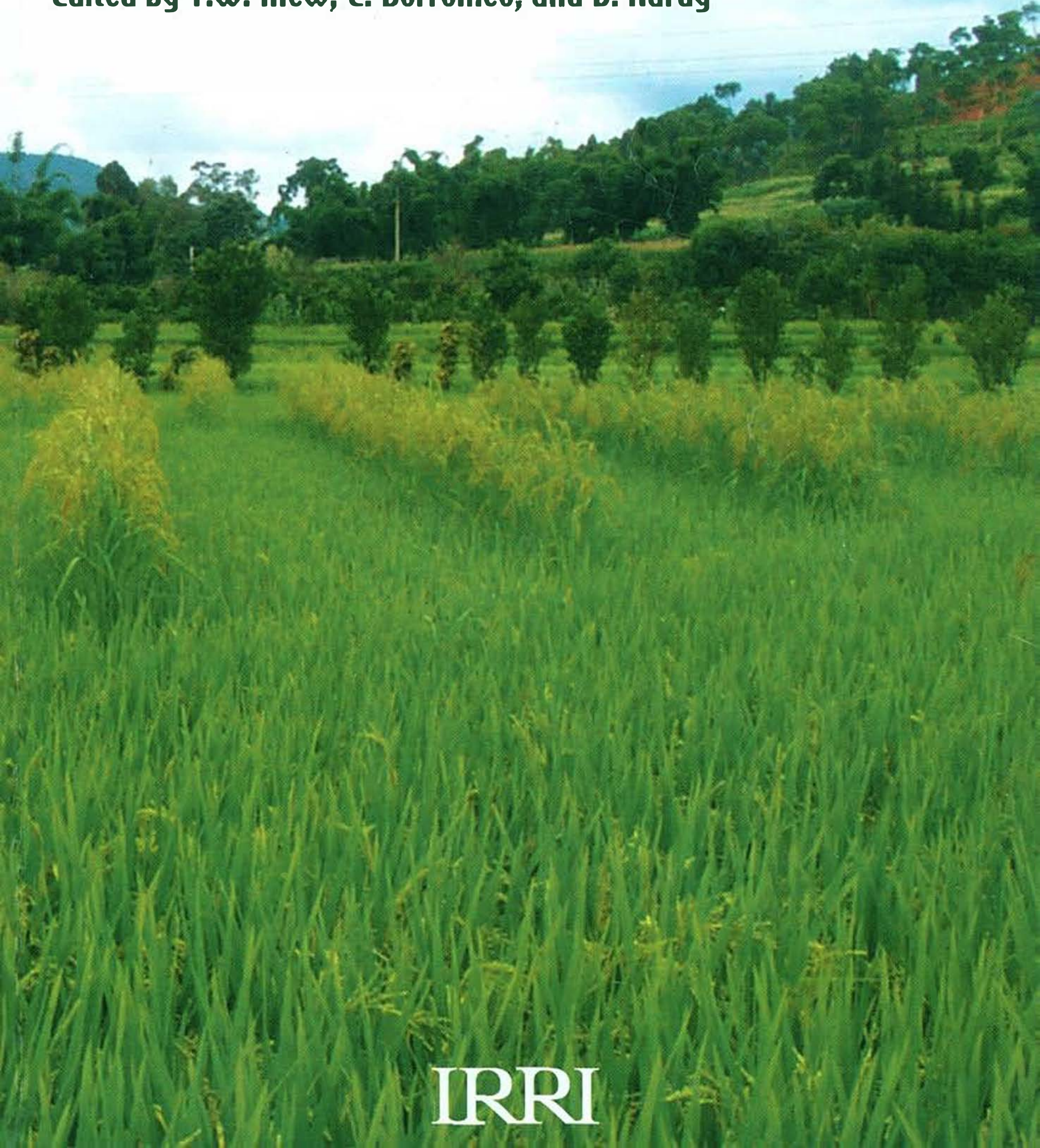
Exploiting Biodiversity for Sustainable Pest Management

Edited by T.W. Mew, E. Borromeo, and B. Hardy



IRRI

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IRRI

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About the cover: Rows of glutinous rice (tall) interplanted among rows of hybrid indica rice, Yunnan Province, China. Photo by I.M. Revilla.

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Foreword

An increased awareness that rice fields must be considered in totality as an ecosystem, rather than considering only one or two target populations of insects or pathogens, has led to growing interest in the application of ecological concepts to pest management. There is now explicit recognition that pest populations can be kept below damaging levels if the fine balance that exists among the biotic components in these human-managed agricultural systems can be maintained or enhanced. It was from this perspective that IRRI embarked upon a research project on the use of biodiversity for sustainable pest management. The goal was to reduce pests and diseases by manipulating or augmenting the habitat, microbial, and genetic diversity present in the rice ecosystem. Here, habitat diversity refers to the rice and nonrice crops in the landscape of rice fields, microbial diversity refers to the microbes associated with the rice crop, and genetic diversity refers to the rice crop only.

This research was conducted in collaboration with national agricultural research system partners in Yunnan Province and the Yangtze Delta in China; Chiang Mai, Thailand; the Mekong Delta, Vietnam; and Ilocos Norte, Philippines. During the three years of the project (1997-2000), researchers developed tools for analyzing biodiversity, accumulated baseline information on the nature and extent of biodiversity pertinent to pest management, and field-tested biodiversity enhancement or deployment approaches to pest management. These research outputs were presented in the impact symposium held in Kunming, Yunnan, China, on 21-23 August 2000, together with the insights solicited from other scientists working on the same subject.

This book compiles the research results and insights presented during the symposium. It underscores IRRI's commitment to developing pest management strategies that are environment- and farmer-friendly, and that fully integrate indigenous biological resources such as diverse plant germplasm, natural enemies of insects, and beneficial microbes.

IRRI is grateful to the Asian Development Bank for financial support for this work.

RONALD P. CANTRELL
Director General
International Rice Research Institute

Preface

Biodiversity may be another buzzword, but, in both theory and practice, it has relevance in addressing many problems of contemporary agriculture. Biodiversity allows the formation of functional groups, at the genotypic or species level, that drive key ecosystem processes. And a key process we are particularly concerned with in agroecosystems is pest regulation. In stable agroecosystems, differential interaction among functional groups of organisms imposes checks and balances so that potentially damaging species usually never become abundant enough to become actual pests. But in an agroecosystem that has lost much of its biodiversity because of intensification, imbalance among functional groups creates instability, which makes the system vulnerable to pest and disease outbreaks.

Intensive rice cultivation has led to substantial increases in yield, but at the cost of losing much biodiversity and, consequently, vulnerability to outbreaks of pests and diseases. It also led to widespread use of pesticide when plant resistance is unavailable or inadequate to contain pests, thus taking a heavy toll on the environment. To sustain high productivity, the challenge before us is to develop approaches that will contain pest populations without damaging the resource base and without compromising the yield levels already attained. The approach we have taken is to look back at biodiversity to develop schemes by which biodiversity can be preserved, introduced, or used to enhance natural pest regulation in intensive rice production systems.

This ADB-funded project, *Exploiting Biodiversity for Sustainable Rice Pest Management* (1997-2000), was implemented at five sites: Yunnan Province, China; Yangtze Delta, China; Mekong Delta, Vietnam: Chiang Mai, Thailand; and Ilocos Norte, Philippines. We called these sites “lighthouses” to symbolize our intent to generate knowledge at these sites and then radiate it afterward as far as possible. To systematize operations and provide focus, important pest problems were identified at these sites, and work concentrated on the aspect of biodiversity—habitat diversity, microbial diversity, or genetic diversity—most relevant to the control of the target pest problem.

The chapters in this book describe part of the work undertaken and were presented in a symposium held in Yunnan, China, 21-23 August 2000. The book also features chapters from several internationally known experts who were invited to share their insights on certain topics. The chapters are grouped into four sections—habitat diversity, microbial diversity, genetic diversity, and technology diffusion—corresponding to the four main topics of the symposium.

Although it may sound easy, exploiting biodiversity for rice pest management poses an enormous challenge. The number and complexity of interacting components of the rice ecosystem make the identification of the components or processes vital to pest management a daunting task. With insect pests, for example, the task requires intensive sampling of arthropod communities to determine relative abundance in space and time on various habitats that make up the rice ecosystem, and then field experimentation to follow up on the clues provided by sampling results. Once these components are identified, preserving or manipulating them is even harder.

The complexity of how biodiversity operates in pest management is further illustrated in one of the chapters on microbial diversity. Although most of the work has focused on enumerating and screening microorganisms with biocontrol properties associated with the rice plant, interestingly, this chapter posited that crop genetic diversity and habitat diversity affect the diversity of microorganisms inhabiting plant surfaces. This is significant in the sense that a more diverse phylloplane population reduces the likelihood that pathogenic microorganisms could gain a foothold and cause disease.

The implementable outputs reported in this proceedings vary in stages of development. The most advanced, which can be considered a mature technology, is genetic diversification by interplanting susceptible glutinous varieties with resistant hybrid indica rice to control rice blast. This has passed the transition from experimental plots to a widespread farmers' practice. Such rapid progress may be attributed to the considerable amount of knowledge that has already accumulated from similar work with other cereal pathosystems in Europe and the United States. One paper presented dealt with experiences with and the theoretical underpinnings of disease control by genetic diversification.

Chapters in the habitat diversity section describe how certain mixes of a rice and nonrice habitat favor the proliferation of beneficial insects that can control the population of insect pests. Caution must be taken, however, in extrapolating these results into other environments as the dynamics of arthropod interrelationships is still scarcely understood and may differ substantially from field to field, season to season, and country to country. Nevertheless, these results deserve serious consideration in further investigating or developing habitat management approaches to pest control.

The chapters on technology diffusion complete the from-concept-to-practice story of applying biodiversity in pest management. The role of farmers in knowledge dissemination was given prominence. The mixture planting approach for the control of rice blast in Yunnan represents a classic case of knowledge evolution and diffusion from farmers to scientists and then back to the farmers. Scientists built on farmers' indigenous knowledge to develop the experimental approach. Once the approach proved to be effective, it was quickly disseminated. Knowledge diffuses rapidly and effectively as farmers share knowledge with other farmers.

We hope that this book will serve as an important resource for researchers, practitioners, and advocates of sustainable pest management. We acknowledge the invaluable contributions of our colleagues from the lighthouse sites, other participants at the symposium, and our invited speakers. We also thank Ms. Santi Culala for ably handling the organizational aspects of the symposium and Mr. George Reyes for putting the manuscripts in order. We conclude by quoting from one of our speakers, who, when referring to this project, said, "...scientists, farmers, extension workers, policymakers, and funding agencies got together for a common goal. No one group could have done it alone. Therefore, I conclude that this project is not just a scientific achievement. It has made a difference in the lives of those who have less in life. In my own pedestrian mind, this represents the triumph of the human spirit."

Habitat diversity

Approaches to a relevant understanding of biodiversity for tropical irrigated rice pest management

M. Way and G. Javier, Jr.

> "We must grasp the essence of the thing,
> this is the only reliable and scientific method of analysis"
> – Mao Tsetung (1930)

The known biodiversity of tropical irrigated rice from the genetic to field community level is greater than that of any other arable crop or wild plant. At the genetic level, there have been outstanding successes in exploiting biodiversity in rice plant resistance to pests and in prospects through genetic engineering. Although these advances have largely been applied to nondiverse monocultures of particular cultivars, relatively few practical advances have been made in using genetic biodiversity as part of an integrated pest management approach, such as manipulating the patterns of rice cropping to counter resistance-breaking strains of pests.

At the rice crop community and ecosystem levels, it is recognized that, for insect pests in particular, an extraordinary biodiverse complex of natural enemies can be crucially important in biological control of key pests. Yet, there is a priority need to define which particular natural enemies are really important, how they can be preserved, and how their role can be enhanced. In this context, there has been much emphasis on sampling arthropod communities in space and time on rice, associated crops, and other habitats including the noncrop bunds of rice fields that comprise the rice agroecosystem. Now, the key question is, What does this mean for pest management and, ultimately, how might such knowledge be exploited by farmers?

It is suggested that a further time-consuming generalized accumulation of biodiversity data is likely to be unrewarding until presently available information is first examined for integrated pest management (IPM) clues that should then be experimentally tested. These should then point to more sampling, which can then focus on key species in particular crop conditions. Examples are given of sampling data that must contain important clues, and of one experimental approach for testing the role of bunds as a source of early arriving natural enemies regarded as essential for biological control of some important insect pests of tropical irrigated rice.

Biodiversity embraces the entire variety and range of variation of living things and its magnitude and cause are one of the key problems of science as a whole

(Hawksworth and Ritchie 1993). In the context of pest (by pests, we mean harmful animals, diseases, and insects) management, there are three major considerations: (1) that relevant pest management components of biodiversity are not only preserved but could also be manipulated to enhance natural pest management, integrated where necessary with artificial controls such that the latter are complementary in action; (2) that biodiversity components of IPM are acceptable to farmers as safe, cost-effective, and sustainable (Castillo and Ooi et al, this volume, Way and van Emden 2000); and (3) that pest management practices based on manipulating biodiversity do not unduly harm components of biodiversity that are considered to be environmentally important.

In terms of rice pest management, the emphasis must be on *functional* biodiversity involving pest-controlling biodiversity components as part of an IPM approach. This raises the key question of how to "*grasp the essence*" by undertaking the right kinds of relevant research and development work and is therefore the challenge for the project "Exploiting Biodiversity for Sustainable Pest Management" funded by the Asian Development Bank (ADB).

Table 1 briefly outlines the levels of biodiversity relevant to pest management of rice. At the genetic level, there are continuing and outstanding practical successes in manipulating the unique diversity that exists within rice species, which may now be revolutionized by genetic modification technologies. Successes have arisen primarily from straightforward releases of new cultivars but also from the manipulation of diversity, especially in disease management, such as through varietal diversification (e.g., Koizumi, Mundt, and Zhu Youyong, this volume, Youyong et al 2000), for which there are important future opportunities (Borromeo et al, this volume) and economic advantages for farmers (Castillo and Revilla et al, this volume).

Table 1. Levels of biodiversity relevant to rice pest management.

1. Genetic diversity
    - Within rice cultivars (e.g., tolerance of pests)
    - Within strains of a particular pest or beneficial natural enemy (e.g., in the pest's virulence to rice or natural enemy resistance to pesticides)
2. Crop community diversity of rice and other crops in localized groups of fields
    - In groups of rice monoculture fields with associated pest/natural enemy species (e.g., involving cultivar mixtures of rice within, and also between, fields based on diversity in space and time)
    - Same as above for polycultures of rice with other crops
3. Agroecosystem diversity
    - This includes components 1 + 2 above and also other noncrop habitats within the area or region, which is conceptually large enough to "explain" the long-term natural regulation of pests and therefore the causes of pest incidence. However, the part of the ecosystem relevant to control of some weeds may be little larger than a single field, but for some migratory pests may be huge (e.g., parts of China plus Japan for brown planthopper).

At the level of the community and agroecosystem, the complexity of trophic interactions that can affect insect pest management and the ability of insects to exercise choice create enormous difficulties in understanding and using biodiversity. This in part explains the limited progress exemplified by only one study on varietal rotation to preserve resistance (Sama et al 1991) and unverified models suggesting that crop mosaics may not be useful (Tabashnik 1994) but that within-crop mixes could be beneficial (Bennett et al 2001).

Although it is well recognized that a complex of natural enemies is critically important in the control of insect pests of tropical irrigated rice (Way and Heong 1994), little has been done to *examine* how such complexes can best be used. Highlights of relevant work include the pioneer research of Kenmore (Kenmore et al 1984), the demonstration of the vital importance of a complex of early arriving arthropod predators (Heong et al 1991), the potential of nonpest sources of food for attracting and retaining predators (Settle et al 1996), the important role of spiders (Sigsgaard et al 1999), and the potential significance of some noncrop habitats within the rice ecosystem (Liu Guangjie et al and Yu Xiaoping, this volume).

The most forbidding challenge is that, at the ecosystem level and even within one rice field, there seem to be intractable difficulties in determining the dynamics of *relevant* biodiversity linkages for diseases (Youyong et al 2000) as well as insects. Moreover, understanding so far has given only a glimpse of those links that may be functionally important in pest management. There is also the challenge of how relevant elements can be manipulated to enhance insect pest management. This is especially daunting for tropical irrigated rice, which faunistically is uniquely biodiverse compared with dryland annual crops. This chapter therefore aims to indicate some progress on rice insect pests, leading up to, and including, the current ADB project.

Magnitude of the insect pest problem at the community and ecosystem levels

The challenge is twofold:

1. Within tropical irrigated rice crops, there can be >9,000 trophic links among >645 mainly arthropod taxa (Cohen et al 1994).
2. The dynamics of interrelationships must differ from field to field, season to season, and country to country.

So, in what ways is it humanly feasible to understand better what is needed and to use this knowledge to improve farmer-acceptable practices? The following two different but necessarily interlinked approaches have been adopted.

Accumulation and analysis of basic sampling data

This general approach based on intensive sampling is like that adopted by environmentalists interested in biodiversity for its own sake in that it seeks to determine the species and their relative abundances in particular systems. The crucial difference, however, is that the sampling must be designed with clear pest management objectives. Current work is represented by studies on arthropod communities in rice and associated crops at “lighthouse” sites of the ADB project (e.g., Marcos et al, this volume) and by other impending research in Southeast Asia (M. Thomas, 2000, personal communication). Anticipated outcomes include

1. More detailed information on the makeup of pest and beneficial populations in particular systems that could provide better clues to species that may be especially important for IPM in rice systems in different countries and climatic conditions, such as natural enemies. As such, this approach could provide information for critical experimental approaches on the value of particular species or groups of species in pest management. Present examples include sampling data on biodiversity along transects in Ilocos Norte in the Philippines involving the distribution in time and space of different taxa in rice fields and associated crops (Marcos et al, this volume) (Table 2). These data indicate how different crops and noncrops might act as reservoirs for each other's pests and natural enemies, but they still require much more analysis and more focused research, namely on the spatial and temporal distribution of potentially key species of natural enemies and hosts or prey that provide alternative sources of food at times when rice is unavailable or unattractive. Such work could be vital in providing clues for relevant experimental work.
2. More detailed information on food webs aiming to satisfy the objectives of those who consider that food webs should provide a basis for improved pest management (e.g., Cohen et al 1993). Examples of recent research include generalized food web differences in different ecosystems (Schoenly et al 1996) and differences in food web structure between unsprayed and insecticide-sprayed fields (Cohen et al 1994), though the fact that food webs are simplified by insecticidal destruction of some natural enemies and pests is implicit from much previous field evidence (e.g., Kenmore et al 1984). Cohen et al (1994) showed for a single crop in a single season that it was possible to expose the seemingly more important food web elements, but there has been no follow-up academically or, most importantly, in linking the approach to improving pest management (Way and van Emden 2000).

The experimental approach

Crucially, the experimental approach has been the basis for virtually all advances in scientific knowledge, both fundamental and applied (Wiggles worth 1955). It involves

Table 2. Arthropod taxa and abundances of herbivores and natural enemies in different habitats along a transect at Badoc, Philippines (L.B. Flor, personal communication).

	Rice	Rice bund	Rice fallow	Garlic	Onion	Maize	Mungbean
Herbivores							
No. of taxa	28	19	19	7	3	3	11
Total no.	3,705	215	326	23	5	3	98
Natural enemies							
No. of taxa	125	79	62	30	9	8	33
Total no.	3,363	927	897	178	12	20	252

experiments to test hypotheses based on existing information, perhaps from simple observations or from quantitative sampling such as in Table 2. In the context of applied biodiversity, it can be envisaged as a step-by-step approach toward optimal use for pest management in rice and other crops. This is exemplified by the work on varietal diversification for rice disease management and for insects. Recent ADB-funded work at IRRI is now used as an example where the starting point is the knowledge that early arriving natural enemies seem to be vitally important (Heong et al 1991). Critical questions include: Where are they coming from, how can their role be suitably maintained and enhanced to improve pest management, and can new practices be made farmer-feasible (Ooi et al, this volume)? Table 3 shows that some natural enemies can arrive within two to three days after transplanting as shown by direct visual examination, earlier than indicated previously by suction trapping (Way and Heong 1994). The bunds around a rice field are by far the closest possible source, particularly for the wingless predatory spiders. This prompted an experimental examination of the contribution of bunds.

Bund fauna. Year-long records of the flora and fauna were kept for several irrigated rice fields where replicated lengths of bunds were treated differently. In one set of experiments, vegetation was cut periodically, left uncut, or herbicide-treated to remove virtually all vegetation cover. One example shows the effects of such treatments on the flora (Table 4), which in general are similar to those of Marcos et al (this volume). In other experiments, a herbicide + insecticide treatment was included to remove both plants and resident arthropods (Table 5). All the results agreed in confirming that the vegetated bunds supported many herbivore species, though few were rice pests. Brown planthopper (BPH) and some leafhoppers as well as a few adult rice stem borers were sometimes recorded as adults. However, the herbicide and insecticide treatments dra-

Table 3. First arrivals in newly transplanted rice.

		Days after transplanting	
		Suction-trapped	Visual
Pest	Homoptera	15 (6–27)	>3
Natural enemies	Heteroptera	15 (6–21)	2–3
	Mature spiders, spiderlings, and others	13 (6–21)	3

Table 4. Plant species per 10-m lengths of bunds treated with herbicide, with vegetation periodically cut, and with vegetation left uncut throughout the crop season.

Treatment	Mean no. of species	% cover
Herbicide	5.0 (68%)[a]	1.6
Cut vegetation	20 (41%)	84
Uncut vegetation	4 (43%)	95

[a](%) = % dicotyledons.

Table 5. Total numbers of arthropod species and mean numbers per 0.4-m^2 suctioned sample from bunds treated with herbicide + insecticide, herbicide only, and untreated.

Treatment	Herbivores		Predators			
			Spiders		Total predators	
	Total spp.	Mean no. of individuals	Total spp.	Mean no. of individuals	Total spp.	Mean no. of individuals
Herbicide + insecticide	0	0	1	1	4	1.7
Herbicide only	0	0	1	1	2	1.3
Untreated	28	28	5	42	18	60

matically affected the numbers of suction-sampled predators, particularly spiders, though the results do not show that, in fact, some spiders occurred in soil cracks of bunds treated only with herbicide.

Effect of bund treatment on biological control of BPH in the rice crop. Replicated 12 x 12-m plots were set up adjacent to differently treated bunds in an experiment involving five 60 x 24-m fields. The plots with herbicide + insecticide-treated bunds were surrounded by a 1-m-high transparent plastic fence painted with an outside band of grease. This combination prevented access by crawling arthropods except from the adjoining 12-m length of bund, although it still permitted colonization by flying insects and wind-blown spiderlings. In each plot, groups of five “control” hills infested with BPH nymphs were caged to exclude all other insects.

Initial experiments showed good evidence that bunds can be an important source of biological control agents. For example, after 5 d, 72% of BPH survived on one set of rice plants and 18% on another both with herbicide + insecticide-treated bunds, 6.1% survived where herbicide only was applied to the bunds, and 5.5% where bunds were untreated. An indication of the relative natural enemy pressures was given by the mean number of spiders per five hills—7 immature spiders, 22 immature spiders, 43 adults and immatures, and 41 adults and immatures, respectively. This indicated the importance of bunds as sources of adult spiders, which quickly reached the infested rice plants where bunds had not been insecticide-treated, whereas, in contrast, only windborne immatures reached plots where all predators in bunds had been killed by the insecticide. However, this result was not borne out by an example of another set of experiments on a relatively late-sown crop. Here (Fig 1), exposed BPH populations initially remained similar in all treatments although increasingly there was an indication that the fewest BPH were killed in plots with the herbicide + insecticide-treated bunds. Such differences were mostly insignificant statistically and would also be unimportant in terms of crop damage. They contrasted strikingly with the protected controls where BPH populations increased to hopper-burn levels (Fig. 1). All the predator-exposed treatments confirmed past evidence of outstanding biological control by natural enemies. This is illustrated by the development of spider (Fig. 2) and *Cyrtorhinus lividipennis* (Fig. 3) populations. In particular, windborne spiderlings, but not adults, quickly reached the previously insecticide- “sterilized” plots by air from outside the field as did adult *C. lividipennis* and some other winged predators.

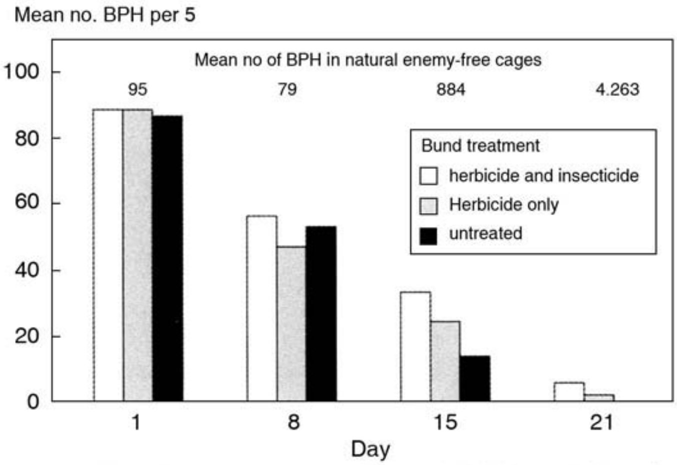

Fig. 1. Effect of bund treatment on changes in initial populations of 100 brown planthopper (BPH) nymphs per five irrigated rice hills.

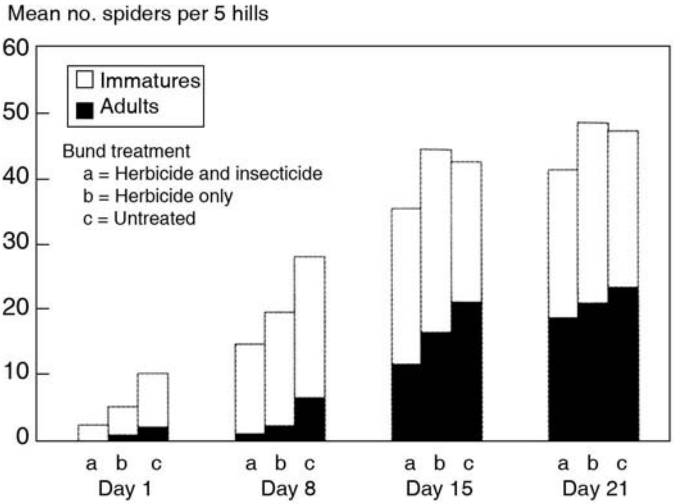

Fig. 2. Effect of bund treatment on numbers of spiders arriving and developing on irrigated rice initially infested with 100 brown planthopper nymphs per five hills.

With current evidence, we conclude that several factors determine the importance of bunds as key sources of natural enemies, in particular the sowing date of the crop. Thus, bunds may be especially important for the early sown crop, whereas, for later sown crops, other earlier sown crops can be the richest source of natural enemies. In this context, single-season rainfed and irrigated crops may depend crucially on bunds as sources of natural enemies (see Marcos et al, this volume). Furthermore, the role of bunds as sources of natural enemies of pests other than hopper species, including

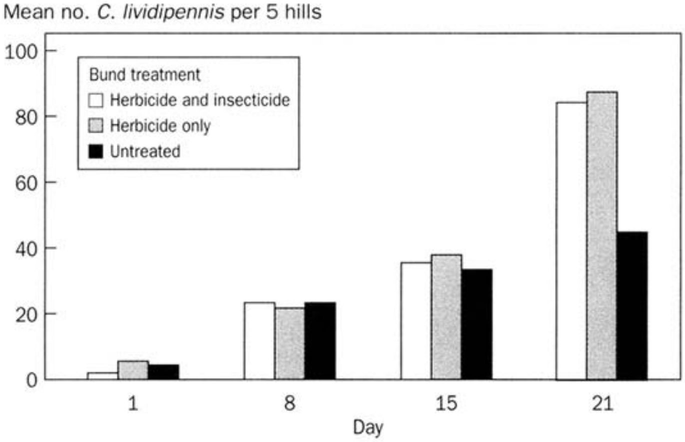

Fig. 3. Effect of bund treatment on numbers of *Cyrtorhinus lividipennis* arriving and developing on irrigated rice initially infested with 100 brown planthopper nymphs per five hills.

leaffolders and stem borers, remains to be examined in more detail (see Yu Xiaoping, this volume). At this stage of understanding, it would be dangerous to underestimate the value of appropriately managed bunds and, in particular, to consider introducing technologies based on removing bunds to increase field size.

Discussion

That biodiversity is of paramount importance in pest management of tropical irrigated rice is evident from the outstanding success in exploiting the unique genetic biodiversity of rice species, and, potentially, genes from nonrice species. However, much remains to be done to use such diversity as a necessary part of the integrated pest management approach (Way and Heong 1994). The successful strip cropping of different rice cultivars in Yunnan highlights the value of approaches that involve mixtures of cultivars within fields as well as opportunities for patterning them between fields in space and time. These approaches for disease management are also potentially significant for insect pest control.

Much work on tropical irrigated rice has indicated the value of natural enemy biodiversity at the community and ecosystem levels for insect pest management. Those concerned have been faced with some virtually intractable problems of complexity such that we still know relatively little about what particular components really matter–knowledge that is essential in terms of improved pest management, particularly in relation to future changes, such as larger rice fields involving less bunds, more nonrice crops in rice bowls, destruction of noncrop habitats, and climatic change. Publications, not necessarily focused on pest management, provide some clues to mechanisms involved (Crawley 1997, Polis et al 1989, Price 1983, Sunderland et al 1997). In particular, Southwood (1977) used a synoptic model that can provide clues on the relative importance of various pest management strategies, and Polis et al (1989) also highlighted the potential significance of interspecies predation, which can be a factor in predator survival when nonpredator prey are scarce. The significance and opportunities for using environmental diversity have been discussed frequently, almost exclusively in relation to dryland crops (e.g., LaSalle 1999, Perrin 1980, Polaszek et al 1999, Way 1977). However, all this work provides little more than clues for irrigated rice, particularly since it mostly highlights conditions in dryland single-season arable crops where lack of continuity tends to dislocate natural enemy–pest interactions (Way 1977), unlike the continuity of two to three crops per year of much tropical irrigated rice (Way and Heong 1994).

There are other important distinctions, for example, between cereals such as wheat and rice. The major insect pests and their transmitted diseases of wheat in its exotic Western European habitats are indigenous wild grassland species (Way 1988) growing in hedgerows (the ecological equivalents of bunds) and in other wild areas and fields. In contrast, the major insect pests of tropical irrigated rice are monophagous or almost all monophagous for rice. So, except for a few polyphagous outbreak pests, such as locusts and armyworms, wild and other nonrice cultivated areas can be regarded primarily as beneficial sources of natural enemies rather than sources of pests. The basis for manipulating biodiversity against tropical irrigated rice pests must therefore be regarded very differently from that of single-season dryland crops, including dryland tropical rice, which has seriously damaging polyphagous pests (Litsinger et al 1987).

The emphasis on a sophisticated knowledge of the ecosystem as a basis for IPM (e.g., Kogan 1998) needs to be questioned in terms of value as well as of practicality. Already an enormous amount of data have been collected on the arthropod fauna of irrigated rice and of associated crops and wild plant systems. These data still need examining, not as an academic exercise in biodiversity or for exercising sophisticated methodologies, such as Schoenly et al (1998), but rather in terms of new components that could add to our ideas on species and cropping situations that could be experimentally examined for improved pest management. So, the question needs to be asked, Is the continued accumulation of raw biodiversity data still desirable? Surely the priority now is for the already accumulated data to be examined in much more detail for potentially important new clues, such as which insect pest and natural enemy taxa are common in fields and wild habitats that can provide linking sources to rice crops in space and time? This could lead to experimental testing, and then to much more focused sampling and other methods of field examination of particular taxa rather than continued unquestioning collection of everything by the ubiquitous suction sampler.

Table 6 lists the range of experimental testing at all levels of biodiversity from genetic to ecosystem. There are already clear and successful precedents for manipulating biodiversity of rice monocultures, notably at the genetic level (Table 1, part 1). However, Table 1 parts 2 and 3 present much greater experimental difficulties, in particular the latter, such as resolving disagreements on the value of diversity in time associated with synchronous versus asynchronous planting. In the humid tropics, the overlapping asynchronous irrigated rice crops favor movement of insect natural enemies directly from rice crop to rice crop; hence, asynchrony seems to be crucially

Table 6. Experimental testing of relevant components of biodiversity in rice-cropping systems.

1. Rice crops
   - Conventional comparisons of cultivars
   - Comparison of cultivar mixtures within fields
   - Comparisons of cultivar mixtures between fields in space and time
   - Studies of synchronous vs asynchronous planting
2. Roles of other arable crop fields grown in the rice system
3. Roles of flora, fauna, and size of rice field bunds
4. Roles of scattered patches of mixed cultivated and wild herbs and trees within a rice habitat and also adjoining it

important in biological control of insect pests (Way and Heong 1994). But is this applicable to single-season irrigated and rainfed rice and to pathogens? Experimental comparison of asynchrony versus synchrony is a formidable task, but empirical evidence (Way and Heong 1994), most significantly that of Sawada et al (1992), highlights the value of asynchrony, though this is questioned for insect-transmitted tungro disease, for which the critical epidemiological research is lacking (Way and Heong 1994).

It seems likely (Way and Heong 1994) that relatively few of the very biodiverse natural enemies that are associated with tropical irrigated rice are of real importance in biological control. This concept is supported by empirical and theoretical evidence that simple ecosystems can be stable and, hence, that much of the species complexity of tropical irrigated rice systems may be irrelevant to IPM (e.g., May 1975, Pimm 1984, Way and Heong 1994). There is therefore an urgent need to experimentally test (e.g., Cohen et al 1994) this and other hypotheses that could make manipulation of biodiversity very much simpler and farmer-friendly. This seems the only practical approach to the use of biodiversity for insect pest management and no doubt for other pests (see Youyong et al 2000).

In conclusion, based on the mass of present sampling data, we must surely use more imagination to define more relevant questions and then design feasible experiments for answering them. This requires thought—"a painful process" (Wigglesworth 1955)!

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Role of **Saccharosydne procerus** on *Zizania caduciflora* as an alternate host for **Anagrus nilaparvatae,** the egg parasitoid of the brown planthopper **Nilaparvata lugens,** which attacks temperate rice

Yu Xiaoping

In the Yangtze River Delta, *Saccharosydne procerus* (Delphacidae: Homoptera) on the vegetable *Zizania caduciflora* L. is a major alternate host of the egg parasitoid *Anagrus nilaparvatae* (Mymaridae: Heteroptera), which is an important biocontrol agent of planthoppers on single-season japonica rice. *A. nilaparvatae* and related *Anagrus* species can overwinter in *S. procerus* eggs in *Zizania* fields during the rice off-season. In April, *Anagrus* spp. disperse to and build up on hopper hosts on newly transplanted rice fields. Most movements of *Anagrus* spp. were found between *Zizania* fields and rice during the early rice season, especially in June. In a free-choice test, *A. nilaparvatae* oviposited in and emerged from both *S. procerus* and the brown planthopper (BPH) *Nilaparvata lugens* eggs although it preferred BPH. Although parasitism of *S. procerus* by *A. nilaparvatae* was more than 15%, *S. procerus* could not complete its lifespan on rice, indicating no damage by this hopper to rice. This chapter shows that proper deployment of rice and *Zizania* fields will enhance the efficiency of the egg parasitoid *A. nilaparvatae* against both planthoppers in rice and *S. procerus* in *Zizania* fields. Habitat manipulation based on this research could be incorporated into integrated pest management in the temperate rice ecosystem.

Zizania caduciflora L. (Gramineae) is a favorite vegetable crop for the residents of the Yangtze River Delta in China. It is traditionally planted together with rice or surrounding rice fields (Ding and Yang 1982). A planthopper, *Saccharosydne procerus* (Matsumura) (Delphacidae: Homoptera), severely damages *Zizania* when its population is very high (Zhang 1983, Ding and Yang 1982). However, the eggs of *S. procerus* serve as an alternate host of the egg parasitoid *Anagrus nilaparvatae,* which is an important biocontrol agent of the rice brown planthopper *Nilaparvata lugens* (Yu et al 1999). In this temperate japonica single-season rice ecosystem, the parasitism of *A. nilaparvatae* on brown planthopper is usually low and fluctuates sharply throughout the year because of the frequent disturbance of farmers' practices such as spraying and harvesting. Additionally, the population growth of *A. nilaparvatae* and other relevant *Anagrus* spp. is interrupted in winter because rice is absent (Lou and Zhu 1980). The role of some delphacids serving as alternate hosts in grassy areas surrounding rice fields was studied recently (Yu et al 1995, Yu and Heong 1997a). Sampling showed that the parasitism of brown planthopper by *A. nilaparvatae* was notably higher where rice fields were surrounded by *Zizania* crops (Yu et al 1999). Laboratory and field work was therefore done to examine links between *A. nilaparvatae* in rice and neighboring *Zizania* as a refuge for this parasitoid. *S. procerus* was reported to be a pest of rice (Zhang 1983), so effects of *S. procerus* on rice were also studied.

Materials and methods

Adults of *N. lugens* and *S. procerus* were collected from japonica rice and *Zizania* fields and reared in the laboratory on the rice variety TN1 and on *Zizania*, respectively. They laid eggs that were parasitized by *A. nilaparvatae. A. nilaparvatae* emerged from rice and *Zizania* plant leaves that contained the parasitized eggs of *N. lugens* and *S. procerus.*

Ten *S. procerus* of different instars were introduced into mylar cages containing a rice plant or *Zizania* leaf in culture solution. All treatments were replicated 12 times and set up at temperature of 25 ± 1 °C. Hopper survival was recorded every 6 h.

In another test, at 25 ± 1 °C, a newly hatched nymph of *S. procerus* was introduced into each of 120 test cages, each with a fresh *Zizania* leaf. The development and mortality of the nymphs were observed and recorded daily until all the hoppers were dead. Female and male adults were mated to produce the F_1 progeny. The experiment was repeated for the F_1 and F_2 generations.

The development and survival of *A. nilaparvatae* were determined using 20 potted rice plants bearing hopper eggs, placed separately in both rice and *Zizania* fields for trapping egg parasitoids. After 2 d, the potted plants were brought back to the lab and put in black cloth-covered mylar cages topped by glass vials for collecting emerging adult parasitoids.

Zizania leaves were collected monthly from fields for dissecting and counting the parasitized eggs of *S. procerus.*

Wooden plates (50 cm^2) covered on each side with yellow sticky paper (5 parts of caster oil:4 of colophony: 1 of glycerol) (Yu et al 1999) were used for monitoring egg parasitoid dispersal in April and July 1997. Twenty sticky plates were placed vertically in bunds between rice and *Zizania* fields, one side facing the *Zizania* and the other facing the rice field. The sticky plates were angled northwest/southeast to avoid direct effects of prevailing wind. The sticky papers were replaced every 6 h and the trapped *Anagrus* spp. were identified and counted.

For preference tests, *A. nilaparvatae* were obtained from *N. lugens* and *S. procerus* collected in rice and *Zizania* fields. A pair of *A. nilaparvatae* was released onto each test cage, in which various egg ratios of *N. lugens* and *S. procerus* were set up on rice and *Zizania* plants. One hundred pairs of parasitoids were replicated in each treatment and all were kept at 25 ± 1 °C and a 12-h day. The *A. nilaparvatae* adults were removed 24 h later. The unparasitized and parasitized eggs were counted by dissecting the rice and *Zizania* plants after 4 d at high humidity.

The times for 50% mortalities (LT50) of *S. procerus* on rice and *Zizania* plants were assessed using Quant 3.0 probit analysis software.

Deviations of the parasitoid numbers from random choice of their prey were measured using a_i, the vector with the ith component (Chesson 1983). Since *A. nilaparvatae* is a solitary egg parasitoid, the estimate for preference when food is depleted is important, and was given by

$$\alpha_i = \frac{\mathrm{Ln}\,(n_{io} - r_i/n_{io})}{\mathop{\mathrm{Ln}}\limits_{j=1}^{m}\,(n_{jo} - r_j/n_{jo})} \quad i = 1,2,\ldots,m$$

where n_{io} is the number of items of type i present at the beginning of a foraging bout and r_i is the number of items of food type i in the consumer's diet (Manly 1974). The chi-square (χ_c^2) was used to test the differences of values from a fixed ratio. Such values of α range from 0 to 1 with 0.5 representing no preference.

Results

No *S. procerus* of any instar could complete its life cycle on rice. The LT50 of nymphs on rice was little longer than that of adults, lasting 1-3 d, and was significantly shorter than the LT50 of *S. procerus* on *Zizania* (3-20 d) (Table 1). It seems that the 1st-instar nymph was more adaptable than other stages.

The nymphal longevity of *S. procerus* on *Zizania* was not significantly different over three generations (Table 2). About 55% of the nymphs became adults in all three generations.

Saccharosydne procerus laid on *Zizania* plants an average of 200-400 and sometimes up to 1,000 eggs. Field surveys showed that parasitism of *A. nilaparvatae* on

Table 1. The LT50s of various stages of *S. procerus* reared on the rice variety TN1 and on *Zizania caduciflora*.

Host plant	Insect stage	Time-survival correlation	LT50 (d)
Rice	1st instar	Y = 9.9948 – 1.2175x	2.52
	2nd-3rd instar	Y = 7.7969 – 0.8113x	1.31
	4th-5th instar	Y = 9.2601 – 1.1231x	1.85
	Male adult	Y = 6.2186 – 0.7635x	1.74
	Female adult	Y = 5.7971 – 0.5740x	1.72
Zizania	1st instar	Y = 7.1137 – 0.7168x	19.08
	2nd-3rd instar	Y = 11.5904 – 3.0580x	8.63
	4th-5th instar	Y = 7.5025 – 1.3996x	5.98
	Male adult	Y = 8.4829 – 1.3889x	12.28
	Female adult	Y = 7.0924 – 1.6616x	3.52

S. procerus fluctuated in sampling locations and seasons from 0 to 75%. In June-October, there was around 30% parasitism, which declined sharply to 2—5% in November-January, probably because *Zizania* fruit harvesting and the cold weather decreased parasitoid activity (Table 3). With the large fecundity of *S. procerus* and high parasitism, a large population of *Anagrus* spp. developed in *Zizania* fields during and between the rice seasons.

The pot-plant-trap data (Fig. 1) in *Zizania* and neighboring rice fields showed that, from mid-June to early August, more *Anagrus* spp. were attracted into *Zizania* fields than into rice fields and field bunds. In early season rice, the number of *Anagrus* in rice fields neighboring *Zizania* was 2.26 times that in rice fields that were relatively isolated from *Zizania* plants, which suggests that *Zizania* plants are a major source of *Anagrus* spp. in fields after the winter season when rice is absent. In late-season rice, however, the population of *Anagrus* spp. in both rice and *Zizania* fields was low, probably due to heavy spraying of insecticides.

The sticky trap catches from bunds between habitats showed that, in April, the number of *Anagrus* spp. on the side facing *Zizania* fields was significantly higher than that on the side facing rice fields; most *Anagrus* spp. were trapped between 0800

Table 2. Duration of nymphal stages of *S. procerus* on *Zizania caduciflora*.

Instar	Developmental period (d)		
	1st generation	2nd generation	3rd generation
1st	3.11 ± 0.52	3.48 ± 0.76	2.98 ± 0.48
2nd	2.19 ± 0.59	2.67 ± 0.51	2.84 ± 0.48
3rd	2.76 ± 0.55	3.12 ± 0.66	3.13 ± 0.53
4th	3.21 ± 0.73	3.24 ± 0.59	3.49 ± 0.61
5th	4.30 ± 0.67	4.40 ± 0.76	4.18 ± 0.55
Nymphal period	15.57 ± 4.20	16.90 ± 1.92	16.60 ± 5.05

Table 3. Seasonal variation in the percentage parasitism by *A. nilaparvatae* of *S. procerus* eggs in *Zizania* fields.

Sampling date	No. of eggs inspected	Mean % parasitism[a]	Range
15 Jun	5,522	31.46 ± 17.55 a	13.24 – 62.66
15 Jul	4,670	27.11 ± 11.34 a	5.33 – 57.21
15 Aug	2,161	30.03 ± 16.23 a	5.10 – 75.69
15 Sep	4,310	27.84 ± 14.61 a	10.65 – 52.85
15 Oct	4,540	29.52 ± 14.45 a	5.16 – 55.33
15 Nov	3,514	5.36 ± 4.30 b	1.20 – 23.29
15 Dec	3,449	2.15 ± 2.71 b	0 – 17.20
15 Jan	4,289	4.45 ± 3.33 b	0 – 22.53
15 Feb	4,294	3.35 ± 1.75 b	0 – 8.05
15 Mar	4,899	4.00 ± 3.88 b	0 – 13.15
15 Apr	829	23.16 ± 9.13 a	3.52 – 75.86

[a] In a column, means followed by the same letter are not significantly different at the 5% level based on Duncan's multiple range test.

and 1400, suggesting the greatest activity and emergence of this parasitoid. In June, however, the traps facing rice trapped more *Anagrus* spp. than on the side facing *Zizania* fields. The trend of parasitoid activity in June was similar to that in April (Fig. 2). This shows that there was more *Anagrus* dispersal from *Zizania* fields to newly transplanted rice fields in April but the reverse occurred in June when parasitized planthoppers were abundant in rice fields.

Manly's preference indices of *A. nilaparvatae* from both rice and *Zizania* (Table 4) showed a significantly stronger preference for BPH eggs on rice than *S. procerus* eggs on *Zizania* plants ($a_B = 0.74^{**}$, $c_c{}^2 > c_{0.01}{}^2$). Meanwhile, *A. nilaparvatae* trapped from *Zizania* fields appeared significantly to prefer BPH eggs to *S. procerus* eggs ($a_B = 0.70^{**}$, $c_c^2 > c_{0.01}{}^2$). There was no significant density-dependent influence in the preference tests of this egg parasitoid.

Discussion

Anagrus spp. are considered to be an important biocontrol agent for suppressing rice planthoppers in most Asian countries (Chandra 1980, Dyck and Thomas 1979). Insecticide spraying, harvesting, and plowing, however, cause a severe fluctuation of the parasitoid population because of the scarcity of host eggs at critical times. The gramineous grasses supporting related homopterans in surrounding field bunds that serve as refuge areas for egg parasitoids were studied recently (Yu et al 1999); but, for sanitary purposes, the farmers usually remove and burn the wild vegetation on field bunds. This caused us to examine the role of other plants, especially gramineous crops, for conserving natural enemies when there are disadvantageous conditions for natural

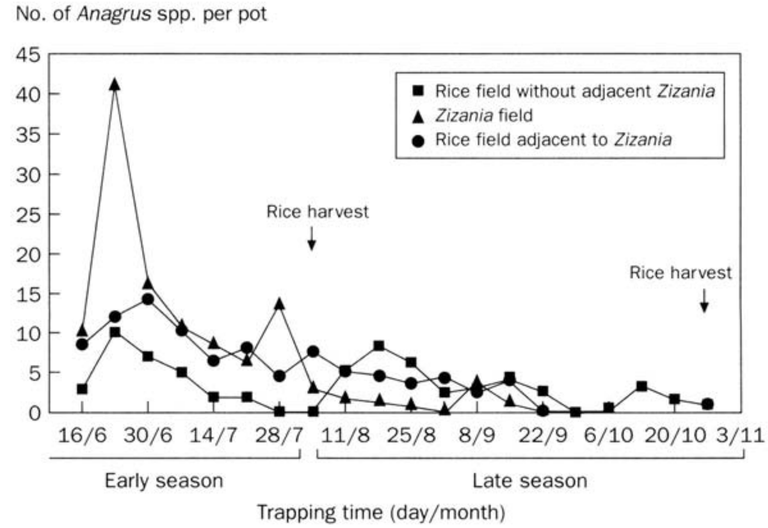

Fig. 1. Pot-plant trap catches of *Anagrus* spp. in rice fields with and without adjacent *Zizania* fields during rice seasons.

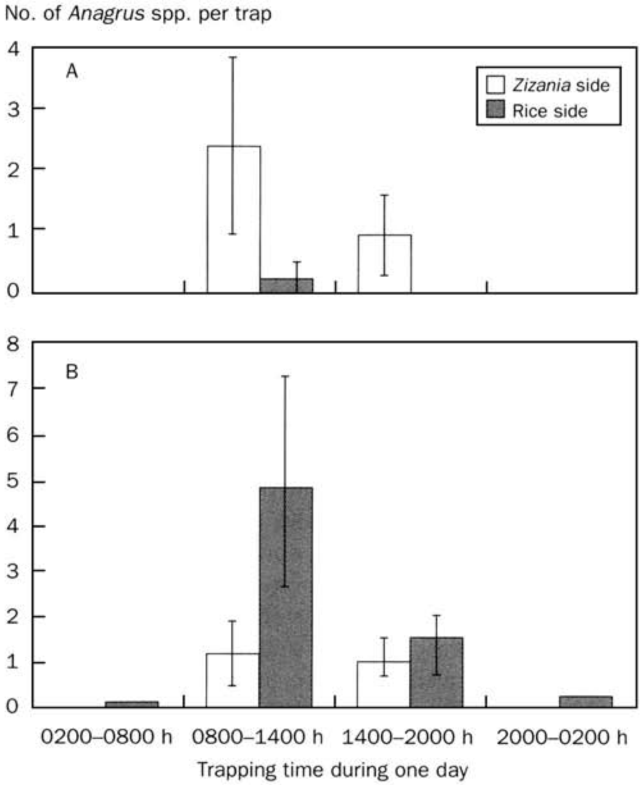

Fig. 2. Movement of the egg parasitoid **Anagrus nilaparvatae** and other **Anagrus** spp. between rice and **Zizania** habitats based on two-sided yellow sticky trap catches. (A) Numbers trapped in April; (B) numbers trapped in June. The vertical line on each column is the standard error.

Table 4. Choice of *A. nilaparvatae* for *N. lugens* and *S. procerus* eggs as shown by preference indices based on chi-square tests for the hypothesis of no preference.

Preference indices	From *N. lugens* eggs in rice field	From *S. procerus* eggs in *Zizania* field
n[a]	51	56
α	0.74 ± 0.31	0.70 ± 0.30
χ_c^2	$16.09 > \chi_{0.01}^2$	$14.76 > \chi_{0.01}^2$
Degree of freedom = 1, $\chi_{0.01}^2 = 6.63$		

[a] n = mean number per preference test; α = preference indices of *A. nilaparvatae* for *N. lugens* and *S. procerus* eggs.

enemies within the rice field. Sugarcane, maize, and particularly *Zizania caduciflora* are planted in a mosaic pattern with rice fields in the Yangtze delta, which is the largest rice-producing area in China. This study showed that *S. procerus,* a major insect pest of *Zizania,* is a bridge host of *A. nilaparvatae* between different rice seasons and rice fields. Rice planthoppers such as *N. lugens* and *Sogatella furcifera* Horvath (Delphacidae:Homoptera) cannot overwinter in most of the temperate area and usually emigrate to the southern rice area (Kisimoto 1979). Egg parasitoids *A. nilaparvatae* and other *Anagrus* spp. have to overwinter in nonrice hoppers, for instance, as pupae in *S. procerus* eggs on *Zizania* leaves in the Yangtze delta area (Yu, unpublished data). The results from sticky traps indicate that, in April, there is dispersal of *Anagrus* spp. from *Zizania* to neighboring rice fields. In June, however, there is a frequent exchange of *Anagrus* spp. between habitats where abundant egg parasitoids exist. It seems that *Anagrus* spp. emerging on *Zizania* after winter synchronized with rice transplanting and promoted populations of *Anagrus* spp. in rice fields.

Preference tests revealed that *Anagrus nilaparvatae* emerging from both *S. procerus* and *N. lugens* eggs prefer parasitizing *N. lugens* eggs rather than *S. procerus.* Perhaps the white wax filament cover on eggs of *S. procerus* adversely influences searching and ovipositing by the parasitoid. However, a female *A. nilaparvatae* can parasitize 15-20 *S. procerus* eggs within 24 h when given no choice.

It is important to study the dispersal capacity of *A. nilaparvatae* and other *Anagrus* spp. between habitats in their search for new hosts and refuge areas. In this context, *A. flavelous,* the dominant egg parasitoid of *N. lugens* in Laguna, Philippines, can move >30 m in 1 wk from a grassy area to rice fields (Yu et al 1995, Yu and Heong 1997b). *Anagrus epos* can disperse more than 4 km from an overwintering refuge (wild *Rubus)* into commercial vineyards (Doutt and Nakata 1965, 1973). The effects of such dispersal among habitats should be studied further.

Apart from benefits, the possible harmful effects of rice pests as well as the role of natural enemies from *Zizania* fields should be examined further in relation to *Zizania* habitat manipulation as a tool for rice integrated pest management. As regards pests, *S. procerus* was once reported to be a pest of rice (Zhang 1983); however, this study has shown that *S. procerus* cannot complete its life cycle on rice plants. Some stem borers related to *Scirpophaga incertulas, Chilo suppressalis,* and *Sesamia inferens,* which are major pests of rice, attack *Zizania,* but preliminary data show that there are biological differences in stem borers on rice and *Zizania* (Yu et al 2001). Konno and Tanaka (1996) also mention that stem borers from rice and *Zizania latifers* are dissimilar.

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Relationships between pests and natural enemies in rainfed rice and associated crop and wild habitats in Ilocos Norte, Philippines

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Pest and natural enemy abundances in rice and nonrice habitats along transects at Batac and Badoc were monitored to characterize herbivore and natural enemy assemblages in rice and surrounding nonrice habitats during different cropping seasons. The data were analyzed to determine arthropod associations, population shifts, and habitat compatibilities with rice. A bund management study was then made to validate characterization results and design a strategy whereby natural enemy action can be conserved and enhanced to reduce pest abundance.

The characterization study yielded a total of 119 arthropod families with 363 confirmed taxa. At Batac, 120 herbivore and 158 natural enemy taxa were identified, whereas Badoc had 113 herbivore and 159 natural enemy taxa. Cicadellids were the predominant herbivores in both rice and nonrice habitats. Spiders, formicids, and coccinellids were the dominant predators and Mymaridae, especially *Mymar taprobanicum*, and the trichogrammatid *Oligosita* sp. were the dominant parasitoids. Natural enemies had the most taxa in most habitats. The measurement of habitat compatibility generally showed bunds and fallow similar to those of rice in terms of herbivore and natural enemy assemblages.

Insect pests and natural enemies were more abundant and had more taxa in rice paddies with weeds on bunds than in paddies without bund weeds. They were also more abundant in bunds than in paddies. Natural enemies were most abundant in bunds with only broadleaf weeds. Among the 36 species of weeds monitored in bunds, seven—*Alysicarpus vaginalis*, *Sphaeranthus africanus, Vernonia cinerea, Ipomoea triloba, Heliotropium indicum, Digitaria sanguinalis, and Ischaemum rugosum*—were associated with insect pests, whereas all species except *A. vaginalis* harbored natural enemies. Where the bund weeds *Panicum repens*, *Cynodon dactylon*, *Dichanthium aristatum*, and *Commelina diffusa* were infected with sheath blight, the adjoining edges of rice paddies were sometimes also infected.

The results indicate that some other crops, notably cowpea, are especially important reservoirs of natural enemies of rainfed rice pests during the nonrice season. So, appropriate crop manipulation might be feasible for enhancing the role of insect natural enemies in integrated pest management of rice. Similarly, it is evident that bund manipulation, particularly the enhancement of broadleaf weeds, can provide vital sources of insect natural enemies as well as prevent the spread of rice sheath blight from grassy hosts on bunds.

Much attention has been given to pest management in tropical rice because of serious damage by particular pests exacerbated by the complexities of crop losses caused by pest damage to the crop. Thus, increasing rice yields requires development of sustainable pest management practices (Teng 1996). The dominant pest control strategies in tropical rice over the past 30 years have been the use of resistant varieties and especially chemical insecticides. Most research related to arthropods in tropical rice has been directed toward only a few pest species without examining the biotic linkages to the rest of the system (Settle et al 1996). The findings of Kenmore et al (1984) suggested that integrated pest management (IPM) strategies in tropical rice should integrate biological control, host-plant resistance, and cultural practices to keep pests at low densities, with insecticides applied as a last resort. One of the main challenges facing agroecologists today is identifying the types of heterogeneity (at either the field or regional level) that will yield desirable agricultural results (e.g., pest regulation) given the unique environment and entomofauna of each area. This challenge can be met only by further analyzing the relationships between vegetation diversification and the population dynamics of associated damaging and beneficial species (Altieri 1994).

Arthropods in the rice ecosystem vary widely depending on the distribution of rice and other crops and wild habitats. The dynamics of pests and natural enemies in the area is influenced by how the crop is managed but, despite this complexity, relatively few manageable components of natural diversity are probably the key to insect pest management in irrigated rice in the tropics (Way and Heong 1994). Some studies have suggested that the size and composition of nonrice habitats adjacent to rice fields may have positive effects on natural enemies in rice fields (Stapley 1975, Bentur and Kalode 1985, as cited by Xiaoping et al 1995). Whether or not the nonrice habitats in the rice ecosystem are beneficial sources of rice pests (Hibino and Cabunagan 1986, Way and Heong 1994), they need to be explored for their preservation and possible manipulation to favor natural control of rice pests. These studies aimed to characterize assemblages of herbivores and natural enemies in rice and surrounding nonrice habitats over different cropping seasons in order to determine their associations, population shifts, and habitat compatibilities with rice. This included a bund management study to validate the characterization results and to provide a basis for strategies whereby natural enemies are conserved to reduce insect pest and disease incidence.

Methodology

Characterization of arthropods

Sampling points. Two transects were established, one in the municipality of Batac and the other in Badoc, Ilocos Norte. Ilocos Norte is situated in the northwestern part of Luzon island between 17°48′ and 18°29′N latitude and 120°25′ and 120°58′E longitude. These sites were selected on the basis of their differences in cropping sequence during the wet and dry seasons. Each transect comprised 40 sampling points 50 m apart along a gradient of diverse cropping patterns and practices and vegetation. The 3.6-km Badoc transect spanned four barangays, Paltit, Pasuc, Mabusag Sur, and Mabusag Norte, whereas the 3.6-km Batac transect crossed two barangays, Magnuang and Tabug. There were 38 farmer cooperators at each site.

Sampling and arthropod identification. Arthropods in each sampling point were collected using a blower-vac suction sampling machine covering an area of 1,590 cm^2 (Cariño et al 1979, Arida and Heong 1992). Monthly sampling was done for 17 months, from August 1997 to December 1998. Arthropods were collected for 2–3 min depending on the age of the crop. Collected specimens were sorted, identified, and counted in the laboratory. Specimens were identified to family level and the common ones to genera or species. Dr. A.T. Barrion, of the International Rice Research Institute, verified taxonomic identities.

Data analysis. Arthropod abundances were lumped to the same taxonomic and functional groups and habitat zones between sampling dates and seasons following the standardized taxonomic list describing their respective functions, using the LUMP program developed by Schoenly and Zhang (1999a). This was done for all the arthropods collected in the habitats along the transect sites. The differences in taxonomic composition of lumped herbivores, natural enemies, and detritivores were then analyzed using the statistical sofware program SPPDISS, with Clarke's R-test (Schoenly and Zhang 1999b). This compares between-community and within-community dissimilarities for all $n(n - 1)/2$ pairwise dissimilarities between n sites and computes the average rank of the between-community (rb) and within-community (rw) pairs having a test statistic of

$$R = \frac{4\,(rb - rw)}{n(n-1)}$$

If R = 1, the two communities are taxonomically different and, if R approaches 0 (R~0), the two communities are taxonomically similar.

Bund management for pest control

Three sets of farmers' rice fields in each of three barangays, Mabusag East, Mabusag West, and Paltit, municipality of Badoc, Ilocos Norte Province, were identified for a bund management study as a follow-up to the transect characterization study. Three treatments were made at each site: (1) rice paddy with weeds on bunds (RWWB), (2) rice paddy without weeds on bunds (RWOWB), and (3) rice paddy without grasses on bunds (RWOGB). Sampling was also carried out on bunds and the following treatments were considered: (4) bund with weeds (BWW), (5) bund without weeds (BWOW), and (6) bund without grasses (BWOG). The bunds for treatments without grasses were sprayed with fluazifop-P-butyl (Onecide) herbicide, whereas those without weeds were sprayed with glyphosate (Roundup). Onecide kills only grasses while Roundup kills all kinds of weeds. Both treatments were sprayed twice to prevent the growth of unnecessary weeds and maintain the treatments throughout the cropping season. The weed species found along the bunds in all the treatments were recorded before the set-up of the experiment and in every sampling period. Numbers of insect pests and natural enemies and incidence of diseases were monitored in alternate weeks starting 2 wk after transplanting until crop maturity. A blower-vac sampler was used as indicated above.

Insect pests and natural enemies associated with weed species on bunds were collected from individual plants that were enclosed in plastic bags and then cut off at the base.

Results

Arthropod fauna in rice and nonrice habitats

Collected samples from rice and nonrice habitats along the transects (Batac and Badoc) yielded 119 arthropod families with 363 confirmed taxa. Table 1 lists the different habitats sampled along the transects. The 17-month arthropod collection yielded 22,025, 5,971, 7,786, 403, 70,201, 805, 26, 85, 34, and 38 individuals in rice, bund, fallow, garlic, onion, maize, mungbean, tomato, bell pepper, tobacco, and cowpea, respectively, totaling 37,444 individuals at the two transect sites (Table 2). In Badoc, the abundance of arthropods in decreasing order in the habitats was ranked as rice > bund > fallow > mungbean > garlic > onion, whereas, in Batac, arthropod abundance in rank was as follows: rice > fallow > bund > mungbean > maize > bell pepper > garlic > cowpea > tobacco > tomato. Generally, more herbivores and natural enemies were found in Badoc than in Batac and more arthropod catches were made in rice, bunds, and fallow (Tables 2 and 3). More arthropods were caught in the rice season presumably because the sampling points during this wet season (WS) were mostly in the predominant rice paddies and associated bunds, which had abundant weed species at the 7 sampling points in Badoc and 11 in Batac (Table 1).

Herbivore taxa. Abundances of herbivores in the habitats along the transect were dominated by the Cicadellidae, especially *Nephotettix* spp. *Nephotettix malayanus, N. virescens,* and *N. nigropictus* were collected in rice, bund, fallow, and mungbean and a few on maize, garlic, onion, and bell pepper (Tables 1 and 2). Other abundant herbivores collected in rice and nonrice habitats were the delphacids (mostly *Nilaparvata and Sogatella* spp.) and aphids. Aphids were found in rice, bund, fallow, maize, cowpea, bell pepper, mungbean, and garlic.

