress a cooked rice kernel. Stickiness and adhesiveness were defined as the maximum force and work done to separate a compressed rice kernel from a sample probe. Balance, the ratio of stickiness to hardness, has been commonly used as the index of cooked rice texture in Japan. A modified balance is defined as the ratio of adhesiveness and toughness and was used to differentiate the texture of cooked Koshihikari samples.

# Fertility studies on Japanese varieties of rice: Akitakomachi and Koshihikari

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California growers and marketers are increasingly interested in producing Japanese rice varieties for the potentially lucrative export market. To capitalize on this market, rice must be of a consistent high quality with specific grain characteristics. Careful nitrogen management is a key to producing the desired quality attributes. The objectives of this study were to (1) determine the optimal rate and time of nitrogen application to optimize yield and grain quality and (2) ascertain the relationship of leaf nitrogen content at key growth stages to specific grain quality components.

Akitakomachi and Koshihikari were planted in replicated experiments conducted in 1996, 1997, and 1998. Nitrogen treatments of 0, 20, 40, 60, 80, 100, and 120 lb N acre$^{-1}$ were applied either entirely preplant or as a split application between preplant and a specific growth stage. In all years, split applications were applied at panicle initiation and heading. Split applications at other growth stages varied slightly between years but did not produce significant results. The results presented below represent those treatments common to all experiments across years. Plant growth and development, yield components, yield, grain protein, and amylose and fatty acid content were measured and Satake taste scores determined every year. In 1998, leaf nitrogen concentrations were tracked throughout the season, by both chemical analyses and the Minolta chlorophyll meter, which was calibrated in 1996 and 1997.

Averaged data from four field experiments indicated that total nitrogen (100 and 80 lb acre$^{-1}$) applied preplant produced the highest yields in Akitakomachi vis-à-vis various split application combinations. In contrast, the best yields in Koshihikari resulted from split applications of nitrogen (20/20 and 40/120 lb N acre$^{-1}$ preplant/heading split). Preplant/panicle initiation or preplant/heading split applications produced similar results in Koshihikari. The highest-yielding treatments produced average taste scores of 82 and 77 in 1997 for Koshihikari and Akitakomachi, respectively. The highest yields were observed when leaf tissue nitrogen was near 2.5% at panicle initiation. Taste scores were negatively correlated with grain protein content ($R^2 = 0.85$), whereas leaf tissue nitrogen at heading was positively correlated with grain protein content ($R^2 =$ 0.96 and 0.85 for preplant and preplant/panicle initiation split applications, respectively).

# Influence of nitrogen fertilizer rate and timing on rice grain and milling yields

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Arkansas for many years exclusively recommended that nitrogen (N) fertilizer be applied in three topdress split applications to dry-seeded delayed-flood rice. When the rice reached the four- to five-leaf stage, 50% to 65% of the N fertilizer was applied preflood onto dry soil followed immediately by the establishment of the permanent flood. The remaining 35% to 50% of the N was applied directly into the floodwater around panicle differentiation in two topdress applications. Since the release of semidwarfs and short-stature rice that does not lodge easily, Arkansas has been moving away from applying the N fertilizer in split applications to applying all of the N fertilizer in a single preflood application. Research has clearly shown that the rice cultivars currently grown obtain either a higher grain yield or a similar grain yield with less N fertilizer when it is applied in a single preflood application compared with split applications. Some have questioned how milling yield is affected by N application timing and whether N applied at booting can affect milling yield as well as total grain yield. The following study was conducted to compare rice grain and milling yield when N fertilizer was applied in split applications and in a single preflood application with and without an N application at booting.

The study was conducted in 1996 and 1997 at the University of Arkansas Rice Research and Extension Center near Stuttgart. The soil was a Crowley silt loam (Typic Albaqualfs) that had been in a rice-soybean rotation for the past 10 years. A split-split plot experimental design with four replications was used. The main plot was varieties (LaGrue and Cypress); the subplot was N rate (0, 90, 134, or 179 kg N ha$^{-1}$ applied in a single preflood and a three-way split application scheme); and the sub-subplot was a boot N application (0 or 45 kg N ha$^{-1}$). Experimental parameters measured were grain and milling yields. The rice was drill-seeded at 112 kg ha$^{-1}$ in nine-row plots (row spacing of 18 cm), 4.6 m in length. All plots were flooded when the rice was at the four- to five-leaf stage and remained flooded until the rice was mature. At maturity, 3.66 m of the center four rows of each plot were hand-harvested, threshed, dried to 120 g $kg^{-1}$ moisture content, and grain weight was determined. After drying, a 150-g sample of rough rice from each plot was milled. The milling procedure consisted of hulling the rough rice with a McGill huller, milling the brown rice for 30 seconds with a McGill No. 2 miller, and separating the white rice into head rice (kernels having three-fourths or more original kernel length) and brokens using a Seedburo sizing machine with a No. 13 top screen and a No. 12 bottom screen. Total white rice yield and head rice yield were expressed as a percentage of the original rough rice sample. Statistical analyses were conducted with SAS and mean separations were based on protected LSD where appropriate.

LaGrue and Cypress had similar grain yields in 1996, but LaGrue had significantly higher grain yields in 1997. Rice grain yields increased as N fertilizer rate increased up to 134 kg N $ha^{-1}$ when the N was applied in a single preflood application and up to 179 kg N $ha^{-1}$ when the N was applied in split applications. In both years with both varieties, the three-way split application method required more N fertilizer to produce grain yields comparable with those of the single preflood N application method. The application of N fertilizer at booting resulted in significantly higher grain yields for both varieties in 1996, but had no effect on grain yield in 1997. Cypress had a higher percent total white rice and head rice than LaGrue in both years. As N rate increased and grain yield increased, so did milling yields. Milling yields were highest at the N rate that produced maximum grain yield. Since the grain yields produced with the split application method lagged behind those of the single preflood method until the optimum rate of N for maximum grain yield had been applied, so did the milling yields. Until an optimum rate of N was applied to produce maximum grain yields, the single preflood method resulted in higher total white rice and head rice percentages than the split application method. The application of N fertilizer at booting had no significant effect on percent total milled white rice and head rice for either variety in 1996, but did increase both significantly in 1997 for both varieties. Kernel weight also increased in both years from the N application at booting.

