Elimination of Aflatoxin Contamination in Peanut

Editor: Ralf G. Dietzgen

A collaborative workshop project
between the
Queensland Department of Primary Industries, Australia
and
Bogor Agriculture University, Indonesia

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Cover: Drought-affected peanut cultivar NC-7 near Kingaroy, Queensland.
And (inset) Aspergillus flavus growing on peanut kernels and pods.

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INTRODUCTION AND OVERVIEW

Elimination of Aflatoxin Contamination in Peanut

R.G. Deitzgen[1]

THIS workshop was an initiative of the Technology Diffusion Program 'Targeting Research Alliances' (TRA) of the Department of Industry, Science and Resources. It was conducted under the auspices of COSTAI (Collaboration in Science and Technology, Australia – Indonesia) in the priority area of 'Agricultural Biotechnology'.

The Technology Diffusion TRA Program is a major initiative by the Australian Government to give industry access to leading edge technologies. This workshop project enabled industry to become familiar with and discuss implications of potential agricultural biotechnology applications to eliminate aflatoxin contamination in peanut.

Aflatoxin is the highest priority disease problem of the Australian peanut industry. National and international alliances (with the USA, Indonesia and India) were strengthened between the peanut industry, funding bodies and researchers, which resulted in the development of three international, collaborative research proposals as one of the major workshop outcomes. These should also include China as the world's largest peanut producer with an active research program in aflatoxin control, and with already established excellent linkages through current ACIAR projects.

Access to national and overseas leading edge technologies to solve the worldwide problem of aflatoxin contamination in peanut can be achieved by funding one or more of these approaches. Harvest of aflatoxin-free peanuts from the field would strongly contribute to the competitiveness of the Australian peanut industry. Such new technology approaches and their value-added products are also likely to create investment opportunities.

The workshop was held at the TAFE College in Kingaroy, Queensland from March 2–5, 1999 and was attended by 33 participants from Australia, Indonesia, USA and India.

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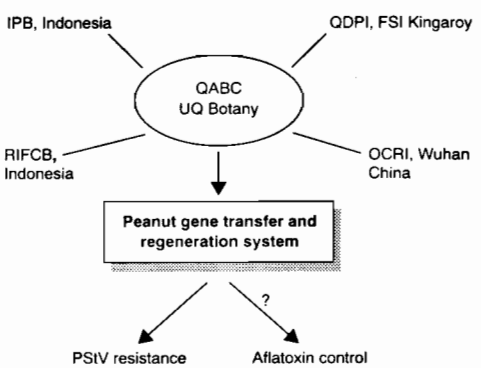
These proceedings contain summaries of the workshop presentations that cover the current status of aflatoxin control and emerging biotechnological approaches.

Detailed proposals for collaborative research projects using integrated agricultural biotechnology, solutions and conditions required to proceed with such projects, were another outcome of the workshop. These proposals are considered commercial-in-confidence and are therefore not included in these proceedings.

The Australian Centre for International Agricultural Research (ACIAR) has been involved in molecular peanut improvement through molecular biology and tissue culture technologies. Project 9439 involves research institutes and researchers at the Queensland Department of Primary Industries (QDPI) and University of Queensland in Australia, Bogor Agriculture University and Research Institute for Food Crops Biotechnology in Indonesia and the Oil Crops Research Institute in Wuhan, China.

One of the major outcomes of this collaborative research is a reliable and efficient system for peanut gene transfer and regeneration. This system has been used successfully to introduce a viral 'resistance gene' and is available for introducing other economically valuable traits into peanut germplasm.

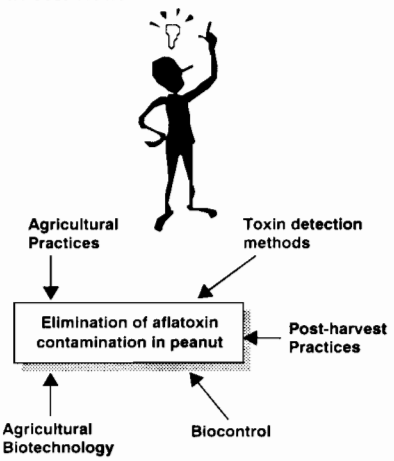
ACIAR 9439



The Aflatoxin Problem

- Aflatoxin is produced by strains of *Aspergillus flavus* and *A. parasiticus* in peanut kernels.
- Aflatoxin is a potent toxin and carcinogen.
- Serious human and animal health hazard.
- Recognised as a global problem.
- Leads to reduced income for farmers and reduced health for consumers.
- Resistance to other fungal pathogens does not appear to effect *Aspergillus* sp.

Potential solutions



Some important issues to be addressed

- Availability of *resistance genes* in the peanut germplasm?
  - — which cultivars?
  - — DNA markers?
  - — resistance to fungal infection and/or toxin production?
- Reliable peanut *transformation systems*
  - — U Georgia, QDPI/UQ
  - — ICRISAT? Others?
  - — speed and general applicability?
- Action of *novel 'resistance' genes*
  - — inhibition of fungal infection
  - — prevention/inhibition of toxin production
  - — detoxification of aflatoxin
  - — others?
- Promoters for *gene expression*
  - — when, where, how much?
- *Intellectual property* and *patent* issues
- *Acceptance* by growers and consumers?

AFLATOXIN—IMPORTANCE AND CURRENT CONTROL STRATEGIES

Economic Importance of Aflatoxin to the Australian Peanut Industry

R.B. Hansen and K.L. Norman[1]

CONTAMINATION of peanuts with aflatoxins is a worldwide problem (Will et al. 1994). Within the Australian peanut industry, aflatoxin represents one of the major challenges to the industry's future, especially so for farmers with rainfed farms. Based on current estimates, management and treatment of peanuts contaminated with aflatoxin is costing the Australian peanut industry between $3 million and $6 million annually. Aflatoxin is an issue that must be addressed by the *entire* peanut industry.

Aflatoxin is a known animal and human carcinogen and therefore peanut processors must go to major lengths to keep their peanut products free from this contaminant.

Aflatoxin is a mycotoxin that may form when the pod is invaded by one of two fungi, *Aspergillus flavus* and/or *Aspergillus parasiticus.*

For detection of aflatoxin, Peanut Company of Australia (PCA) uses a rapid chemical test known as the mini-column method to detect the presence and level of aflatoxin contamination in farmers' stock peanuts. Visual detection of aflatoxin is completely unreliable.

In the USA, rather than use chemical detection, most buying points still predominantly rely on visual detection of the *A. flavus* and *A. parasiticus* fungi. However, various USA laboratories have found that peanut samples that passed a visual detection test may actually contain very significant aflatoxin concentrations (Dorner and Cole 1997), even up to 1000 parts per billion (T. Whittaker, pers. comm.).

Farmers' stock peanuts with aflatoxin are segregated into various levels based on the concentration detected. Shelling, grading and sorting may remove some contamination, but is not in itself reliable enough under Australian conditions to meet the Australian Food Standards of less than 15 parts per billion (ppb).

[1]Peanut Company of Australia, Haly Street, Kingaroy, Qld 4610

Blanching and colour sorting is the main strategy used to indirectly remove kernels affected by aflatoxin but significantly reduces the edible content and therefore increases costs. This strategy can be very effective depending on the variety of peanut, the grade, the level of contamination and other factors such as seasonal impacts (Whittaker 1997).

As the level of contamination increases, efficiency falls and losses (and therefore costs) may increase dramatically.

Prior to the 1994–95 growing season, the cost of aflatoxin contamination was 'subsidised' to some extent by all growers across all regions and PCA. However, since 1996 PCA's pricing for aflatoxin contaminated farmers' stock peanuts closely reflects the *true* cost of removing aflatoxin from contaminated loads and supplying consumers with healthy Australian peanuts.

Many growers will be familiar with the economic impact of aflatoxin on their returns. The value of a load of peanuts can be dramatically reduced if high levels of aflatoxin are detected. It is not uncommon for a grower to lose more than $A7000 on a single semi-trailer load of peanuts that has been classified as Segregation 4. (Segregation 4 is the highest on a scale of 1 to 4 and indicates high levels of aflatoxin contamination).

Across the various growing regions in Australia, the historic levels of aflatoxin contamination are as follows:

- Dryland South and Central Burnett — 42%;
- Atherton Tableland and Lakeland — 11%;
- Fully irrigated areas — 8%;
- Northern Territory — 17%.

In some extreme seasons, nearly 100% of loads from the dryland South and Central Burnett may contain some level of aflatoxin contamination in the edible kernel grades.

Of those loads that contain aflatoxin from the irrigated areas, usually less than 5% of these loads have aflatoxin concentrations greater than 80 ppb.

By comparison, of those loads from dryland production areas that are detected with aflatoxin, 20% or more of these loads have aflatoxin concentrations greater than 80 ppb.

All peanuts are susceptible to the potential contaminant, but irrigation will significantly lower the risk of contamination and the likely level of contamination.

Table 1 illustrates the relative costs to farmers of aflatoxin contamination. Growers with very high levels of aflatoxin may receive only 50% of the returns of those growers with no aflatoxin.

In the irrigated production areas, peanut growers with good yields and quality would expect gross margins of more than $1000 per hectare in the absence of aflatoxin. By contrast, the average gross margin of peanut growers in the South Burnett with aflatoxin is $168 per hectare (Norman and Hansen 1998).

Very few peanut growers will be able to survive in the industry with such low returns for a sustained period. To ensure the long-term viability and sustainability of the Australian peanut industry, agronomic, varietal and management solutions to aflatoxin must be further investigated and developed.

The risk to consumers in Australia is low from peanuts, as the Australian peanut industry since the late 1970s has taken a very proactive approach to minimising aflatoxin in the products sold. The average of the entire product PCA distributes is less than 2 ppb and is probably closer to 1 ppb. This is not the case in Indonesia, Philippines and Thailand (Lubulwa and Davis 1991), India, Africa (PCA analysis) and possibly the USA where aflatoxin concentration in consumer-ready peanuts is higher.

The social and economic cost is detailed in Table 2.

This does not allow for the immuno-suppression effects of hepatitis or malaria.

Conclusion

Australian peanut farmers bear a significant proportion of the risk and economic cost of aflatoxin minimisation. The risk to Australian consumers and the economic cost to consumers is very low, if not close to zero. The economic cost to the Australian peanut producers ranges between $A3.0 million and $A6.0 million due to the cost of detection, storage and elimination. If a more economical solution can be found, the productivity and sustainability of the Australian peanut industry will improve dramatically.

In Southeast Asia, the economic and social cost is high causing both liver cancer deaths and increased cost of production for intensive livestock producers. Any reduction in aflatoxin in the food chain in Southeast Asia may significantly reduce liver cancer and improve the efficiency of the intensive livestock industry.

Table 1. Relative costs to farmers of aflatoxin contamination.

Segregation level	Segregation 1	Segregation 2	Segregation 3	Segregation 4
Aflatoxin conc. in each segregation	<8 ppb	8–80 ppb	80–160 ppb	>160 ppb
% Value of Seg. 1	100%	91–95%	78–85%	50–55%

Table 2. Social and economic cost of aflatoxin ($A millions).

	Cost of extraction, elimination and post harvest losses	Intensive livestock production	Loss of human life	Total $A/year	Estimated human mortality due to aflatoxin ingestion/year
Australia	4.0				
USA	210.0?				
Indonesia	90.0	13.0	229	332.0	22509
Philippines	13.5	37.0	39	89.7	2640
Thailand	4.1	27	24	55.3	672

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Aflatoxin Reduction in the Blanching Process

C. Ganzer[1]

THE Australian peanut industry faces challenges to become a cost-effective and profitable industry which can become world competitive. The main issues preventing the industry from achieving these goals are the need to focus on customers' requirements, the ability to exploit the genetic potential of the peanut plant in relation to yield, and the management of aflatoxin.

Aflatoxin reduces returns to farmers by deductions made by peanut processors, which therefore gives no security to future supply of a locally-grown crop. Financial returns to growers are poor because aflatoxin adds cost to a low-quality product because of the large amount of resources required by dedicated blanching facilities to remove aflatoxin. The final reason aflatoxin in peanuts is a problem is the most important—aflatoxin peanuts are simply not suitable for human consumption due to the risk of liver disease.

Kingaroy Blanching is a joint venture company between Jorgenson Waring Foods, G. Crumpton and Sons, and Clifton Farming Company, producing peanuts for the peanut butter, confectionery and wholesale peanut markets. The purpose of Kingaroy Blanching is to produce safe, high quality blanched peanuts and to maximise the use of aflatoxin peanuts to achieve this purpose.

The blanching process involves the roasting of raw peanuts with the aim of loosening skins for easy removal by the blanching rollers. These rollers have an abrasive surface which removes the skins. Not only does this process remove the skin for better presentation, but defective peanuts become easier to observe by the colour sorters. The colour sorters electronically sort peanuts by identifying those peanuts which have a different colour from the acceptable peanuts. Those peanuts that have a different colour are removed from the process by a jet of compressed air. Therefore, peanuts with mould (which may contain aflatoxin), will be detected by the colour sorters and removed from the peanut stream. This action will reduce aflatoxin levels to below the legal standard of 15 ppb. Packaging is normally in 1 tonne bags or 25 kg bags for distribution throughout Australia.

For the past season, prices to farmers have been at a low level and achieving a return on investment has become more difficult. The presence of aflatoxin also results in high financial deductions to the growers and in many cases there has been no income to growers from their peanut crop. While the cost of raw materials are reduced by aflatoxin deductions, this is only an indication of the additional costs required to remove aflatoxin affected peanuts. The higher the aflatoxin level, the slower the process due to the need to remove mould-effected peanuts.

Cost of Virginia peanuts, 1998 crop.

Peanut size	Prize/tonne (A$)
Jumbo	1100
1	1100
2	950
Splits	750

Aflatoxin deductions.

Aflatoxin category	Aflatoxin range (ppb)	Deduction/ tonne (A$)
I	0–5	0
II	6–40	150
III	41–100	300
IV	100+	450

There is a strong relationship in roasted peanuts, between aflatoxin content and production losses due to the removal of defective peanuts (Figure 1). Those peanuts with high aflatoxin have large amounts of reject type peanuts which must be removed to meet legal aflatoxin requirements, while peanuts with low aflatoxin have a minimum of reject peanuts and therefore have less losses.

[1]Kingaroy Blanching, Bunya Highway, Crawford, Qld 4610

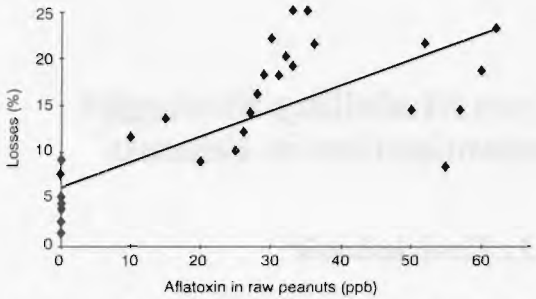

Figure 1. Relationship between blanching losses and aflatoxin.

Legal requirements in Australia (Australia and New Zealand Food Authority, ANZFA) require peanuts for human consumption to contain less than 15 ppb of aflatoxin. Many industrial customers have become more demanding with more stringent specifications ranging from 5 to 10 ppb. Of the peanuts that were available to Kingaroy Blanching (KB) in 1998, 82% of batches had aflatoxin levels greater than 15 ppb. All of these batches required significant further processing to reduce the aflatoxin to below 5 ppb by the use of electronic colour sorters.

Levels of aflatoxin available to be blanched.

Aflatoxin category	Aflatoxin level (ppb)	% Available to KB
SEG I	0–5	18
SEG II	6–40	36
SEG III	41–100	34
SEG IV	100+	12

There is a strong relationship between reject peanuts (discoloured and mouldy peanuts) and aflatoxin level. To remove aflatoxin as a food safety risk is a critical control point in the blanching process. To ensure that this point is controlled, there must be <1% defective peanuts after colour sorting. This is clearly demonstrated in the graph (Figure 2).

Of 1030 samples, only 4 results were identified as above 15 ppb, when the level of rejects after colour sorting was below 1%. With a standard deviation of 3.3 ppb, 96% of all samples were less than 9 ppb. Despite very heavy levels of aflatoxin, including batches as high as 300 ppb, it is possible to reduce the aflatoxin levels in these peanuts to <15 ppb, and regularly below 5 ppb.

The process of accessing critical control points in a production environment to ensure product meets specifications consistently is known as HACCP (or hazard and critical control point) analysis. Most customers demand this system and it will become a legal requirement in the next 5 years. However, the process is cost-effective because it prevents problems occurring.

Aflatoxin is a significant problem in the Australian peanut industry and has the potential to jeopardise the future of this industry. The blanching process, with its use of colour sorters, takes advantage of an opportunity to use peanuts which would not normally be available for human consumption and turns them into a high quality, high value-added product.

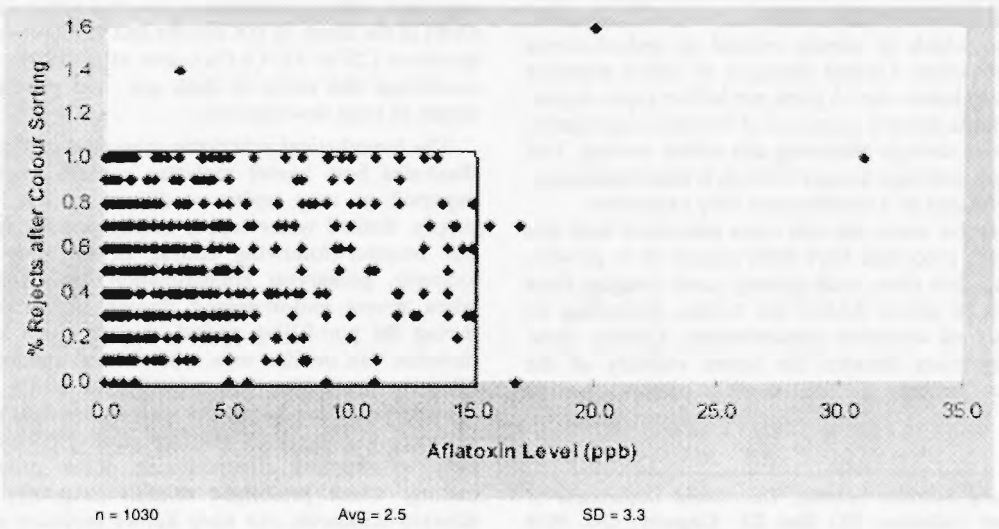

Figure 2. Correlation between aflatoxin and reject peanuts after colour sorting.

Agronomic, Genetic and Crop Modelling Strategies to Minimise Aflatoxin Contamination in Peanuts

G.C. Wright and A.L. Cruickshank[1]

IT IS BELIEVED that aflatoxin contamination can be minimised 'on-farm' using a combination of agronomic and genetic strategies including: optimal harvesting management, including monitoring of thermal time + kernel moisture content; optimal postharvest management, including short cutting–thrashing intervals and drying; good crop management, including control of soil insects, use of rotations, appropriate plant density; and use of peanut varieties that escape end-of-season drought stress.

Simulation modelling approaches can assist in defining aflatoxin risk across regions and countries. Such information can be useful for growers, industry, researchers and policy makers, when defining management strategies, machinery and plant investment and future research priorities.

Background

The major aflatoxin problem confronting the Australian peanut industry relates to preharvest contamination, which is closely related to end-of-season drought stress. Current strategies to reduce aflatoxin levels to below the 15 parts per billion (ppb) regulatory limits involve a process of selective segregation achieved through blanching and colour sorting. This strategy, although being effective, is time consuming, wasteful and as a consequence very expensive.

In recent years, the real costs associated with this recovery procedure have been passed on to growers for the first time, with penalty costs ranging from $A150 to above $A450 per tonne, depending on severity of aflatoxin contamination. Clearly, these penalty costs threaten the future viability of the peanut industry in the rainfed peanut growing regions of Australia where aflatoxin incidence has a high probability of occurrence.

There is therefore an urgent need to find solutions to the aflatoxin problem via the implementation of management, genetic and/or crop modelling strategies that can minimise its 'on-farm' impact for growers. These strategies depend on a thorough understanding of the environmental and associated crop conditions leading to high aflatoxin incidence.

Factors Affecting Aflatoxin Production in Peanut Kernels

It is only in the past decade that a thorough understanding of the environmental and associated crop conditions leading to high aflatoxin incidence have been made (Diener et al. 1988; Dorner et al. 1989). Aflatoxin production will only occur in peanut kernels when the *Aspergillus flavus/parasiticus* fungus is present under conditions of lowered water activity (Aw) in the range of 0.8 to 0.95 and favourable temperatures (25 to 32 °C) (Schearer et al. 1999). Such conditions can occur at both pre- and postharvest stages of crop development.