The proportion of herbivores varied from 15% to more than 44% of the total arthropod populations in the different habitats in Badoc and from 18% to 55% in Batac (Table 2). Overall, the total herbivore taxa ranged from 30.2% to 42.5% in Badoc and from 22.2% to 54.5% in Batac (Table 3). Other herbivores found in other nonrice habitats were delphacids, aphids, and Chrysomelidae.

Natural enemy taxa. In Badoc, the total number of taxa of natural enemies in rice is 132, constituting 52.6% of all arthropod taxa collected from rice. Bunds had 85 (51.83%) natural enemy taxa; 62 (42.5%), 30 (47.6%), 9 (34.6%), 8 (50%), and 34 (45.3%) natural enemy taxa were caught in fallow, garlic, onion, maize, and mungbean, respectively (Table 3). Similarly, the total number of natural enemy taxa collected in Batac in the different habitats along the transect was generally greater than the herbivore and detritivore compositions. Natural enemies in Badoc ranged from 24.4% to 55.6% in rice and nonrice crops along the transect, whereas, in Batac, they ranged from 14% to 46.2%. This conforms with Schoenly et al (1998), who showed that in typical irrigated rice in the Philippines natural enemies had the most taxa in both the canopy and floodwater, followed by herbivores and detritivores. Natural enemies were more abundant in the habitats than the herbivores, except in rice, the bunds in Batac, and onions in Badoc, which were slightly lower in number (Table 2). The total natural enemy taxa in rice was 46.5%, in bunds 44.4%, in fallow 40.0%, in garlic 46.0%, in maize 41.0%, in mungbean 35.7%, in tomato 44.4%, in bell pepper 43.8%, and in tobacco 18.2%. The dominant predators in nonrice habitats were spiders, formicids (mostly *Tapinoma* sp. and *Solenopsis* sp.), coccinellids (mostly *Micraspis* sp.), *Metioche* sp., *Anaxipha* sp., and *Conocephalus* sp. In the Badoc transect, they constituted 25.2%, 92.5%, 74.1%, 72.5%, 100%, 70%, and 72.7% of the total natural enemies collected in rice, bund, fallow, garlic, onion, maize, and mungbean, respectively. In the Batac transect, the dominant predators comprised 44.0%, 73.0%, 51.9%, 57.1%, 84.1%, 93.8%, 8.3%, 60.0%, 27.3%, and 100% in rice, bund, fallow, garlic, maize, mungbean, tomato, bell pepper, tobacco, and cowpea, respectively (Table 4). At both transect sites, spiders were in all habitats except in Batac, where no spiders were caught in tomato and cowpea (Table 1). The spider population was dominated by *Tetragnatha, Pardosa,* and *Oxyopes* species, especially in rice and bunds. Formicids and spiders occurred in bunds throughout the sampling duration. *Micraspis* sp. occurred at the maximum tillering stage of the rice crop until harvest time and was on bunds and mungbean during the nonrice season from December to February.

No formicids were collected from tobacco in Batac. There were few sampling points in tobacco, cowpea, and tomato (Table 1), which might explain why they were not observed/collected at sampling. Overall, the characterization study along the transect showed more natural enemies in most habitats.

Compatibility of herbivore and natural enemy taxonomic composition in rice and nonrice habitats

The seasonal measurements of compatibility of the herbivore and natural enemy taxonomic compositions in rice and different nonrice habitats using Clarke's R-test method

Table 1. Monthly sampling of crops, fallows, and bunds and occurrence of commonest herbivores and natural enemies in the Batac and Badoc transects.

Transect Crop/habitat	1997								
	Aug[a]	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Badoc									
Rice	33 */f	33 */sfm	31 */smc	2 *sc	3 *s	5 *s	4 *sm	3 *	3
Bund	7 */f	7 */sfm	7 */mc	7 *sfc	7 *sf	7 *sf	7 *sf	7 *f	7 sf
Onion	–	–	2 *	3 */	3 f	–	–	–	–
Fallow	–	–	–	16 *sfc	15 *sfc	15 *sf	19 *sf	20 *f	21 *sf
Garlic	–	–	–	12 *s	12 *s	11 */s	–	–	–
Mungbean	–	–	–	–	–	2	10 */sf	9 */af	9 */sf
Maize	–	–	–	–	–	–	–	1	–
Batac									
Rice	21 */f	26 */afm	26 */asfmc	–	–	–	–	–	–
Bund	11 */f	11 */asfm	11 */asfmc	11 *sf	11 *sf	11 *sf	11 *sf	11 */f	11 *sf
Fallow	8 *	3 */asf	1 s	25 s	14 *sf	11 *f	11 *sf	13 */f	25 *sf
Bell pepper	–	–	2	3	3 s	3	3 *f	3 *	–
Maize	–	–	–	1	1 fs	1 *f	1 f	5 *f	1 *fs
Mungbean	–	–	–	–	4 fs	5 *fs	4 *f	2 */	1
Tomato	–	–	–	–	2 *f	2	2 *f	2 *	–
Tobacco	–	–	–	–	1	2	2 *s	2 *	1 *
Garlic	–	–	–	–	5 *fs	5 *fs	1 *fs	–	–
Cowpea	–	–	–	–	–	–	1 f	1 */	–

[a]– = not planted with the crop during the month. * = *Nephotettix* spp., / = delphacids, a = aphids, s = spiders, f = formicids, m = mymarids, c = *Micraspis* sp.

are shown in Table 5. In the Badoc transect, results during the 1997-98 WS and 1997-98 dry season (DS) croppings were similar. The herbivore and natural enemy taxonomic compositions in the bunds showed similarity with rice, which implies that the bund is not compatible with rice as herbivores found in rice are also found along the bunds, which may serve as sources of insect pests. However, the bunds harbored natural enemies similar to those of rice, implying that they are associated with each other. Garlic showed a weak association of herbivores and natural enemies during the 1998 DS. The Batac transect shared a similar composition of herbivores and natural enemies. Bell pepper appeared to be compatible with rice during the 1998 DS cropping with dissimilarity in herbivore taxonomic composition and similarity with rice during the 1997-98 WS and DS croppings (Table 5).

In comparing the differences in the herbivore and natural enemy taxonomic compositions of rice and fallow before and after the rice season using Clarke's R-test method (Tables 6 and 7), the herbivore compositions at fallow sites showed dissimilarity with rice at the Batac and Badoc transect sites. In the 1998 WS rice vs 1998 DS fallow in Batac, however, results showed similarity in taxonomic composition. For the natural enemies in the fallow period before the 1998 rice-cropping (July-October 1998) season compared with the 1998 WS rice cropping, the herbivore and natural enemy taxonomic compositions were highly significantly different (Table 8).

No. of sampling points							
1998							
May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
–	–	17 */s	32 */sfmc	33 */sfmc	31 */sfmc	3* /smc	13 */smc
7 *sf	7 *sf	7 */sf	7 */sfmc	7 */sfm	7 */sfmc	7 */sfmc	7 */sfm
–	–	–	–	–	–	1 s	1 sf
25 *sf	31 *sfc	14 */sf	1 *sf	–	2 */sfm	27 */sfmc	15 */scfm
–	–	–	–	–	–	2s	3 *sm
8 */sf	1 f	1 /f	–	–	–	–	–
–	1 *f	1 */asf	–	–	–	–	1 s
–	–	7 */sf	9 */asfc	17 */asfc	20 */asfmc	9 */asmc	3 */smc
11 *sf	11 *sfc	11 */asfm	11 */asf	11 */asfmc	11 */asfm	11 */asfmc	11 */sfmc
27 *sf	27 */sfmc	18 */asfm	20 */asfmc	12 */asfc	7 */sfmc	17 */asfmc	2 */sfmc
–	–	–	–	–	2 /s	2	–
1 *s	2 fscm	4 */fscm	–	–	–	–	–
–	–	–	–	–	–	–	–
–	–	–	–	–	–	1	2
1 *	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–

Bund management experiment

Insect pests. Insect pests in both paddies and bunds were most abundant in rice paddies with weeds on bunds, whereas they were least abundant in paddies without weeds on bunds except at Paltit (Fig. 1, Table 9). The same trend was observed in bunds. It was noted, however, that insect pests were more abundant in bunds than in paddies, as observed at the three locations. The number of taxa followed the same trend (Fig. 2). In contrast, however, more taxa were found in paddies than on bunds. This shows that more species of insect pests were infesting the rice crops than the weeds on bunds. Although there were fewer natural enemy species on bunds, they were more abundant.

Natural enemies. Natural enemies were also monitored in both paddies and bunds (Fig. 1, Table 9). As with insect pests, paddies with weeds on bunds had the most abundant natural enemies and they were least abundant in paddies without weeds on bunds. This was observed in Mabusag East and Paltit. Among the bunds, however, those without grasses had the most abundant natural enemies, which shows that natural enemies were closely associated with broadleaf weeds. The number of taxa was again highest in paddies with weeds on bunds, followed by paddies without grasses on bunds. They were least in paddies without weeds on bunds. Generally, however, more taxa were in paddies than on bunds, notably insect pests because of their association with rice.

Table 2. Total numbers of arthropods in rice and nonrice habitats along Batac and Badoc transect sites, Ilocos Norte. August 1997 to December 1998.

Site	Arthropods	Habitat[a]										
		Rice	Bund	Fallow	Garlic	Onion	Maize	Mungbean	Tomato	Bell pepper	Tobacco	Cowpea
Badoc	Herbivores	4,863 (35.0)	539 (23.8)	712 (32.7)	49 (15.2)	28 (40.0)	9 (25.0)	263 (44.7)	–	–	–	–
	Natural enemies	3,394 (24.4)	963 (42.5)	897 (41.2)	178 (55.1)	16 (22.9)	20 (55.6)	253 (43.0)	–	–	–	–
	Detritivores	5,645 (40.6)	762 (33.7)	568 (26.1)	96 (29.7)	26 (37.1)	7 (19.4)	73 (12.4)	–	–	–	–
	Total	13,902	2,264	2,177	323	70	36	589				
Batac	Herbivores	1,509 (18.6)	1,295 (34.9)	2,523 (45.0)	16 (20.0)	–	61 (37.0)	68 (31.5)	7 (26.9)	31 (36.5)	13 (38.2)	21 (5.3)
	Natural enemies	1,192 (14.6)	1,279 (34.5)	1.213 (21.6)	35 (43.8)	–	69 (41.8)	96 (44.4)	12 (46.2)	30 (35.3)	11 (32.4)	8 (21.2)
	Detritivores	5,422 (66.8)	1,133 (30.6)	1,873 (33.4)	29 (36.3)	–	35 (21.2)	52 (24.1)	7 (26.9)	24 (28.2)	10 (29.4)	9 (23.7)
	Total	8,123	3,707	5,609	80		165	216	26	85	34	38
Total		22,025	5,971	7.786	403	70	201	805	26	85	34	38
Grand total		37,444										

[a] – = no crops planted in these transects. In parentheses are the percentage compositions of the total arthropods in each grouping.

Table 3. Total numbers of taxa found in rice and nonrice habitats along Badoc and Batac transects.[a]

Site	Arthropods	Rice	Bund	Fallow	Garlic	Onion	Maize	Mungbean	Tomato	Bell pepper	Tobacco	Cowpea
Badoc	Herbivores	90 (35.9)	62 (37.8)	62 (42.5)	19 (30.2)	9 (34.6)	6 (37.5)	30 (40.0)	–	–	–	–
	Natural enemies	132 (52.6)	85 (51.8)	62 (42.5)	30 (47.6)	9 (34.6)	8 (50.0)	34 (45.3)	–	–	–	–
	Detritivores	29 (11.6)	17 (10.4)	22 (15.1)	14 (22.2)	8 (30.8)	2 (12.5)	11 (14.7)	–	–	–	–
Batac	Herbivores	73 (33.6)	63 (36.8)	62 (37.4)	6 (25.0)	–	16 (41.0)	12 (42.9)	2 (22.2)	12 (37.5)	6 (54.5)	6 (50.0)
	Natural enemies	101 (46.5)	76 (44.4)	73 (44.0)	11 (45.8)	–	16 (41.0)	10 (35.7)	4 (44.4)	14 (43.8)	2 (18.2)	1 (8.3)
	Detritivores	43 (19.8)	32 (18.7)	31 (18.7)	7 (29.2)	–	7 (18.0)	6 (21.4)	3 (33.3)	6 (18.8)	3 (27.3)	5 (41.7)

[a] – = no crop planted along these transects. In parentheses are the percentage compositions of the total taxa collected in each grouping.

Table 4. Percentages of dominant predators and parasitoids in rice and nonrice habitats in the transects.

Habitat	Percentages	
	Predators	Parasitoids
Batac		
Rice	44.0	10.2
Bund	73.0	5.8
Fallow	51.9	6.6
Garlic	57.1	0.0
Maize	84.1	4.4
Mungbean	93.8	0.0
Tomato	8.3	0.0
Tobacco	27.3	0.0
Bell pepper	60.0	3.3
Cowpea	100.0	0.0
Badoc		
Rice	25.2	16.2
Bund	92.5	5.7
Fallow	74.1	5.1
Garlic	72.5	6.6
Onion	100.0	0.0
Maize	70.0	15.0
Mungbean	72.7	5.9

Table 5. Measurement of compatibility of rice and nonrice habitats by season in terms of the taxonomic composition of herbivores and natural enemies using Clarke's R-test at the Badoc transect site.

Season	Herbivores			Natural enemies		
	Observed R	*P*	Rank	Observed R	*P*	Rank
1997 wet season (Aug-Oct)						
Rice vs bund	0.1452	0.0100[a]		0.0559	0.9431	
1998 wet season (Jul-Oct)						
Rice vs bund	0.1348	0.0789		0.1610	0.0390[a]	
1998 dry season (Nov-Dec)						
Rice vs bund	0.2273	0.1279	3	0.0649	0.2547	1
Rice vs fallow	0.3470	0.0130	2	0.5520	0.0020	3
Rice vs garlic	0.5000	0.2517	1	0.5000	0.2697	2

[a]Different at the 5% level of significance.

Insect pests and natural enemies associated with weeds in bunds. Among the 36 weed species monitored in the bunds, only seven had insect pests (Table 10). The insect pests were Cicadellidae, Aphididae, and Chrysomelidae. Cicadellidae were found on *Alysicarpus vaginalis, Sphaeranthus africanus,* and *Vernonia cinerea;* Aphididae on *S. africanus* and *Ipomoea triloba;* and Chrysomelidae on *Heliotropium indicum, Digitaria sanguinalis,* and *Ischaemum rugosum.* Only two grass species had insect

Table 6. Measurement of compatibility of rice and nonrice habitats by season in terms of the taxonomic composition of herbivores and natural enemies using Clarke's R-test at the Batac transect site.

Season	Herbivores			Natural enemies		
	Observed R	*P*	Rank	Observed R	*P*	Rank
1997 wet season (Aug-Oct)						
Rice vs bund	0.3353	0.0020[a]		0.0457	0.8402	
1998 wet season (Jul-Oct)						
Rice vs bund	0.0137	0.4975	2	0.0913	0.9051	1 equal
Rice vs fallow	0.0673	0.2208	1	0.1465	0.1429	2 comp
1998 dry season (Nov-Dec)						
Rice vs bund	0.0867	0.2008	3	0.0272	0.3127	2
Rice vs fallow	0.1751	0.9890	2	0.0030	0.3846	1
Rice vs bell pepper	0.7500	0.0629	1	0.1667	0.2717	3

[a] Different at the 5% level of significance.

Table 7. Differences in the herbivore and natural enemy taxonomic compositions of rice and fallow before and after the rice season using Clarke's R-test in the Badoc transect.

Season	Herbivores		Natural enemies	
	Observed R	*P*	Observed R	*P*
Aug-Oct 1997 rice vs Nov 1997-Jan 1998 fallow	0.3501	0.0010[a]	0.0413	0.9001
Feb-May 1998 fallow vs Jul-Oct 1998 rice	0.6857	0.0010[a]	0.7584	0.0010
Jun 1998 fallow vs Jul-Oct 1998 rice	0.8158	0.0010[a]	0.7584	0.0010
Jul-Oct 1998 rice vs Nov-Dec 1998 fallow	0.4516	0.0010[a]	0.1877	0.0130

[a] Different at the 5% level of significance.

pests, notably Chrysomelidae. No insect pests were observed in sedges. The Cicadellidae and Chrysomelidae are insect pests of rice.

All the weed species except *A. vaginalis* harbored natural enemies. The ones most commonly encountered were the formicids, spiders, and coccinellids. Formicids were the most commonly encountered and were recorded in 30 species of weeds, followed by spiders in 14 species. Coccinellids were found only on *Sida rhombifolia*, *I. rugosum*, and *Fimbristylis littoralis*. Since only the shoot system of weeds was sampled, no

Table 8. Differences in herbivore and natural enemy taxonomic compositions of rice and fallow before and after the rice season using Clarke's R-test in the Batac transect.

Season	Herbivores		Natural enemies	
	Observed R	*P*	Observed R	*P*
Aug-Oct 1997 rice vs Nov 1997-Jan 1998 fallow	0.6671	0.0001[a]	0.0906	0.0849
Apr-Jun 1998 fallow vs Jul-Oct 1998 rice	0.4698	0.0010[a]	0.7168	0.0010
Jul-Oct 1998 rice vs Nov-Dec 1998 fallow	0.0703	0.1508	0.1217	0.0629

[a] Different at the 5% level of significance.

species associated with the root system were recorded and those on the soil surface were not included as they were recorded in the suction samples from bunds in the transect study.

Disease incidence. At all locations, only the paddies with weeds on bunds were infected with rice sheath blight disease (caused by *Rhizoctonia solani*), which was observed near the bunds where weeds were infected. Weeds on bunds that were infected were *Panicum repens*, *Cynodon dactylon*, *Dicanthium aristatum*, and *Commelina diffusa*. Only those rice plants near the bunds were infected. In the transect characterization study, 14 species of weeds along bunds, fallow, and in paddy rice and dry-season crops were infected with sheath blight (Marcos et al 2000). These included the four species referred to above. Such weeds have been listed as hosts of rice sheath blight (Dath 1990).

Discussion

Nonrice habitats as sources of pests and natural enemies

Insect pests. Nephotettix spp. that are vectors of rice tungro virus diseases were recorded, particularly on bunds and fallow during the nonrice seasons. *Nephotettix virescens* is known to transmit tungro virus disease in rice (Hibino and Cabunagan 1986). Outbreaks of tungro virus in the Philippines in recent years suggest outbreaks of green leafhopper (GLH) *Nephotettix virescens* (Dyck and Hsieh 1972). *Nephotettix* spp. were observed to occur at the early stage of the rice season. Elsewhere, GLH often dominated in fields planted with rice (Heong et al 1991) with *Nephotettix* spp., especially *N. virescens,* accounting for more than 70% of the total leafhoppers. The same is true with the planthoppers (Delphacidae) in rice, where outbreaks have occurred in several places, especially in Asia, damaging many hectares of rice fields.

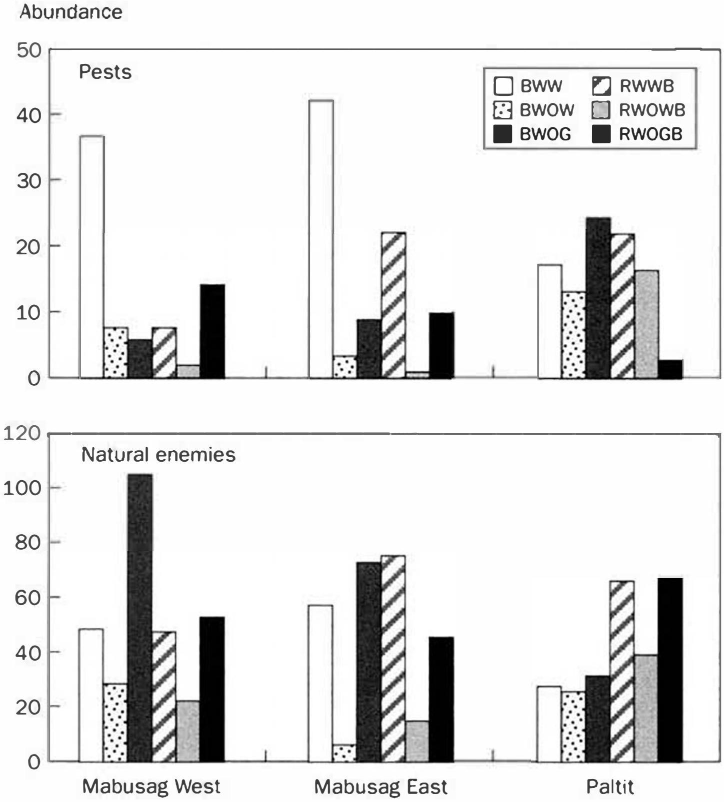

Fig. 1. Insect herbivore and natural enemy abundance on rice paddies and bunds In three locations of Ilocos Norte, 2000 dry season. BWW = bunds with weeds, BWOW = bunds without weeds, BWOG = bunds without grass, RWWB = rice paddy with weeds on bunds, RWOWB = rice paddy without weeds on bunds, RWOGB = rice paddy without grass on bunds.

Table 9. Total abundance of herbivores and natural enemies on rice paddy and bunds at selected sites of Badoc, Ilocos Norte, 2000 dry season.

Treatment[a]	Insect pests		Natural enemies	
	Total	Mean ± SE	Total	Mean ± SE
BWW	98	32.67 ± 12.34	140	46.67 ± 19.60
BWOW	23	7.67 ± 5.03	62	20.67 ± 12.10
BWOG	41	13.67 ± 10.02	214	71.33 ± 37.45
RWWB	53	17.67 ± 9.64	195	65.00 ± 15.52
RWOWB	19	6.33 ± 8.39	87	29.00 ± 15.39
RWOGB	28	9.33 ± 5.69	170	56.67 ± 10.02

[a] BWW = bunds with weeds, BWOW = bunds without weeds, BWOG = bunds without grass, RWWB = rice paddy with weeds on bunds, RWOWB = rice paddy without weeds on bund, RWOGB = rice paddy without grass on bunds.

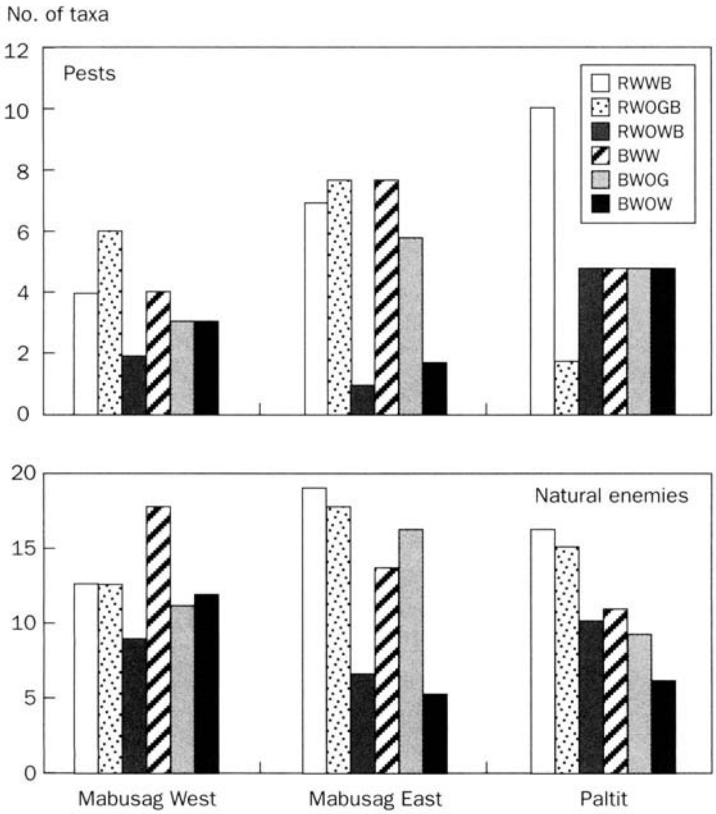

Fig. 2. Total number of arthropod taxa on rice paddies and bunds in three locations, December 1999 to March 2000 dry season. BWW = bunds with weeds, BWOW = bunds without weeds, BWOG = bunds without grass, RWWB = rice paddy with weeds on bunds, RWOWB = rice paddy without weeds on bunds, RWOGB = rice paddy without grass on bunds.

Several studies on the ecology, occurrence, and control of planthoppers have been conducted (Kenmore 1980, Kisimoto 1981, Kenmore et al 1984). The pest's presence in nonrice habitats could possibly be an important factor in the transmission of virus diseases that the insect can transmit.

The occurrence of a rice pest in nonrice crops bordering rice fields can serve as good refuges of these pests, thus sustaining the insect's occurrence in the field, especially during the nonrice season. Cicadellids occur throughout the year in bunds and they were observed to invade rice paddies as early as July, the start of the planting season in the Ilocos area. Several species of weeds present in areas adjacent to crops may also serve as alternative hosts for crop pests (Van Emden 1965).

Natural enemies. It is recognized that natural enemies are important in preventing pest build-up (Heinrichs 1994), so nonrice habitats could be a vital reservoir and source of appropriate natural enemies. For example, mungbean is used as a relay crop

Table 10. Herbivores and natural enemies associated with weeds on bunds at selected sites of Badoc, Ilocos Norte, 2000 dry season.

Herbivores and natural enemies	Weeds	Rice insect pest/ natural enemy[a]	Host of rice sheath blight
Herbivores			
Cicadellidae	*Alysicarpus vaginalis*	+	–
	Sphaeranthus africanus		–
	Vernonia cinerea		–
Aphididae	*Sphaeranthus africanus*	–	–
	Ipomoea triloba		–
Chrysomelidae	*Heliotropium indicum*	+	–
	Digitaria sanguinalis		–
	Ischaemum rugosum		+
Natural enemies			
Formicidae	*Ipomoea congesta*	+	+
	Lippia nodiflora		+
	Elephantophus spicatus		–
	Sphaeranthus africanus		–
	Ipomoea triloba		–
	Phyllanthus amarus		–
	Cucumis melo		–
	Heliotropium indicum		–
	Vernonia cinerea		–
	Portulaca oleraceae		–
	Cardiospermum halicacabum		+
	Chromolaena odorata		–
	Sida rhombifolia		–
	Synedrella nodiflora		–
	Panicum repens		+
	Dicanthium aristatum		+
	Imperata cylindrica		+
	Eleusine indica		+
	Leersia hexandra		+
	Brachiaria mutica		–
	Digitaria sanguinalis		–
	Pennisetum clandestinum		–
	Cyperus rotundus		+
Tapinoma sp.	*Commelina diffusa*	+	+
	Alternanthera sessiles		–
	Alternanthera tenella		–
	Elephantophus spicatus		–
	Portulaca oleraceae		–
	Sphenochloa zeylanica		–
	Cardiospermum halicacabum		+
	Synedrella nodiflora		–
	Cynodon dactylon		+
	Dicanthium aristatum		+
	Eleusine indica		+
	Ischaemum rugosum		+
	Echinochloa colona		+
	Pennisetum clandestinum		–
Trichogrammatidae	*Lippia nodiflora*	+	–

continued on next page

Table 10 continued.

Herbivores and natural enemies	Weeds	Rice insect pest/ natural enemy[a]	Host of rice sheath blight
Salticidae	*Ipomoea congesta*	+	+
	Heliotropium indicum		–
Theridiidae	*Ipomoea aquatica*	–	–
	Ipomoea congesta		+
	Sphaeranthus africanus		–
	Vernonia cinerea		–
	Chromolaena odorata		–
	Digitaria sanguinalis		–
Clubionidae	*Eclipta alba*	–	–
	Heliotropium indicum		–
	Sphenochloa zeylanica		–
Thomisidae	*Eclipta alba*	–	–
	Vernonia cinerea		–
	Sphenochloa zeylanica		–
	Cardiospermum halicacabum		+
	Ischaemum rugosum		+
Araneidae	*Phyllanthus amarus*	+	–
Oxyopes	*Phyllanthus amarus*	+	–
	Ischaemum rugosum		+
Tetragnatha	*Heliotropium indicum*	+	–
	Imperata cylindrica		+
	Brachiaria mutica		–
Pardosa	*Vernonia cinerea*	+	–
Gnaphosidae	*Imperata cylindrica*	–	+
Coccinellidae	*Ischaemum rugosum*	–	+
Micraspis sp.	*Sida rhombifolia*	+	–
	Fimbristylis littoralis		–

[a]+ = is a rice insect pest/natural enemy and host of rice sheath blight. – = not a rice insect pest/natural enemy and host of rice sheath blight.

with rice in the Philippines and both pests and natural enemies of rice were associated with the crop (Litsinger et al 1988). Spiders, which were abundant on many nonrice crops, are recognized as important for biological control (Hassell 1978, Murdoch et al 1985) and some species were abundant in rice. They also occurred commonly in bunds and fallows during the nonrice season. It is especially important that they arrive very early on rice (Way and Heong 1994) from the bunds and also as wind-blown juveniles from other habitats. Many suggestions have been made on how spiders can be better managed as biological control agents, either by enhancing their habitats or by reducing their enemy habitats (Riechert and Lockley 1984, Riechert and Bishop 1990, Cohen et al 1994).

The formicids were found abundantly in the different habitats along the transect. Way et al (1998) showed that the bunds around tropical irrigated rice fields usually support an abundant and sometimes very diverse ant community with nesting populations limited to the dryland bunds when fields are flooded. Many ants may prey on insects in rice fields, both on the canopy and during fallows. *Solenopsis geminata* can be a “keystone” species with major impact as a predator of other arthropods. The parasites/parasitoids found mostly in the different rice and nonrice habitats in the transects that are dominant in number are the hymenopterans, mymarids (mostly *Anagrus* sp. and *Mymar taprohanicum*), trichogrammatids,and scelionids. They ranged from 5% to 16% of the total dominant natural enemies collected in the transects (Table 4). Mymarid wasps are the most abundant parasitoid collected on rice, bunds, and fallow and they have even occurred in mungbean, maize, and garlic. An analysis made by Schoenly et al (1998) showed that this wasp gave the best fit to all tested surrogate taxa in irrigated rice fields.

The early occurrence of plankton feeders and detritivores, together with abundant populations of generalist predators, has been observed (Heong et al 1991, Schoenly et al 1994a, 1998) and experimental studies support the finding that predators actually feed on the detritivores and plankton feeders (Settle et al 1996). It is possible that early arriving natural enemies prey on detritivores when pest abundances are low, then switch to pests after the detritivore population declines (Schoenly et al 1994a). The tropical rice agroecosystem has complexes of natural enemy species (predators, parasites, parasitoids, and detritivores) that live in the plant canopy, on the water surface, in the floodwater, and on or in the waterlogged soil. Thus, biological control in this agroecosystem involves many species, spans multiple trophic levels, and acts along spatiotemporal gradients (Heong et al 1991, 1992, Cohen et al 1994, Schoenly et al 1994b, 1998).

Herbivore and natural enemy association

The results show that rice and nonrice habitats at the transect sites have similarity in terms of the herbivore and natural enemy compositions. The variability in the nonrice habitat's compatibility with that of rice may depend on the time of sampling as influenced by the growth stage of the crop in both habitats being compared, seasonal abundance of arthropods, and the farmers' cultural practices employed on their farms. Movements of natural enemies will determine whether interplanted vegetation will act as a source of the natural enemy and will determine the spatial extent of enhancement. Clearly, the movement behavior of natural enemies has a strong influence on their response to agroecosystem diversification (Corbett 1998). With the results obtained, the natural enemy compositions in a diversified cropping pattern are generally more than the herbivores and several of them are associated with each other. It is important to take into consideration the underlying ecological mechanisms of their compositions in a diversified field. The nonrice habitats, particularly the narrow vegetation-covered bunds surrounding each field, seem especially important as a source of natural enemies, particularly early arriving species such as spiders, and are an important source of some predators such as *Cyrtorhinus lividipennis* and Gryllidae that can seasonally concentrate on rice (Way and Heong 1994). Crop diversification may increase generalist enemy effectiveness by increasing alternate food or prey availability (Sheehan 1986). Several researchers have shown that vegetation in adjacent areas can provide the alternative food and habitat essential to perpetuate certain natural enemies of pests in crop fields. Several other studies indicate that the abundance and diversity of entomophagous insects within a field are closely related to the nature of the surrounding vegetation (Altieri 1994). Conservation of natural enemies through habitat management in rice has not received much emphasis although the limited data and many empirical observations point to its potential (Bottrell 1992). The observed changes in pest and natural enemy populations must also be accounted for to fully understand the effects of agricultural diversification before proper management can be employed.

Weeds are important hosts of insect pests and pathogens in agroecosystems. Van Emden (1965) cites 442 references relating to weeds as reservoirs of pests. More than 70 families of arthropods affecting crops were reported as being primarily weed-associated (Bendixen and Horn 1981). However, certain weeds are important components of agroecosystems because they positively affect the biology and dynamics of beneficial insects. They offer many important requisites for natural enemies such as alternative prey/hosts, pollen, or nectar as well as microhabitats that are not available in weed-free monocultures. Herbivore-natural enemy interactions occurring in a crop system can be influenced by the presence of herbivores on associated weed plants (Altieri and Letourneau 1982).

According to Wainhouse and Coaker (1981), weedy plants near crop fields can provide the requisites for pest outbreaks, whereas certain grasses can act as hosts for cereal pests (Burn 1987). Dambach (1948) concluded that the more closely border vegetation is related botanically, the greater is the danger of its serving as a potential source of infestation by injurious insects. Thus, less crop-pest risk is involved in the use of woody border vegetation in areas where the crops are grain, vegetables, and forage plants. But, again, several studies documented the importance of adjoining wild vegetation in providing alternate food and habitat to natural enemies that move into nearby crops (Van Emden 1965, Wainhouse and Coaker 1981, Altieri and Schmidt 1986). For instance, shelter belts and hedgerows frequently provide refuges for beneficial parasites and predators that can invade crops and pastures to help control insect pests and thereby reduce the need for pesticides (Altieri et al 1987, Paoletti et al 1989).

Conclusions

Our results show that natural enemy compositions in a rice-based cropping system diversified in time and space are generally more complex than the herbivores and also more abundant. So, it seems that many nonrice habitats must be important for carryover of natural enemies that are significant in rice pest management. These include vegetation-covered bunds surrounding each field, which seem especially important as a source of natural enemies, particularly early arriving species such as spiders and some other predators. Our work on bund manipulation shows that encouraging broadleaf plants benefits natural enemies of rice pests while minimizing the role of grasses as hosts of rice insect pests, and also sheath blight disease. Moreover, it is evident that crop diversification increases generalist natural enemies, which may be very important for control of rice pests (Sheehan 1986). Such results suggest the need for more work on habitat manipulation in rice-based cropping systems (Bottrell 1992). In particular, detailed work is needed on crop patterning, especially where rice is grown in only one season as in Ilocos Norte such that survival of natural enemies in adequate numbers depends on intervening nonrice habitats.

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Notes

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Managing insect pests of temperate japonica rice by conserving natural enemies through habitat diversity and reducing insecticide use

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Population changes of major insect pests, natural enemies, and detritivores in rice habitats were monitored at monthly intervals in insecticide-free and insecticide-treated habitats for an entire rice season. Based on our present studies with temperate japonica rice, the following conclusions could be drawn:

1. Soybean neighboring paddy fields is a beneficial habitat for conserving natural enemies of insect pests of rice without sharing the insect pest species with the rice habitat.
2. Planting planthopper-resistant rice varieties without early sprays of insecticide could effectively and efficiently suppress pest populations.
3. Decreasing numbers of insecticide sprays in an entire rice season is possible and acceptable though this needs to be examined further by more detailed work.

The conservation and use of natural enemies of insect pests for sustainable crop management have long been practiced on some crops. Many studies on how to conserve the natural enemies of insect pests of rice have been conducted in tropical and temperate rice-growing areas (Cohen et al 1994, Wu et al 1994, Guo et al 1995). The use of resistant varieties with reduced insecticide sprays can play an important role in conserving natural enemies (Feng et al 1999).

Varietal resistance has been widely used as one of the major control measures in integrated pest management (IPM) for rice in Asia. For instance, IR36 and IR64, resistant to the brown planthopper *Nilaparvata lugens,* are successful examples in tropical areas (Cohen et al 1997). In China, numerous cultivars with resistance to ***N.*** *lugens* and the whitebacked planthopper *Sogatella furcifera* have been released since 1980 (Liu 1998). The resistant Xiang-Zao-Xian 3 has been planted on 0.7 million hectares in Hunan Province (Wei et al 1988).

Although insecticides are considered as a last resort to keep populations of insect pests below economic thresholds, many rice farmers largely rely on routine spraying of insecticides even if they do not know when this is necessary. Outbreaks of insect pests have then occurred after insecticides were overused. For example, in 1991, an outbreak of rice planthoppers occurred in many provinces, even as far north as Tangshan, Hebei (Feng et al 1992). Numerous reports demonstrated that spraying insecticides could cause a serious decrease in natural enemies and consequently lead to the outbreak of *N. lugens* (Wang et al 1994, Gu et al 1997).

Hangzhou is located in the east coast region of central China where japonica rice is the major type. *N. lugens, S. furcifera,* and the striped stem borer *Chilo supressalis* are the important insect pests, and spraying insecticides is one of the major management measures. Farmers may spray 5–8 times per rice season, though recently the use of economical and environment-friendly management techniques has attracted more attention. Therefore, we evaluated the effects of different neighboring crop habitats on the population changes of natural enemies in paddy fields, and the effect of a reduction in insecticide use on population changes of insect pests and their natural enemies in order to identify beneficial habitats and the possibility of reducing insecticide use without significantly affecting yields.

Materials and methods

The experimental site was located in the village of Xintang (30°27.37´N, 120°19.90´E), Yuhang, Zhejiang, China (approximately 45 km from Hangzhou City). The experiments were conducted during June and November 1999. Flooding occurred during the season, which consequently was prolonged for 7–10 d compared with average years.

The two experiments occupied approximately 3,000 m^2. In one on effects of habitat diversity and varietal resistance, no insecticides were applied and there were three pairs of habitat combinations: (1) rice-cotton, (2) rice-vegetables, and (3) rice-soybean. Cotton and rice have a similar growth duration, but soybean matured a month earlier. We monocultured the Chinese vegetable Zao-Shu no. 5 (*Brassica campestris* subsp. Terimensis Olsson) in a nonrice habitat. Each nonrice habitat and paddy field had 4 and 5 random sampling points, respectively. Plant species and vegetation coverage were sampled with an “H” pin frame using 50 pins per sampling site. Arthropods were sampled using a motorized blower-vac machine for 2–3 min in an enclosure of 0.25 m^2. Vegetation and arthropods were sampled on the 20th of every month. Rice yields were obtained from three samples per field. Three rice varieties were used: Chujiang 11 and Xiushui 47 (moderate resistance to brown planthopper) and Chunjiang 15 (resistant). All three are agronomically similar and have similar maturation times.

In the second experiment, on insecticide treatment, buprofezin and “Shachongshuang” were mixed and sprayed once or three times at 1.5 kg ha^{-1} for buprofezin (25%) and 3.0 kg ha^{-1} for Shachongshuang (18%). Untreated plots served as controls. Arthropods were sampled on the same day and in the same way as mentioned above at three sampling points per treatment. Average rice yields for each treatment were obtained from three samples in the area of 400–640 m^2 for each treatment.

A total of 81 arthropods were identified in rice and nonrice habitats though only 30 major arthropods were categorized into three groups: (1) insect pests: Cicadellid sp., Delphacidae (immature), Aphididae, *N. lugens, S. furcifera,* and *Nephotettix virescens;* (2) natural enemies: Lycosidae (immature), Diapriidae, Braconidae, Phoridae, Tetragnathidae, *Itoplectis naranyae, Ummeliata insecticeps, Coleosoma octomaculata, Tetragnatha japonica, Gnathonarium dentatum, Cyrtorhinus lividipennis, Pirata subpiraticus,* and *Erigone prominens;* and (3) detritivores: Psychodidae, Chironomidae, Sciaridae, Ceratopogonidae, Chironomidae, Empidae, Tomoceridae, Anthomyzidae, Ephydridae, and Entomobryidae.

Arthropod counts and yield data were analyzed by analysis of variance with STATGRAPHICS. Means were analyzed by the test of least significant difference (LSD). Dissimilarity of natural enemies and insect pests between habitats or treatments was analyzed using software developed by Schoenly and Zhang (1999).

Results

Effect of habitat diversity and varietal resistance on natural enemy conservation

During the rice season, the foliage cover in paddy fields increased to >75% before harvesting (Fig. 1). There was relatively low diversity. In contrast, in the cotton, vegetable, and soybean habitats, there was more diversity and more variation in vegetation coverage.

In the insecticide-free habitats, the populations of insect pests increased in July, reaching about 48 individuals per 0.25 m^2 (Fig. 2). *N. virescens* and aphids were major pests in the growing and milking stages, respectively, where cultivars resistant to brown planthopper were used. The populations of their natural enemies followed the insect pests and reached a peak of 56 individuals per 0.25 m^2. The populations of detritivores declined as the natural enemies increased and the populations of insect pests in the paddy fields declined as rice ripened. On the other hand, the number of the natural enemies *Ummeliata insecticeps* and *Pirata subpiraticus* increased after harvesting along with an increase in the detritivores Entomobryidae and Tomoceridae. Nonrice habitats with more than 50% of foliage coverage provided similar shelter for arthropods.

Based on dissimilarity analyses of arthropod community structures in the paddy fields and their corresponding neighboring crop habitats, the habitat pair soybean-rice (Chunjiang 15) had a significantly higher dissimilarity in herbivore species and higher similarity in natural enemies (Table 1) than did the other crop combinations, particularly cotton-rice.

Among the tested rice varieties, there was a significantly high similarity in herbivores and less similarity in natural enemies (Table 1).

Chunjiang 11, Xiushui 47, and Chunjiang 15 yielded 4.7, 4.3, and 5.9 t ha^{-1}, respectively.

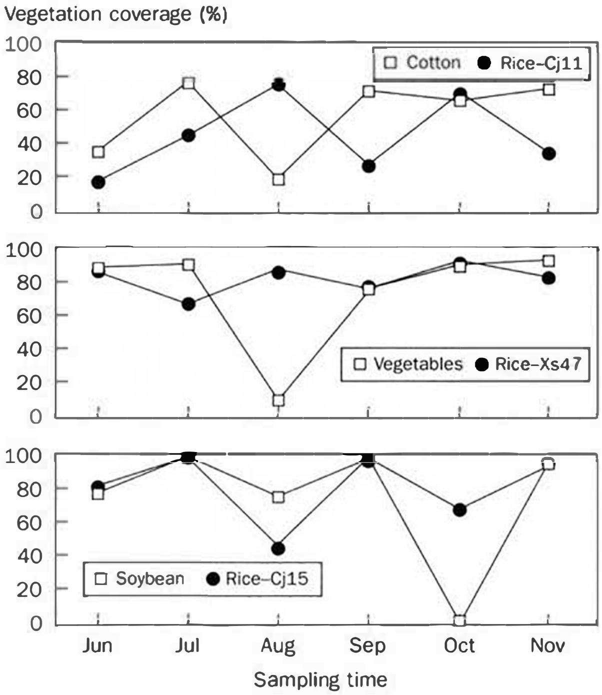

Fig. 1. Changes in vegetation coverage in insecticide-free habitats, Yuhang, Yangtze Delta, China, 1999.

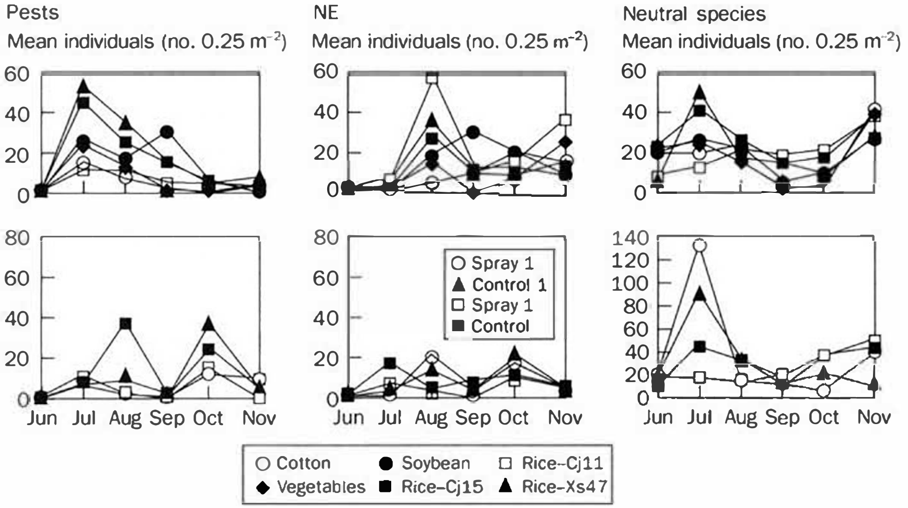

Fig. 2. Changes in mean numbers of different insect groups on unsprayed rice during the cropping season. Upper row = mean numbers of pests, natural enemies (NE), and other species on different rice cultivars and associated crops. Lower row = mean numbers of different insect groups on unsprayed control Cj11 rice compared with rice sprayed once or three times with insecticides.

Table 1. The similarity analysis of arthropod herbivore and natural enemy community structures in insecticide-free and insecticide-treated pairs of crops, Yuhang, Yangtze Delta, 1999[a].

Herbivores			Natural enemies		
Crop pairs	R value	Dissimilarity rank	Habitat pairs	R value	Similarity rank
Insecticide-free habitat pairs					
Soybean–rice, Cj15	0.009	1	Soybean–rice, Cj15	0.039	1
Rice, Cj11–bund	0.011	2	Vegetables–rice, Xs47	0.026	2
Vegetables–rice, Xs47	0.015	3	Rice, Cj11–rice, Xs47	0.014	3
Rice, Cj11–rice, Xs47	0.016	4	Rice, Cj11–bund	0.013	4
Rice, Cj11–rice, Cj15	0.022	5	Rice, Cj11–rice, Cj15	0.011	5
Cotton-bund	0.026	6	Cotton–rice, Cj11	0.005	6
Cotton-rice, Cj11	0.030	7	Cotton–bund	0.004	7
Insecticide-treated habitat pairs					
Cj11 spray 3–control[b]	0.008	1	Spray 1–control	0.013	1
Cj11 spray 1–control[b]	0.012	2	Spray 3–control	0.012	2

[a] Cj11 = Chunjiang 11; Xs47 = Xiushui 47; Cj15 = Chunjiang 15. [b] Spray 1 and spray 3 = rice was sprayed with insecticides 1 and 3 times, respectively.

Table 2. Insect pest incidence and rice yields in insecticide-treated and untreated paddy fields in Yuhang, Yangtze Delta, China, 1999.

Treatment	Pest damage				Rice yield[a] ($t\ ha^{-1}$)	Yield increase compared with controls
	N. virescens (no. m^{-2})		Leaf rolling by *C. medinalis* (%)			
	July	August	August	September		
Spray 3	25.3	1.3	2.1	1.6	6.39 b	23.94
Unsprayed control	13.3	153.3	8.0	19.6	4.86 a	
Spray 1	8.0	2.6	0.9	13.7	5.24 ab	14.07
Unsprayed control	5.3	32.0	5.8	16.3	4.48 a	

[a] Means followed by the same lowercase letters are not significantly different at the 95% level.

Effect of the reduction in insecticide use on natural enemy conservation

The major insect pests of rice *N. virescens* and *Cnaphalocrocis medinalis* were monitored before and/or after insecticide sprays. Table 2 shows that the population of *N. virescens* and the percentage of leaf rolling caused by *C. medinalis* declined remarkably after spraying compared with the unsprayed control. At the same time, spraying insecticides also decreased the populations of natural enemies and detritivores (Fig. 2). The more the insecticide sprays, the more serious the negative effect on the beneficial insects. However, it is concluded that spraying the rice crop three times in a single season is sufficient to suppress the insect pests and give yields as good as the six sprays of insecticide applied by rice farmers.

According to the similarity analysis of insect community structures in rice habitats treated with insecticides, the rice habitat treated with the selected insecticides three times showed less similarity in herbivores and better similarity in natural enemies than the controls (Table 1).

Discussion

The use of natural control measures is demonstrably one of the most effective and efficient tools in sustainable pest management of irrigated rice in the tropics. Habitat diversity, especially species diversity, could be helpful in conserving and enhancing the natural enemies of insect pests of rice (Heong et al 1991). Our study demonstrated that, in the temperate rice-growing area, the soybean habitat neighboring a paddy field was beneficial in conserving natural enemies yet was not a source of insect pests. We found that, in conserving arthropods, vegetation coverage seems more important than plant species diversity. The dissimilarity analysis of major insect pests and their natural enemies among rice varieties indicates that similar habitats share similar arthropod species.

The minimum use of insecticides in the temperate rice ecosystem could be vitally important for conserving natural enemies (Hu et al 1996) as indicated by heavy and continuous use of insecticides, which can create outbreaks and resurgences of insect pests (Heinrichs and Mochida 1984, Gu et al 1997) associated with negative effects on natural enemies (Cuong et al 1997, Chen et al 1999, Tanaka et al 2000). The present study shows that it is not necessary to spray insecticides within 50–60 d after sowing of rice and that, compared with rice farmers' six insecticide sprays during the crop season, three sprays gave similar rice yields.

In summary, the conclusions drawn from this study on temperate japonica rice are that

1. Crops, notably soybean neighboring paddy fields, can be beneficial for conserving natural enemies of insect pests of rice without sharing the pest species.
2. Planting planthopper-resistant rice varieties without early sprays of insecticide favors early buildup of natural enemies, which can contribute to effective and efficient suppression of pest populations.
3. Reducing the numbers of insecticide sprays during the rice season is possible and acceptable.

We plan to conduct further experiments in a well-selected village of Zhejiang to implement all the available techniques, particularly emphasizing the importance of habitat diversity for the conservation of natural enemies of rice insect pests.

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Notes

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Habitat diversity: an approach to the preservation of natural enemies of tropical irrigated rice insect pests

L.P. Lan, N.P.D. Huyen, N.H. Quang, and N.V. Minh

The relationships between components of rice and nonrice habitats were examined in relation to rice pest management based on ecology and biological control. The diversity of flora and fauna was therefore investigated on a 10-km transect along the Kinh Xang canal in Chau Thanh District (Tien Giang Province) in the Mekong Delta, Vietnam. The crops included rice monocultures and nonrice multicultures of two to three crops per year from September 1997 to December 1998.

Overall species components of the nonrice habitats were more diverse, but the development of the arthropod population was more stable in the rice habitats than in other individual crops. There were about 253 species in the rice field and 283 in the nonrice habitat. Many species of arthropods exist concurrently in two habitats. Similarity indices changed with the rice growth stage, from ≤.5 at the tillering stage to ≥.5 at the ripening stage.

Homoptera Auchenorrhycha contributed a large component to the total of arthropods collected in rice and nonrice habitats. Cicadellidae were less abundant than Delphacidae and there were large populations of spiders and other predators in both rice and nonrice habitats.

The floral diversity of nonrice habitats around rice fields is considered to be important in biological control of rice pests.

The population development of some species in rice fields seems to be related to nonrice habitats adjacent to rice fields (Chiu 1979) and their relationships have been discussed (Tang et al 1996, Xiaoping et al 1996). The biodiversity of fauna and flora is important in integrated pest management of rice (Way and Heong 1994). Besides other arable crops, crop production by farmers comprises other nearby vegetation including fruit tree gardens and noncultivated areas. This can greatly increase the diversity of vegetation and arthropods.

This study was conducted to understand the arthropod fauna in rice fields and nonrice habitats, particularly to search for available nonrice habitats that support and are sources of natural enemies of rice insect pests.

Materials and methods

The study site was located in Long Dinh Village, Chau Thanh District, Tien Giang Province, and was an approximately 10-km transect line along each side of the Kinh Xang canal. Ten sampling sites (P1-P10) about 1 km apart were selected along the transect. At each sampling site, 20 samples were taken in different rice and nonrice habitats on each side of the canal.

A modified suction cleaner was used to collect all the arthropods within sampling areas 40 cm in diameter. A total of 160 samples were collected monthly in different habitats. The collected arthropods were identified and species verified by Dr. A.T. Barrion of the International Rice Research Institute.

Weed coverage in nonrice habitats was estimated by point sampling. Data were analyzed using ecological software developed by Schoenly and Zhang (1999).

Results

Characterization of the site

Habitat diversity. The relative areas of crop habitats showed that rice occupied about 80-90% at the P4-P10 sites (Table 1). The proportion of rice was about 18-45% at sites P1-P3 where there was rice with vegetables, fruit tree gardens, flowers, and noncultivated areas. There was an increase in rice area from north-south to east-west along the canal. During the year, farmers cultivated different crops, especially at the P1, P2, and P3 sites (Fig. 1.), whereas the cropping pattern was more consistent at the P7-P10 sites.

Faunal diversity. A total of 304 species were collected in 34 different habitats, including rice. They were grouped into (1) natural enemies with 82 species of parasitoids and 95 species of predators (which included 41 species of spiders); (2) herbivores, 90 species; (3) detritivores, 19 species; and (4) tourists and omnivores, 18 species. The overall species richness of the nonrice habitats seemed slightly higher than that of the rice habitats. Many of the same species were collected in both rice and nonrice habitats (Table 2). The species richness ranged from 2 to 20 per 0.1-m^2 sampling unit.

Floral diversity. Seventy-seven plant species were recorded along the transect including 17 grasses, 25 species belonging to other families, and 30 crop species. Grass weeds such as *Panicum repens*, *Echinochloa* sp., *Cynodon dactylon*, and *Brachiaria* sp. were common in some nonrice habitats. with *C. dactylon* predominant on levees but *B. mutica* and *Leersia hexandra* in other noncultivated areas (Table 3).

Table 1. The percentages of rice habitat along the transect in 1997-98.

Time	Site							
	P1	P2	P3	P4	P7	P8	P9	P10
March 1998	44.0	42.8	18.1	91.5	99.3	99.3	97.9	86.9
July 1998	61.9	12.3	27.9	99.6	99.7	99.6	99.3	80.8

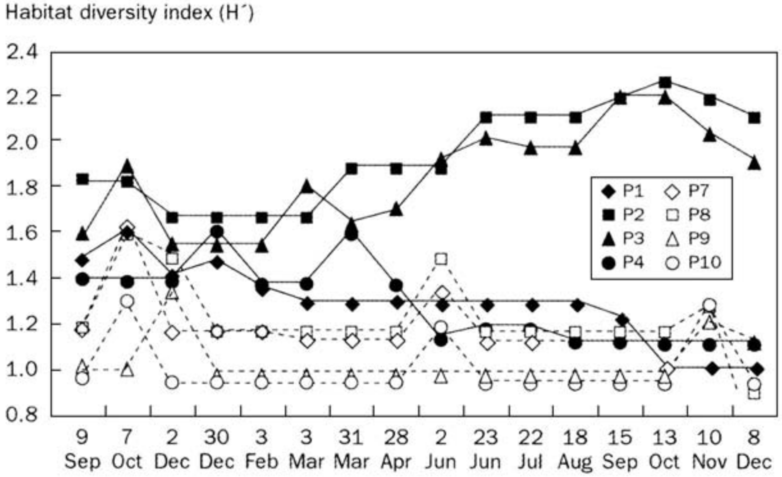

Fig. 1. The changing cropping pattern along the transect line, Long Dinh village, Chau Thanh District, Tien Giang Province. P1 to P9 are sampling sites: P1, P2, P3 = three crops per year mixed with upland crop; P4, P7, P8: three to four rice crops per year; P9, P10 = two rice crops per year, flooded in September to November.

Table 2. Species richness of arthropods in rice and nonrice habitats adjacent to the rice fields of the transect, September 1997 to December 1998, Tien Giang.

Functional groups	Rice	Nonrice	Overlap taxa	Total
Parasitoids	71	76	65	82
Predators[a]	76 (34)	90 (40)	71 (33)	95 (41)
Herbivores	75	83	68	90
Detritivores	16	16	13	19
Tourists, omnivores	15	18	15	18
Total	253	283	232	304

[a] Spiders are in parentheses.

Key pests and natural enemies

Among the 253 species in rice habitats, 10 were abundant, notably Homoptera, including brown planthopper *Nilaparvata lugens* (Fig. 2), especially in March, July, September, and October. Symptoms of hopper burn were recorded in September, when rice plants were at the flowering to milky stage.

In the natural enemy group, *Cyrtorhinus lividipennis, Microvelia d. douglasi,* and the spiders *Pirata subpiraticus, Dischiriognatha* sp., *Tetragnatha* sp., *Coelosoma* sp., and *Atypena formosana* were dominant. The populations of *C. lividipennis* and *M. d. douglasi* seem to show the same trend as the total population. The lycosid *P. subpiraticus* was the commonest spider.

Table 3. Relative abundance (%) of vegetation encountered in some main nonrice habitats, Tien Giang Province. The calculation of relative abundance is based on weed coverage aboveground of a given habitat by using the pointing method. Superscript numbers are ranks of relative abundance values.

Vegetation	Habitat						
	Open area	*Polianthes*	Fruit garden	Bunds	Vegetables	Coconut	Banana
Ruella tuberosa	4.3^{7}	–	–	–	–	–	1.7
Alternanthera sessilis	–	0.3^{9}	1.9^{12}	1.5^{8}	0.6^{28}	6.5^{6}	1.7^{14}
Ageratum conyzoides	–	–	0.1^{35}	0.7^{19}	–	7.1^{4}	–
Commelina communis	0.4^{13}	0.3^{8}	5.4^{6}	0.7^{17}	–	3.1^{9}	–
Cyperus rotundus	–	–	0.6^{23}	0.7^{16}	–	–	–
Jussiaea erecta	–	–	–	0.3^{26}	–	6.9^{5}	0.4^{19}
Axonopus compressus	1.0^{10}	–	–	0.8^{15}	–	4.4^{7}	7.2^{5}
Brachiaria distachya	–	–	–	0.8^{14}	0.7^{25}	–	–
Brachiaria mutica	26.5^{1}	0.9^{4}	0.5^{25}	13.5^{3}	0.7^{24}	14.6^{2}	8.5^{4}
Cynodon dactylon	6.5^{5}	–	3.3^{9}	37.5^{1}	3.1^{7}	2.5^{11}	4.3^{10}
Digitaria sp.	–	–	0.5^{24}	1.4^{9}	2.0^{10}	0.4^{20}	–
Echinochloa colona	–	–	–	0.2^{28}	0.3^{32}	–	1.3^{16}
Echinochloa crus-galli	0.6^{12}	1.8^{4}	6.4^{5}	0.7^{18}	0.6^{27}	0.8^{16}	3.4^{11}
Leersia hexandra	20.0^{2}	–	2.5^{10}	0.9^{11}	–	–	9.8^{3}
Leptochloa chinensis	1.2^{9}	–	2.4^{11}	1.6^{7}	1.8^{11}	–	0.9^{18}
Oryza sativa L.	3.5^{8}	4.4^{3}	4.6^{7}	9.7^{4}	3.7^{6}	3.1^{8}	4.3^{*}
Panicum repens	16.5^{3}	0.6^{6}	0.6^{22}	4.3^{5}	0.9^{20}	2.5^{10}	6.8^{7}
Panicum sp.	–	–	1.1^{15}	1.4^{10}	–	–	–
Ground touches	13.5^{4}	10.0^{2}	29.4^{1}	15.9^{2}	34.3^{1}	11.7^{3}	22.1^{1}
Total of weed species in habitat	15	9	32	32	32	23	20

Relationship between habitat diversity and selected pests

In all groups, only two species were significantly positively correlated with all canopy taxa: the brown planthopper (BPH) and the tetragnathid *Dischiriognatha* sp. The veliid M. *d. douglasi* and mirid *C. lividipennis* numbers did not linear-fit the data. However, the numbers of veliids, the lycosid *P. subpiraticus,* and the mymarid *Anagrus* sp. were positively correlated with the numbers of BPH. Total spiders best fitted the numbers of BPH, but the correlation was not significant at $P>0.05$ (Table 4).

There was no correlation between the habitat diversity index (H') and the abundance of each feeding group (Fig. 3) based on the data of each sampling time at each site. Pooled data were also not significantly different (Fig. 4). However, by using the population trends of all taxa in relation to habitat diversity, we found that, instead of the changing of habitat diversity (location), the component of each feeding group did not change so much, but they changed over time; this may depend on rice plant stage and farmers' practices (Figs. 5 and 6). For all sampling sites, there was correlation only between entomobyiids (detritivores) and habitat diversity (Table 5). In general, the arthropod population did not change in time and space according to habitat (Fig. 7). This could be due to the diverse vegetation attracting more species of arthropods (Fig. 8). However, the development of their populations may depend on factors such as

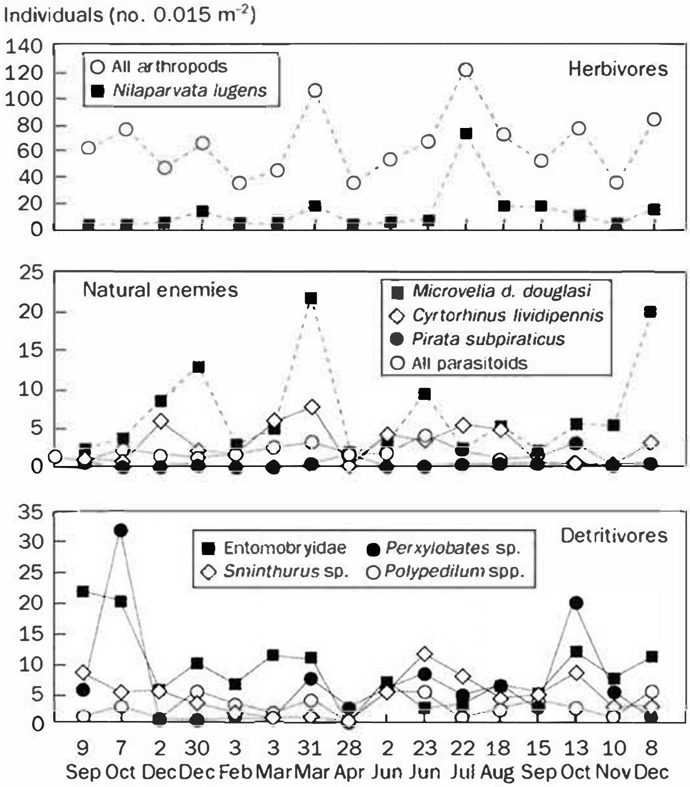

Fig. 2. Population dynamics of the dominant taxa in rice fields along the transect from September 1997 to December 1999, Long Dinh, Tien Giang.

Table 4. Correlations between numbers of commonest natural enemy species with total arthropod species and with numbers of brown planthopper.