# Effect of potassium fertilization on rice yield and agronomic characteristics

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The large potassium extraction caused by rice monoculture and the harvest system has made Chilean soils deficient in potassium. It is not uncommon to find rice fields with extractable K contents lower than 40 mg $kg^{-1}$.

From 1990 to 1992, several field experiments were performed to evaluate rice response to K fertilization alone and/or in combination with nitrogen and phosphorus. Potassium varied from 0 to 100 kg K $ha^{-1}$. Nitrogen and P varied from 0 to 240 kg N $ha^{-1}$ and from 0 to 52 kg P $ha^{-1}$. Potassium was applied as potassium sulfate (40% K) and/or potassium chloride (50% K), whereas, for N and P, urea (45% N) and triple superphosphate (20% P) were used. The K and P fertilizers and half of the N rate were broadcast applied on dry soil and incorporated with a rototiller. In all cases, a randomized block design with three replications was used. The experiments were planted with Oro (short grain) and Diamante-INIA (long grain) varieties. The plot size was 15 $m^2$, of which 4 $m^2$ were harvested for grain yield. Evaluations involved grain yield and yield components, plant height, stem rot (caused by *Sclerotium hydrophyllum* Sacc.) severity, and flag-leaf potassium content at heading.

Results showed a significant effect of K applications on rice yield because of an increase in the number of grains per panicle and grain weight. However, the effect of K was dependent on nitrogen level. Larger responses to K fertilization occurred with higher N rates. No interaction between K and P was observed. On soils infested with stem rot, K applications drastically decreased the severity of the disease, thus allowing the crop to produce normal yields. No differences were observed between potassium sulfate and potassium chloride in any of the evaluated variables.

Potassium fertilization has been a standard practice in rice production in Chile since the 1992-93 growing season. Application rates vary from 25 to 50 kg K $ha^{-1}$. Potassium is added as potassium chloride in NPK fertilizer mixtures.

# First report of bacterial sheath brown rot of rice in Uruguay

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In recent years, necrotic leaf and sheath symptoms and grain discoloration have been observed on rice (*Oryza sativa* L.) in Latin America.

Bacterial sheath brown rot of rice plants was reported in 1976 from Hokkaido, Japan, to be caused by *Pseudomonas fuscovaginae*. Since 1985, this pathogen has been described on rice in Burundi, in Madagascar, and in Latin America.

To check infection by *P. fuscovaginae* in the field, samples were collected and tested in the laboratory. Diseased sheaths, leaves, and grains were collected and ground separately in sterile distilled water (10 mL for 1 g of fresh tissue), streaked on King's B (KB) medium, and incubated at 28 °C. After 2 to 5 days, single fluorescent colonies were subcultured to fresh plates of KB. Colonies were fluorescent under ultraviolet light, up to 2–3 mm in size, white in color, and convex. Pathogenicity was proved by completing Koch's postulates on rice seedlings of cultivars El Paso 144, INIA Cuaró, and Cebollati grown in the greenhouse. Pathogenic strains underwent the following physiological tests: Gram stain, oxidase, arginine dihydrolase, starch hydrolysis, protease (gelatin liquefaction), growth on sorbitol neutral red agar, levam from sucrose, soft rot of potato, and hypersensitivity in tobacco (Cv. Butley).

On the basis of pathogenicity and physiological tests, the bacterium was identified as *Pseudomonas fuscovaginae.* This study reports for the first time *P. fuscovaginae* causing bacterial sheath brown rot of rice in Uruguay.

# A comparison of anther culture response of commercial rice varieties of Sri Lanka

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Anther culture response of eight indica rice varieties (including six leading commercial Sri Lankan varieties), six japonica varieties, and their crosses were studied with the objective of developing a suitable protocol for rice improvement through the doubled-haploid technique. Agar-solidified media based on SK and N6 salts, supplemented with naphthalene acetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D), and kinetin (Kin) were used for callus induction. Sterilized anthers plated on the media were incubated at 28 ± 1 °C in the dark. The induced calli, after 2 months in culture, were transferred to a proliferation medium, also based on SK salts. After 10 days, the proliferated calli were transferred to two MS-based regeneration media. Eight-wk-old plantlets were transferred to half-strength solid MS medium and after 1 wk to sterilized soil in humid chambers for acclimatization. The highest callusing percentage was observed on SK medium supplemented with NAA 0.75 mg $L^{-1}$, 2,4-D 2.5 mg $L^{-1}$, and Kin 0.75 mg $L^{-1}$. Among the genotypes studied, three indica varieties—AT 354, IR46, and BG 403—recorded 13.3%, 6.1%, and 4.2% callusing, respectively, versus 7.6% in Fujisaka, a japonica variety. Total plant and green plant regeneration were more in the solid MS medium supplemented with NAA 1 mg $L^{-1}$ and Kin 1 mg $L^{-1}$. However, in the most responsive AT 354 variety, a higher concentration of NAA (1.5 mg $L^{-1}$) produced better results.

The protocol was used to attempt the production of doubled-haploid lines from different cross combinations. So far, anthers from crosses involving the varieties AT 354, Bg 352, Bg 850/2, and Pokkali have produced calli at rates ranging from 0.42% to 3.42%.

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# Desirable characteristics for lowland and upland rice varieties for the cool environment of Hungary

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Rice cultivation has only a 70-year history in Hungary (including the introductory trials), starting with a simple technology (water seeding, broadcasting by hand) and a variety with good seedling vigor, Dunghan-Shali, in the region of 47–48°N. Production was rather risky in these disadvantageous environmental conditions (cold and blast damage); therefore, the government subsidized both production and research to develop highly adaptable varieties and production technologies. Since the 1950s, self-sufficiency has been an important economic aim.