The hypothetical schematic presented in Figure 1 illustrates how kernel moisture content would be expected to vary under conditions of full water supply, limited water supply during pod-filling and wet weather following cutting of the crop. For example, preharvest contamination can eventuate when severe end-of-season drought stress occurs during the pod-filling period, during which kernel moisture can decline to a critical level suitable for aflatoxin production (around 18% to 28%). Such contamination can be present well before final maturity occurs in the crop (Dorner et al. 1989). Similarly, postharvest aflatoxin can occur following cutting, where prolonged rainfall in poorly constructed windrows can keep kernel moisture in the critical range for aflatoxin production (Figure 1).

[1]Farming Systems Institute, Queensland Department of Primary Industries, PO Box 23, Kingaroy, Qld 4610 Australia

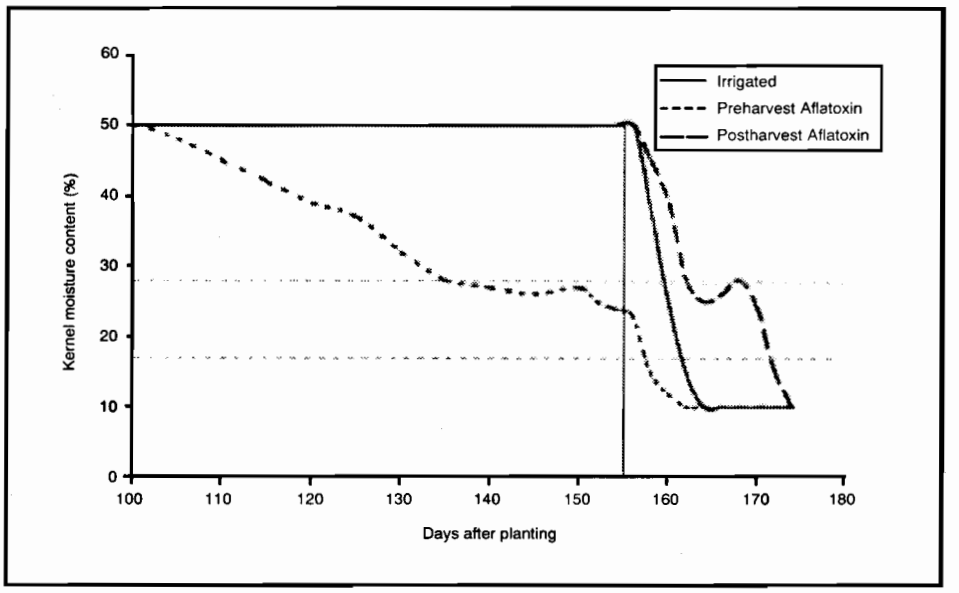

Figure 1. The change in kernel moisture content with time for a hypothetical irrigated crop, end-of-season drought stress and wet weather after cutting.

The critical role of kernel moisture in controlling aflatoxin production (Figure 1) highlights how management and/or genetic strategies can assist in reducing the aflatoxin risk. For example, early cutting in a severe drought year may minimise the period spent in the critical aflatoxin moisture range and thereby minimise aflatoxin contamination.

Crop Management Strategies to Minimise Aflatoxin Contamination in Peanuts

(a) Maturity time

In years of severe end-of-season drought, 'on-time' or even early cutting can minimise time in the 'risk' kernel moisture range. In recent 'on-farm' experiments at Kingaroy, cutting of the crop by up to two weeks prior to normal maturity time significantly reduced aflatoxin levels in the variety 'VB-97' (Figure 2). Growers need to consider that this strategy should only be considered in severe drought years, otherwise significant pod yield and/or quality losses could occur.

Current research is focussed on better defining this cutting decision for growers, including monitoring of kernel moisture content and calculation of thermal time for maturity prediction to determine 'optimal' cutting time.

(b) Short cutting — thrashing intervals

In order to minimise the risk of wet weather following cutting and hence exposure of the crop to the 'critical' kernel moisture range for aflatoxin, it is essential to shorten cutting-thrashing intervals. Recent data collected from 'on-farm' experiments investigating the influence of time spent in the windrow on aflatoxin contamination clearly showed that pods/kernels that spent long periods in the windrow were significantly more contaminated than crops where pods were thrashed within a couple of days following cutting (Figure 3).

This management strategy would, however, require growers to acquire the capability of harvesting/thrashing at high moisture contents (>25%), in addition to investment in substantial artificial drying facilities which would involve additional expense. With the current aflatoxin price penalty, such investment may be well be an economic proposition.

(c) Planting arrangement (skip rows peanuts)

In severe terminal drought years, it is proposed that a management strategy involving the use of wide rows (i.e., every third row skipped) may help to reduce the rate of crop water use and hence preserve soil water late in the season. This strategy would ensure that kernel water stress and hence aflatoxin development

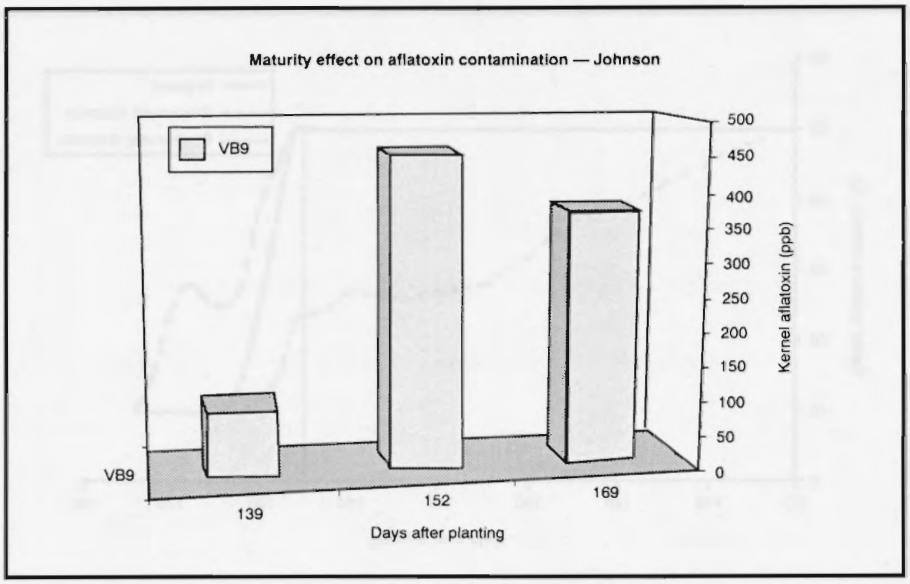

Figure 2. The effect of early cutting on aflatoxin levels in a crop of VB-97 grown under severe end-of-season drought stress.

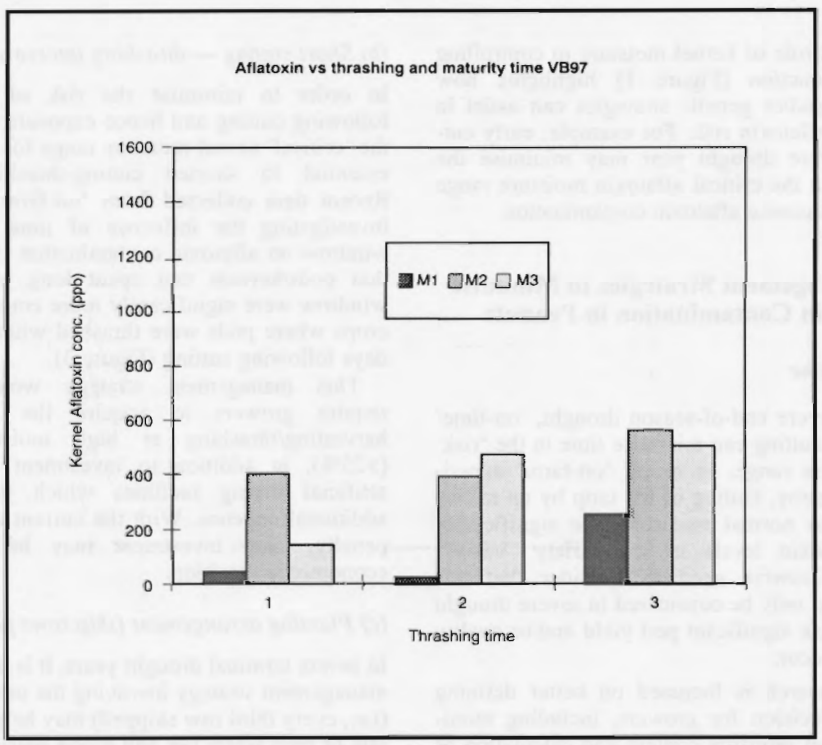

Figure 3. The effect of thrashing following 3 (1), 10 (2) and 20 (3) days after cutting for crops cut at three maturity times (M1, M2 and M3) (Var VB97).

would be minimised. It is also likely that kernel quality (size) would be improved.

Growers need to be cautioned that such a management strategy will result in a substantial yield penalty in good seasons, and would need to be considered as an overall farm risk management approach to minimising aflatoxin. Research is underway this season to evaluate the value of such an approach using a range of commercial varieties.

(d) Minimising damaged pods/kernels

A significant proportion of the total aflatoxin contamination in a peanut sample can occur in insect damaged pods (e.g., white grub, etiella). A recent analysis of aflatoxin contamination in various kernel grades collected during 'on-farm' experiments clearly demonstrated that large concentrations can occur in damaged kernels (Figure 4), as found previously by other workers (Dorner et al. 1989). While current sorting techniques used by shellers can adequately reject obviously damaged kernels (and hence high aflatoxin kernels), growers can also employ appropriate management practices to control pod-damaging insects and hence minimise this aflatoxin risk.

(e) Other management practices

Any management practice that improves soil physical, biological and chemical health will assist in reducing potential aflatoxin contamination in the crop. For example, large organic matter additions will improve soil structural stability, which improves rainfall infiltration and hence water available to the crop. The practicing of sound rotations will also tend to reduce *A. flavus* populations in the soil and hence reduce the risk of aflatoxin development in kernels.

The bio-control approach involving the addition of non-toxigenic strains of *A. flavus* and *A. parasiticus* (Pitt 1989) should prove a valuable long-term management solution to aflatoxin contamination provided reliable systems of application can be developed. John Pitt (this workshop) covers this approach in more detail.

Genetic Options to Minimise Aflatoxin Contamination

Despite considerable research effort around the world (USA, India), widespread genetic resistance/tolerance to aflatoxin contamination has not been

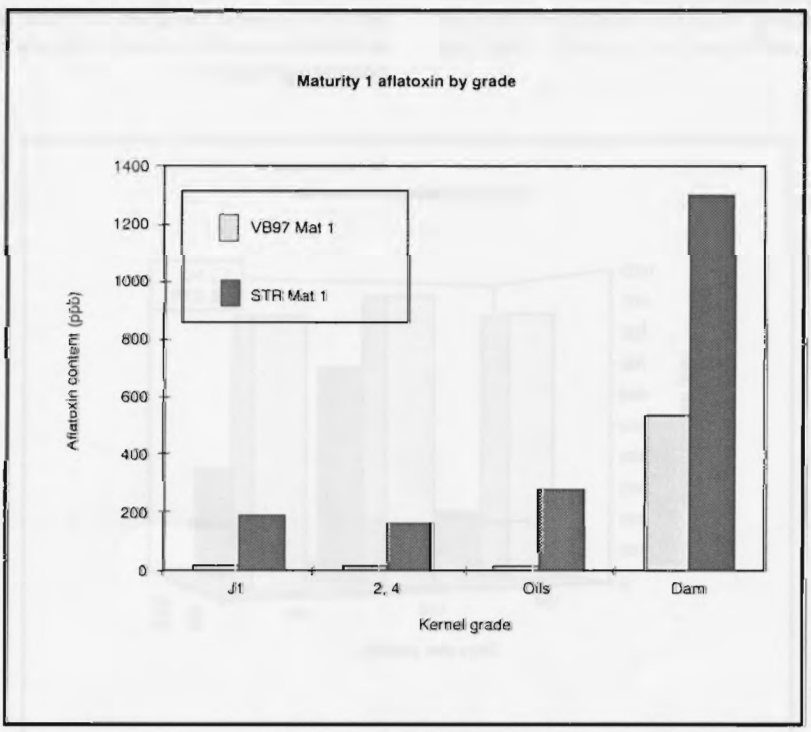

Figure 4. Aflatoxin concentration by grade for kernels collected from recent 'on-farm' trials for varieties VB-97 and Streeton.

identified (Anderson et al. 1995; Upaphyaya et al. 1997). This is not surprising considering the complex 'plant-soil-environment' interaction required for *A. flavus* growth and aflatoxin production. It is believed that with better physiological understanding of this complex, the search for resistance/tolerance traits suitable as selection criteria in breeding programs should be improved.

Plant *escape* and *tolerance* mechanisms can potentially be utilised to minimise aflatoxin contamination, using the physiological principles proposed in the kernel moisture model illustrated in Figure 1.

(a) Escape traits

Wright and Hansen (1997) proposed that earlier maturing varieties which can escape severe terminal drought stress may substantially reduce aflatoxin incidence in the Kingaroy peanut growing region. By fitting the peanut crop phenology to 'most probable' drought pattern, it was shown that in 70% of years that such varieties would have lowered aflatoxin levels. Early evidence suggests this approach may be successful, with a number of promising short maturing varieties currently being evaluated (e.g., Ruby, TAG 24) in multi-location trials across the Burnett region this summer. It is believed that a suite of variable maturity varieties should give dryland growers a powerful tool to 'spread' yield and aflatoxin risk.

(b) Resistance/tolerance traits

In Queensland, the newly released Streeton variety has been shown to have significantly lower aflatoxin incidence. Intake data at shellers' plants have, for example, shown that aflatoxin incidence can be up to 50% lower than other commercial varieties such as NC-7 and Florunner. Figure 5 also shows aflatoxin levels measured in recent 'on-farm' trials, where Streeton had consistently lower levels compared to NC-7.

Studies are currently underway at QDPI, Kingaroy, to understand the physiological basis of this tolerance. Early results indicate that two mechanisms are involved. Streeton, with better rooting and water uptake capacity from drying soils is able to maintain higher crop water status than other varieties. For example, in a recent study comparing crop water status under a severe terminal water stress, Streeton had canopy temperatures some 5–10 °C lower than NC-7 indicating superior rooting capacity (Wright, unpublished data). The other mechanism involves Streeton's capacity for rapid drying following cutting. Laboratory studies have clearly shown that following cutting, Streeton kernels dry down to a safe moisture content (i.e., Aw < 0.8) in quicker time compared to NC-7 (Figure 6). Further studies on the extent of genetic variation for these traits are aimed at defining suitable selection criteria for inclusion in breeding programs.

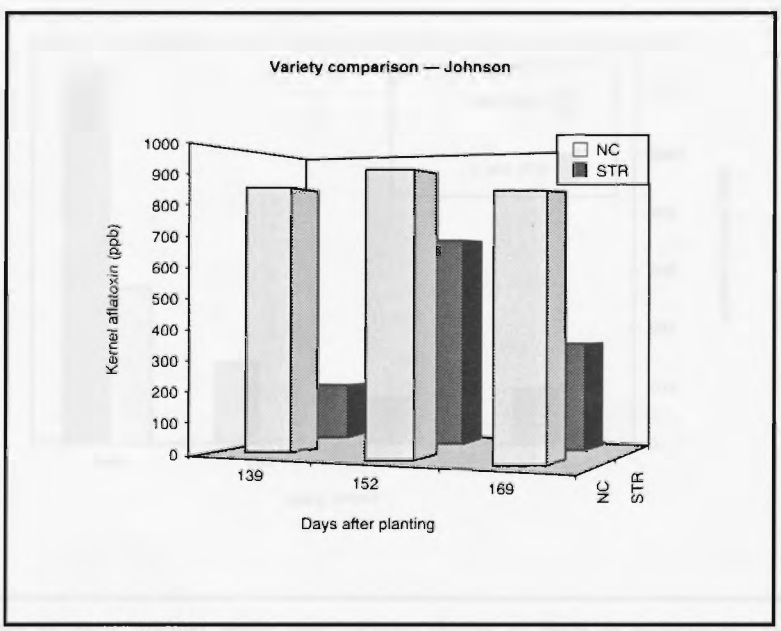

Figure 5. Comparison of aflatoxin levels in Streeton and NC-7 varieties cut at 139, 152 and 169 days after planting.

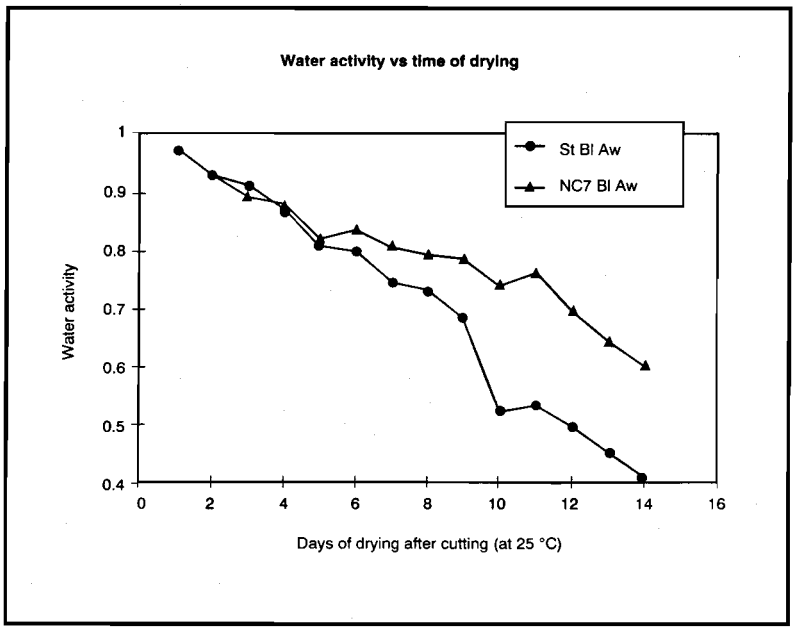

Figure 6. The change in kernel water activity over time after cutting, for Streeton and NC-7 varieties.

Simulation Modelling of Aflatoxin Contamination

Wright and Hansen (1997) have recently described a crop modelling approach to assist in defining aflatoxin risk on a regional basis and aflatoxin research priorities on a country/regional basis, with the use of long-term climate data in a probability analysis framework. Readers are referred to this paper for further details.

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Aflatoxin Toxicity and Reduction in Contaminated Commodities

W.L. Bryden[1]

THE TOXIC effects of aflatoxin are either acute or chronic, depending largely on the dose and duration of exposure. The liver is the organ most severely affected by aflatoxin and there are considerable epidemiological data that dietary aflatoxin B_1 is an important risk factor for human hepatocellular carcinoma. Exposure may also occur from inhalation of respirable grain dust, contaminated by aflatoxin. There is considerable species variation in aflatoxin susceptibility and this reflects differences in aflatoxin metabolism.

Metabolism of aflatoxin B_1 is mediated principally by hepatic and extrahepatic microsomal cytochromes P-450, although other conversions have been described. Most of the metabolic products are less toxic than aflatoxin B_1. Contamination of crops with aflatoxin B_1 is often unavoidable, even with best agricultural practices. Several strategies have been developed to reduce postharvest product contamination by aflatoxin B_1. Some of these involve identification and segregation of contaminated commodities. Ammoniation has shown considerable promise as a means of nearly completely eliminating aflatoxin B_1 from contaminated commodities.

Another successful strategy is the use of inorganic adsorbent feed additives, which prevent absorption of aflatoxin in animals. The development of ELISA from aflatoxin detection has greatly assisted in the monitoring of this mycotoxin in the food chain.

The aflatoxins are a group of secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The most toxic compound of this group is aflatoxin B_1 (AFB_1) which is also most commonly found in nature. Aflatoxins have been detected in a wide range of commodities destined for both human and animal consumption. The commodities most likely to be affected are peanuts, maize and cottonseed. Contamination of crops with AFB_1 is often unavoidable, even with best agricultural practices.

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This brief overview discusses the toxicity and metabolism of AFB_1, monitoring aflatoxin in the food chain and approaches to toxicity reduction.

Acute and Chronic Toxicity of Aflatoxin

The toxic effects of aflatoxin are both dose and duration of exposure dependent. Furthermore, species and breed of animal differ significantly both in susceptibility and manifestation of aflatoxicosis. These differences are directly related to the ability of individual animals to metabolise AFB_1. Acute aflatoxin intoxication is less likely to occur than chronic intoxication. In all species the principal target organ is the liver. Numerous liver functions are affected and the cumulative impact can be fatal.