	All taxa		*Nilaparvata lugens*	
Dominant species	R	Probability of the linear regression	R	Probability of the linear regression
Nilaparvata lugens	0.583	<0.001	–	–
Sogatella furcifera	0.224	0.063	–	–
Nisia atrovenosa	0.105	0.221	–	–
Recilia dorsalis	0.040	0.222	–	–
Thrips sp.	0.041	0.453	–	–
Microvelia d. douglasi	0.214	0.071	<0.001	0.943
Cyrtorhinus lividipennis	0.214	0.071	0.117	0.194
Dischiriognatha sp.	0.805	<0.001	0.319	0.022
Coelosoma sp.	0.342	–[a]	0.273	0.038
Atypena formosana	0.162	0.121	0.097	0.239
Tetragnatha sp.	0.229	0.060	0.040	0.455
Pirata subpiraticus	0.010	0.707	<0.001	0.950
Total of spiders	0.587	<0.001	0.328	0.020
Anagrus sp.	0.282	0.034	0.743	<0.001
Oligosita sp.	0.099	0.236	0.195	0.086

[a] No data.

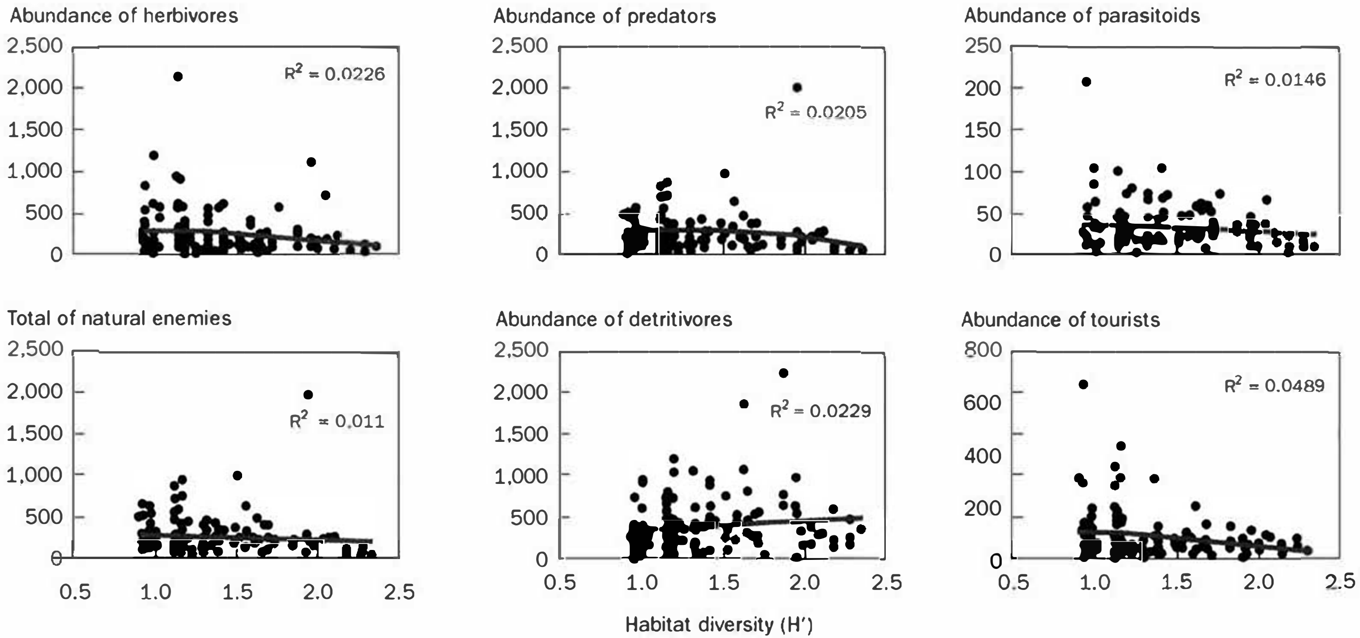

Fig. 3. Correlation between the habitat diversity (H′) of the sampling site (P level) and the abundance of all taxa of each feeding group based on the data of each sampling date.

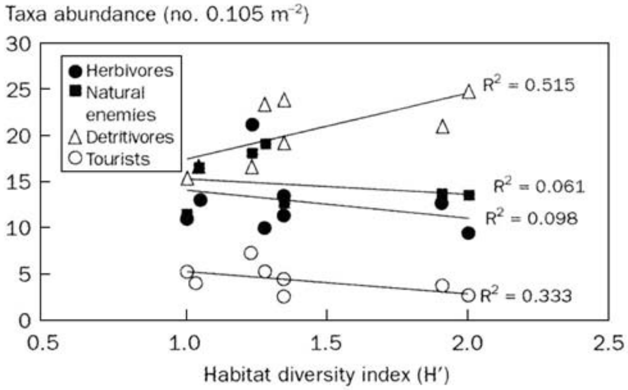

Fig. 4. Correlations within sampling sites between the habitat diversity index and abundance of all taxa belonging to different functional groups. The tabulated significance at $P \leq 0.05$ is 0.707 at df = 6.

natural biological control, the habitat diversity of the location, and others. High populations of BPH were recorded only at the P8-P10 sites.

Nonrice habitats with high biological control potential

There was little difference between the overall abundances of total taxa based on Clark's test for evaluating compatibility between the 33 nonrice habitats and the rice habitat in terms of the similarity of herbivores and natural enemies (Tables 6 and 7). The habitat of levees with grass weeds, noncultivated areas, fruit gardens, coconut gardens, and banana had natural enemy components similar to those of the rice habitat. The high similarities of herbivores indicated a movement and shift of arthropods between adjacent habitats.

Approach to managing weeds on levees

At the P9-P10 sites, where the rice habitat occupied about 80-90% of the land area, farmers usually applied herbicide to control grass weeds such as *Brachiaria mutica* on levees. We suggested that they cut the grass instead of spraying herbicide. The testing was replicated in three adjacent rice fields with no insecticide spraying in September 1999. A plastic barrier was used to separate the rice and levees into two sides. Pitfall traps and sticky traps were used to collect the arthropods that moved from one side to the other.

Most of the arthropods collected by pitfall traps were ants, spiders, and some carabids. Except in controls, more spiders were collected by pitfall traps on levees than on rice, but the differences were not significant between treatments. Lycosids were the major spider component on both sides of the plastic barrier. The data also suggest that disturbing the grass on levees could force predators to move to rice fields (Fig. 9). Cutting of grass instead of using herbicides should maintain and provide

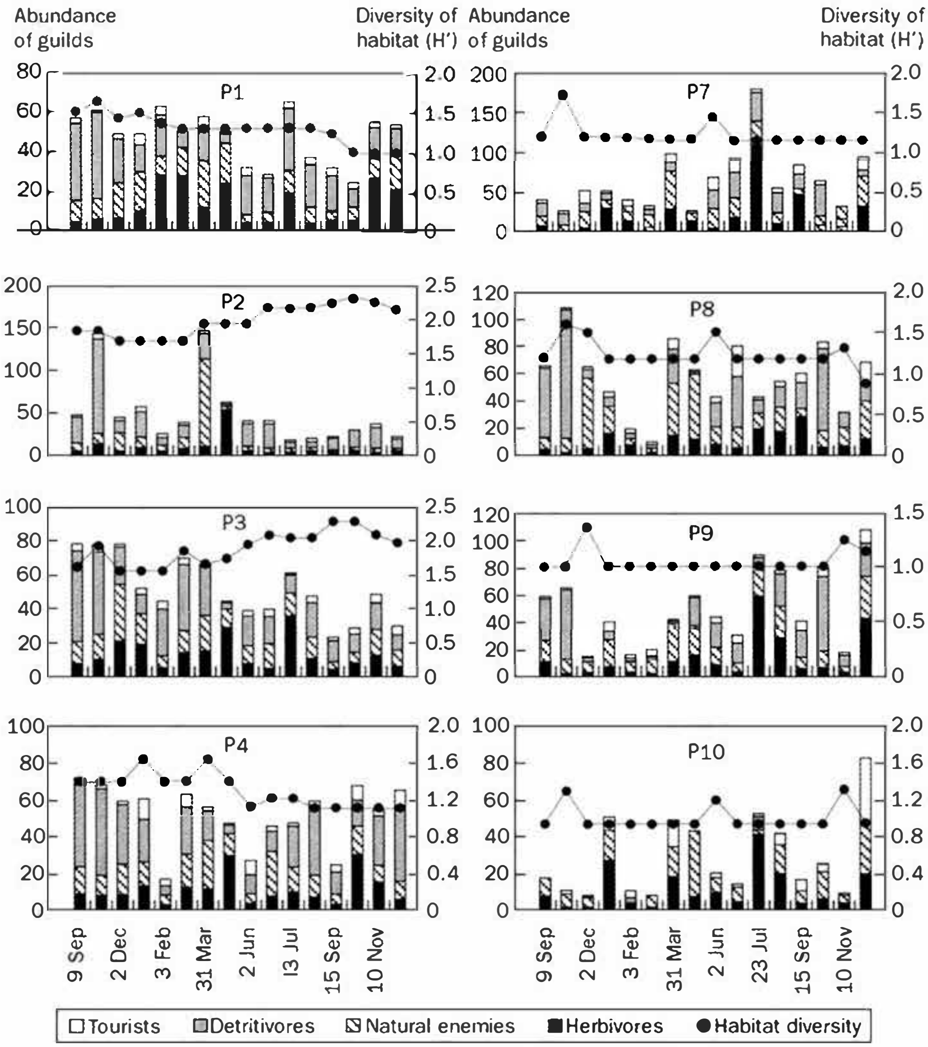

Fig. 5. Correlation between the habitat diversity (H') of the sampling site (P level) and the abundance of all taxa of each feeding group. The primary vertical axis is the abundance of all taxa belonging to each feeding group; the second is the diversity index (H') of the sampling site by time intervals.

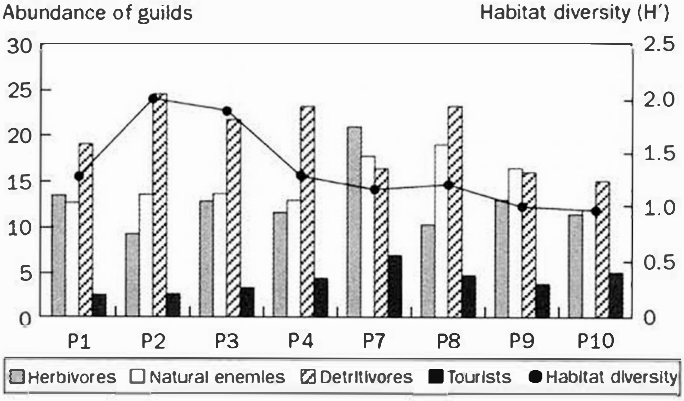

Fig. 6. The abundance of trophic levels in relation to habitat diversity at different locations along the transect.

Table 5. Correlation in space between the commonest arthropod species and habitat diversity.

Dominant taxa	R	Intercept	a	Probability
Nilaparvata lugens	0.123	14.3	-3.9	0.394
Sogatella furcifera	0.149	0.1	0.2	0.345
Recilia dorsalis	0.328	0.1	0.2	0.138
Nisia atrovenosa	0.201	0.9	-0.3	0.265
Pirata subpiraticus	0.610	0.9	-0.3	0.022
Tetragnatha sp.	0.351	0.8	-0.3	0.121
Atypena formosana	0.033	0.6	-0.05	0.664
Cyrtorhinus lividipennis	0.017	1.9	-0.22	0.754
Entomobryidae	0.644	-9.6	14.7	0.016
Sminthuricidae	0.040	4.8	-0.6	0.633

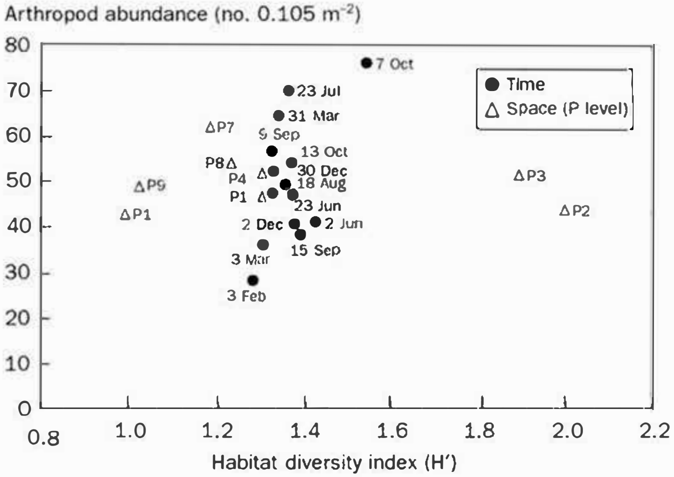

Fig. 7. Plotting diagram of the abundance of all arthropods and diversity of habitats in time and space.

Table 6. **Compatibility of nonrice habitats with rice in terms of totals of herbivore and natural enemy species.**

Habitat	Numbers of samples	Natural enemy species				Herbivore species				
		No. of taxa	Total numbers	% similarity with rice	Rank[a]	No. of taxa	Total abundance	% similarity with rice	Rank[a]	Sum of rank
Rice	1,147	146	14,173			73	21,234			
Bund with grass weeds	545	119	3,661	81.5	1	61	3,591	83.6	33	34
Bund with potato	9	17	50	11.6	18	14	51	19.2	18	36
Bamboo	16	27	149	18.5	14	11	31	15.0	16	30
Banana	44	57	400	39.0	5	34	200	46.6	30	35
Coconut	95	81	560	55.5	3	43	375	58.9	31	34
Eucalyptus	44	63	456	43.1	4	30	450	41.0	27	31
Mixed fruit trees	38	31	140	21.2	12	23	56	31.5	22	34
Orchard	32	46	162	31.5	9	29	293	39.7	26	35
Chiku fruit	64	57	371	39.0	6	33	320	45.2	29	35
Jujube fruit	36	38	141	26.0	10	32	112	43.8	28	38
Open land	96	95	1,100	65.1	2	44	3,195	60.2	32	34
Allium	37	31	122	21.2	13	17	104	23.3	20	33
Brassica chinensis	28	35	110	23.9	11	27	158	36.9	23	34
Capsicum annuum	10	25	92	17.1	16	15	33	20.5	19	35
Brassica juncea	5	23	38	15.7	17	14	39	19.2	17	34
Raphanus sativus	18	15	32	10.3	19	11	54	15.0	15	34
Polianthes tuberosa	68	51	227	34.9	8	27	110	36.9	24	32

[a] Rank (1 = best, 33 = worst). Based on 3 nonrice habitats.

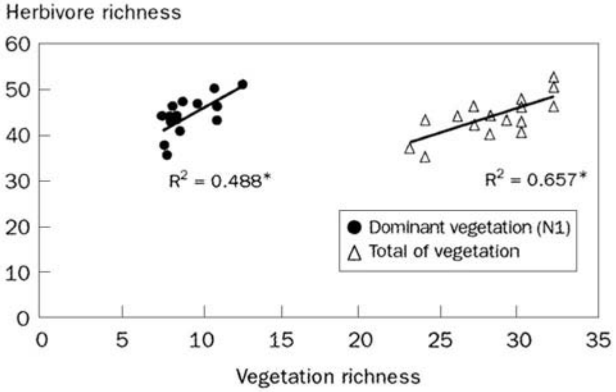

Fig. 8. Correlation in time series between vegetation richness and all herbivore taxa collected by suction sampling.

Table 7. Rank of habitats supporting natural enemies of rice pests[a].

Habitat	Natural enemies (abundance)	*P* value	Rank[b]	Herbivores	*P* value	Rank[b]	Sum of rank
Bunds with grass weeds	0.413	0.001	5	0.283	0.587	13	18
Bunds with sweet potato	0.251	0.100	2	0.374	0.002	12	15
Open land	0.501	0.002	7	0.409	0.001	10	17
Flowers—*Polianthes* sp.	0.511	0.001	9	0.592	0.001	4	13
Mixed fruit trees	0.409	0.001	4	0.508	0.001	9	13
Orchards	0.512	0.001	10	0.586	0.001	6	16
Chiku fruit	0.510	0.001	8	0.589	0.001	5	13
Jujube fruit	0.513	0.001	11	0.593	0.001	3	14
Bamboo	0.513	0.001	11	0.594	0.001	2	13
Banana	0.512	0.001	10	0.593	0.001	3	13
Coconut	0.511	0.001	9	0.593	0.001	3	12
Eucalyptus	0.514	0.001	12	0.571	0.001	7	19
Allium	0.513	0.001	11	0.592	0.001	4	15
Vegetables[c]	0.705	0.001	13	0.753	0.001	1	14
Cucurbitaceae[d]	0.135	0.055	1	0.274	0.007	14	15
Tomato, chili, mungbean	0.447	0.001	6	0.537	0.001	8	14
Mentha plants[e]	0.291	0.003	3	0.408	0.001	11	14

[a] The observed R by using Clarke's R test in measuring the compatibility of rice and nonrice habitats. [b] The rank of R value, lowest is the best. [c] Pak-choi, Chinese mustard, cutting lettuce, *Raphanus sativus*. [d] Cucumber, benicasa. [e] *Coleus aromaticus, Corandium sativum, Limnophila oromatica, Mentha arvensis, Perilla frutescens, Petroselium sativum.*

shelter for the spiders. Rice yields were not significantly different; they ranged from 3 to 4 t ha^{-1} (Fig. 10).

Conclusions and suggestions

- The agricultural habitat changed along the 10-km transect with rice predominating (approximately 95%) for about 65 km and rice (35%) with other crops in the remaining 35 km.

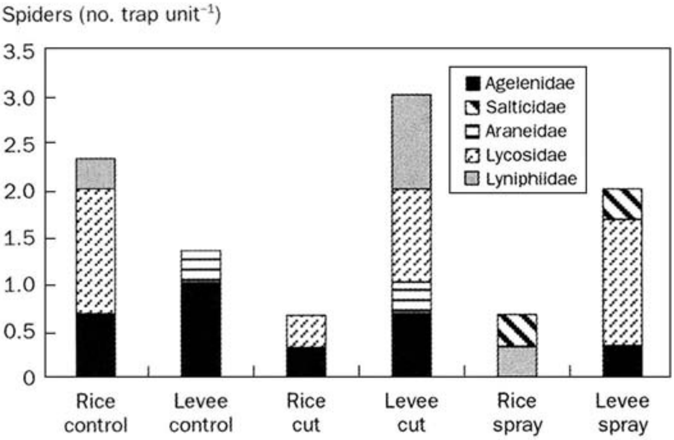

Fig. 9. Different groups of spiders collected in pitfall traps by method of control of grass weeds on levees.

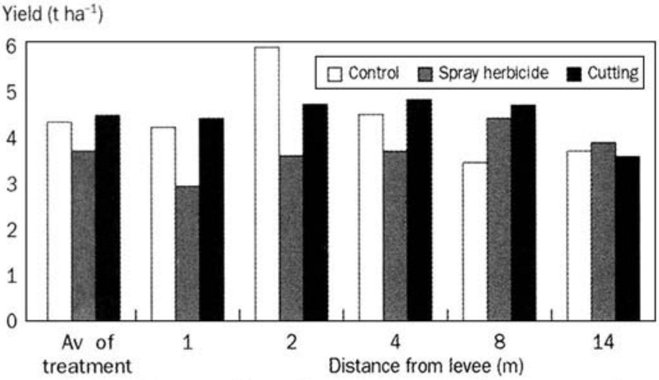

Fig. 10. Rice yields as affected by control method for grass weeds on levees.

- A total of 304 arthropod species were identified: 82 parasitoids, 95 predators, 90 herbivores, and 37 others.
- The species richness of arthropods in fruit garden levees and noncultivated areas was greater than in the rice habitat. Arthropods tended to decrease from multiple-cropped areas to rice monocropping. However, the development of their populations was not strongly linked to change in habitat diversity.
- Nonrice habitats adjacent to the rice field could be a vital source of rice pest natural enemies, but this could change over time depending on farmers' management practices. The habitats of coconut, fruit tree gardens, noncultivated areas, and levees with grass weeds seem to be the best as sources of natural enemies of rice pests.

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Microbial diversity

The role of microbial immigration in disease management by enhancement of plant diversity

S. Lindow

The process of immigration of microbes to plants will be discussed in the context of establishing inoculum of plant pathogenic bacteria on plants. Bacteria of all types move freely from one plant to another and inoculum of a plant pathogen frequently originates in distal plants. Subsequent multiplication of bacterial cells on leaf surfaces can establish a large epiphytic population size that is required before infection can occur. The use of plant variety mixtures ensures that the initial immigrants to a plant are a diversity of microorganisms, only a small proportion of which might be pathogenic to that plant. This reduces the likelihood that the initial immigrants to a leaf are compatible pathogens and therefore reduces the probability that such pathogens can establish a population large enough to cause disease. The local plant composition can therefore strongly influence the composition of microbial communities on a plant.

Plant disease management by the use of variety mixtures and spatial deployment of different crop species and varieties on a regional basis has proven to be an effective control method. Unfortunately, the biological basis for the reduced disease in such biologically diverse cropping systems is still under debate. The purpose of this review is to address what is known about the role of the processes of emigration and immigration of microorganisms in the development of microbial communities on plants. Much of the review will focus on plant-associated bacteria. Such an analysis is pertinent to the issue of disease management in several ways. Important plant pathogenic bacteria such as *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial blight of rice, occur as epiphytes on the surface of plants before infection occurs. In fact, the likelihood of disease caused by such plant pathogenic bacteria has been shown to be proportional to the epiphytic population size on a plant (Hirano and Upper 1983, 1990). Thus, plants can often tolerate substantial populations of potential plant pathogenic bacteria on their leaves without risk of disease. Likewise, factors that influence the size of bacterial populations on plants are important determinants of disease. Plants do not always support large populations of microorganisms, particularly plant pathogens. It is thus important to understand the processes that dictate the large seasonal variations in population sizes of particular microbial species that are usually seen on plants. Although many plant pathogenic bacteria have the potential to multiply rapidly on plant surfaces (Hirano et al 1995, Hirano and Upper 1989), the process by which plants become inoculated by such bacteria will play an important role in determining the eventual composition of bacteria on a plant. The process of immigration of microbes to plants will thus be discussed in some detail so that a better appreciation of this phenomenon in establishing inoculum of plant pathogenic bacteria on plants can be gained. I hope to make it clear that bacteria of all types move freely from one plant to another and that inoculum of a plant pathogen frequently originates in distal plants. The local plant composition can therefore strongly influence the composition of microbial communities on a plant.

Emigration of microbes from plants

Cells of epiphytic bacteria have been demonstrated to be removed from plants in at least two different ways. The most attention has been placed on rain as a vector by which cells are removed from leaves. Rain can wash off a substantial proportion of the cells from a leaf (up to 50%) (Constantinidou et al 1990, Haas and Rotem 1976, Lindemann and Upper 1985, Upper and Hirano 1991). Although a large fraction of epiphytic bacterial cells can be washed from leaves during heavy rain, this loss is small relative to the magnitude of variation in population size normally seen on leaves at a given time. For example, the population size of a given bacterial species such as *Pseudomonas syringae* can vary by as much as 1 million-fold on a collection of leaves from a single plant population (Hirano et al 1995, Hirano and Upper 1989). While rain can wash some of the cells from leaves, a large variation in bacterial population sizes (although a little smaller overall) will remain among these leaves (Hirano et al 1995). In addition, the reduction in bacterial population that occurs during some rain events (Ercolani et al 1974) is sometimes small compared with the magnitude of increases that occur following rain (Hirano et al 1995). The relatively small reductions in bacterial populations during rain may be due in part to the relative efficiency with which bacterial cells attach to plants. Although epiphytes are relatively easy to dislodge from leaves by washing or sonication (O'Brien and Lindow 1989), suggesting that they are not irreversibly attached to plant surfaces, cells in aqueous suspensions often attach to leaves within a few seconds (Romantschuk 1992). Although many epiphytes may be dislodged during rain, many may reattach to other leaves before they are lost from the plant canopy. Some of the bacteria that are dislodged from plants during rain become incorporated into small droplets of water that can be dispersed away from the plant on which they were generated (Venette and Kennedy 1975, Venette 1982). Some of these aerosol droplets can be recaptured by adjacent plants by impaction and sedimentation, thereby minimizing the loss of bacteria from the phylloplane during rain (Lindemann and Upper 1985, Lindemann et al 1982, Upper and Hirano 1991, Venette 1982).

It has recently been appreciated that epiphytic bacteria leave the surface of plants in substantial numbers in dry aerosol particles. A net upward flux of bacteria has been noted above plant canopies, especially during midday, when plants are dry and winds are at their maximum velocity (Lighthart and Shaffer 1995, Lindemann and Upper 1985, Lindemann et al 1982, McInnes et al 1988, Upper and Hirano 1991). In fact, the upward flux of bacteria from dry plants is much greater than that from either wet plants or from bare soil (Lindemann and Upper 1985, Lindemann et al 1982, Upper and Hirano 1991). A net daily introduction to the atmosphere of about 5×10^6 patricles bearing viable bacteria per m^2 of canopy of plants having large epiphytic bacterial population sizes (ca. 10^6 cells cm^{-2} of leaf) has been observed (Lindemann et al 1982). If one assumes a leaf area index of 5.0 for the bean canopies under which such fluxes were estimated, and assumes that the entire soil surface was covered by plants, then the total epiphytic bacterial population in the canopy is about 5×10^{10} cells m^{-2}. Thus, the loss of about 5×10^6 cells m^{-2} represents an emigration of only about 0.0001% of the total epiphytic population each day (assuming that aerosol particles harbored one viable cell). Even if the number of viable cells per aerosol particle were as high as 10 (based on the average size of the particles that contained viable bacteria [Lindemann and Upper 1985, Lindemann et al 1982, Upper and Hirano 1991]),the fraction of the epiphytic bacteria that would be lost as aerosol particles would still be very small. Thus, although an impressive number of bacteria can be released from a plant canopy having a high epiphytic bacterial population during dry conditions, emigration away from a plant appears to contribute little to the reduction in epiphytic bacterial populations on a plant. Other modes of removal of bacteria from plant surfaces, such as by insect vectoring or physical removal by abrasion of adjacent leaves, are probably inconsequential, but have not been quantified.

Immigration of microbes to plants

Immigration of bacteria to a leaf is coupled strongly to their emigration from another leaf since most epiphytes have leaves as their primary habitat. Thus, for bacteria to immigrate to a leaf, they must first emigrate from another plant having epiphytic bacterial populations. Although, as discussed above, emigration of bacteria from a plant has been shown to be a common phenomenon, it is also probably small in magnitude compared with the population sizes that are often observed on plants.

Mechanisms of immigration

Immigration to a leaf can occur via several modes of transportation. First, many bacteria can be transported to a leaf via rain splash. Although quantitatively important in releasing bacteria from plants, rain deposits a large percentage of the bacteria released from plants onto the soil (Constantinidou et al 1990, Ercolani et al 1974, Lindemann and Upper 1985, Lindemann et al 1982, Upper and Hirano 1991). Nonetheless, substantial lateral movement of bacteria can occur during rain (Venette 1982). For example, deposition of a *P. syringae* strain increased more than 5,000-fold during rain compared with deposition during dry conditions (Lindemann and Upper 1985).

The retention of immigrant bacteria in rainwater during a rainstorm (as opposed to the number washed from leaves) has not been estimated, however. In general, it is believed that there is a net decrease in bacteria on leaves during a rain event. More bacteria are removed from a plant with an established epiphytic microflora than are deposited from adjacent plants (Hirano et al 1995, Upper and Hirano 1991). Second, several phytopathogenic bacteria can be transferred from infected plants to healthy plants by insect vectors. Although some bacterial pathogens are disseminated due to intimate associations of the pathogen with the insect vector (Harrison et al 1980, Venette 1982), bacteria are most often transmitted via insects that are contaminated during their foraging or nectar-collecting activities (Harrison et al 1980). This latter phenomenon is most well studied in the case of the vectoring of *Enwinia amylovora* from cankers or infected flowers of pear and apple trees to newly opened flowers where infection can occur (Harrison et al 1980). Although such transmission has been demonstrated, the number of cells that are transferred is unknown, but probably very small. Third, plant pathogenic bacteria might be disseminated with infected leaves that become airborne. Since bacteria can survive for long periods of time on dead and/or dry infected leaves (Henis and Bashan 1986, Leben 1981), it is likely that, as leaf fragments are dispersed in the wind, cells of phytopathogens could be transferred to healthy leaves (Venette 1982). Again, the prevalence of such a phenomenon and the number of bacteria that might potentially be transferred to new leaves by this process are unknown. Fourth, many bacterial plant pathogens produce exopolysaccharides. This slime can be quite substantial in the case of certain pathogens such as *E. amylovora*. These slimes can produce strands that are as much as 10 cm long in infected tissues and it has been speculated that these strands could fragment as they dry and disperse as small particles to other plants (Eden-Green and Billing 1972). Fifth, aerosols can be produced under both wet and dry conditions. As noted above, wind blowing over dry foliage is a source of substantial numbers of aerosol particles. The number of dry aerosol particles containing bacteria increased in proportion to wind speed and thus exhibited a diurnal periodicity, being highest at midday (Lindemann and Upper 1985). The production of dry aerosol particles is proportional to the source strength of the plants over which the wind has blown (Lindemann and Upper 1985, Lindemann et al 1982, Upper and Hirano 1991). The deposition of dry aerosol particles has been rather well studied. On average, about 10^3 particles containing viable bacteria were deposited in an area the size of a bean leaf (ca. 100 cm^2) each day (Hirano et al 1995, Lindemann and Upper 1985, Upper and Hirano 1991, Lindow, unpublished). Small water droplets containing bacterial cells can also be generated by raindrops hitting healthy foliage containing epiphytic bacterial populations or infected leaves. Although many aerosol droplets are scrubbed from the air by other raindrops, some can disperse beyond the immediate site of release and potentially can be deposited onto other plants (Hirano et al 1995, Lindemann and Upper 1985, Upper and Hirano 1991). The release of such wet aerosol particles is apparently less frequent than the release of dry aerosol particles. The contribution of immigrant wet aerosol particles to the size of epiphytic populations has not been well studied, but is probably less than that of dry particles.

Significance of immigration

Immigration is significant for populations of epiphytic bacteria in at least two different ways: (1) immigrant cells are a source of inoculum of bacteria that subsequently multiply on leaves and (2) immigrant cells contribute directly to the population size of leaves on plants on which little growth is possible.

The role of immigrant cells as inoculum of plant pathogens that subsequently proliferate on plants has been examined closely. A popular model of the microbiology of leaves is based on the "island biogeography" theory (Andrews et al 1987). In this model, the population structure of living beings on an island is strongly influenced by the nature and size of immigrants to the islands that are isolated from each other and are initially void of colonists (Andrews et al 1987). Many leaves emerge from buds or seeds with few if any bacterial colonists (Andersen et al 1991, Beattie and Lindow 1994, Hirano et al 1991, 1995, Hirano and Upper 1989, Jacques et al 1995, Lindow 1982,1983,1985,1987, Lindow et al 1978a,Lindow and Panopoulos 1988, Mew and Kennedy 1982). Such leaves usually have resources that can support the growth of bacteria inoculated onto them (Andersen et al 1991, Beattie and Lindow 1994, Lindow 1982, 1983, 1985, 1987, Lindow et al 1978b. 1983, Lindow and Panopoulos 1988, Mew et al 1984). Such leaves thus have similarities to isolated islands in their initial lack of colonists. The relatively low population size of newly emerged tissues is thus often apparently due to a lack of inoculum of appropriate epiphytic bacterial strains. Adjacent leaves often harbor quantitatively and qualitatively different resident microflora (Hirano et al 1982, 1995). Similarly, the diversity of bacteria on a given leaf may be either very high or very limited (Hirano et al 1995). Therefore, the colonization of leaves may be a rather stochastic process involving the repeated (but limited) introduction of genotypes of bacteria capable of colonization of leaves. Occurrence of a particular species (such as a plant pathogen) on a particular leaf will be dependent on its successful immigration (at least once) from another source. This has led to the interest in identifying "collateral hosts," plants that can support epiphytic growth of the pathogen but not lesion development, which can serve as a source of immigrant inoculum of plant pathogenic bacteria (Hirano and Upper 1983, 1990, Venette 1982). Such alternate hosts may be very important in the epidemiology of plant pathogens in enabling the pathogen to survive epiphytically for extended periods, even in the absence of susceptible hosts (Beattie and Lindow 1995, Henis and Bashan 1986, Hirano and Upper 1983, 1990, Venette 1982).

Conceptually, the effect of immigrant bacterial cells in determining the composition of populations on a leaf is influenced by the number of bacterial cells already present on the leaf. As the number of established bacterial cells increases, the contribution of an immigrant cell to the relative population size of the leaf will decrease. Similarly, each new immigrant to a leaf would contribute progressively less and less to the number of genotypes on a leaf. If it is assumed that each strain on a leaf is similar in competitiveness (probably a poor assumption) and the population sizes of each strain each increase rapidly and at the same rate, then it is clear that the first immigrants to a leaf will (at least initially) be well represented in the phylloplane population. Obvious differences in competition, changes in physical environment, changes in host resources, and other perturbations will cause the proportion of different strains on a leaf to change with time, that is, succession will occur. Therefore, immigration to uncolonized leaves is very important in providing inoculum to exploit that habitat. Immigration to more colonized plants is less important quantitatively, but may be particularly important in contributing inoculum of strains that might exploit unfilled niches on leaf surfaces or of strains that are superior competitors or phytopathogenic.

Although immigration has been recognized as contributing qualitatively to the composition of phylloplane bacterial populations, there has been little appreciation of its contribution to the size of these populations. Some communities, including those of some fungal epiphytes, respond directly to enhancements in recruitment or immigration; by definition, these communities are said to be immigration-limited (Kinkel 1991, Kinkel et al 1989). For example, Kinkel et al (1989) found that *Aureobasidium pullulans* population sizes on apple leaves in the field to which large numbers of this species had been inoculated were significantly larger than population sizes on leaves that had not been inoculated, even after substantial apparent growth of this species on the leaves. This direct response in population size to an increase in the numbers of immigrants indicated that *A. pullulans* is immigration-limited (Kinkel et al 1989). Similarly, Jacques et al (1995) found that population sizes could be increased by up to 100-fold when the exposure of emerging leaves to air was increased, particularly later in the growing season when the abundant epiphytic bacterial populations that existed on adjacent plants could apparently serve as a source of immigrant inoculum. Unfortunately, few other studies have addressed this immigration of fungal or bacterial species on leaves.

Immigration may be relatively more important in determining population size on some plant species than on others. A wide range of bacterial population sizes occurs on plants, even after leaves have been inoculated with epiphytic strains and allowed to incubate under moist conditions conducive to growth for long periods of time (O'Brien and Lindow 1989, Kinkel et al 1995, Lindow et al 1978b, Lindow 1982, 1985). Although maximum population sizes for some plants such as bean and cucumber exceed 10^7 cells cm^{-2}, some plant species, such as citrus species and conifers, support less than 10^3 cells cm^{-2} (O'Brien and 1989 , Lindow et al 1978b, Lindow 1982, 1985). The rate of deposition of bacteria in landscapes that include an abundance of plants having high epiphytic bacterial populations has been estimated to be about 10^3 cells 100 cm^{-2} d^{-1} (Hirano et al 1995, Lindemann and Upper 1985, Upper and Hirano 1991, Lindow and Andersen 1996). This rate of deposition would yield a cumulative immigrant population of about 10^4 cells per month on an average-sized leaf (such as a bean leaf). If we assume that most leaves in nature are subject to immigration of about 10^4 total cells per month, then immigrant cells could account for a substantial fraction of the bacterial population observed on plants having a relatively low population size but only a very small proportion on plants such as bean and cucumber having relatively high population sizes. For example, navel orange leaves in California seldom harbor more than about 10^5 cells (Lindow 1982). Since these leaves are long-lived and have been shown to be exposed to an average of about 10^4

immigrant cells per month (Lindow and Andersen 1996), most of the cells on their leaves may be attributable to immigration. Further support for such a conjecture is obtained from examining the growth of genetically marked bacterial strains on citrus leaves after inoculation. Little growth of common epiphytic bacteria such as *Pseudomonas syringae*, *Erwinia herbicola*, and *P. fluorescens* was observed (Lindow and Andersen 1996). Thus, many plant species having small epiphytic bacterial population sizes may not support the epiphytic growth of bacteria. Instead, such plant species may simply harbor “casual” occupants (immigrants) that did not arise by growth on the plants. As shown by Jacques et al (1995), immigration may be important in determining the epiphytic population size on emerging leaves of plant species that harbor large populations on their mature leaves.

Implications of the role of immigration and other processes in determining bacterial populations on leaves

The epiphytic bacterial populations on many plant species may be expected to be immigration-limited. Such immigration limitation has already been demonstrated for several crop plants such as almond, maize, bean, potato, navel orange, tomato, pear, and broadleaf endive (Andersen et al 1991, Beattie and Lindow 1994, Jacques et al 1995, Lindow 1982, 1983, 1985, 1987, Lindow et al 1978a, 1983, Lindow and Panopoulos 1988). Some of these plant species have relatively large carrying capacities, suggesting that immigration limitation will not be limited to those plants in which little epiphytic growth occurs. Immigration limitation may be most prominent early in the development of an epiphytic microflora, before many of the resources that the plant provides are depleted. For example, newly opened flowers of trees such as pear apparently possess nutrient resources capable of supporting the development of large (> 10^6 cells flower^{-1}) populations. Frequently, such populations are not achieved for many days or weeks unless flowers are inoculated with a suitable bacterial strain (Andersen et al 1991, Lindow 1982, 1983, Lindow, unpublished).

Knowing whether epiphytic bacterial populations are immigration- or growth-limited will have important implications for the strategy of management of these populations. For example, the populations of bacteria on plants that do not support much bacterial growth or the young leaves of flowers of many plants may be expected to be immigration-limited. The manipulation of immigrant inoculum may alter the epiphytic microflora on a plant. Growing of crop plants in areas distant from the presence of bacterial pathogens on crop or noncrop plants has been recognized as a way to limit their presence on the crop (Venette 1982). In a classic example of this phenomenon, brown spot disease of bean has been shown to be associated with proximity to vetch, a nonhost plant that harbors *P. syringae* (Ercolani et al 1974). Likewise, epiphytic populations of *P. syringae* and brown spot disease were lower on beans planted in regions where other beans were absent than in a bean-growing region (Lindemann et al 1984). Quantitative effects of nearby sources of immigrant bacteria on epiphytic populations on crop plants have also been observed. For instance, total culturable bacteria and ice nucleation-active (Ice$^+$) bacteria were more numerous on navel orange trees grown near plants having high epiphytic bacterial populations (Lindow and Andersen 1996). Many cover crop plant species grown under pear trees in California had much higher epiphytic bacterial populations than pear, especially early in the spring when pear flowers and leaves were just emerging from buds (Lindow, unpublished). The population sizes of both total culturable bacteria and Ice$^+$ bacteria were higher on trees grown above such cover crop plant species than on trees grown above bare soil (Lindow, unpublished). Furthermore, the population sizes of bacteria on pear were highly correlated with the population sizes of bacteria on different plant species grown under the trees (Lindow, unpublished). Thus, an appreciation of the contribution of immigrant bacteria to epiphytic bacterial population sizes can be used to devise management strategies that minimize the development of large populations of deleterious bacteria on plants. Studies of processes determining population sizes of epiphytes can thus contribute directly to improving control procedures.

Although there have been no direct studies of the microbial immigration processes that occur in variety mixtures or mixed plantings of crops such as rice, it is not hard to speculate how the process of leaf colonization would differ in such situations from that of plants in large monocultures based on the examples of immigration-mediated plant colonization noted above. Although appropriate genotypes of plant pathogenic bacteria such as *X. oryzae* would likely be in highest abundance on plants susceptible to that genotype, such genotypes would probably also colonize the surface of incompatible hosts if they were dispersed to such hosts. Likewise, if incompatible genotypes of the pathogen were among the initial immigrants to new leaves of a plant (along with nonpathogenic bacteria from distal plant sources), they would have maximum potential to exploit the resources that that leaf had to offer. If they are able to rapidly and fully exploit the new leaf surface as a habitat, the initial colonists could effectively preclude the growth of subsequent immigrants by a process of preemptive competitive exclusion. Thus, reducing the likelihood that the initial immigrants to a leaf are compatible pathogens reduces the probability that such pathogens can establish a large enough population size to cause disease. The use of plant variety mixtures thus ensures that the initial immigrants to a plant are a diversity of different microorganisms, only a small proportion of which might be pathogenic to that plant.

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Sheath blight management with antagonistic bacteria in the Mekong Delta

P.V. Du, N.T.P. Lan, P.V. Kim, P.H. Oanh, N.V. Chau, and H.V. Chien

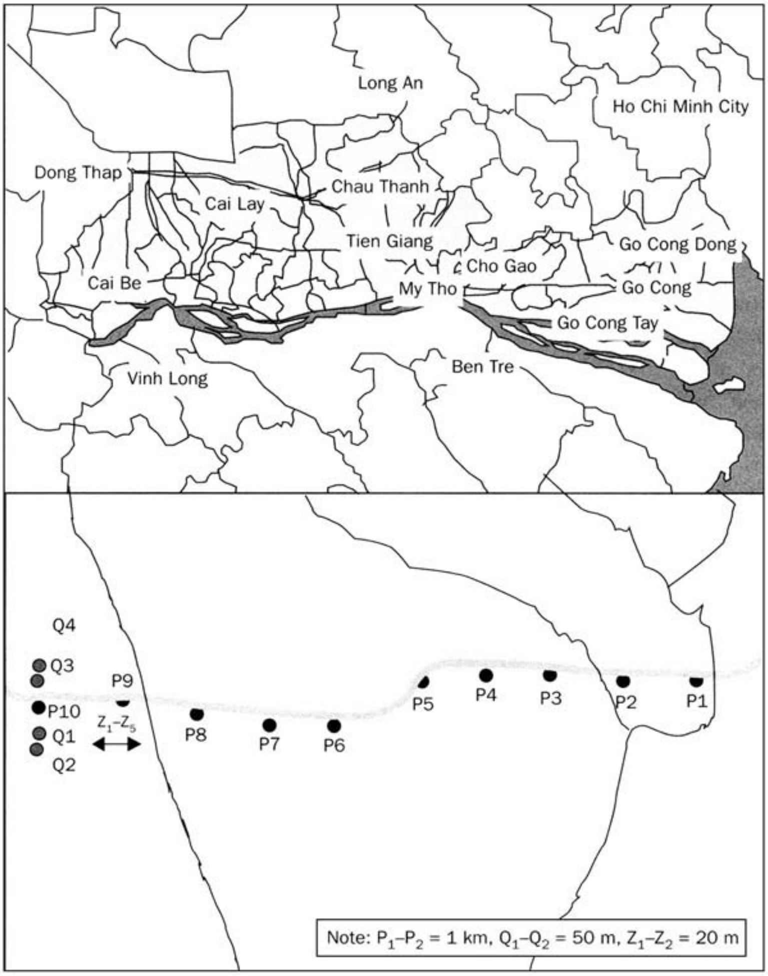
A survey of sheath blight (ShB) disease and antagonistic bacteria on nonrice plants, soils, and rice seeds was conducted for one year starting in August 1997 at a "lighthouse" site in Tien Giang Province. Seventy-two isolates of sheath blight showed 5 morphotypes of colony appearance and 4 anastomosis groups that belong to AG 1 with 11 isolates, AG 2-2 with 2 isolates, and AG 4-HG-1 with 1 isolate. Another 50 isolates did not show any type of anastomosis with AG tester isolates; therefore, these isolates were temporarily grouped as AG-UNK. Pathogenicity testing determined that all sheath blight isolates found on 32 weed species can infect rice. The main source of sheath blight inoculum is water contaminated with sclerotia; mycelium in rice plant debris can also act as a primary source. The possibility of employing antagonistic bacteria to control sheath blight was studied. Approximately 1,000 strains of beneficial bacteria were isolated from seeds and other rice components; 10 of these possessed high levels of antagonistic properties and enhancement of plant growth. The selected strains significantly reduced sheath blight development and spread in both greenhouse and field conditions. Four strains, NF49, NF52, TG17, and TG19, were particularly active. A mixture of two strains in a ratio of 1:1 gave significantly better control of the disease than each of the strains used individually. Under conditions of high disease pressure, however, an antagonistic formulation amended with Validacin 5SP (validamycin) at 25% of the recommended rate has been shown to increase the efficacy of biological control of *Rhizoctonia solani* in natural conditions. To maintain an effective density of biological control agents (BCA) on the rice plant surface, bacterial suspension should not be applied before the maximum tillering stage because the ShB pathogen rarely works at that time under flooded paddy soil conditions. To enhance BCA in the rice field, weed management should be linked with water management and crop residue management to limit incoming inoculum sources. Demonstrations of BCA application for the suppression of sheath blight disease in rice farmers' fields have been conducted and will be continued. The optimization of fermentation and suitable formulations with low production costs need to be assessed to scale up the technology.

Sheath blight disease caused by *Rhizoctonia solani* is a major rice disease in intensive rice production systems in Vietnam. In Vietnam, China, Japan, and Korea, disease control has largely depended on fungicides. Research on alternative disease management, especially in Vietnam, is meager. Since 1995, we have conducted preliminary research on the population fluctuation of microbes from the rhizosphere, rhizoplane, surface of leaves, stems, and seed of different rice varieties. We found that the paddy rice system in the Mekong Delta is rich in antagonistic bacteria. Many of them have a broad spectrum to suppress the development of more than one disease in vitro. However, biological control agents (BCA) are living things and should not be treated as chemical fungicides. We should recognize their ecology status and relation to other organisms, including microorganisms and host plants. To be effective, biocontrol depends not only on suitable biocontrol organisms but also on methods and strategies for introducing and maintaining the organism in the crop. A decentralized BCA production system should be established using “indigenous strains for solving a local problem with the resources available locally” (Mew et al 1998). To improve biological control and the effectiveness of BCA/PGPB (plant growth-promoting bacteria), it is necessary to find out the optimal conditions for BCA/PGPB function, particularly in the intensive irrigated rice system, together with the limited sources of incoming disease inoculum. Therefore, to prepare for BCA application, suppression of sheath blight disease needs to be done in rice farmers’ fields. Our study aimed at using microbial biodiversity for a better understanding of the following: the pathosystem of disease spread, water and crop residue management, the effect of BCA on reducing sheath blight foci over time in farmers' rice fields, and BCA and fungicides amended with lower recommended rates. Finally, the optimization of fermentation and suitable formulations of BCA need further study.

Materials and methods

Diversity of the sheath blight pathogen *(Rhizoctonia solani)*

Diversity of the sheath blight pathogen was studied in two different cropping zones for two to three rice crops at the Tien Giang “lighthouse” site because of floodwater during the wet season and an area of rice interplanted with upland crops. Sheath blight disease was severe in rice and weeds. In the 10-km primary transect described in Figure 1, sampling points were determined at each primary P transect; however, under each sampling point, two other sublevels of the transect were also determined as secondary transect Q and tertiary transect Z. At each transect Z, there were five subsampling points across five different crop establishments, that is, the rice field, bund, orchard garden, vegetables, and backyard area. Diseased samples were collected at five transects (Z_1-Z_5) twice a month including sclerotia from soils, diseased weeds, and diseased rice plants. Sampling started on 8 September 1997. Diseased samples were isolated in the laboratory. Anastomosis grouping was tested using AG testers of 14 isolates from Japan to determine groupings among isolates. Pathogenicity testing of all isolates was done using leaf-stem cuttings of the rice plant at the booting and heading stages and artificial inoculation with sclerotia was conducted in



Note: P_1–P_2 = 1 km, Q_1–Q_2 = 50 m, Z_1–Z_2 = 20 m

Fig. 1. Descriptive analysis of transect at lighthouse site, Tien Giang Province, Mekong Delta.

petri dishes to determine cross-infection from different nonrice plant species. Assessment was made at 24, 48, and 72 h after inoculation (Premalatha 1978).

Sources of initial inoculum for rice sheath blight in irrigated rice in the Mekong Delta

To clarify whether the initial inoculum came from sheath blight infection in rice fields or from other sources, we examined the percentage of infected plants in an experimental field generated with different sources of inoculum of *R. solani* such as sclerotia, mycelium in the diseased rice straws, infected weeds, and water contaminated with sclerotia from infected nonrice plants and plant debris. For the check plots, the water supply was filtered to prevent contamination of floating sclerotia and mycelium in plant debris. Results were collected from the field experiment, which was repeated twice.

Population of BCA in the rice field

Antagonistic bacteria were isolated from samples collected at the lighthouse site. Samples of plants (weeds and rice) and soil were collected at P sampling points from September 1997 to August 1998 on the eighth day of the month. Bacterial antagonists were isolated in the laboratory, 5 g of samples (plant, soil, seed) were soaked in 50 mL of 1.2% $MgSO_4$, the filtrate was shaken for 20 min, and a serial dilution of filtrate was prepared in 10^{-0}, 10^{-2}, 10^{-4}, and 10^{-6} dilutions. An amount of 0.1 mL of each filtrate was plated onto nutrient agar (NA) or King's B medium (KMB). Fluorescent colonies were detected by examining KMB plates under ultraviolet light after 14, 28,and 48 h at 28-31 °C. About 10-30% of the colonies differentiated by shape and color were selected. Single colonies were stored in YGCA (yeast glucose chalk agar) slants. Antagonism tests were conducted for each bacterial strain to find out their ability to inhibit mycelial growth of *Rhizoctonia solani.* The estimated percentage of antagonistic colonies of each group (total number of bacteria on each plate, antagonistic bacteria per plate, % antagonistic bacteria for each sample) was calculated.

Antagonistic bacterial strains. Four strains of antagonistic bacteria, TG17 and TG19 (Can Tho University laboratory) and NF52 and NF49 (Cuu Long Delta Rice Research Institute laboratory), were used as biocontrol agents for sheath blight of rice. These strains were selected from among 1,000 strains of antagonistic bacteria, which were isolated from seeds and other components of the rice ecosystem and showed strong antibiosis. The strains were grown on nutrient agar medium containing 5 g peptone, 3 g beef extract, 5 g NaC1, and 15 g bacto agar per liter for 24 h at room temperature. The concentration of bacterial suspension was adjusted to log 8 cfu mL^{-}1. Bacterial suspensions (10^8 cfu mL^{-}1) were sprayed with a mixture consisting of these strains used individually when sheath blight lesions were visible in the field. The spray was applied three times per crop to test its ability to suppress rice sheath blight fungus in greenhouse and field conditions. To enhance the effective control, Validacin 5SP (validamycin) was added to the bacterial suspension. Each experiment was repeated two times.

The pathogen. Rhizoctonia solani belonging to anastomosis group AG 1 - 1A isolated from infected rice fields in the Mekong Delta was used in the study as a source of pathogen inoculum. For in vitro tests, sclerotia that were harvested from potato dextrose agar (PDA) medium were used as a primary source of inoculum, whereas mycelia in the rice hull and rice grain medium were used for greenhouse and field tests.

Suppression of rice sheath blight by using a combination of antagonistic bacteria and fungicide

Rice cultivar OM 2031 was planted in the greenhouse (1 × 1-m^2 plot) and in the field (50-$m_2$plot) to evaluate the biocontrol agents. Rice plants at the maximum tillering stage were sprayed with 48-h-old cell suspensions of antagonistic bacteria alone and combined with chemical fungicide (Validacin 5SP) at 1/2, 1/4, 1/6, and 1/8 of the recommended rate 2 d before and after artificial inoculation with the sheath blight pathogen. The percentage of infected hills at different distances from the focus (or sheath blight expansion) from an initial inoculum source was recorded at 7 d after inoculation and at 7-d intervals until harvesting. A control was also maintained in three replications.

The effectiveness index (EI) was calculated on the basis of percentage of infected hills (P) as

$$\mathrm{EI}\ (\%) = \frac{P_{\mathrm{Control}} - P}{P_{\mathrm{Control}}}$$

The EI value can range from 0 (no effect) to 100 (total effectiveness).

Long-term effect of BCA in greenhouse and field conditions

To assess the biocontrol suppression of sheath blight by using antagonistic bacteria in combination with fungicide in the field for a long-term effect, antagonistic bacteria and fungicide used to control rice sheath blight were evaluated for durability under greenhouse and field conditions. In the first crop, the plants were inoculated with inoculum of *R. solani* at the maximum tillering stage. Bacterial suspensions were applied at 1 and 2 wk after inoculation for every rice crop. After each crop, diseased rice straw and plant debris were buried in the plots to maintain inoculum. In the greenhouse as well as in field conditions, plots were completely protected from incoming sheath blight inoculum. Disease suppression was observed over time to compare with the inoculated control but without application of BCA.

Long-term demonstrations of BCA by rice farmers

Rice farmers will be trained how to use BCA to manage sheath blight in their fields. Natural infection of sheath blight in 10 fields with high severity was selected in two different districts: 5 fields in Cai Lay and 5 others in Tan Phuoc. Each field is 1,000 m^2. The field is divided into 10 plots with 100 m^2 for each plot. BCA or BCA in combination with fungicide (Validacin) was sprayed 2–3 times at 5–7-d intervals. The two controls were (1) no spray of BCA and fungicide and (2) two sprays of Validacin. BCA were applied for the first time at 40 d after seeding at a volume of 600 L ha^{-1} of bacterial suspension with a concentration of 10^6 bacterial cells mL^{-1}. Infected tillers were assessed in 80 fixed hills of each plot. The demonstration is to be continued over seasons for at least 2 y, starting in April 1999.

Results and discussion

Diversity of sheath blight pathogen *(Rhizoctonia solani)* at the lighthouse site

More than 100 diseased samples of soil and plants were collected from the Tien Giang lighthouse site and 72 isolates of *Rhizoctonia solani* were obtained. Based on the cultural appearance of these isolates, 5 morphotypes were found, in which 4 morphotypes had only a single isolate. For the rest of the isolates, mycelial growth was normal on PDA. This type was dominant among the 72 isolates. For growth rate and sclerotia production, two isolates, I_{28} and I_7, had mycelial growth slower than that of the others, 8 isolates formed sclerotia 48 h after incubation, and 3 isolates (I_{26}, I_6, and I_7) produced sclerotia in 78 h, 84 h, and 240 h, respectively. For the rest of the isolates, sclerotia were formed in 54 to 72 h (Tables 1 and 2).

From anastomosis grouping, four types were observed on PDA culture: (1) when mycelial growth overlapped, and hyphal tip contact occurred between isolates, the mycelial zone was found to produce a sclerotia belt or aerial mycelial growth, (2) to form a "killing zone," (3) to lose capacity to anastomose or not develop further, and (4) to anastomose with each other in all combinations. Twenty-two isolates were grouped into three AG groups: AG 1 with 11 isolates, AG 2-2 between isolates with two isolates, and AG 4-HG-1 with one isolate. Fifty isolates did not show any type of anastomosis with AG tester isolates; therefore, these were temporarily grouped as AG-UNK. Some isolates had an anastomosis group with AG 1 and AG-UNK distributed in all sampling points of the transect (Table 3). Three types of hyphal anastomosis were found: (1) cytoplasmic fusion (S reaction), (2) semicytoplasmic fusion (K reaction), and (3) noncytoplasmic fusion. Most of the isolates contained four to eight

Table 1. Morphology of colony of *Rhizoctonia solani* isolates collected from two different cropping areas of the lighthouse site.

Cropping area	Type 1		Type 2		Type 3		Type 4		Type 5	
	No. I[a]	%	No. I	%	No. I	%	No. I	%	No. I	%
Rice-based	24	33.3	1	1.4	1	1.4	0	0.0	1	1.4
Rice-rice	44	61.1	0	0.0	0	0.0	1	1.4	0	0.0
Total	68	94.4	1	1.4	1	1.4	1	1.4	1	1.4

[a] No. I = number of isolates.

Table 2. Time lapse for sclerotia forming of different groups of *Rhizoctonia solani* isolates collected from two different cropping areas of the lighthouse site.

Cropping area	Time lapse for sclerotia forming (h)					
	Group 1 48 h	Group 2 54–60 h	Group 3 61–69 h	Group 4 72 h	Group 5 78–84 h	Group 6 240 h
Rice-based	4	13	5	2	2	1
Rice-rice	4	22	11	8	0	0
Total	8	35	16	10	2	1

Table 3. Anastomosis grouping of sheath blight isolates collected in the cropping area of the transect.

AG group	Cropping area			
	Rice-based		Rice-rice	
	No. I[a]	%	No. I	%
AG 1-1A	4	18.2	7	31.8
AG 1-1B	2	9.1	2	9.1
AG 1-1C	0	0.0	4	18.2
AG 2-21V	1	4.5	1	4.5
AG 4-HG-1	0	0.0	1	4.5
Unknown (AG-UNK)	20	40.0	30	60.0

[a]No. I = number of isolates.

nuclei in the mycelium cell belonging to *Rhizoctonin solani*. Pathogenicity testing indicated that almost all *R. solani* isolates collected from 32 nonrice plant species and in soil were also pathogenic to rice except isolate I_7 isolated from *Allium tuberosum*. Although cross-infection took place with all isolates, most of the isolates established infection 48 h after inoculation. These plants with sheath blight disease might be alternate hosts that served as a source of inoculum for rice. In areas with a rice-based cropping system, sheath blight isolates expressed more diversity relative to rice-rice croppings in terms of colony types, duration of sclerotia forming, and virulence to rice (Table 3).

BCA population in the rice fields

Enumeration of antagonistic bacteria against *R. solani* was carried out at the transect of the lighthouse site for one year from September 1997 to August 1998 (Fig. 2). Isolation was done in all samplings of rice plants, nonrice plants, and soil. Results from the antagonism test indicated that the BCA population was very high in the rice field. More than 1,000 isolates were found; however, most of the isolates showed a weak antagonistic effect based on the dual-culture test (Fig. 2). Some isolates that showed a strong inhibition against the pathogen (*R. solani*) were selected for further experiments such as TG17, TG19, NF49, and NF52. The isolate TG19 belongs to the genus *Pseudomonas*; isolates TG17, NF49, and NF52 belong to the genus *Bacillus*. During the isolation at the transect, when the peak of flooding in the wet season occurred in November, the population of BCA was very low in plants as well as in the soil. After flooding, BCA reestablished and a higher population was found in July, August, and September (Fig. 2). The BCA population was not significantly different from that of plant surfaces, or rice soils, or both at the sampling sites. Fluctuation of BCA in the system was most affected by high and low tides of the Mekong River. The intensive rice-cropping system supports more sheath blight spread. Although the BCA population was high, efficiency of sheath blight reduction was not observed. Unknown parameters need to be identified for efficient deployment of these bioagents in the rice ecosystem.

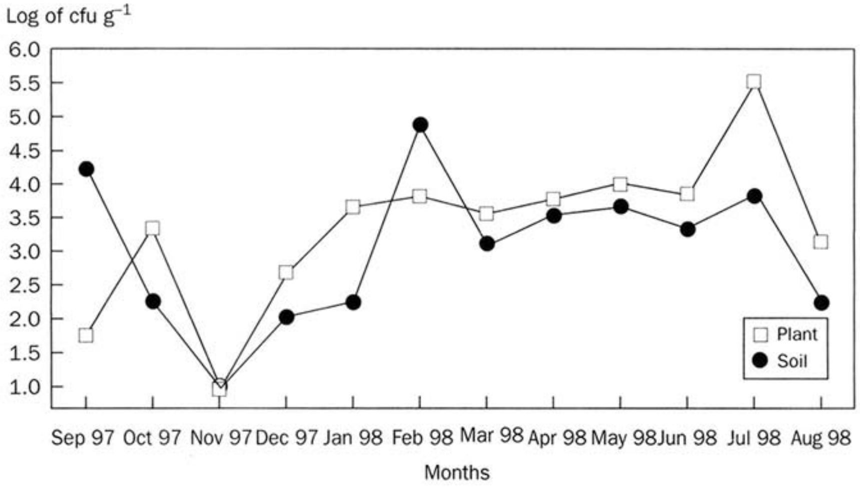


Fig. 2. Variation in average BCA population over 12 months in plant and soil samples collected at site.

Sources of initial inoculum for rice sheath blight in irrigated rice in the Mekong Delta

In the Mekong Delta, rice cultivation with small-scale landholding and intensive cultural management is a key feature. Rice fields are surrounded by many canals as the main source of water supply to the field. In many canals, however, weed (*Monochoria* spp., *Eichhornia crassipes, Brachiaria* sp.) growth occurs throughout the year and these weeds are infected by the sheath blight pathogen. Moreover, farmers are not advised to clean up these weeds during the cropping season. In paddy fields, farmers manage rice straw after harvest in many ways. This needs to be considered seriously for managing rice sheath blight disease. In areas where common practices involve no or minimum tillage, rice straw is burned right after harvest in the same field. The quick burning of rice straw can remove mycelia but not sclerotia in the soil, or rice straw is used for mushroom cultivation and soon afterward it becomes organic manure for orchard gardens and vegetables. Some farmers bury rice straw in their fields during tillage operations. Experiments were therefore conducted to simulate common practices to determine where the main sources of sheath blight inoculum are and to take into consideration weed and water management, tillage practices, and crop residue management. From these experiments, our data indicated that sclerotia floating on the water surface are a main source of primary inoculum. Mycelium on the diseased rice straw, however, may also act as a primary inoculum depending on the situation. Diseased weeds, especially water hyacinth, were not considered as an initial inoculum but they may cause contamination of the water supply to the rice field (Fig. 3). A clear understanding of the specificity and mode of action of antagonists as well as the epidemiology of the pathogen can lead to improved biocontrol.

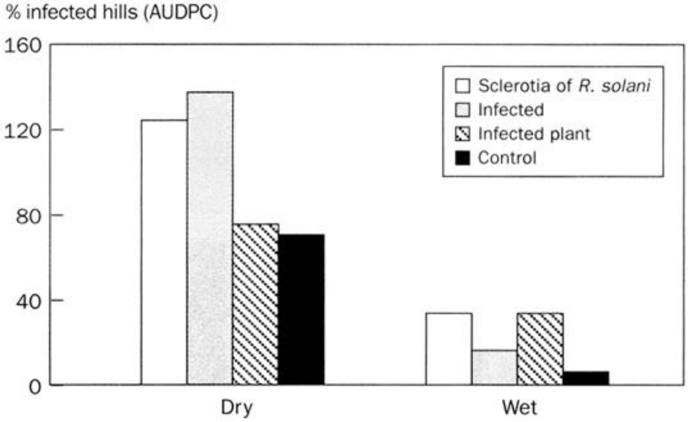


Fig. 3. Percentage of diseased hills in plots with different sources of *R. solani* inoculum in the Mekong Delta. AUDPC = area under disease progress curve.