Rice production was carried out on about 1 million hectares till the late 1980s. Concentration of the rice area and improved technology have increased production (fully mechanized large-scale rice production) and highly adaptable and good-quality rice varieties have been released and cultivated.

Since the early 1990s, a drastic change has occurred and the future of rice production is uncertain. Mostly, the ceasing of government subsidies, continuous cheap imports, and the increase in production costs resulted in a decrease in profitability and area of rice to a minimum level (from 1997 to mid-1999, only 2,000–3,000 ha). To maintain rice culture in Hungary, growers, processors, and researchers collaborate to find better profitability.

For breeding, the main emphasis has been on maintenance of the recently released rice varieties (Ringola, Sandora, Dama, and Risabell) and a good supply of basic seed. We used our research conditions to test and evaluate the biotic and abiotic stress reactions of our breeding material. On the basis of these experiments, the variety characters required among the disadvantageous conditions of our country were determined together with the features required for an increase in production, crop safety, and a decrease in production costs.

The desirable characteristics change slightly depending on the production system, that is, selection is carried out for the dry or flooded production system. Our findings demonstrated eight desirable characteristics, such as seedling vigor, earliness, lodging resistance, and tolerance for cold, drought, and leaf and neck blast.

# Cold tolerance of short-season rice cultivars in Uruguay

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The reproductive phase of rice growth, including panicle development and anthesis, is very susceptible to low temperatures that cause grain sterility. Cold periods during the reproductive phase are common in Uruguay and were identified as one of the main reasons for yield instability. The warmer months of summer, January and February, have an average of 10 and 9.6 days, respectively, with minimum temperatures below 15 °C. The development of short-season cold-tolerant cultivars with good milling and cooking quality has been a priority for the local breeding program. The short-season high-yielding cultivars INIA Yerbal and INIA Tacuarí were released in 1989 and 1992, respectively. Their cold tolerance performance in trials from 1989-90 to 1992-93 compared with check cultivars Bluebelle and El Paso 144 was demonstrated. INIA Caraguatá, a semidwarf, long-grain, and high-yielding variety, was released in 1995. It has superior milling and cooking quality, but, according to preliminary information, it was considered as susceptible to low temperature. All these cultivars, except El Paso 144, an indica-type variety, are tropical japonica types.

The information from time of seeding experiments from 1989-90 to 1996-97, including 36 trials, was used to compare the cold tolerance of INIA Tacuarí and INIA Caraguatá to that of the check cultivars Bluebelle and El Paso 144. Minimum and mean preflowering temperatures for a 20-d period (20 DPF) and 12-d period (12 DPF), mean temperatures during flowering (10 DF), mean temperatures during the whole reproductive phase (12 DPF + 10 DF), as well as mean temperatures during a 20-d postflowering period (10–30 D + F) were recorded for each plot and related to the observed grain sterility, adjusting regression equations.

The higher determination coefficients ($R^2$) for grain sterility were obtained with mean temperatures during 20 DPF and 12 DPF

+ 10 DF. The $R^2$ values for the different varieties ranged from 17% to 59% in the first case and from 11% to 66% in the second case. INIA Tacuarí had the smallest $R^2$ values. At the same time, its regression equations showed less incidence of mean temperature on grain sterility than INIA Caraguatá and the check cultivars.

At 20 DPF and 12 DPF + 10 DF, the grain sterility of El Paso 144, Bluebelle, and INIA Caraguatá showed a sharp increase with mean temperature lower than 23 °C. INIA Tacuarí maintained moderate sterility (about 30%) at the lowest temperatures, which resulted in high sterility in the cold-susceptible cultivars (60–70%).

Selection under natural conditions in late-seeded populations has been effective for developing cold-tolerant cultivars that may help to increase and stabilize grain yield in the country.

# RFLP mapping of rice blast-resistance QTLs in a recombinant inbred line population derived from Lemont × Teqing

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A set of recombinant inbred lines (RILs) was developed from a cross between Lemont, a tropical japonica variety grown widely in the southern United States and throughout the world, and Teqing, a very high yielding Chinese indica. A framework restriction fragment length polymorphism (RFLP) map containing 175 loci was developed from analysis of DNA extracted from 284 $F_{10}$ progeny lines whose $F_9$ parental lines appeared homogeneous in the field. Ancestral $F_8$ plants were spray-inoculated and evaluated for complete resistance to five individual races of the blast pathogen: IC-17, IB-54, IB-49, IE-1, and IG-1. Linkages between the mapped markers and genes affecting disease resistance were detected using both Chi-square and Fisher's exact test. Four major resistance genes were identified and located on chromosomes 2, 6, 11, and 12. Each gene was effective against more than one race of the pathogen. The gene on chromosome 6 appears to be a new gene, while the other three genes may be allelic to previously reported major genes.

$F_8$ plants were also grown in field plots that were naturally inoculated by a mixture of races and evaluated for quantitative measures of disease reaction: the standard evaluation system (SES) for rating leaf blast, percentage diseased leaf area (%DLA), and the area under a disease progress curve (AUDPC). MapMaker/QTL was used to conduct interval analysis of correlations between the marker data and the quantitative disease data. Nine putative quantitative trait loci (QTLs) associated with one or more of the quantitative measures of field resistance were located, one QTL on each of chromosomes 1, 2, 3, 4, 6, 7, and 9 plus two linked QTLs on chromosome 12. Teqing contributed the resistance allele for all but one of these putative QTLs. All nine QTLs were associated with

AUDPC, while only six were associated with both %DLA and SES. Individual QTLs accounted for 5–32% of the observed phenotypic variation and combined QTL models accounted for 43–53%. Three of the QTLs mapped similarly with three of the four major genes that were mapped using the qualitative single-race response data. Four of the putative QTLs mapped similarly with genes and/or QTLs that had been reported by others elsewhere involving different populations and field environments. These genes/QTLs that were found to be effective under different genetic and environmental conditions may be more widely useful in providing varietal resistance.