Chronic aflatoxicosis can result when low levels of toxin are ingested over a prolonged period. In general affected livestock exhibit decreased growth rate, lowered productivity and immunosuppression. It is this latter aspect of chronic intoxication along with carginogenicity that are important in human populations exposed to AFB_1.

Impairment of the immune system by AFB_1 occurs through two primary effects. There is interference with the development of acquired immunity and this involves effects on cell-mediated immunity. A serious consequence can be the failure of vaccination programs. The second immunosuppressive effect is impairment of native resistance to infection. Decreased phagocytic activity of macrophages and circulating concentrations of nonspecific humoral substances such as complement and interferon have been demonstrated.

Aflatoxin is carcinogenic in several species including rats, ducks, mice, trout and subhuman primates. Dietary levels of AFB_1 as low as 15 ppb fed chronically to rats cause a high rate of liver tumours (hepatic carcinoma). The covalent binding of an AFB_1 metabolite (AFB_1-8, 9-oxide) to DNA provides the basis for altering gene expression which

results in the development of carcinomas. Numerous epidemiological studies link aflatoxin consumption to human liver cancer. Liver cancer rates increase with increasing levels of exposure to aflatoxin. The risk of liver cancer from aflatoxin is significantly higher in individuals who are hepatitis B carriers.

Aflatoxin Metabolism and Pharmacokinetics

Species differences in susceptibility to AFB_1 intoxication reflect variations in hepatic enzyme activity, and particularly cytochrome P_{450} activities. Aflatoxin is metabolised to an active epoxide (AFB_1-8, 9-epoxide). The epoxide metabolite can also be produced by tissue lipoxgenases. A major detoxification pathway is conjugation of AFB_1-8, 9-epoxide with glutathione. Of lesser importance is the conversion of the epoxide to a dihydrodiol. The hydroxylated metabolites such as AFM_1, AFQ_1 and AFP_1 form glucuronides and sulphate conjugates which are excreted.

In all species studied, AFB_1 has been shown to be distributed throughout the body with major concentrations occurring in the liver and kidneys. The milk toxin (aflatoxin M_1, AFM_1, a derivative of aflatoxin B_1) is invariably found in milk of lactating animals. Studies in both rats and chickens demonstrate that the toxin is rapidly excreted with a half-life of approximately 72 hours. Initially, urine is the major route of excretion in the rat reaching a peak during the first few hours after injection. Faecal elimination does not reach a peak for some hours after the urine elimination peak. The slower appearance of metabolites in faeces, reflects the passage rate of digesta through the gastrointestinal tract as maximum biliary excretion of aflatoxin occurs 20 minutes after injection.

Aflatoxin Surveillance

Surveillance of both domestic and imported commodities provides a way of reducing the occurrence of aflatoxin in the food chain by monitoring the effectiveness of aflatoxin control measures. In addition, monitoring human populations will determine level of exposure. These are costly exercises but provide essential data on which authorities determine regulatory limits.

Methods of aflatoxin analysis in feedstuffs fall into three categories: (1) presumptive tests that identify commodities that are probably contaminated, (2) rapid screening tests, designed to establish the presence or absence of aflatoxin, that are used to determine whether a lot should be accepted or rejected, and (3) quantitative methods that are used to determine the levels of aflatoxin in a commodity (Table 1). Presumptive tests are usually based on a visual inspection of the feedstuff—sometimes under a microscope or ultraviolet (UV) light. The characteristic bright greenish yellow fluorescence (BGYF) under UV light (365 nm) of cottonseed and corn infected with *Aspergillus flavus* is the basis of a presumptive test for aflatoxin. It should be remembered, however, BGYF is an indication of the presence of the fungus and not the toxin, so many false positive results can occur. A 'minicolumn' is used in rapid screening for aflatoxin.

Table 1. Analytical methods for aflatoxin analysis

Thin-layer chromatography (TLC)
Gas chromatography (GC)
High-performance liquid chromatography (HPLC)
Gas chromatographic-Mass spectrometric analysis (GC-MS)
Mass spectrometry—Mass spectrometry or Tandem Mass spectrometry (MS-MS)
Immunoassay

Of the methods presently available, immunoassays appear to offer the best opportunity for the development of rapid, repeatable and sensitive assays. These techniques are based on the highly specfic reaction between an antibody and antigen and require the development of an antibody against aflatoxin. Aflatoxins are non-antigenic but are able to illicit an antibody response after conjugation to a protein or polypeptide carrier. Enzyme-linked immunosorbent assays (ELISA) for the detection of aflatoxin in feedstuffs and residual toxin or metabolites in body fluids have been developed. The ELISA technique is adaptable to field use and commercial ELISA kits are available for aflatoxin.

Approaches to Reduction of Aflatoxin Contamination

Once aflatoxin has formed in a commodity, it is difficult to remove and its concentration should be reduced below that allowed by legislation. This can be achieved by physical removal (e.g., colour sorting, density segregation) or by dilution with uncontaminated commodities. Methods and strategies to reduce the concentration of aflatoxin contamination are listed in Table 2.

Table 2. Aflatoxin reduction strategies.

Food and feed processing	Chemical degradation	Biological reduction
Thermal inactivation	Ammonia	Competitive exclusion
Irradiation	Bisulphate	Microbial inactivation
Solvent extract	Catalytic degradation	Chemisorption
Mechanical separation	Hydrogen peroxide	
Density segregation	Ozone	
Adsorption	Alkali	
	Formaldehyde	

Complete degradation or removal of aflatoxin is difficult while retaining the nutritional and functional qualities of the treated commodity. Ammoniation is now used commercially to destroy aflatoxin present in peanuts, cottonseed and maize. Ammonia reacts with AFB_1 forming an ammonium salt, which is decarboxylated by heat to form numerous products including AFD_1. The compounds so formed are several orders of magnitude less toxic than AFB_1. Another strategy that is being used increasingly in the animal feed industry is the incorporation of non-nutritive adsorbent compounds into diets. The most promising is hydrated sodium calcium aluminosilicate (HSCAS), a sorbent compound obtained from natural zeolite. Aflatoxin and HSCAS form a stable complex that is not absorbed from the gastro-intestinal tract and the bioavailability of aflatoxins is reduced in a dose-dependent manner.

Other approaches that have not been developed commercially include the destruction of aflatoxin by microorganisms and the incorporation of microbial enzymes into contaminated commodities. Much of the technology is available for the latter approach as enzymes are routinely added to animal diets to remove the antinutritive effects of pentosans and phytate.

Whatever reduction strategies are used they should be designed to meet the following FAO criteria for decontamination processes:

- destroy, inactivate, or remove the mycotoxin;
- not produce or leave toxic or carcinogenic/mutagenic residues;
- retain the acceptability and nutritive value of the product;
- not significantly alter important technological properties;
- destroy fungal spores and mycelia which could grow and form new mycotoxins.

Conclusion

The problems associated with fungal growth and aflatoxin production in agricultural commodities can have serious economic consequences. However, recent developments in mycotoxin analysis and contamination reductions provide an opportunity to minimise these problems provided that adequate research is conducted into their refinement and application.

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Controlling Aflatoxins in Peanuts by Competitive Exclusion of Toxigenic Fungi

J.I. Pitt[1]

DURING the past 20 years, a number of approaches have been advocated and tested for reduction of aflatoxins in peanuts: resistant cultivars, thickened shells, waxy testa, improved farm management techniques and postharvest procedures involving drying and storage. Some of these approaches have merit, but despite the expenditure of large sums of money excessive aflatoxin in peanuts remains a serious commercial problem. Other approaches are still needed.

One of the more promising is the concept of biocontrol by competitive exclusion. This involves the use of competitive fungi to reduce the possibility of toxigenic fungi present in the soil entering developing peanuts and then producing aflatoxins in them.

Aflatoxins are produced by the common fungi *Aspergillus flavus* and *A. parasiticus* when they grow in suitable substrates, including peanuts. These fungi are present in high numbers in cultivated soils. *A. flavus* (and *A. parasiticus*) are able to grow as commensals in developing peanut plants (Pitt et al. 1991) and from there can invade developing peanuts.

Entry to the peanut plant may be from roots, flowers, leaves damaged by insects, and from air or dust. The most important point of entry to the developing peanut, however, is directly from the soil surrounding it. So the point of application of any biocontrol measure such as competitive exclusion should be in the soil in peanut growing fields.

Although it is possible to conceive of a number of different genera and species of fungi which could be used as biocompetitors in this system, strains of *A. flavus* and *A. parasiticus* which do not produce aflatoxins were used. This appears to be the least complex, most readily understood and readily controlled approach. Even so, the factors involved make this a remarkably complex process.

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First, it is necessary to have strains of these fungi which are known not to produce aflatoxins. Although many isolates of *A. flavus* from nature are nonproducers, nontoxigenic isolates of *A. parasiticus* are very rare.

Second, it is preferable that isolates used have genetic defects to positively prevent reversion to aflatoxin production.

Third, it is important to know whether control requires the use of both *A. flavus* and *A. parasiticus*, or whether use of one or the other will be effective. Then the strains must be competitive in the open field. They must be capable of multiplication in soil and preferably persist, so that addition to the soil need not be made each year. It is desirable, though not essential, that such strains be readily recognisable in culture, to permit easy tracking in the field.

The method of application requires great care. Because *A. flavus* is a weak human pathogen, it is not considered to be acceptable practice to add the fungus to fields or plants by spraying or dusting. Hence, the fungus must be added directly to the soil in an environmentally acceptable manner. It must be safe to use by farmers, on the farm, using readily adapted farm machinery. The inoculum to be added to soil must be stable for at least several months. The process used must be capable of scale-up to commercial peanut farms and be economically feasible. In addition, the competitive fungi must not affect the peanut seed at planting nor the plant during growth.

Further questions relate to distribution and numbers of the introduced fungi in the soil. What levels of nontoxigenic strains, in relation to the toxigenic strains already present, will provide effective competition? Will the introduced nontoxigenic strains compete only for a single season, or for long periods? Where in the field should the nontoxigenic strains be introduced? Is it sufficient to add them around the root base, or must they cover the whole field?

Over the past few years, a great deal of work has been carried out at Food Science Australia in North

Ryde in collaboration with Peanut Company of Australia, and recently with Queensland Department of Primary Industries, in Kingaroy. A dedicated glasshouse at North Ryde has been used for small-scale experiments, with peanuts being grown to maturity in large garbage bins under controlled temperature and moisture. Soil used for growing the peanut plants has been brought from Kingaroy, and contains controlled numbers of added fungi, both toxigenic and nontoxigenic.

Nontoxigenic strains of *A. flavus* and *A. parasiticus* isolated from throughout the South Burnet region were initially screened for toxicity by growth on coconut cream agar (Dyer and McCammon 1994), then by sensitive HPLC systems. Some promising strains were examined biochemically (by Dr T.E. Cleveland, USDA, New Orleans) or by molecular techniques (by Dr D. Carter, University of Sydney) to find those least likely to revert to toxicity. At first, *A. flavus* cultures mutated with UV light to produce coloured spores were used, as these mutants are readily tracked and monitored, a big advantage under field conditions.

Promising isolates and colour mutants were screened initially in the glasshouse, by assessing growth under competitive conditions in young peanut plants, which was found to be a very useful screening system requiring only a few weeks (Pitt et al. 1991). It was found that mutants were generally noncompetitive, so more recent work has used only naturally occurring nontoxigenic isolates. Glasshouse trials were also extended to growth of peanut plants through the whole life cycle, assessing the ability of nontoxigenic strains to invade developing peanuts under controlled conditions.

The influence of parameters such as drought stress on invasion was also investigated. The glasshouse studies established that the best nontoxigenic strains competed equally with toxigenics, neither type enjoying a competitive advantage. It was concluded that use of nontoxigenic strains under field conditions is strictly a numbers game: to be effective, high numbers of nontoxigenic spores, perhaps as many as 100 times the numbers of toxigenic spores already present, need to be introduced into the field.

To accommodate all of those factors, we chose to introduce nontoxigenic *A. flavus* and *A. parasiticus* into soil on millet or other small seeds, as small seed provides the highest number of particles per tonne, an important economic consideration. Conditions used ensure that growth of the fungus in the millet seed is substantial, to provide for effectiveness and stability, but where sporulation is inhibited, to minimise the possible hazard to handlers. Commercial scale preparation has been carried out at Peanut Company of Australia, using a coffee roaster to sterilise the seed and a commercial cement mixer, with intermittent rotation, as a growth chamber. Two tonne batches suitable for commercial trials have been produced in this manner.

The use of millet or similar seed as inoculum has a very large additional benefit. Experiment has shown that once the millet seed is added to moist soil, the fungus grows in the seed, and within two weeks produces large number of spores, providing a multiplier factor of 100 to 1000 fold over the number of added seeds.

In early field trials, millet seed was sown in the fertiliser drill of a peanut planter, providing a high inoculum around the plant base. However, this technique, effective on the small scale, was not effective in field trials. So this year the more difficult approach of adding nontoxigenic strains throughout the field before planting is being investigated. This latter technique requires use of higher levels of inoculated millet seed per hectare. However, the even distribution throughout the field which results has the advantage that the nontoxigenic strains are likely to be effective for more than a single season, which would be a real bonus.

United States scientists have recently shown very promising results in small-scale trials of competitive exclusion (Dorner et al. 1998). It is believed that competitive exclusion is a feasible technique for reducing aflatoxin in the Australian peanut industry, within a short time frame.

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Aflatoxin Research in Indonesia

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THE HUMID tropical conditions of Indonesia and its agricultural practices favour rapid growth and widespread infestation of crop plants by pathogens, including *Aspergillus flavus*, the aflatoxin-producing fungus. The danger of aflatoxin to human and animal health has been known in Indonesia, but special attention was initiated only in 1969. A research program that has been completed in the first phase of the study, 1969–1974 included three aspects, (1) a survey of *A. flavus* invasion and aflatoxin contamination in peanut, (2) studies on prevention of aflatoxin production in peanut, and (3) assessments of relationship between aflatoxin content in the diets and human hepatic cancer. Since then, aflatoxin research has been done in other crops such as rice, maize, soybean, spices, and medicinal plants. Peanut samples collected from the field, farmers' storage, collectors, distributors and markets were invaded by *A. flavus* ranging from 60–80%, with aflatoxin contents 40–4100 μg/kg. Postharvest handling also affected invasion by *A. flavus* in freshly harvested peanut. Seed moisture is highly correlated with *A. flavus* population and aflatoxin contents. Eighty percents of peanut seed samples with moisture contents ranging from 3.6–11.0% contained >30 ppb of aflatoxin B_1. Peanuts and peanut products such as fried peanut, roasted peanut, peanut flour, and peanut paste sampled from markets, stores and food manufacturers also contained aflatoxin from trace amounts to more than 1000 μg/kg.

A study on control of aflatoxin in peanut showed harvest time, drying and storage affected aflatoxin contamination in variety Gajah. Aflatoxin was first detected in home stored, good quality peanut seeds 10 weeks after storage, and the level increased rapidly to over 500 μg/kg due to increase in seed humidity from 8% to 11.4%. The aflatoxin contained in peanut were aflatoxin B_1 and G_1.

Different fungal pathogens were isolated from stored maize kernels including A. *candidus*, *A. flavus*, *A. niger*, *A. penicillioide*, *A. tamarii*, *A. versicolor*, and *A. wentii*. *A. flavus* invasion and aflatoxin contamination in maize could occur while it was in the field. Aflatoxin B_1 was detected from maize kernels at levels from 5.3–291.4 ppb. The fungal population and aflatoxin content in stored maize grains were increasing with storage periods. The fungal population and aflatoxin contents were not affected by fumigation of maize seeds. Freshly harvested kernels collected from farmers, from village collectors, middleman and distributors in Lampung province were invaded by *A. flavus* and contained aflatoxin from 5.3–291 ppb. Maize and chicken feed from manufacturers and maize products from manufacturers and supermarkets which usually have moisture contents between 12.5–14.5% were invaded by *A. flavus* and contained aflatoxin B_1 from 0–100 ppb.

A. candidus, A. flavus, A. niger, A. versicolor were reported as the dominant fungi on black and white pepper. The *A. flavus* population on black pepper was lower than that on white pepper. No aflatoxin, however, was detected from either samples of black or white pepper. Aflatoxin was also detected from animal feed herbs and traditional medicines (*jamu*). These materials contained aflatoxin B_1 ranging from 8 to 1190 μg/kg and aflatoxin G_1 from 4 to 690 μg/kg. Aflatoxin was also detected in forage crops.

A. candidus, A. flavus, A. niger, A. penicilloides, A. sydowii, A. tamarii, and *A. wentii* were isolated from stored phosphine-fumigated soybean meal. *A. candidus* and *A. flavus* were most frequently found. Aflatoxin content in the samples ranged from 8–34 ppb.

The association of aflatoxin with human and animal diseases has been known for a long time. The incidence of liver cancer in humans and animals in Indonesia is high. Among 81 patients surveyed including 66 males and 15 females ranging in age from 7 to 75 years, 80 were suffering from primary

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liver cancer. Aflatoxin was detected in 58% of the patients suffering from liver cancer. Aflatoxin B_1, G_1, and M_1 were detected in liver specimens at levels from trace to over 400 µg/kg. The food commonly consumed by the contaminated patients were peanut or soybean cake (*oncom* and *tempe*), fried peanut, peanut sauce, soy sauce, and salty fish.

Carbon dioxyde (CO_2) at a rate of 2 kg/ton of stored maize kernel did not reduce the population of *A. flavus* and other fungi, but lowered aflatoxin content in the sample. CO_2 treatments at concentrations from 20–80% affected some biological aspects of *A. flavus* including mycelial growth, fungal sporulation, spore germination in vitro, and aflatoxin production. Phosphine, a chemical commonly used for insect control of stored grain, affected growth and sporulation of *A. flavus* as well as spore germination and aflatoxin contents. Detoxification of aflatoxin from maize kernel using ammonium hydroxide was the best when its concentration was 1.5% and the incubation period 36 hours. Plastic bags were better suited to store maize kernels than jute bags.

Proposed future research on peanut aflatoxin in Indonesia as it relates to the mandate of the Research Institute for Food Crops Biotechnology:

1. Survey for updating information on *A. flavus* distribution and aflatoxin contamination in peanut in farmers' crops in the field, on peanut seeds and peanut products at farmers', collectors, distributors and retailers' level.
2. Collection of *A. flavus* from different crops and localities. Strain identification based on their virulence to peanut cultivars and the ability to produce aflatoxin.
3. Effect of *A. flavus* and aflatoxin on chemical composition of peanut seed and peanut products.
4. Control of *A. falvus* invasion and aflatoxin contamination at pre- and postharvest, using physical, biological and chemical means, detoxification of aflatoxin in peanuts and peanut products, and the development and evaluation of a rapid and sensitive test kit for aflatoxin in legumes and cereals.

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AFLATOXIN RESEARCH IN INDIA

Research on Aflatoxin at ICRISAT

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A GROUNDNUT Improvement Program was initiated in 1976 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Center in India, with special emphasis on tackling the problem of aflatoxin contamination in the peanut crop, although vulnerability of sorghum, another of the four ICRISAT mandate crops, to aflatoxin contamination was recognised at that time.

In most of the semi-arid tropic (SAT) countries, where groundnut is a cash export crop, there has been a tendency to concentrate efforts on ensuring the acceptability of the commodity to the importing country, while little attention has been paid to aflatoxin contamination in produce for local consumption. While it is appreciated that loss of export income is of great importance, it is also appreciated that it is very difficult for many groundnut-producing countries in the semi-arid tropics to meet the stringent regulations currently imposed by importing countries.

A decade of ICRISAT's research has been summarised in an international workshop on 'Aflatoxin Contamination in Groundnut' (ICRISAT 1989). In addition to this workshop proceedings, there are two aflatoxin-related publications produced by ICRISAT, i.e., a global review and literature database (Mehan et al. 1992) on the groundnut aflatoxin problem, and a summary proceedings of the First Asia Working Group Meeting facilitated by ICRISAT during 27–29 May, 1996 at Hanoi, Vietnam (Mehan and Gowda 1997). The Asia working group involved participation from Bangladesh, China, India, Malaysia, Philippines, Thailand and Vietnam. A similar working group has been initiated in 1997 by ICRISAT in West Africa involving Mali, Ghana, Nigeria and Senegal.

ICRISAT's approach to aflatoxin research has been focussed mainly on identification and utilisation of genetic resistance to seed invasion by *Aspergillus flavus* and toxin production rather than pre- and postharvest management practices to reduce aflatoxin. The agronomic management of aflatoxin was considered to be the responsibility of national programs. Thus, research on genetic resistance alone may seem to be a somewhat limited approach to tackling a problem such as aflatoxin. However, ICRISAT feels that seed-based technologies (rather than more expensive and local specific agronomic management) would have a wider impact on smallholder SAT farmers. Further, ICRISAT would also have a comparative advantage in concentrating on genetic resistance, particularly in view of ICRISAT's access to the world collection of groundnut and wild *Arachis* germplasm.