Suppression of rice sheath blight using a combination of antagonistic bacteria and fungicide in long-term experimental plots

The results presented here provide evidence that antagonistic bacteria that originated from seeds and other components of the rice ecosystem were more effective in reducing infection of *R. solani.* Both strains demonstrated a similar capacity. A concentration of 10^8 cfu mL$^-$1 of the antagonist was the most effective in inhibiting infection of *R. solani.* When applied together to the plant surface, the combination of isolate NF52 and isolate NF49 resulted in better disease suppression compared with the single strains in both the greenhouse and field trials (Fig. 4). Under conditions of high disease pressure, however, a BCA alone was not adequate to suppress disease development or to enable the introduction of enough bacterial cells to allow the expression of their beneficial activities in natural conditions. Supplementation of bacterial suspensions with 25% of the recommended rate of Validacin 5SP further enhanced the suppressive effects. The EI was greater than 50%, particularly in preventing disease expansion from the focus under flooded paddy soil conditions (Fig. 5).

These strains reduced the number of plants with sheath blight symptoms, leading to a decreasing formation of sclerotia compared with the nontreated control (Fig. 6). From a control microplot, the data indicated lower sheath blight incidence in a second crop relative to a first crop if the plots continued to receive BCA strain NF52 + 1/4 Validacin. This is also one of the promising aspects of the long-term use of BCA in field conditions (Fig. 7). The study also obtained more detailed information on the interactions among host plants, pathogens, and isolated beneficial organisms for reducing sheath blight of rice in field conditions. We also found that a bacterial suspension should not be applied before the maximum tillering stage because the sheath blight pathogen rarely attacks the host plant at that time, especially under flooded paddy soil conditions. A stronger effect of applying BCA was found in low planting

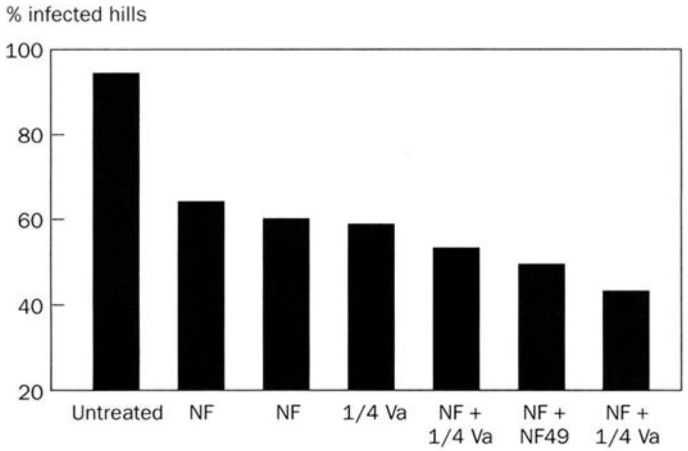


Fig. 4. Effects of different treatments of antagonistic bacteria on the suppression of sheath blight in the long-term field experiment, second crop. AUDPC = area under disease progress curve of sheath blight incidence over time.

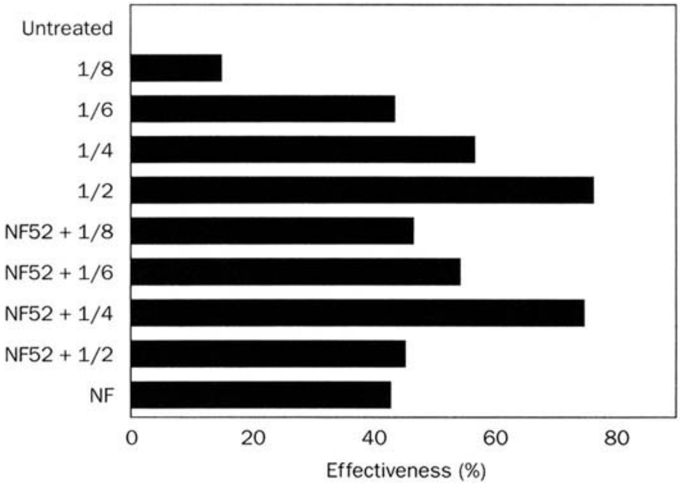


Fig. 5. Influence of Validacin 5SP amendments on biocontrol of sheath blight by antagonistic bacteria (isolate NF52) in field conditions.

density relative to high planting density (Figs. 8 and 9). The indirect effect of the rice plant canopy leads to an increase in sheath blight spread (Du 1995). This should also be considered in enhancing the effect of BCA activities. In Jiangsu, China, BCA strain 916 (*Bacillus subtilis*) was introduced with a high inoculum density at the maximum tillering stage when sheath blight lesions were visible in the field. The disease control value ranged from 70% to 80% (Mew et al 1998). In Thailand, a downward trend of

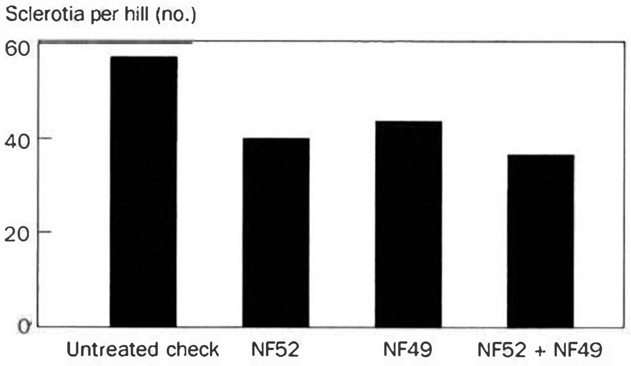


Fig. 6. Effects of BCA application on sclerotial formation of *R. solani*.

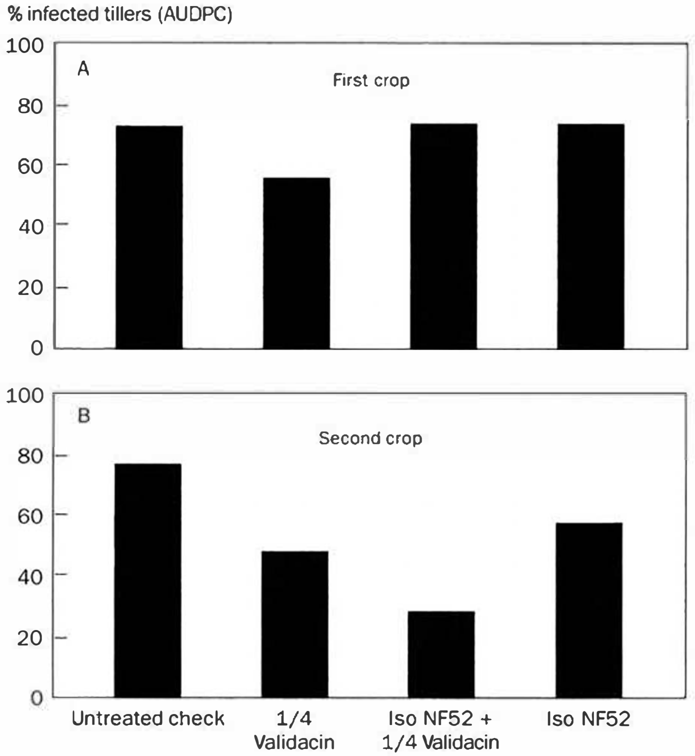


Fig. 7. Reduction in sheath blight severity (%) in second crop at plots inoculated and treated with Validacin (1/4 dose), isolate NF52 + Validacin, and a combination. AUDPC = area under disease progress curve of sheath blight incidence over time.

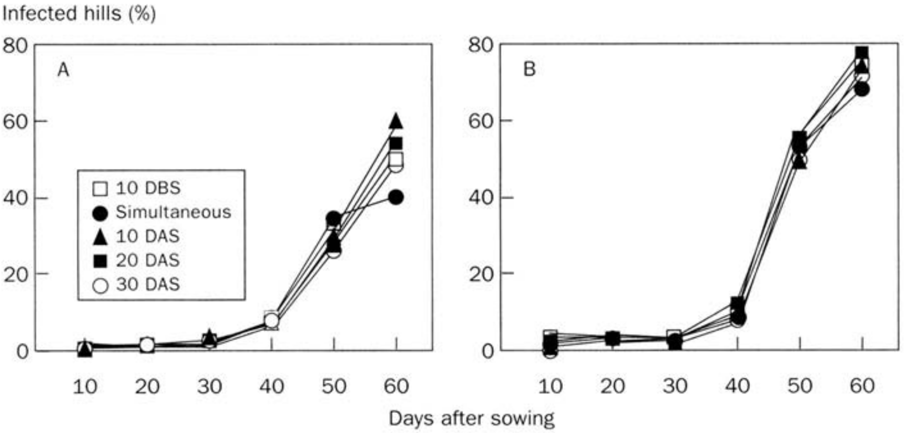


Fig. 8. Interaction effect between time of inoculation of sheath blight at different rice growth stages and planting density of 80 kg (A) and 100 kg (B) seed ha^{-1} on sheath blight severity. DBS = days before sowing, DAS = days after sowing.

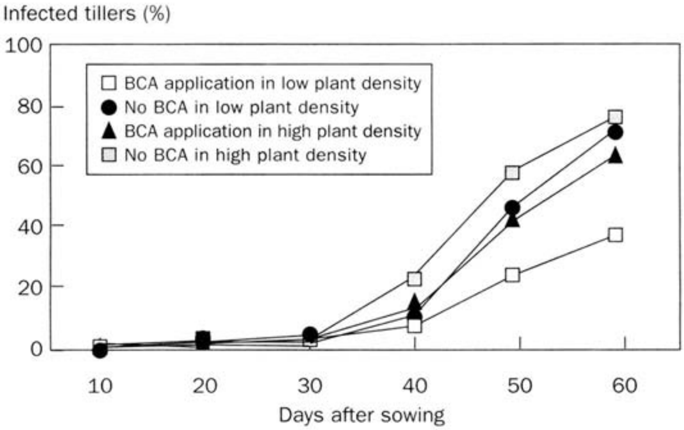


Fig. 9. Effect of BCA application against sheath blight of rice at two different planting densities.

sheath blight foci from 77% in the first crop to 20% in the fifth crop cycle was noted, whereas sheath blight incidence in neighboring fields remained very high. These researchers believe that "local products with a local strain for local use" may be a model to scale up the biological control technology for rice disease management (Mew et al 1998).

Demonstration of the long-term effect of BCA by rice farmers

The effect of BCA used by rice farmers was measured in three subsequent crops from the early season 1999, wet season 1999, and dry season 1999-2000 in two different districts, Cai Lay and Tan Phuoc. In the wet season 1999, significant differences in diseased hills and yield in treatments treated with BCA + 1/2 commercial dose of Validacin were observed among demo-plots heavily infected by rice sheath blight earlier. No differences were observed, however, in the early wet season and third dry season, although BCA were applied two to three times with 5- to 7-d intervals per crop (Fig. 10). The area is flooded every year. Incoming inoculum from outside fields, for example, was carried by water contaminated with the sclerotia from weeds (water hyacinth). This is one of the potential means for sheath blight spread from the source in the wet season. Results from 10 rice farmers' fields showed that the area where BCA were applied needs to be expanded and continued for some more time. The provincial authority of Tien Giang approved support for the continuation of farmers applying BCA to manage sheath blight disease. We will organize a group of farmers to use BCA for the long term and establish farmers' training at the Regional Plant Protection Department, Long Dinh, while we try to produce BCA at a lower cost.

Prospects for adoption by rice farmers

Sheath blight disease of rice is becoming more severe every year under the intensive irrigated rice ecosystem of the Mekong Delta. However, losses in the rice crop are caused not only by disease but also by other pests. A lot of pesticides had been used, but these could not explain yield variation (Du et al 1997). The overuse of pesticides was problematic for the environment and health. The use of biodiversity for sustainable disease management offers new options and it can be a potentially valuable component in integrated pest management. Antagonistic bacteria are abundant in the flooded paddy field system. The proportion of strong antagonism is very low, so this may be one reason for spread of the pathogen. Antagonistic bacteria can be effectively applied to manage sheath blight disease in rice when used at the right formulation and

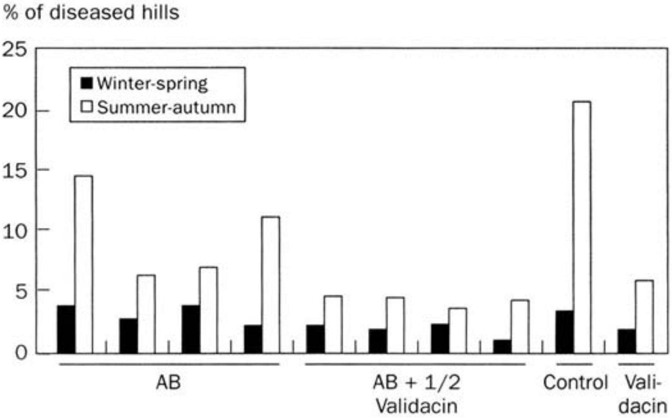


Fig. 10. Effect of BCA in a combination with 1/2 commercial dose of Validacin in rice farmers' fields in Cai Lay District. AB = antagonistic bacteria.

applied at the right epidemiological stage of the disease. To be effective, biocontrol depends not only on suitable biocontrol organisms but also on methods and strategies for introducing and maintaining the organism in the crop. To enhance BCA in rice fields, weed management together with water management and residue management need to be linked to limit the source of inoculum.

Biocontrol can present an attractive new technology for pest management. However, the optimization of fermentation and formulation processes remains a constant goal in order to keep the cost low and produce a stable formulation that would make the product profitable.

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Notes

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Biological control agents against major pathogens of rice in the Yangtze Delta area of China

Xie Guanlin, Yu Xuefang, and Ren Xiaoping

Biocontrol agents (BCA) of bacteria from rice of three cultural patterns (direct row seeding, direct broadcast seeding, and transplanting) were evaluated, detected, and identified in the Yangtze Delta area of China during 1997-2000. Some 8,993 nonpathogenic bacterial isolates were isolated from 1,050 samples of leaf sheath and grain of rice collected at six different growth stages and tested for antagonistic effects against major pathogens of rice. The number against *Rhizoctonia solani* was the highest. Seed yielded the lowest number among the three parts of the rice plant. The population of BCA decreased with rice plant maturity in the three cultural patterns. A higher fluctuation of the BCA population was noted in some stages in each cultural pattern. The higher number of BCA detected was closely related to the severity of rice sheath blight in the field. Some promising BCA were found in field evaluations against sheath blight and bakanae disease of rice. Seven genera of 17 species or types were involved in antagonistic activity against the six major pathogens of rice.

Rice cultivation has more than 6,000 years of history in the Yangtze Delta area, which is now one of the major bases of rice production in China (You 1979). Since this area is located in the coastal area of the country, recent economic development has signified great changes in cultural practices for rice. New cultural patterns such as direct row seeding and direct broadcast seeding have emerged although transplanting of rice has been practiced for several thousand years. In some counties, direct row seeding has covered more than 80% of the total rice cultivated area. Varieties resistant to blast and bacterial blight were widely distributed there. The most important diseases in the past were seldom found in recent years. However, sheath blight of rice caused by *Rhizoctonia solani* AG 1 is more severe than before because of the higher density of rice plants in the cultural patterns in which microclimate is more favorable for the disease to develop. Yield loss from sheath blight in the area is about 20-40% every year, which is threatening rice production.

Jinggangmycin, a fungicide used in this area for more than 20 years, is the only chemical fungicide for control of rice sheath blight. The rate of Jinggangmycin has increased from 1.5 to 4.5 kg ha^{-1} now. The frequency of fungicide spraying also increased from 2 to 3-4 times. Because the traditional transplanting of rice was replaced by direct row seeding and direct broadcast seeding, farmers sprayed the chemical once a week after the tillering stage in some villages. This has resulted in resistance of *R. solani* to the chemical (Chen et al 2000). The pathogen's resistance to the chemical was also observed in other countries (Baby and Manibhushanrao 1993, Sarker et al 1992). Moreover, because of environmental pollution and health hazards from chemical control, biological control has become more and more valuable in plant disease management in the area. Many studies have been made in screening and evaluating biological control agents (BCA) against *R. solani* (the causal organism of sheath blight of rice) in rice-growing countries using transplanting (Mew and Rosales 1986, Devi et al 1989, Gnanamanickman and Mew 1992, Mew et al 1994, Xie et al 1999). No information is available for the BCA in the new cultural patterns in the Yangtze Delta area. The diversity of the naturally occurring BCA is not known. As we cannot rely on a single control tactic, an understanding of these BCA in relation to the cultural patterns of rice production in the area is essential.

BCA diversity against *R. solani* in different cultural patterns

We isolated 8,993 bacterial isolates from the three cultural patterns and compared their antagonistic bacteria. Direct broadcast seeding supported the highest number of antagonistic bacteria against *R. solani,* followed by transplanting. The total number of antagonistic bacteria in the first pattern is significantly different from that of direct row seeding (Table 1). The number among the nonfluorescent antagonistic bacteria was not significantly different among the three cultural patterns. The major difference in broadcast seeding from the other two practices is the high percentage of fluorescent antagonistic bacteria. The higher number of antagonistic bacteria was closely related

Table 1. Comparison of antagonistic bacteria against *Rhizoctonia solani* from three different cultural patterns.

Item	Cultural pattern[a]		
	DRS	DBS	Transplanting
Total number of bacterial isolates tested	2,866	2,135	3,992
Number of fluorescent bacteria tested	334	244	478
Total number of antagonists obtained	158	217	298
% antagonists of total	5.51 b[b]	10.16 a	7.46 ab
Number of fluorescent antagonists	52	74	106
% f-antagonists to total f-bacteria[c]	15.56 b	30.32 a	22.18 ab
Number of nonfluorescent antagonists	106	143	196
% n-f-antagonists to total n-f-bacteria	4.19 ns	7.56 ns	5.57 ns

[a]DRS = direct row seeding, DBS = direct broadcast seeding. [b]In a row, treatment means followed by a common letter are not significantly different by LSD at $P<0.05$ level. [c]f = fluorescent, n-f = nonfluorescent.

to the severity of rice sheath blight in the field, which was most severe in broadcast seeding after the tillering stage because of high canopy density. This could be explained by several reports on biological control of the disease (Mew and Rosales 1986, Lin et al 1992, Chen et al 2000).

Biological control agents against *R. solani* appear to occur widely in rice fields. They were isolated from the rhizosphere, phyllosphere, rice seeds, soil, paddy water, and even on the sclerotia (Mew and Rosales 1986, Devi et al 1989, Chen and Mew 1998). BCA could be detected from healthy and infected plants. More were found on sclerotia collected in flooded rice fields than in dryland rice fields. However, all BCA associated with rice were from the transplanting practice. We also compared the BCAs on different parts of the rice plant from the three different cultural patterns. The highest number of BCA was obtained from the sheath of the rice plant in all cultural patterns (Fig. 1). The density of BCA in a unit of fresh sheath was significantly higher than that in fresh leaf and grain. The lowest number of BCA was from grain. The cultural patterns did not significantly affect BCAs on the same part of the rice plant in the study. This indicated that the rice sheath harbors a larger number of BCA than other parts of the rice plant. The conditions of the leaf sheath are more stable than those of the leaf surface at the tillering stage since the sheaths are shaded by leaves. These conditions are favorable for bacteria as well as BCA to reside and develop on the leaf sheath, whereas BCA on the leaf and grain of rice are easily disturbed by rainfall, wind, ultraviolet light, and water stress (Wilson and Pusey 1985). The population of BCA on these parts of the rice plant is lower than that on the leaf sheath as the niche of microbial establishment. The conditions on the leaf and grain fluctuate more frequently than on the leaf sheath.

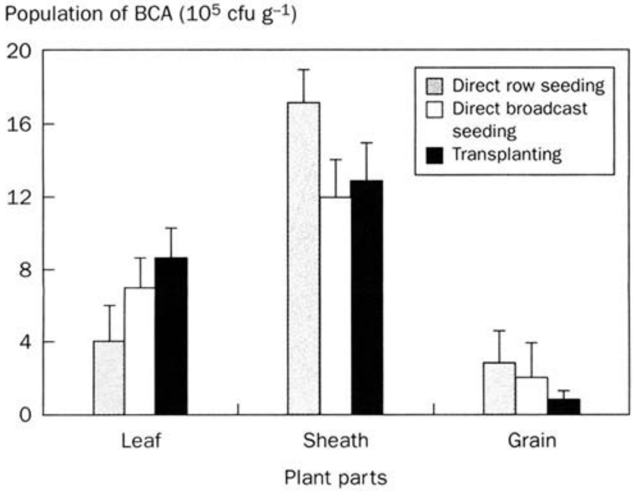

Fig. 1. Distribution of BCA population against *Rhizoctonia solani* on different parts of rice plants in three cultural patterns.

Bacterial populations against R. solani at different growth stages of the rice plant

Published data indicated that the frequency distribution of BCA in an ecosystem is affected by the cropping system, seasonality, genotypes, plant nutrition, and moisture conditions (Xie 1996, Latour et al 1996). No information is available, however, on the distribution of BCA against *R. solani* at different growth stages with the new cultural patterns. Our data showed a trend that the population of BCA decreased as the rice plant matured in the three cultural patterns (Fig. 2). Nevertheless, the BCA population fluctuated according to the stage of crop growth in each cultural pattern. In direct row seeding, the BCA population was maintained at about 10^6 cfu g^{-1} at the seedling and tillering stage. It reached the maximum at the booting stage, then gradually declined. The population of BCA under direct broadcast seeding increased from the seedling to tillering stage. The population then decreased rapidly. In transplanted rice, the BCA population was 5 x 10^6 cfu g^{-1} at the seedling stage. The population then decreased after transplanting. It increased markedly, however, from the tillering to booting stage. The BCA population climaxed at the booting stage in direct row seeding and transplanting and at the tillering stage in direct broadcast seeding. The highest level of BCA isolation coincided with the severity of rice sheath blight in the fields. The more severe the disease, the higher the BCA population detected/isolated in most cases. In direct broadcast seeding, disease severity was higher at the maximum tillering stage because of the higher density of rice plants. The peak of the BCA population and disease severity was earlier than in the other two cultural patterns.

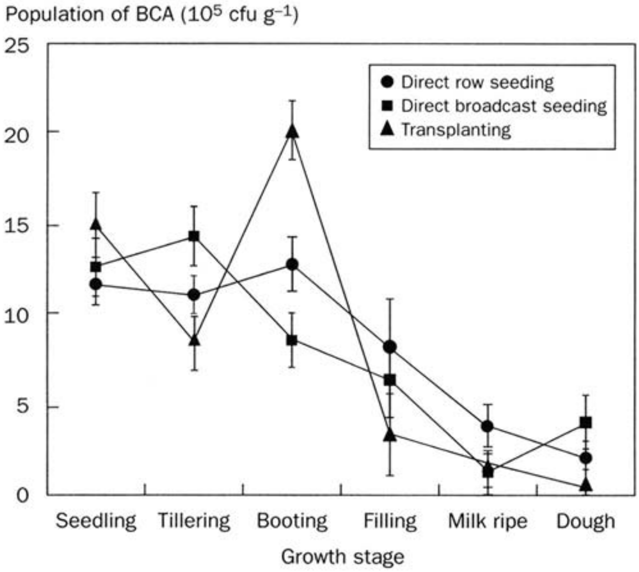

Fig. 2. Distribution of BCA population against *Rhizoctonia solani* at different growth stages of rice in three cultural patterns.

Functions of BCA against some rice pathogens

We tested other characteristics of the BCA in addition to their antagonistic effect. Six fluorescent and two nonfluorescent bacterial isolates antagonistic to *R. solani* were selected for further evaluation on sclerotial germination, leaf segment infection, and the promoting effect of seedling vigor. Seven of the eight antagonistic isolates promoted the growth of seedling roots compared with the control (Table 2). Six isolates inhibited 40-60% of sclerotia germination. All eight isolates showed a protection effect when the isolates were sprayed onto the leaf segments before inoculation of the fungal pathogen. The fluorescent bacteria exhibited both inhibition of the pathogen and growth promotion of seedling roots. The data revealed that the bacteria antagonistic to *R. solani* in vitro do not necessarily correspond to their ability in growth promotion and inhibition of sclerotia germination. An antagonistic bacterium may not exhibit growth-promoting effects.

The eight bacterial isolates were also evaluated for their biocontrol effect in the field against *R. solani* and *Gibberella fujikuroi,* the causal organism of sheath blight and bakanae of rice. The control efficiency one day before the inoculation of *R. solani* is better in some cases than that with inoculation and spraying of BCA at the same time. Three of the eight antagonists against *R. solani* in vitro also demonstrated effective control of the disease in the field (Table 3). Five isolates induced a larger inhibition zone against *R. solani* in vitro but their ability to reduce the lesion length in the field was insignificant when compared with the control. BC1145 and BC196-2 were effective against both fungal pathogens. BC178-1 did not suppress sheath blight development but considerably reduced bakanae disease.

Burr et al (1978) and Kloepper et al (1980) reported that plant growth-promoting rhizobacteria (PGPR) enhanced the yield of potato and other crops. The PGPR were fluorescent strains of *Pseudomonas fluorences* and *P. putida*. We also obtained a large amount of these fluorescent strains from rice. Many of them were antagonistic to one or more pathogens of rice and some had an effect similar to that of PGPR since they significantly promoted seedling root growth. However, the growth-promoting

Table 2. Functions of some biological control agents (BCA) against *R. solani* in vitro.

Code number[a] of BCA	Inhibition zone of BCA	Inhibition on sclerotial germination (%)	Promoting root length of seedling (mm)	Infection of rice leaf segments after BCA inoculation (%)
BC34-2	11.3 b	70.8 bc	108.6 c	70.0 d
BC178-1	13.0 ab	62.5 ab	128.7 b	70.0 d
BC179-2	11.3 b	66.7 ab	122.1 bc	10.0 a
BC180-3	13.7 ab	41.7 a	152.5 a	70.0 d
BC196-2	13.3 ab	50.0 ab	152.9 a	20.0 ab
BC2378	10.1 c	62.5 ab	101.8 c	50.0 c
BC2619	12.2 b	70.8 bc	82.1 d	40.0 c
BC1145	15.1 a	50.0 ab	102.2 c	20.0 ab
Check	0.0 d	95.8 c	86.1 d	100.0 e

[a] BC2619 and BC1145 are nonfluorescent and the other six are fluorescent bacteria. In each column, means followed by a common letter are not significantly different at $P = 0.05$ by Duncan's multiple range test.

Table 3. Field evaluation of some biological control agents against *R. solani* and *Gibberella fujikuroi*, the causal organism of sheath blight and bakanae of rice.

Code of BCA	Time for treatment[a]	Sheath blight lesion length (cm)	Bakanae-[b] infected seedlings (%)
BC34-2	IBS-1	15.7 ab	15.3 ab
	IBS-0	16.1 ab	
BC178-1	IBS-1	15.5 ab	8.3 c
	IBS-0	16.4 ab	
BC179-2	IBS-1	7.1 cd	15.0 ab
	IBS-0	7.6 cd	
BC180-3	IBS-1	17.3 a	17.3 a
	IBS-0	18.3 a	
BC196-2	IBS-1	8.2 c	5.0 d
	IBS-0	9.7 c	
BC2378	IBS-1	14.3 b	15.3 ab
	IBS-0	16.7 a	
BC2619	IBS-1	16.5 ab	14.6 b
	IBS-0	17.0 a	
BC1145	IBS-1	5.0 d	8.0 c
	IBS-0	8.7 c	
CK	IBS-1	19.8 a	18.3 a
	IBS-0	17.8 a	

[a] IBS-1 means inoculation one day before spraying of BCA, ISB-0 means inoculation and spraying of BCA on the same day. In one replication, 100 rice plants were measured. In each column, means followed by a common letter are not significantly different at $P = 0.05$ by Duncan's multiple range test. [b]Nature-infected seeds were used.

effect diminished after the seedling stage. Many biotic and abiotic factors in the paddy field affect the colonization, multiplication, and development of BCA.

Bacterial species having antagonists against major pathogens of rice

Six hundred and seventy-three out of 8,993 bacterial isolates from rice plants in the three cultural patterns were antagonistic to one or more major pathogens of rice. These target pathogens are *R. solani, Sarocladium oryzae, G. fujikuroi, Acidovorax avenae* subsp. *avenae, Xanthomonas oryzae* pv. *oryzicola,* and *X. oryzae* pv. *oryzae,* the causal organisms of sheath blight, sheath rot, bakanae, bacterial brown stripe, bacterial leaf streak, and bacterial leaf blight of rice, respectively. Rosales et al (1993, 1995) reported that members of at least four distinct genera *(Bacillus, Pseudomonas, Erwinia,* and *Serratia*) were responsible for antagonistic activity against some rice fungal pathogens (*R. solani* and *G. fujikuroi*). Xie (1996) found that, out of more than 4,000 bacteria isolated from rice seed, at least 12 bacterial species of 5 genera were involved in antagonistic activity against 2 fungi and 3 bacterial pathogens of rice in the Philippines. In the Yangtze Delta area of China, 17 species or morphotypes of 7 genera had an antagonistic effect against 1 or more of the 6 major pathogens of rice (Table 4). Eleven species were nonfluorescent and 4 were fluorescent. In each of the 17 species or types, there were antagonists against *R. solani*. Strains from the nonfluorescent

Table 4. Bacterial species from rice plants having antagonists against the six major pathogens of rice[a] in the Yangtze Delta area of China.

No.	Biolog identity	Gram stain	Biolog similarity	1	2	3	4	5	6
01	*Acinetobacter baumannii genospecies* 2	–	0.61–0.82	+	–	–	–	–	–
02	*Acinetobacter calcoaceticus genospecies* 13	–	0.60–0.91	+	+	–	+	–	–
03	*Alcaligenes faecalis* subsp. *aecalis*	–	0.63–0.73	+	–	–	+	–	–
04	*Bacillus brevis*	+	0.53–0.68	+	–	+	–	–	–
05	*B. megaterium*	+	0.50–0.66	+	–	+	–	–	–
06	*B. pumilus*	+	0.57–0.65	+	+	–	–	–	–
07	*B. subtilis*	+	0.65–0.75	+	+	+	+	+	–
08	*Burkholderia cepacia*	–	0.64–0.92	+	+	+	+	–	+
09	*Enterobacter cloacae* A	–	0.53–0.75	+	+	+	+	–	–
10	*En. Gergoviae*	–	0.50–0.76	+	–	–	–	–	–
11	*Stenotrophomonas maltophilia*	–	0.70–0.88	+	+	+	+	+	–
12	*Pseudomonas aeruginosa* (F)	–	0.70–0.88	+	+	+	+	+	+
13	*P. fluorescens* C (F)	–	0.60–0.92	+	+	+	+	+	+
14	*P. fluorescens* G (F)	–	0.70–0.82	+	+	+	+	+	+
15	*P. fulva* (F)	–	0.50–0.72	+	+	+	+	+	–
16	*P. putida* A1 (F)	–	0.66–0.96	+	+	+	+	+	+
17	*P. putida* B1 (F)	–	0.56–0.86	+	+	+	+	+	+

[a] At least 10 bacterial isolates in a species were tested for each target pathogen. 1 = *R. solani*, 2 = *Sarocladium oryzae*, 3 = *Gibberella fujikuroi*, 4 = *A. avenae* subsp. *avenae*, 5 = *Xanthomonas oryzae* pv. *oryzicola*, 6 = *X. oryzae* pv. *oryzae*. (F) = fluorescent.

species *Bacillus subtilis, Burkholderia cepacia,* and *Stenotrophomonas maltophilia* were not only antagonistic to the three fungal pathogens and *A. avenae* subsp. *avenae* but were also effective against one of the pathogens of *Xanthomonas.* No strains from the other eight nonfluorescent species were antagonistic to the pathogens of *Xanthomonas.* The strains from the four species of *Bacillus* were antagonistic to two to three fungal pathogens. This revealed that the number of nonfluorescent strains antagonistic to the three bacterial pathogens was low. However, more strains from the six species or types of fluorescent bacteria were antagonistic to almost all of the six pathogens. This indicates that the fluorescent bacteria have a wider range in antagonism, whereas the nonfluorescent bacteria exhibited a relatively narrower range or were specific to certain pathogens.

The genera of *Pseudomonas* and *Bacillus* were the most important group of bacteria associated with rice. Some 65% of the total bacterial species that are antagonists belong to these two genera and about 35% belong to the other five genera tested. Antagonistic bacteria from the rice plant are quite diverse. Our data have shown that the rice plant has a huge reservoir of biological control agents. We identified 17 nonpathogenic bacterial species or types having strains antagonistic to one or more rice pathogens isolated from the Yangtze Delta area of China. The identity of these bacterial isolates from the rice plant is still unknown.

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Populations of rice sheath blight in the Ilocos Norte rice based ecosystem

J.O. Manalo, T.W. Mew, P.S. Teng, A.R. Velilla, and E.S. Borromeo

A total of 378 isolates from plants and soil were collected in Batac and Badoc, Ilocos Norte, Philippines. Comparisons between the two populations based on pathogenicity, anastomosis, and DNA banding pattern were made. Major differences in the isolates were evident in all the tests performed, indicating that the soil and plant populations are separate strains. Plant isolates were virulent to IR64 rice and 88.4% of these were vegetatively compatible with the AG 1 isolate. Soil isolates, however, were generally nonpathogenic and did not anastomose with the AG 1 from rice. Molecular characterization confirmed that the soil isolates were a different strain, although morphological features closely resembled those of the *Rhizoctonia* spp.

Characterization tests of the isolates suggest that the predominating *Rhizoctonia*-like isolate in the Ilocos Norte soil is not damaging to the rice crop. Primary sources of infection were believed to be the weeds along the bunds and maize that was grown in sequence with rice. Cluster analyses indicated that the isolates from plants were homogeneous throughout the sites regardless of the host sources, but grouping tendencies can be observed as the isolates move closer to each other along the transect.

Spread of the disease in the field is apparently within the canopy through plant-to-plant contact. Identification of important weed species was included in this study.

Rice sheath blight disease is caused by a soilborne pathogen, *Rhizoctonia solani* Kuhn (*Thanatephorus cucumeris* Donk.). It is one of the major diseases inflicting heavy losses in rice in most Asian countries. and one of the two niost serious fungal diseases worldwide (Cu et al 1996). Its wide occurrence is brought about by intensive methods of rice cultivation involving the use of short-culmed, high-tillering, high-yielding varieties and high inputs of nitrogen fertilizer. These favor the development of the disease by promoting a more dense environment with high relative humidity. The causal fungus is able to survive in the soil and overwinter in the form of sclerotia or as mycelia in infected rice materials left in the field from the previous crop. In the absence of rice, the sclerotia usually remain in the soil. The sclerotia and infected crop residues are the known primary sources of infection in the field (Premalatha Dath 1990, Castilla 1998, Rosales 1985). Many studies have positively correlated the significant increase in sclerotial population in the soil with disease severity on infected plants. Disease severity is often associated with increasing viability and sclerotial size (Naiki and Ui 1977, Damicone et al 1993). In some cases, however, sclerotial bodies were not successfully recovered from the field where sheath blight is known to occur. These were believed to be colonized by bacteria or fungi that are antagonistic to the fungus (Mew et al 1980). The sheath blight pathogen may also survive in rice stubbles after the cropping season. Infected stubbles are left in the field and eventually buried into the soil. The pathogen remains viable in rice straw for varying periods depending on the soil moisture condition (Mew et al 1980, Premalatha Dath 1990).

Because of the persistent nature of the pathogen, this study aims to characterize the populations of *Rhizoctonia* in different host sources and plant debris by phenotypic and molecular methods and assess their differences according to their distribution in the Ilocos Norte rice-based cropping system. This is important because it allows us to determine the primary sources of inoculum and why it efficiently spread the infection in the field, and propose appropriate methods for managing the disease.

Methodology

Sheath blight isolates were collected from infected plants and plant debris along the transects in Batac and Badoc, Ilocos Norte, Philippines. The disease was monitored for 17 monthly sampling periods. Tissues of infected plant samples were plated in PDA medium. Mycelia emanating from the tissue were isolated and purified in agar slants. Similar growths from soil samples were collected using the method of Boosalis and Scharen (1959). Soil was seived through the mesh and the remaining soil particles and plant debris were collected. These were placed in sterile agar medium and observed for mycelial growth.

A 3-d-old culture was used to inoculate 4-wk-old IR64 plants. A sclerotium is inserted inside the leaf sheath, bound with strips of gauze cloth, and retained for 3 d to hold some moisture. In the absence of an infective sclerotium, the isolate is added with sorghum seeds into the culture medium and the sorghum seed is inserted in the leaf sheath in lieu of the sclerotium. The inoculated plants are observed for infection 4 d after inoculation.

Anastomosis is the fusion of hyphae between closely related strains of *Rhizoctonia solani.* A rice sheath blight causing a strain of *Rhizoctonia* belongs to anastomosis group 1 (AG 1) (Agoshi 1987). Each isolate from a nonrice host was placed at the same time on a clean glass slide with an isolate from rice. The glass slide was incubated inside a moist chamber for at least 24 h or until such time that the hyphal growth of one isolate touched the other. The slide was viewed under the microscope and observed for hyphal fusion.

Pathogenic and nonpathogenic isolates were grown separately in sterile rice straw medium. After 5 d, equal amounts of the cultures were added to the soil and mixed thoroughly. Soil sampling was done at least 1 wk after the inoculation. Recovery of isolates from the soil samples was done by baiting with mungbean seeds.

A mini-scale preparation of DNA using the CTAB/NaC1 protocol for blast has been used to extract the sheath blight DNA. At least 50 mg of ground lyophilized mycelia was placed in a 1.5-mL eppendorf tube. The mycelia were suspended in 500 mL extraction buffer and vortexed until evenly suspended. Fifty mL of 10% SDS was added to the mixture and gently shaken for 1 h at 37 °C. Then 75 mL of 5M NaCl was added and followed by 65 mL CTAB/NaCl. The mixture was placed in a water bath at 65 °C for 10–20 min. Chloroform:isoamyl (24:1) was added in equal volume (700 mL) and the solution was shaken vigorously and spun at 10,000 rpm for 12 min. The aqueous, viscous supernatant was transferred to a fresh tube, added with 450 mL cold 2-propanol to precipitate the nucleic acid, and mixed. Again the solution was centrifuged at 10,000 rpm for 12 min. The supernatant was removed and the pellet was rinsed with 70% ETOH. The pellet was then allowed to dry before adding 40 mL 1XTE.

Following the REP-PCR protocol using the ERIC primer, a 23-mL reaction mixture was prepared for each sample tube:

Reagent	Volume (mL) per tube
Filtered, autoclaved, HPLC-grade water	11.05
Gitschier buffer	5.00
BSA (bovine serum albumin)	0.20
DMSO (dimethylsulfoxide)	2.50
DNTPs 1.25	
Primer ERIC 1R	1.00
Primer ERIC 2	1.00
Taq polymerase	1.00
Total	23 mL

Cluster analyses were done using PC-ORD.

Results

More than 300 total isolates were collected from infected plants and soil in 17 mo of sampling along the Batac and Badoc transects in Ilocos Norte. Twenty-eight of these were from rice, 68 from weeds, 1 from maize, and 281 from plant debris. Infected weeds were usually found along the bunds. A cultural characteristic of these isolates is associated with extensive mycelial growth with varying intensity of brown color. The soil isolates, however, differed because these were not producing the typical sclerotia, although a microscopic view of these isolates showed that these had a hyphal growth and multinucleate similar to those of the plant isolates.

All plant isolates formed sheath blight infection on IR64, whereas only four of the soil isolates gave this type of symptom. About 30 of the soil isolates were less aggressive on rice and all the others were not able to infect at all (Fig. 1).

The sheath blight-forming plant isolates generally belonged to AG 1 and 88% of the test isolates anastomosed with the rice isolate. Table 1 shows the reactions of the

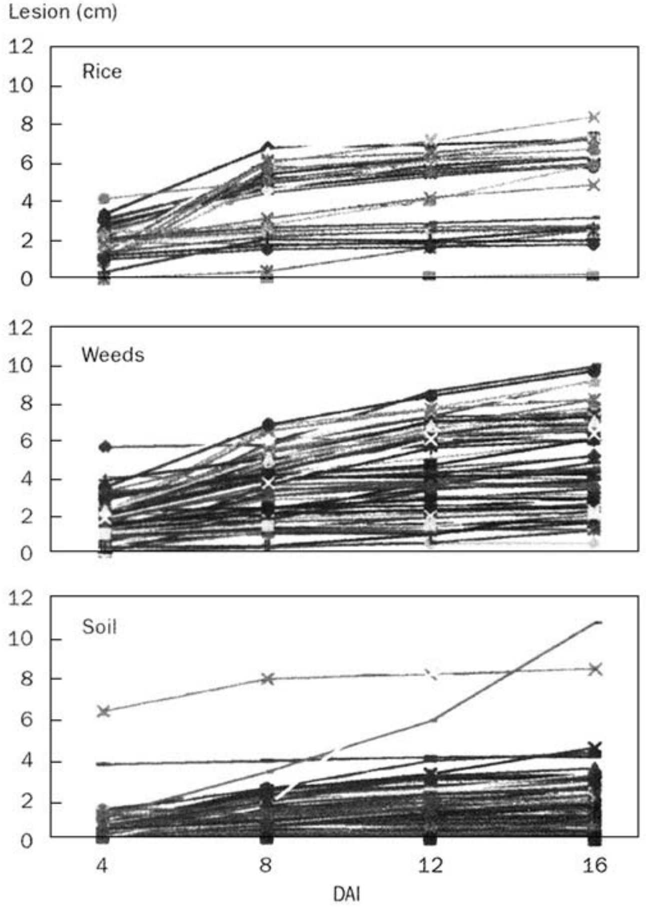

Fig. 1. Graphs showing lesion length (cm) of isolates from various sources. DAI = days after inoculation.

weed and maize isolates. Complete fusion of the vegetative hypha was evident among these compatible isolates. Anastomosis among related *Rhizoctoria* isolates is commonly associated with the transfer of cytoplasmic contents and hyphal constriction of the fusing cells. Selected soil isolates did not anastomose with the rice sheath blight, including the ones that gave positive infection on rice.

A dual-culture test was conducted to determine the probable interaction between the pathogenic and nonpathogenic soil isolates. These were mixed into the soil and allowed to incubate for some time. Recovery of isolate is shown in Table 2. Virulent isolate is significantly suppressed with the presence of the nonvirulent one. Recovery of the pathogenic form is very low in dual culture compared with when it is grown alone.

Table 1. Hyphal interaction of weed and maize isolates with an AG 1 rice sheath blight.

Isolate	Reaction[a]	Isolate	Reaction	Isolate	Reaction
C1	+	W23	+	W48	–
W1	+	W24	–	W49	+
W2	+	W25	–	W50	+
W3	+	W26	+	W51	+
W4	+	W27	+	W52	+
W5	+	W28	+	W53	+
W6	+	W29	+	W54	+
W7	–	W30	+	W55	+
W8	+	W31	+	W56	–
W9	+	W32	+	W57	+
W10	+	W33	+	W58	+
W11	+	W34	+	W60	–
W12	+	W35	+	W61	+
W13	+	W38	+	W62	+
W14	+	W39	+	W63	+
W15	–	W40	+	W64	+
W16	+	W41	+	W65	+
W17	+	W42	+	W66	+
W18	+	W43	+	W67	+
W19	+	W44	+	W68	+
W20	+	W45	+	W69	+
W21	–	W46	+	W70	+
W22	+	W47	+	W71	+

[a] + = positive, – = negative.

Table 2. Recovery of isolates from a dual-culture test using the baiting technique on mungbean seeds.

Treatment	% infection[a]
R12	100.0 a
S136	100.0 a
S136 + S1	45.0 b
S136 + S201	47.5 b
S1	35.0 bc
R12 + S1	32.5 bc
R12 + S201	37.5 bc
S201	10.0 c

[a] Means with the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test, based on transformed arsin data.

DNA fingerprinting of plants and soil isolates was conducted by PCR using the ERIC primer. The DNA banding pattern was run in PC-ORD and the cluster dendrogram is shown in Figures 2-4. Cluster analysis of the isolates in Badoc showed no clear grouping of the isolates in the transects, but isolates in closer transect points sometimes tended to go together regardless of their host source. The same is true for the Batac transect. There is apparently a close association or similarities between isolates as they move closer in the transect. Although the observation is not always true for all the isolates, some tendencies can be observed.

Moreover, when the isolates in two transects were compared in a cluster analysis (Fig. 2), some isolates at one site joined with another at the other site. Some isolates in Batac show similarities with those present in Badoc. Host species of these isolates also have wide variation. In one group in the cluster (Fig. 3), similar isolates could have come from rice and different weed species such as *Ischaemum rugosum, Imperata cylindrica, Brachiaria mustachia, Paspalidium flavidum, Cynodon dactylon, Distachia aristatum,* and *Saccharum spontaneum.* This confirms the wide host range of the rice sheath blight pathogen.

Discussion

More than 300 isolates were collected from infected hosts and plant debris under different field conditions along the Batac and Badoc transects. These were identified based primarily on morphological and cultural characteristics on PDA medium. A pathogenicity test of the sheath blight isolates from various hosts (rice, weeds, and maize) showed that there is cross-infection on IR64. However, the soil isolates gave a varying degree of disease severity. Only four of the total population gave the desired symptom; some were less aggressive but most of them were not pathogenic to rice. Contrary to many studies showing that the main sources of sheath blight infection were the soil and plant debris, this study showed otherwise. A low number of soil isolates were infective to rice. This number is not significant considering the total sampling distance of 7 km for the two sites, and that the sampling procedure was followed three times over different field conditions: fallow, planting, and fallow after harvest.

Results of the anastomosis test indicated that the weed isolates were vegetatively compatible with the rice isolate and belonged to the AG 1 group of *R. solani*. Soil isolates, however, did not show the same reaction, including those that formed infection. This confirms that the soil population does not belong to the pathogenic strain and that their dominating presence in rice soil does not pose any threat to the staple crop. Moreover, a profile of the DNA fingerprints indicated that the plant and soil populations were separate groups of fungi. Although the morphological features of the soil isolates have a very close resemblance to the pathogenic fungi, major differences exist between the soil isolates and those collected from sheath blight in terms of their morphology, pathogenicity, and AT-DNA restriction fragment length polymorphism. Even if soil isolates were reported to infect young rice plants, there is a slight difference in visible symptoms from those of plant isolates (Banniza et al 1999).

Presence of the *Rhizoctonia*-like fungi is common in plant debris, not only in Ilocos Norte soil. These fungi were also found frequently in soil debris of tall *Festuca* sp. Recovery of this saprophytic fungus appeared more often than the target species, *R. solani* (Martin et al 1983). Apparently, the survival of the pathogenic fungi in soil debris is overcome by the *Rhizoctonia*-like saprophytic fungi. This is supported by the results of this study. Preliminary results indicated that the nonpathogenic isolate

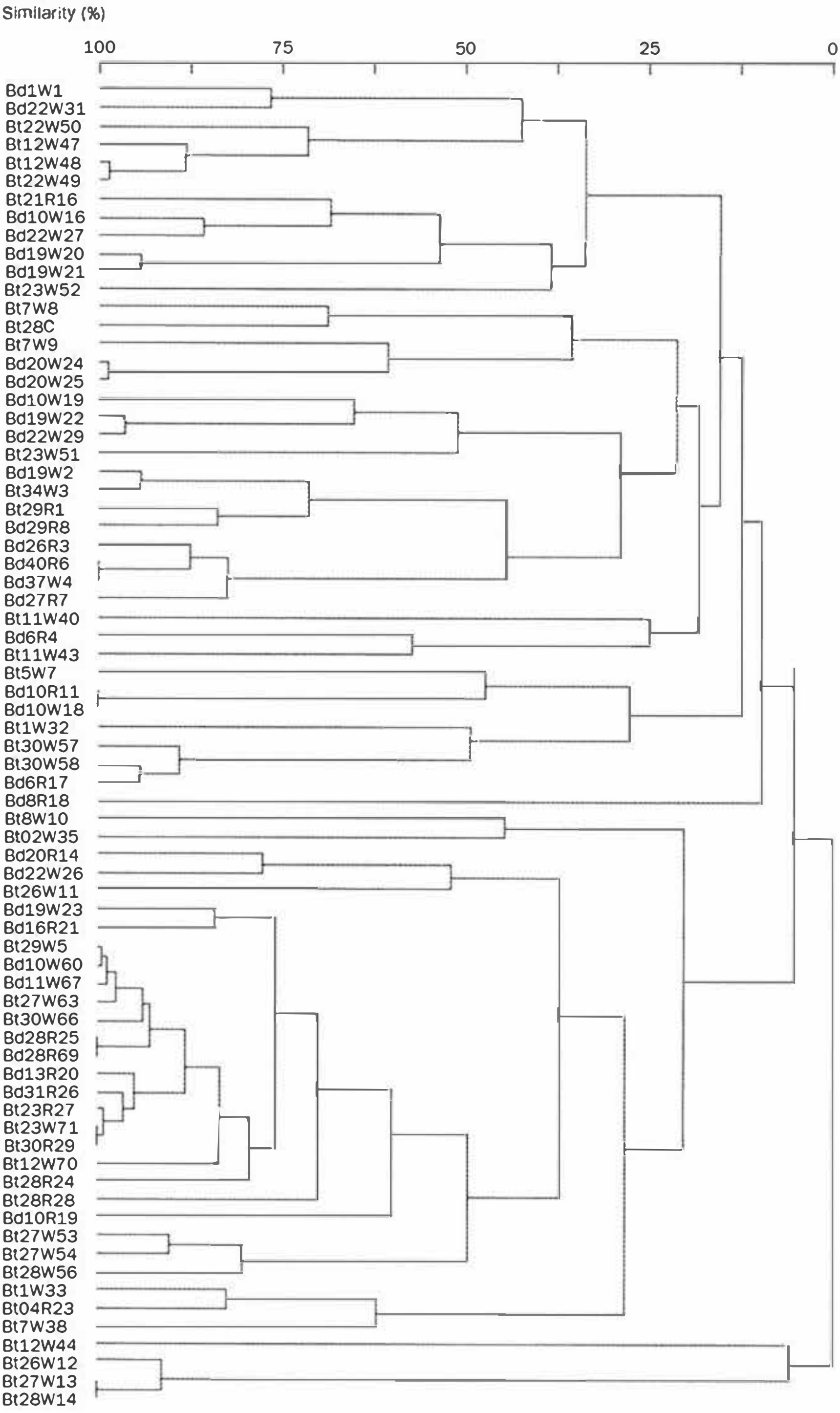

Fig. 2. Cluster analysis of the sheath blight isilates from the Badoc and Batac sites.

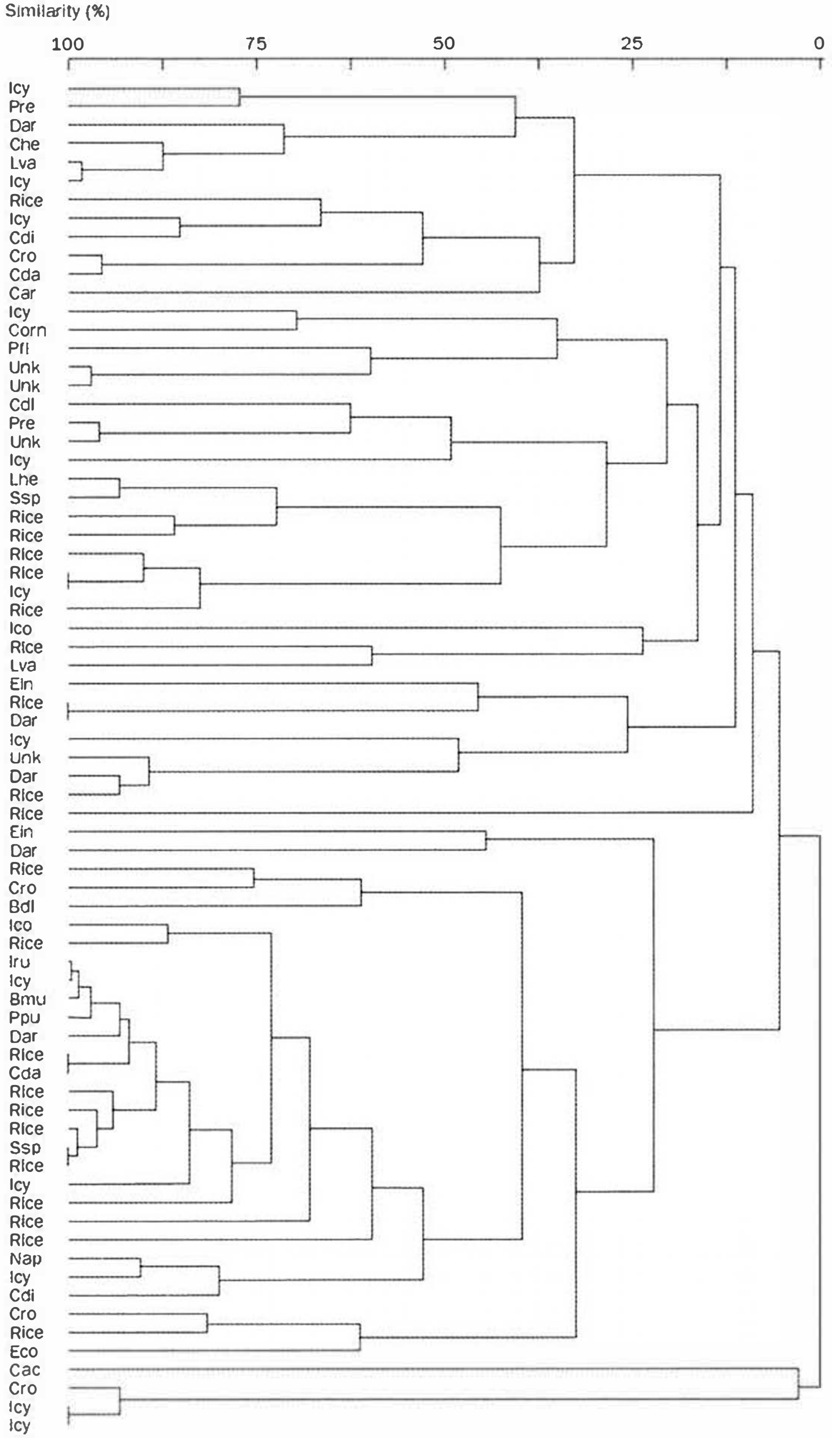

Fig. 3. Cluster dendrogram of the sheath blight isolates showing the different host sources.

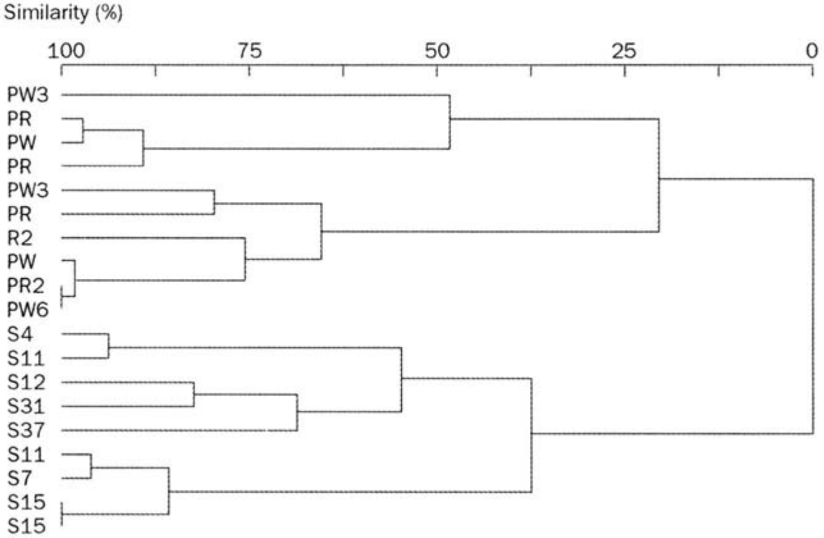

Fig. 4. Cluster analysis of DNA banding pattern of plant soil isolates.

was more competitive than the pathogenic one. This suggests that, even if the infected crop residues were initially viable, the low recovery of the virulent isolate from plant debris indicates that the group of plant isolates possesses a lower fitness for survival in the soil than the soil isolates. Plant isolates may just be part of the soil population or some plant isolates temporarily spread into the soil without establishing themselves in large quantities (Banniza et al 1999).

The number of sclerotia under tropical conditions is comparatively lower than in temperate or subtropical climates. If such is the case for the Ilocos Norte rice ecosystem, any attempt to alter the sheath blight population structure by soil incorporation of antagonistic microorganisms, soil solarization, or other cultural or chemical practices would not seem appropriate for controlling the disease. Considering the poor establishing capacity of plant isolates in rice soil despite the presence of sheath blight infection in predominating vegetation in Ilocos Norte, perhaps the number of infection units in the soil is of minor importance for the initial development of the disease. Instead, the spread of infection within the canopy seems to be more important in sheath blight epidemiology.

This study found that populations of sheath blight from rice, weeds, and maize were significantly homogeneous throughout the transects at two sampling sites. This is based on their pathogenicity, anastomosis, and DNA banding pattern. Cultural practices in Ilocos such as crop sequencing, cropping pattern, and crop rotation, which vary in different sampling points, would unlikely be important in this case. Sheath blight infection is present in the weeds along the bunds and this ensures propagation of the pathogen even in the absence of rice. During the wet season, and incidentally also the time for planting by farmers. the disease rapidly spread to adjacent young rice plants. This study enumerates the important weed species for the pathogen: *Dicanthium aristatum, Imperata cylindrica, Saccharum spontaneum, Cynodon dactylon, Cyperus rotundus, Ipomoea congesta, Commelina diffusa, Chrysopogon aciculatus, Panicum repens, Eleusine indica, Paspalidium flavidum, Brachiaria distachya, Leersia hexandra,* and *Ischaemum rugosum.* Dominant at both sites were *D. aristatum* and *I. cylindrica.* Alternate hosts of the pathogen cover the major weeds in rice fields and removal of these weeds seems inappropriate because most of these serve as a refuge for many natural enemies against the insect pests of rice (Flor et al 2000). Effective control is more likely to be achieved by preventing plant-to-plant spread of the fungus through bacterization of seeds, fungicide treatments, or the development of resistant varieties (Banniza et al 1999).

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Bacterial antoganist against *Rhizoctonia solani* AG1 in irrigated rice ecosystems

Wanzhong Tan and T.W. Mew

Bacterial antagonists against the rice sheath blight pathogen, *Rhizoctonia solani* AG 1, were estimated in irrigated rice ecosystems and their potential for disease management was evaluated. Bacterial isolations were done using an improved method of trapping bacteria from the washings of plant tissues, paddy soil, and weeds in and around the field. Total and antagonist populations varied with source of isolation and collection sites. A majority of the 4,785 bacterial isolates obtained were isolated from rice plant tissues with the leaf blades and sheath harboring a higher proportion. Eighty-seven percent (4,159 isolates) of these bacteria were neutral to the pathogen, 12.8% (613 isolates) showed antagonism, and only 0.27% appeared to promote pathogen growth. Ninety percent of the antagonists exhibited strong inhibition to the fungal pathogen either by inhibiting the germination of pathogen sclerotia or by inhibiting the size of the colonies in agar media. Similarly, these antagonists also affected germination and seedling growth of the rice plant to various degrees. A total of 91 isolates demonstrated strong inhibition to the pathogen but promoted rice seed germination and seedling vigor.

Evaluation of the effectiveness of these antagonists for sheath blight management revealed that population density, pathogen inoculum level (disease pressure), and crop management practices such as time of application of antagonists or spray of fungicides are factors that influence the efficacy of the antagonists. The data also suggest that the value of antagonist application for sustainable sheath blight management may lie more in its potential to reduce inoculum efficiency, resulting in a decrease in infection foci and limited disease spread.

Vulnerability of the modem agricultural ecosystem to pests and diseases has been attributed to the erosion of biodiversity, which can disturb the naturally occurring biological control mechanisms. Biodiversity maintains the balance of different habitats and organisms. A tropical rice field offers a biologically diverse and dynamic environment for microbial populations to flourish. Microbial diversity refers to the diverse microorganisms associated with the rice plant, paddy soil, and water. Rice ecosystems support abundant microorganisms, which may have either a positive or negative impact on rice cultivation. Those that have a negative effect on rice production are the pathogens. And those that potentially may be related to a positive effect on rice growth may have the ability to suppress a broad range of other microorganisms that cause rice diseases. Among those microorganisms that have a beneficial effect on crop production are the biological control agents (BCA).

Many types of BCA are associated with agricultural systems. Bacteria and fungi are dominant among the BCA. In the literature, reports of BCA in relation to plant disease management or control are numerous. Most of the reports deal with individual bacterial or fungal strains isolated from either related or unrelated ecosystems. These reports evaluate the potential for biological control on a target pathogen and crop disease. Eventually, the objective was to formulate a commercial product to scale up the application. Relatively little effort has been made to assess components of the microbial diversity within an ecosystem to understand the relationship to pest management in general and disease control in particular. In recent years, we have attempted to understand the microbial community within the rice ecosystems (targeting irrigated rice) and assess the functions of the different components in relation to disease management. In rice seed, for instance, Cottyn et al (2001) indicated that the predominant bacteria associated with rice seed were *Enterobacteiaceae* (25%), *Bacillus* spp. (22%), and *Pseudomonas* spp. (14%). Other bacteria regularly isolated were *Xanthomonas* spp., *Cellulomonas flavigena,* and *Clavihacter michiganense.* Four percent of the total number of isolated strains exhibited in vitro antifungal activity. Besides the bacteria, about 120 identified species of fungi were also associated with rice seed.

In the current project funded by the Asian Development Bank (ADB), our main objectives are to estimate and evaluate bacterial antagonists in irrigated rice ecosystems. The target rice pathogen is *Rhizoctonia solani* AG 1, which causes sheath blight of rice. *Rhizoctonia* sheath blight is an important disease in intensive rice production systems. It has caused significant yield losses over large areas with high inputs and under favorable production situations. The management tactics for control of this disease are limited. Chemical control, although effective, is limited to farmers in a few countries in Asia. Most rice farmers in Asia have not applied any control measures to minimize the damage caused by sheath blight. As part of the natural resources within the rice ecosystems, naturally occurring biological control agents, especially the bacterial antagonists, should be tapped for potential disease management in general and sheath blight in particular.

Methods of trapping bacterial antagonists against *R. solani*

There is a community of bacteria on the rice plant in the irrigated rice ecosystem. The functional bacteria antagonistic to *R. solani* AG 1 may consist of only a small fraction of the total. It is unlikely that we could estimate the total number of the bacterial cells and the species associated with a rice ecosystem. To achieve the project objective, it is vital to find a way to isolate and estimate the functional group that is antagonistic to *R. solani* AG 1. It is therefore essential to devise a method to estimate or isolate these bacterial antagonists. We tested a trapping method using sclerotia as a possible method to bait a large part of the bacteria that would be antagonistic to *R. solani* AG 1 and suppress sheath blight development.