Both the durable resistance exhibited by Lemont and the complete and broad-spectrum resistance exhibited by Teqing in the U.S. were attributed to a combination of major genes capable of inducing hypersensitive reactions and minor genes causing less distinctive phenotypic differences. Interactions were noted between QTLs and major genes. Our findings support the strategy of pyramiding major genes and QTLs in carefully selected combinations to develop improved varieties with resistance to the blast fungus that is both broad in spectrum and durable.

Transgressive segregation has been noted for all phenotypic characters observed to date including height, heading time, resistance to two fungal diseases, seedling vigor, leaf size, tiller number, panicle and kernel size, and chemical and physical measures of grain quality. Segregation has been observed even for characters for which the two parents are not apparently different. Although 10% of the Lemont/Teqing RILs require intense management to maintain because of their high sterility and/or low germination rates, as a whole, this population exhibits less sterility and less non-Mendelian genetic skewing than other available rice mapping populations. Seed and molecular data from this self-replicating population are being shared upon request (contact Dr. Pinson at srpinson@tamu.edu).

# Status of Alismataceae weeds in Australian rice crops and potential for their biological control

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The Alismataceae family of aquatic plants, consisting of the natives *Alisma plantago aquatica* and *Damasonium minus* and three introduced species, *A. lanceolatum, Sagittaria montevidensis,* and *S. graminea,* are considered the most important aquatic broadleaf weeds of rice in Australia. The introduction of aerial sowing, irrigation development, and the emergence of herbicide-resistant biotypes have increased the importance of these weeds. The three introduced species have entered the rice-growing regions within the last 20 to 30 years and are spreading rapidly, having not yet reached their potential distribution.

Current control methods rely largely on the use of a single herbicide, Londax®. Intensive herbicide programs probably contributed to the development of Londax-resistant populations of *S. montevidensis* and *D. minus* and alternative means of control are being sought. The endemic fungal pathogen *Rhynchosporium alismatis* is being investigated as a potential biological control agent for these weeds. *R. alismatis* produces necrotic and chlorotic lesions on mature leaves and less frequently on petioles and inflorescence stalks of *D. minus* and *Alisma* species. By increasing the pathogenicity and widening the host range of the fungus, the use of a fungal bioherbicide to control broadleaf weeds in Australian rice fields would become a viable option.

The application of emerging technologies in Australian rice weed control is directly relevant to rice production in the United States, where both climate and rice culture are similar to those of Australia.

# Rice variety development in New South Wales, Australia

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The diversity of rice varieties emanating from the rice breeding program at Yanco has increased markedly. Over the past 25 years, the New South Wales rice industry has moved from producing two quality types—soft-cooking long-grain and medium-grain types—to producing rice in six distinct quality classes. The trend toward increased quality diversification began in 1990, driven by market demand both domestically and internationally for rice of specific qualities. This direction is likely to be maintained in the future, and genetic improvement will be enhanced by the continued development of rapid objective tests for all aspects of grain quality.

# Evaluation of *Bacillus thuringiensis* strains for control of rice insect pests

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*Bacillus thuringiensis* strains have been used to develop several biopesticides, which have been used to protect agronomically important crops. For instance, rice (*Oryza sativa* L.) is exposed to intense insect pressure from Coleopteran and Lepidopteran pests. In addition, toxin genes from these biopesticides can serve as a source of insecticidal genes for the development of enhanced germplasm conferring resistance to injurious insects. Methods to rapidly identify and classify insect-specific toxin genes (Cry toxins from *Bacillus thuringiensis*) would facilitate their implementation and use in crop protection. Recent approaches to the characterization of novel isolates involve the use of the polymerase chain reaction (PCR) to rapidly identify and classify Cry toxins based on the presence of diagnostic fragments when employing specific classes of oligonucleotide primers. I have extended this approach by developing new primers for the rapid detection of a potentially new and useful gene [vegetative insecticidal protein (Vip3A)] from various *B. thuringiensis* strains. *B. thuringiensis* strains were then identified that contain different gene-toxin combinations (i.e., different mixtures of Cry1, Cry3, Cry5, and Vip3A(a) toxins). Field trials were then conducted using *B. thuringiensis* isolates that contain different toxin combinations to evaluate their potential for controlling rice water weevil (RWW) larval populations. Several *B. thuringiensis* strains were capable of causing a reduction in RWW larval densities.

# Water-use efficiency in two flood management systems

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Water is the most valuable renewable natural resource. Irrigation water management is becoming critically important worldwide. As freshwater for agriculture becomes increasingly scarce, greater efficiency of water use is essential. Rice *(Oryza sativa),* like any other crop, requires an adequate supply of water to grow and develop. Unlike other crops, rice is usually grown in flooded soil.

Rice irrigation management in Uruguay normally consists of a dry period after emergence of about 45 days. During this period, flushing is required to prevent water stress (average 2 flushes). Following 45 days after emergence (DAE), a flooding is established until at or near harvest.

The study was conducted at the National Institute of Agricultural Research (INIA) at Treinta y Tres, Uruguay, during the 1996-97 and 1997-98 growing seasons. Two macroplots, each 2.5 ha, were seeded with cultivar INIA Tacuarí (an early maturing, high-yielding, long-grain variety). Water use and yield were measured and water-use efficiency [yield (kg ha$^{-1}$)/total water use (m$^3$ ha$^{-1}$)] was calculated for the traditional flood timing at 45 DAE and an early flood timing at 15 DAE in both years.