Although the project has been revised a number of times since its inception, the research has centred on the following major objectives:

- development of rapid methods to quantify aflatoxin contamination in groundnut;
- identification of sources of resistance to seed invasion by *A. flavus* and aflatoxin production;
- understanding factors influencing pod and seed invasion by *A. flavus*, especially preharvest invasion and aflatoxin production; and
- genetic enhancement for aflatoxin resistance.

Progress

Since significant infection of intact pods by *A. flavus* and production of aflatoxin is known to occur in the field, identification and possible use of genetic resistance to invasion of seeds by the fungi has been given a high priority. In the absence of rapid and economical tools to quantify aflatoxin, the extent of seed infection by *A. flavus* has been used as an index to possible resistance to aflatoxin production. A large number of genotypes have been screened in laboratory and field to identify resistance to seed invasion and colonisation by *A. flavus*.

Resistance to Seed Invasion and Colonisation by *Aspergillus flavus*

The genotypic resistance to in vitro seed colonisation by *A. flavus* has been assessed by surface inoculation

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of seed by conidial suspension of an aflatoxigenic strain of *A. flavus*. A number of germplasm lines were screened in which the percentage of seed colonisation among the genotypes ranged from 6%–100% representing a large genotypic variation. Genotypes (PI 337394F, PI 337409, UF 71513, Ah 7223, J 11, U 4-47.7, Var 27, Faizpur and Monir 240-30) with <15% seeds colonised were identified as resistant (Mehan and McDonald 1980). Three genotypes resistant to seed colonisation by *A. flavus* (C55-437, 73-30 and 73-33) were reported from ICRISAT Center, Niger, West Africa (Waliyar and Bockelee-Morvan 1989).

Resistance to *A. flavus* Seed Infection in the Field

The association between drought intensity and infection by *A. flavus* in the field has been established (Mehan et al. 1988) and a number of germplasm and advanced breeding lines were screened for *A. flavus* infection under simulated drought conditions in the field. Stability of resistance has been assessed in multilocational studies, and only genotype J11 was found to show stable resistance to *A. flavus* infection.

Sensitivity of drought-tolerant genotypes to *A. flavus* infection in the field has been examined in a limited number of genotypes. Most Valencia market types found tolerant to end-of-season drought were susceptible to infection by *A. flavus*. It was interesting to note that C-55-437, a drought-tolerant Spanish cultivar, showed tolerance to seed infection by *A. flavus*. It is possible that the weak pod shell structure of Valencia market types might be a contributing factor for susceptibility of drought-tolerant genotypes to seed infection. Limited information is available on the role of pod wall structural characteristics in resisting *A. flavus* infection.

In the studies on in vitro colonisation by *A. flavus*, the protective role of seed testa has been implicated with genetic resistance. However, it was also realised that the testa-related resistance will be of little value in the case of decorticated seed, or in the case where the testa is damaged. In studies relating to field tolerance to invasion by the fungus, a possible role of the pod exudates in inhibiting the fungus development in the pod zone has also been suggested. A significant genotypic difference in the effect of pod exudates on growth and germination of *A. flavus* has been reported (Nahdi 1989).

Resistance to Aflatoxin Production

Genotypic variability for resistance to aflatoxin production has been examined by assessing aflatoxin B_1 production following inoculation of sound mature kernels with *A. flavus* conidial suspension. About 500 germplasm lines were screened and two genotypes (U 4-7-5 and VRR 245) were found to support low levels of aflatoxin (Mehan 1989). About 16 wild *Arachis* species were screened for aflatoxin production but none were found to be resistant to the toxin production. It was interesting to note that the genotypes, which showed resistance to in vitro colonisation by *A. flavus* supported aflatoxin production when seeds were inoculated with *A. flavus*. No correlation was observed between fungal growth and aflatoxin production.

Genetic Enhancement for *A. flavus* Resistance

Because of the lack of easy, rapid and economical selection tools for either *A. flavus* resistance or aflatoxin production, the genetic enhancement efforts for aflatoxin resistance in groundnut have been concentrated on incorporating pod, seed-coat and cotyledonary resistance to *A. flavus* into high yielding and agronomically desirable material.

The resistance sources identified at ICRISAT, and elsewhere (viz., J11, PI 337394F, PI 337409, UF71513, Ah 32, Faizpur 1-5) were used in the breeding program (Rao et al. 1989). The selection of progenies was based on both yield and seed resistance to invasion by *A. flavus* in the field. The selected lines were evaluated for both yield and *A. flavus* resistance in replicated multilocation tests. The G × E analysis for resistance to *A. flavus* indicated some scope for stable resistance across environments.

Recently, two lines (ICGV 88145 and 89104) have been released as improved germplasm with tolerance to *A. flavus* infection in the field (Rao et al. 1995). Promising breeding populations with *A. flavus* tolerance are being made available to National Agricultural Research Systems in Asia and Africa by collaborative international links. Some lines have shown superior performance for yield and *A. flavus* infection. For example, ICGVs 91278, 91279, 91283 and 91284 have shown superior performance in Thailand and Vietnam. ICGVs 87084, 87094 and 87110 have shown superior performance and *A. flavus* resistance in West and Central African countries (Waliyar et al. 1994). However, the ability of these genotypes to resist aflatoxin production under end-of season drought conditions has yet to be established.

Aflatoxin Detection Technologies

During 1997–98, significant progress was made in the development of rapid, accurate and cost-effective

techniques to detect aflatoxin, using enzyme-linked immunosorbent assay methods (Reddy et al. these Proceedings). Availability of rapid detection technologies has enabled ICRISAT scientists to re-visit the aflatoxin problem with more confidence.

Current aflatoxin research at ICRISAT is focussed on conducting comprehensive surveys for aflatoxin contamination of groundnut produce in major groundnut-based production systems in India, and to understand the physiological basis for genotypic variation and G × E interaction for *A. flavus* resistance, aflatoxin production using selected genotypes.

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Current Research on Aflatoxin Detection and Genetic Transformation in Peanut at ICRISAT

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GROUNDNUT or peanut (*Arachis hypogaea*) is one of ICRISAT's mandate crops. It is one of the most common food crops in which aflatoxin contamination occurs. Groundnut is grown under different environments that often favour *Aspergillus flavus* infection and aflatoxin contamination. In addition, postharvest conditions are also conducive for aflatoxin production. Research at ICRISAT is focussed on developing cost-effective tools for aflatoxin estimation and sustainable methods to reduce aflatoxin contamination under pre- as well as postharvest conditions.

Diagnostic Tools

High titered polyclonal as well as monoclonal antibodies have been produced for aflatoxin B_1-BSA conjugate. The monoclonal antibodies selected so far have shown differing specificities, recognising either B_1 alone, or B_1 and G_1 or B_1, B_2 and G_1. These clones will be used for generation of recombinant antibodies for use in the development of inexpensive ELISA-based tests for quantitative aflatoxin estimation.

Genetic Resistance

The genotype × drought interaction for seed infection by *A. flavus* has been conclusively shown in field experiments. The results from these experiments have been adequately described by Nageswara Rao et al. in these Technical Reports.

Genotypes J-11, UF 71513, PI 337409 and U4-7-5 have earlier been shown to have resistance to seed colonisation as well as aflatoxin production. These have been used to develop several advanced breeding lines with a combination of resistance to *A. flavus* infection and high yield. These include ICGVs 88145, 89104, 91278, 91283 and 91284. Efforts are currently focussed on the development of genotypes which can resist either *A. flavus* invasion or aflatoxin production or preferably both. Future strategies aim to explore additional sources of resistance to aflatoxin and to examine the basis for genotypic variation for resistance to aflatoxin production in order to pyramid the genes for resistance.

Arachis cardenasii has been shown to possess resistance to aflatoxin contamination. Currently, 13 accessions of *A. cardenasii* are being tested for aflatoxin contamination under natural conditions. The majority of the lines did not show any contamination in initial tests. They are being multiplied and will be tested under laboratory and field conditions. Depending on the results, interspecific derivatives will be generated.

Genetic Transformation in Peanut

Efficient protocols for the regeneration and genetic transformation of *A. hypogaea* have been developed. Various morphogenic responses in diverse explants from mature seeds of several genotypes have been optimised. These include shoot regeneration from cotyledon and leaflet explants and somatic embryogenesis from leaflet and embryo axis explants of mature seeds. Shoot regeneration and somatic embryogenesis in 90% to 100% of the explants can be obtained. Among these systems, de-embryonated cotyledons of pre-imbibed seeds and leaflets from 1 to 3 day-old seedlings have been successfully utilised in *A. tumefaciens*-mediated transformation.

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Efficient transfer of marker genes and the coat protein gene of Indian peanut clump virus-Hyderabad isolate (IPCV-H cp) carried in binary vectors have been achieved with frequencies up to 45% of the recovered plants. The IPCV-cp-containing transgenic plants are in their third generation and are currently being tested for the stability of genes and gene expression. Putative transformants from the first sexual generation have been characterised. Based on in vitro regeneration on selection, PCR amplification of the introduced genes, and Southern blot hybridisation, it was apparent that stable transformants were obtained.

Work on confirmation by northern and western blot hybridisations is in progress. Field evaluation of selected transformed groundnuts will be undertaken this year. Efforts to incorporate the coat protein gene of groundnut rosette assistor virus (GRAV) in groundnut cultivars are under way. The authors are also interested in regulating the expression of introduced genes in a tissue-specific manner. This would eventually be useful in proposed projects on incorporation of genes to prevent *A. flavus* invasion and aflatoxin production.

GENETIC TRANSFORMATION OF PEANUT

Direct Gene Transfer and Regeneration of Peanut

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PEANUT (*Arachis hypogaea* L.) has a narrow germplasm base which lacks resistance genes to protect it from many economically important diseases, especially those caused by viruses and fungi. Peanut stripe virus (PStV) has been identified as the most important viral pathogen infecting peanuts in Asia. Among fungal infections, leaf spot diseases and aflatoxin-producing strains of *Aspergillus flavus* and *A. parasiticus* are of particular concern.

Current commercial peanut cultivars are allotetraploids, apparently derived from a single hybridisation event between diploid parents *A. duranensis* and *A. ipaensis* (Kochert 1996).

Introgression of desirable traits, if available, from wild species is often difficult due to genetic incompatibility. Thus, introduction of beneficial resistance traits from outside the compatible germplasm using genetic engineering techniques directly into commercial cultivars would allow the development of superior disease-resistant lines. However, peanuts have proved recalcitrant to genetic transformation. There have been few confirmed transgenic peanut plants following microprojectile or *Agrobacterium*-mediated gene transfer and reported transformation methods are often limited by low efficiency, cultivar specificity, chimeric or infertile transformants, or availability of explants. An overview of peanut tissue culture and transformation was recently presented by Higgins et al. (1997).

Built on the work of Ozias-Akins et al. (1993), we have developed a practical and efficient gene transfer and plant regeneration system for peanut by particle bombardment into embryogenic callus derived from mature, dry seeds (Livingstone and Birch 1999). This transformation system has been successfully applied to cultivars of Spanish (cv. Gajah) as well as Virginia (cv. NC-7) market types and fertile, non-chimeric plants were obtained. We have expressed in transgenic peanuts a firefly luciferase reporter gene (Livingstone and Birch 1999) and different modified versions of the PStV capsid protein to provide virus resistance (C.M. Higgins et al., unpublished). The transformation system is suitable for the efficient transfer into commercial peanut cultivars of other agronomically important traits such as genes to eliminate aflatoxin contamination.

This peanut transformation system takes a minimum of 12 months to complete from initiation of explants to transformed plants in the glasshouse. Mature peanut seeds are surface sterilised and embryo axes excised, disinfected and the radicle removed. Embryos are then placed on medium containing picloram to initiate and proliferate embryogenic callus (8–12 weeks). DNA of two plasmids, one allowing selection of transformed callus using the antibiotic hygromycin and the other carrying the desired gene, are co-precipitated onto tungsten particles. Embryogenic callus is bombarded with DNA-coated particles which are accelerated by a helium pulse in a vacuum chamber. Callus is placed on an osmoticum prior to and after bombardment. The co-transformation rate of the two plasmids is about 90%. Transformed peanut embryos are selected escape-free (min. 12 weeks) on media containing picloram and 20 mg/L of hygromycin B. Between 1–10 independent transformants (average 3) can be obtained per bombardment of 10 cm^2 of embryogenic callus.

Regular subculture and selection of the 'right' callus type are important at this stage. Proliferated transformed embryos can be tested by PCR to confirm the presence of the introduced gene(s). Shoots are initiated on medium containing activated charcoal, shoots proliferated and roots initiated by cytokinin treatment. Plantlets are grown on hormone-free medium until they reach a size suitable for transplantation into soil and acclimatisation to glasshouse conditions.

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Comparison of *Agrobacterium*-Mediated and Particle Inflow Gun Mediated Transformation of Peanut

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DEVELOPMENT of genetic transformation protocols for some legume crops is the main activity in the Department of Agronomy, Bogor Agricultural University (IPB). In line with this activity, we have developed efficient protocols for inducing somatic embryos from embryo axis (Edi 1997) and embryonic leaflets (Sulichantini 1997).

This paper summarises preliminary results of experiments to develop *Agrobacterium*-mediated transformation and subsequent regeneration of peanut via induction of somatic embryos. The objectives of our experiments were (1) to determine factors affecting the effectiveness of *Agrobacterium*-mediated gene transfer and (2) to compare the effectiveness of established *Agrobacterium*-mediated and Particle Inflow Gun mediated transformation of peanut.

Materials and Methods

Mature peanut seeds were surface sterilised and the embryo axis dissected. Subsequently, the embryonic leaflets, the axis without radicle, and/or axis without leaflets or radicle were used as explants for the transformation and for regenerating somatic embryos. To induce somatic embryos, explants were cultured on MS medium containing 16 μM picloram and subcultured onto fresh medium every two weeks until somatic embryos formed.

Agrobacterium tumefaciens LBA4404 carrying pTOK233 (hygromycin resistance, ß-glucuronidase (GUS) marker) or pSAQ (hygromycin resistance, green flourescent protein (GFP) marker) were co-cultivated with peanut explants at 25 °C for 2 days in the dark (Newton 1997). Selection of transformed tissue and removal of *Agrobacterium* was on medium containing 300 mg/L cefotaxime and 20 mg/L hygromycin. Peanut tissue was bombarded using the protocols developed by Livingstone and Birch (1995, 1999) and transformed tissue celected on 20 mg/L hygromycin.

Putative transformed embryos were analysed by PCR of their extracted DNA or by 'tissue soak PCR' (Thomson and Dietzgen 1995) using the appropriate primers to detect the presence of *hpt* (codes for hygromycin resistance), *uid*A (codes for GUS), or *mgfp*5-ER (codes for GFP) genes.

Results and Discussion

All peanut explants tested (embryonic leaflet, embryo axis without radicle, or embryo axis without radicle or leaflet) required long term pre-culture prior to co-cultivation with *Agrobacterium* to yield transformed embryos. Immediate *Agrobacterium* transformation of peanut tissues or pre-culture for 6 or 12 days did not yield any embryos on hygromycin selection.

Following 7 weeks of culture prior to *Agrobacterium* co-cultivation and after 10 weeks on selection, 21 hygromycin-resistant somatic embryos regenerated from a total of approximately 1900 initial explants (Table 1).

Table 1. Effect of protocols for regenerating somatic embryos and long term pre-culture treatment on effectiveness of *Agrobacterium*-mediated transformation of peanut with binary plasmid pTOK233.

Regeneration strategies	Type of explant	# of initial explants	# of hyg^R embryo
Protocol I	EA-R	750	7
Protocol II	EA-RL	550	7
Protocol III	Embryonic leaflet	605	7

Note: Protocol I: Livingstone & Birch, 1995. Protocol II: Edi, 1997. Protocol III: Sulichantini, 1997.

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Table 2. Comparative effectiveness of *Agrobacterium*-mediated and Particle Inflow Gun mediated transformation of peanut.

Transformation strategies	# of initial explants	# of hyg^R embryos	# of PCR (+) for *hpt* gene	# of PCR (+) for *mgfp5-ER* gene
Particle inflow gun	1200	54 (4.5%)	19 out of 20	19 out of 20
Agrobacterium	1000	18 (1.8%)	12 out of 18	13 out of 18

The effectiveness of *Agrobacterium*- and particle inflow gun mediated transformation to give rise to hygromycin-resistant somatic embryos was evaluated. In both transformation strategies, embryogenic callus cultures derived from embryo axis of peanut were used as target tissues. Particle bombardment yielded 54 hygromycin-resistant somatic embryos from a total of 1200 initial explants. *Agrobacterium* treated explants yielded 18 hygromycin-resistant somatic embryos out of 1000 initial explants (Table 2). The DNA extracted from regenerated somatic embryos was subjected to PCR to determine the presence of the marker genes.

Particle bombardment appeared to be more efficient because 95% of tested embryos carried both the *hpt* and *mgfp* genes, whereas this was only the case in ~70% of embryos after *Agrobacterium*-mediated gene transfer. Furthermore, particle bombardment resulted in larger numbers of embryos on selection. However, the experiment demonstrated that embryogenic callus can serve as target for *Agrobacterium*-mediated gene transfer and give rise to transformed embryos. An efficient regeneration system for embryos already exists (Livingstone and Birch 1999). More research will need to be done to increase the effectiveness of *Agrobacterium*-mediated transformation in peanut.

Conclusions

Although further optimisation is still required, our experiments indicated that *A. tumefaciens* can be used to transfer genes into peanut tissues capable of regeneration. Since this approach is more suitable to the research conditions in Indonesia, optimising the protocol for *Agrobacterium*-mediated transformation of peanut will be a priority at the Institut Pertanian Bogor.

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POTENTIAL GENES FOR AFLATOXIN CONTROL

Plant Antimicrobial Peptides and Their Potential for the Control of Aflatoxin in Peanuts

J.M. Manners[1]

MOST organisms produce proteins which inhibit the growth of pathogenic microbes in vitro. These proteins are believed to play an important role in host defence processes. In many instances defensive proteins that inhibit microbial growth contain less than 100 amino acid residues and are often referred to as antimicrobial peptides (AMPs). Antimicrobial peptides have been isolated from a wide range of sources including animal, plant and microbes (Broekaert et al 1997; Hancock 1997). They have been proposed to represent a wealth of new antibiotic molecules that might find application in the agricultural, veterinary and pharmaceutical industries (Boman and Broekaert 1998).

In plant agriculture, the interest in AMPs is mainly in their potential for the design of new fungicides and in the possibility that AMP-encoding transgenes might provide enhanced disease resistance in transgenic crop plants (Broekaert et al. 1997). The latter application is of particular interest where there is little or no natural resistance in the gene-pool for the development of disease resistant varieties using conventional breeding.

Antimicrobial peptides appear to play an important role in plant disease resistance as described in Figure 1. In plants, AMPs are produced constitutively in some tissues (Terras et al. 1995) while in other tissues the production of AMPs can be induced after pathogen attack (Penninickx et al. 1996; Manners et al. 1998). Many of the established classes of plant AMPs have been initially isolated from seeds (Marcus et al. 1997; Broekaert et al. 1997). Presumably, seeds contain high amounts of antimicrobial peptides because the imbibition and germination stages are usually carried out in microbially rich soil environments and represent a particularly vulnerable stage of plant development. Evidence for the active role of the antimicrobial peptide class of plant defensins in forming a defensive barrier around germinating seeds has been reported in radish (Terras et al. 1995). Obviously, fungi such as *Aspergillus flavus*, that successfully colonise plant seeds may have resistance to seed defence processes and this is discussed later in relation to the utility of using AMP genes for the control of aflatoxin production in peanuts.

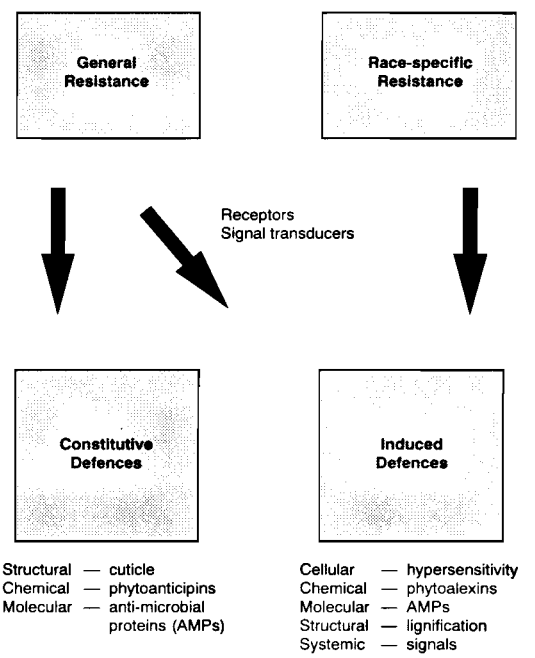

Figure 1. Illustration of the role that AMPs play in both constitutive and induced defences in general and race-specific plant resistance to microbial pathogens. It is evident that AMPs are one component of a range of cellular, chemical, structural and molecular defences employed by plants to resist bacterial and fungal pathogens. Examples of these types of defences are listed.