The method was based on the hypothesis that existing functional antagonists *in situ* would colonize the sclerotia of the pathogen present in the system. Two methods were used in a series of experiments to test this hypothesis. Plant samples were washed in an appropriate solution with the assumption that most of the bacteria would be washed off from the plant surface. The antagonists were then isolated from the plant washings using two approaches. The first was to isolate antagonistic bacteria from sclerotia soaking in the plant washes and then plate them directly on agar media such as potato dextrose agar (PDA). The second approach was, after soaking the sclerotia in the plant washes, to wash them in sterile distilled water and make a serial dilution with the water and plate on PDA to isolate antagonistic bacteria. In both methods, a dual-culture test of the bacteria and the fungal pathogen was followed to screen out the antagonists. By doing so, we might have reduced significantly the total number of culturable bacteria carried by the plant washing and we might have isolated only the functional group of antagonists.

Using these two methods of sclerotia-baiting techniques, when the sclerotia were soaked in plant washings, most of the sclerotia (87-100%) were colonized by bacteria (Fig. 1). The germination and growth of the soaked sclerotia (62%) were inhibited to various degrees and a few of the sclerotia failed to germinate. The results indicated that the sclerotia were colonized easily by the antagonistic bacteria from the plant washings.

The efficiency of the two methods to obtain antagonistic bacteria against *R. solani* AG 1 was also similar in the experiments (Tables 1 and 2). Compared with the conventional method, that is, serial dilution plating of the plant washings directly on agar media, only a very few antagonistic bacteria (0—4 isolates per sample) were obtained, or less than 10% of the isolated bacteria on average. The efficiency of the new methods for isolating antagonistic bacteria was thus improved by plating the sclerotia soaked in the plant washings. More antagonistic bacteria were obtained (8.4 isolates per sample) and the overall efficiency was 54.9% and 39.4%, respectively, in the two experiments. However, the isolation efficiency was significantly improved when plating the sclerotia directly on PDA after soaking them in plant tissue washings. The isolation efficiency was 84.6% and 87.2% of the total bacterial isolates in the two experiments. This was followed by isolation made by plating the suspension of the sclerotia

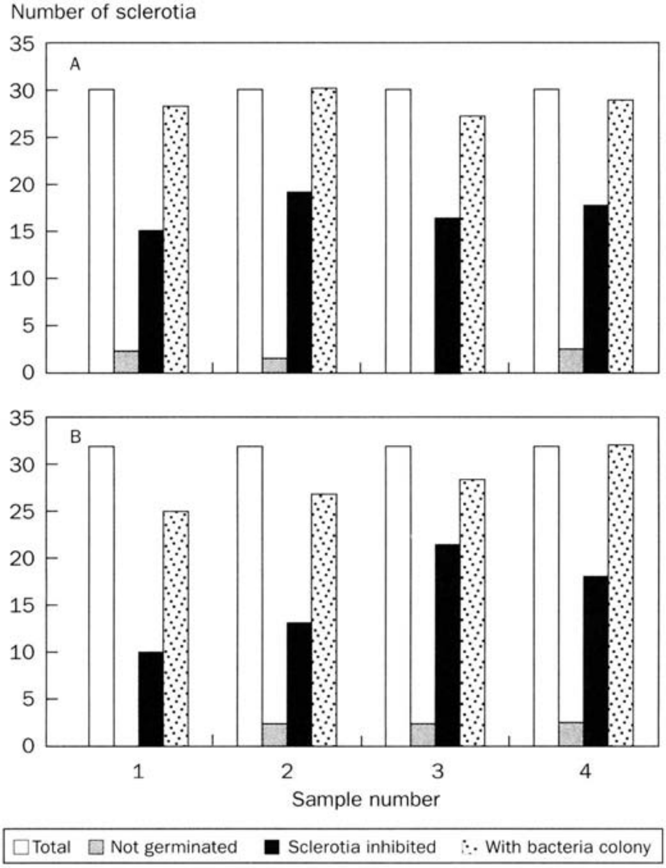

Fig. 1. Effects of plant tissue washing treatment on sclerotia of *R. solani* in two experiments (A and B).

being soaked in the plant tissue washings. As the dual-culture test is a test of mycelial growth, there is no indication that only those bacteria antagonistic to sclerotia germination were obtained. The methods are therefore efficient for isolating bacteria antagonistic to *R. solani* AG 1.

Population diversity and function of bacterial antagonists

Our purpose was to gain understanding of the population diversity of bacterial antagonists against the sheath blight pathogen. Experiments were carried out to compare total culturable bacteria from a rice plant and those that were antagonists to *R. solani* AG 1. We used a varietal demonstration plot at the IRRI experiment station as the site for sampling rice plants (leaves, stems, roots, spikes), plants of different varieties, healthy as well as diseased tissues, weeds of the sampling site, and also plants

Table 1. Number of bacterial isolates recovered with the different isolation techniques (experiment I).

Sample number	Original tissue washing			Soaked-sclerotia washing			Soaked sclerotia plated directly		
	Total	Inhibitory	Inhibitory (%)	Total	Inhibitory	Inhibitory (%)	Total	Inhibitory	Inhibitory (%)
I	28	4	14.3	23	11	47.8	14	12	85.7
II	19	1	5.3	15	8	53.3	10	9	90.0
III	31	3	9.8	21	14	66.7	15	11	73.3
IV	25	2	8.0	22	6	27.3	13	12	92.3
Total	103	10	9.7	81	39	54.9	52	44	84.6
Mean	25.3	2.5		17.8	9.8		13.0	11.0	

Table 2. Number of bacterial isolates recovered with the different isolation techniques (experiment II).

Sample number	Original tissue washing			Soaked-sclerotia washing			Soaked sclerotia plated directly		
	Total	Inhibitory	Inhibitory (%)	Total	Inhibitory	Inhibitory (%)	Total	Inhibitory	Inhibitory (%)
I	22	3	13.6	18	6	33.3	7	7	100.0
II	16	0	0	17	7	41.2	9	8	88.9
III	18	1	5.6	15	5	33.3	10	7	70.0
IV	26	2	7.7	21	10	47.6	13	12	92.3
Total	82	6	7.5	71	28	39.4	39	34	87.2
Mean	20.0	1.5		17.8	7.0		9.8	8.5	

treated and untreated with fungicides. Based on these samples, bacteria were isolated using the method of sclerotia plating from tissue washings. Functions of the bacteria were tested for antagonism against *R. solani* AG 1 on PDA plates, and their effect on rice seed germination.

The following plants and plant parts were used to sample and isolate the bacteria:

1. *Rice varieties.* Four rice varieties grown in the varietal demonstration plot at the IRRI farm were taken at the anthesis stage. The samples were replicated three times, that is, three samples per sampling of each variety at the anthesis stage. Only the leaves (leaf sheath and leaf blades) were processed for bacterial isolation.
2. *Endophytic and epiphytic bacteria of rice leaves.* Five samples were taken at the tillering stage from var. PBSRc-52 grown from different fields at the IRRI farm. The leaves (leaf sheath and leaf blades) of each sample were cut into small pieces (2—3 cm). For epiphytic bacteria, the leaf tissues were placed in phosphate buffer saline (PBS, pH 7.3) and washed by aeration for 6 h. The aliquot was diluted for bacterial isolation. To isolate endophytic bacteria, the leaf tissue was surfaced-sterilized with 95% ethanol for 1 min and then trans-

ferred to 25% sodium hypochloride for 5 min followed by washing the leaf segments in sterile distilled water a few times. The leaf tissues were then macerated with a sterile mortar and pestle. The macerated tissues were suspended in 100 mL PBS for serial dilution and isolation.

3. *Sampling plant parts.* Samples were taken from var. IR72 grown in the screenhouse. Three samples (10—15 tillers per sample) of the plant were taken at the anthesis stage. For each sample, the plants were divided into the stem, leaf blades, leaf sheath, and roots to isolate the bacteria.
4. *Sampling a transect site of the rice-field from rice to nonrice habitat of the IRRl farm.* A transect site was mapped along Pili Drive East on the IRRI experimental farm. Samples were taken from 5 sites along the transect at about 150 m between each site. The samples included the rice plant, weeds, and paddy soil. The rice crops were of different varieties in the fields and different growth stages at the time of sampling.
   a. Site 1–rice variety unknown, anthesis stage
   b. Site 2–variety unknown, crop had been harvested, ratoon seedlings were sampled
   c. Site 3–variety PSBRc-52, tillering stage
   d. Site 4–variety IR64, ripening stage
   e. Site 5–PSBRc-18, ripening stage

The soil samples were taken at each site and all possible plant tissues were removed. The samples were air-dried on filter paper. The rice leaves and weed plants were cut into 1—2-cm pieces for bacterial isolation.

A total of 4,785 bacterial isolates were obtained from different parts of the rice plant, paddy soils, and weeds grown in and around the fields. Using the dual-culture test, the bacteria could be classified into three broad groups: the antagonists, which composed 613 isolates or 12.8% of the total; 4,159 isolates or 86.9% apparently neutral to the fungal pathogen; and 13 isolates or 0.27%, which appeared to promote growth of the pathogen (Fig. 2).

There are significant differences in antagonistic bacteria present in the different parts of the rice plant grown in irrigated conditions of the tropics. Among the parts of the rice plant, leaf blades and sheaths appear to harbor a higher total bacterial population and more antagonists than other parts (Fig. 3A). Antagonistic bacteria appear to be present on all parts of the rice plant. However, the number of bacterial antagonists was about 10—20% of the total culturable bacteria. Like the total population, leaf blades and sheaths also harbored the largest number of antagonistic bacteria compared with other plant parts.

The plant samples were taken from the four rice varieties at the anthesis stage. All four rice varieties harbored different numbers of bacteria and antagonists (Fig. 3B). IR40 and IR72 appeared to have a larger bacterial population than PBSRc-52 and PBSRc-18. The difference was significant. Bacterial antagonists were also isolated from all four varieties, with more from PBSRc-18 (av 11 isolates per sample) than PBSRc-52 (av 5.7 isolates per sample).

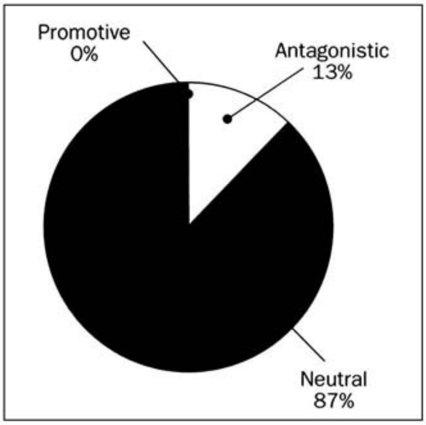

Fig. 2. Functional groups of bacteria isolated from rice systems based on dual-culture tests on potato dextrose agar plates. Only 13 isolates exhibited promotive effects of the total of 4,785 isolates; hence, the 0% reflected in the chart.

It appears that the number of bacteria detected from healthy and diseased tissues was different, as well as the morphotypes. The number of antagonists from the two types of leaf tissue was also different (Fig. 3C). The bacterial population from tissues with sheath blight lesions was 6 times (6.6 x 1010 cfu g^{-1}) more than that from healthy tissues (1.1 ´ 10^{10} cfu g^{-1}). The number of antagonists was significantly higher on diseased tissues (12 per sample) than on healthy tissues (9 per sample).

Most of the bacteria were epiphytic; however, one population clearly appeared to be endophytic based on the method used to eliminate the epiphytic population (Fig. 3D). Among the antagonists, the number of morphotypes from the endophytic population was less (5 per sample) than that from the epiphytic population.

Bacteria and antagonists were isolated from rice plants, grasses, and soil at all sampling sites along the selected transect. There was a significant difference in the population of total bacteria and the antagonists between the sources and the sites (Table 3). Rice seems to harbor the highest number of total bacteria, antagonists, and morphotypes, whereas the soil has the lowest. The proportion of antagonists in the total bacterial population in cfu g^{-1} of the sample was quite constant at all sampling sites. On average, the rice plant was a rich habitat of antagonists (22.3%), and the poorest was the soil (7.5%) and the grass weeds (17.5%). The difference among sites along the transect was also obvious. Site 5 had 41 morphotypes and the other sites had about 24—29 types.

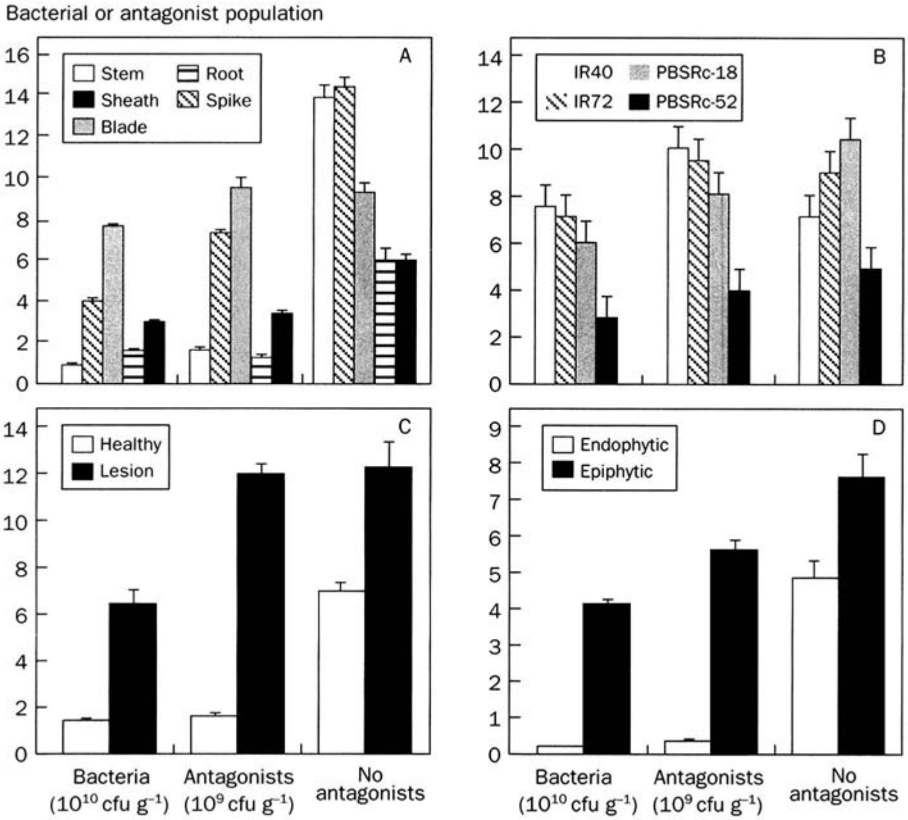

Fig. 3. Populations of bacteria and antagonists against *R. solani* AG 1 on rice plants. (A) On different plant parts, (B) on different varieties, (C) on healthy and lesion tissues, (D) on surface and inside of leaves.

Diversity of antagonistic bacteria in irrigated rice ecoystems

In relationship to *R. solani* AG 1

From all the experiments indicated above, a total of 4.785 bacterial isolates, with 613 isolates exhibiting antagonism against *R. solani,* were obtained from rice plants, paddy soils, and weeds in the rice fields and nonrice habitat adjacent to the rice fields. These antagonists were shown to have a different relationship to *R. solani* AG 1 and they also exerted a different effect on rice seed germination and growth of young rice seedlings (Table 4).

Most of the bacteria (87%) associated with the irrigated rice ecosystem appear to be neutral to *R. solani* AG 1. These bacteria did not show any observable effects on pathogen growth on PDA. About 613 or 13% of the bacteria suppressed mycelial growth or colony development of the pathogen on PDA plates. They could be further distinguished into two subgroups. One subgroup composed those isolates that exhib-

Table 3. Transect analysis of bacterial and antagonist populations.[a]

Sample site	Bearer	Bacterial population ($\times 10^{10}$ cfu g^{-1})		Number of antagonist morphotypes
		Total[b]	Antagonists	
P1	Rice	7.7156	1.1573	9
	Grass	1.5630	0.2735	11
	Soil	0.6492	0.0487	5
P2	Rice	5.5828	0.9770	14
	Grass	1.2492	0.2186	9
	Soil	0.7068	0.0530	6
P3	Rice	5.7068	0.9987	12
	Grass	1.1776	0.2061	8
	Soil	0.5068	0.0380	7
P4	Rice	4.3240	0.7567	8
	Grass	1.0972	0.1920	7
	Soil	0.3068	0.0230	9
P5	Rice	3.5762	0.6258	18
	Grass	0.6492	0.1136	15
	Soil	0.3560	0.0267	9
Mean	Rice	4.2397	0.9031	12.2
	Grass	1.1452	0.2004	10.0
	Soil	0.5052	0.0379	7.6

[a] The transect was located along Pili Drive East, IRRI experiment farm, Los Baños, Laguna, Philippines. The distance between sampling sites was about 150 m. Pigment production medium was used for plating bacteria and maintaining isolates in slants; potato dextrose agar was used for the inhibition test. [b] Each number is the mean of 3 replicating plates.

Table 4. Antagonist grouping based on their effects on the growth of young rice seedlings.

Group[a]	Number of isolates	%
S+R+	49	11.2
S+R0	47	10.8
S+R–	0	0
S0R+	36	8.2
S0R0	180	41.2
S0R–	20	4.6
S–R+	7	1.6
S–R0	30	6.9
S–R–	68	15.6
Total	437	100.0

[a] S and R refer to shoots and roots; +, 0, and – refer to growth-enhanced, not affected, and suppressed, respectively. S+R+ means both shoot and root growth are enhanced.

ited strong inhibition to the fungal pathogen. The inhibition zone may vary in size but is clearly measurable on the agar plates. More than 90% of the 613 fall into this subgroup. The other subgroup composed the fast-growing bacteria that prevented colony development of the fungal pathogen. Although there were very few yet, some bacterial isolates tended to promote the growth of the fungal pathogen on PDA. It appears that the bacteria induced fast and prosperous mycelial growth and thus made the fungal pathogen form very large colonies with a thick mycelial mat.

The proportion of each group or subgroup varies according to the sources of their isolation. For instance, in the transect analysis, about 21% and 17% of the antagonists were isolated from rice plants and weeds, respectively, whereas only 7% were isolated from the paddy soils. Likewise, the proportion also varies by plant part as well as between seasons and perhaps among crop management practices. This is very evident from the experiments (Table 5) that showed the effect of fungicide application in relation to the detection of antagonists. The results indicated that untreated plants yielded a significantly higher number of bacterial antagonists than the plants treated with validamycin, an effective fungicide for sheath blight control. Interactions occur among antagonists. When *Bacillus subtilis* B-916 was applied to the rice crop, other antagonist populations appeared to be lower than that of the untreated crop.

In relation to the germination of *R. solani* AG 1 sclerotia, the antagonists may be categorized into several groups: those that inhibited sclerotia germination completely, partially, or weakly. About 72 isolates (11 %) could inhibit sclerotia germination completely. Although in some cases a few sclerotia might eventually be able to germinate, the mycelial mat appeared to be very weak and thin. A large number of antagonistic isolates inhibited more than 50% of sclerotia germination. Although 50% or more of the isolates in this group may be able to germinate, the growth and vigor of the mycelia from the germinated sclerotia were again weak and thin. The third group of antagonists would not inhibit the germination of the tested sclerotia but the size of the colonies decreased significantly (Fig. 4).

The suppression of fungal colony development by all the antagonists was quantitative and the reduction in colony size of the pathogen differed from isolate to isolate.

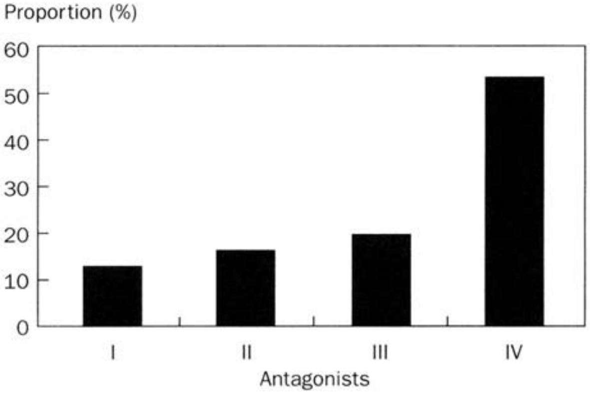

Fig. 4. Distribution of 613 antagonists based on their effect on *R. solani* sclerotium germination. I: 0 to 20% sclerotia germinated; II: 21% to 60%; III: 61% to 90%; IV: 91% to 100%.

In relationship to rice seed germination

Considering the function of the antagonistic bacteria for rice seed germination, an early report indicated that a majority of the bacteria showed no effect, whereas the antagonists were demonstrated to be neutral, growth-promoting, or deleterious (Rosales and Mew 1997). After careful analysis of the 613 antagonists, however, their effects on seed germination based on enhancement or suppression of shoots (hypocotyl), roots (radicle), or both of germinated seed, we distinguished nine possible types. Table 4 presents the details. Briefly, 65 isolates (10%) promoted seed germination, 60 isolates (10%) suppressed germination, and 264 isolates (43%) had no effect on seed germination, that is, no significant effect on shoot and root growth. However, 60 isolates (10%) promoted shoot growth but were neutral to root growth, 49 isolates (8%) improved root growth but had no effect on shoot growth, and 27 isolates (4.4%) reduced root growth without affecting shoot development. Only a very few isolates (1%) suppressed root growth but promoted shoot growth.

Based on antagonism and seed germination tests, 91 antagonists demonstrated both strong inhibition of the fungal pathogen and also promoted seed germination and seedling vigor. These isolates may offer a high potential for deployment in the field for rice production and disease control.

Functions of antagonists for sheath blight management

For antagonists to be functional in disease management, it is necessary to consider the efficacy of the population density of the applied antagonist and the inoculum density of the pathogen in actual rice production situations. Results obtained from the ADB-funded project indicated that there was a close relation between the efficacy of the antagonist and the inoculum concentration of the pathogen. Even if the antagonist is applied at a higher density, the effect on disease control may not be achieved if the disease pressure measured by the amount of inoculum at the infection site is also high. In such a case, other crop management practices may be more important for reducing disease pressure, such as a one-time fungicide application at a reduced dose to minimize the activity of pathogen pressure.

In this study, the experiments were designed to look at the immediate effect of the antagonists. As a result, the effectiveness of antagonists was demonstrated at different times of application after inoculation of the fungal pathogens. The results seem to indicate that, in the context of the sheath blight epidemic process, the antagonists are more effective in reducing inoculum efficiency for secondary spread than in reducing sheath blight severity after the pathogen has established in the plant tissues. The data also suggest that the value of antagonist application in sustainable sheath blight management may lie more in its potential to reduce disease foci instead of achieving an immediate effect with a one-time application of the antagonist to control the disease in a single crop season. Hence, the application of an antagonist for disease control should take into account the epidemic parameters of the target disease and for a long-term instead of short-term effect as shown with most fungicides (T.W. Mew, unpublished data). Therefore, the antagonist should not be treated like a fungicide.

Rice ecosystems are rich in microbial resources. Some of the microbes could be captured for disease management as well as crop management. However, the population of total bacteria and antagonistic bacteria in the irrigated rice ecosystems was affected by crop management practices. Validamycin, a common fungicide used to control sheath blight of rice, reduced the total population of bacteria as well as the antagonists significantly (Table 5). Some of the antagonists also influenced the population of other bacteria in the system. For instance, *Bacillus subtilis* strain B-916 also reduced the total bacterial and antagonistic bacteria population on the rice plants, whereas *Pseudomonas resinovorans* strain P9918 apparently had no effect on these bacteria.

The function of antagonists applied into the canopy or through seed bacterization eventually established in the canopy exposes the target site to antagonist sprays, thus allowing a higher probability of lesion-antagonist contact. In direct-seeded rice, the dense leaves could trap more of the applied antagonists, thus making antagonist-lesion contact less possible. Introduced antagonists could thus reduce inoculum efficiency for secondary spread of infection from plant to plant or leaf to leaf. Therefore, the function of antagonists is to restrict focal point expansion in the process of sheath blight development (Savary et al 1995). As most of the antagonists, except those endophytes under the present study, are all saprophytes by nature, they would unlikely function to reduce sheath blight severity measured by lesion length or relative lesion height once *R. solani* invades the plant tissues. The potential of antagonists thus seems to be related to limiting disease spread.

Pathogen inoculum and antagonist density

All antagonist strains reduced sheath blight severity significantly compared with the check, but a slight difference in their biocontrol activity was detectable. Evidently,

Table 5. Effects of fungicide and antagonist application on total bacteria and antagonistic bacteria.[a]

Measurement	Treatment	I	II	III	Mean	SE
Total bacteria (10^{10} cfu g^{-1})	Validamycin	0.8479	1.0342	1.0021	0.9614 c	0.0575
	B-916	1.8562	2.3153	2.1058	2.0924 b	0.1327
	P9918	2.8561	2.5647	2.6231	2.6813 a	0.0890
	Check	2.2511	3.0127	2.6056	2.6231 a	0.2200
Antagonist bacteria (10^{10} cfu g^{-1})	Validamycin	0.1536	0.2341	0.0895	0.1591 b	0.0418
	B-916	0.2274	0.2138	0.2804	0.2405 b	0.0203
	P9918	0.3951	0.5012	0.4749	0.5571 a	0.0319
	Check	0.4467	0.5128	0.4280	0.5625 a	0.0257
Number of antagonist morphotypes	Validamycin	3	3	2	2.6667 c	0.3333
	B-916	4	3	6	4.3333 bc	0.8819
	P9918	5	7	6	6.0000 ab	0.5773
	Check	8	6	6	6.6667 a	0.6667

[a] Data were collected from greenhouse experiment. Pigment production medium was used for plating bacteria and potato dextrose agar was used for inhibition test. Numbers followed by the same letter were not significantly different based on the Student multiple range test (SMRT) at 5%.

the larger the inoculum size of the pathogen, the less effective are the antagonists applied at manageable density to the crop (Table 6). Disease severity decreased with an increase in antagonist density. Analysis of variance with the !esion area data demonstrated very significant differences in disease severity between density of the antagonists ($P<0.001$) and the inoculum of the pathogen. For all three antagonists, applications with lower densities (10^3-10^5 cfu mL^{-1}) did not influence lesion area significantly and produced almost no control of disease development compared with the check ($P>0.05$). When rice leaves were sprayed with 10^6 cfu mL^{-1} of B-916, lesion area declined markedly ($P<0.05$) and so disease severity (area under the disease progress curve, AUDPC) decreased by 30.4%. The other three densities (10^7-10^9 cfu mL$_{-1}$) significantly lowered disease lesion area and the AUDPCs decreased by 52.5-77.3% compared with the check; however, analysis failed to show constant significant differences among the three densities of antagonists (Table 7).

The application time of the antagonist is equally important for achieving biocontrol of rice disease. Four antagonistic strains of different species (*Bacillus subtilis* strain B-916. *Pseudomonas fluorescens* strain 7-14, *P. resinovorans* strain 9409, and *P. malculicola* strain 10353) were tested for sheath blight control. Each antagonist was sprayed 2, 1, and 0 d before and 1 and 2 d after inoculation and one treatment was sprayed when lesions were visible on the leaves. Different antagonists and time of antagonist application resulted in greater and different effects on sheath blight control. With B-916 and 9409, the control was most effective when sprayed 1 d after inoculation and less effective in other treatments. For 7-14, spraying 1 d before inoculation was the most effective, but no significant control was observed in the D-2 and D+L treatments. Strain 10353 had the best control effect when sprayed immediately following inoculation, but was less effective in other treatments. The linear function was the best ($P<0.05$) for simulating the temporal dynamics of sheath blight development with the most effective time application. This indicated that antagonists could control sheath blight by altering the disease development pattern when they were applied at the right time (Table 8).

Discussion

The lowland rice ecosystem represents an environment rich in plant-associated microorganisms. Microbial communities varied significantly among crop species and agroecosystems. MacInoy and Kloepper (1995) obtained 34 genera of bacteria in 1,029 isolates from the roots and stems of cotton and sweet corn, and they postulated that these two crops might have more diverse communities than other plants. Germida and Theoret (1997) found that the rhizoplane communities of canola and wheat grown at the same site differed significantly. In our study, we found that the differences in bacterial and antagonist populations on plant leaves were highly significant among rice varieties. The numbers of bacteria and antagonists recovered from IR40 and IR72 were about 2.5 times those from PBSRc-52 plants. This result indicates that bacterial and antagonist populations and diversity are associated with crop genotypes.

Table 6. Effect of sclerotium size of *R. solani* on rice sheath blight development.[a]

BCA	Sclerotium size (mm)	Mean lesion area (cm^2) recorded at day after inoculation							
		3	4	5	6	7	8	9	AUDPC
B-916	2.5	0.0829 a	0.2108 a	0.7046 a	1.2930 a	2.203 a	3.287 a	4.242 a	9.861
	2.0	0.0229 b	0.0771 b	0.2838 b	0.7180 b	1.473 b	2.298 b	3.160 b	6.441
	1.5	0.0133 c	0.0571 b	0.1775 c	0.4570 c	0.967 c	1.596 c	2.216 c	4.369
	1.0	0.0028 c	0.0104 c	0.0838 d	0.1680 d	0.496 d	0.882 d	1.373 d	2.328
7-14	2.5	0.1533 a	0.3846 a	0.7854 a	1.579 a	2.200 a	3.564 a	4.371 a	10.770
	2.0	0.0621 b	0.2192 b	0.4642 b	0.772 b	1.281 b	2.275 b	3.163 b	6.624
	1.5	0.0475 b	0.1342 c	0.2829 c	0.520 c	0.883 c	1.512 c	2.075 c	4.393
	1.0	0.0096 c	0.0588 d	0.1367 d	0.277 d	0.486 d	0.845 d	1.285 d	2.383
9409	2.5	0.1350 a	0.2704 a	0.6583 a	1.307 a	2.087 a	2.833 a	3.493 a	8.970
	2.0	0.0563 b	0.1263 b	0.3625 b	0.811 b	1.341 b	1.789 b	2.272 b	5.544
	1.5	0.0350 c	0.0888 c	0.2533 c	0.471 c	0.858 c	1.286 c	1.654 c	3.802
	1.0	0.0058 d	0.0330 d	0.0800 d	0.238 d	0.458 d	0.814 d	1.212 d	2.232
Check	2.5	0.1956 a	0.3467 a	1.2080 a	2.360 a	3.664 a	4.854 a	5.356 a	15.210
	2.0	0.0878 b	0.2378 b	0.4911 b	1.560 b	1.991 b	2.727 b	3.586 b	8.844
	1.5	0.0711 c	0.1444 c	0.3233 c	0.820 c	1.374 c	1.803 c	2.772 c	7.273
	1.0	0.0089 d	0.0700 d	0.1956 d	0.500 d	0.993 d	1.510 d	1.806 d	5.079

[a] Each number of disease lesion area is a pooled mean of the eight antagonist density treatments. Means followed by the same letter within the same column are not significantly different ($P<0.05$) based on Duncan's multiple range test. AUDPC = area under the disease progress curve.

Table 7. Effect of application density of antagonists on sheath blight development.[a]

BCA	cfu mL^{-1}	Lesion area (mm^2) recorded at different day after inoculation							
		3	4	5	6	7	8	9	AUDPC
Check		0.1325 a	0.1575 a	0.4800 a	0.9118 a	2.1375 a	2.7468 a	3.6293 a	8.3145
B-916	10^3	0.0775 a	0.1658 a	0.4468 a	0.9608 a	1.7768 a	2.5168 a	3.3693 a	7.5904
	10^4	0.1175 a	0.1383 a	0.4658 a	0.8008 a	1.8683 a	2.5150 a	3.3968 a	7.5454
	10^5	0.0850 a	0.1558 a	0.4925 a	0.8533 ab	1.7175 a	2.4568 a	3.0458 b	7.2413
	10^6	0.0475 b	0.0675 b	0.3133 b	0.7558 b	1.0318 b	2.0718 b	2.7808 b	6.1544
	10^7	0.0025 c	0.0225 c	0.1293 c	0.5725 b	0.8168 b	1.5143 c	2.2883 c	4.2008
	10^8	0 c	0 c	0.0508 c	0.2633 c	0.5418 c	1.5075 c	1.8500 cd	3.2885
	10^9	0 c	0.0033 c	0.0433 c	0.1575 c	0.3993 c	0.6468 d	1.5200 d	2.0102
7-14	10^3	0.0868 ab	0.2693 ab	0.5910 a	1.0975 a	1.5143 a	2.2008 a	2.9508 a	7.1918
	10^4	0.1018 a	0.3318 a	0.5868 a	1.0693 a	1.4258 a	2.4443 a	2.9408 a	7.3793
	10^5	0.0950 a	0.2533 b	0.5108 ab	0.9333 ab	1.3668 ab	2.5275 a	3.1543 a	7.2163
	10^6	0.0700 ab	0.2008 b	0.3988 b	0.6768 b	1.1608 bc	1.8200 b	2.7378 b	6.1621
	10^7	0.0408 b	0.1033 c	0.2050 c	0.4383 c	0.8543 c	1.5918 bc	2.1150 c	4.1705
	10^8	0.0325 b	0.1075 c	0.2493 c	0.4893 bc	0.9683 c	1.8950 b	2.6250 b	5.0382
	10^9	0.0125 b	0.0775 c	0.1708 c	0.3983 c	0.7343 c	1.3708 c	2.0057 c	3.7608
9409	10^3	0.0750 a	0.1750 a	0.5183 a	1.1208 a	1.6083 a	2.5150 a	2.9918 a	7.4721
	10^4	0.1175 a	0.2258 a	0.5543 a	1.0193 a	1.7500 a	2.0351 a	2.8250 a	7.0558
	10^5	0.0850 a	0.1983 a	0.4853 a	0.8058 b	1.5283 a	1.7825 b	2.5615 b	6.1235
	10^6	0.0475 b	0.1000 b	0.1732 b	0.4333 b	0.7616 b	1.0175 c	1.5200 c	3.2694
	10^7	0.0025 c	0.0535 b	0.1350 b	0.3225 c	0.7025 b	0.9825 c	1.3150 c	2.8548
	10^8	0 c	0.0450 b	0.1465 b	0.2293 c	0.4983 c	0.9375 c	1.5093 c	2.6113
	10^9	0 c	0.0308 b	0.1025 b	0.2450 c	0.4793 c	0.9358 c	1.3900 c	2.4884

[a] Each number of disease lesion area is a pooled mean of the four *R. solani* density treatments. Means followed by the same letters are not significantly different ($P<0.05$) based on Duncan's multiple range test compared with the check treatment. AUDPC = area under the disease progress curve.

Table 8. Simulation models of lesion expansion dynamics obtained for treatments with different antagonists at the most effective time.[a]

BCA	Treatment	Function	Simulated model	R^2	S_{xt}	Significance
B-916	D+1	Linear	$x = -0.1332 + 0.0544\,t$	0.9169	0.0299	$P<0.01$
		Logistic	$\text{logit}\,(x) = 5.0431 - 0.5923\,t$	0.8826	0.3944	$P<0.05$
		Gompertz	$\text{gompit}\,(x) = 1.8992 - 0.2312\,t$	0.8984	0.1428	$P<0.05$
9409	D+1	Linear	$x = -0.1588 + 0.0626\,t$	0.9745	0.0185	$P<0.001$
		Logistic	$\text{logit}\,(x) = 5.0540 - 0.6874\,t$	0.8783	0.4673	$P<0.05$
		Gompertz	$\text{gompit}\,(x) = 2.0281 - 0.2677\,t$	0.9304	0.1250	$P<0.01$
7-14	D–1	Linear	$x = -0.2893 + 0.1046\,t$	0.9347	0.0505	$P<0.001$
		Logistic	$\text{logit}\,(x) = 8.0637 - 0.9574\,t$	0.8367	0.7724	$P<0.05$
		Gompertz	$\text{gompit}\,(x) = 2.4735 - 0.3969\,t$	0.9003	0.2410	$P<0.05$
10353	D–0	Linear	$x = -0.0972 + 0.0487\,t$	0.9653	0.0170	$P<0.001$
		Logistic	$\text{logit}\,(x) = 4.3692 - 0.4872\,t$	0.8749	0.3365	$P<0.05$
		Gompertz	$\text{gompit}\,(x) = 1.6782 - 0.1966\,t$	0.9025	0.1054	$P<0.05$
CK		Linear	$x = -0.2606 + 0.1251\,t$	0.8942	0.0792	$P<0.05$
		Logistic	$\text{logit}\,(x) = 4.3300 - 0.7205\,t$	0.9694	0.2336	$P<0.01$
		Gompertz	$\text{gompit}\,(x) = 2.0963 - 0.4134\,t$	0.9733	0.1250	$P<0.001$

[a] x in the models refers to sheath blight severity (%) at time t, logit $(x) = \ln[(1 - x)/x]$, gomit $(x) = \ln/\ln(x)$. Level of significance of r: $P_{3,0.05} = 0.878$, $P_{3,0.01} = 0.959$.

Within a cropping system, the pathogen should be more closely related to its antagonists than to other microbes coexisting in the ecosystem. Chen et al (1999) reported that many more bacterial antagonists against rice sheath blight could be isolated from sclerotia of the pathogen and the diseased tissue than from the other tissues of rice plants. Mew et al (1993) described similar results. In our study, the populations of total bacteria and antagonists on plant tissues with sheath blight lesions were about 6 to 10 those on healthy sheath tissues and there were significantly more antagonist morphotypes on the diseased tissues. These results indicate that the sheath blight pathogen could be very closely correlated with its antagonists in their rice habitats.

There was a significant difference between the populations of epiphytic and endophytic bacteria/antagonists. The endophytic bacterial antagonist populations were only about 5% of the epiphytic populations. Taxonomic identification of the bacterial isolates was not performed in our project but initial distinction of the antagonists based on morphological characters of the colonies on pigment production medium (PPM) plates and slants was similar. The study of Germida and Theoret (1997) on canola revealed that the interior bacterial populations were a subset of the exterior community. However, more information on accurate identifications of the isolates is obviously needed to establish the relationship.

The transect analysis showed that both neutral bacteria and pathogen antagonists were present on rice plants and weeds and in the paddy soil. Significant differences existed in total bacterial populations and the antagonists among rice, grass, and soil, and also among sampling sites along the transect. Overeas and Torsvik (1998) compared the microbial diversity and community in two different agricultural soils and elucidated significant differences in both total and culturable bacterial populations between the organic and sandy soil. Germida and Theoret (1997), however, failed to show any difference in bacterial diversity among canola samples from different sampling sites. With our study, therefore, the difference in total bacteria/antagonists on rice crops might have resulted from variations in rice varieties or growth stages; the difference in weeds was probably more related to different weed species. The antagonist populations in the paddy soils from different sites were also significantly different and this was probably correlated with the soil fertility and rice varieties at these sites.

The functions of bacteria can be described in various ways according to their relationships with different organisms coexisting in the same niche. In our study, three functional groups were described with special reference to their relation to the sheath blight pathogen. In crop disease biocontrol, most effort has focused on antagonists with inhibition to the target pathogen, those that are highly competitive with the pathogen due to fast growth rate. As a result, research has neglected strong competition for nutrient and space. The literature has no information concerning bacteria with a pathogen growth-promoting effect. Pathogen-promoting bacteria might be related to the fast buildup of disease inoculum and thus contribute to the synergistic development of disease epidemics on field crop plants.

Effects of antagonists on the pathogen and plant growth through sclerotia and seed germination suggested that antagonistic bacteria associated with the rice ecosystem were rather diverse on the pathogen and on rice plants. Four major categories can be distinguished. Many isolates of the first type were less effective in inhibiting sclerotium germination or the subsequent mycelial growth of the pathogen, and of no influence on rice seed germination and seedling development. Normally, these isolates should be excluded from the list of antagonistic isolates to be further examined. The second group had some isolates that were very effective in suppressing the pathogen but were also deleterious to rice plant development. Similarly, this type of isolate would not be considered for further selection tests. The third type of isolate was able to promote plant seedling growth but was weak in suppressing the pathogen. The fourth group, the rest of the isolates, could suppress pathogen development very effectively and slow promotion of or have no effect on seed germination and seedling growth. This fourth group of antagonists could be the ideal candidates for field tests and application in a screening program for sheath blight management.

Pesticides used in crop production affected the total as well as antagonistic bacterial population. On wheat and barley crops, mancozeb reduced nontarget fungal populations significantly and prevented recolonization by some of these fungi for more than 24 d after spraying, but triademefon had only a minor effect on the fungal community (Southwell et al 1999). Some sheath blight management strategies were also examined for their effects on bacterial and antagonist populations in our study. Our results showed that the validamycin fungicide reduced the populations of both bacteria and antagonists on rice plants significantly. When the two biocontrol agents were sprayed, *Bacillus subtilis* strain B-9 16 also reduced bacterial and antagonist populations markedly on rice plants, but *Pseudomonas resinovorans* strain P9918 had no apparent effect. We might hypothesize here that the differences in populations of bacteria and antagonists among the four rice varieties, described and discussed earlier in this chapter, could be correlated with disease resistance and some other traits of these varieties. The effect of management strategies on microbial and antagonist populations and diversity would be an important issue in sustainable crop disease management. The results discussed here could have some implications in this respect, but more detailed investigations are necessary to further address the relationships between microbial/antagonist diversity and disease management.

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Notes

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Genetic diversity

Varietal diversification and disease management

C. Mundt

One of several strategies to extend the longevity of host-plant resistance is to grow mixtures of plants that possess different race-specific resistance genes. After many years of discussion, the diversity approach is beginning to be applied in commercial agriculture. The efficacy of mixtures for disease control is affected by spatial scale, with mixtures performing better in commercial production than in small-scale experimental plots. Limited field observations have not detected rapid selection for pathogen races with multiple virulence genes in host mixtures, though mixtures should be managed to avoid such an occurrence. Identification of useful variety combinations with functional diversity requires knowledge of both host and pathogen populations, as well as significant field testing. Diversity of crops, diversity of people, and diversity of lighthouse approaches are all needed, at different spatial scales, to contribute to the sustainability of disease management through host diversity.

The use of host-plant resistance is one of the most desirable disease control methods, as it is of low cost to crop producers and very sound environmentally. Unfortunately, the single-gene resistances that can be manipulated most easily in breeding programs are often specific to particular races of a pathogen and, therefore, the use of resistance in pure stands can sometimes result in rapid selection for new, virulent races of the pathogen, nullifying the effectiveness of the resistance (Browning et al 1969, Wolfe and Schwarzbach 1978, Kiyosawa 1989, Bonman et al 1992). This sometimes ephemeral nature of disease resistance is especially serious given that many years are required to produce a new variety of a crop, and that a limited supply of resistance genes is available for use in agriculture.

One of several strategies to extend the longevity of host-plant resistance is to grow mixtures of plants that possess different race-specific resistance genes (Browning and Frey 1969, Mundt and Browning 1985, Wolfe 1985). Multiline varieties (mixtures of lines bred to be phenotypically uniform for important agronomic traits) and variety mixtures (mixtures of cultivated varieties with no attempt to breed for phenotypic uniformity) have been shown to reduce disease, increase yields, and improve yield stability (Browning and Frey 1969, Mundt and Browning 1985, Wolfe 1985). Most mixture research has been with biotrophic pathogens that cause polycyclic, foliar diseases of small grains (Browning and Frey 1969, Mundt and Browning 1985, Wolfe 1985). However, more recent data (summarized in Garrett and Mundt 1999) suggest that mixtures can sometimes be useful with larger plants such as potatoes and trees, against nonspecific pathogens, and even for monocyclic, soilborne pathogens.

Several mechanisms have been postulated to explain the reduction in severity of disease caused by polycyclic, foliar pathogens in host mixtures (Wolfe 1985, Garrett and Mundt 1999). There seems to be agreement, however, that increased distance between plants of the same genotype, which dilutes pathogen inoculum during dispersal, is likely the most important mechanism (Wolfe 1985). Leonard (1969) derived equations to predict rates of pathogen increase in mixtures as compared with monocultures in simple model systems consisting of a single pathogen genotype and two host genotypes, with one host being susceptible and the other being resistant to that pathogen genotype. He concluded that the apparent infection rate will be proportional to the logarithm of the proportion of susceptible plants in a mixture, and this relationship has been confirmed experimentally for several polycyclic, foliar diseases of small grains (Leonard 1969, Burdon and Chilvers 1977, Luthra and Rao 1979a,b, Elliot et al 1980, Koizumi and Kato 1987). In commercial agriculture, host and pathogen populations will usually be more complex, but such simple mixtures provide an excellent model for testing whether host diversity affects epidemic development for a given host/pathogen system (Garrett and Mundt 1999). Other mechanisms could play an important role, such as induced resistance (Chin and Wolfe 1984b, Calonnec et al 1996) or changes in microenvironment (Zhu et al 2000).

After many years of discussion, the diversity approach is beginning to be applied in commercial agriculture. Multiline varieties have been used to control rust diseases of coffee (Ruiz and Zapata 1990), oats (Browning and Frey 1981), and wheat (Borlaug 1981, Allan et al 1983). Currently, more emphasis is being given to the use of variety mixtures (Wolfe 1985). Approximately 300,000 ha of barley (*Hordeum vulgare*) variety mixtures were grown in the former East Germany to control powdery mildew (caused by *Erysiphe graminis*) (Wolfe 1992). Wheat variety mixtures are gaining popularity in the Pacific Northwest of the United States, for several reasons, and are increasing in popularity each year (Mundt 1994). In fall 1998, 10% of the soft white winter wheat area of Oregon was sown to variety mixtures, for a total of 32,000 ha of mixtures (Korn 1998). In the state of Washington, 12.7% of the soft white winter wheat area was sown to variety mixtures in fall 1998, for a total of 96,000 ha of mixtures. In addition, 62,000 ha of club wheat were sown to the multiline variety Rely, which represents 76% of the Washington club wheat area (http://www.nass.usda.gov/wa/annual98/wheat98.htm). More recently, wheat variety mixtures have been investigated in Kansas, with very positive grower reaction (Kessler 1997). In the 1998-99 winter wheat season, 6.1% of the Kansas wheat crop was sown to variety mixtures, for a total of 227,000 ha (Bill Bockus, Kansas State University, personal communication). Variety and species mixtures are also gaining significant popularity in several European countries (Wolfe 1997).

Mixtures and spatial scale of deployment

The efficacy of mixtures for disease control is affected by spatial scale, with mixtures performing better in commercial production than in small-scale experimental plots (Wolfe 1985). For example, the proportion of the barley area sown to variety mixtures in the former East Germany gradually increased from 0 in 1980 to 92% by 1990. Correspondingly, mildew severity gradually declined from 50% in 1980 to 10% in 1990 (Wolfe 1992). Similar, but less extensive, observations have been made for wheat stripe rust in Oregon, USA (Mundt 1994). More recently, rice (*Oryza sativa*) variety mixtures were tested experimentally on 812 and 3,342 ha in Yunnan Province, China, in 1998 and 1999, respectively. Mixtures reduced the severity of blast (caused by *Magnaporthe grisea*) on susceptible sticky rice varieties by an impressive 94% compared with monocultures of the same varieties, and increased crop productivity substantially (Zhu et al 2000).

The discrepancy between mixture performance in experimental plots versus commercial production has several explanations. First, mixtures seem to be much more sensitive to the effects of interplot interference than are many other types of experimental treatments (Wolfe 1985, Mundt 1994, Garrett and Mundt 1999). Second, artificial inoculation at unnaturally high levels can reduce mixture performance (Wolfe 1985, Garrett and Mundt 1999). Third, as discussed below, mixture performance at larger spatial scales may improve relative to monocultures for pathogens that are dispersed in turbulent air.

Minogue and Fry (1983) and van den Bosch et al (1988a) developed mathematical theories of epidemic spread, which, due to assumptions and model construction, always predict a constant velocity (v, $m\ d^{-1}$) of epidemic increase in time and space, that is, $v = r/b$, where r is the temporal rate of disease increase and b is a measure of the steepness of the pathogen's dispersal gradient in space. This theory would imply that differences in rate of epidemic velocity between variety mixtures and monocultures would be constant with time and distance. Through consideration of previous theoretical models (van den Bosch 1988a,b) and field studies with wheat stripe rust, van den Bosch et al (1990) concluded that, "In an ideal mixture of susceptible and resistant plants, the radial velocity of focus expansion increases linearly with the logarithm of the proportion of susceptible plants." This logarithmic relationship was confirmed in a subsequent study with bean rust, caused by *Uromyces appendiculatus* (Assefa et al 1995). What is not clear from the work of van den Bosch et al (1990) and Assefa et al (1995), however, is whether velocities were constant with distance from the source. This in fact, would be very difficult to determine for these two mixture studies, as the plot sizes used allowed epidemic development to be followed for a maximum of only 1.65 m (van den Bosch et al 1990) and 2 m (Assefa et al 1995). Further, several experimental artifacts can cause one to incorrectly conclude that epidemic velocity is linear with time and distance (Mundt, unpublished).

In contrast, Ferrandino (1993) presented an analysis that questions the assumption of a constant velocity of epidemic increase. He noted that a traveling wave of constant velocity should be expected only if the tail of the dispersal distribution follows an exponential function. Because of the physics of turbulent air movement, an exponential function is expected only if there is insignificant dispersal of inoculum out of the canopy in the vertical direction (Aylor 1987, Ferrandino 1993), which would result in a constant rate of reduction in spore deposit per unit of distance, that is, $dy/dx = -b$, where y = inoculum concentration, x is distance from the source of inoculum, and b is the steepness of the gradient. However, if spores do escape the canopy, the probability of doing so will increase with distance from the point of production (Aylor 1987, Ferrandino 1993). This would then result in a dispersal gradient that is steep near the source and becomes increasingly flattened with distance, as is the case with the "power law," that is, $dy/dx = -b/x$, and an epidemic velocity that increases in time and space, that is, $v = rx/b$. A "first principles" model of spore dispersal applied to data sets from six different rust epidemics on three different crops indicated that the velocities of epidemics were predicted to increase with distance from the initial inoculum source (Ferrandino 1983). This would suggest that the rate of epidemic velocity between mixtures and monocultures would be greater at a large spatial scale than at a small spatial scale (Mundt, unpublished).

Theory suggests that variety mixtures become less effective for disease control as plants of the same variety become more aggregated (Mundt and Leonard 1986), such as random mixtures of plants versus row mixtures versus interfield diversification. However, this also depends on the degree of aggregation of the pathogen population, with less effect of aggregation for focal patterns than for uniform patterns of initial inoculum (Mundt and Leonard 1986, Mundt et al 1986). Further, even for uniform patterns of inoculum and high host aggregation, diversification may increase with total size of the host population under consideration (Mundt and Brophy 1988, Mundt et al 1996).

Mixtures and pathogen evolution

A concern with the use of mixtures is that they may select for complex races able to overcome multiple host-resistance genes. Limited field observations have not detected rapid selection for complex races in mixed populations (Segal et al 1980, Mundt and Browning 1985, Wolfe 1985). Unfortunately, neither mathematical (Leonard and Czochor 1980, Marshall 1989, Lannou and Mundt 1996, 1997) nor empirical (Chin and Wolfe 1984a, DiLeone and Mundt 1994, Huang et al 1994, Kolmer 1995) approaches have provided a definitive answer to this question. Most models of pathogen complexity have assumed that there will be a cost of virulence to counter directional selection toward increased pathogen complexity (e.g., Marshall 1989), though the consistent presence of such a cost is doubtful for agricultural systems (Parlevliet 1979). Other mechanisms of selection against pathogen complexity may be operative (Mundt and Browning 1985, Chin and Wolfe 1984a, Lannou and Mundt 1996, 1997), though their importance is not yet clear. Regardless of the mechanisms involved in influencing selection for pathogen complexity in mixtures, variety mixtures should be managed to prevent or slow the evolution of such complexity (Wolfe 1985).

Implementation considerations

Varieties grown in mixtures must be compatible in height, duration, and quality. There are many potential ways to ensure such compatibility (Wolfe 1985, Mundt 1994, Zhu et al 2000), depending on the specific farming situation. For example, rows of glutinous and nonglutinous rice could be alternated in Yunnan Province, China, because the crop is hand-harvested (Zhu et al 2000). For mechanized production of wheat and barley, varieties with similar agronomic and quality characteristics have successfully been mixed and harvested together (Wolfe 1985, Mundt 1994). The quality of mixed grain can be maintained in mixtures, even for demanding markets such as the malt industry (Wolfe 1985).

Effort is required to implement a successful varietal diversification program. Identification of useful variety combinations with functional diversity (Schmidt 1978, Mundt and Browning 1985) requires knowledge of both the host and pathogen populations, as well as significant field testing. Implementation of varietal diversity will benefit from a balance of basic and applied research; understanding the underlying mechanisms by which variety mixtures function will increase the chances of identifying favorable variety combinations, whereas applied research will provide opportunities to identify further basic research questions. Successful mixtures must not only control disease but also provide high yields and favorable crop quality. Thus, interaction of pathologists, breeders, and agronomists is critical. The importance of involving farmers and extension personnel is crucial to ensure that a practical approach is chosen, and this cannot be overemphasized.

The “lighthouse”concept has been a key factor in the Asian Development Bank-funded project on “Exploiting Biodiversity for Sustainable Pest Management.” It is important to note that the lighthouse concept, like the concept of diversification itself, applies at different spatial scales. Ultimately, implementation requires farmer-to-farmer transfer of knowledge, and early adopters of diversity provide a lighthouse for neighboring farmers. At the other extreme, reporting of the impacts of diversity in the international scientific literature provides the potential to transfer concepts across continents and cropping systems. Lighthouse activities at intermediate scales are relevant to knowledge transfer regionally and nationally. Diversity of crops, diversity of people, and diversity of lighthouse approaches are all needed, at different spatial scales, to contribute to the sustainability of crop production.

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Rice blast control with multilines in Japan

S. Koizumi

The current situation of rice blast control with multilines and its studies in Japan are summarized for durable and effective control of the disease. From 15 different recurrent rice cultivars, near-isogenic lines with different genes with complete resistance to blast have been developed or are being developed to control the disease in Japan. One group of these, the Sasanishiki multilines, has been registered and cultivated in northern Japan. Mechanisms of blast reduction in multilines are also discussed. A reduction in susceptible plants, the barrier effect of resistant plants, and induced resistance are considered to contribute to blast reduction in multilines and cultivar mixtures. The effectiveness of the Sasanishiki multilines for blast control and the changes in blast races in them were investigated. Results indicated that an increase in the number of components and proportion of resistant components is necessary for effective blast control with multilines. Since available genes with complete resistance to blast are limited in Japan, multilines with high levels of partial resistance are required.

Because of conducive environmental conditions during the rice-growing season, blast caused by *Pyricularia grisea* (telemorph *Magnaporthe grisea*) (Rossman et al 1990) is the most destructive disease (0.5–5.6% annual yield losses) in Japan (Koizumi 1998). To control the disease, many rice cultivars with complete resistance have been developed. However, their resistance has broken down within several years after their release because of the increase in new blast races virulent to the resistance (Kiyosawa 1974, Yamanaka and Yamaguchi 1987). To prevent this breakdown in resistance, the use of multilines proposed by Jensen (1952) and Borlaug (1959) was suggested, and a reduction in blast development in mixtures of rice cultivars and near-isogenic lines with different complete resistance has been reported (Koizumi 1983, 1994, Shindo and Horino 1989, Koizumi and Fuji 1994, Koizumi et al 1996, Nakajima et al 1996a).

Sasanishiki multilines were the first registered rice multilines to control blast under the conditions in Japan and they were released in 1995 (Matsunaga 1996). The multilines have been effective for blast control although their composition has changed. In this chapter, the current situation of blast control with multilines and its studies in Japan will be discussed.

Development of multilines

Table 1 shows the near-isogenic lines (NILs) being used to control rice blast with multilines in Japan. Among 15 genes with complete resistance to blast identified in rice cultivars in Japan (Kiyosawa 1997, Hayashi et al 1998), 13 genes (*Pik-s, Pik-p, Pik-h, Pia, Pii, Pik, Pik-m, Piz, Piz-t, Pita, Pita-2, Pib,* and *Pit*) were used to develop NILs to compose multilines (Table 1). Genes with partial resistance to blast are not yet employed to develop NILs since they have not been completely analyzed.

Sasanishiki multilines, named Sasanishiki BL, were the first registered multilines to control rice blast in Japan (Matsunaga 1996) and they were released in 1995. A leading rice cultivar, Sasanishiki, was used as the recurrent parent to develop the multilines. Sasanishiki holds a gene, *Pia,* with complete resistance to blast, but the level of partial resistance to the disease is low. Nine NILs were developed from the recurrent parent Sasanishiki by five to eight backcrosses at the Miyagi Prefectural Furukawa Agricultural Experimental Station. One (*Pik-s* line) of the nine NILs lacks genes with complete resistance effective against almost all Japanese strains of rice blast fungus, and each of the other NILs has a different gene with complete resistance (*Pii, Pik, Pik-m, Piz, Pita, Pita-2, Piz-t,* and *Pib*) to blast in addition to the *Pia* gene derived from the recurrent parent, although the *Pia* gene in the *Pita-2, Piz-t,* and *Pib* lines has not been confirmed yet since we do not have the blast fungus strains to check it in them. Seven NILs, except for the *Pik-s* line, are currently registered. Three lines, *Pik, Pik-m,* and *Piz,* of the seven were first mixed in a proportion of 4:3:3 and used as a multiline in 1995, and the mixed proportion was changed to 3:3:4 in 1996. Beginning in 1997, the *Piz-t* line was added to the three. Now, the *Pik, Pik-m, Piz,* and *Piz-t* lines are mixed in a proportion of 1:1:4:4 and cultivated as a multiline (Matsunaga 1996). The multilines have been effective for blast control and fungicide is applied only one time for panicle blast control, whereas fungicides are commonly applied four to five times to ordinary rice cultivars. The multilines were cultivated on 5,453 hectares in Miyagi Prefecture of northern Japan in 1997 and their cultivated area is now about 1,400 ha.

NILs of Nipponbare and Toyonishiki are already developed; however, they are not yet cultivated as multilines in farmers' fields, although their mixtures were proved to reduce blast development (Shindo and Horino 1989, Koizumi and Fuji 1994). Other NILs are now being developed and most of their recurrent parents are leading rice cultivars whose levels of partial resistance to blast are low, although they have the good eating quality requested by consumers (Table 1).

Mechanisms for reducing rice blast in multilines

A reduction in the number of susceptible plants. the barrier effect of resistant plants, and induced resistance by incompatible blast races are considered to contribute to

Table 1. Near-isogenic lines used to control rice blast with multilines in Japan.

Recurrent parent	Complete resistance genotype of recurrent parent	Resistance genes introduced into NILs	Breeding place	Year released (developed)
Sasanishiki	*Pia*	*Pik-s, Pii, Pik, Pik-m, Piz, Piz-t, Pita, Pita-2, Pib*	Miyagi Pref.	1995
Hitomebore	*Pii*	*Pik, Pik-m, Piz, Piz-t, Pib, Pita, Pita-2*	Miyagi Pref.	–[a]
Manamusume	*Pii*	*Pik, Pik-m, Piz-t, Pib, Pita*	Miyagi Pref.	–
Nipponbare	*Pik-s/Pia*	*Pii, Pik, Piz, Piz-t, Pita-2, Pib*	Natl. Agric. Res. Cent.	(1984)
Hokkai 241		*Piz, Pib, Pita-2, Piz-t, Pit*	Hokkaido Natl. Agric. Exp. Stn.	(1981)
Toyonishiki	*Pia*	*Pii, Pik, Pita, Pita-2, Piz-t*	Tohoku Natl. Agric. Exp. Stn.	(1988)
Koshihikari	*Pik-s*	*Pia, Pii, Pik, Pik-p, Pik-m, Piz, Piz-t, Pita, Pita-2, Pib*	Niigata, Toyama, and Fukui Pref.	–
Hinohikari	*Pia, Pii*	*Pik-m, Pita, Pita-2*	Miyazaki Pref.	–
Maihime	*Pia*	*Pii, Pik-h, Pik-m, Piz, Pita, Pita-2, Piz-t, Pib*	Aomori Pref.	–
Hanaechizen	*Piz*	*Pik, Piz-t, Pita, Pita-2, Pib*	Fukui Pref.	–
Etsunan 157	*Pia*	*Pii, Piz, Piz-t, Pita-2*	Fukui Pref.	–
Mineasahi	*Pia, Pii*	*Pik, Pik-m, Piz, Piz-t, Pib, Pita, Pita-2*	Aichi Pref.	–
Chubu 64	*Pii*	*Pik, Pik-m, Piz, Piz-t, Pib, Pita, Pita-2*	Aichi Pref.	–
Kinuhikari	*Pii*	*Piz-t, Pib, Piz, Pita-2*	Hokuriku Natl. Agric. Exp. Stn.	–
Akitakomachi	*Pia, Pii*	*Pik, Pik-m, Piz, Piz-t, Pita, Pita-2, Pib, Pit*	Akita Pref.	–

[a] Near-isogenic lines that are being developed.

reducing rice blast in multilines and cultivar mixtures (Koizumi 1983, Nakajima et al 1996b). Stabilizing selection of the pathogen population (van der Plank 1963) is also supposed to cause a reduction in rice blast in multilines. However, it is not yet clear whether the stabilizing selection operates in multilines or not.

Reduction in susceptible plants

In multilines, a reduction in susceptible plants decreases the multiplication foci of the pathogen and reduces disease development (Chin and Wolfe 1984, Mundt 1994). This mechanism operates in multilines and cultivar mixtures to control rice blast also.

In mixtures of susceptible rice cultivar Toyonishiki and its resistant NIL, an increase in the resistant NIL decreased leaf blast severity (Shindo and Horino 1989; Fig. 1). Moreover, in upland nursery experiments, the equation $r_m = r_s + c \log_e m$, proposed by Leonard (1969), where r_m is the apparent infection rate (r) in mixtures of susceptible and resistant rice cultivars, r_s is the single planting of susceptible plants, m is the proportion of susceptible plants in the mixtures, and c is a constant, was applicable to leaf blast (Fig. 2; Koizumi and Kato 1987, Koizumi 1994).

Barrier effect of resistant plants

In multilines and cultivar mixtures, resistant plants block spore dispersal of the causal fungus among susceptible plants. Figure 3 shows that the resistant plants interrupt the horizontal spread of leaf blast from the inoculum source in the mixtures of resistant and susceptible rice cultivars (Koizumi 1994).

The resistant plants also obstruct the vertical spread of leaf blast in rice canopies of the mixtures of resistant and susceptible plants (Fig. 4; Koizumi et al 1999). Since leaf blast lesions in the upper layer of rice canopies are important inoculum sources for panicle blast, the obstruction to the vertical spread of leaf blast reduces panicle blast severity in multilines (Ashizawa et al 2000).