The planting dates were 17 and 8 October and emergence was established on 6 November and 26 October for the 1996-97 and 1997-98 growing seasons, respectively. Management practices (seeding rate, fertilization, herbicides, fungicides) were the same in both treatments. Both treatments were harvested with a commercial combine on 19 and 23 March in the 1996-97 and 1997-98 growing seasons, respectively.

The water supplied in each irrigation treatment was divided into three components: the water that was needed to flush the plots (flushes), the water necessary to flood the plots (flood), and the water needed to maintain the flood (flood maintenance).

The total amount of water supplied to each treatment was 6,220 $m^3$ $ha^{-1}$ and 8,062 $m^3$ $ha^{-1}$ in 1996-97 and 3,383 $m^3$ $ha^{-1}$ and 4,531 $m^3$ $ha^{-1}$ in 1997-98 for the early and traditional flooding treatment, respectively.

Since the early flooding treatment was flooded 15 DAE, flushes were not required. With traditional flooding, three flushes were necessary in the 1996-97 growing season and two flushes in the 1997-98 growing season. The amount of water needed for flood maintenance was higher in the early flooding treatment than in the traditional flooding treatment in both years because of a longer flood period. In spite of this, the difference in these totals (flood maintenance) between treatments was smaller than the amount of water needed to flush the traditional flooding treatment during the "dry" period.

Rice yields were similar for both treatments: 7,340 and 7,065 kg $ha^{-1}$ in 1996-97 and 6,297 and 6,435 kg $ha^{-1}$ in the 1997-98 growing season for early and traditional flood timing, respectively. Water-use efficiency, defined as kg of rice produced per $m^3$ of total water use, was higher in the early flood timing than in the traditional flood timing treatment in both years, mainly because of the lower amount of water received in the first treatment, since yields were almost the same.

# A soil nitrogen test for flooded rice: soil NIR spectra and the prediction of soil nitrogen supply and soil properties

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In the rice fields of Australia, nitrogen (N) fertilizer applied before flooding is twice as efficient as that applied on the growing crop. However, adoption of preflood N is limited because of the lack of a suitable soil test. Such a soil test needs to be rapid and highly correlated with soil N supply, as measured by the N uptake of rice tops at panicle initiation (PI) or maturity. We describe a rapid presowing soil nitrogen (N) test for flooded rice (*Oryza sativa*). The test, a physical one using soils near infrared reflectance (NIR) spectral properties, was evaluated for its ability to predict aboveground rice N uptake at PI from unfertilized field plots at 24 sites over four years. Analysis of NIR soil spectra revealed a very strong relationship with PI ($R^2 = 0.85$). Furthermore, soil NIR spectra were correlated strongly ($R^2 > 0.70$) with several important soil properties such as total soil N, total soil C, S, P, K, Ca, Mg, pH, Na, and EC. These properties were generally poorly intercorrelated. We conclude that rapid soil analysis using soil spectral properties is possible, currently needed, and will become more urgent as new management technologies, such as precision farming and remote sensing, gain popularity.

# Susceptibility of rice varieties to molinate under water-seeded rice culture

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Molinate (*S*-ethyl hexahydro-1 *H*-azepine-1-carbothiote) preplanted incorporated with water-seeded rice is one of the most efficient methods to control red rice. In 1997 and 1998, the phytotoxicity of molinate on domestic rice varieties was studied at INIA Treinta y Tres, Uruguay. Untreated and treated large plots (10.5 × 9.5 m) with molinate were broadcast with seed of four rice varieties: INIA Tacuarí (short-season japonica type), INIA Caraguatá (mid-season japonica type), INIA Cuaró (mid-season indica type), and El Paso 144 (long-season indica type). These varieties interacted in a factorial arrangement with two seed treatments: dry or pregerminated seed in small plots (2 × 4 m). An alley of 0.5 m was left among small plots to help in seeding and data collection. The split-plot design was a randomized complete block design with three replications. Molinate was applied at 4.48 kg ai ha$^{-1}$ with a CO$_2$ pressurized sprayer with a 4-nozzle boom with Teejet 8002 tips. A volume of solution of 140 L ha$^{-1}$ was applied. The herbicide was incorporated right after the application. Dates of herbicide application, establishment of flooding, and rice seeding were 17 November, 20 November, and 26 November for the experiment in 1997 and 28 October, 9 November, and 13 November for the experiment in 1998, respectively. Six hundred and fifty viable seeds m$^{-2}$ in each small plot were seeded in water. One half of the rice seed was soaked from 36 to 48 h and after that drained under shade from 36 to 48 h. In 1997, all rice varieties were evenly sprouted before seeding; however, in 1998, seeds germinated slowly and unevenly. The other half was seeded with dry seed (without pregermination). Every large plot was flooded and drained individually and pinpoint water management was used. A layer of 10-cm water depth was used. To avoid damage to the rice stand by birds and ducks, nets were placed. An assessment of injury from herbicide was done by counting seedlings at 15 days after seeding (DAS). Three and six samples per small plot were taken in 1997 and in 1998, respectively. The recovery of normal seedlings was statistically analyzed using the GLM procedure of SAS without data transformation. Mean separation was done using the Tukey honestly significant difference (HSD) at 5% of alpha level. The average of the rice stand was similar for both experiments. It was 232 (36% of recovery) and 243 (37% of recovery) seedlings $m^{-2}$ at 15 DAS in 1997 and 1998, respectively. There was no significant main effect of the herbicide molinate on the rice stand. Pooled over years, the rice stand was 202 seedlings $m^{-2}$ at 15 DAS for the herbicide-treated plots and 278 seedlings $m^{-2}$ at 15 DAS for the plots without herbicide treatment. The tendency was to obtain more seedlings without molinate than with molinate. No rice variety difference was obtained and neither was an interaction observed among molinate, rice variety, and seed treatment. There was an interaction between year of the experiment and seed treatment. The difference between dry seed and pregerminated seed in the rice stand (236 vs 228 seedlings $m^{-2}$ at 15 DAS) was not significant in 1997, but was significant (183 vs 303 seedlings $m^{-2}$ at 15 DAS) in 1998.