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In Australia in recent years, there has been an increased incidence of diseases caused by necrotrophic fungal pathogens attacking important grain and oilseed crop plants. In general, this has been associated with an increased diversity of crops used in cereal cropping rotations together with favourable climatic conditions for epidemics. These necrotrophic pathogens include *Aschochyta rabiei* on chickpeas, *Colletotrichum gloeosporioides* on lupins, *Mycosphaerella pinodes* on field peas, *Alternaria helianthi* on sunflowers, *Leptosphaeria maculans* on canola, *Aschochyta lentis* on lentils and in 1998, *Pyrenophora tritici-repentis* on wheat and *Pyrenophora* teres on barley were serious problems. Some necrotrophic pathogenic fungi have very wide host ranges and examples are *Sclerotinia* spp. and *Rhizoctonia* spp.

In many instances, there is a dearth of highly effective natural resistance to these necrotrophic pathogens and it is generally believed that the development of novel methods of control will be necessary. At the Cooperative Research Centre for Tropical Plant Pathology, researchers are working towards the testing and evaluation of genes encoding plant AMPs for the control of foliar necrotrophic pathogens in grain crops with particular emphasis on pathogens of canola and sunflowers. The assessment of the potential of these genes is currently at the proof of principle stage and it is not yet clear whether they provide a viable option for providing durable resistance to fungal pathogens.

Aspergillus flavus and *Aspergillus parasiticus* are soil-inhabiting microorganisms that can colonise peanut seeds. Although there is debate on whether these are true pathogens, it is known that they can cause discolouration of the nut kernel and produce aflatoxin, which respectively affect the marketability and human safety of the food product. It would appear that there is little highly effective resistance to colonisation by these microorganisms in the current peanut germplasm and the most effective controls in use at the moment are management options in the field and postharvest sorting to remove nuts with *Aspergillus* infestation and high aflatoxin content. Clearly, there is a need for new methods of control of both the colonisation of the nut by the fungus and the production of aflatoxin.

One possible strategy would be:

1) to identify an antimicrobial peptide that has high activity against *Aspergillus* spp. in vitro using a high throughput screening method;
2) characterise the peptide and clone the gene; and
3) express the gene that encodes this peptide in a transgenic peanut in sufficient quantity at the right time and place in the developing nut to inhibit the growth and development of colonising *Aspergillus* fungi. This strategy is illustrated in Figure 2.

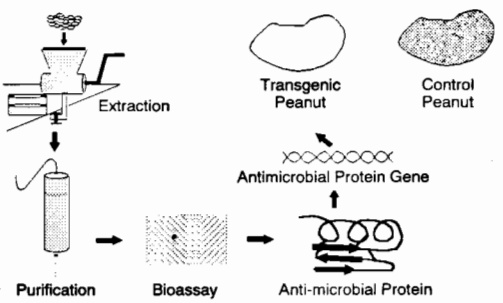

Figure 2. A flow chart illustrating the strategy of screening for an antimicrobial protein or peptide that can inhibit the growth of *Aspergillus flavus* and then transferring the gene to transgenic peanuts to prevent fungal colonisation, discolourisation and the production of aflatoxin.

Prospects for the Use of Plant Antimicrobial Peptides for Control of *Aspergillus flavus*

A preliminary investigation of the sensitivity of *Aspergillus flavus* to known antimicrobial peptides has been undertaken in a pilot project by researchers at the Cooperative Research Centre for Tropical Plant Pathology (CRCTPP) and the Queensland Agricultural Biotechnology Centre based in Brisbane (QABC). A series of plant AMPs for testing were kindly provided by Professor W. F. Broekaert and Dr B.A.P. Cammue of the Katholeike Universiteit Leuven (KUL). These included AhAMP1 from *Aeschulus hippocastanum,* DmAMP1 from *Dahlia merckii,* HsAFP1 from *Heuchera sanguinea,* IbAMP3 from *Impatiens balsamina* and RsAFP1 from *Raphanus sativus* (Osborn et al. 1995; Terras et al. 1995; Tailor et al. 1997). In addition, three antimicrobial peptides from Australian native plants, including MiAMP1 from macadamia (Marcus et al. 1997), all identified at CRCTPP were included. Drs C. Higgins and R. Dietzgen of QABC provided an isolate of *Aspergillus flavus* from the Kingaroy region in Australia and Dr. K. Goulter of CRCTPP undertook the tests for inhibition. The results obtained by Dr. Goulter are shown in Table 1.

These results show that the fungus was not substantially inhibited by any of the peptides at the concentrations tested. Previous published work has shown that most of these peptides are capable of substantially inhibiting the growth of a wide range of fungi at concentrations <10 µg/mL. These results suggest that *Aspergillus flavus* is highly resistant to a range of plant antimicrobial peptides that originate

from plant seeds. It is tempting to speculate that the resistance of *A. flavus* to peptides originating from seeds may be a specific adaptation to its ecological niche as a soil microbe capable of infesting plant seeds in the soil.

Table 1. Preliminary results testing the inhibitory effect of a range of plant antimicrobial peptides on the growth of *Aspergillus flavus* in culture (Ken Goulter, pers. comm.).

Antimicrobial Peptide	Source	% Growth inhibition after 72 hours in culture	
		Peptide concentration 50 μg/mL	Peptide concentration 10 μg/mL
MiAMP1	CRCTPP	0	2
CRCAMP2	CRCTPP	6	3
CRCAMP6	CRCTPP	30	25
AhAMP1	KUL	5	9
DmAMP1	KUL	8	8
HsAFP1	KUL	45	27
IbAMP3	KUL	Not tested	40
RsAFP2	KUL	31	22

Terras et al. (1995) demonstrated that an expression level of RsAFP2 at about 0.2%–0.6% of total protein in transgenic tobacco was necessary to detect significant resistance to the pathogen *Alternaria longipes*. This radish peptide results in 50% growth inhibition of this pathogen at concentrations of 2 μg/ml in vitro. By comparison with Table 1, it is clear that none of the peptides tested (including RsAFP2) showed equivalent levels of growth inhibition of *A. flavus* in vitro and the potential for their use in transgenic plants is therefore very low. Although this comparison is not encouraging for the direct application of current plant antimicrobial peptides to the control of *Aspergillus flavus,* it does not rule out the possibility that peptides that strongly inhibit the growth of *Aspergillus flavus* may be found from sources other than plant seeds. Recently, it has been reported (De Lucca et al. 1998) that a synthetic antimicrobial peptide can substantially inhibit the growth of *A. flavus* in vitro with a 100% growth inhibition being observed at approximately 12.5μM (approximately 20 μg/mL). This is an encouraging result for a strategy aimed at the discovery of more novel and potent peptide inhibitors from alternative sources that may be expressed in transgenic peanuts.

There is some evidence in the literature to indicate that AMPs may have a role in the natural resistance of some maize genotypes to infestation (Chen et al. 1998). It was reported that maize seeds that were resistant to colonisation by *A. flavus* contained very high levels of a 14 kDa protein. In resistant lines the protein was present at concentration between 13 and 22% of total protein while in susceptible lines this protein was only present at levels of 0% to 6%. The purified protein inhibited the growth of *A. flavus* in culture but only at very high concentrations (>500 μg/mL). These results suggest that if antimicrobial seed proteins are to be effective in limiting the growth of *A. flavus* then they have to be present at very high concentrations. The expression of antimicrobial proteins a levels in excess of 10% of seed protein in peanuts would be very demanding and may also affect the nutritional and other properties of the kernel. This might not be quite so much of a problem if the protein was highly expressed in specific tissues within the peanut where *A. flavus* initially establishes itself. However, little is known of the stages of colonisation of peanuts by *A. flavus* and at the moment it is unknown if this approach is possible.

Licensing, Regulatory and Marketing Considerations

Ultimately, the commercial success of a strategy using AMPs for aflatoxin control will rely on 1) being able to develop transgenic peanuts as a commercial product, 2) meet the appropriate requirements for biosafety and food safety and 3) obtain acceptance with consumers in the market.

At the moment, the methods and materials used to produce a transgenic plant are often subject to patent applications. These patented technologies, and the associated rights to use them, are mostly controlled by multinational companies in the agricultural biotechnology sector. Patents are specific to particular countries and therefore the patents held in potential export markets need to be considered closely as well as the point of production. It is therefore essential that the final product has a clear path to market and does not infringe patents controlled by others.

Two strategies are emerging to deal with this issue. The first is to develop one's own enabling technologies that allow one to bypass any proprietary technology; the second is to form an alliance with the organisation that controls the enabling technology under licensing terms that are of mutual benefit.

The commercialisation of transgenic peanuts will also need to meet national biosafety and food safety regulatory requirements. The issues that will be considered for environmental safety will be the potential effect of both the transgenic plant and the transgene if they were to escape the peanut production system. For example, could the plant become weedy or could the gene outcross into weedy species and interfere

with their ecological adaptation. Probably of greater concern in peanuts are the food safety issues and the final product would have to undergo significant toxicological analysis. Many of the genes discussed above are seed proteins and may be potential allergens. It is worth noting that a company in the USA had to cease work on the expression of a sulphur-rich protein from brazil nut in transgenic soybean because of the allergenicity of the protein.

Finally, there is still considerable debate on the acceptance of food products derived from genetically modified organisms by consumers. The technology of genetic engineering is relatively new in plant improvement and unfamiliar to most consumers. Like all technologies, it is capable of delivering benefits to both producers and consumers but also requires close regulation. The acceptance of foods from GMOs is very uneven globally with widespread use of products from genetically modified soybean, canola and maize in the North America. However, there is currently a low level of acceptance in Europe.

If the food products from genetically modified plants are carefully regulated and no safety issues emerge, then it would appear likely that over time, a greater familiarity with the technology will lead to increased acceptance. However, in the short term any business plan concerning a food product from a genetically modified plant will have to take the uncertainty of market acceptance into account.

Conclusions

Scientifically, it would appear that the expression of antimicrobial peptides in transgenic plants may be a viable strategy for the control of aflatoxins by restricting infestation by *Aspergillus* spp. However, to implement the strategy it will be necessary to undertake the following:

- New peptides especially potent on *Aspergillus* spp. will need to be discovered and their genes cloned. Current results suggest that this should focus on sources other than seeds.
- High and stable levels of expression of the antimicrobial peptide at appropriate sites will need to be achieved in transgenic plants of high commercial productivity.
- Freedom to operate will be needed for all proprietary enabling technologies used in the strategy.
- The transgenic plant and the peanuts must meet all biosafety and food safety requirements.
- A viable consumer market for genetically modified peanuts with lower aflatoxin levels must exist.

Acknowledgments

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Extra and Intra-Cellular Signals Regulating Aflatoxin Production by *Aspergillus* spp.

N.P. Keller[1]

A MAJOR constraint to peanut production worldwide is contamination of peanut seed with the carcinogenic secondary metabolite, aflatoxin. Aflatoxin is produced in peanuts when they are infested by *Aspergillus* spp., ubiquitous seed-infesting fungi. Because traditional plant pathology control strategies have not worked well in reducing *Aspergillus* colonisation of peanuts and subsequent aflatoxin contamination, recent studies have focused on identifying the molecular signals required for *Aspergillus* infestation and subsequent aflatoxin production.

One important advance has been the discovery of an *Aspergillus* G-protein intracellular signalling pathway that genetically links *Aspergillus* sporulation to mycotoxin production. Induction of different components of this pathway can lead to stimulation or repression of either spore or aflatoxin production. Extracellular factors that effect sporulation and mycotoxin production are seed fatty acids, in particular linoleic acid and seed lipoxygenase derivatives of linoleic acid which are associated with plant resistance to other fungal pathogens. The recent cloning of a peanut seed lipoxygenase gene that is induced by *Aspergillus* infection holds promise in identifying—and ultimately regulating—host components important in the seed/*Aspergillus*/aflatoxin interaction.

Aflatoxin (AF) and the related mycotoxin sterigmatocystin (ST) cause liver necrosis and cancer in mammals, birds and other vertebrates. Both mycotoxins are products of the same lengthy biosynthetic pathway (Figure 1) where ST is the end metabolite of *A. nidulans* and *A. versicolor* but the pentultimate precursor of AF, the end metabolite of *A. flavus* and *A. parasiticus*. These carcinogenic compounds are produced and accumulate in *Aspergillus* colonised seed such as corn, peanuts, cottonseed and treenuts. Aside from the losses arising from AF/ST related health problems for both humans and animals, international concerns have resulted in establishing AF (more commonly found than ST) limits on food products which have also resulted in economic losses and trade wars.

The long term goal of research is to provide methods for removing this international health concern by identifying novel means for controlling fungal AF and ST production. Significant progress has been made in understanding ST/AF biosynthesis using the genetically well studied *A. nidulans* as a model organism. Results in the past several years have clearly established that the genes involved in ST and AF biosynthesis, including an ST/AF regulatory gene *aflR*, are conserved among *A. nidulans*, *A. flavus* and *A. parasiticus* (Brown et al. 1996a, b; Fernandes et al. 1998). The latest studies (Hicks et al. 1997) have established that ST/AF production is a normal *Aspergillus* developmental process that is genetically linked to the ability of the fungus to produce spores (Hicks et al. 1997). This coupling of spore production to toxin production promises a potential control strategy that could simultaneously reduce both aflatoxin concentrations as well as inoculum production.

Intracellular Signals

The molecular components linking *Aspergillus* sporulation to ST/AF production are members of a G protein signalling pathway. It has been determined that FadA (the α subunit of a heterotrimeric G protein) and FlbA (a GTPase protein that modifies FadA activity by enhancing its endogenous GTPase activity) are both required for normal sporulation and toxin production (Hicks et al. 1997).

When FadA is in its activated state both sporulation and ST/AF production are repressed. When FlbA is over-expressed (and thus *de*activating FadA activity), both spore and ST/AF production are induced early and inappropriately.

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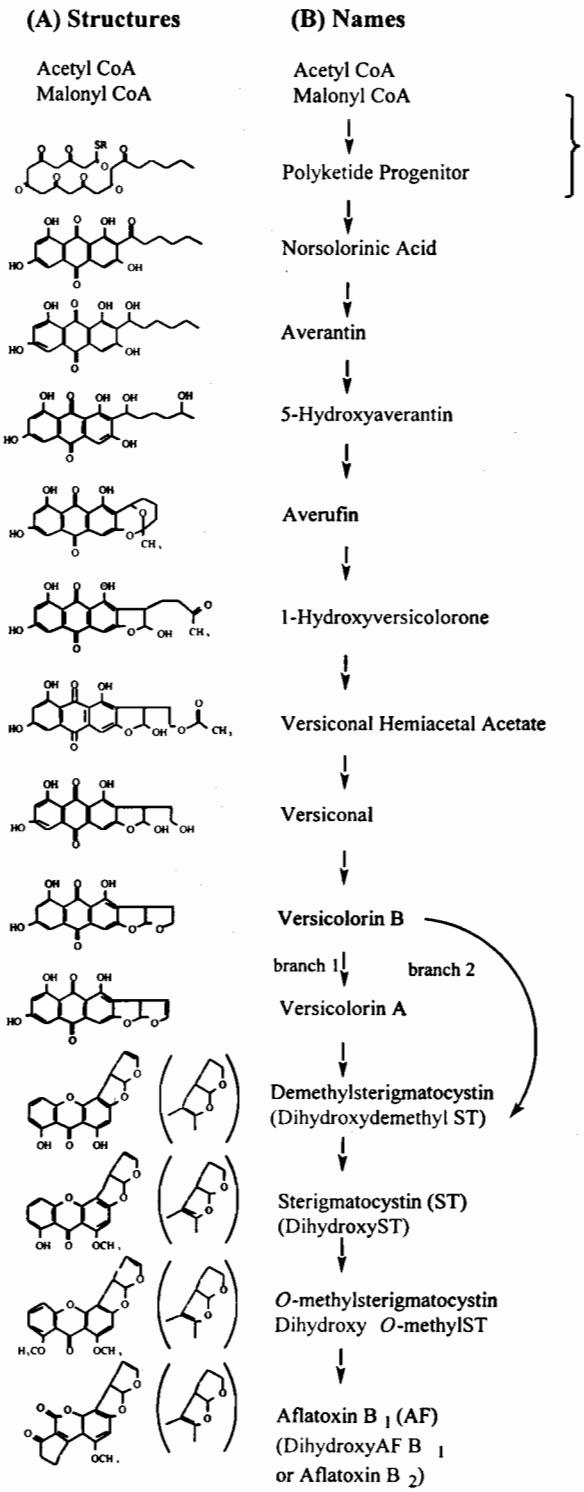

Figure 1. Aflatoxin biosynthetic pathway.

Researchers are currently looking for other members of this G protein signalling pathway and have tentatively identified other molecules (i.e., kinases, Shimizu and Keller, unpublished) which uncouple sporulation and ST/AF biosynthesis. Strategies aimed at regulating these *Aspergillus* intracellular molecules could result in novel means to control both colonisation and aflatoxin contamination of peanut and other *Aspergillus* susceptible seed.

Extracellular Signals

All G protein pathways are only operational upon stimulation by suitable environmental signals. One class of extracellular molecules that affects both *Aspergillus* sporulation and AF/ST production is polyunsaturated fatty acids. Specifically, 18 carbon fatty acids including linoleic acid, linolenic acid and their lipoxygenase derivatives act as *Aspergillus* sporogenic factors (Calvo et al. submitted).

All of these fatty acids are common constituents of oil seeds. Lipoxygenases are ubiquitous plant enzymes that are associated with resistance to pathogen and pest infestations. These enzymes add molecular oxygen to either the 9^{th} or 13^{th} carbon of both linoleic and linolenic acid to produce 9 and 13 hydroperoxy fatty acids respectively (9 and 13-HPODE). These hydroperoxy fatty acids can be further reduced to hydroxy fatty acids and other molecules associated with pathogen resistance (i.e., jasmonic acid). Because *Aspergillus* spp. produce endogenous sporogenic compounds from linoleic acid called psi factors (a mixture of hydroxy derivatives of linoleic acid), it is hypothesised that the sporogenic effects of seed fatty acids are due to their mimicking and/or interfering with the endogenous *Aspergillus* psi factors.

Additionally, 9- and 13-HPODEs have a differential effect on AF/ST production in vitro such that 9-HPODE extends the length of time AF/ST genes are expressed and 13-HPODE inhibits AF/ST gene expression (Burow et al. 1997). To determine whether seed lipoxygenase expression may play a role in the seed/*Aspergillus*/aflatoxin interaction, a peanut seed lipoxygenase, Pnlox1 has been cloned and characterised (Burow et al. submitted). *Pnlox1* is induced by *Aspergillus* infections in mature seed and the protein, Pnlox1, produces approximately 70% 13-HPODE and 30% 9-HPODE.

One future goal would be to deactivate this gene in the peanut and determine if elimination of this enzyme would have an effect on *Aspergillus* colonisation and AF production in the peanut seed. Because other studies have also indicated that there are at least two more peanut seed lipoxygenases (one predicted to produce primarily 13-HPODE and one primarily 9-HPODE), the intention is also to clone, characterise and ultimately deactivate these other peanut seed lipoxygenases. If these fatty acids are important extracellular sporulation and AF/ST signals for *Aspergillus*, regulation of peanut seed lipid metabolism may also prove a means to control the aflatoxin problem.

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Albicidin Detoxification—A Case Study in Plant Genetic Engineering to Destroy Toxins from Microbial Pathogens

R.G. Birch[1]

ALBICIDIN toxins, produced by the pathogen *Xanthomonas albilineans*, selectively block DNA replication in bacteria and plastids, and result in the characteristic chlorotic symptoms of sugarcane leaf scald disease. Transgenic sugarcane lines expressing a novel gene for detoxification of albicidins do not develop chlorotic disease symptoms. Plants with albicidin detoxification capacity equivalent to 1–10 ng AlbD enzyme per mg cellular protein show greatly reduced systemic multiplication of the pathogen, the precursor to economic disease. This demonstrates that genetic modification to express a toxin resistance gene can confer resistance to both disease symptoms and multiplication of a toxigenic pathogen in its host. It also shows that a single enzyme can be effective in plant cells for removal of all members of a family of toxic secondary metabolites produced by a microbial pathogen. In the past, the detoxification strategy has been successfully applied to generate crop plants resistant to several classes of herbicides. With the recent demonstration of an efficient transformation system for diverse peanut cultivars, the same approach is feasible for the development of peanut cultivars with 'in-built' capacity for removal of contaminating aflatoxins.