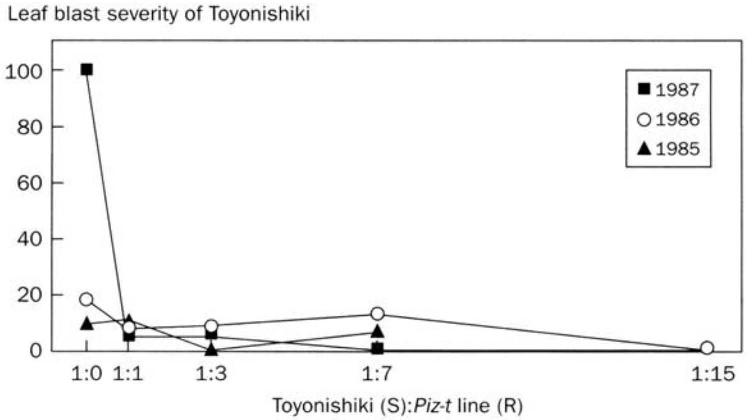


Fig. 1. Reduction in leaf blast severity in mixtures of rice cv. Toyonishiki (susceptible) and its near-isogenic *Piz-t* line (resistant) (Shindo and Horino 1989).

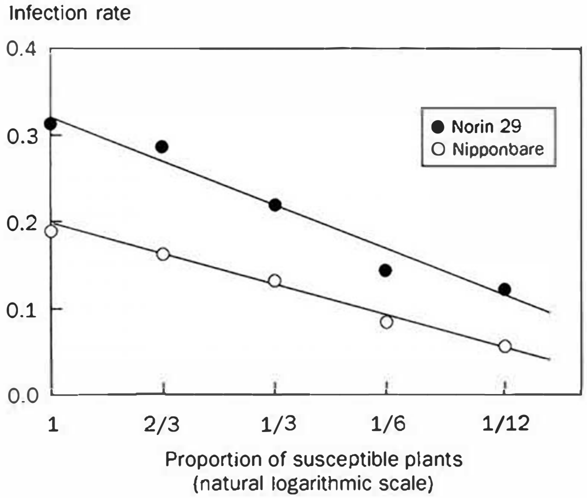


Fig. 2. Relationship between infection rates of rice leaf blast and proportion of susceptible plants in mixtures of susceptible and resistant plants in the upland nursery experiment in 1981. Mixtures consisted of rice cultivars Norin 29 or Nipponbare for susceptible plants and Toride 1 for resistant plants.

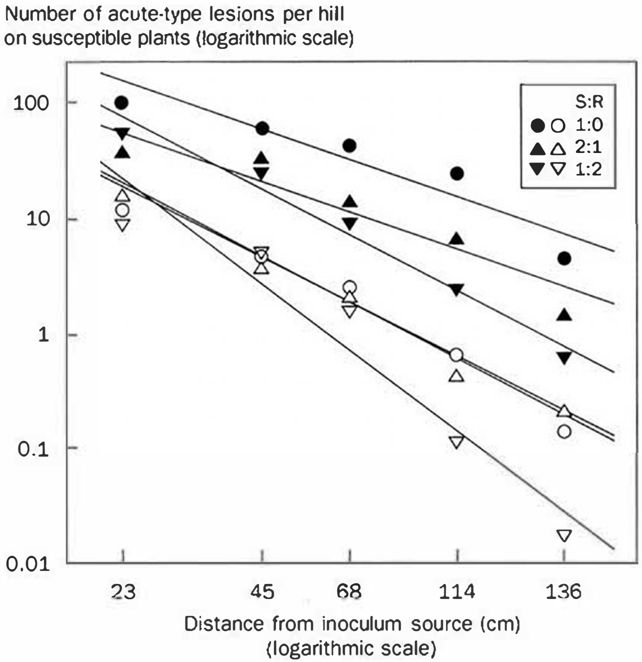


Fig. 3. Gradients of number of acute-type rice leaf blast lesions per hill on susceptible plants from the inoculum source in mixtures of susceptible (S) and resistant (R) plants and pure stands of susceptible plants in the paddy field experiment in 1981. Mixtures consisted of rice cultivars Norin 29 or Nipponbare for susceptible plants and Fukunishiki for resistant plants. For the inoculum source, diseased rice seedlings were transplanted in the center of each plot. Disease severity was evaluated on 20 August. Solid symbols represent the values of Norin 29 and open symbols denote those of Nipponbare.

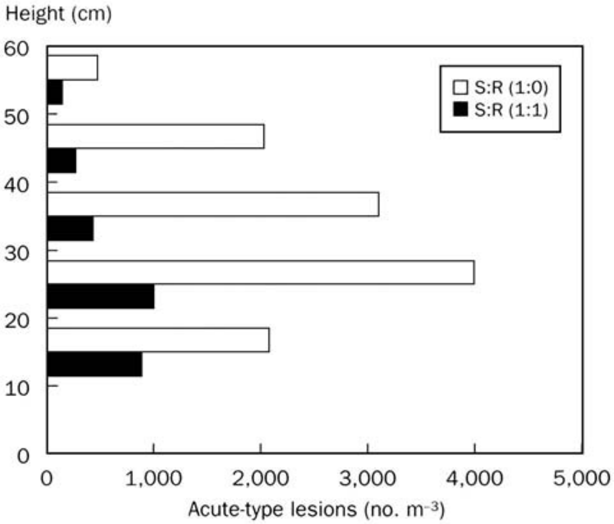


Fig. 4. Vertical distributions of leaf blast lesions within rice canopies in mixture of susceptible rice cultivar Sasanishiki (S) and its resistant near-isogenic *Piz-t* line (R) and a pure stand of S in a paddy field trial in 1998. The number of acute-type lesions that occurred naturally was recorded on 15 July.

Induced resistance

Preinoculation with incompatible strains of blast fungus causes induced resistance in rice plants and it reduces the severity of leaf and panicle blast by compatible blast fungus strains (Iwano 1987, Ashizawa et al 1999). Nakajima et al (1996b) believe that the induced resistance operates to depress leaf blast in the Sasanishiki multilines since the disease severity in the multilines with the inoculum sources inoculated with compatible and incompatible blast strains was less than that in those having inoculum sources from compatible blast strains (Fig. 5).

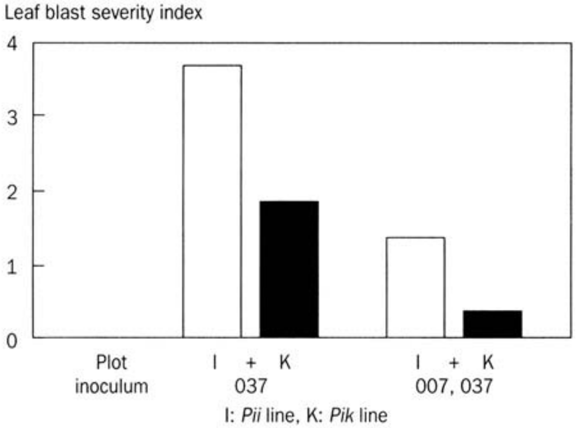
The induced resistance works only on the leaves preinoculated with incompatible strains for leaf blast (Iwano 1987), however, and a reduction in panicle blast occurs when panicles are preinoculated with a high-concentration spore suspension of incompatible strains (Ashizawa et al 1999).

The presence of diseased plants caused by incompatible strains is necessary in multilines when the induced resistance operates in them. Japanese farmers expect a high reduction in rice blast severity for multilines. The contribution of the induced resistance to rice blast in multilines is considered to be less important than that of the reduction in susceptible plants and the barrier effect of resistant plants.

Effectiveness of multilines for rice blast control

Blast reduction in rice cultivar mixtures and multilines

Mundt (1994) summarized data on blast reduction in rice cultivar mixtures. I followed his paper and summarized data on blast reduction in rice cultivar mixtures and



	Pii line	*Pik* line
Race 037	S	S
Race 007	S	R

Fig. 5. Reduction in leaf blast severity by induced resistance in Sasanishiki multilines. (Adapted from Nakajima et al 1996b.)

multilines, which were reported in Japan and were not summarized by him (Table 2). As Mundt (1994) did, data from rice cultivar mixtures and multilines of equal proportions of components were only used for the calculation of blast reduction in them. Mean percentages of leaf blast reduction and yield increase were similar to those of his summarization. However, the mean percentage of the reduction in panicle blast of my summarization was smaller than that of Mundt's. Most of the trials that I summarized were conducted using small plots under natural heavy blast epidemics. These conditions might affect the results.

Large effects of interplot interference on panicle blast development, which require high levels of inoculum reduction to decrease panicle blast, are supposed to cause less effectiveness of cultivar mixtures in controlling panicle blast (Mundt 1994). In addition to the causes, the following factors may affect the lower effectiveness of panicle blast control in multilines and cultivar mixtures. Incompatible blast fungus races can induce panicle blast lesions on resistant rice cultivars and NILs (Table 3; Yamanaka and Yamaguchi 1989, Ohba et al 1999). It is supposed that levels of autoinfection are higher for panicle blast than for leaf blast and those of the barrier effect are lower for panicle blast than for leaf blast (Fig. 6).

Multilines with 10 components (mixtures of equal proportion of Sasanishiki and its nine NILs) showed more stable and higher effectiveness in controlling blast than multilines containing 2, 3, and 4 components. In all our mixture trials from 1993 to 1996 using the Sasanishiki multilines, there were statistically significant negative correlations (P<0.01) between rough rice yields and percentages of diseased spikelets with panicle blast (Koizumi and Tani 1996).

Table 2. Blast reduction and yield increase relative to mean of component pure stands for rice cultivar mixtures and multilines in Japan.

Author(s)	Number of components in mixture	Kind of components[a]	Mixing method[b]	Reduction (%)		Yield increase (%)
				Leaf blast	Panicle blast	
Shindo (1977)	3	Cv	Intra	85	86	16.5
	3	Cv	Intra	72	65	2.8
Tokairin et al (1982)	2	Cv	Intra	75	53	–
	3	Cv	Intra	–40	52	–
	3	Cv	Intra	75	34	–
	3	Cv	Intra	90	53	–
	3	Cv	Extra	20	14	–
	3	Cv	Extra	81	–15	–
	3	Cv	Extra	93	19	–
Yokoo and Saito (1982)	2	Cv	Extra	–	13	3.3
	2	Cv	Extra	–	25	15.5
Nakajima et al (1989)	2	NIL	Intra	89	77	–
Ise (1990)	2	NIL	Intra	–	15	12.4
Koizumi and Fuji (1994)	2	NIL	Intra	39	15	20.6
	2	NIL	Intra	50	19	3.4
	2	NIL	Intra	48	18	9.3
	2	NIL	Intra	55	24	18.6
	2	NIL	Intra	67	–3	11.1
	2	NIL	Intra	2	51	4.5
Koizumi et al (1996)	2	NIL	Intra	15	–15	20.6
	2	NIL	Intra	38	36	10.7
	2	NIL	Intra	11	–23	–2.9
	10	NIL	Intra	59	46	13.5
	2	NIL	Intra	63	39	18.5
	2	NIL	Intra	76	24	14.4
	2	NIL	Intra	28	4	9.3
	4	NIL	Intra	28	6	7.1
Koizumi and Tani (1997)[c]	2	NIL	Intra	35	21	28.1
	2	NIL	Intra	–51	–3	–11.4
	2	NIL	Intra	40	9	13.2
	2	NIL	Intra	30	21	15.5
	4	NIL	Intra	33	–44	6.3
	10	NIL	Intra	63	16	14.1
Hayashi and Tani (1998)[c]	2	NIL	Intra	89	48	23.5
	2	NIL	Intra	58	68	10.4
	2	NIL	Intra	94	39	2.3
	2	NIL	Intra	93	27	–4.2
	4	NIL	Intra	–	37	2.0
	10	NIL	Intra	97	47	8.8
Hayashi and Tani (1999)[c]	2	NIL	Intra	50	–6	–6.7
	2	NIL	Intra	50	39	2.9
	10	NIL	Intra	75	53	0.2
Mean	2			48	24	9.7
	3			60	39	9.7
	4			31	0	5.1
	10			74	41	9.2
Total				52	26	9.2

[a]Cv = rice cultivars. NIL = near-isogenic lines. [b]Intra = near-isogenic lines and cultivars mixed within hills. extra = mixtures composed of genetically uniform hills. [c]Unpublished.

Table 3. Avirulent races of *Pyricularia grisea* isolated from panicle blast lesions of Sasanishiki near-isogenic lines and panicle blast severity on them in the field trial.

Near-isogenic line	Isolated avirulent races (%)	Diseased spikelets (%)
Pita line (pure stand)	89	55
Piz line (pure stand)	45	12
Pik line mixed with *Pii* line	13	6
Pi-k line mixed with Sasanishiki	19	5

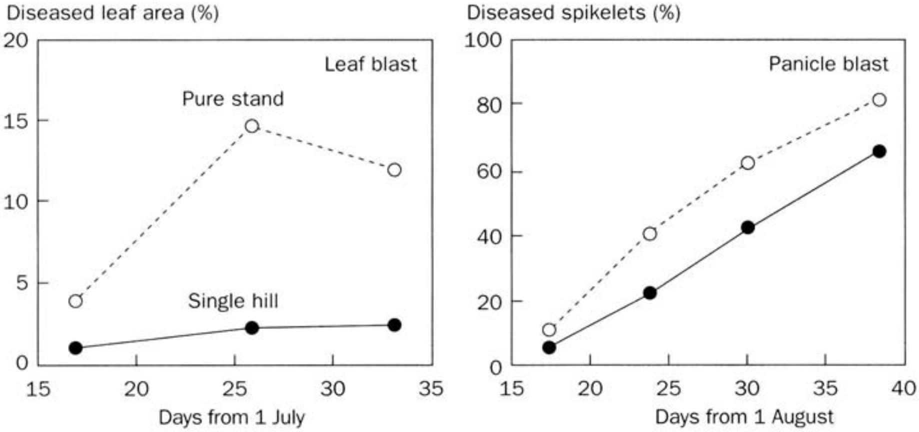


Fig. 6. Blast development in a pure stand of Sasanishiki and on a single hill of Sasanishiki in pure stands of its resistant near-isogenic lines. A single hill of Sasanishiki was transplanted in the center of each plot of the pure stands of Sasanishiki and its resistant near-isogenic lines.

Comparing blast control between multilines and fungicide treatment

To compare the effectiveness of multilines for rice blast control with that of fungicide treatment, we also conducted mixture trials under natural heavy blast epidemics. Sasanishiki and its near-isogenic *Piz-t* line were used for the mixture trials. Sasanishiki was susceptible to all of the races of *Pyricularia grisea* distributed in our trial fields and the *Piz-t* line was resistant (immune) to all of them (Table 4). Sasanishiki was mixed with the *Piz-t* line in proportions of 1:1 and 1:3 and blast severity in the mixtures was compared with that in pure stands of Sasanishiki treated and untreated with fungicides. The mixtures of an equal proportion of Sasanishiki and its nine NILS (TML, total mixture lines) were also used in these trials (Table 5).

The mixtures of the *Piz-t* line with Sasanishiki inhibited leaf blast, and leaf blast severity in the mixtures including 50% and 75% *Piz-t* lines was approximately equal to that in the fungicide-treated Sasanishiki pure stand. However, panicle blast severity in the mixture of the 50% *Piz-t* line was greater than that in the fungicide-treated pure stand, although the severity in the mixture with the 75% *Piz-t* line was statistically equal to that in the chemical-applied pure stand (Table 5). TML also reduced

Table 4. Near-isogenic lines developed from rice cultivar Sasanishiki and their reactions to main races of *Pyricularia grisea* in field trial.

Near-isogenic line	Genotype with complete resistance	Japanese race[a]			
		007	037	077	107
Tohoku 1 (*Pik-s* line)	*Pik-s*	S	S	S	S
Tohoku 2 (*Pii* line)	*Pii Pia*	S	S	S	S
Tohoku 3 (*Pik* line)	*Pik Pia*	R	S	S	R
Tohoku 4 (*Pik-m* line)	*Pik-m Pia*	R	S	S	R
Tohoku 5 (*Piz* line)	*Piz Pia*	R	R	S	R
Tohoku 6 (*Pita* line)	*Pita Pia*	R	R	R	S
Tohoku 7 (*Pita-2* line)	*Pita-2**	R	R	R	R
Tohoku 8 (*Piz-t* line)	*Piz-t**	R	R	R	R
Tohoku 9 (*Pib* line)	*Pib**	R	R	R	R
Sasanishiki	*Pia*	S	S	S	S

[a] R = resistant, S = susceptible. **Pia* unknown.

Table 5. Blast severity (RADPC[a]) in mixtures of rice cultivar Sasanishiki and its near-isogenic lines, and in pure stands of Sasanishiki treated and untreated with fungicides.

Blast	Trial	Sasanishiki:*Piz-t* line		TML[b]	Sasanishiki	
		1:1	1:3		Fungicide-treated[c]	Nontreated
Leaf	1994			0.6[d]	0.5	4.2
	1995-1	1.2[d]	0.5	0.6	1.7	11.1
	1995-2	1.5		0.8	0.5	6.9
	1996-1	0.5	0.2	0.3	0.1	1.9
	1996-2	1.3		0.7	0.3	6.2
Panicle	1994			15.2	8.6	51.3
	1995-1	34.2	15.5	29.1	16.2	84.2
	1995-2	17.7		12.5	4.0	49.0
	1996-1	27.2	12.9	17.1	13.0	69.3
	1996-2	24.6		25.6	20.5	81.0

[a] Relative area under the disease progress curve (Bonman et al 1989). [b] Mixture of equal proportion of Sasanishiki and its nine near-isogenic lines. [c] Probenazole granule (24 g ai 100 m^{-2}) was submerged for leaf blast control and tricyclazole suspension concentrate (× 1,000, 3 g ai 100 m^{-2}) was sprayed twice for panicle blast control, respectively. [d] Underlined RADPC values in the mixtures are not significantly different from that in pure stand of Sasanishiki treated with fungicides in each trial.

blast development. Leaf blast severity in TML was equal to that in the fungicide-treated Sasanishiki pure stand, although panicle blast severity in TML was greater than that in the chemical-applied pure stand (Table 5). The most predominant Japanese race of rice blast fungus in TML was 007, which can attack three components (Sasanishiki, and *Pik-s* and *Pii* lines) of TML (Table 4).

These results indicate that NILs with 75% resistance are required in the Sasanishiki multilines to reduce blast severity to the level of fungicide treatments. The level of partial resistance to blast in the Sasanishiki multilines is low. If multilines have a high level of partial resistance to blast, the percentage of resistant NILs in them may change.

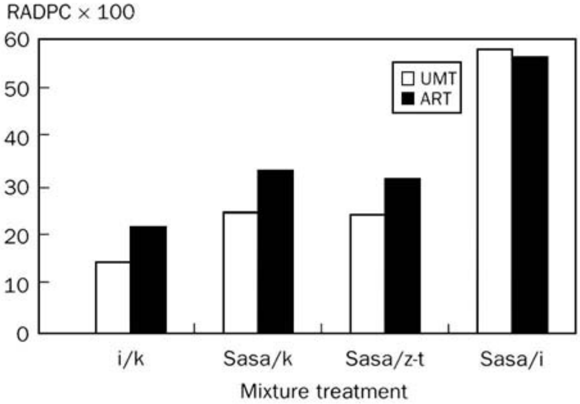


Fig. 7. Effect of mixing pattern in the Sasanishiki multilines on panicle blast development. Two components of the mixtures were uniformly mixed in equal proportions in UMT and they were planted in alternate rows in ART. RADPC = relative area under the disease progress curve; Sasa = Sasanishiki, i = *Pii* line, k = *Pik* line, Z-t = *Piz-t* line. RADPC × 100 value in pure stand of Sasanishiki was 167.

Mixing pattern

The effect of mixing pattern of NILs in multilines on rice blast reduction was also studied using Sasanishiki and its NILs. In one treatment (ART), two components of the mixtures were planted in alternate rows; in another treatment (UMT), two components were uniformly mixed in equal proportions within hills and planted. The effectiveness of ART for panicle blast control was lower than that of UMT (Fig. 7), although differences in leaf blast reduction between ART and UMT were not clarified.

Change in blast races in multilines

We examined the change in blast races in the Sasanishiki multilines and pure stands of their components from 1993 to 1995 using Japanese differential rice cultivars (Yamada et al 1976). The examination was conducted in field trials using small plots under natural heavy blast epidemics.

Figure 8 shows a part of the results. Japanese race 007 was constantly predominant in the pure stands of Sasanishiki, *Pik-s* and *Pii* lines, and TML from 1993 to 1995, although some kinds of isolated races were greater from TML than from the pure stands of Sasanishiki and *Pik-s* and *Pii* lines. Race 007 is virulent to Sasanishiki and *Pik-s* and *Pii* lines (Table 5).

On the other hand, at the second cropping of the mixture of *Pii* and *Pik* lines, blast races 037 and 077, which can attack both of the NILs (Table 5), predominated. Races 037 and 077 were also predominant in the mixture of equal proportion of *Pik, Pik-m, Piz,* and *Piz-t* lines from 1995 to 1996. Race 077 has virulence against three of the four NILs and race 037 can attack two of them (Table 5). The “super race” capable of attacking all components of TML was not isolated from every examined plot (Fig. 8).

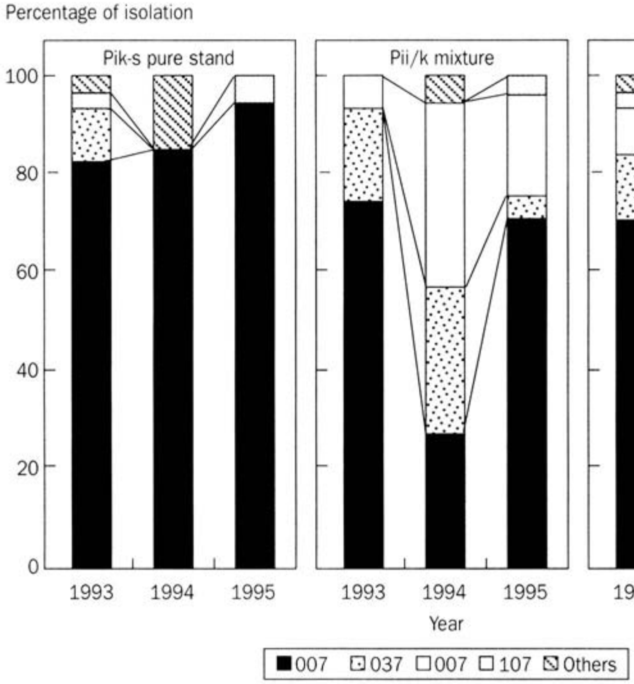


Fig. 8. Change in Japanese races of *Pyricularia grisea* isolated from a pure stand of a near-isogenic line (NIL) of sasanishiki and mixtures of its NILs. Pii/k mixture = mixture of equal properties of *Pii* and *pik* lines; TML= mixture of equal proportion of Sasanishiki and its nine NILs.

Change in blast races in the Sasanishiki multilines was also followed from 1995 to 1998 in farmers' fields of Miyagi Prefecture of northern Japan, where the multilines were released (Ohba et al 1999, Tsuji et al 1999). In 1996, two years after the release of the multilines, blast race 077, which can attack all three components of the multilines, was isolated from panicle blast lesions in the multilines (Tables 5, and 6). Afterwards, their composition changed and the *Piz-t* line was introduced into the multilines from 1997. In 1998, however, blast race 407 with virulence to the *Piz-t* line was isolated in a field and there were no components with complete resistance in the multilines to all of the blast races distributed in Miyagi Prefecture (Table 6). Owing to the low distribution rates of blast races virulent to the multilines, unfavorable weather conditions for blast development, and a fungicide application for panicle blast control, there has been little damage from blast in the multilines.

Analysis of rice blast reduction in multilines using simulation models

We used DISBL, a computer model, to simulate the dispersal and deposition of spores of *Pyricularia grisea* in and over the rice canopy (Koizumi and Kato 1991) and analyzed deposition of the spores within a rice canopy of a mixture of Sasanishiki and its

Table 6. Races of *Pyricularia grisea* isolated from panicle blast lesions in the Sasanishiki multiline[a] cultivated in farmers' fields.

Year	No. of places examined	Isolated Japanese races										
		007	037[b]	047	407	*003, 007*	*007,* 013	*007,* 037	033, 037	037, 047	*003, 007,* 037	*007, 037,* 077
1995	20	4[c]	7			2	1	4	2			
1996	23	7	4			1		6		1	3	1
1997	3	2	1									
1998	23	18	1	1	1	1		1				

[a] Composition of the Sasanishiki multilines; Pik line:*Pik-m* line:*Piz* line = 4:3:3 (1995): Pik line:*Pik-m* line:*Piz* line = 3:3:4 (1996); Pik line:*Pik-m* line:*Piz* line:*Piz-t* line = 1:1:4:4 (1997, 1998). [b] Underlined races were virulent to a part of the components of the Sasanishiki multilines. [c] Number of places where respective blast races were isolated.

resistant NIL, which were uniformly distributed within hills. The calculated results suggested that the deposition rate of the spores on the susceptible leaves was higher than that on the resistant leaves (Koizumi et al 1999).

We are now modifying BLASTL, a systems analytical model for leaf blast epidemics (Hashimoto et al 1984), to simulate leaf blast development in multilines. Simulations with the modified BLASTL may give us effective methods of rice blast control with multilines.

Conclusions

Our field trials using the Sasanishiki multilines indicated that an increase in the number of components and proportion of resistant components is necessary for effective rice blast control (Koizumi and Fuji 1994, Koizumi and Tani 1996). However, available genes with complete resistance are limited for rice blast control with multilines in Japan, although 15 genes with complete resistance to blast were identified (Kiyosawa 1997, Hayashi et al 1998). Multilines with high levels of partial resistance to blast (especially panicle blast) should be urgently developed for effective blast control with rice multilines for a long period, although it is difficult to combine high levels of partial resistance to blast and good eating quality, which is a required and important characteristic in Japanese rice cultivars.

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Current status and prospect of mixture planting for control of rice blast in Yunnan

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Under the project funded by the Asian Development Bank on "Exploiting Biodiversity for Sustainable Pest Management," scientists from the Philippines-based International Rice Research Institute (IRRI) and the Yunnan Agricultural University began a farmer participatory project to control blast by diversifying varieties in farmers' fields.

Large-scale field tests for two consecutive years showed that the highly susceptible glutinous rice varieties (Huangkenuo and Zinuo) suffered less disease when interplanted with the generally resistant indica hybrid varieties (Xianyou 63 or Xianyou 22). The field design was a repeating pattern of one row of glutinous rice interplanted with four or six rows of indica rice. Helped by a vigorous extension campaign, farmers in Yunnan rapidly adopted the scheme, with area planted to the mixture now covering 42,500 hectares. This rapid adoption can be attributed to strong support from technicians and leaders of counties, relevance to the needs (problems) of farmers in the area, simplicity and effectiveness of the technology, and improvement in the yield and income of farmers. Plans are now in place to implement the technology in other provinces of China, and to widen the options for varieties that can be used for diversification.

Yunnan Province is located in Southwest China, from 20° to 29° N latitude and from 97° to 196°E longitude. It has an area of about 383,000 km^2. The elevation is highest at 2,700 m in Lin Lang County and lowest at 76 m in Hekon County. The Yunnan plateau has many plains. These plains are the main places for agriculture, especially for rice production. There are 1,442 plains, most of which are small (about 1 km^2); only 47 plains are large, each with more than 100 km^2.

Blast is the main disease of rice in Yunnan. The wet, cool climate of Yunnan Province is highly favorable for the development of rice blast epidemics. Throughout the history of rice cultivation in this area, blast outbreaks have caused serious losses. Crop losses from blast accounted for 91%, 96.6%, and 98.6% of total disease losses in 1974, 1978, and 1990, respectively. Generally, the average loss per year for a 21-y period (1971 to 1992) was 57.8% of total disease losses.

Because of the incorporation of resistance genes, rice blast is no longer a serious problem for the widely grown hybrid indica rice. However, it remains a serious problem for glutinous rice (32% losses), japonica rice (5–12% losses), and upland rice (losses could reach 100%). Farmers growing susceptible varieties use fungicide to control blast, making as many as 3–8 spray applications per season.

It is now widely recognized that increasing the intraspecific genetic diversity of crop populations provides an ecological approach for suppression of epidemics. Deployment of multilines or mixtures of varieties has been successfully used to manage serious diseases of some cereals such as stripe rust of wheat and powdery mildew of barley on a commercial scale. Based on this knowledge, and drawing from the experiences of our own farmers, we experimented with a diversification approach to control rice blast. In this chapter, we will describe the results of mixture experiments, the current status of mixture planting by farmers, and the prospects for mixture planting in other provinces of China.

Beginning and early results of diversification project

Under the project funded by the Asian Development Bank on "Exploiting Biodiversity for Sustainable Pest Management," a collaborative project was established between the Yunnan Agricultural University and IRRI to explore diversification strategies for controlling rice blast.

When we started this project in 1997, several diversification approaches were considered, including seed mixtures and planting different varieties in adjacent fields. Farmers, however, did not like the idea of seed mixtures. We eventually set up an interfield diversification experiment in Bao Xiu, Shiping County. which was similar to that done in 1980-84. Each farmer's field of about 0.067 ha was considered as one plot. We took 3∞5 rows of adjacent plots and planted a different variety in each plot. The results were not very good. Disease was severe in plots where susceptible varieties were planted.

At about the same time, a similar experiment was conducted in Zhang Gui Zhai, also in Shiping County; however, the farmers modified the design. Drawing on their own experience, diversification was done within their fields. Rows of the susceptible glutinous variety were planted between every four rows of the relatively resistant indica rice in a repeating pattern throughout the field. The results were remarkable. The mixture of a glutinous variety (Huangkenuo or Zinuo) with an indica hybrid variety (Shianyou 63 or Shianyou 22) significantly reduced the incidence and severity of rice blast disease. Apparently, farmers in this area had been interplanting susceptible glutinous rice among the relatively resistant indica rice to escape from serious blast disease. This practice started in about 1983, although on a limited scale, and the pattern of interplanting is somewhat random or irregular.

Large-scale field trials

In 1998, mixtures of varieties were planted in a 812-ha area consisting of all rice fields in five townships (Baxing, Baoxiu, Songchun, Maohe, and Yafanzi) of Shiping County, Yunnan Province. Four different mixtures of a glutinous and a hybrid variety were planted within this area. Rice fields were managed by farmers, using agronomic practices standard for the area. For mixtures, the same row spacing of hybrid rice was used as in monoculture, but one row of glutinous rice was added between each group of four rows of hybrid rice in an "addition" approach (Fig. 1).

To monitor disease. survey plots were established at 15 sites, three in each of the five townships participating in the diversification program. All plots were treated in the same manner as the surrounding mixed variety plantings, including fungicide application. Row spacings were those commonly used by rice farmers in Yunnan Province (Fig. 1). At each of the 15 survey sites, a field was divided into three plots. One plot was planted to the mixture that local farmers were most commonly growing, whereas the remaining two plots were monocultures corresponding to the glutinous and hybrid variety that were included in that mixture. Plots ranged from 100 to 450 m^2 each depending on field size. No fungicides were applied to control leaf blast in the survey plots. However, a one-time spray for neck blast was made for the rest of the demonstration plots. Survey plots were assessed for the severity of blast symptoms, expressed as the percentage of panicle branches that were necrotic.

The mixture planting area was expanded to 3,342 ha of rice fields in 1999. This area consisted of all rice fields in 10 townships that spanned Jianshui and Shiping

Fig. 1. Rows of a glutinous rice variety interplanted among rows of a hybrid indica variety in a repeating pattern of one row of glutinous rice for every four rows of hybrid rice.

counties, with five participating townships per county. Procedures were identical to the 1998 experiment, except that no foliar fungicide applications were made for either leaf or panicle blast. In addition, some farmers chose to plant mixtures in a ratio of 1:6 glutinous:hybrid rather than 1:4. There were 15 survey sites per county (three in each of the five townships).

Results

Mixture planting had a substantial impact on rice blast severity (Fig. 2) and yield (Fig. 3). In the 1998 planting in Shiping, panicle blast severity on the susceptible glutinous varieties was 94% to 99% less severe when the varieties were grown in mixtures than when grown in monoculture (Fig. 2A). Panicle blast on the more resistant hybrid varieties was 1% to 25% less severe in mixed plots than in monoculture (Fig. 2B), even though hybrids were planted at the same density in both mixture and monoculture survey plots (Fig. 1). Results from 1999 were similar to those of the 1998 season for panicle blast severity on the susceptible varieties (Fig. 2A), showing that the impact of diversification was very robust among mixtures and between seasons and counties. In contrast, the effects of crop diversification on blast severity of the hybrid varieties were larger in 1999 than in 1998, with panicle blast severity averaging 59% less in mixed populations than in monoculture (Fig. 2B). By the second year of the project, no foliar fungicides were needed for blast control in the diversified area. Though elimination of foliar sprays may not be possible in all seasons, varietal diversification can clearly result in a very substantial reduction in fungicide use.

Mixture interplanting of hybrid and glutinous varieties not only reduced the production loss caused by rice blast but also increased total rice yield per hectare compared with monoculture fields (Fig. 3). The average yield of mixture planting of Xianyou 63 with Huangkenuo was 825.8 kg more than that of pure cropping of Xianyou 63. The average yield of mixture planting of Xianyou 63 and Zinuo was 833.9 kg more than that of pure cropping of Xianyou 63. The average yields of mixture planting of Xianyou 22 with Huangkenuo and Xianyou 22 with Zinuo were 916.8 kg and 852.5 kg more than the pure cropping of Xianyou 22, respectively.

Current status

The Yunnan diversification program has resulted in great interest by farmers and a commitment from government officials to help expand the practice. At the start of the 2000 cropping season, the interplanting scheme was implemented in 42,500 hectares in 40 counties in Yunnan (Fig 4).

The rapid adoption of the mixture planting technique can be attributed to a vigorous and systematic knowledge dissemination scheme. At the county level, the first step was to hold a meeting with county government officers including the vice mayor in charge of agriculture, the chief of the county science and technology commission, and the chiefs of plant protection and agrotechnology stations. Once support is received from the officers, the next step is to conduct a workshop with the technicians

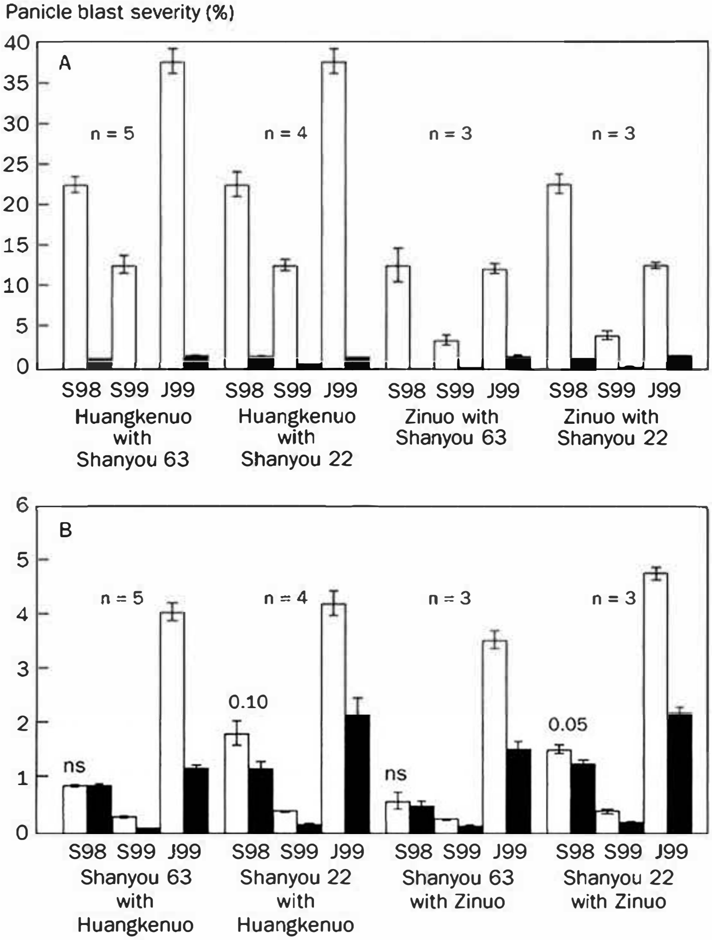

Fig. 2. Panicle blast severity (mean percentage of panicle branches that were necrotic due to infection by *Magnaporthe grisea*) of (A) susceptible glutinous rice varieties and (B) resistant hybrid rice varieties when grown in mixed culture and in monoculture. Each open column indicates blast severity for a variety grown in monoculture control plots and the adjacent black column indicates blast severity of the same variety when grown in mixed culture plots in the same field. Error bars indicate one SEM. Values of n indicate the number of plot means that contribute to individual bars for each of the four combinations of susceptible and resistant varieties. All differences between pairs of monoculture and mixture bars are significant at *P*<0.01 based on a one-tailed t-test unless indicated by 0.05 (significant at *P*<0.05), 0.10 (significant at *P*<0.10), or ns (not significant at *P* = 0.10).

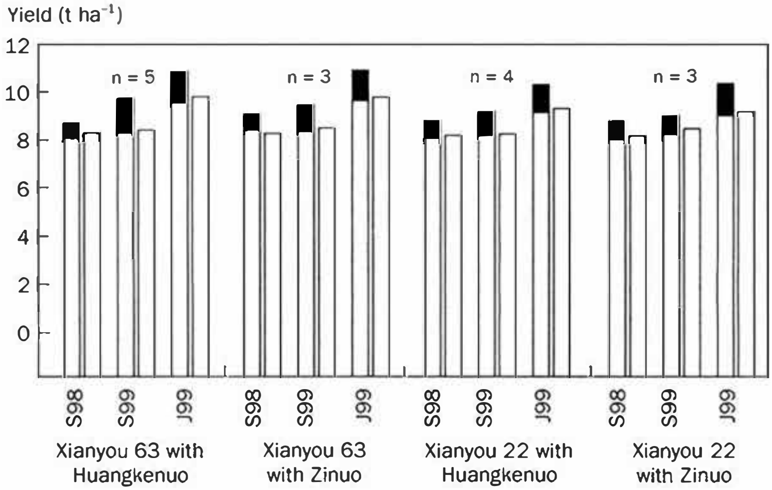

Fig. 3. Comparative yield of mixture crops of indica and glutinous varieties versus monoculture crops of the indica variety per hectare during the 1998 and 1999 seasons in Jiangshui and Shiping. Each open column indicates yield of the indica variety and the black column indicates yield of the glutinous variety when grown in mixed culture with the indica variety in the same field.

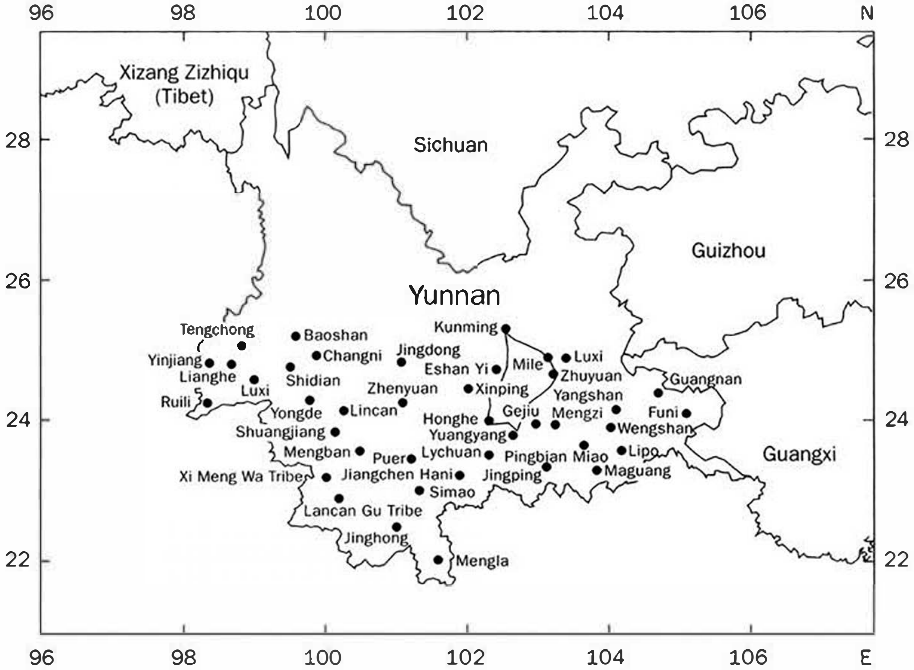

Fig. 4. Counties in Yunnan Province covered by the diversification scheme during the 2000 crop season.

in agricultural technology and county plant protection offices. These trained technicians then organize through village officers small group discussions among farmers. During planting time, the more skilled farmers took the lead to demonstrate to other farmers, with the agrotechnicians as the supervisors. In 2000 alone, we have held 53 regular training courses for about 400 agricultural technicians from various plant protection stations of Yunnan Province and other provinces. More than 7,000 farmers have been subsequently trained in echo seminars and field demonstrations.

Other extension methods include video clips provided to the local cable television network. These are usually shown during commercial breaks of popular television programs.

Prospects and future plans

Expansion to other provinces in China

The area covered by mixture planting is expected to expand beyond Yunnan Province. China's Ministry of Science and Technology is very keen to extend the germplasm diversification approach for blast control to other provinces in China because this project serves well the purpose of the Chinese Central Government’s Poverty Alleviation Program for the southwest and northwest. An agreement on mixture planting demonstrations has been signed with nine other provinces of China: Sichuan, Chongqing, Hunan, Hubei, Jiangxi, Guangdong, Shanxi, Jiangshu, and Zhejiang (Fig. 5).

Testing new varieties suitable for mixtures

As the area covered by the mixture planting scheme expands, it becomes necessary to introduce additional diversity into the system to preclude pathogen adaptation, which might lead to disease outbreaks in the future. We screened 208 varieties for functional diversity using both field data and molecular data generated in our laboratory using resistance-gene analog–polymerase chain reaction tools. Twenty new rice varieties, including high-quality rice, have been included for a new mixture interplanting trial. We have started evaluating the agronomic performance of 11 high-quality aromatic rice varieties for possible inclusion in the mixed planting. All these 11 varieties are rarely planted now because they are highly susceptible to blast.

We are also evaluating various mixture combinations of japonica by japonica varieties. Because of the substantially larger area planted to japonica varieties than glutinous varieties, developing an effective diversification scheme for japonica varieties is expected to have a greater impact than the current success in mixture planting of indica by glutinous varieties.

Understandingthe mechanism of disease control in mixtures

The team has also set up a few important experiments in the field to find out why some varieties are effective in mixtures and others are not, and also the potential mechanism of blast control using interplanting, such as microclimatic changes, spore dispersal, and induced resistance. Understanding this mechanism will enable us to predict the sustainability of the system.

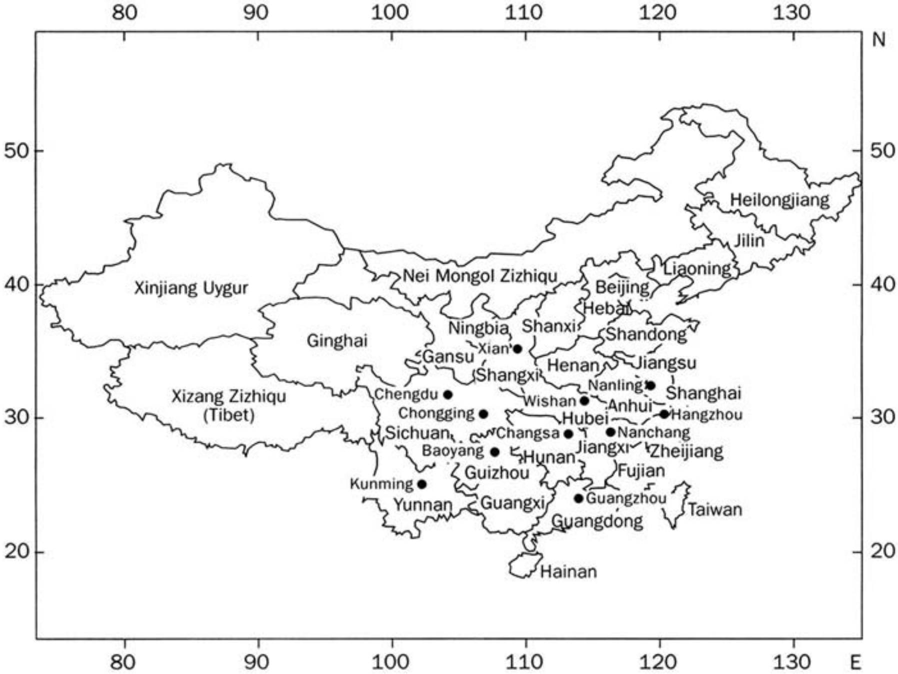

Fig. 5. Provinces in China where demonstration trials for mixture planting have been carried out in 2000.

Conclusions

Among the knowledge that can be derived from the implementation of this project, three important lessons stand out. First, our results support the view that crop diversification can provide highly effective disease suppression when practiced on a large spatial scale. Second, our results demonstrate that collective efforts from groups of scientists, institutions, and farmers are vital to the development and dissemination of an effective diversification technology. Finally, wide adoption of a diversification technology depends on simplicity, effectiveness, and ability to bring about obvious economic benefits to farmers.

Notes

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Biodiversity of blast pathogens and implications for blast management

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Rice blast, caused by *Pyricularia grisea* Sacc., is so diverse that it makes control of the disease difficult. Research on the host range, biodiversity, and interaction *of* various rice genes with resistance to this fungus was carried out in Chiang Mai, Thailand. *Leptochloa chinensis* and *Panicum repens* were found to be potential hosts and served as sources of inoculum. Results from the DNA fingerprinting of the 90 blast isolates selected from 597 isolates obtained from rice, weeds (*Brachiaria rnutica, Digitaria sanguinalis, Eleusine indica, Penniseturn polystachyon, Panicum repens*), and barley confirmed that certain weed isolates were classified in the same group as those from rice. Moreover, some were 90% similar to rice isolates. Although the barley isolates were totally different from those of rice, they were 75% similar to *P. repens* isolates and 70% similar to *E. indica* and *D. sanguinalis* isolates. The barley isolates, however, could infect rice under glasshouse conditions; therefore, growing susceptible rice varieties after or near barley should be avoided. Testing 23 selected blast isolates against a set of IRRI's near-isogenic lines containing 16 resistance genes showed that *Pita, Pik-p,* and *Pita-2* were resistant to the tested isolates, thus indicating their possible use in rice breeding programs.

Rice blast is identified as a key pest and is known to occur in every region of the world where rice is grown. It is especially serious in humid rice-producing areas. Although it is principally a foliage disease, the symptoms are also found on the leaf sheath, joint of the culm, rachis, and even the glumes. Severe foliage infection detected in the seedling stage as well as in the posttransplanting stage leads to the total destruction of the leaf. However, infection of the culms causes more damage than leaf infections. Moreover, neck blast infection could cause half-filled or totally chaffed panicles, which lead to low rice yield. The frequent introduction of new resistant rice cultivars is used as one control measure, but rice blast still occurs in chronic epidemic cycles. The absence of durable blast resistance in the field has been attributed to high levels of virulence forms of polymorphism in pathogen populations. Thailand was also affected by the outbreak of blast disease, which caused significant damage to rice production throughout these years, especially in the Chiang Mai Valley, one of the major rice production areas where the temperature and relative humidity were favorable for the outbreak and development of pathogens. The most severe neck blast epidemics occurred in north and northeastern Thailand in 1992 with 90% of the damage reported in the north. Among the losses in this area, yield loss in Chiang Mai was 16%, causing a monetaly loss of approximately US$12.45 million.

The rice blast fungus, *Pyricularia grisea* (Cooke) Sacc. (syn. *P. oryzae* Cavara), or the perfect telemorph *Magnaporthe grisea* (Hebert) Barr., an important pathogen of rice in most rice–growing countries, is a heterothallic ascomycete that is primarily haploid and reproduces asexually in nature. Furthermore, this pathogen is noted for expressing a large number of virulence forms or genotypes, especially in tropical areas where conditions favor its outbreak and development, such as in the Philippines. Ou (1980) has reported 250 pathotypes among the field isolates. Besides rice, this fungus can infect more than 50 species of grass (Ou 1985), many of which are important weeds found in rice fields. It is possible that the grass weeds harbor different pathogenic lineages that may or may not parasitize the local rice varieties but produce enough conidia to be disseminated by air currents to infect crops in other localities. It is therefore necessary to investigate the genetic variability in populations of the blast pathogen to understand the host coevolution. In addition, information on the interaction of the resistance genes and different blast isolates will benefit breeding programs for rice blast resistance. Information obtained is expected to improve sustainable rice blast management.

Materials and methods

Pyricularia spp. from rice and weed samples were isolated using the single–spore isolation method and cultured on prune agar. The mycelium stock cultures were kept in slant culture for further investigation.

DNA extraction for all *Pyricularia* isolates was carried out following the methodology used by Lee and Taylor (1990) , after which the polymerase chain reaction (PCR)-based method (IRRI protocol) was used for fingerprinting. The Gel Compar II Software (Applied Maths) was used to calculate and construct the dendrogram.

A set of 34 resistance genes obtained from IRRI (IRBL set) was tested with 9 selected isolates. Twenty–one–day–old seedlings were inoculated with spore suspension of these isolates at a concentration of approximately 50,000 spores cc^{-1}. Seedlings were kept under moist and cool conditions for 7 d, after which the seedlings were assessed for leaf blast reaction using IRRI's SES guidelines (IRRI 1996).

Results and discussion

Blast pathogens were isolated from rice and weed hosts and collected on the slant agar. To prevent the loss of virulence, the pathogens were preserved on filter paper discs. A total of 597 blast isolates were preserved as listed in Table 1.

Biodiversity of blast pathogens

Blast pathogens, 342 isolates (isolated from rice varieties), and weed hosts at different locations could be separated into 50 groups using the Gel Compar II Software (Applied Maths) at 60% similarity. The genetic variation calculation based on Nei and Li (1979) was very high and most of the pathogens isolated from the same kind of host could be placed in different lineages, as listed below and in Figure 1. However, the pathogens isolated from rice and weeds were identified as the same groups in certain lineage groups: 5 (DS, EI, NSPT, HR), 6 (RD23, BM), 22 (EI RD6), 38 (HR, RD6, RD10, RD23, NSPT, BM, PP, PR), 40 (RD6, RD10, BM, PR), 43 (B1–6, RD6, UN), and 44 (M, EI). The only rice isolates could be grouped in the lineage groups 13, 19, 28, 34, 35, 36, 37, 42, 43, 45, 46, 47, and 48. The weed isolates were grouped in the following lineages: 1 (DS, BM), 2 (DS), 4 (PP), 7 (DS), 8 (BM, EI), 9 (BM), 10 (BM, DS), 11 (DS, EI), 12 (PP), 14 (PP), 15 (DS), 16 (PP), 17 (PP), 18 (DS), 20 (PP), 21 (EI), 23 (EI), 24 (PP, EI), 25 (BM), 26 (BM), 27 (BM), 29 (EI), 30 (PR), 39 (PR), 41 (BM, PP, PR), and 49 (UN). The similarities of the pathogens were scattered from within the same or different locations. Remarkably, the weed isolates could also be grouped by host.

Table 1. Sources of *Pyricularia* spp. collected in the laboratory.

Hosts	No. of isolates
Rice cultivars	
KDML	57
Lom Karn (LK)	6
Mong Rice 1 (M1, upland rice)	4
Mong Rice 2 (M2, upland rice)	7
NSPT	25
RD 6	95
RD 10	45
Highland paddy rice # 28 (HR)	2
Barley	50
Upland rice, Chiang Dao	3
Weeds	
Brachiaria mutica (BM)	62
Pennisetum polystachyon	41
Panicum repens (PR)	46
Digitaria sanguinalis (DS)	31
Eleusine indica (EI)	30
Leptochloa chinensis (LC)	15
Unknown	10
Indicator boxes (RD 23)	68
Total	597

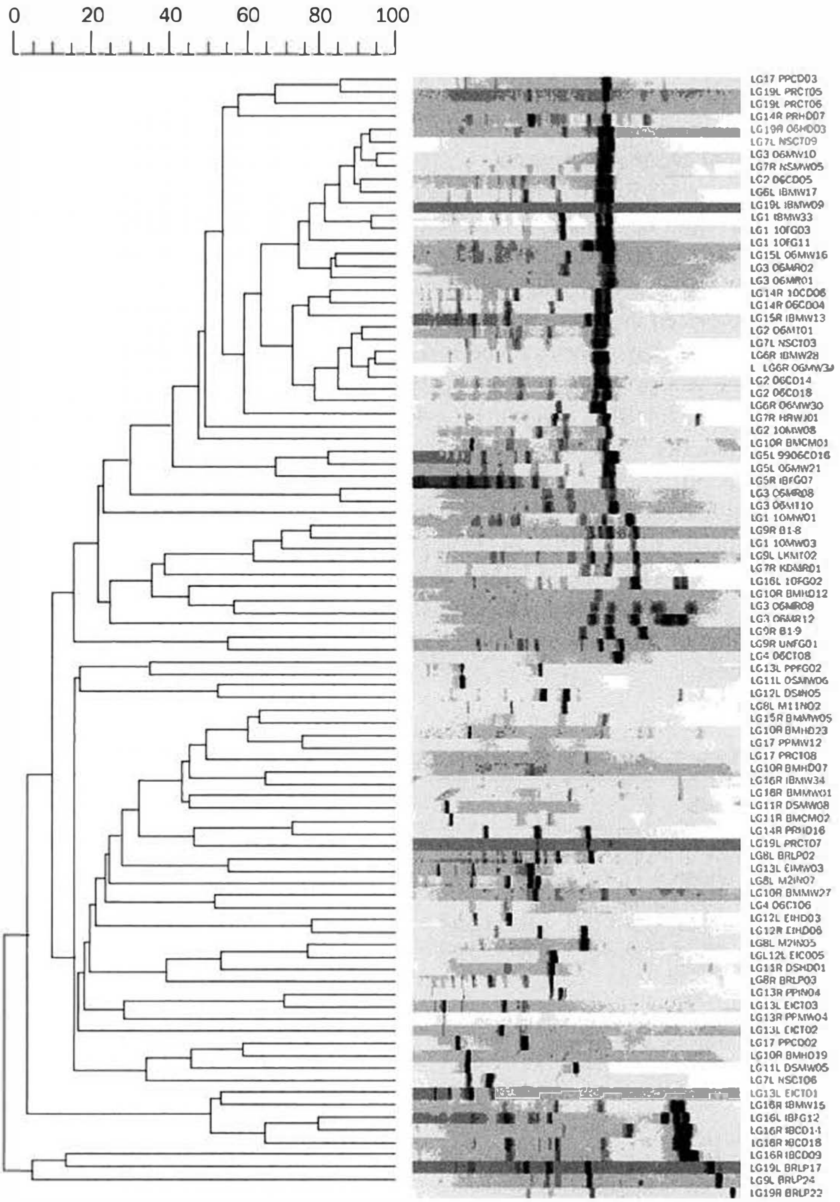

Fig. 1. Some results from phylogenic analysis of 90 isolates from rice, weeds, and barley.

Similarity groups of blast pathogens isolated from barley, rice, and weed hosts could be listed as follows:

1. DSHD 08 and BMCMU 02
2. DSCT 04
3. BRLP 03–13 (leaf blast and flag leaf blast)
4. PPCD 02
5. EIHD 13, 14, 16, 17, 19, 20, 21, NSCT, 06, DSWJ 01, 02, DSCT 02, HRCD 02
6. IBMW 34 (RD 23), BMMW 01
7. DSMW 07, 08
8. BMHD 20, EICT 01
9. BMCMU 01
10. BMHD 19, DSMW 01–05
11. DSCD 01–04, EICD 01-03, 05, DSHD 01, 04, DSCT 01
12. PPFG 04–07, 09- 11, 18
13. M2IN 07
14. PPFG01–03
15. DSMW 06
16. PPCD 04, 06-08
17. PPMW 05
18. DSIN 01, 04–05
19. M1IN 02–03
20. PPIN 04
21. EICT 02
22. EIHD 05, 07-08, 12, 15, 18, 06,CT06
23. EIHD 01–03
24. PPCD 09, PPMW 04, EICT 03
25. BMHD 04, 12
26. BMHD 06, 07
27. BMMW 27
28. M2IN 01, 03
29. EIMW 0 1, 03
30. PRMW 04, 06*, 09*, 10*, PRCT 02, 07, PRMR 01, PRCD 02–04, PRHD 02, 12–16.20 *another subgroup
31. BRLP 01, 02 (leaf blast)
32. BRLP 15–16, 18, 20, 23, 24 (flag leaf blast), 27% similar to group 31
33. BRLP 17 (flag leaf blast), 22% similar to groups 32 and 31
34. 0MW 03, 04 BI–8 KDMR 01
35. B1–1–3, LKMT 01–03
36. 06MR 07-10, 12
37. B1–9
38. HRWJ 01–02, 10FG 01, 03, 05–09, 11, IBMW 9, 10, 12, 13, 16, 17, 18, 19, 20, 21, 23, 24, 27, 28, 30, 31, 33, B1-5, NSMW 01, 04–07, 10, NSCT 03–05, 07–12, BMMW 2 1, 28, 06HD 01–04, 07-12, 06 MT 01–06, PPCD 03,

PRCT 05, 10CD 03, 07-10, 06CT 01–02, 04, 05, 10, 11, 06CD01, 03, 04, 07, 08, 10, 11, 17, 18, 06MW 02, 03, 07, 08, 10, 11–13, 16, 24, 25, 27, 28, 29–34, 06MR 01–03

39. PRCT 06
40. 10MW 08, BMMW 05, 06, 11–13, 15, 18, 24, 25, PRHD 05, 07
41. PPMW 12, BMHD 03, 23, BMMW 22, PRCT 08
42. 06MW 17–19, 15, 20–23 (another subgroup), 06HD 13–15, 06CD 16, M1IN 01, 06MT 08–10, IBFG 01, 02, 04, 06, 07, 11, IBCD 07, 15, 16
43. B1-6, 06CT 07, 08, UNFG 01, 03
44. M2IN 05, M1IN 02, 04, EIHD 06, 09, 10
45. 10FG 10, 12–14 10MW 01, 06, M2IN 04
46. IBCD 01, 02, 11, 14, 18, IBFG 10, 12, 14, IBMT 07, IBMW15
47. 10CD 01, 02, 04, 05
48. IBCD 04, 05, 09
49. UNHD 01
50. BRLP 22 (flag leaf blast)

Notes:
06 = RD 6
10 = RD 10
B1–1 = Srisaket, Kantararak
B1–2 = Surin, Lumduan
B1–3 = Burirum, Satuk
B1–4 = Nopangkai, Ratanavapi
B1–5 = Srisaket, Rasisalai
B1–6 = Roi-et, Suwannapum
B1–7 = Roi-et, Suwannapum
B1–8 = Roi–et, Suwannapum
BM = *Brachiaria mutica*
BR = barley
DS = *Digitaria sanguinalis*
EI = *Eleusine indica*
IB = indicator box
PP = *Pennisetum polystachyon*
PR = *Panicum repens*
KD = KDML

Similarity of blast pathogens isolated from rice and barley hosts

At 60% similarity, the rice and barley isolates could be grouped into 25 groups. The barley isolates obtained from Lam Pang Province could be classified into five groups (1, 2, 3, 8, and 10), which were totally different from the other rice isolates. The highland rice isolates (M2IN, Mong rice, Inthanon, group 4, 5, 22, 24, 25) were very similar if the number of bands was higher; however, the migration of bands was slightly different. This could possibly be caused by some errors during the experiments. The largest group was group 19, which consisted of isolates from various districts of the Chiang Mai Valley: Chiang Dao (CD, 20 isolates), Mae Wang (MW, 38 isolates), Mae Tang (MT, 10 isolates), Jom Thong (CT, 13 isolates), Mae Rim (MR, 3), Wat Chan (WT, 2), Fang (FG, 7 isolates), and B1–5 (Srisaket, Rasisalai, 1 isolate). Isolates from Srisaket, Kantararak (B1–1), Surin, Lumduan (B1–2), Burirum, and Satuk (B1–3), the northeast isolates, were remarkably similar to the Lom Khan isolates from Mae Tang, group 15. Roi–et (B1-6), Suwannapum District, was similar to the Jom Thong isolates in group 21.

The similarity of rice and barley isolates could be listed as follows:

1. BRLP 15–16, 18, 20, 23–24 (flag leaf blast)
2. BRLP 17 (flag leaf blast)
3. BRLP22 (flag leaf blast)
4. M2IN0I, 03
5. M2IN07
6. 06CT06
7. HRCD02, IBMW34
8. BRLP03–13 (leaf blast and flag leaf blast)
9. NSCT06
10. BRLP01–02
11. IBCD01–02, 11, 14, 18, IBFG10, 12, 14, IBMT07, IBMWI5
12. 10CD01, 4-05, 10FG02
13. IBCD04–05, 09
14. 10MW03–04, B1–8, KDMR01
15. B1–1–3, LKMT01–03
16. 06MR07–10, 12
17. B1-9
18. 10MW08
19. 10CD03, 06, 07, 08, 09, 1006CD 01, 03, 04, 05, 07, 10–14, 17, 18 06MW 02, 03, 07, 08, 10, 12, 13, 16, 24-34, 06MT 01– 06, IBMW 10, 12, 13, 16–21, 23, 24, 27, 28, 30, 31, 33, IBMT 01, 03, 04, 06, IBCD 06, 17, NSCT 03–05, 07, 09–12, 10FG 01, 03, 05–09, NSMW 01, 04–07, 10, 06HD 01, 03, 04, 07–12, 06CT 01, 02, 04, 05, 10, 11, 06MR 01–03, B1-5, HRWJ 01, 02
20. 06MW 15, 17–23, 06HD 13–1 5, 06CD 16, M1IN 01, 06MT 08–10, IBFG 01, 02, 04, 06, 07, 11, IBCD 07, 15, 16
21. B1–6, 06CT 07–09
22. M1IN 04, M2IN 05
23. 10FG 10, 12, 13, 10MW 01, 06
24. M2IN 04
25. M1IN 02.03

Reaction of selected blast isolates to resistance genes

Thirty–two resistance genes. Three of these resistance genes (*Pia–1*, *Pik–p*, and *Pita–2*) provided adequate germinated seedlings for all nine isolates tested and the resistant reaction “R” was observed in all cases (Table 2). For the other 29 genes, some nongerminated seeds caused missing data in some cases (Table 3). Germinated seeds with resistance genes provided a resistant reaction in most cases. Four resistance genes, *Pita* (C105TTP2L9),*Pis–h* (Shin2), *pi–1* (C101LAC), and *Pi–5(t)* (RIL249 (Moro.)), were compatible with some isolates, thus resulting in the “S” reaction. Several short brown streaks had joined together, causing drying leaves in some cases; therefore, the “S” reaction was given in such cases.