# Characterization of temperate rice germplasm in terms of salinity resistance: a study of 240 European varieties

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Stringent requirements for water holding for European rice producers who use pesticides have resulted in a loss of stand and visible symptoms of leaf damage for some growers. A field survey and subsequent series of experiments were conducted to determine the range of salt tolerance among 240 varieties of rice (*Oryza sativa* L.) that are common to European rice-growing areas. Phenotypic resistance to salinity is expressed as the ability to survive and grow in a salinized medium. Some subjective measure of overall performance has normally been used in plant breeding programs aimed at increasing salinity resistance, not only to evaluate progeny but also to select parents. Salinity resistance has at least implicitly been treated as a single trait. Physiological studies of rice suggest that a range of characteristics (such as low sodium concentration, compartmentation of salt in older rather than in younger leaves, tolerance for salt within leaves, and plant vigor) would increase the ability of the plant to cope with salinity.

# The role of endosperm reserves in the growth response of submerged rice seedlings

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Elongation activity, carbohydrate supply, and energy reserves are the key traits of submerged rice seedlings. The present study examines the relations of seedling age at submergence to elongation activity, dry matter, and survival for several rice varieties with a wide range of seed sizes.

Twelve lowland rice varieties were tested: Oochikara, Husayoshi (large seed size: 38.3–46.8 mg seed$^{-1}$), Koshihikari, Sasanishiki, Kinuhikari, Dontokoi, Akihikari, Hukunohana, IR24, Milyang 23 (medium seed size: 23.4–30.6 mg seed$^{-1}$), Syouryujinrikihen, and Shinkin × Aikoku (small seed size: 12.3–15.1 mg seed$^{-1}$). Pregerminated seeds were sown daily from 11 to 18 May 1998 in trays filled with nursery soil. After the seedlings attained the age of 5–12 days, they were completely submerged in a tank (482 × 140 × 31 cm deep). The water level was maintained at 31 cm for 6 days and then lowered.

Seedling length increased 1.7–8.8-fold during submergence and the elongation ability of most varieties tested tended to decrease when the seedlings were submerged around the middle of the 2nd-leaf stage. However, most seedlings survived complete submergence for 6 days, irrespective of age prior to submergence, elongation activity, and variety. This could be associated with the carbohydrate supply from endosperm reserves because the submerged seedlings had 18–78% residual endosperm reserves. Thus, if the submergence stress was more severe because of increasing duration, for example, 10 d, seedling survival might decrease and vary with seedling age or variety. The increase in shoot dry weight of most varieties tested during submergence was largely influenced by seedling age, and the average calculated over the seedling age for each variety was highly correlated to seed size. However, a larger increase in shoot dry weight during the 10 d after submergence was observed in both aged seedlings prior to submergence and in varieties with a larger seed size. As a result, shoot dry weight of submerged plants at 10 d after submergence increased with seedling age to varying degrees for each variety. There was a direct relationship between seed size and average shoot dry weight calculated over the seedling age for each variety. This average shoot dry weight obtained for submerged plants was calculated as a percentage of that obtained for nonsubmerged plants and the shoot dry weight percentage was not correlated with seed size.

# Irrigation systems, water-holding times, and salinity affect water quality in California rice

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In the early 1980s, herbicide residues from rice-field tail-water caused fish (carp) mortality in agricultural drains and off-taste problems in the City of Sacramento water supply. To solve these problems, regulatory agencies required growers to block field water outlets and hold irrigation water on fields for up to 30 days after herbicide applications. This improved water quality by allowing the herbicide to dissipate in the field, but limited grower water management flexibility. Some growers have shifted from conventional irrigation systems to closed irrigation systems to hold their water and improve their water management flexibility. During these long water-holding periods, some growers have had seedling establishment problems and have expressed concern about possible salinity problems.

A field-scale water quality demonstration project, funded by the United States Department of Agriculture, was established near Colusa, California, to assess and show the effectiveness of several rice irrigation systems for controlling herbicide residue discharges from rice fields from 1991 to 1994. Conventional, recirculating, and static systems were compared.

Herbicide concentration and water flow data were collected and used to calculate accumulated and total mass discharges for each system. Discharge differences among the systems were evident in some years, but not in others. The static and recirculating systems were able to keep pesticide residue discharges into public waterways very low during most years, whereas discharges from the conventional system were very low in some years and higher in other years depending on how water was managed. Each system was subject to crop production and weather problems, which resulted in emergency releases of water and pesticide residues. Our experience indicates that recirculating and static systems may re-

duce discharges compared with conventional systems sometimes, but that they are vulnerable to emergency release, just like conventional systems. In addition, conventional systems contained pesticide residues about the same as the alternative systems when little or no spillage was allowed. They just required more careful management than the other systems.

Most of the discharges from the demonstration fields occurred within a few days of the initial peak outflow following a herbicide application. The earlier the outflow, the higher the discharge. Discharges were lower as water-holding time increased. Our findings provide supporting evidence for the use of water-holding periods to control residue discharges from rice fields. The use of early "emergency" releases from rice fields in recent years has been rare and limited to weather-related problems such as unseasonable rainfall, cool weather, and wind problems. Recently, emergency release provisions for salinity problems have been developed. Growers should have some provision for the emergency release of water when weather or salinity problems arise; otherwise, economic loss could occur.

Salinity problems in rice were assessed in a survey conducted on the wide side of the Sacramento Valley from 1993 to 1995. It showed that rice-field soil and water salinity levels were significantly higher in bottom basins than in top basins, especially during the early season. Intensive monitoring in selected fields showed that water salinity levels were the highest during water-holding periods, especially in bottom basins. Water salinity levels in bottom basins of some fields increased to damaging levels during the water-holding period, while salinity in other fields was much lower and not a problem. Rice stand establishment and growth declined as water salinity increased. In higher EC fields, adding water to the bottom basin during the water-holding period helped reduce salinity levels. Ponding water on fallow land or recirculating it may also help. If water management adjustments do not reduce salinity levels below damaging levels, an emergency release may be needed.