Phytotoxins Can Play a Key Role in Plant Disease Development

Many bacterial pathogens of plants produce low molecular weight toxins that induce symptoms characteristic of the associated disease (Rudolph 1976). These toxins are not the primary determinants of host range, as sometimes occurs with fungal phytotoxins (Walton and Panaccione 1993). However, there is increasing evidence that the toxins may be primary determinants of disease, not only by inducing the economically damaging symptoms, but also by suppressing plant resistance mechanisms at critical stages of the host-pathogen interaction. For example, mutants of *Pseudomonas syringae* pv. *phaseolicola* that have lost the capacity to produce phaseolotoxin not only fail to induce the characteristic chlorotic halos around leaf lesions of bean halo blight, but also fail to invade systemically to cause economic disease (Patil et al. 1974). The toxin suppresses plant defence responses (Patil and Gnanamanickam 1976) and there is a selective advantage in invaded plant tissues for bacterial cells with restored or increased toxin production (Birch et al. 1981).

Detoxification may be Developed as a Plant Disease Resistance Mechanism

Such observations indicate the potential to genetically modify plants for increased resistance to toxins involved in pathogenesis, as a novel mechanism of disease resistance. The identification of the first cloned plant disease resistance gene as a reductase effective against a host-selective fungal phytotoxin demonstrated that some plants naturally employ this mechanism (Johal and Briggs 1992). The ability of plants that express an introduced detoxification gene to protect themselves against exogenously applied toxins has been exploited commercially, in the development of cultivars resistant to herbicide damage (Stalker 1991).

However, there have been difficulties in testing the idea of novel toxin resistance genes for control of diseases caused by toxigenic pathogens. Tabtoxin-resistant transgenic tobacco is protected from the chlorotic halo around infection sites of the leaf spot pathogen *Ps. syringae* pv *tabaci*, but the effect on bacterial population development has not been reported (Anzai et al. 1989). By growth under debilitating conditions, tobacco plants can be rendered susceptible to the bean pathogen *Ps. syringae* pv. *phaseolicola*. Phaseolotoxin-resistant

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transgenic tobacco lines proved less susceptible to *Ps. syringae* pv. *phaseolicola* under these conditions, but it has not been possible to test the approach in a host species for lack of a suitable transformation system (De la Fuente-Martínez et al. 1992).

Albicidin Toxins Play a Key Role in Sugarcane Leaf Scald Disease

The role of albicidin toxins in leaf scald disease, caused by a systemic vascular pathogen, *Xanthomonas albilineans*, in sugarcane (*Saccharum officinarum*, polyploid interspecific hybrids) has been investigated. This host-pathogen interaction is finely balanced, with prolonged latent infection often preceding the development of damaging chronic symptoms or the devastating acute phase of the disease (Ricaud and Ryan 1989). The xylem-invading pathogen produces a family of low molecular weight toxins (albicidins) that selectively block prokaryote DNA replication, and cause the characteristic chlorotic symptoms by blocking chloroplast development. Tox– mutants fail to cause disease, indicating that albicidins may be also a key factor in systemic invasion or the unpredictable transition from latent infection to disease (Birch and Patil 1983, 1985a, 1987a, b).

The major toxin, named albicidin, has been partially characterised as a novel low molecular weight compound with several aromatic rings. Because albicidin is rapidly bactericidal to a range of Gram-positive and Gram-negative bacteria at concentrations as low as 1 ng/mL it is also of interest as a potential clinical antibiotic (Birch and Patil 1985b). As is commonly the case for secondary metabolites, albicidin biosynthesis involves multiple genes in several clusters, and is subject to complex environmental regulation (Wall and Birch 1997; Zhang et al. 1998).

Albicidin Detoxification Confers Toxin and Disease Resistance

To test the detoxification approach against sugarcane leaf scald disease, an efficient sugarcane transformation system was developed, and gene control sequences suitable for expression of foreign genes in sugarcane cells identified (Bower and Birch 1992; Bower et al. 1996; Rathus et al. 1993; Birch et al. 1996; Hansom et al. 1999).

Various bacterial genes for resistance to albicidins were also cloned, and resistance mechanisms and gene products characterised as candidates for use in transgenic sugarcane. Mechanisms of albicidin resistance include elimination of active antibiotic uptake in *Eschericia coli* (Birch et al. 1990); production of a protein which reversibly binds the antibiotic in *Klebsiella oxytoca* (Walker et al. 1988) and in *Alcaligenes denitrificans* (Basnayake and Birch 1995); and irreversible detoxification in *Pantoea dispersa* (Zhang and Birch 1996).

One gene designated *albD*, encodes an esterase that detoxifies albicidins under conditions that indicate potential to destroy albicidin toxins in transgenic sugarcane (Zhang and Birch 1997a). The gene contributes to the strong activity of *P. dispersa*, a secondary invader of leaf scald diseased sugarcane, as a biocontrol agent against *X. albilineans* (Zhang and Birch 1997b).

As a preliminary test of application of *albD* to protect sugarcane against leaf scald disease, we introduced the gene into the pathogen, *X. albilineans*. Expression of this novel gene for albicidin detoxification abolished the capacity of *X. albilineans* to release albicidin toxins and to incite disease symptoms in sugarcane (Zhang and Birch 1997a).

It has recently been shown that expression in transgenic sugarcane of the albicidin detoxification gene *albD* can confer a high level of resistance to chlorotic symptom induction, multiplication and systemic invasion by *X. albilineans* (Birch and Zhang 1996; Zhang et al. 1999). Plants with albicidin detoxification capacity equivalent to 1–10 ng of AlbD enzyme per mg of leaf protein did not develop chlorotic disease symptoms in inoculated leaves, whereas all untransformed control plants developed severe symptoms. Transgenic lines with high AlbD activity in young stems were also protected against systemic multiplication of the pathogen, which is the precursor to economic disease. This low level of an introduced gene product is not expected to impose any substantial metabolic load. Genetic transformation to obtain clones with an appropriate pattern of *albD* expression is therefore a promising approach to rescue agronomically outstanding sugarcane cultivars that would otherwise have to be discarded because of leaf-scald susceptibility.

These results confirm that toxins first recognised because of the disease symptoms that they elicit can play a larger role in pathogenesis by rendering the host more susceptible to invasion. It has been demonstrated that genetic modification to express a toxin resistance gene can confer resistance to both disease symptoms and multiplication of a toxigenic pathogen in its host.

It is likely that this anti-pathogenesis approach can be developed to target pathogenicity factors other than toxins; for example signal molecules used in quorum sensing by pathogens, or compounds such as hormones and enzymes that elicit host responses favourable to the pathogen. An advantage of the

approach is that in most cases such resistance is expected to be stable, because it can only be overcome through a gain of function by the pathogen.

Can the Detoxification Approach be Applied in Peanut?

The recent demonstration of an efficient transformation system for diverse peanut cultivars lays the foundation for application of gene technologies for peanut improvement (Ozias-Akins, et al. 1993; Livingstone and Birch 1999).

The approaches described above for detoxification of albicidins can equally be applied to the cloning and characterisation of genes for detoxification of aflatoxins. They will need to be combined with research to obtain sequences directing appropriate patterns of introduced gene expression, and identification or engineering of enzymes capable of essentially complete destruction of aflatoxins in peanut pods around the time of harvest.

The combination of these molecular biotechnologies makes feasible the development of peanut cultivars with 'in-built' capacity for removal of contaminating aflatoxins.

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Enzymatic Bioremediation Technologies for Mycotoxins

J.G. Oakeshott[1], R.L. Harcourt[1], T. Sutherland[1] and R.J. Russell[1]

SEVERAL uses for enzymatic detoxification technologies are now emerging in agriculture. The major use to this point lies in the development of transgenic crops resistant to herbicides like glyphosphate, bromoxynil, 2,4-D, Basta and phenyl carbamates using genes encoding specific detoxifying enzymes (Streber et al. 1994; Powles et al. 1997). Enzymes that detoxify a range of insecticides, herbicides and fungicides are also under development as formulated fermentation products for bioremediating contaminated waste water streams and the surfaces of contaminated fruit and vegetables (Russell et al. 1998; 1999). Some phytotoxins produced by plant pathogens are also amenable to detoxification through the expression of appropriate detoxifying enzymes in transgenic crops (Zhang and Birch 1997). This paper examines the prospects for developing detoxifying enzymes for mycotoxins like aflatoxins.

Depending on the crop and to a lesser extent the toxin, an appropriate mycotoxin degrading enzyme could be deployed either pre- or postharvest. Preharvest intervention would most likely take the form of transgenic cultivars expressing the detoxifying enzyme at the appropriate tissue and time. In the case of aflatoxins in peanuts, this would clearly be in the peanut itself. Postharvest intervention might be by addition of formulated enzyme during processing, for example, during preparation of peanut pastes, sauces or stockfeed meals. Regulatory and consumer acceptance would clearly be critical in each case but there is no a priori reason to expect adverse side effects and the alternative may be a risk of mycotoxin consumption.

There would be several threshold performance criteria for effective mycotoxin detoxification enzymes whether they are deployed through transgenic cultivars or as fermentation products. A first and foremost prerequisite would be that there must be essentially qualitative reduction in toxicity. This means not just qualitative breakdown of the mycotoxin but also that the breakdown products are not themselves toxic. A second prerequisite would be that the reaction be carried out by preferably one and probably not more than two or three enzymes; beyond this, the technological challenges and the cost may become prohibitive. Substrate specificity is also a key issue.

On one hand, the enzymes(s) should detoxify all the relevant variants of a particular toxin class, for example, all the active forms of aflatoxin. On the other hand, adverse side effects must be avoided, so the enzyme should be effectively inactive against other potential physiological substrates in the microenvironment of its use. Finally, the kinetics of the enzymes for their mycotoxin substrates are also critical. Since the enzyme must be effective down to low ppb concentrations, both affinity and turnover need to be high (i.e., K_m must be low and k_{cat} high).

There are also some additional performance criteria that will be specific for the method of use. In the case of transgenic cultivars, this means, for example, that the enzyme should be capable of functioning effectively in the microenvironment where toxin accumulates, which, in the case of aflatoxins in peanuts, might be quite hostile to effective enzyme function. This is because of the low metabolic activity, limited water and high oil content of a peanut.

Functionality must also be retained for significant periods between harvest and processing, during which risks of further fungal infection remain. Such constraints impact on the properties of the promoters used to drive enzyme expression as well as the properties of the enzyme(s). In the case of fermentation products, one key requirement is independence from cofactors, some of which (e.g., NADPH) would be prohibitively expensive and others of which (e.g., metal ions) may represent unacceptable contaminants in themselves.

While the above constraints are clearly demanding, they have apparently been satisfied for one mycotoxin, fumonisin, which is of particular concern in maize and for which three enzymes have

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already been identified as having the requisite properties (Duvick et al. 1998). Although not closely related to one another, all three enzymes are from the one multigene family of carboxyl/cholinesterases, which has also furnished several of the enzymes being trialled for use in making herbicide resistant crops and formulated for pesticide bioremediation (Oakeshott et al. 1999). The fumonisin degrading enzymes are patented by a major maize seed company. Only a few details on their performance in planta are publicly available.

There is good reason to expect that appropriate enzymes can be developed for several other mycotoxins, including aflatoxins. Most mycotoxins contain one or more bonds/moities that are intrinsic to their toxicity but susceptible to oxidation, conjugation or hydrolysis. Several cytochrome P450s are known to attack aflatoxin (Aoyama et al. 1990) and some glutathione-s-transferases (GSTs) are known to conjugate the oxidised products (Van Ness et al. 1998). There may be problems in supplying regeneration enzymes for the P450s and cofactors for the GSTs in ex vivo fermentation product approaches but these issues may be less problematic in in vivo transgenic cultivar applications. Hydrolysis of the aliphatic or cyclic carboxylester bonds in many mycotoxins like aflatoxins would also achieve detoxification.

Many carboxylesterases and other hydrolytic enzymes do not require regeneration or cofactors, which explains in part why they feature prominently among the detoxification enzymes under development for various bioremediation functions. While cyclic carboxylester bonds like those in aflatoxins are energetically less amenable to hydrolysis than aliphatic carboxylesters like fumonisins above, there are nevertheless many enzymes known with efficient cyclic carboxylesterase capability (Oakeshott et al. 1999). Use of in vitro mutagenesis, including structure-based design and in vitro evolution, has also proven effective in creating and/or improving desired substrate specificities and kinetics for various such esterases (Moore and Arnold 1996; Millard et al. 1995, 1998).

In the case of aflatoxins, there are also some key experimental reagents and assays already available that would be invaluable in the identification of effective detoxification enzymes. These include sensitive immunochemical and fluorometric assays that should be readily adapted to high through-put microbial plate formats (Gilbert 1991). These assays should enable effective screens of natural microbial sources of candidate enzymes and would be equally valuable in in vitro mutagenesis to create or improve appropriate enzymes.

In conclusion, there is good reason to be confident that enzymes capable of detoxifying aflatoxins and other mycotoxins can be obtained. Whether they would function effectively in planta may be more problematic, given the need for sustained activity in seeds such as peanut pre- and postharvest. These constraints must be carefully considered in the approaches taken both to obtain and to deploy the enzymes. However, no insurmountable problems have been identified in either regard and the precedent work with fumonisin esterases for use in maize gives good cause for optimism.

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Trends in Agricultural Biotechnology: Implications for Australia and Opportunities in Peanut Improvement

R.G. Birch[1]

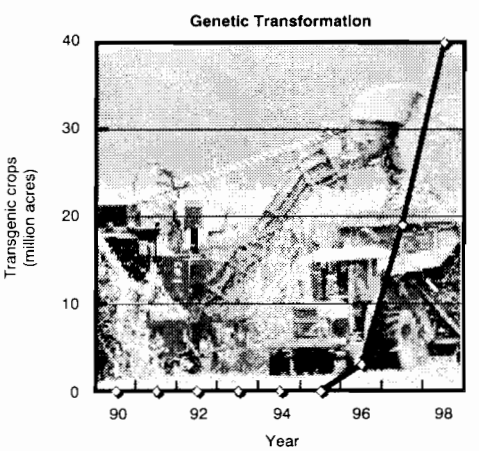
DURING 1998, just 15 years after production of the first transgenic plants, plant molecular biology delivered multi-billion dollar commercial outcomes from 30 million ha of transgenic crops, with an exponential growth projection. Efficient transformation systems are in place for the major economic plant species, and two of the world's most powerful technologies (molecular genetics and computing) are being focused on the discovery of genes with commercial potential. The full genetic sequence of a model plant species will be delivered by 2002 at a cost of around US$100 million. High throughput techniques are beginning to reveal the subtle patterns of expression of individual genes that determine important agricultural traits.

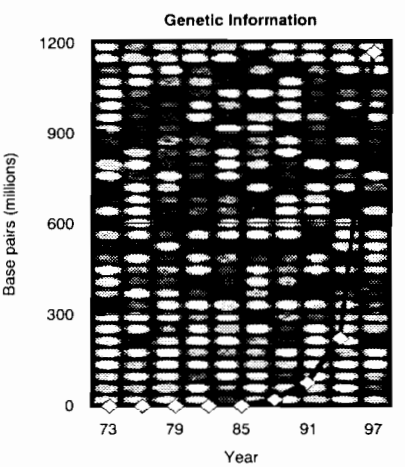
As yet, we are skating over the thinnest ice of understanding over the lake of plant biology. For example, clumsy introduction of foreign genes often causes 'collateral genetic damage', and plants commonly detect and silence introduced foreign genes, by mechanisms that we do not fully understand. A sequenced genome will be barely the start of efforts to understand and control the subtle processes controlling the conversion of encoded genetic information into the diverse biological activities essential to any crop. As with any major new technology, there is also a vital need to establish, and to obtain public acceptance of, the safety and benefits of the outputs.

However, the advances of the past 15 years show how rapidly these challenges may be overcome. Biotechnology will be the driving force of sustainable and profitable agriculture through the next century. Innovations in agricultural biotechnology require substantial investment. They are commonly subject to intellectual property protection, which allows a period for investors to capture value, while delivering long-term benefits to the community. Access to these emerging technologies will be vital for profitability of agricultural enterprise in a global economy. Agricultural biotechnology is a 'critical strategic technology' for every nation that hopes to profit from its own agricultural enterprises during the next decades. To remain competitive, they must urgently identify priority targets, and allocate sufficient resources to power the strategic research that will allow them to capture a share of the benefits from innovation.

Agricultural Biotechnology

Convergence of functional genomics and plant transformation biology





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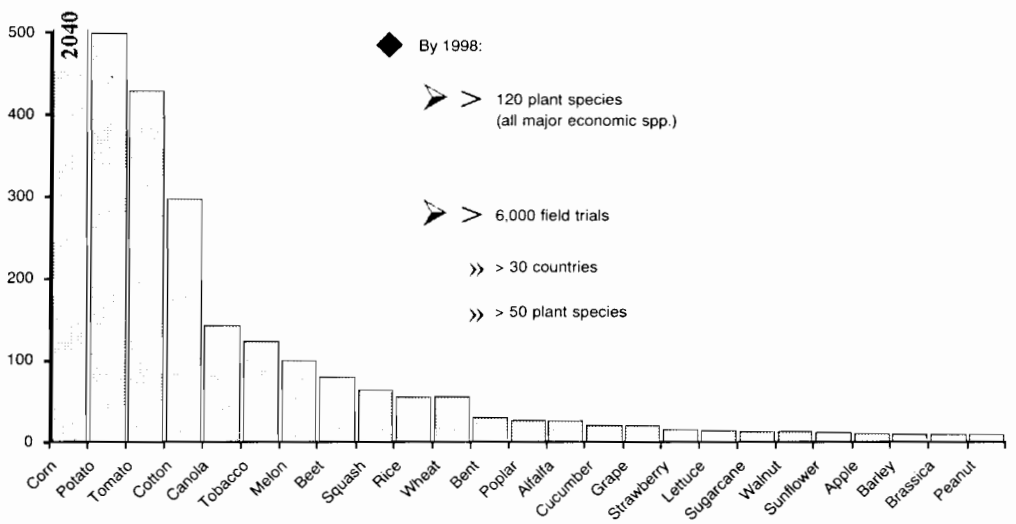
Extraordinary advances often arise from the convergence of powerful new technologies. This is true in the field of molecular genetics, where the number of identified genes has grown exponentially following the convergence of automated sequencing and computing technologies. Sequencing is only a first step towards understanding gene function. The term functional genomics is sometimes applied to the larger task of understanding the roles, interactions and control of the hundreds of thousands of genes in any organism. This is about to explode through the convergence of high throughput methods including microarray and Expressed Sequence Tags (EST) technologies with computer-linked bioinformatics. In agricultural biotechnology, the area of transgenic crops has grown exponentially following the convergence of molecular genetics and transformation biology. Advances in functional genomics and transformation biology appear set to reshape agriculture during the coming decades, delivering new products, higher product quality, more efficient production, and more sustainable production systems.

Plant Genetic Transformation

First transgenic plants described in 1984

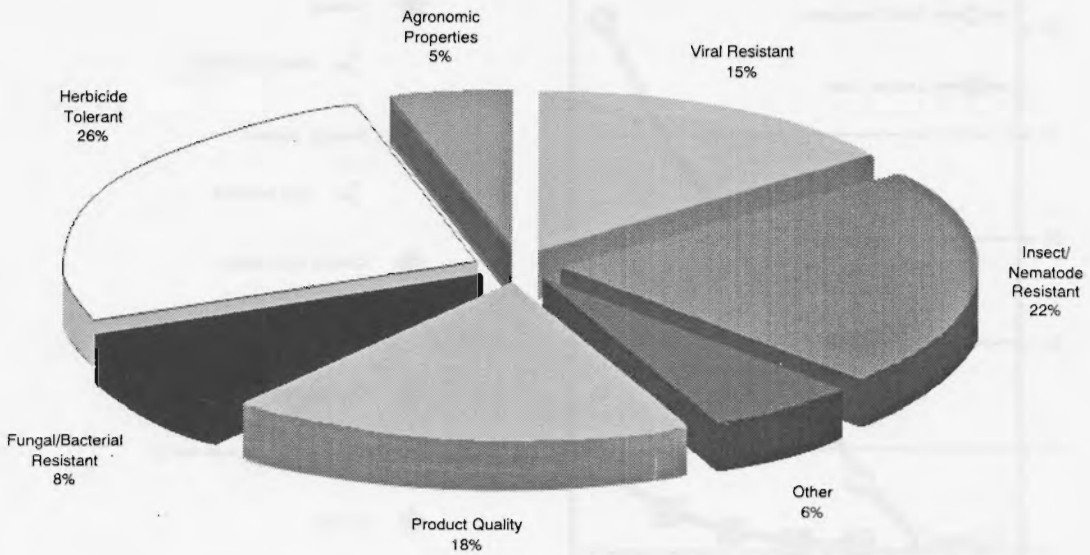


The first plants expressing a novel trait introduced by genetic transformation were reported in 1984. By 1998, techniques were developed for gene transfer into almost all major economic plant species. In most major crops, tens (and in some cases hundreds) of field trials have been conducted around the world, including plants improved in major economic traits including resistance to serious pests and diseases.