Table 2. Reaction of nine blast isolates from rice and weed hosts to three resistance genes from IRRI.

Entry no.	Designation/ cross combination	Gene	Donor/seed source	Isolate[a]								
				M11N03	PRHD16	M21N04	06MR12	M1N04	UNHD01	PPIN04	EICD01	BMMW22
IRBL2	IRBLa-C	*Pia-1*	C039	R[a]	R	R	R	R	R	R	R	R
IRBL7	IRBLkp-K60	*Pik-p*	K60	R	R	R	R	R	R	R	R	R
IUF$_5$ 37	IR24/C101PKT //6*IR24	*Pita-2*	IUF$_4$ 48-3	R	R	R	R	R	R	R	R	R

[a] R = resistant.

Table 3. Reaction of nine blast isolates from rice and weed hosts to 29 resistance genes from IRRI.

Entry no.	Designation/ cross combination	Gene	Donor/seed source	Isolate								
				M11N03	PRHD16	M21N04	06MR12	M1N04	UNHD01	PPIN04	EICD01	BMMW2
IRBL 1	IRBLa-A	*Pia*	Aishi Asahi	–	–	–	–	R	–	–	R	R
3	IRBLi-F5	*Pi-1*	Fujisaka 5	–	R	–	R	R	R	R	–	R
4	IRBLksF5	*Pik-s*	Fujisaka 5	–	–	–	R	R	–	R	–	–
5	IRBLks-S	*Pik-s*	Shin 2	R	R	R	–	R	R	R	R	
6	IRBLk-ka	*Pik-s*	Kanto 51	R	R	–	–	R	–	R	–	
9	IRBLz-Fu	*Piz-(?)*	Fukunishiki	R	R	–	–	–	R	–	–	–
10	IRBLz5-CA	*Piz-5*	C101A51	R	–	–	–	R	R	R	–	–
11	IRBLzt-T	*Piz-t*	Toride1	R	–	–	–	–	R	R	–	R
12	IRBLta-K1	*Pita*	K1	–	–	–	–	–	–	–	–	R
13	IRBLta-CT2	*Pita*	C105TTP2L9	S	–	–	–	–	S^1	–	S^1	S^1
14	IRBLb-B	*Pib*	BL1	–	R	–	–	–	R	R	–	R
15	IRBLt-K59	*Pita*	K59	–	–	–	–	–	–	–	–	R
16	IRBLsh-S	*Pis-h*	Shin2	R	–	S	–	–	–	–	–	–
17	IRBLsh-B	*Pis-h*	BL1	R	R	–	–	–	R	–	R	–
18	IRBL1-CL	*Pi-1*	C101LAC	R	R	S	R	–	R	R	R	R
19	IRBL3 CP4	*Pi-3*	C104PKT	R	R	R	R	–	R	–	R	–
20	IRBL5-M	*Pi-5(t)*	RIL249 (Moro.)	S^1	S	R	R	R	S^1	–	R	S
21	IRBL7-M	*Pi-7(t)*	RIL29 (Moro.)	R	R	R	R	R	R	–	R	R
22	IRBL9-W	*Pi-9(t)*	WHD-IS-75-1-127	–	R	–	R	–	R	–	R	–
23	IRBL12-M	*Pi-12(t)*	RIL10 (Moro.)	R	–	–	–	–	R	–	–	–
IUF$_5$ 1	IR24/Fujisaka5//6*IR24	*Pii*	IUF$_4$ 2-1	R	R	R	R	–	R	–	R	R
10	IR24/Kusabue//6*IR24	*Pik*	10-3	R	R	R	R	–	R	–	R	–
IUF$_5$ 22	IR24/PI NO.4//6*IR24	*Pita-2*	IUF$_4$ 26-2	R	R	R	R	R	R	–	R	R
28	IR24/C101LAC//6*IR24	*Pi-1*	39-2	R	R	R	R	R	R	–	R	R
43	IR24/C104PKT//6*IR24	*Pi-3*	54-13	R	R	R	R	R	R	–	R	R
52	IR24/C101A51//6*IR24	*Piz-5*	87-8	R	R	R	R	R	R	–	R	R
61	IR24/RIL249//6*IR24	*Pi-5(t)*	98-6	R	R	R	R	–	R	–	R	R
70	IR24/RIL29//6*IR24	*Pi-7(t)*	119-4	R	R	R	–	–	R	–	–	R
79	IR24/WHD-IS-75-1-127//6*IR24	*Pi-9(t)*	111-2	R	R	R	R	R	R	–	R	R

[a]R = resistant, S = susceptible, – = no germination, S^1 indicates only a small black spot on the infected leaf. S^1 = scale 1 (short streak) joined together to form scale 9, causing dried leaf for most leaves.

Sixteen resistance genes. The inoculation test against another set of rice lines containing 16 resistance genes having more fertile seeds was also carried out following the same procedures mentioned above. Some lines contained the same gene but differed from those in the first set in terms of donors. Four distinct isolates were selected for this test.

Most of the genes were incompatible with the isolates tested, resulting in the "R" reaction including *Pik–p* and *Pita–2*, also reported to give a resistant reaction in the first set. This indicated a potential use for these two genes in the blast resistance breeding program (Table 4).

Conclusions

The biodiversity of blast pathogens in the Chiang Mai Valley was quite diverse, although there was a high similarity of the pathogens from the same locations or hosts. The pathogens obtained from barley were quite different from the rice and weed hosts; they were also specific to the same group, such as leaf blast and flag leaf blast. Isolates from the northeast showed similarity to the local rice variety Lom Khan and most of the Mae Wang isolates as well as Wat Chan groups. Some similarity of the weed isolates and rice isolates was found, especially in the highland rice varieties. Considering the isolates obtained from the indicator boxes in which RD 6 was used, the isolates from Chiang Dao, Fang, Mae Tang, and Mae Wang were grouped in the same group.

Table 4. Reaction of four selected blast isolates from rice and weed hosts to 16 resistance genes from IRRI.

Entry no.	Donor	Gene	Isolate[a]			
			10CD05	KDMR0	1LKMT01	06CT08
1	C101LAC	*Pi-1*	R	R	R[a]	R
2	C104LAC	*Pi-1(t)*^{lac}	R	R	S[b]	R
3	C103TTP	*Pi-1(t)*^{ttp}	R	R	R	R
4	C101A51	*Piz-5*	R	R	S[b]	R
5	C104PKT	*Pi-3*	R	R	S[b]	R
6	C101PKT	*Pi-4*^{a}*(t)*	R	S[b]	R	R
7	C102PKT	*Pi-4*^{a}*(t)*^{pkt}	R	R	R	R
8	C101TTP	*Pi-4*^{a}*(t)*^{ttp}	R	R	R	R
9	C105TTP-4-L23	*Pi-4*^{b}*(t)*	R	R	R	R
10	C039	*Pia*	R	R	R	R
11	F98-7	*Pik-m*	R	R	R	R
12	F124-1	*Pita*	R	R	R	R
13	F128-1	*Pita-2*	R	R	R	R
14	F145-2	*Pib*	R	R	R	R
15	F129-1	*Pik-p*	R	R	R	R
16	F80-1	*Pik*	R	R	R	R

[a] R[a] = scale 1 was predominant but covered 30% of the total area; S[b] = scale 1 with 1–2 lesions of scale 5.

The resistance genes involved would be useful for breeding purposes and were the same as previously reported: *Pia–1*, *Pik–p*, and *Pita–2*. The other genes, *Pita*, *Pis–h*, *Pi–1*, and *Pi–5(t)*, showed some resistance to certain isolates and some of the susceptible reaction noted was the short brown streaks that merged to form large lesions. This could depend on the favorable environment for disease development in the tested periods.

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Genetic diverstity of the rice blast pathogen and rice varieties and sustainable control of the disease in Jiangsu, China

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Based on reactions on 7 Chinese rice differential varieties, 7 race groups and 11 races of *Magnaporthe grisea* were detected from rice in Jiangsu Province located in the Yangtze Delta of East China in 1997-99. Race group ZG has always been dominant since 1993. Most of the tested isolates of *M. grisea* can overcome resistance of *Pik-s, Pita-2, Pit,* and *Pita* genes. DNA fingerprints of *M. grisea* by reppolymerase chain reaction (PCR) showed that all tested samples from Jiangsu could be amplified by 6-17 bands and 23 bands were scored in cluster analysis. A dendrogram demonstrated that tested isolates could roughly be classed in 6-7 similarity genetic groups (lineages) at a 55% similarity level by cluster analysis of *M. grisea* DNA fingerprints. Resistance gene *Piz-t* demonstrated resistance to all seven typical Chinese races and was very significant for rice production in Jiangsu. Resistance-gene analog (RGA)-PCR analysis of 59 varieties from Jiangsu (65-90 bands scored) showed abundant polymorphism. A mixture-variety-planting experiment with five varieties with a markedly different genetic background and different resistance demonstrated different rules of regulating blast disease. Control effects varied among different variety groups and the maximum reached 46-97% compared with susceptible variety Suyunuo. Eleven races included in 6 race groups were identified from Wujiang in 1980-94. Data on the resistance reaction of rice differential varieties in Wujiang show that race variation is almost identical to that of artificial inoculation. Serious susceptibility of Tetep from 1989 to 1991 is relative to race group ZA. Xiushui 04 and others with gene *Pita-2* or *Pik-m* easily lost resistance after cultivation for 1-4 years. Variety 93-31 and others with the *Piz-2* gene present maintained their resistance. The recent use of resistant varieties in Wujiang is also a cause for the low occurrence of rice blast.

Rice blast disease (caused by *Magnaporthe grisea*) has long been recognized as one of the most damaging diseases of the rice crop. Resistance to blast is easily lost in 3 to 5 years because *M. grisea* populations are composed of complex pathotypes (physiological races or race groups) (ACCRPPRO 1980, Ou 1980, Valent 1990). DNA fingerprinting research based on repeated DNA sequences and random amplified polymorphic DNA revealed that a complex genetic background exists in *M. grisea* populations (Hamer et al 1989, Valent and Chumley 1991, Levy et al 1991, 1993, Xia et al 1993, Huff et al 1994). *M. grisea* populations are composed of different genetic lineages in which each includes isolates with higher-level genetic similarity. Some research showed that *M. grisea* genetic lineages are related to pathotypes with different cultivar specificity (Hamer et al 1989, Zhu et al 1994). Repetitive element-based polymerase chain reaction (rep-PCR) obtains fingerprints by amplifying sequences between randomly dispersed copies of the element in the plant pathogen genome. The technique is simpler than restriction fragment length polymorphism (RFLP) and has been used in genetic studies of many pathogenic bacteria and fungi (Vera Cruz et al 1996, Shen et al 1996, George et al 1997). The rep-PCR technique using the *Pot2* gene sequence (Kachroo et al 1994) is widely used in genetic similarity studies of *M. grisea* (George et al 1998, Fan et al 1998, Wu et al 1999). Sequence comparisons among cloned resistance genes reveal structural similarities of host-crop varieties (Bent et al 1994, Dixon et al 1996). Although the overall sequence homology among the genes is low and not sufficient to be detected by cross hybridization using RFLP, the conserved motifs in the genes offer opportunities for PCR amplification and the isolation of similar sequences in other plant species (Kanazin et al 1996, Leister et al 1996). Based on conserved motifs of disease resistance genes, PCR primers have been developed as resistance-gene analog (RGA) markers to characterize genetic diversity in crop species (Chen et al 1998). In this chapter, rep-PCR and RGA-PCR were used, respectively, to study the genetic diversity of *M. grisea* and host rice varieties.

A new direction in plant protection research is to exploit biodiversity for sustainable crop pest management. The conservation and manipulation of biodiversity within and around crop fields can be used to develop sustainable, low-pollution, and environmentally compatible crop protection technology for wide dissemination. In this study, through research on genetic diversity analysis of *M. grisea* isolates and rice varieties, rice varieties with different resistance-gene types will be employed in a rational arrangement and in the mixture-variety-planting method according to the distribution of *M. grisea* pathotypes (e.g., physiological races or genetic lineages) (Mundt 1994). Different pathotypes of the blast pathogen will be maintained in equilibrium with no dominant pathotype.

Materials and methods

Around 120 samples per year were collected from Wujiang, Yixin, Tongzhou, Gaoyou, and Ganyu counties of Jiangsu Province from 1997 to 1999. *M. grisea* isolates tested were isolated from the samples by monoconidial isolation. All rice varieties were collected from rice breeding institutions in Jiangsu.

Rice seedlings at the 3-leaf stage were artificially inoculated by spraying suspension with 10^6 spores of *M. grisea* isolates. Details for the method of sporulation, inoculation, and scoring of disease were based on Sun et al (1989) and Zhou et al (1999). Reactions of rice varieties to *M. grisea* isolates were scored for identification of blast race groups (ACCRPRPO 1980) and for identification of resistance-gene types of rice varieties (Lu et al 1999). Seven Chinese rice differential varieties (Tetep, Zhenglong 13, Sifeng 43, Dongnong 363, Guandong 51, Hejiang 18, and Lijianghegu) were used to classify race groups. Thirteen mono-resistance-gene differential varieties (Shin 2, Aichi-Asahi, Fujisaka 5, Kusabue, Tsuyuanke, Fukunishiki, K1, Pi-4, Toridel, K-60, BLI, K59, and K3) from Japan or differentials of rice near-isogenic lines (NILs) (CO39 and LTH NILs) from IRRI (Chen and Ling 1995) were used for pathogenicity of isolates and resistance-gene-type markers of tested rice varieties.

Methods of DNA extraction from *M. grisea* and rice were based on George et al (1998) and Murray et al (1980).

DNA of *M. grisea* and rice varieties was amplified by using rep-PCR (George et al 1998) and RGA-PCR (Chen et al 1998), respectively. Two primers (*Pot2-1* 5′ CGGAAGCCCTAAAGCTGTTT3′ and *Pot2-2 5′* CCCTCATTCGTCACACGTTC 3′) were applied for rep-PCR. Primer pairs XLRR for/rev, S I/AS3, and Pto kin 1/kin2 were used in RGA-PCR for analysis of rice variety resistance genes.

Ten to 12 **m**L of *M. grisen* DNA product amplified by rep-PCR was electrophoresed in a gel containing 0.5% agarose and 0.75% Synergel™ and in 0.5x TBE buffer for 7–9 h at 120V (George et al 1998). After the PCR product was stained by EB, fingerprint results were read and recorded under ultraviolet light. Five **m**L of product amplified by RGA-PCR was run in 0.5x TBE buffer by high-resolution denatured PAGE for 2–2.5 h at ~ 1,400V at 50 °C. The gel was silver-stained and results were recorded by a photo-scanner (Chen et al 1998). All solutions for DNA extraction and electrophoresis were made as in Sambrook et al (1989). To determine the genetic relationships among isolates or rice varieties, the presence or absence of bands was converted into binary data (1 for presence and 0 for absence of each band). Similarity matrices were calculated with Dice's coefficient and the SIMQUAL program of NTSYS-pc version 1.7. Cluster analysis was done within the SAHN program by using the UPGMA method. Some results were analyzed by soft Gel Compar version 4.2.

Five rice varieties with very different genetic backgrounds on the basis of RGA-PCR analysis and different resistance to rice blast were selected for the MVP experiment. Six mixture-planting combinations were randomly arranged in the MVP trial with four replications for each treatment and a buffer interval of susceptible variety Suyunuo. Leaf blast was scored four times and neck blast one time. Yield was measured according to five grade scales.

Results

Pathogenicity and genetic lineages of *M. grisea* from rice in Jiangsu

Pathogenicity of M. grisea *isolates from rice in Jiangsu.* One hundred to 115 isolates of *M. grisea* were isolated from five rice regions of Jiangsu each year in 1997-99. Based on the reactions on 7 Chinese rice varieties, 7 race groups and 9 races were

Table 1. Resistance of 13 mono-gene varieties to tested isolates from Jiangsu in 1997 and 1998.

Year	No. of isolates tested	Gene type of mono-resistance-gene varieties												
		Pik-s	*Pia*	*Pii*	*Pik*	*Pik-m*	*Piz*	*Pita*	*Pita-2*	*Piz-t*	*Pik-p*	*Pib*	*Pit*	*Pik-h*
		Frequency of compatible reactions (%)												
1997	107	90	54	10.3	16.8	20.6	20.6	66.5	69.2	1.9	11.2	11.2	66.4	12.2
1998	109	93	19	6.4	13.8	18.4	0.9	56	72.5	0.0	11	0.0	13.8	1.8

obtained in 1997, 6 race groups and 11 races in 1998, and 6 race groups and 9 races in 1999. Since 1993, race group ZG was always dominant but race group ZA was barely present. Results of pathogenic analysis with mono-resistance-gene varieties are shown in Table 1. Most of the 107 isolates of *M. grisea* from rice in 1997 can overcome the *Pik-s, Pita-2, Pit,* and *Pita* resistance genes. There is a low infection rate in the varieties with *Piz-t, Pii, Pib, Pik-p,* and *Pik-h* genes. Most of the 109 isolates in 1998 can overcome *Pik-s, Pita-2,* and *Pita* resistance genes. There is a low infection rate in the varieties with *Pii, Piz-t, Pib,* and *Pik-h* genes.

Population structure and lineage analysis of M. grisea from five rice-planting regions in Jiangsu using rep-PCR analysis. DNA fingerprints of *M. grisea* by rep-PCR showed 6–17 bands. A dendrogram demonstrated differentiation of all isolates collected in 1996-98 from Jiangsu. All isolates could roughly be classed in 6–7 similarity genetic groups (lineages) at a 55% similarity level. Different fingerprint patterns exist in the isolates from the same rice cultivation region and are distributed in 2–4 lineages. The isolates collected from major cultivated varieties belong to similar lineages. DNA fingerprints showed that most of the isolates (more than 85%) from Jiangsu Province contained a front band (around mw = 440) that barely existed in the isolates from other provinces of China. Isolates from the same lesions are similar.

Resistance and genetic diversity of rice varieties in Jiangsu

Resistance of major cultivated rice varieties in Jiangsu. The resistance to *M. grisea* of 17 cultivated varieties and 21 candidate varieties in Jiangsu was investigated. Five varieties have high resistance to *M. grisea* in the field, seven have moderate resistance, three have moderate susceptibility, and two have high susceptibility in the main cultivated varieties; 13 have high resistance, five have moderate resistance, and three have moderate susceptibility in candidate varieties. Using 13 mono-resistance-gene varieties as a control, 21 tested varieties from Jiangsu could be divided into 6 gene types. Seven of 21 varieties carrying resistance-gene *Piz-t* demonstrated resistance to all 7 typical Chinese races. Analysis of 28 varieties from Jiangsu with inoculation of 6 typical isolates of the Philippines showed that (1) reaction to Philippine isolates varies highly between varieties and single plants of varieties and (2) varieties susceptible and resistant to blast of Jiangsu displayed similar reactions to typical isolates from the Philippines (e.g., Suyunuo, Shanyou 63).

Resistance-gene analysis of rice varieties in Jiangsu by RGA-PCR. Genomic DNA samples of 59 rice varieties from Jiangsu including all varieties used in the mixture-variety-planting experiment and 6 C039 NILs were amplified by RGA-PCR with primer pairs XLRR for/rev, S1/AS3, and Pto kin1/kin2. High-resolution electrophoresis analysis showed abundant polymorphism in the 59 varieties from Jiangsu (65–90 bands scored) (Fig. 1) and poor polymorphism in the NILs. Variety clusters formed by RGA analysis closely corresponded to pedigree and their affinity to japonica, indica, and hybrid varieties. RGA-PCR analysis of four varieties and one buffer variety of the mixture-variety-planting experiment in 1999 at Wujiang, Jiangsu, showed a high degree of variation in five varieties for polymorphism and the genetic background of the varieties was distant among them.

Plot experiment of mixture-variety-planting for control of rice blast disease

Five varieties with different genetic backgrounds and different resistances were selected for MVP in Wujiang, Jiangsu, in 1999 and 2000. Four scorings for leaf blast showed that 95-22 and Zhendao 2 have higher resistance than Taihujing 2 and Zhendao 6. Severity of blast disease among the susceptible varieties increased in the early stage and declined in the late stage. This demonstrated different ways to regulate blast disease in the MVP. The control effect in the MVP was about 10–37% more than the

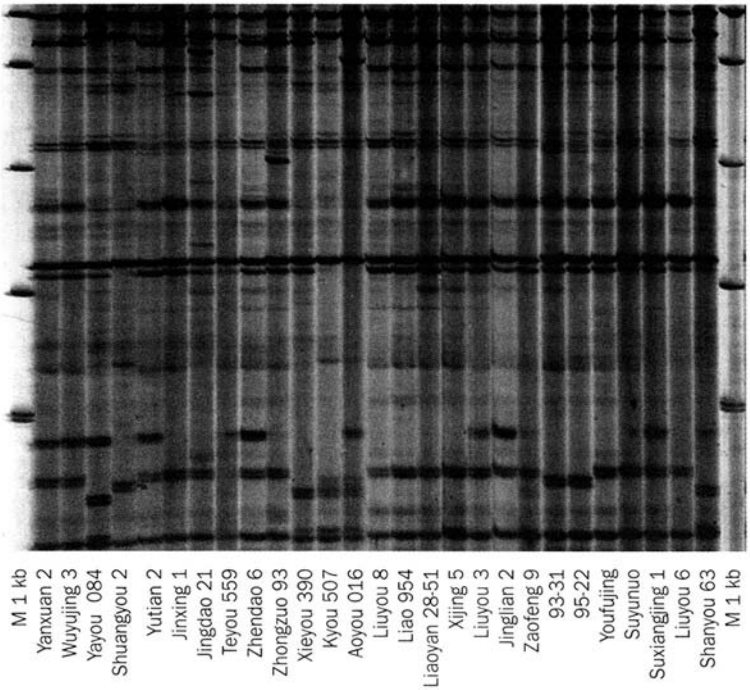

Fig. 1. Polymorphism of fingerprint patterns for rice varieties from Jiangsu by RGA-PCR.

mean of two varieties compared with mono-variety-planting, 8–46% more than that of one of two varieties compared with mono-variety-planting, and 46–97% more than that of susceptible variety Suyunuo. Severity of blast disease in the treatments with two susceptible varieties increased in the early stage and declined clearly in the late stage, but the degree of severity of blast disease in the treatments with one resistant variety and one susceptible variety barely increased in the early stage. This demonstrated that the MVP with different resistant varieties could effectively control the spread of blast in susceptible varieties. Yield analysis showed a similar trend in the control effect of rice blast.

Analysis for race variation of *M. grisea,* its relationship with rice varieties, and the use of resistant varieties for sustainable control of the disease in Wujiang

The types, appearance frequency, and variation of M. grisea races from rice in different years in Wujiang. On the basis of reactions on 7 Chinese rice differential varieties, 11 races included in 6 race groups were identified from 133 monoconidial M. grisea isolates in Wujiang in 1980-94: ZA_{19}, ZA_{49}, ZA_{51}, ZB_{17}, ZB_{31}, ZD_1, ZD_3, ZE_1, ZE_3, ZF_1, and ZG_1. Figure 2 showed that the appearance frequency of ZG_1 is highest with 46.5%, followed by race group ZE with 18.1%, ZD with 15.1%, ZF with 12.0%, ZA with 7.7%, and ZB with 0.6%. The appearance frequency of the races varied markedly with different years. ZG_1 and ZF_1 were only isolated in 1980-82, ZE and ZD were dominant in the isolates in 1983-88, ZA appeared in 1989-92 and was superior in 1990, ZD and ZE showed dominance in 1991 and 1992, and ZA disappeared and ZG recovered as a dominant race group since 1993.

Loss of rice variety resistance and use of resistant rice varieties. Data on the resistance reaction of rice varieties in a diseased garden (Table 2) shows that race variation is almost identical to that of artificial inoculation. Lijianghegu has always been susceptible since 1980 for the presence of race group ZG. Guandong 51 and Dongnong 363 became susceptible because of the presence of ZE and ZD. The serious susceptibility of Tetep from 1989 to 1991 is relative to ZA. Xiushui 04, Xiushui

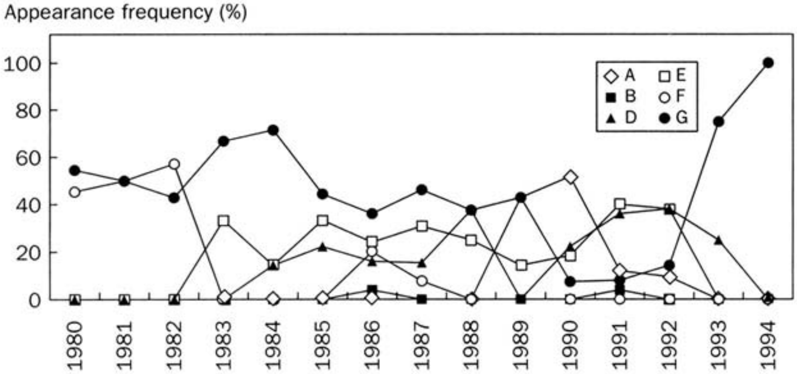

Fig. 2. Variation trend of *M. grisea* races in Wujiang, Jiangsu, China.

Table 2. Resistance reaction of rice varieties in a diseased garden in different years in Wujiang.

Variety	Resistance reaction of rice varieties[a]																			
	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
Indicator varieties																				
Tetep	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	–	–	/	/
Zhenglong 13	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	/	/
Sifeng 43	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	/	/
Dongnong 363	–	–	–	–	–	–	+	–	–	+	+	+	+	+	+	+	+	+	/	/
Guandong 51	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	/	/
Hejiang 18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	/	/
Lijianghegu	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	/	/
Variety change																				
Xiushui 48	/	/	R	R	S	S	S	/	/	/	/	/	/	/	/	/	/	/	/	/
Xiushui 06	/	/	/	/	R	S	S	S	/	/	/	/	/	/	/	/	/	/	/	/
Xiushui 04	/	/	/	/	/	R	R	R	R	S	S	S	/	/	/	/	/	/	/	/
8524	/	/	/	/	/	/	/	/	R	S	S	S	/	/	/	/	/	/	/	/
Bing 620	/	/	/	/	/	/	/	/	/	/	R	S	/	/	/	/	/	/	/	/
88-122	/	/	/	/	/	/	/	/	/	/	R	S	S	S	/	/	/	/	/	/
91-17	/	/	/	/	/	/	/	/	/	/	/	/	/	R	R	R	R	/	/	/
93-31	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	R	R	R	R
Wuyujing 5	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	R	R	R	S
9522	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	R	R	R
9325	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	R	R

[a] + = infected. – = resistant. R = resistant. S = susceptible. / = not planted.

Table 3. Loss of resistance of major rice varieties and replacement for control of blast in Wujiang.

Variety	Year released to year variety resistance lost	Proportion of planted area (%)	Diseased area (ha)	Rate of infected panicles (%)	Severity[a]	Isolated race	Gene type
Xiushui 48	1982-84	42.7 (1984)	6,000	26.5	11.8	B_{31}, E_3, G_1	*Pita-2*
Zhejing 66	1984-86	7.8 (1986)	6,000	32.2	17.8	D_1	
8006	1984-86	4.0 (1986)	6,000	58.9	34.6	D_1, E_1, E_3	
Xianghu 47	1984-86	3.8 (1986)	6,000	61.0	38.4	D_1	
Xiushui 04	1985-89	73.2 (1988)	9,133	99.2	55.0	A_{49}, A_{19}, A_{51}, B_{17}, D_1, D_3, E_1, E_3, G_1	*Pita-2*
8524	1988-89	26.6 (1989)	1,333	98.4	65.0	A_{49}, A_{19}, A_{51}, D_3, E_3, G_1	
88-122	1990-92	92.7 (1992)	17,067	88.1	53.3	A_{49}, D_1, D_3, E_1, G_1	*Pik-m*
Bing 91-17	1993-	75.8 (1994)	0	0	0	No	*Piz-t*
Wuyujing 5	1995-99	4.3 (1998)	400	34.7	19.2	No	*Piz-t*
93-31	1996-	89.1 (1996)	0	0	0	No	*Piz-t*
9522	1996-	89.7 (1999)	0	0	0	No	*Piz-t*
93-25	1998-	3.0 (1998)	0	0	0	No	*Piz-t*

[a] Disease index on a scale of 0–100.

48,88122, and others with gene *Pita-2* or *Pik-m* lost resistance after cultivation for 1–4 years. Varieties 97-17, 93-31, 9522, and Wuyujing 5 planted at present maintain resistance. Table 3 shows the use of resistant varieties and resistance loss in Wujiang. The use of resistant varieties is also a cause for the rare occurrence of rice blast in Wujiang.

Discussion

Jiangsu Province is located in the Yangtze Delta of East China. About 3 million ha of rice are cultivated there. Rice blast is always an important disease. Since 1980, pathogenicity of the blast pathogen has been monitored. Results show that races of pathogenicity exist in diverse forms. Cluster analysis of DNA fingerprints demonstrates that abundant genetic diversity, including 6–7 lineages in 55% similarity, exists in the *M. grisea* population structure from rice in Jiangsu too (Wu et al 1999). Lineages of *M. grisea* are very relative to the varieties from which pathogen samples are collected. The variation of lineages varies slowly with years (Wu et al 1999). Abundant resistance diversity and genetic diversity exist in rice varieties from Jiangsu (Wang et al 1999). Rice blast is a disease that follows the gene-for-gene concept (Flor 1971, Valent 1990, de Wit 1992, Silué et al 1992). The pathogenicity of the *M. grisea* structure varies with cultivated rice varieties (Lu et al 1999, Yan et al 1999). A varietal diversification program carried out in Jiangsu Province for the past 15 years has apparently reduced the occurrence of the disease. The use of genetic diversity for the sustainable control of blast includes the replacement of resistant varieties, rotation of resistant and susceptible varieties, rational arrangement of different resistance-gene

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typical varieties, and mixture-variety-planting with very different genetic backgrounds. These measures have been employed widely in Jiangsu and have given farmers huge benefits. The MVP experiment shows that the use of genetic diversity is beneficial to not only the sustainable control of blast but also to yield increases. Understanding the genetic diversity of *M. grisea* and cultivated rice varieties has important significance for the sustainable management of rice blast disease in Jiangsu.

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Notes

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Prospects of a marker-aided varietal diversification strategy for disease control

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and Hei Leung

Polymerase chain reaction (PCR) primers corresponding to the conserved motifs of prototype resistance genes were used to characterize germplasm in traditional and current commercial varieties that are candidates for varietal diversification. The predictive value of PCR markers on the phenotypic expression of disease resistance among germplasm was evaluated. Our results showed that, although the markers were useful in grouping genetically related germplasm and varieties, they are not sufficiently predictive of specific resistance phenotypes. Based on analyses of segregating populations from other controlled crosses, we conclude that specific sequences corresponding to candidate resistance or defense genes have better potential for predicting functionality.

The study of Zhu Youyong et al (this volume) has demonstrated that intrafield varietal diversification can be an effective tool for controlling rice blast. The combination of the highly susceptible glutinous rice and the relatively resistant hybrid indica rice, planted in a repeating pattern of 1 row of glutinous rice for every 4–6 rows of indica rice, has been particularly effective in reducing blast in the susceptible variety. However, results of mixed planting experiments involving resistant and susceptible japonica rice have not shown significant disease reduction, suggesting that functional differences, in either disease resistance or other agronomic traits, are important factors in determining the effect on disease reduction.

One approach for identifying the appropriate combination of genotypes would be to evaluate experimentally the effect of disease control and yield gain in different combinations in the field. Although this could be guided by historical agronomic data, determining the right genotypes and combinations by screening and field testing is a daunting task, particularly if the number of varieties to be evaluated is large. Although a variety of genetic and molecular markers are available in rice for use in diversity studies, and in marker-aided breeding, no study applies markers to determine diversification.

Molecular markers can be used to aid this process in two ways depending on the nature of these markers. First, anonymous markers can be used to assess the relatedness of the test materials. Second, markers that are associated with a putative function can be used to predict performance in a particular trait. We reason that it may be possible to use molecular markers to predict which combination will be effective and to increase the efficiency in selecting candidate varieties for diversification. We further hypothesize that functional diversity in resistance genes possessed by the varieties in a mixture is the determining factor in mixture effectiveness to control the spread of the disease. Following this line of thinking, we focus on the molecular markers that are most likely related to resistance genes.

Disease resistance genes

We can broadly group genes involved in host resistance into two categories. First are the major resistance genes (R) that are commonly used for disease control; these genes are involved in pathogen recognition, which triggers downstream signal transduction pathways. Cloning and molecular analyses of R genes have revealed that most R genes share similar structural motifs: the nucleotide-binding site (NBS), leucine-rich repeats (LRR), and kinases (Song et al 1995, Dixon et al 1996, Yu et al 1996). Such sequence similarity suggests that many plants adopt a similar, though not identical, structure-functional scheme in defending against invading pathogens. For disease control, sequence conservation has offered opportunities to develop markers that are indicative of function (Leister et al 1996). Conserved DNA primers can be designed to amplify sequences by polymerase chain reaction (PCR) that correspond to the conserved motifs of resistance genes. These amplified sequences are called resistance-gene analogs or RGA. Resolving the amplification products in a polyacrylamide gel would reveal polymorphic RGA fingerprints. RGA markers have been used to characterize germplasm and breeding lines in barley, wheat, and rice (Chen et al 1998). This strategy is becoming more powerful with the increasing amount of sequence information available in public databases. It is now possible to inspect the entire genome of an organism to identify sequences that have NBS-LRR motifs. For example, it is now known that the *Arabidopsis* genome has 168 NBS-LRR sequences. A larger number of such sequences have been found in rice, although not all these sequences are necessarily functional resistance genes.

The second category of host resistance genes includes those that are downstream from the initial recognition. These are genes that control the intermediate steps in the signal transduction pathways and genes that encode products that directly restrict pathogen growth. These are generally considered as defense response genes that mediate resistance to invasion and function collectively to restrict pathogen growth. Thus, it is generally assumed that these genes are related to quantitative resistance. However, there is increasing evidence that some major genes can also function quantitatively in conferring resistance to pathogens (Li et al 1999).

In this study, we used PCR primers corresponding to the conserved motifs of prototype resistance genes to characterize germplasm in traditional and current commercial varieties that are candidates for varietal diversification. We evaluated whether the PCR markers have predictive value on the phenotypic expression of the germplasm with respect to disease resistance. Our results showed that the markers are useful in grouping genetically related germplasm and varieties. However, they are not sufficiently predictive on specific resistance phenotypes. Based on analyses of segregating populations from other controlled crosses, we conclude that specific sequences corresponding to candidate resistance or defense genes are more useful in predicting functionality.

Experimental approach to relate RGA polymorphism and diversity in blast resistance

Blast epidemics used to be of common occurrence in Jiangsu Province. But, for the past 15 years, starting when farmers in the province grew diverse rice varieties in different districts, blast epidemics have rarely occurred. We hypothesized that this could be attributed to the diversity of rice varieties planted in the area. To test the hypothesis, we characterized the level of diversity present in the widely grown varieties using molecular markers. We then tested the level of association between molecular and functional diversity in disease resistance.

We collected 36 elite varieties extensively cultivated in different districts of Jiangsu. Historical production data and agronomic characters are shown in Table 1. To evaluate the spectrum of resistance, these varieties were inoculated with seven Philippine isolates (PO6-6, IK81-3, IK81-25, CBN9219-25, JMB840160, C9240-1, V850196) that were commonly used to differentiate many blast resistance genes among rice germplasm in the tropics.

Three primer pairs (XLRRfor/XLRRrev, S1/AS3, and Ptokin 1/Ptokin2) corresponding to each of the three conserved motifs (LRR, NBS, and kinase domains) were used in this study (Table 2). We determined in earlier studies with 41 rice varieties from Yunnan Province (unpublished data) that three to four primer pairs were sufficient to reveal the genetic relationships among rice varieties.

The procedures for PCR amplification, electrophoresis, and silver staining were adopted from Chen et al (1998). PCR solution consisted of 50 mM KCL, 20 mM Tris-HCI, 5 mM $MgC1_2$, 0.2 mM of each dNTP, 60 ng of each primer, 20 ng of template DNA, and approximately 2.5 units of *Taq* polymerase for each 25 mL of reaction. Amplification was performed using 40 cycles of 1 min at 94 °C, 1 min at 45 °C, and 2 min at 72 °C. Final extension of 72 °C followed for 7 min.

PCR products were resolved in 4% polyacrylamide gels in 1X TBE buffer. Electrophoresis was carried out using a sequi-gen nucleic acid sequencing cell (Biorad, USA) for 2 h at 8 watts. Bands were detected by a silver sequence DNA staining system (Promega, USA) with a 1-kb ladder as a DNA marker.

To infer the genetic relatedness among varieties, dendrograms based on a polymorphic banding pattern were constructed using the numerical taxonomy system (NTSYS-pc, version 1.80, Rohlf 1993) and Gelcompar software (Applied Maths).

Table 1. Historical data and agronomic characteristics of varieties used in Jiangsu Province, 1983-97.

Variety	Type[a]	Pedigree	Year released	Area planted[b]	Region planted[c]	Yield (t ha⁻¹)	Plant height (cm)	Growth period (d)	Field resistance[d]
Shanyou 63	I-H	Zhenshan 97A/Minghui 63	1985	9,776.7	AYH, LXH, NHR, HS	7.5	115–120	142	R
Shanyou 559	I-H								
Teyou 559	I-H	Longtefu A/Hui 559	1996	50	AYH, LXH, NHR, HS	8.3	110		R
Zhenxian 232	I	Xiangzaoxian No.3/IR54	1996	3.3	AYH, LXH, NHR, HS	8.3	110–115	147	R
Siyou 422	J-H	Sidao No. 8A/422	1993	6.7	TH, HS	10.5	105	155–160	MR
Liuyou No. 6	J-H								
Jiuyou 138	J-H	9201A/N138	1996	13.2	–	8.3	105–110	145–150	MS
Xiushui 04	J	Ce21///Funong 709// Funong 709/Dan209	1987	733.3	Southeastern TH	7.5	105	162	R
Xiushui 122	J								
Huaidao No. 2	J	3017/Shennong 1033	1992	3.3	NHR	7.5	90–95	136	S
Huaidao No. 3	J	02427/Sidao No. 8	1996	10	NHR	8,3	95	145	MR
Sidao No. 9	J	Sidao No. 8/Zhongdan No. 3	1993	166.7	NHR	7.5	85	141–147	MR
Sidao No. 10	J	Suxiejing /Yanjing No. 2	1996	10	LXH	8.3	95	150	R
Yandao No. 4	I	3021/665	1995	3.3	AYH, LXH, NHR, HS	8.3	115	140	R
Yandao No. 5	I	Teqing/4011 Nuo	1997	–	AYH, LXH, NHR, HS	8.3	110	141	R
Zhendao No. 2	J	Guihuanuo mutant	1992	100	TH, HS, South AYH	7.5	95	150	MS
Zhendao No. 3	J	7030/Chengte232//Xiushui 04	1995	1.3	TH	8.3	95	160–165	S
Zhendao No. 4	J	Xinguang/Nanjing 11	1997	3.3	LXH	8.3	85–90	150	S
Zhendao 88	J	Yueguang/Wuxiangjing No. 1	1997	240	NHR	8.3	95	145	R
Yangdao No. 2	I	BG902	1985	521.4	AYH, LXH, NHR, HS	7.5	110	145–149	R
Yangdao No. 4	I	Yangdao No.2/7101	1990	127.5	AYH, NHR, HS, LXH	7.5	120	140	R
Yangdao No. 6	I	665/3021 (Co60 radiation mutant)	1997	3.3	AYH, LXH, NHR, HS	11.3	115	145	R
Yanjing No. 4	J-H	20229/Shennong 1071	1993	203.3	LXH	7.5	95–100	150	MS
Yanjing No. 5	J	Yanjing No. 2/Suxiejing	1997	66.7	LXH	8.3	80–85	148	MR
Wuyujing No. 2	J	Zhongdan No. 1/79-51// Zhongdan No. 1/Yangjing No. 1	1989	1,455.3	TH, HS, South AYH	8.3	95–100	155	MR
Wuyujing No. 3	J	Zhongdan No. 1/79-51// Zhongdan No. 1/Yangjing No. 1	1992	2,440.7	LX, South AYH, HS	8.3	94	150	MR
Yangjing 186	J	Yang 201/Huangjinqing	1996	33.3	LXH, HS	8.3	100	145	S
Yangjing 201	J	Nanjing 11/Nanjing 32	1989	80	LXH	7.5	107	140–145	S

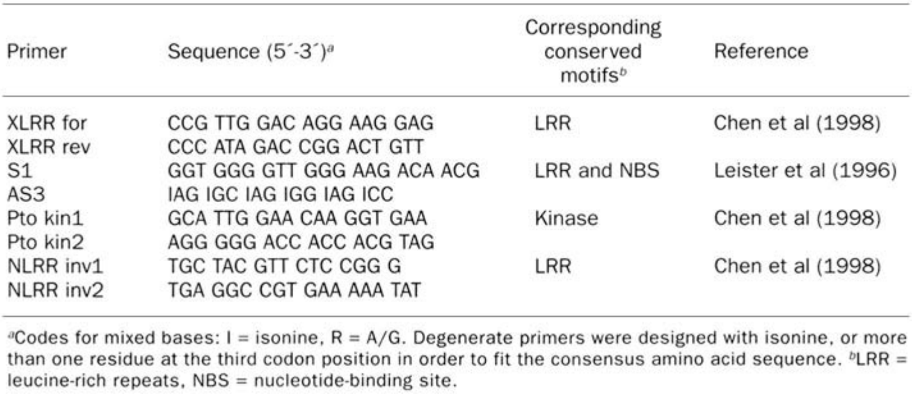
continued on next page

Table 1 continued.

Variety	Type[a]	Pedigree	Year released	Area planted[b]	Region planted[c]	Yield (t ha^{-1})	Plant height (cm)	Growth period (d)	Field resistance[d]
Taihujing No. 2	J	Taihunuo/Xiushui 04	1994	33.3	South TH				MR
Yangfunuo No. 1	I	IR29 radiation mutant	1990	16.7	AYH, LXH, NHR, HS	7.5	97	130–135	R
Yangfunuo No. 4	I	IR1529-681-3-2	1995	3.3	AYH, LXH, NHR, HS	8.3	102	139	R
Yangfuxian No. 2	I	IR1529-68-3-2 radiation mutant	1991	6.7	AYH, LXH, NHR, HS	7.5	110	143	R
Ce 48	J								
Tong 109	J	Suyinjing No. 1		66.7	South AYH				MR
93-25	J	Wuyujing No. 2/Wuxiangjing No. 1//Xiushui 04///I22		200	TH, HS, South AYH				MR-MS
9522	J	Jia 48/9121//Bing 815		6.7	TH, HS, South AYH				R

[a] I = indica, J = japonica, H = hybrid. [b] In thousand ha. [c] LXH = Lixiahe rice region, NHR = northern Huaihe River rice region, HS = hill rice region, TH = Taihu rice region, AYH = along Yangtze River and Huanghai Sea rice region. [d] R = resistant, S = susceptible, MR = moderately resistant, MS = moderately susceptible.

Table 2. PCR primers used to characterize rice varieties from Jiangsu Province.



Primer	Sequence (5´-3´)[a]	Corresponding conserved motifs[b]	Reference
XLRR for	CCG TTG GAC AGG AAG GAG	LRR	Chen et al (1998)
XLRR rev	CCC ATA GAC CGG ACT GTT		
S1	GGT GGG GTT GGG AAG ACA ACG	LRR and NBS	Leister et al (1996)
AS3	IAG IGC IAG IGG IAG ICC		
Pto kin1	GCA TTG GAA CAA GGT GAA	Kinase	Chen et al (1998)
Pto kin2	AGG GGG ACC ACC ACG TAG		
NLRR inv1	TGC TAC GTT CTC CGG G	LRR	Chen et al (1998)
NLRR inv2	TGA GGC CGT GAA AAA TAT		

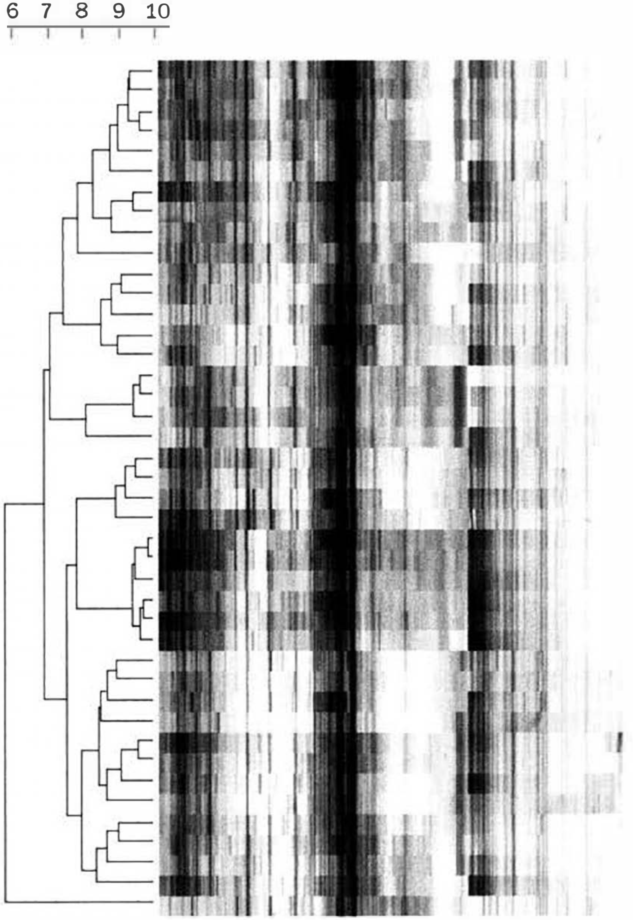
[a] Codes for mixed bases: I = isonine, R = A/G. Degenerate primers were designed with isonine, or more than one residue at the third codon position in order to fit the consensus amino acid sequence. [b] LRR = leucine-rich repeats, NBS = nucleotide-binding site.

Genetic differentiation shown by RGA markers

Abundant RGA polymorphism was observed among the varieties tested. Varietal clusters formed by RGA markers closely corresponded to pedigree and their affinity to japonica, indica, or hybrid rice (Fig.1). Varieties within the same cluster had a similar spectrum of resistance to the blast isolates tested.

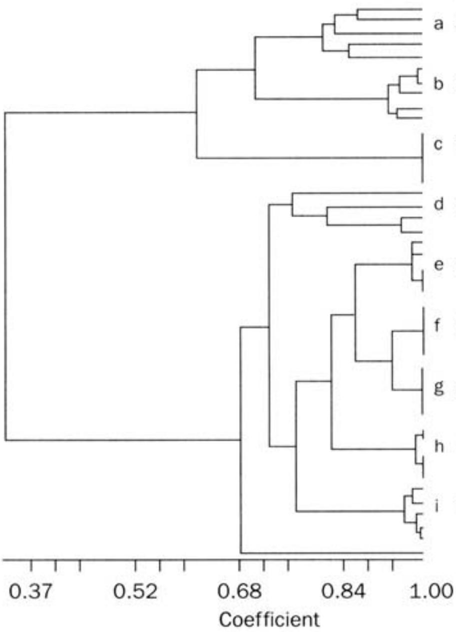
Some varieties showed heterogeneous RGA fingerprints among individual plants. These individual plants also showed a range of disease reaction from susceptibility to resistance. To confirm the observed within-variety variation, single plants (5–15 plants in two separate experiments) from nine varieties showing both a homogeneous and heterogeneous disease reaction were examined further for disease reaction and RGA polymorphism. The result consistently showed that varieties with extensive variation in disease reaction also showed marked polymorphism in RGA markers. In contrast, varieties with uniform disease reaction showed no RGA polymorphism (Fig. 2).

The results suggest that polymorphism in RGA markers is correlated with functional polymorphism in the varieties we sampled from Jiangsu Province. Yet, attempts to genetically relate specific RGA polymorphism with the phenotypic segregation in resistance have not been successful. In one such genetic test, the polymorphic RGA marker did not co-segregate with resistance in the F_2 progeny. It is possible that many other resistance genes are present in the variety and that the RGA profile does not capture the molecular variation related to disease resistance. Thus, although diversity in disease resistance and molecular markers exists in commercial varieties, pinpointing the precise relationship between specific genes and function may require additional genetic markers and diagnostic pathogen strains to discern specific resistance genes.



		P06-6	IK8-25	CBN9219-25	JMB840160	C9240-1	V850196
Wuyujing No. 2	J	S	S-R	R-MR-S			
Taihujing No.2	J	R	S-MS-MR	R	R	R	R
Yangjing No. 186	J	S	S-MS	S-MR-R	MS-MR-R	R-MR	R-MR
Yangjing 201	J	S	S-MS-MR-R	S	S	S-MS-R	S-MR-R
Yanjing No. 5	J	R	MS-MR	R-MR-S	R	R	R
Yanjing No. 4	J-H	R	S-MS-MR-R	R	R	R	R
Yangfunuo No. 1	I	S	S-MS-MR	R-MS	R	R	R
Yangfunuo No. 4	I	S	S-MS-MR	R-MR-MS	R	R	R
Yangfuxian 2	I	S	S-MS-MR-R	S	R-MS	R	S-MR-R
Wuyujing No. 3	J	R-MR-MS	R-S	R-MR-S	R-MR-S	R-MR-MS-S	R
Zhendao 88	J	R	MS-MS-MR-R	R	R-MR-S	R-MR	R
Yangdao No. 2	I	S	S-MS-MR-R	S-MS-R	R-S	R-S	R-MR-S
Yangdao No. 6	I	S	S	R-MS-S	R-MR-MS-S	R-MR	S-MR-R
Yandao No. 4	I	S	S-MS-MR	R	R-MS-S	R	R
Yandao No. 5	J	S-MS-MR-S	S-MS-MR-R	S-MRS-MR-R	R	R	R-MR-S
93-25	J	S-MR-R	R	R	R	R	R
9522	J	R	R	R	R	R	R
Tong 109	J	R	S	R	R	R	R
Ce 48	J	R	S-MS-MR-R	R	R	R	R
Shanyou 559	I-H	R-S	S-MS-MR-R	R-MR-MS	R	R	R-S
Teyou 559	I-H	R-MR-S	S-MS-MR-R	R-MS-S	R	R	R
Zhenxian 232	I	S	R	S	S-MS	R	R
Shanyou 63	I-H	R	R	R	R-MR	R-MR-MS-S	R
C039	I	S	S	S	S	S	S
C101A51	I	R	S	R	S	R	R
C101LAC	I	R	R	R	R	S	R
C104PKT	I	R	S	R	S	S	S
C101PKT	I	S	R	S	S	R	R
C105TTP	I	S	R	S	R	R	R
Siyou 422	J-H	R	S-MS-MR-R	S-MS-R	R-MR-MS-S	R-MR	R
Jiuyou 138	J-H	R	R	S	R-MR-MS-S	R	R
Liuyou No. 6	J-H	S-MS-MR-R	S-MS-MR-R	S-MR-R	R-MR-MS-S	R	R
Xiushui 04	J	R	R	R	R	R	R
Huaidao No. 3	J	R	R-MR	R	R	R	R
Sidao No. 9	J	R	R-MR	R	R	R-MR	R
Huaidao No. 2	J	R	R-MR	R	R	R	R
Xiushui 122	J	R-MR	R	R	R	R	R
Sidao No. 10	J	R	R-MR-MS	R	R-MS-S	R-MR-MS	R
Zhendao No. 2	J	S-MR	MS-MR	S-MR	MS-S	R	R
Zhendao No. 4	J	S-R	R	R	R	R	
Zhendao No. 3	J	S	R	R	S-R	R	R
Yangdao No. 4	I	S	S-MS-R	S	R-MR-S	R	R-MR

Fig. 1. A Gelcompar-generated dendrogram of 36 rice varieties from Jiangsu, China, and six C039 near-isogenic lines based on three resistance-gene analog primer combinations and the corresponding blast resistance. I = indica, J = japonica, H = hybrid, R = resistant, MR = moderately resistant, S = susceptible, MS = moderately susceptible.



	Variation in blast resistance within lines		
	Isolates		
Variety	P06-6	IK81-3	IK81-25
a. Shanyou 63	R-S	R-MR-MS-S	R-MR-MS-S
b. Teyou 559	R-MR-S	R-MR-MS-S	MR-MS-S
c. Zhenxian 232	S	R	R
d. Liuyou No. 6	R-MR-MS-S	R-MR-MS	R-MR-MS
e. Xiushui 122	R-MR-S	R	R
f. Huaidao No. 2	R	R	R
g. Sidao No. 9	R	R	R
h. Yangjing 186	S	S-MS-MR	S
i. Zhendao No. 3	R-MR-S	R	R

Fig. 2. RGA clustering of five single plants within lines and their corresponding blast resistance variation. R = resistant, MR = moderately resistant, S = susceptible, MS = moderately susceptible.

Predictive value of RGA markers for varietal deployment

Although an exact relationship between RGA polymorphism and disease resistance was not established, the overall correlation between genomic variation and diversity in resistance suggests that RGA markers could be used as predictors of functional diversity. If RGA polymorphism reflects function, varieties with similar RGA profiles would be less likely to provide complementary function. It follows that varieties with dissimilar RGA profiles would be more effective in reducing disease by providing complementary function in disease resistance. We set out to test whether RGA fingerprint unrelatedness of varieties is related to performance of the varietal combinations used in diversification experiments.

Working with varieties used in the Yunnan diversification experiments, we found that the glutinous varieties Huangkenuo and Zhinuo are distantly related to the hybrid indica varieties Shanyou 63 and Shanyou 22, compared with the japonica varieties, which were closely related (Fig. 3). The mixtures of glutinous with hybrid rice varieties were effective in controlling blast, whereas the japonica mixtures were ineffective. The ineffectiveness of japonica mixtures may be due to the genetic relatedness

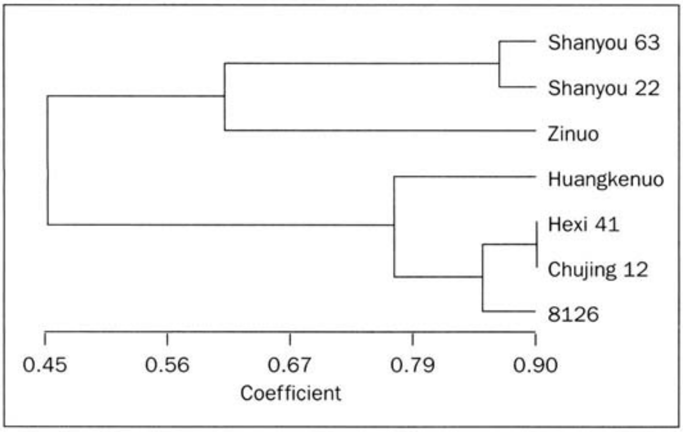


Fig. 3. Dendrogram constructed using RGA profiles generated with the PCR primer pairs XLRRfor/XLRRrev, Pto kin1/Pto kin2, and S1/AS3 for varieties used in interplanting trials in Yunnan. Variety 8126 is a sister line of Luxuan 1, the japonica variety used in interplanting experiments, which is genetically similar to Hexi 41 and Chujing 12.

of the component varieties as revealed by RGA markers. The preliminary data suggest that RGA markers may be useful for designing varietal combinations that will be effective against blast. However, the causal relationship cannot be well established by these observations alone. Further comparison between markers and combinations of effective and ineffective varieties is needed to validate the predictive value of RGA markers.

Prospects of improving functional prediction using candidate resistance genes

For many years, neutral markers have been used to characterize germplasm and varieties. Although neutral markers are useful in showing genetic differentiation, they generally do not reflect functionality. Our results indicate that, as with neutral markers, RGA markers are useful in differentiating groups of varieties. However, they are not ideal predictors of phenotypes. A main limitation of PCR-based RGA markers is that many small amplified fragments are not related to disease resistance. For instance, many LRR sequences in the genome are involved in protein-protein interaction but not defense response. Thus, considerable noise is present in the RGA profile. On the other hand, sequences with contiguous NBS and LRR motifs are more likely to be involved in disease resistance. We therefore expect that the predictive value on phenotypes will increase by using sequences with both NBS and LRR motifs in developing a DNA profile of the germplasm.

In separate studies, we have used a set of candidate genes including NBS-LRR sequences and defense response–related sequences to characterize backcross breeding lines and recombinant inbred lines. The results indicate that candidate gene profiles of recombinants from a breeding or mating population are associated with the quantitative expression of the phenotypes (Jianli Wu, Liu Bin, and H. Leung, unpublished data). For example, using 30 candidate genes to characterize a recombinant inbred population that segregates in quantitative blast resistance, we observed a clustering of high-performing (resistant) lines with similar candidate gene profiles. In contrast, the use of random restriction fragment length polymorphism or microsatellite markers did not reveal any functional relationship with the DNA profiles.

Thus, by using a set of candidate genes that can more precisely reflect function, it is possible to determine the functional complementarity of varieties to be used in a diversification strategy. This approach will be used in molecular characterization of resistant varieties in deploying diversification schemes in the future.

Conclusions

We have attempted to use RGA markers to infer function in germplasm and varieties with historical records on phenotypic performance. PCR-based markers were chosen because of their simplicity in application. However, these markers are limited because some of the amplified fragments are not related to disease resistance. To improve the utility of molecular markers in inferring function, full sequences corresponding to putative disease resistance genes are needed. We will use these sequences to characterize candidate varieties for diversification. Further, we will reexamine the varietial combinations that are effective and ineffective in diversification experiments. Results from these experiments should give us the means to design varietal combinations and validate the predictive power of the markers by testing the varietal combinations in the field. If candidate gene markers can indeed predict function, this concept could be of great general utility for implementing diversification strategies on a variety of agronomic traits.

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Notes

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Technology diffusion

Farmer scientists in IPM: a case of technology diffusion

P.A.C. Ooi, Warsiyah, Nanang Budiyanto, and Nguyen Van Son

In the past decade, farmer education through an integrated pest management (IPM) field school approach has been recognized as an effective way to help farmers better understand the complex agroecosystem they have to work in. An important aspect of sustaining the knowledge gained in the field school is to encourage farmers to continue doing research. Such follow-up activities of the FAO Programme for Community IPM in Asia have resulted in many cases of farmers making informed decisions on matters relating to pest management in their villages. Three case studies are discussed to highlight the role of farmer scientists in facilitating technology diffusion. The first case study refers to farmer studies in Dalat, Vietnam, that led to an understanding of the selective action of the microbial insecticide *Bacillus thuringiensis* in managing the diamondback moth. With the information from experiments and pilot studies, farmers were able to contemplate organizing village-wide activities to conserve the population of *Diadegma semiclausum*, a key larval parasitoid of *Plutella xylostella*. This is only possible with the support of the local government and IPM trainers. The second case study concerns rat management in Kalensari village, Indramayu District, West Java, Indonesia, where farmers were able to mobilize local residents to reduce the rat population by catching the rats at night and feeding them to ducks. Reducing rat populations to very low levels before the planting season resulted in very low rat populations and hence less damage to the crop. The third case study provides an example of farmers working with IPM trainers in setting up a farmer learning facility in Sleman District of Yogyakarta, Indonesia. Rice farmers experimented with growing vegetables and enhanced their understanding of soil and microbials to manage insects and plant pathogens together with evaluating new cultivation practices. These three case studies reflected the strategy of the FAO Programme in promoting science and farmers. It has resulted in farmers who are more confident in solving field problems. These farmer scientists also share their knowledge with other farmers in the spirit of technology diffusion.

The integrated pest management (IPM) farmer field school (FFS) is the primary learning approach developed and used by the Indonesian National IPM Programme (Dilts and Pontius 2000). The rice IPM field school is a season-long learning experience. The adjacent rice field is the classroom for participants. Farmers learn to carry out experiments to understand biological concepts, leading to a better understanding of the agroecosystem. Lessons learned from implementation of FFS in more than 12 countries in South and Southeast Asia by the Food and Agriculture Organization (FAO) of the United Nations confirmed that farmers are capable of understanding scientific concepts associated with IPM. Farmer field schools provided an entry point for farmers to learn science.

Because the FFS is an entry point, it is important that, after the FFS, there be follow-up activities to sustain farmer curiosity about the agroecosystem (Ooi 1998). Hence, "science and farmers" are an important activity of the FAO Programme for Community IPM in Asia. Case studies of farmer field research are available to demonstrate that farmer scientists come into creation when given the opportunity to experiment and be creative (Ooi et al 1999). As important as understanding scientific concepts and generating knowledge, community IPM also encourages the sharing of knowledge through farmer-organized activities that will reach more Asian rice farmers (Matteson 2000). Some farmers are farmer trainers skilled in helping other farmers understand science in FFS. Some farmers prefer to organize meetings, seminars, or workshops to share experiences and knowledge. Whatever the approach, community IPM activities highlight technology diffusion via farmer empowerment. Three case studies are presented in this chapter to demonstrate this.

Case study 1: diamondback moth management in Dalat

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), is the most important pest of crucifers in tropical areas. In the highlands of Dalat, Vietnam, farmers grow crucifers year-round and spray insecticides as many as 24 times per season. Besides the large number of sprays, farmers often resort to mixtures of three or more different chemicals at concentrations far exceeding the recommended rates. This exacerbated the problem of insecticide resistance in *P. xylostella*, which was first reported in 1950 from Indonesia (Ankersmit 1953). Excessive use of chemical insecticides on crucifers was reported from the highlands of Malaysia (Ooi and Sudderuddin 1978, Sudderuddin and Kok 1978) and the Philippines (Poelking 1992).

In response to the desperate measures used to control the diamondback moth, the Plant Protection Department of the Ministry of Agriculture and Rural Development, Vietnam, embarked on an ecological approach involving the importation of effective parasitoids to manage the pest population. CAB International was contracted to introduce parasitoids of the diamondback moth and the FAO Intercountry Programme on Vegetable IPM supported FFS for vegetable farmers in the country. Both *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae) and *Diadromus collaris* (Gravenhorst) (Hymenoptera: Ichneumonidae) were imported from Malaysia and released in Dalat in 1997 (Lim 1997). A rearing facility was set up in Dalat and large numbers of both parasitoids were successfully produced. Despite regular releases, both parasitoids failed to establish because farmers continued spraying chemical insecticides on crucifers (Ooi 1999).

In late 1999, discussions with farmers from a vegetable IPM ecological group in Commune 7 suggested that farmers had some ideas about how parasitoids kept pest populations in check and their regular sprays prevented parasitoids, particularly *D. semiclausum*, from exerting control. Similar results were reported from the Philippines (Poelking 1992), Indonesia (Sastrosiswojo and Sastrodihardjo 1986), and Malaysia (Ooi 1992). However, interventions with the microbial insecticide *Bacillus thuringiensis (Bt)* rather than very toxic chemical insecticides have helped to conserve the ichneumonid parasitoids in Malaysia (Ooi 1992).