# Identification and transfer of blast resistance from *Oryza rufipogon to O. sativa*

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A marker-assisted advanced backcross strategy was used to identify and transfer QTLs controlling blast resistance from the wild species *Oryza rufipogon* into an elite rice cultivar, Jefferson. A total of 353 $BC_2F_2$ families derived from a cross between Jefferson and *O. rufipogon* (IRGC 105491) have been analyzed for resistance to some of the major races of blast found in the southern U.S. rice-growing areas (IC17, IE1k, IG1, and IB49). Preliminary phenotypic data indicate that *O. rufipogon* contains useful genetic resistance to race IB49 that is not found in Jefferson. Using microsatellite and RFLP-based markers, the loci controlling this resistance have been mapped.

# Rice water management in Egypt

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Water resources in Egypt are limited to 55.5 × $10^9$ $m^3$ a year. With the tremendous increase in population, rice production has to be increased and irrigation water has to be well managed and ways found to increase water-use efficiency.

Several field experiments were conducted to rationalize water use. These experiments involved (1) replacing long-duration varieties with short-duration varieties, (2) using a different irrigation interval, (3) withholding water at certain growth periods that are not sensitive to water deficit, and (4) using different methods for planting combined with land preparation. The available results indicated that, by replacing long-duration variety Giza 171 (160 d) with Giza 177 or Sakha 102 (120 d), this could save about 20% of the irrigation water.

Results showed also that irrigation every 6 d during the growing season gave reasonable production compared with continuous flooding or irrigation every 3 d. Rice varieties showed higher sensitivity at the early tillering stage and panicle initiation stage. Most of the varieties were less sensitive in the later tillering stage and late maturity stage. Using mechanical transplanting could save about 3–5% water, which was used for seedlings in the nurseries. Data showed also that dry leveling using the laser technique helped to achieve good distribution of the irrigation water in the rice field, thus preventing water accumulation in some areas while other areas were dry. Drill-seeded rice could save some irrigation water compared with wet direct-seeded methods such as broadcast seeding and dibbling.

The investigation concluded that long-duration varieties must be replaced by short-duration varieties. Drought-tolerant variety Giza 178 is recommended in the most water-short areas.

# Effect of no-tillage on nutrient absorption and growth of rice plants cultured on clay soil by using controlled-availability nitrogen fertilizer

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No-tillage is not only an energy-saving form of agriculture but it may also have many advantages over the conventional tillage system. For rice cultivation, no-tillage may have several benefits besides energy saving. This study has been conducted to find the beneficial effect of no-tillage through the analysis of nutrient absorption and yield components by the use of controlled-availability nitrogen fertilizer attached to the roots of seedlings as the sole fertilizer source. The paddy field was divided into four plots in 1995. One plot received a no-tillage treatment in 1995. Each year, no-tilled plots have expanded. In 1997, we had 1-, 2-, and 3-year-old no-tilled plots. Rice seedlings (cv. Kinuhikari) were grown in the seedbed with controlled-availability fertilizer (CAF), LPS100, at the rate of 225, 450, and 675 mg N hill$^{-1}$. LPS100 (Chisso Asahi Ltd.) released only 2% to 3% of the N by the time when the accumulated temperature reached 750 degree-days, then the sigmoidal N release occurred. About 80% of the N is released by 2,500 degree-days after the fertilizer absorbs water. The 35-day-old rice seedlings were transplanted to the soil with a V-shaped cut, which was made on the no-tilled paddy by a small blade. The same seedlings were also transplanted on the puddled plot.

Nutrient absorption in the no-tilled and tilled plot was compared at the same nitrogen absorption rate. Phosphorus and calcium were absorbed in the same way by both treatments. However, silicon and potassium were absorbed slightly more in the tilled plot than in the no-tilled plot. The grain yield of both plots was compared at the same N application rate and indicated better yield in the tilled plot. However, the yield response curve against the nitrogen absorption rate gave a similar curve for both plots. Dry matter production vs nitrogen absorption also gave the same response curve between the tilled and no-tilled plot. The number of spikelets for both plots also showed a similar response curve. However, in the no-tilled plot, grain filling was better when the spikelet number increased. The soil surface of the no-tilled plot accumulated easily decomposable nitrogen more than the tilled plot. However, the accumulated nitrogen does not release much of its nitrogen when submerged without puddling. Thus, the conversion of the no-tilled plot to a tilled plot after 3 years showed more yield than the conventionally tilled plot. In conclusion, rice plants with CAF attached to their hill can absorb nutrients in proportion to the nitrogen absorption rate, although no-till management of paddy soil reduces the solubility of nutrients such as phosphate and silicon in the soil. Thus, the efficiency of the dry matter production of no-tilled rice plants was similar to that of the tilled plot. Grain filling of no-tilled rice plants was better than that of the filled plot. The uppermost layer of the no-tilled plot accumulated easily decomposable nitrogen more than the tilled plot; however, the accumulated nitrogen may not be mineralized until the plot is tilled and puddled. No-till management of rice fields has the potential to sustain a high and stable rice yield with less energy input.

# Genotypic variation in salinity tolerance among commercial New South Wales rice cultivars

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Salinity is an increasing problem for rice production in New South Wales (NSW) as groundwater is saline, water tables are within 1.5 to 2 m of the soil surface, and the use of saline bore water to supplement surface irrigation is increasing. The associated salinity problems currently affect up to 10% of the rice area. Furthermore, there is increased retention of water on-farm for environmental and economic reasons, and this practice contributes to salinity in irrigation water. There is a need to establish the relative salinity tolerance among NSW cultivars so that genetic differences can be used where possible to mitigate these adverse effects.