By 1995, the first transgenic crops had completed safety and economic evaluations and gained approval for commercial use. The area of transgenic crops has increased exponentially since then, with North American producers and major agricultural companies like Monsanto taking the lead in commercial development. In countries including the USA, transgenic varieties of the major crop species with improved characteristics have been shown to be identical to conventionally bred products in terms of consumer and environmental safety, and accordingly released from any regulations imposed simply because they were produced using the tools of molecular genetics.

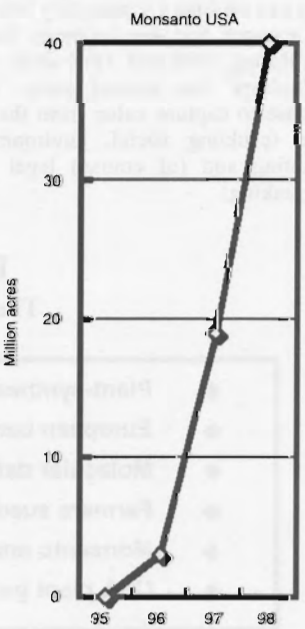
Field Releases by Trait

Major economic characteristics targeted

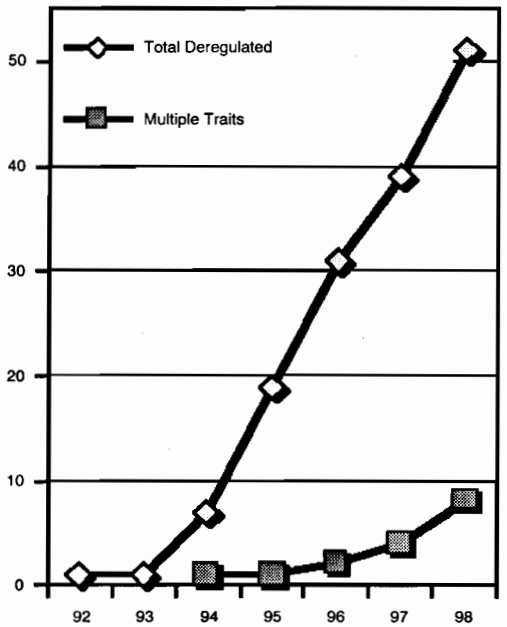


Commercial Outputs — 1998

Country *and Crop*	% Crop	Million hectares	Billion US $
Canada *Canola*	40%	2.2	$1.24
USA *Soybean*	40%	11.3	$7.04
USA *Cotton*	35%	2.3	$2.24
USA *Corn*	20%	6.5	$5.0
GLOBAL *TOTAL*	—	> 26	$ > 20



Deregulated Products (USA)



- **Tomato**
  - delayed ripening
- **Squash, Papaya**
  - virus resistant
- **Cotton, Corn, Beet**
  - insect, herbicide resistant
- **Canola, Soybean**
  - oil quality, male sterile
- **Potato**
  - virus, insect resistant

Many analysts have observed these remarkable developments in molecular biology at the end of the 20th century, and concluded that the next century will bring extraordinary community benefits from biological research and development. Some of the headlines during 1998 and 1999 show that the 'Century of Biology' has started early. The international response to capture value from these developments, while resolving social, environmental, technical, marketing and (of course) legal issues has been breathtaking.

One reason for this level of interest can be seen in the consequences for international agriculture. The discoveries from plant molecular biology, and their applications through agricultural biotechnology, will reshape agricultural industries and determine competitive efficiency. However, these developments are inventions, eligible for protection under intellectual property laws. The implications for agricultural economies are profound, and not yet fully appreciated by government or industry in some countries.

Plant Biotech '98 Headlines

The 'century of biology' has begun early

- **Plant-synthesised vaccines and plantibodies entered clinical trials**
- **European bans on transgenic crops — rules illegal by EC**
- **Molecular detection of germplasm theft — brings $300M penalty**
- **Farmers sued for planting 'saved seed'**
- **Monsanto and DuPont continue $Billion acquisitions**
- **USA plant genomics research > $Billion**

Commercial Realities

No prizes for 2nd place in the IP races

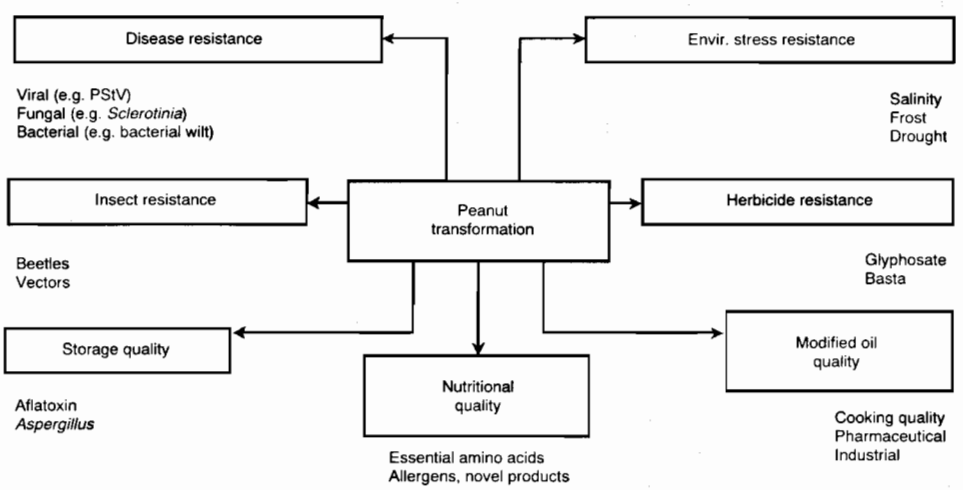
- **Future leading varieties will be transgenic**
- **Gene technologies will**
  - ➢ **reshape agricultural industries**
  - ➢ **determine competitive efficiency**
- **Sequences and technologies patented**
- **IP will be virorously protected**
- **Investors will maximise 'captured value'**
- **Access to gene technologies = profitability**

Australian investment is dangerously low

Some of the economic opportunities created by the development of an efficient genetic transformation system can be illustrated using the example of peanuts. In each case, achievement of these outcomes requires substantial investment in research, development and commercialisation.

Peanut Transformation

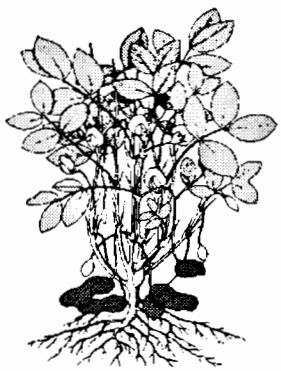
Opportunities



Peanut Transformation

Prerequisites for commercialisation

- **Controlled transgene expression**
  - ➢ **desired expression patterns**
  - ➢ **stability and inheritance**
- **Field evaluation/adverse effects**
  - ➢ **minimise collateral genetic damage**
- **Identify priority targets**
  - ➢ **local/global**
- **Access useful genes and promoters**
  - ➢ **ensure IP position**
- **Meet regulatory and educational needs**
- **Demonstrate value**



No player can develop all of the possible opportunities. It is possible to define criteria by which the best opportunities can be identified as priority targets for a particular industry. Intellectual property (IP) is a form of currency, which is commonly traded and cross-licensed between owners. This can enable access to key technologies, which may otherwise be denied or offered only at a cost that would make growers non-competitive.

Not all research investments will generate commercial returns to investors (there are risks of innovation, which need to be spread over a sufficient scale of investment). There are far higher risks of failure to innovate (or failure to innovate in time). On a national scale, the stakes are very high: continued profitability of agricultural industries. For example, Australia is unlikely to remain competitive as an agricultural exporter based on geographic advantages. Competition based on lowest wages is unappealing. With ownership of substantial IP, agriculture becomes a value-added industry in which we can develop as a full partner in the future global agriculture. Without ownership of substantial IP in agricultural biotechnology, the prospects are very poor: inability to access vital competitive technologies, or farming as virtual servants under license to the owners of such technologies.

Peanut Transformation

Identification of priority targets

- **Solve problems or create opportunities**
- **Features of 'immediate' targets**
  - ➢ **Major commercial impact**
  - ➢ **Not achievable by breeding**
- **For highest probability of success**
  - ➢ **Base on results in other species**
  - ➢ **Ensure IP position**
- **For highest potential return**
  - ➢ **Generate new IP from exploration**

Agricultural Biotechnology

Australia — preparing to win or lose?

- **The plant biotechnology IP races are on**
- **The prize is agricultural profitability**
- **Australia cannot leave the race**
- **The stakes are our agricultural industries**
- **The races are won through 'research power'**

Insufficient strategic 'research power' will make Australian farmers servants instead of partners in the future global agriculture

The capacity for development and ownership of IP is determined by several characteristics that can be summarised in the concept of 'research power'

Research power is generated by combining:

- **Intellectual capability**
- **Research and development skills**
- **Research infrastructure**
- **Industry support for strategic directions**
- **Government policy encouraging strategic research**
- **Strategic investment sufficient to spread risks of innovation**

Urgent action is required where this check-list reveals weaknesses for any nation that hopes to profit from its own agricultural enterprises during the next decades.

OUTCOMES OF WORKSHOP DISCUSSIONS

Approaches to the Elimination of Aflatoxin Contamination in Peanuts

R.G. Dietzgen[1]

AFLATOXIN contamination in peanuts is not a health issue in Australia, but an issue of yield losses, reduced income to growers due to downgrading of contaminated seeds, and high technology and costs for removal of contaminated kernels during processing. In developing countries, the main issue is the risk to human and animal health through consumption of aflatoxin-contaminated peanut products.

Current strategies for aflatoxin control are containment and minimisation, whereas the identified need is elimination of aflatoxin contamination. The problem needs to be fixed at the source, i.e., on the farm (preharvest) and postharvest.

Dr Ken Reed, Director of the Queensland Agricultural Biotechnology Centre (QABC), defines biotechnology as 'the art of optimising utility of genetic information'. In the global economy of rising costs and falling commodity prices, agricultural biotechnology aims to reduce costs by protection of crops from pests and diseases through reduced chemical usage, and to increase the value of the commodities.

The optimal outcome would be the elimination of aflatoxin contamination in peanuts, i.e., to have peanuts with predictable zero levels of aflatoxin. Taking into account the reservations of some sectors of the public regarding genetically modified food, it may be advantageous, if zero levels could be achieved without expressing novel genes in the edible nut itself. The workshop participants explored a broad spectrum of potential avenues to achieve these desired outcomes.

Biocontrol

Proof of concept has been demonstrated for the use of non-toxigenic strains of *Aspergillus flavus* to counteract toxin-producing strains in the environment by 'competitive exclusion'. Research so far has determined the characteristics of non-toxigenic strains and limited success has been achieved in the USA and Australia.

Further field evaluations are required in Australia. Commercial applications, formulations, and delivery and scale-up production need to be investigated. Proof of reliability, efficacy pre- and postharvest and safety of this approach, and further research on survival of the competitor strain in the soil and stability of the lack of toxin production will be required.

Advantages of biocontrol include minimal disturbance of the ecosystem, positive consumer perception, no gene technology or food safety regulations, and the potential for readily transferable technology to other countries. Disadvantages include that the current extensive aflatoxin testing systems and farm management will still be required, that aflatoxin may be minimised, but not eliminated. Questions about the risk of failure and efficacy for postharvest contamination were also raised.

Use of Endogenous Germplasm in Breeding

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) holds the world collection of wild and cultivated *Arachis* species. Limited knowledge of 'low aflatoxin' genotypes exists, and mutagenised germplasm is available. Rapid and high throughput antibody-based screening assays for sensitive aflatoxin detection have been developed recently. Research is required to determine the basis of 'resistance' to either aflatoxin production, or fungal infection or proliferation (independent from drought tolerance—cultivar *Streeton* has been identified as having lower aflatoxin incidence due to tolerance to drought through deeper roots and quicker drying in the windrow during harvest).

[1]QDPI Queensland Agricultural Biotechnology Centre, Level 4, Gehrmann Laboratories, The University of Queensland, St Lucia, Qld 4072

Increased knowledge is needed of genotype × environment interactions, peanut × fungus interactions and peanut kernel lipid composition and metabolism. An extensive breeding and backcrossing program would also be required to identify and incorporate useful traits. This could be accelerated by the use of molecular markers.

Advantages of this approach include the lack of regulations, ease of consumer acceptance, well established plant breeding accelerated with DNA markers and the gain of new useful knowledge on peanut germplasm and biology. Disadvantages include the long time frame involved, the extensive amount of research, uncertain outcomes and the low degree of compatibility between *Arachis* species.

Transgenic Peanuts Carrying Novel Genes

Biodegradation (enzymatic detoxification)

Genes for aflatoxin detoxifying enzymes would need to be identified from fungal or bacterial sources. Potential candidates include *A. niger* and *Rhizopus* sp. Screening assays would need to be developed. To be successful in pre- and postharvest biodegradation, enzymes would need to be expressed in high concentration in seed cotyledons. Enzymes would need to be active in low water content seed, be highly specific and safe in food. Besides aflatoxin, intermediate products of the biosynthesis pathway could also be targeted; this would be essential for any toxic intermediates.

Antifungal peptides

Screening of already available or new antifungal peptides (AFPs) for activity towards *A. flavus* and *A. parasiticus* would need to be done first. *A. flavus* could be included in the testing of available AFPs in the CRC Tropical Plant Pathology (a preliminary screen revealed only low activity). Alternatively, new AFPs could be identified by additional bioprospecting. Proof of principle that transgenic expression of AFPs will yield durable resistance needs to be established in other crop/pathogen interactions. The question of where in the plant to deploy AFP genes will need to be decided depending on the activity and action of the AFP. It was recommended that this was not a viable option at this stage and one should keep a watching brief on further developments in this field.

Host-fungus interactions

This approach aims to influence the expression of plant genes that are involved in plant-fungus interactions leading to or preventing aflatoxin production be the fungus. An increased knowledge of such interactions is emerging and needs to be expanded.

A target could be the suppression of enzymes involved in lipid metabolism which could also lead to improvements in fatty acid composition as a positive side effect. Down or up regulation of appropriate plant genes would need to be achieved in the seed and needs to be active in dried, mature seed. Functional genomics and micro array technologies would assist in identifying appropriate targets.

COLLABORATIVE RESEARCH FUNDING

Postharvest Technology in Asia: ACIAR's Framework for Collaborative Research and Development

G.I. Johnson[1]

ON THE brink of the 21st century, the need to become more effective in bringing home the harvest of the Green Revolution has become increasingly apparent. The Strategic Plan of the Australian Centre for International Agricultural Research (ACIAR) for 1997–2001 contains the following statement:

'Recognising the urgent need to reduce losses and maintain acceptable quality of harvested agricultural products, ACIAR will increase its investment in research on postharvest technology relevant to crops, livestock, aquatic resources and forest products. Resources will be diverted from research on production aspects of these commodities if necessary.' (ACIAR, 1997a)

In seeking to implement this part of the Strategic Plan, ACIAR has devoted a considerable effort to defining the rationale for this investment and developing a framework for collaborative research and development in postharvest technology with partner countries. This paper outlines the rationale and framework for the plan, and indicates the opportunities for interface between developing country partners and other agencies involved in international agricultural research for development. Collaboration and co-operation can maximise effective use of those agri-research resources that postharvest scientists and marketing specialists glean from the production specialists!

The Need for Postharvest Research and Development

Over the past 40 years, remarkable increases in agricultural production have occurred world-wide. During the same period, socio-economic changes driven by population growth, land shortages, urbanisation and community standards have increased product handling and storage standards, and the requirements for product quality and safety. The changes in standards and expectations have occurred across the spectrum of products harvested for use as food, fibre or shelter. As well, food security policies, and the drive for disposable income from the export of commodities, have also increased the demand for storage and transportation of both perishable and durable products.

So: There has been a dramatic change in the rationale and requirements for progress. Much more research in the postharvest sector is needed to address the real changes in community/world priorities. Among the critical issues that urgently require attention by researchers are the following:

Loss management

Postharvest losses remain unacceptably high, particularly in developing countries. More than 20% of agricultural produce world-wide does not reach consumers or is of unsatisfactory quality, resulting in increased costs.

Chemical replacement

Changing regulatory standards have seriously reduced the availability of highly effective agents for control of pests and spoilage.

Production surpluses and trade

In many countries in the Asia-Pacific region, the Green Revolution has led to the production of grain surpluses which can be stored, or exported. However, both require additional technological inputs. Trade is the means by which farm surpluses are converted into income. Food reserves and savings from income provide security against crop failure, natural disasters and health emergencies. And it is postharvest technology that enables both storage of produce and trade.

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Market access

Export markets have become more competitive and discriminating, leading to increased emphasis on Quality Assurance, and food hygiene.

Nutritional security

Achievement of 'rice security' coupled with urbanisation and moves away from the land has led to the need for 'nutritional security'—the transport and consumption of fruit and vegetables, dairy products, seafood and meat as sources of vitamins, minerals and protein. These more perishable commodities need effective postharvest technologies.

Food hygiene/environmental impact

Aquaculture and livestock feed lotting have increased the potential for production of fish and meat, but intensive production and processing creates new challenges in managing food hygiene, transport and storage, as well as waste disposal.

Quality maintenance and contaminant reduction

Poor product quality impairs human health and nutrition, and reduces the durability of clothing and shelter. Contamination by mycotoxins, excessive levels of agricultural chemicals and microbes is particularly undesirable. Microorganisms can cause serious and acute health problems. Mycotoxins accumulate in body organs over a number of years, weakening the immune system and predisposing consumers to carcinomas.

The annual social cost of mycotoxins in maize and peanuts in Southeast Asia alone exceeds $500 million (Lubulwa and Davis 1996). Pesticides and other undesirable chemicals in produce can arise from both direct application to the agricultural system or from non-target accumulation in agricultural soil or water followed by uptake by crops or livestock.

Cost efficiencies

A major portion of producer and community costs occurs in the post-production sector. These include the impact of postharvest handling and processing on the environment. For example, postharvest processing facilities can produce waste products which pollute agricultural land and water systems. As well, aging populations and labour shortages in rural areas have increased the need for cost-effective and labour-saving postharvest technologies.

Role of the Australian Centre for International Agricultural Research (ACIAR)

ACIAR-funded research aims at helping developing countries to help themselves, thus contributing to their well-being and general economic growth.

ACIAR allocates its bilateral research resources to developing countries in Australia's area of interest on a regional basis, which reflects the geographical focus of Australia's broader aid program. During the next five years, ACIAR will maintain a regional allocation of research resources within the following percentile bands (Table 1).

In developing research projects for these regions, ACIAR places emphasis on priorities determined in consultation with partner countries (Table 2), and communicates these priorities to research providers in Australia. Allocation of ACIAR's resources to partner countries in Southeast Asia reflects their changing circumstances. As partner countries become more affluent, technical cooperation will replace development cooperation as the primary mode of assistance. It is anticipated that such countries will contribute more of the cost of the joint research projects, and will increasingly help us to help less affluent countries.

Table 1. Regional allocation percentile bands of resources for ACIAR projects (ACIAR, 1997a).

Region	% of bilateral research budget
Southeast Asia	50–60
Papua New Guinea and Pacific Island Nations	10–20
China	10–20
South Asia	10–20
Africa	5–10

Will an increase in postharvest research lead to greater capture of benefits?

Cost-effective postharvest technologies at an appropriate scale have a very high rate of adoption because they are driven by demand. Population growth, urban migration, trade liberalisation and income growth are spurring demand for improved transport and storage of harvested products. There are strong links between postharvest technologists and the marketing, trading and processing sectors and domestic consumers. These ensure that research is market-driven and that technologies are relevant to the marketplace.