During discussions with trainers and farmers in Dalat, it was reported that *Bt* was ineffective, as it did not kill the diamondback moth (DBM) immediately. Further discussions suggested that farmers based their decisions on a chemical insecticide model. As part of a follow-up activity to encourage farmers to generate knowledge, the National IPM Programme in collaboration with a scientist from the FAO Programme for Community IPM facilitated studies to understand the mode of action of *Bt*.

Farmers had to first learn the correct method of handling fragile caterpillars. After farmers learned how to collect DBM caterpillars without harming them, they proceeded to bioassay five different commercial *Bt* formulations that were available in Dalat. Each treatment had three replicates. In each replicate, 10 young caterpillars were dropped onto leaf discs kept separately in small plastic cups.

In Commune 7, IPM trainers facilitated the study with DBM. Initially, farmers evaluated the study using a bioassay method for chemical insecticides. Based on a 24-h evaluation, they concluded that *Bt* was not effective (Table 1).

When farmers in the vegetable IPM ecological group used a scoring system that involved an understanding of how *Bt* works, they came to a different conclusion (Table 2). In the second method of evaluation, farmers were asked to score for (1) damage to the leaf discs, (2) amount of frass produced, and (3) state of the larvae in the cup. To visualize the results obtained, a simplified statistic resembling the conventional F-test at $P<0.05$ with three replicates was used. Farmers drew horizontal lines linking the minimum and maximum score for each treatment. The result based on this scoring system encouraged farmers to discuss the effects of *Bt*. Many were convinced that *Bt* was effective and they had the option of selecting what they found to be the most effective commercial *Bt* product. They were able to relate to the action

Table 1. Results of a study of five commercially available microbial insecticides (*Bt*) against diamondback moth using a conventional method of counting the number of dead caterpillars at 6 h and 24 h after feeding with cabbage leaves treated with recommended dosages of *Bt* as shown on the label.

Treatment	Water			Xentari			Delfin			Biobit			Aztron			V-*Bt*		
Replicate no.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
At 6 h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
At 24 h	1	1	0	0	0	0	2	1	0	0	1	0	0	0	0	1	0	0

of the endotoxin in producing stomach paralysis resulting in low production of frass. They noticed that caterpillars that fed on treated leaves did not eat much. Table 3 suggests that Biobit was effective. Xentari often showed good effects at 24 h after treatment but overlap with the water treatment suggested that there may not be a convincing difference.

This experiment was repeated in Commune 3 with similar results. Farmers in Commune 3 also carried out a study to evaluate the effects of different concentrations of a commonly available insecticide on DBM. They found that, 48 h after treating the DBM caterpillars with a commonly used insecticide at ten times the recommended rate, the caterpillars did not die. These results helped explain the concept of insecticide resistance. Farmers concluded that, although chemical insecticides were ineffective against DBM, they killed parasitoids. Hence, the Commune 3 vegetable IPM ecological group decided not to use chemical insecticides and relied on *Bt* instead. This resulted in lower populations of DBM and often farmers did not apply *Bt* subsequently.

The vegetable IPM ecological groups in communes 3 and 7 also realized that, for effective management of DBM, the entire community of farmers had to know about

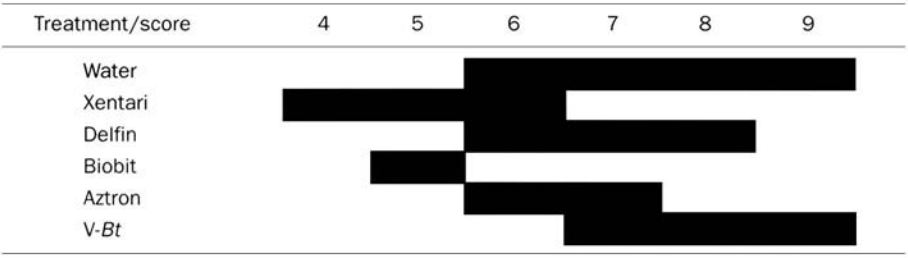
Table 2. Results of a study of five commercially available *Bt* products against diamondback moth using a scoring system[a] that measures the effect of *Bt* on the caterpillars tested.

Treatment	Water			Xentari			Delfin			Biobit			Aztron			V-*Bt*		
Replicate no.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Leaf damage	3	3	2	2	1	1	2	2	3	2	2	2	2	2	2	3	2	3
Frass production	1	3	2	2	1	1	2	2	3	2	2	2	2	2	2	3	2	3
State of larvae	3	3	2	2	2	2	2	2	2	1	1	1	2	2	3	3	3	3
Total score	7	9	6	6	4	4	6	6	8	5	5	5	6	6	7	9	7	9

[a]Scoring system:

Leaf damage	Low 1	Some 2	Severe 3
Frass production	None 1	Some 2	Much 3
State of larvae	Dead 1	Moribund 2	Active 3

Table 3. Visualizing results of the *Bt* study using data from Table 2 with a simplified statistic adapted from van den Berg (personal communication 2000).



the results of their studies. With the support of the Lam Dong Plant Protection Subdepartment (PPSD), they organized meetings and field days with other farmers and commune leaders. One such meeting was held on 31 May 2000 in Commune 3 and attended by officials of the PPSD, Department of Agriculture, research institutions, universities, and the farmers' union around Dalat together with farmers from other communes. Farmers from Commune 3 organized the field day and farmers from both communes 3 and 7 presented their findings. A field visit was made to a farmer who sprayed his 2-mo-old crop three times with *Bt.* There was evidence of *D. semiclausum* activities in the field and, as a result, many farmers better appreciated the role of parasitoids in managing DBM.

Case study 2: rat management in Indramayu, Indonesia

The farmer action research group in the village of Kalensari was set up in 1995. By 1996, the farmers had a good idea about how the rice white stem borer, *Scirpophaga innotata* (Walker) (Lepidoptera: Pyralidae), invaded rice fields in the first season following an extended drought (Warsiyah et al 1999). They were able to share their knowledge first with other farmers in the village and then with farmers elsewhere in the district. Indeed, their achievements included obstructing the scheme to provide farmers with insecticides to control the white stem borer. The district officer agreed to an alternative plan whereby farmers monitored the flight of moths that emerged from diapause. Farmers were encouraged to prepare seedbeds after the peak of moth flights and this avoided serious pest damage.

After this initial success with managing white stem borers, farmers in the group continued with field research to better understand the rice ecosystem, meeting regularly to discuss issues related to their fields. One of the issues discussed was that of rice field rats. The farmers learned that ducks could feed on fresh rat meat if trained to do so. In the village of Kalensari, many farmers rear ducks (about 50 ducks per farmer) to supplement their income. Hence, the group decided to investigate the possibility of using rats as food for ducks. An outcome of this investigation is that farmers go out at night during the dry season when no crop is in the field. By hunting rats before the season starts, farmers actually reduce the risk of rat damage to their crops. In some cases, farmers purchase live rats caught in other villages. As a result, the population of rice field rats was reduced to a very low level in Kalensari and neighboring villages too. The lower rat population meant less rat damage during the rice season. When asked about the strategy to control rats by feeding them to ducks, Pak Warsiyah remarked that, since the natural enemies of rats have been reduced by farming activities, it was necessary to find ways to kill the rats without forcing farmers to hunt them. Rats provided good protein food to ducks, particularly during drought. Moreover, ducks fed with rat meat tend to be healthier and produce more eggs, resulting in more income for farmers.

Case study 3: farmers' learning capacity

In July 1999, the IPM Farmer Trainers Association of West Sleman, Yogyakarta, Central Java, discussed the possibility of establishing a "school" to encourage farmers, particularly those who have graduated from the IPM farmer field school (FFS), to continue to do research. This "school" was called the farmers' learning facility (FLF). In the following month, a plan was presented to the local government to set up this FLF.

The village government of Margoluwih provided a piece of land (6,000 m^2) to enable farmers to carry out research. This meant that the IPM FFS alumni in Yogyakarta had a place to meet, share experiences, and develop enterprises based on direct field studies. The FLF was placed under the management of the IPM Farmer Trainers Association of West Sleman. Farmers from seven subdistricts were invited to join in the activities of the FLF. By March 2000, 29 IPM farmers from five adjoining subdistricts participated in the facility.

The research farm was converted from rice to grow other crops in line with the desire to expand knowledge and opportunity so that farmers in the program could realize their potential. The farm was organized into several blocks for experiments with different vegetable crops. Initially, the rice farmers faced many problems when they cultivated crops such as tomato, shallots, chillies, crucifers, brinjals (eggplant), etc., for the first time. However, their desire to avoid the use of chemical poisons as far as possible allowed them to explore and experiment with alternative methods of agriculture.

A follow-up activity of the FLF was to carry out studies to better understand soils and how they affect crop growth. These studies were not taught at the FFS. Therefore, it was important that farmers had the opportunity to continue to experiment and expand their knowledge (Nanang Budiyanto 2000).

To avoid the use of chemical pesticides to control insect pests and plant pathogens, farmers turned to the local government–operated plant protection laboratory for microbials. Experiences with using the microbials suggested that farmers did not fully understand the nature of the microbials nor did they appreciate how they work. At this stage, farmers at the FLF moved to the next phase: preparing a protocol to ensure that they better understand the microbials they want to use by discovering more about how they work and understanding symptoms, etc.

The FLF has as its objectives the process of learning from nature and encouraging cooperation among farmers to achieve the following aims:

- Develop farmers' framework of thinking—to be more critical, analytical, and systematic;
- Encourage traditional agriculture-based institutions to support rural agricultural development;
- Build a farmer organization based on farmer leadership and mutual cooperation;
- Initiate a network of farmers to develop a mutual working relationship between farmers and other agencies; and
- Enhance farmers' capacity for advocacy.

On 9-11 July 2000, the IPM Farmer Trainers Association of West Sleman organized a national symposium of IPM farmer trainers (including former IPM trainers who returned to their original government job of pest monitoring following termination of a World Bank-funded project). About 150 of the former IPM trainers (government employees) and 50 IPM farmer trainers from 11 provinces attended the event. Besides discussing the change in the role of government IPM trainers, the meeting recognized the wisdom of developing farmer-based programs at village, district, and provincial levels. This case provided an example of how farmers play an important role in sustaining the diffusion of science and technology.

Conclusions

The three case studies were selected to reflect recent attempts by groups of farmers in both Vietnam and Indonesia to organize themselves so that the knowledge they generated could be shared with other farmers and institutions involved in rural development. The FAO Programme for Community IPM in Asia maintains that farmers can empower themselves through knowledge generation and this is clearly shown in two groups of farmers involved in diamondback moth management in Dalat. Based on their discoveries, these farmers were able to synthesize the different aspects of DBM management and they concluded that, unless other farmers stopped using chemical pesticides, the parasitoid *D. semiclausum* would not be able to keep DBM populations in check. Farmers in Commune 3 were pleasantly surprised when they recovered *Cotesia glomeratus* (L.) (Hymenoptera: Braconidae) from *Pieris* sp. (Lepidoptera: Pieridae) from fields kept free of chemical insecticides *C. glomeratus* was imported in 1991 from Florida (USA) by the University of Dalat and was assumed not to have established itself.

The experience of organizing an area-wide campaign to manage the rice white stem borer in the district of Indramayu encouraged farmers from the village of Kalensari to continue looking for answers to their field problems. When they discovered that rats could be fed to ducks, farmers realized that this approach could be used to manage rat populations as well as promote a profitable home industry. As farmers in Kalensari were proud to acknowledge, a more sustainable method of rat management is when catching rats becomes profitable and reduces crop losses. Rat control campaigns forced upon farmers by outsiders are often rejected.

The need to continue field studies or research after FFS, as well as the necessity to share or diffuse the information generated, is also reflected in the setting up of a farmer learning facility in Sleman. Even in its early stage, the FLF has shown that it can bring together farmers who would like to learn more about cultivating crops other than rice. Farmers in the group were able to learn about healthy soil and explore the use of biological control agents. The FLF provided an opportunity for farmers, trainers, and researchers to work together to synthesize from a large pool of information some useful activities that would benefit farmers.

Technology diffusion by farmers based on knowledge they generated by working together with trainers and researchers as equal partners is proving to be a more sustainable way to modernize agriculture. As the concept of science and farmers expands to all the FAO IPM participating countries, more innovative and creative ways to learn science will emerge for the benefit of the farming communities. The Programme will continue to encourage seminars by farmers for this is "a good means of systematizing local knowledge and of spreading IPM experiences throughout local farmer networks" (Dilts 1999).

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Notes

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Adoption of mixture planting for biodiversity in China: its impact on pest management and farmers' income

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This chapter presents an overview of the technology adoption and diffusion of mixture planting in Yunnan Province, promoted by a China-IRRI collaborative research project, and assesses its impact on pest management and farmers' income. One hundred four farmer-adopters of mixture planting in four villages and 30 nonadopters from three villages of Shiping and Jianshui counties, Hong He Prefecture, were personally interviewed from 12 to 28 July 2000 to generate information on household characteristics, farm management practices, input use, yield, costs, and income. Data were then analyzed using "before and after" and "with and without" project comparisons to determine the impact of mixture planting.

The rapid adoption of mixture planting can be attributed to a vigorous and systematic scheme implemented by the Yunnan Plateau lighthouse team in collaboration with the local extension agency. Results show that farms under mixture planting have a lower incidence of blast disease and farmer-adopters on average spend only \$10.50 ha^{-1} for pesticides compared with nonadopters' pesticide cost of \$42.92 ha^{-1} in 1999. Farmer-adopters had higher yields in 1999 than in 1996. In comparison with the yield on nonadopters' farms, glutinous rice yield is 84% higher on mixture farms. The yield of hybrid rice was almost the same with 20% less land that was reallocated to plant one row of glutinous rice in between four rows of hybrid rice. Overall, the yield was 7% higher and gross return was 14% more since the price of glutinous rice, because of its higher quality, was twice that of hybrid rice. The net gain in farm-operator surplus was estimated at 25%.

Farmers' perceptions of the impact of mixture planting in general tend to support the quantitative analyses done for this study.

Modern rice production in Asia has more than doubled since the Green Revolution began in the 1960s (Pingali et al 1997). Nearly 80% of this increase in production came from the increase in yield caused by the spread of improved modem rice varieties. Because the modem varieties are highly profitable compared with the traditional landraces, their introduction has led to an erosion of cultivar diversity with a large proportion of rice area being allocated to a few varieties (Smale 2000). The increased intensity of cropping that followed the development of irrigation has increased pest pressure, leading to farmers using harmful agrochemicals to control pests (Kenmore 1980, Rola and Pingali 1993). Although the modem varieties developed later incorporated host-plant resistance against major insects and diseases, farmers still depend on chemical control for pest management (Fischer and Cordova 1998, Rola and Widawsky 1998, Heong et al 1995).

Pest scientists have been trying to develop environmentally sustainable methods of pest management using natural biological control such as habitat diversity and pest-predator relationships (Way and Heong 1994, Yasutmatsu 1967, Islam and Heong 1998, Yu et al 1996). Research has been conducted in developed countries to understand the mechanism of pest control with mixed planting of varieties (Wolfe 1985, 1997, Mundt 1994, Mundt et al 1996, Zhu et al 2000 and this volume). This project aimed to apply the scientific knowledge on mixed planting of rice to control the endemic blast disease by growing popular glutinous rice varieties in China through adaptive research and farmer participatory experiments.

Chinese farmers had indigenous knowledge of using mixed planting of varieties for control of diseases. Scientists have built on the farmers' indigenous knowledge to develop the proper ratio of mixtures between traditional glutinous rice and modem hybrid rice and crop management techniques for optimum control of pests and maximized yields, using resources provided by the Asian Development Bank (ADB)-funded project on “Exploiting Biodiversity for Sustainable Pest Management,” in partnership with scientists from the International Rice Research Institute (IRRI) and advanced research institutions. Some of the resources are used to train farmers in the modern methods of mixture planting to facilitate dissemination of knowledge about the technology and its transfer to farmers’ fields.

This study was initiated to evaluate the impact of the project in terms of the adoption of the technology and its effect on the use of pesticides and farmers’ income. Its objectives are

1. To present a historical perspective of how the technology of mixture planting diffused in Yunnan Province,
2. To assess the impact of mixture planting on pest management and farmers’ income, and
3. To estimate the rate of return to investment in the project.

Methodology

One hundred four farmer-adopters and 30 nonadopters from seven villages in Shiping and Jianshui counties, Hong He Prefecture, were randomly selected and personally interviewed from 12 to 28 July 2000. These two counties represent the areas where the technology of mixture planting was first introduced in 1997.

Twenty-six households each from four villages in Shiping and Jianshui were randomly selected as farmer-adopters. To have a control group for comparison, 10 households each from three villages in the two counties were also selected at random to represent the nonadopters.

A pretested questionnaire was used to generate information on farmers' demographic characteristics, landholding, access to information, aspects of the decision-making process in relation to varietal choice, yield, farm management practices, input use, and income.

Adopters were asked detailed questions about their farming situation or practices before and after the adoption of mixture planting. The "before" situation refers to 1996, the year before the farmer participatory experiment started. The "after" situation refers to the 1999 rice-cropping season. Similarly, data from the 1999 cropping season were obtained from nonadopters.

Data from 1996 and 1999 were compared to assess the impact of mixture planting on inputs, labor use, yield, costs, and income of adopters. Likewise, the performance of the nonadopters was analyzed in comparison with that of the adopters.

Results and discussion

Background to the project and the technology

In early 1996, the Entomology and Plant Pathology Division of IRRI submitted to the Asian Development Bank (ADB) a project proposal on using biodiversity for sustainable pest management. While waiting for the Bank's approval, opportunities were explored to involve scientists from developing member countries (DMCs) of ADB using a "lighthouse" concept in which sites were selected for their potential to demonstrate impact. Five lighthouse sites representing different ecosystems in four DMCs were identified: the Mekong Delta in Vietnam, Chiang Mai Valley in Thailand, Ilocos Norte in the Philippines, and the Yangtze Delta and Yunnan Plateau, both in China.

The IRRI research team visited Yunnan Agricultural University (YAU) in 1996 to check whether YAU would be interested in working on blast as the target pest problem. In 1997, Drs. T.W. Mew and P.S. Teng, IRRI team leaders, made a second visit to confirm YAU's participation in the ADB-funded project "Exploiting Biodiversity for Sustainable Pest Management." Professor Zhu Youyong, who had just returned from his postdoctoral stint in Australia, was designated as the team leader of the lighthouse site because, among the YAU staff, he had the longest experience in working with rice blast.

When the project officially began in April 1997, Dr. Chris Mundt, IRRI visiting scientist, first suggested mixing seeds as a varietal diversification technique. Farmers, however, did not like the idea. An experiment that was almost similar to what was done in 1980-84 by YAU was set up in Bao Xiu, Shiping County. Each farmer's field of about 0.067 ha was considered as one plot. The team took 3 rows and 5 columns of adjacent plots and planted different varieties in each plot. The result was not very good and disease was severe in plots where susceptble varieties were planted.

At about the same time, another varietal diversification experiment within farmers' fields was conducted in Zhang gui zhai, also in Shiping County. The farmers, however, modified the experiment by interplanting one row of glutinous rice for every four rows of indica rice. The result was quite remarkable. The mixture of glutinous variety Huangkenuo or Zinuo with hybrid indica rice Shanyou 63 or Shanyou 22 significantly reduced the incidence and severity of rice blast disease.

Little was known about this practice of interplanting, which has been followed by a few farmers in limited areas of Shiping County since 1983, although there was no clear pattern.

After the success in Zhang gui zhai, more demonstration areas were set up and more and more farmers got involved. Farmers in Hong He Prefecture readily accepted the technology. They find the technology to be simple and easy to apply, aside from the perceived benefits of obtaining higher yield because of less pest pressure, growing high-quality rice. and increases in their income.

The technology spread so fast that, after the project was launched in 1997, the area under mixture planting continued to expand. From the original 667 ha planted to mixtures in Shiping County in 1997. the area grew to about 1,333 ha in Shiping and Jianshui counties in 1998.

Factors contributing to technology adoption and diffusion

In July 1999, a field day-cum-workshop was organized to share the results of interplanting glutinous rice with hybrid indica rice for blast management. A video clip of the varietal diversification project, which was produced by the Central China Television Network, was shown to more than 150 people who attended the meeting. This helped enhance the enthusiasm of the participants to adopt mixture planting in their respective areas and draw attention of the senior local government officials regarding the potential of the technology, which was instrumental in mobilizing local support for fast diffusion of the technology.

During the meeting, both the local prefecture and county government leaders strongly expressed their interest in the project. The Director of the Agricultural Bureau of Hong He Prefecture proposed that the mixture planting project be included in the Bureau’s priority project for year 2000 and urged all 13 counties under the prefecture to target 13,333 ha of rice area to be planted to mixtures.

In September 1999, an agricultural task force was organized to prepare the groundwork to implement mixture planting in the different counties. The counties then involved township leaders in implementing the project. The plant protection stations at the county level were tasked to spearhead implementation of the technology in their respective areas of responsibility. Some counties even allocated funds and letters of agreement between the upper and lower government units were signed to ensure a clear understanding of responsibilities among the different units involved.

Because of the commitment and systematic coordination of those involved in the project at different levels of leadership. and the financial support provided by the Asian Development Bank through IRRI and through the Key Phytopathology Laboratory of YAU, the project implementation activities went smoothly.

By mid-May 2000, the entire Hong He Prefecture completed interplanting of 16,667 ha of hybrid and glutinous rice, which was 3,333 ha more than in the original plan.

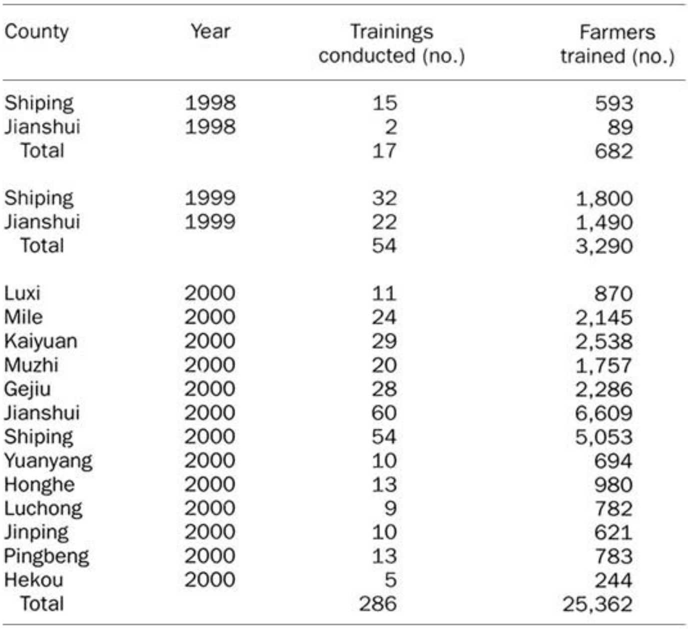
The training process

At the county level, the first step was to hold a meeting with county government officers including the vice mayor in charge of agriculture, the chief of the county science and technology commission, and the chief of plant protection and agrotechnology stations. Once support was received from the officers, the next step was to conduct a workshop with technicians in agricultural technology and county plant protection officers. These trained technicians then organized, through village officers, small group discussions among farmers. During planting time, the more skilled farmers in the village took the lead to demonstrate to other farmers the procedure of interplanting glutinous and hybrid rice, with the agrotechnicians as supervisors. In 2000 alone, 286 training courses were organized in 13 counties of Hong He Prefecture, with 25,362 farmer-participants (Table 1). The total number of farmers using mixtures increased from 20,327 in 1998 to 446,000 in 2000 (Table 2). IRRI provided additional funds to the research team to support the intensive training program. The Chinese government has pledged to provide financial support to continue the training till 2003.

Farm household profile

The average household size was four and the average number of children per couple was 1.76 (Table 3). This indicates that the Chinese policy of one child per family is not effectively followed in rural areas. Farming is done by relatively less educated people. Most of the farm managers have had formal schooling only up to the primary

Table 1. Number of farmers trained on mixture planting by county from 1998 to 2000, Yunnan Province, China.



County	Year	Trainings conducted (no.)	Farmers trained (no.)
Shiping	1998	15	593
Jianshui	1998	2	89
Total		17	682
Shiping	1999	32	1,800
Jianshui	1999	22	1,490
Total		54	3,290
Luxi	2000	11	870
Mile	2000	24	2,145
Kaiyuan	2000	29	2,538
Muzhi	2000	20	1,757
Gejiu	2000	28	2,286
Jianshui	2000	60	6,609
Shiping	2000	54	5,053
Yuanyang	2000	10	694
Honghe	2000	13	980
Luchong	2000	9	782
Jinping	2000	10	621
Pingbeng	2000	13	783
Hekou	2000	5	244
Total		286	25,362

Table 2. Number of farmers using mixture planting and area planted to mixtures, Yunnan Province, China, 2000.

County	Year	Farmers using mixture planting (no.)	Area planted to mixtures (ha)
Shiping	1998	19,975	799
Jianshui	1998	352	13
Total		20,327	812
Shiping	1999	54,300	2,006
Jianshui	1999	48,000	1,336
Total		102,300	3,342
Luxi	2000	22,000	667
Mile	2000	38,000	1,333
Kaiyuan	2000	39,000	1,667
Muzhi	2000	35,000	1,333
Gejiu	2000	47,000	1,533
Jianshui	2000	80,000	3,333
Shiping	2000	84,000	3,333
Yuanyang	2000	17,000	667
Honghe	2000	17,000	667
Luchong	2000	21,000	667
Jinping	2000	23,000	667
Pingbeng	2000	19,000	667
Hekou	2000	4,000	133
Total		446,000	16,667

Table 3. Distribution of households by number of members and by number of children, Yunnan Province, China, 2000.

No. of members	Percent of households	No. of children	Percent of households
0		0	6.8
1		1	24.8
2	3.8	2	57.9
3	15.9	3	7.5
4	57.6	4	2.3
5	15.9	5	0.8
6	6.1		
7	0.8		

level (Table 4). The younger generation, however, is better educated, indicating progress made over time in human capital formation. In some farm households, more than 20% of the third children are high school graduates. Whether this more educated younger generation will remain in farming will depend on the productivity of labor and income from farming compared with alternative occupations.

The average per capita income was estimated at US$207 for the adopter households and $204 for the nonadopter households. Rice farming accounts for 30% of the household income. The World Bank defines as poor those households with a per capita income of less than $1 per day. More than 50% of the farm households' per capita income is about $187.50 per annum, or only half a dollar per day (Table 5). Thus,

Table 4. Distribution (%) of household members by educational attainment, Yunnan Province, China, 2000.

Educational attainment	Head	Spouse	1st child	2nd child	3rd–5th child
No schooling	7.5	13.1	8.9	12.2	
Elementary (did not finish)	9.8	12.3	17.1	15.6	
Elementary (finished)	54.1	46.9	28.5	34.4	47.1
High school (did not finish)	13.5	8.5	20.3	15.6	11.8
High school graduate	12.8	16.2	17.9	13.3	23.5
Vocational	2.3	2.3	4.9	5.6	5.9
College (did not finish)		2.3	0.8	2.2	5.9
College graduate			1.6	1.1	5.9

Table 5. Annual per capita income and share of rice income by type of farmer, Yunnan Province, China, 2000.

Per capita income (US$)	% of adopters reporting	% of nonadopters reporting	Share of income from rice farming (%)
< 187	56	52	39
188–375	35	38	22
376–750	9	10	16
Average income	207	204	

although the basic objective of the project is environmental protection by reducing farmers' dependence on harmful agrochemicals, the project is well targeted to make an impact on poverty alleviation. Since the project area is among the extremely poverty stricken areas in China, an increase in productivity and income from the adoption of the technology will make a direct contribution to poverty alleviation.

Land-use pattern

The predominant crop is rice with almost all area planted to rice during the winter season. Land is mostly left fallow after rice is harvested until the next season. The other occupations are livestock raising and various nonfarm activities.

The average farm size is 0.23 ha and the area planted to rice is about 0.16 ha. Farm size is about the same for the adopters and nonadopters (Table 6). The government provides each adult household member with 0.067 ha of land, so the land is fairly equally distributed among rural households.

The average area planted to glutinous rice for adopters (0.03 ha) is three times the size of the nonadopters' area (0.01 ha), whereas the area planted to hybrid rice is slightly smaller for the adopters (0.13 ha) than for the nonadopters (0.15 ha).

Prior knowledge on mixture farming

Three percent of the farmers already knew about mixture farming as early as 1993 (Table 7). According to them, some farmers in Shiping County first observed that interplanting maize with sorghum within the same field reduced the incidence of insect pests and diseases. In the early 1980s, some farmers in the village tried to inter-

Table 6. Farm size, area under rice, and area planted to glutinous and hybrid rice by type of farmer, Yunnan Province, China, 2000.

Item	Adopters (ha)	Nonadopters (ha)
Farm size	0.23	0.22
Area planted to rice	0.16	0.16
Glutinous	0.03	0.01
Hybrid	0.13	0.15
Average farm size	0.23 ha	
Average area under rice	0.16 ha	

Table 7. Year when farmers learned about mixture planting and actual year of adoption, Yunnan Province, China, 2000.

Year when learned about mixture planting	Percent of farmers	Source of information[a] (%)			Year when adopted mixture planting	Percent of farmers
		PPS	DA	Co-farmer/ relatives		
Before 1996	2.9	33.3	33.3	33.3	Before 1996	2.9
1997	26.9	10.7	82.1	7.1	1997	10.6
1998	70.2	5.5	93.2	1.4	1998	86.5

[a] PPS = Plant Protection Station, DA = Department of Agriculture.

plant hybrid indica rice with glutinous rice and the results were positive. The effect was marginal, however, because of random planting and hence the practice did not spread. About 70% of the surveyed farmers first learned about the technology in 1998 when YAU, with support from the Department of Agriculture, conducted farmers' training on mixture planting.

The rate of adoption increased fast among the sample farmers, from 11% in 1997 to 88% in 1999. That farmers like the technology is indicated by the rapid diffusion rate.

Varieties planted

Table 8 lists the major varieties grown by the adopters. Four different varieties of glutinous rice were distinctly named by adopters. Before 1997, Huangkenuo (75%) and Zigu (15%) were the most popular glutinous rice varieties grown. Two years after the adoption of mixture planting, the number of adopters using Huangkenuo increased to 82%, followed by Zigu at 14%. Farmers may have realized that Huangkenuo can yield more than Zigu when interplanted with hybrid rice.

For hybrid rice, more than 40% of the adopters planted Shanyou 63 in 1996 and about 6.3% reported using Siyou 63. In 1999, the distribution of adopters using Shanyou 63 and Siyou 63 was about the same.

Among nonadopters, Huangkenuo and Shanyou 63 are, respectively, the most popular variety of glutinous and hybrid rice grown (Table 9).

Table 8. Distribution of adopters by name of variety planted and by year, Yunnan Province, China, 2000.

Glutinous rice	Percent reporting		Hybrid rice	Percent reporting	
	1996	1999		1996	1999
Ai'jiaonuo	5	–	Ganyou 63	0.5	0.5
Baikenuo	–	1	Guicao	1.0	–
Huangkenuo	75	82	Shanyou 63	42.3	20.2
Qitougu	5	2	Siyou 22	–	0.5
Zigu	15	14	Siyou 63	6.3	28.9

Table 9. Distribution of nonadopters by name of variety planted, Yunnan Province, China, 2000.

Glutinous rice	Percent of farmers reporting	Hybrid rice	Percent of farmers reporting
Duiduino	16.7	Feyou 63	3.3
Zajiano	16.7	Shanyou 63	63.3
Huangkenuo	66.7	Teiyou 63	3.3
		Yungan14	3.3
		Ganyou 63	3.3
		Siyou 63	16.7
		Siyou 22	3.3
		Others	3.3

Sources of seeds

Table 10 lists the different sources of seed for glutinous and hybrid rice. From the data, it can be gleaned that the government has developed an infrastructure for hybrid seed production and distribution. Seeds of hybrid rice for both farm types come from the Department of Agriculture. However, farmers depend on a local network of farmer-to-farmer exchange of seeds for the traditional glutinous rice because of the absence of formal institutions for the production and distribution of seeds for the traditional varieties.

A majority of the adopters are more than satisfied with the performance of their chosen varieties, whether glutinous or hybrid rice (Table 11). Among the nonadopters, the ratings of glutinous rice are equally distributed from being excellent to just good. It was even rated poor by 13% of the farmers within that group. Hybrid rice likewise is rated either very good (42%) or good (48%).

Criteria for varietal choice

Farmers have different criteria for their choice of certain varieties and we ranked these criteria in terms of the three most important ones (Table 12). Among adopters, the number one criterion for their choice of glutinous rice is its good eating quality. The sticky glutinous rice is a social symbol for the ordinary Chinese farmers. Sticky rice confections are served during Chinese festivals to symbolize closeness among members of the Chinese family. Practically every festival or family occasion is cel-

Table 10. Distribution of farmers by source of variety, Yunnan Province, China, 2000.

Source of variety	Adopters[a]		Nonadopters	
	GR	HR	GR	HR
Department of Agriculture	8	97	17	97
Own produce/previous harvest	17	1		
Other farmers	75	2	83	3

[a] GR = glutinous rice, HR = hybrid rice.

Table 11. Distribution of farmers according to the results of varietal use, Yunnan Province, China, 2000.

Result of usage	Adopters[a]		Nonadopters	
	GR	HR	GR	HR
Excellent	17	8	29	10
Very good	78	77	29	42
Good	4	14	29	48
Poor	0	1	13	0

[a] GR = glutinous rice, HR = hybrid rice.

Table 12. Ranking of reasons for varietal use, adopters, Yunnan Province, China, 2000.

Reason for use	Glutinous rice			Hybrid rice		
	1st	2nd	3rd	1st	2nd	3rd
High yield	23	–	–	99	–	–
Good eating quality	66	9	–	3	11	–
High grain recovery	1	2	–	1	6	–
Insect resistance	–	6	–	–	–	–
Disease resistance	–	–	–	–	29	5
Submergence resistance	–	–	–	–	1	6
Low fertilizer requirement	–	–	5	–	2	14
Marketable	–	7	1	–	–	–
High price	–	10	1	–	–	–
Lodging resistance	8	2	4	1	–	1

ebrated by serving specialty dishes made from glutinous rice. Aside from the eating quality of glutinous rice, farmers choose to grow it because it commands a higher price in the market, has a low fertilizer requirement, and is resistant to lodging. The latter may have been the result of interplanting glutinous rice with hybrid rice.

On the other hand, farmers' first major consideration in the choice of hybrid rice is the potential high yield of the variety. Disease resistance ranks second, followed by low fertilizer requirement.

The same ranking of reasons is observed among nonadopters (Table 13).

Effect of mixture planting on yield

Table 14 presents the average yields of glutinous and hybrid rice. The glutinous rice yield of adopters was 34% higher in 1999 than in 1996, averaging 2 t ha^{-1}. This yield is about 84% higher than the average yield obtained by nonadopters in 1999. The differences in glutinous rice yields between years and between adopters and nonadopters are statistically significant.

On average, the yield of hybrid rice for adopters in 1999 was almost the same as in 1996, although some land has been shifted to glutinous rice to accommodate mixed planting. The yield for nonadopter farms where the entire plot was planted with hybrid rice was 8.6 t ha^{-1}, only 2% higher than that for the adopter farms. The average yield when both varieties are taken into account was about 7% higher for the adopter farms than for the nonadopters.

Mean level of input use

Table 15 reports the mean level of input use in both physical quantities and costs. The major differences in input use by adopters and nonadopters were in seeds, pesticides, and labor used for transplanting and harvesting. The seed rate was about 10% more for nonadopters because of the higher seed rates used for hybrid rice. There was no difference in the use of chemical fertilizers.

Mixture planting has obviously affected the level of pesticide use of the adopters. Because of less incidence of blast, adopters' pesticide expense decreases by 44% when they interplant a glutinous variety that is highly susceptible to blast with hybrid rice. The average number of sprays was only one for the adopters versus three for the nonadopters. The nonadopters spent $42.92 ha^{-1} on fungicides compared with only

Table 13. Ranking of reasons for varietal use, nonadopters, Yunnan Province, China, 2000.

Reason for use	Glutinous rice			Hybrid rice		
	1st	2nd	3rd	1st	2nd	3rd
High yield	2	–	–	29	–	–
Good eating quality	3	2	–	–	16	–
Disease resistance	–	–	–	–	12	3
Low fertilizer requirement	–	–	–	–	–	10
Marketable	1	2	–	–	–	–

Table 14. Mean yield of glutinous and hybrid rice by type of farmer, Yunnan Province, China, 2000.

Rice yield (t ha^{-1})	Adopters		Nonadopters 1999
	1996	1999	
Glutinous rice[a]	1.5	2.0	1.1
Hybrid rice	8.4	8.4	8.6

[a] Differences between yields of adopters in 1996 and 1999 and between yields of adopters and nonadopters for glutinous rice are highly significant at the 1% level of probability.

Table 15. Mean level of input use per hectare by type of farmer, Yunnan Province, China, 2000.

Inputs	Adopters		Nonadopters 1999
	1996	1999	
Seed			
Quantity (kg)			
Glutinous rice	5.4	4.4	3.2
Hybrid rice	35.0	32.0	37.1
Cost (US$)			
Glutinous rice	0.08	0.07	0.28
Hybrid rice	4.60	3.99	4.66
Fertilizer			
Quantity (kg)	1,433	1,433	1,475
Cost ($)	11.18	11.18	12.03
Pesticides			
Cost ($)	18.58	10.50	42.92
No. of sprays	2	1	3
Labor (man-days)			
Seedbed preparation	8.85	9.15	23.85
Land preparation	13.80	14.10	23.70
Transplanting	62.70	70.20	59.70
Fertilizer application	6.60	6.60	16.05
Pesticide application	3.60	2.85	20.25
Weeding	3.05	13.35	16.05
Harvesting	71.10	85.20	78.60

$10.50 ha^{-1} for the adopters, indicating a net savings of $32.42 ha^{-1} from adoption of the technology. The reduction in the number of sprays also brought about additional health benefits since farmers became less exposed to toxic pesticides.

For most farm labor operations, the nonadopters' labor use was always higher than that of the adopters in both years except for labor for harvesting. As expected, labor use for seedbed preparation, transplanting, and harvesting was higher in 1999 than in 1996 among adopters. Labor for pesticide application, however, was 0.75 man-days lower for adopters in 1999. The additional labor cost for mixed planting was due to separate transplanting and harvesting of the glutinous rice, which matures about 2 weeks later than hybrid rice.

Costs and returns analysis

To assess the impact of mixture planting on farmers' income, the costs and returns of farm production were estimated (Table 16). Because of increased yields from glutinous rice, which commands a higher price in the market, the gross value of production realized by the adopters is much higher than that of the nonadopters. Adopters gained an additional $143.98 ha^{-1} compared with the 1996 level.

Contrary to expectations that mixture planting requires more seeds, adopters spent $9.75 ha^{-1} less for seeds in 1999. This may be due to more efficient use of seeds by farmers when they follow the straight row planting of both glutinous and hybrid rice. Again, adopters' seed expense is $10.32 ha^{-1} lower than that of the nonadopters.

Table 16. Per hectare costs and returns analysis by type of farmer and by year, Yunnan Province, China, 2000.

Item	Adopters ($ ha^{-1})		Nonadopters 1999
	1996	1999	
Gross value of production	2,153.85	2,297.83	2,016.66
Price kg^{-1} of			
Glutinous rice	0.50	0.36	0.38
Hybrid rice	0.17	0.19	0.19
Costs of production	692.96	674.62	713.92
Seeds			
Glutinous rice	1.48	1.09	1.89
Hybrid rice	69.06	59.70	69.22
Fertilizer	167.70	167.70	180.52
Pesticides	18.58	10.50	42.92
Hired labor	436.14	435.75	419.36
Net returns above cash costs	1,460.62	1,623.21	1,302.73

There was also a substantial reduction of $8.08 ha^{-1} in pesticide costs among adopters in 1999. Nonadopters spent $32.42 ha^{-1} more on pesticides than did adopters.

Although additional labor costs are incurred for both transplanting and harvesting, the savings from pesticides and seeds and higher price of glutinous rice are more than enough to compensate for the increased labor costs. Thus, we see from Table 16 that the net returns above cash costs obtained by adopters are $162 ha^{-1} higher than in 1996, and $320 more than those of nonadopters in 1999.

Impact perceived by farmers

Table 17 shows the impact of mixture planting based on farmers' perceptions. A majority of the farmers thought that there was a significant increase in labor use for seedbed preparation (63%) and harvesting (75%) as a result of adopting mixture planting. Farmers also perceived that this technology required more seeds (54%) but lower pesticide costs (87%) because of less incidence of blast disease (90%).

Now that farmers are able to plant more rice varieties (86%), genetic diversity is also improved. However, despite the ecological soundness of reduced pesticide use, farmers do not seem to associate it with improvements in soil and water quality as well as reduced mortality of fish and other aquatic products in the rice fields.

Most of the farmers (94%) perceived that mixture planting resulted in higher yields. As yield improved, there was an adequate supply of rice (81%) for home consumption. Most of the farmers (97%) reported that the adoption of mixture planting improved income and quality of life.

Economic impact of the project

As noted from the above findings, this technology adoption had the following effects:

1. Reduced pesticide use.
2. Increased yield from elimination of crop losses from pests and lodging.

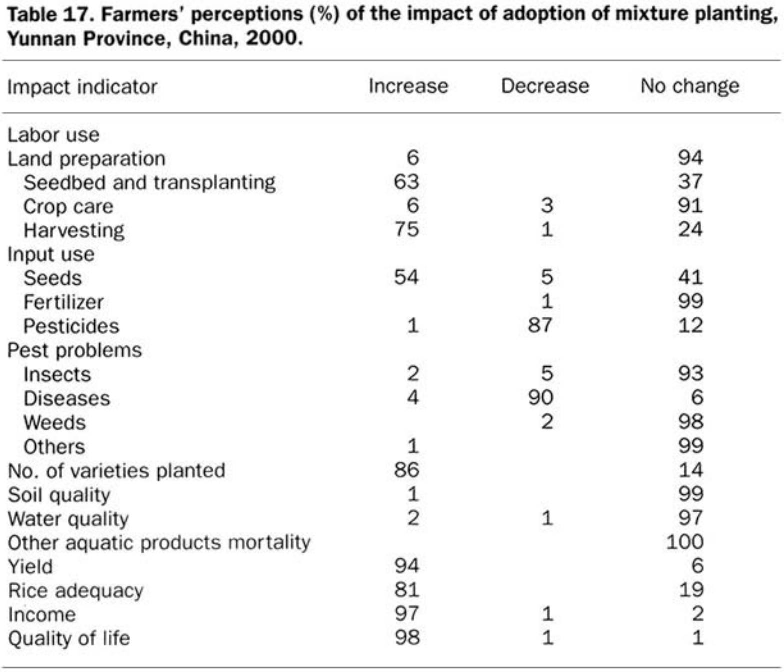


Table 17. Farmers' perceptions (%) of the impact of adoption of mixture planting, Yunnan Province, China, 2000.

Impact indicator	Increase	Decrease	No change
Labor use			
Land preparation	6		94
Seedbed and transplanting	63		37
Crop care	6	3	91
Harvesting	75	1	24
Input use			
Seeds	54	5	41
Fertilizer		1	99
Pesticides	1	87	12
Pest problems			
Insects	2	5	93
Diseases	4	90	6
Weeds		2	98
Others	1		99
No. of varieties planted	86		14
Soil quality	1		99
Water quality	2	1	97
Other aquatic products mortality			100
Yield	94		6
Rice adequacy	81		19
Income	97	1	2
Quality of life	98	1	1

3. Increased production costs on account of higher labor use in planting and harvesting.

Cost savings from reduction in pest pressure

The most direct benefit from mixture planting is the reduction in pest pressure, that is, blast disease, which implies some savings in the use of pesticides and labor for spraying pesticides. The average number of sprays was three for the nonadopters compared with only one for the adopters, which would have a positive effect on health. It has been documented that several diseases are caused by exposure to harmful chemicals during spraying (Rola and Pingali 1993).

Because of the reduced incidence and severity of blast disease, adopters spent only \$10.50 ha^{-1} for pesticides compared with nonadopters' expenditure of \$42 ha^{-1}. There is also a substantial savings in the amount of labor used for spraying pesticides. Comparing the costs incurred by both adopters and nonadopters for pesticides and labor, we estimate that the gains from adopting this technology are about \$72 ha^{-1} (Table 18).

Yield gains from reduction in losses

Nearly 25–40% of the yield of monocropped glutinous rice is usually lost due to blast disease (Zhu 2000 and this volume). The crop also suffers from lodging when there is strong wind because of weak stems and taller plants. The method of interplanting one row of glutinous rice with four to six rows of hybrid rice as a form of varietal diversification helps control blast disease and lodging of the glutinous rice plants. In Table 19, we see that, for nonadopters, only 6% of the area is planted to glutinous rice compared with 19% for adopters.

On mixture farms, the yield of glutinous rice has increased by 82%. This figure is much higher than the 25-40% yield loss from blast. However, the yield of hybrid rice has decreased by about 2%. The combined yield of glutinous and hybrid rice was 10.4 t ha^{-1} for adopters versus 9.7 t ha^{-1} for nonadopters. Thus, the overall yield gain from adopting the technology was about 7.2%.

The gain in gross revenue is even higher at 14% since glutinous rice fetches a price almost double that of hybrid rice because of higher grain quality and the use of glutinous rice on social occasions. The gain in gross value of production from adopting the technology is estimated at \$281 ha^{-1}, almost four times higher than the cost savings in pesticides.

Additional labor costs

This technology is not costless, as shown in the previous analysis and based on farmers' perceptions. Substantial additional costs are incurred, particularly in two farm operations. One is for transplanting labor because adopters have to transplant twice. Another is for harvesting because farmers have to harvest the glutinous and hybrid rice separately. The mixture-planting technology requires additional labor of 17.1 d ha^{-1} and the imputed cost of the additional labor is about \$39 ha^{-1}.

Table 18. Cost savings from reduction in pest pressure, Yunnan Province, China, 2000.

Item	Adopters	Nonadopters
Number of sprays	1	3
Cost of pesticides (\$ ha^{-1})	10.5	42.92
Labor for pesticide application (d ha^{-1})	2.85	20.25
Imputed cost of labor (\$ ha^{-1})	6.49	46.10
Total cost (\$ ha^{-1})	16.99	89.02
Financial benefit (\$ ha^{-1})	72.03	

Table 19. Yield gains from reduction in losses from blast disease and lodging of plants, Yunnan Province, China, 2000.

Item	Adopters	Nonadopters	Percent difference
Percent of rice area under glutinous rice	18.8	6.2	203.2
Rice yield (t ha^{-1})	10.4	9.7	7.2
Glutinous rice	2.0	1.1	–54.5
Hybrid rice	8.4	8.6	–2.3
Gross revenue (\$ ha^{-1})	2,298	2,017	13.9
Financial gain (\$ ha^{-1})	281		

Benefits to farmers from technology adoption

Net income to farmers, after subtracting the additional labor costs of transplanting and harvesting from the value of the two benefits mentioned above, is \$320 ha^{-1} and this is about 14% of the gross revenues from rice cultivation (Table 20).

Farmers' loss of income from reduction in prices of glutinous rice

There is also some expected reduction in farmer-adopters' income because, as more glutinous rice is produced, the market price goes down. This will be a cost to the farmers. On the other hand, consumers stand to benefit from lower prices of glutinous rice.

Note that, with the increased production of glutinous rice, the price has gone down from \$487 t^{-1} in 1996 to \$346 t^{-1} in 1999 for the adopters (Table 21). This will be a major constraint later to the large-scale adoption of the technology as the gain is eroded due to lower prices.

Assuming that, in the long run, the price of glutinous rice would decrease to the level of that of hybrid rice, the minimum net gain would still be \$142 t^{-1}. In the following analysis, this conservative estimate will be used instead of the estimated present net benefits of \$308 ha^{-1} to estimate the rate of return to the project assuming there will be a price reduction for glutinous rice in the long run.

The rate of adoption of the technology

The rate of technology adoption must also be considered in order to estimate the future time stream of benefits. In 1998, mixture-planting technology was adopted in only six counties; by 2000, it had spread to 40 counties (Table 22). Large-scale training of farmers is one of the technology transfer methods implemented to ensure a

Table 20. Benefits to farmers from adopting mixture planting, Yunnan Province, China, 2000.

Net benefits	Values
To farmers (\$ ha^{-1})	320
As % of gross revenue	13.9
As % of family income	25.4

Table 21. Farmers' loss of income from reduction in prices of glutinous rice, Yunnan Province, China, 2000.

Item	Change in rice prices (US\$ t^{-1})		
	Adopters		Nonadopters 1999
	1996	1999	
Glutinous rice	487	346	366
Hybrid rice	163	184	183
Minimum gains (\$ t^{-1})		142	

Table 22. Rate of adoption of mixture planting from 1998 to 2000 and target area coverage for 2003, Yunnan Province, China.

Item	1998	1999	2000
No. of counties	6	10	40
No. of farmers (000)	144	252	927
No. of farmers trained	682	3,290	25,362
Percent of farmer-adopters	14.1	40.6	48.1
Area covered (ha)	812	3,342	16,667
Percent of area covered	15.4	36.3	39.4
Target for 2003	200,000 ha		
Total rice area in Yunnan Province	800,000 ha		
Rice area under hybrid rice	300,000 ha		

faster rate of technology diffusion. The technology spread from a mere 812 ha under mixture planting in 1998 to 16,667 ha in 2000. About 40% of Yunnan Province is now planted to mixtures.

A critical factor that contributed to the fast rate of adoption was the use of local county officials and technicians as trainers. They worked with the farmers closely and actively to demonstrate the technology and this helped encourage more farmers to participate. We therefore need to account for the cost of extension when calculating the rate of return.

The target area for expansion in 2003 is about 200,000 ha. With this kind of extension in Yunnan Province, it is possible to achieve this target, which is about 25% of the area under rice in Yunnan Province. However, we perceive that the downward trend in the price of glutinous rice may constrain the diffusion of the technology at this scale unless the provincial government is able to find an export market for the surplus produce. To be on the conservative side, we assume that at full development the technology will spread to 100,000 ha by 2003 and will remain at that level till 2007, when the technology may become obsolete.

Estimated cost of the project

Table 23 presents the total estimated cost of the mixture-planting project in Yunnan from 1997 to 2003. Investments come from three sources: IRRI, the Chinese government, and the local Chinese partners. IRRI substantially increased its level of support to the Yunnan lighthouse site in 1999 when the project started to make an impact. From $137,000 in 1997, YAU received an additional $83,000 in 1999. Similarly, YAU received increased funding support from the Chinese government to expand the area under mixture planting.

Assuming that IRRI will provide $220,000 each year if a 3-year second phase of the project is approved by ADB, the total investment will be $357,000 in 2002 and $146,000 in 2003. These investments are critical for extending the technology.

Table 23. Estimated cost (US$000) of the mixture-planting project, 1997-2003.

Year	Investment from IRRI[a]	Imputed cost of Chinese partners' time	Chinese government contribution	Total
1997	136.6	14.6	–	151.2
1998	136.6	14.6	36.6	187.8
1999	220.0	14.6	91.5	326.1
2000	220.0	14.6	73.2	307.8
2001	220.0	14.6	122.0	356.6
2002	220.0	14.6	122.0	356.6
2003			146.3	146.3

[a] Includes funds from ADB.

Table 24. Benefit-cost ratio of the mixture-planting project, 1997-2007.

Discount rate (%)	Benefit-cost ratio	
	1997-2000	1997-2007
15	2.7	22.0
50	2.0	8.7
100	1.3	7.8

Benefit-cost ratio of the project

The farmers who already adopted the technology will continue to reap the benefits for many years to come until new pest problems make the technology obsolete. We assume 2007 as the terminal year for the continuation of the benefits.

The investment in the project has already been recovered in just the three years since the project began in 1997 (Table 24). At a 15% discount rate, the benefit-cost ratio is 2.7 for the period 1997-2000. If we take the full time stream of benefits from 1997 to 2007, the project gives a benefit-cost ratio of 22. Even at a 100% discount rate, the benefit-cost ratio will still remain high at 7.8, which means that the actual rate of return on investment is higher than 100%. The internal rate of return from investment in theproject (the China component) is estimated at 160%.

Conclusions

Mixture planting has brought several environmental and economic benefits to the farmers in Yunnan Province. The simple technique of interplanting the glutinous rice that is highly susceptible to blast with hybrid indica rice has reduced the incidence of blast in Yunnan Province. Farmers have reduced their pesticide use and are now able to grow the high-value glutinous rice. Yields of glutinous rice have also increased and this has brought about higher net income for the farmers. Farmers who are already adopting the technology will continue to reap the benefits for many years until new pest problems arise.

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A synthesis of two knowledge-based approaches to pest management

G.T. Castillo

I am a great believer in integrated pest management (IPM) and farmer field schools (FFS), perhaps even when faith in them is not always justified. This fascination with IPM comes from my exposure to the Green Revolution, including the euphoria and criticisms that followed its supposed benefits and unanticipated consequences. To illustrate what I mean, in the early 1970s, the Philippine Rice Production Program prescribed calendar spraying at specified doses and intervals. Pesticide application was uniform in all regions. The total target area and the total value of insecticides required were estimated and the pesticide companies divided the business among themselves. Rice production loans required pesticides. The message of pesticide use was so powerful that farmers could not sleep if they did not spray. Pesticide was their security blanket. They did not know the difference between good insects and bad insects. The only good insect was a dead insect. Research orientation at that time favored pesticide management rather than pest management.

So, in 1980, when IRRS began a pioneer field-based project on IPM, my interest in IPM intensified. Perhaps this was also the first time IRRI had a noneconomist social scientist. I spent practically all my time in villages learning about farmer knowledge, practices, and behavior vis-à-vis pest management. Since then, I have always thought that, because IPM is sufficiently complex and properly avant-garde, if farmers could participate in the further development and internationalization of the concept, they could learn almost anything for the 21st century.

By its very nature, IPM immerses the farmer, researcher, extension worker, and policymaker in a new world of discourse requiring new content of messages, higher levels of understanding, and different ways of defining and solving problems and learning together. It also means going beyond prescriptions to decisions. For the farmer, “seeing is believing” will no longer suffice. Some, if not most, of what is involved in IPM is not going to be directly visible to the naked eye and will not always be immediately experienced. Seeing and thinking not only in the abstract but also in the future must be learned. The pedagogy of this learning process has scarcely begun to be unraveled.

We know, however, that we need to make significant additions to the old adage "to see is to believe." such as

To *do* is to believe,
to *use* is to believe,
to *know* is to believe,
to *understand* is to believe, but
to *adapt* is to succeed for *adaptation* localizes the application
of ecological principles and the practices of technology.

The disease and/or pest resistance imbedded in the rice seed is not seen by the observer. It is a fact that must be made known. The dynamics of plant growth, the significance of varietal characteristics, the life cycle of insects, the symptoms of plant diseases, plant-water and nutrient-pest connections, etc., are as much conceptual as empirical. In IPM, we move from technology diffusion to concept understanding, from the adoption of new rice varieties to farmers learning about science, and from individual decisions to social learning and collective action.

Let me reiterate. Although "to see is to believe" is not sufficient, I think it is necessary. Doing, using, knowing, understanding, and adapting are mutually reinforcive but *seeing* must be there. The intangibles must translate into tangibles that can be eaten, traded, stored, or even given away in the process of living one's life.

The chapter by Peter Ooi and others, "Farmer Scientists in IPM: A Case of Technology Diffusion," illustrates the science learning and technology diffusion connection.

1. Without farmer understanding of what parasitoids do, continued spraying prevented parasitoids from performing their function. Learning about the actions of *Bacillus thuringiensis* and the concept of insecticide resistance is something farmers in the past never had an opportunity to do because they were given prescriptions. It was assumed that they would never understand such complexities.
2. The story of the white stem borer and preparation of the seedbed after the flight of the moths indicates that farmers needed to understand the life cycle of the insect. This life cycle is not a technology. It is information derived from a body of knowledge. The results from the application of this knowledge are very visible.
3. Rats to feed ducks seems to have been a win-win scenario for duck-raising rice farmers. Hopefully, nobody will try to produce more rats to feed more ducks. But this is probably beyond ecology.
4. I noticed that FAO has added "community" to IPM so it is now the FAO Programme for Community IPM in Asia and there is an increased emphasis on sharing results of studies generated in FFS with the *whole* com unity. The whole community is probably a bit exaggerated but sharing with others in the community is a definite phenomenon. Agnes Rola's study in the Philippines has definitely documented this phenomenon ("Economics of IPM-FFS Knowledge Diffusion in Rice-Farming Communities: A Case Study in Iloilo, Philippines," 30 June 2000).

She concludes:

"Our results show that IPM FFS knowledge is diffused. The 72 FFS graduates randomly selected from the study barangays altogether diffused the knowledge to 32 co-farmers in a direct way. This is about 44% direct diffusion.... Given the cost of training one farmer in the FFS (₱1,051 to ₱1,103) and the potential returns currently measured by the insecticide cost savings of the FFS graduates, one can conclude that FFS is a feasible investment, not only in the short term but also in the long run. Benefits could potentially accrue from the diffusion, from the intergeneration effects, and from the social costs saved."

The farmers' learning facility is an attempt to institutionalize continuous science learning among farmers. This is a very forward-looking idea that will enable farmers to rise above the agricultural challenges that await them. There is only one caution at this point. If farmers are to continue being excited about science learning, the facilitators and managers of the learning facility must learn more and faster in anticipation of farmer needs and interests. Farmers get bored easily when taught the same lessons over and over. A study of FFS in India showed that farmers, whether trained or untrained, had higher knowledge scores than extension workers, whether trained or untrained. We must do better than this. Such a learning facility puts a great demand on the research community. There must be a continuous flow of useful and challenging things to learn and a variety of situations to apply them to. Are we up to that?

The project "Exploiting Biodiversity for Sustainable Pest Management" is also knowledge-based like the IPM case but it is different in some ways. Although it is based on ecological principles, *biodiversity for sustainable pest management,* its promotion and translation into farmers' fields is "mixture planting," which is very simple and very visible, although there is much science behind this simple scheme. Where else can you find three rows of hybrid rice interplanted with one row of glutinous rice to be harvested at different times? The project did not push new varieties. The message was to mix them. But what to mix and how to mix them did not come automatically. The farmers had to experiment with different combinations based on commonly grown varieties. Adoption was dramatic, impact immediately evident, and "seeing is believing" worked wonders. But, most of all, rice blast incidence decreased and yield loss declined. The mix of varieties was based on farmer preference because glutinous rice commands a premium price and has sociocultural value. In a way, the *mixture* of varieties was the new technology. It is not a plant breeding product.

It has been argued before that, if the technology fits, is adopted, diffused, and makes a difference in farmers' income, in environmental quality, and in productivity, why do we need to invest in a school, even a farmers' field school? Did farmers learn the ecological principles behind the success of mixture planting? What about the other less visible components of the biodiversity project such as the application of microbial biocontrol agents for sheath blight, the proliferation of natural enemies of insect pests through planting compatible crops, and the nonapplication of pesticide during the early stages of crop growth?

My naïve view is that, precisely because "seeing is believing" is so operative in this case, the atmosphere for science learning from knowing and understanding is probably at its best. How do we build this into the project implementation so that the science is not left behind when the technology takes off?

Although there was no FFS, there was the classic case of scientist-policymaker-farmer collaboration in pushing the technology from concept to actual practice.

The specifics of the diversification scheme were defined based on farmers' practices and inputs. Highly motivated farmers and members of the provincial and county plant protection stations then participated in the implementation of the project in farmers' fields. Initial successes generated from the demonstration plots soon encouraged more farmers to follow the diversification scheme. Over a period of less than three years, yield increased, rice adequacy for consumption increased, income increased, and the quality of life improved.

Impact must be seen, felt, and acknowledged by farmers, not just by scientists. Even when positive gains are computed by economists but farmers do not feel those gains, we probably have not made a noticeable and felt difference. For this reason the chapter by Revilla and others included an analysis of farmers' perceptions of the impact of mixture planting in addition to actual economic calculations. As one Filipino rice farmer remarked upon being told that using IPM has given him a net profit of P768, "But I don't have it in my pocket. It is only in your notebook."

Although mixture planting is based on an ecological concept, it was perceived by farmers as a useful technology adopted in a fantastic *scaling-up process,* which means "bringing more benefits to more people quickly" in an ecologically sound way. However, farmers have not yet seen its impact on soil and water quality and livestock mortality. This should follow.

The chapters by Ooi et al and Revillaet al understandably underscored what farmers learned and what changes in farmer practices have occurred, but there is no mention of what scientists learned in the process and what changes in their science have taken place (if any) as a consequence of their involvement in these two approaches. They made a difference to farmers. Did they also make a difference to scientists? In the case of policymakers in China, the impact seems to be obvious. How dramatic do the results have to be to win their support? What will the Yunnan team do for an encore after the scaling-up of the mixture planting strategy? Are other ecological principles waiting to be tested at lighthouse sites?

What is the possibility of bringing the concept behind the Yunnan experience into the curriculum of FFS?

In summary, let me make the following observations:

1. The scientists played an active role in the research translation process, unlike the usual argument that defines their role as ending at the point where the research report is produced or the publication comes out. Very often they say that research use is the job of extension. This translation of technical research results into farmer participatory learning cannot just be passed on to somebody else. In the health field, they call this *intervention research,* others call it *action*

research, but it is still regarded as research although it involves farmers, extension workers, and policymakers.

2. Farmer participation came early in this project and in a very significant way in mixture demonstrations in farmers' fields.
3. There was a large-scale social mobilization of other actors.
4. Training efforts on-farm were massive. This should be studied further in terms of the content, method, and impact on farmer knowledge, skills, yield performance, and ability to influence others. What was taught and what was learned? Because biodiversity is a very knowledge-intensive concept, training plays a crucial role. How much of the science can be distilled so that farmers can be taught?
5. Dr. Zhu and his colleagues were on the ground, they were not in their ivory towers viewing the entire process from above. For this I salute them. This is a case in which modem science meets local knowledge. How can we make things happen in the Philippines, in Thailand, and in Vietnam?

Being involved with field realities and practicalities enables us to learn not only with our minds but also with our hearts.

Science is a powerful force for the public good when practiced to serve a human purpose. In the project "Exploiting Biodiversity for Sustainable Pest Management," scientists, farmers, extension workers, policymakers, and funding agencies got together for a common goal. No one group could have done it alone. Therefore, I conclude that this project is not just a scientific achievement. It has made a difference in the lives of those who have less in life. In my own pedestrian mind, this represents the triumph of the human spirit.

Notes

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