Six rice cultivars were tested for salt tolerance at the three-leaf stage under glasshouse conditions at the Yanco Agricultural Institute in NSW, Australia. Pokkali, a salt-tolerant standard, was compared with five NSW commercial cultivars. The treatments included 25 and 50 mM of salt and a control and were imposed at the three-leaf stage for 21 days, at which time all aboveground biomass was harvested. The experiment was conducted twice to estimate the repeatability of genotypic performance.

Significant genotypic differences were observed in all salinity treatments and significant differences occurred between long- and medium-grain NSW commercial cultivars in response to the 50-mM treatment. Pokkali had a greater dry weight than all the other cultivars. Within the NSW cultivars, the medium-grain cultivars Amaroo and Jarrah had significantly greater dry weights than the long-grain cultivar Pelde.

In contrast, other researchers found no genotypic differences between California long- and medium-grain cultivars. Although the treatment at which the genotypic differences were observed exceeds the salinity likely to be experienced under field conditions, it is a rapid and repeatable screening protocol to establish genotypic differences. Experiments are being conducted to determine salinity effects in a wider range of commercial cultivars and to determine the effects throughout the growing season.

# Cracks and splits in rice: Can we control them?

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In the Australian rice industry, the problem of cracked grain reduces both yield and quality. The Australian rice industry targets high-quality, whole-grain markets and cracked grain is considered to be of poor quality. Moreover, cracked grain often breaks during milling, which decreases yield. Until now, the severity of cracking was thought to depend primarily upon changes in moisture content because of rain and dewy nights between the time that the grain matures and the time of harvest. The aims of this work are to determine (1) whether different varieties are more prone to cracking, (2) the role of moisture in increasing the propensity of grains to crack, and (3) to identify physical and chemical differences between rice that cracks and rice that does not crack. So far, varieties have been identified that are either tolerant of or susceptible to cracking. In the tolerant and susceptible varieties that we have screened, grains that crack or break differ from whole grains in viscosity and starch structure, and in protein complement. These results indicate that grains that will crack differ from grains that will not crack, and this provides the basis for developing strategies to manage the problem.

# The Germplasm Resources Information Network in the United States

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The Germplasm Resources Information Network (GRIN) is the centralized computer system that provides germplasm information about plants, animals, microbes, and invertebrates within the National Genetic Resources Program (NGRP) of the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS). The NGRP was authorized in 1990 by the U.S. Congress to be in charge of the acquisition, characterization, preservation, documentation, and distribution of germplasm of all life forms important for food and agricultural production. GRIN facilitates the management and operation of the National Plant Germplasm System (NPGS), National Animal Germplasm Program (NAGP), National Microbial Germplasm Program (NMGP), and National Invertebrate Germplasm Program (NIGP) and serves to reduce unneeded and redundant data. It is through GRIN that national and international scientists in these areas of life sciences can locate germplasm with specific characteristics for research purposes. GRIN is managed by the Database Management Unit (DBMU) of the National Germplasm Resources Laboratory.

GRIN can be accessed through the Internet (http://www.ars-grin.gov). PC GRIN is a personal computer version of GRIN that includes a special section of informative data on one crop or several crops. The program for accessing the data follows the same principles of GRIN, but accommodates the PC and floppy diskette so that the data are accessible without the use of a telephone line.

The NPGS is a network of organizations and people dedicated to effectively collecting, preserving, evaluating, enhancing, documenting, and distributing plant genetic resources for continued improvement in the quality and production of economic crops important to the U.S. and world agriculture. Members of the NPGS include federal, state, and private organizations coordinated by the

USDA-ARS. Forty Crop Germplasm Committees (CGC) work under the coordination of NPGS. CGC is the generic name for a specific national working group of specialists that provide analysis and recommendations on genetic resources within a specific crop or group of related crops that are economically important. There are more than 450,000 accessions (distinct varieties of plants) in the GRIN database and these accessions currently represent more than 10,000 species of plants. Wheat has 47,090 accessions and it is the largest crop collection. It includes 24 species and three hybrids.

Rice has 19,878 accessions in the working collection, which includes 10 species of *Oryza.* These accessions have been collected from 110 countries. *Oryza sativa* is the major species in the rice germplasm collection, with 19,577 accessions from 109 countries. The International Rice Research Institute (IRRI) has supplied 3,331 accessions, which is the largest group in the rice collection. The National Small Grains Collection (NSGC) located in Aberdeen, Idaho, contains 17,306 accessions of rice and these accessions are available for distribution. In the NPGS are 1,705 accessions at the Plant Germplasm Quarantine Office (PGQO) that are being processed and 867 accessions in the National Seed Storage Laboratory (NSSL) that are not available for distribution.

# Effects of salinity on yield and yield components of rice at different seeding densities

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Salinity damage in rice production has become an important agricultural problem worldwide. A substantial loss of plant stand and yield reduction have been observed in salt-affected direct-seeded rice. One of the possible management options for growers in dealing with the salinity-induced decrease in rice yield is to compensate for yield loss under salinity by increasing seeding density. Plants were grown in the greenhouse in silica sand irrigated with nutrient solutions. The treatments included three seeding densities of 100, 150, and 180 kg ha$^{-1}$ and three salt levels of 1.0, 3.9, and 6.5 dS m$^{-1}$. Yield components were measured on individual plants and total seed yields were measured on a unit area basis. Salinity effects were highly significant for total seed yield, plant stand, seed weight per plant, seed weight per panicle, and spikelets per panicle at each seeding density, but not significant for panicle density and grain weight. Total seed yield did not increase significantly with an increase in seeding density. Plant stand and panicle density increased significantly while seed weight per plant, seed weight per panicle, and fertility decreased with increases in seeding density. It was concluded that yield loss under moderate salinity may not be compensated for by increasing seeding density above normal levels.

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