Table 2. Areas for priority attention as summarised in ACIAR — Country consultations 1994 to 1998 (dates of consultations are indicated).

The priorities are not considered as officially sanctioned priorities of partner country Governments. They are priorities expressed by participants at a consultation at a particular point in time. ACIAR uses them as a framework when assessing proposals for collaborative projects to be supported by ACIAR, subject to further advice and information from the proposed partner countries.

China 22–23 April 1996

- Processing of dairy products
- Postharvest technologies for vegetables
- Grain storage technologies

India 10–11 July 1997

- Feasibility studies on bulk handling and storage systems for grain, including socio-economic aspects;
- Postharvest technologies for meat, wool, fish products, fresh grapes, mango and dry fruits, including increased cooling efficiency of low-cost environment friendly cool chambers
- Storage pest management for pulses;
- Drying, testing, processing and storing seeds for sowing by farmers, including pre- and postharvest seed quality aspects;
- Postharvest technology for cotton (ginning, cotton seed oil);
- Disinfestation and postharvest technology for tropical fruit including quarantine aspects
- Mycotoxins.

Indonesia 20–21 June 1994

- Improvement of handling or ornamentals;
- Improvement of processing of highly perishable produce (roots and tubers, vegetables, fruits and fish).

Papua New Guinea 5–6 October 1995

- Postharvest technology (with special emphasis on down-stream processing of crops).

Philippines 26–27 February 1998

- Innovative approaches to quality management in plant products;
- Development of appropriate postharvest systems to include:
  - — establishment of sanitary and phytosanitary guidelines and capacity building in pest-risk analysis (PRA) for fruits and vegetables
  - — postharvest technologies for marine fisheries involving community level SMEs processing for value-adding through development of diversified products;
- Pesticide risk reduction strategy (policy, regulation, monitoring, research).

Thailand 14–25 February 1997

- Improved safety and utility of agricultural products, including reduced use of chemicals;
- Improved suitability and efficiency of postharvest systems;
- Understanding and manipulating the biological basis of postharvest quality.

Vietnam 16–17 May 1996

- Minimisation of pests, chemical residues and mycotoxins in agricultural produce;
- Extension of storage life in fruits and vegetables (clean, green food);
- Efficient drying and storage of grain;
- On farm, small scale processing and value adding for farm produce (including mobile units);
- Development of animal feed formulations;
- Co-operative marketing and postharvest technology transfer;
- Animal and seafood products: reduction of spoilage, value adding.

Priorities set at regional meetings also help guide ACIAR in the development of research programs. Those listed below were set at the 18th ASEAN Grain Postharvest Technology Seminar, 'Postharvest technology towards attaining food security', Manila, March 11–13, 1997.

- Strengthening of farmer co-operatives;
- More aggressive and deliberate extension and diffusion of improved postharvest technologies and systems;
- Increased infrastructure and support systems for farmers;
- Encouragement of greater private sector participation in commercial scale farming and buffer stocking;
- Strategic positioning of buffer stocks, transport and communication facilities;
- Better coordination among concerned agencies in the government and the private sector; and
- Commitment of more resources to postharvest research especially in priority areas such as wet grain handling, mycotoxin prevention and control, marketing, processing and education and training.

While post-production research directly benefits traders, marketers and manufacturers, the services and employment opportunities they provide benefit many in the community. In ACIAR, greatest emphasis will continue to be directed towards the early (near-farm) steps in the processing and marketing chain, where public benefits are likely to be high. Benefit/cost analyses of ACIAR's postharvest technology projects in that part of the chain indicate internal rates of return of 21–48%.

R&D potential

Because there has been a low investment in postharvest research relative to commodity production research world-wide, there is a plentiful supply of problems that can be easily solved by research. The adaptation of existing technologies to local conditions offers plenty of scope for innovative research at a level immediately relevant to scientists in developing countries, and chances of technical success are very high. Conversely, the application of advanced research methodologies to postharvest quality control is in its infancy.

The Framework

Interventions to reduce risks associated with these factors to enhance food and nutrition security and minimise costs will involve:

- research and development;
- policy adjustments;
- social or cultural changes; and
- infrastructure development.

ACIAR has developed a strategic plan for postharvest technology (ACIAR, 1997b). The goals and outcomes of the plan are summarised in Figure 1.

Of the goals and outcomes and the strategies for achieving them, four areas can be highlighted:

- The prospects for application of molecular biology;
- Food safety and nutrition;
- Systems approaches to postharvest research and development; and
- Collaboration with other international development assistance agencies, National Agricultural Research Systems (NARS) and the International Agricultural Research (CGIAR) Centres.

Goals

1. To improve application and efficiency of postharvest systems for food, wood and fibre products and animal feeds;
2. To optimise the quality and suitability of produce for market requirements;
3. To assure food security and improve trade and market access, and
4. To minimise losses or undesirable health, environmental and social impacts of the products or technologies.

Outcomes

1. High quality, robust produce suited to market requirements.
2. Postharvest technology systems and packaging which reduce losses, minimise costs and optimise produce suitability and quality.
3. Improved environmental safeguards in postharvest systems.
4. Enhanced food security, trade and market access, delivering improved returns to producers, traders and processors, and better value to customers.
5. Improved consumption of healthy and nutritious food and animal feed.
6. Increased postharvest research capability in the National Agricultural Research Systems (NARS) and international agencies.

Figure 1. ACIAR Postharvest Technology Strategic Plan: Goals and Outcomes.

Applications of Molecular Biology

The techniques of molecular biology offer particular promise in relation to maintenance of produce quality. Molecular techniques can be used to:

- turn off undesirable physiological processes such as browning of fruit following chilling injury or oxidation;
- slow down processes such as ripening;
- enhance mechanisms such as the resistance of fruit, grain or wood to pests and decay; and
- ensure a closer match between product and processing requirements such as he development of wheat cultivars more suited to noodle manufacture.

Developments arising this way can be delivered widely provided intellectual property and patent rights are managed. ACIAR is currently supporting

two molecular biology projects in the Postharvest Technology Program. One is on the control of physiological browning and crown deterioration in pineapples (PHT/1994/007). The other concerns the regulation of ripening in papaya and mango (PHT/1994/045). Both projects offer prospects for producing fruit that is less subject to postharvest deterioration, a benefit to producers, marketers and consumers.

Food Safety and Nutrition

Food safety

The contamination of food by chemicals, mycotoxins and microorganisms, and the presence of toxic constituents in food such as cassava can seriously affect the health of consumers and livestock. Research to develop simple tests for detecting the contaminants, or treatments to eliminate them, along with the establishment of reliable monitoring networks and appropriate regulatory mechanisms, will reduce risks to consumers and enhance export opportunities.

ACIAR recently supported research on the development of simple test kits to detect pesticide residues in plant foods (PHT/1993/009), in a collaborative project involving the Central Food Technology Research Institute (CFTRI) in India and the CSIRO in Australia.

ACIAR also supports research on mycotoxins in grains and cyanide in cassava. Information on ACIAR publications and previous projects concerning mycotoxin detection and management is summarised as Appendix 1. Information on some other initiatives in mycotoxin detection or management is summarised as Appendix 2.

Nutrients in food

Two important factors contribute to a decline in nutrition, despite the progress towards food security. These are the reductions in vitamin content during storage and processing, and changes in human dietary patterns. To address the decline in consumption of subsistence/artisanally produced food crops of high nutritional values in some Pacific Island Nations ACIAR will support collaborations which address the sociological and technical factors contributing to the trend.

Systems Approaches to Postharvest Research and Development

The postharvest area involves producers, transport providers, marketers and processors as well as consumers. In addition, Government and semi-government authorities may have significant responsibility for marketing and storing all or some portion of the total production of important commodities.

In many agri-enterprises, the whole system approach to managing produce quality and matching consumer expectations is being recognised as a means of consolidating and improving returns (Anon., 1996, Johnson et al. 1997). Quality assurance systems are being developed and implemented in many Australian enterprises and have become an important theme in partner countries as they become more involved in storing and trading produce. Implementation in practice is a challenge, and will be addressed within ACIAR activities which aim at improving the adoption of agricultural policy and technology using participatory processes (ACIAR 1997b).

In Asia, grain storage and marketing is an important aspect of food security. Government and semi-government authorities are involved in grain storage and marketing. Managing grain quality and pests in storage is a complex and important issue, and involves large numbers of personnel. In recognition of the complexity and importance of the issue, ACIAR has funded collaborative research (PHT/1993/021) involving the CSIRO, the Indonesian grain authority BULOG and the Queensland Department of Primary Industries in the development of pest control recommendations and training materials for use in large centrally managed storage facilities. The training material has been developed in a CD-ROM package in both Bahasa and English, and is being used to 'train the trainers'. The advantage of a CD-ROM based system is that text, sound, drawings and photographs and video clips can be incorporated and referred to as necessary to ensure accurate diagnosis and treatment of pests (Figure 2). The training package has attracted the interest of other grain authorities in the region, and funding is been provided from ACIAR and the Australian Agency for International Development (AusAID) to extend the scope and uptake of the project outcomes to Vietnam and the Philippines (Project 1997/131). Within the small project, a module on mycotoxins will be added to the training package.

Postharvest Technology for Grains, Fruit and Vegetables

ACIAR has an ongoing commitment to support R & D that will benefit the Australian rural industries, through initiatives to improve quality and reduce losses in Australia and improve the capability of partner countries for storage, transportation and management of their grain reserves. Recent, current and pipeline (Table 3) initiatives include:

Table 3. ACIAR Postharvest Technology portfolio with projects grouped by postharvest strategy outcome (and proposed projects under development in italics).

1) Enhancement of produce quality and suitability (PHT Outcome 1)	2) Postharvest systems improvement (PHT Outcome 2 & 4)
Projects	***Projects***
PHT	PHT
94/045 Shelf-life extension by molecular transformation of papaya and mango (Philippines and Malaysia)	94/037 Grain drying in China (China)
94/007 Pineapple quality improvement (Malaysia)	93/877 Low cost disinfestation systems (Thailand, Vietnam)
94/016 Shelf life of leafy vegetables (China)	95/136 Cocoa fermentation, drying and quality evaluation (PNG)
Small projects	***Small projects***
	97/131 Computer aided learning to improve grain store pest management (Co-funded under AusAID APEC) (Vietnam; Philippines; Indonesia)
	97/161 *Constraints to banana industry development (Indonesia)*
Improvement of pest & disease control (PHT Outcome 2)	**Reduction of contaminant risks or environmental impact (PHT Outcomes 3 & 5)**
Projects	***Projects***
94/015 Phosphine resistance in stored grain pests (China, India)	94/004 *Reducing mycotoxins in pesticide residues in food (Vietnam)(phase 2)*
95/134 *Phytophthora* in durian (Thailand/Vietnam)	96/009 *Replacement of methyl bromide for timber fumigation (Malaysia; PNG) (phase 1)*
97/094 *Fruit resistance to disease (Sri Lanka) (phase 1)*	97/017 *Reducing aflatoxin in peanuts using biocontrol and management strategies (Indonesia).*
98/137 *Enhancing the efficacy of phosphine fumigation (China, Vietnam) (phase 1)*	
Small projects	***Small projects***
96/152 Postharvest diseases in melons (China)	98/059 *Pesticide risk reduction strategies for sustainable pest management (Philippines)*
96/193 *Phytophthora in Southeast Asia (ASEAN) (phase 4)*	

Recently completed projects:

Applications of in-store drying in the grain industry in Southeast Asia (Project PHT/1990/008). Completed 31 December 1996. This project extended two-stage grain-drying strategies for cereal grains in the humid tropics from large-scale, mainly government operations to the small-scale private sector (mainly traders, millers and farmer cooperatives) (Srzednicki 1996; Champ et al. 1996). **Commissioned Organisation:** University of New South Wales (R. Driscoll and G. Srzednicki). **Collaborators:** National Postharvest Institute for Research and Extension, Philippines; King Mongkut's Institute of Technology, Thailand; University of Agriculture and Forestry, Vietnam.

Development of quarantine disinfestation protocol for an oriental fruit fly (*Bactrocera papayae*) with hot air (Small Project PHT/1994/937) Completed 30/6/1997. This small project developed an oriental fruit fly quarantine disinfestation protocol based on the hot air treatment, to the standards required for fruit export to Japan and New Zealand. Working in Malaysia, the scientists tested a hot water protocol on Australian mango as an early response to the 1995 Australian outbreak of *B. papayae*. They compared the disinfestation

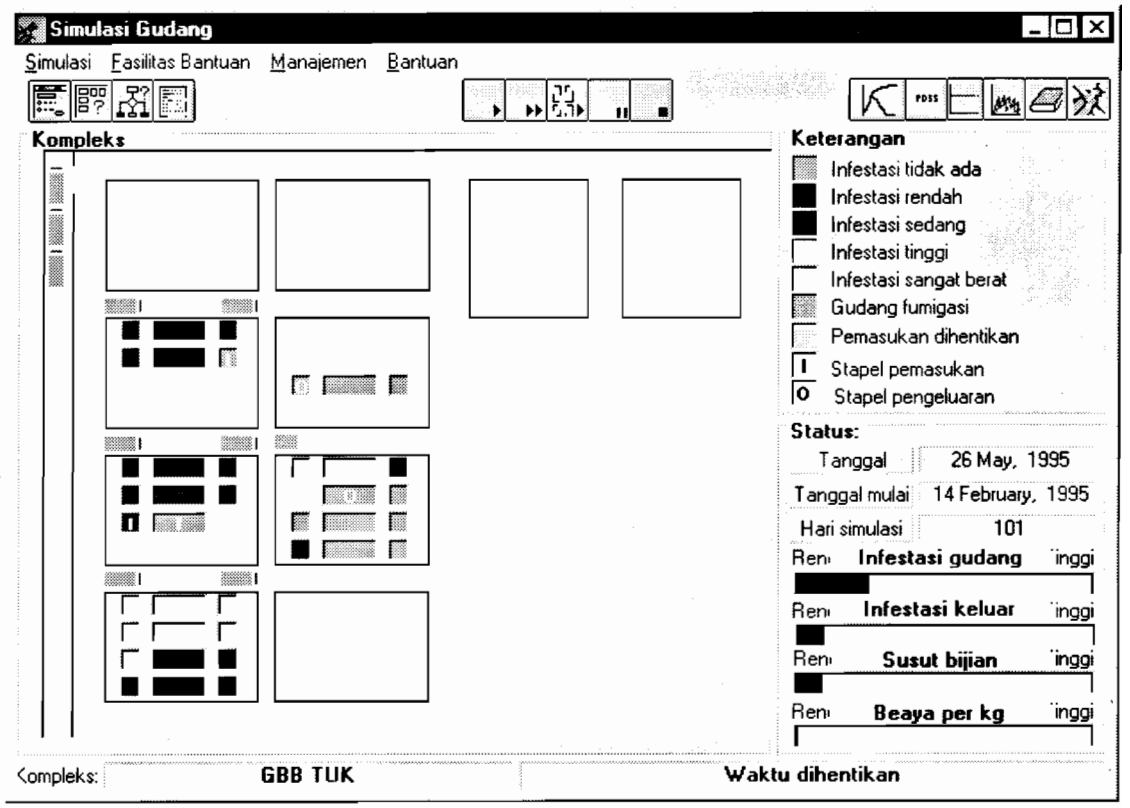

Figure 2. A typical screen from the Simulator program in the training package, showing the layout of a warehouse complex in Indonesia. Each stack within each warehouse is monitored by the software, so that management processes, such as inloading and outloading of grain, sampling, chemical treatment and fumigation are recorded. A population growth model runs within each stack and insect densities are indicated by different colours. Grain losses and treatment costs are shown in the lower right of the screen (Longstaff 1997).

parameters established for another fruit fly species, *Bactrocera tryoni*, to determine the suitability of these protocols for *B. papayae*, and looked for side effects on fruit quality that could arise from application of the disinfestation schedules. **Commissioned Organisation:** Queensland Department of Primary Industries (R. Jordan); **Collaborators:** Australian Quarantine and Inspection Service (AQIS). Malaysian Agricultural Research and Development Institute (MARDI), Malaysia.

Development and application of simple test kits for pesticide residues in plant-derived foods (Project PHT/1993/009). Completed 30 June, 1998. This project developed and introduced a range of simple, affordable tests using specific antibodies and enzyme–pesticide complexes for individual pesticides that already cause concern in the collaborating countries (India and Australia) (Skerritt 1997; Karanth 1998). **Commissioned Organisation:** CSIRO Division of Plant Industry (J. Skerritt). **Collaborators:** University of Sydney; Central Food Technological Research Institute, Mysore (Dr N.G.K. Karanth); Osmania University, Hyderabad (Dr B. Sashidhar Rao), India.

Disease control and storage life extension in tropical fruit (Project PHT/1993/013). Completed 31 December 1997. Previous research between Australia and Thailand (projects PHT/1988/044 and 1983/056) has identified disease agents in some tropical fruits. This project built on that research, combined with new findings by other research groups, to improve prospects for developing non-chemical controls for the diseases anthracnose and

stem end rot. Project scientists also studied how postharvest storage potential was affected by environmental stresses that occur during development, and also focus on factors determining disease incidence and severity (Hofman et al. 1998; Joyce et al. 1998). **Commissioned Organisation:** CSIRO Division of Horticulture (L. Coates QDPI). **Collaborators:** Queensland Department of Primary Industries; Kasetsart University, Thailand; Chiang Mai University, Thailand.

The development of decision-support tools for managing pests in grain storages (Project PHT/1993/021). Completed 31 December, 1997. This project established information on best practices for pest management in gain storage facilities in Indonesia and Australia. The data formed the basis for decision-support systems to help practitioners to make the correct choices for maintaining grain quality through judicious use of grain protectants and fumigants. A CD-ROM based training package was produced, with the intention of refining and implementing it in a follow-on small project (PHT/1997/131) co-funded under the AusAID APEC program (Longstaff 1997). **Commissioned organisation:** CSIRO Division of Entomology, Canberra. (B. Longstaff). **Collaborators:** Centre for Tropical Pest Management, Queensland; Queensland Department of Primary Industries; Badan Urusan Logistik (BULOG), Indonesia.

Active projects and projects under development for possible commencement in 1999 to 2001

1) Enhancement of produce quality and suitability (PHT Outcome 1)

Pineapple quality improvement (Project PHT/1994/007). (Active 1 January 1996 to 30 June 1999). This project will first determine the enzyme(s) responsible for blackheart injury, then apply successive techniques of molecular biology to develop a genetically transformed pineapple that inhibit the expression of blackheart. In separate studies scientists will use conventional methods to determine the physiology of tissue breakdown that leads to crown deterioration, then develop strategies to prevent its occurrence. The project objectives include production of a transgenic pineapple ready for glasshouse evaluation, a molecular transformation system for pineapple and practical solutions to reduce the incidence of crown deterioration. **Commissioned Organisation:** Queensland Horticulture Institute, Department of Primary Industries (M. Smith). **Collaborators:** Malaysian Agricultural Research and Development Institute (MARDI); CSIRO Division of Horticulture, Adelaide; Queensland Agricultural Biotechnology Centre.

Control of ripening in papaya and mango by genetic engineering (Project PHT/1994/045). Active 1 July 1997 to 30 June 2000. This project will be the use of genetic engineering techniques to extend fruit storage-life of papaya. The project researchers will identify, isolate and clone two important genes in the ripening process: 1-aminocyclopropane 1-carboxylate (ACC) synthase and polygalacturonase (PG). They will then prepare antisense constructs, and introduce the altered genes back into the plant using a previously developed transformation system. As well tissue culture and embryogenesis systems that will facilitate genetic manipulation of mango will be produced. **Commissioned Organisation:** University of Queensland, Botany Department (J. Botella). **Collaborators:** Malaysian Agricultural Research and Development Institute (MARDI); Institute of Plant Breeding, University of the Philippines at Los Banos (UPLB); Queensland Department of Primary Industries.

Shelf-life extension of leafy vegetables (Project PHT/1994/016). Active 1 July 1998 to 30 June 2001. This project aims to improve procedures in the Chinese handling system and thus reduce postharvest wastage of leafy vegetables. The scientists will determine which agronomic and postharvest handling procedures increase shelf life, and identify what physiological factors limit shelf-life so that they can focus on them in future biotechnological control and breeding strategies. The specific commodities for study are Chinese cabbage, pak choi, broccoli, asparagus and oriental bunching onions. Saving will occur through reduced wastage and access to further markets, and minimal processing will add value to these commodities. **Commissioned Organisation:** Queensland Horticulture Institute, Department of Primary Industries (T.O'Hare). **Collaborators:** Beijing Vegetable Research Centre, Beijing; Hangzhou Institute of Commerce, Hangzhou, China; University of Adelaide; Institute for Horticultural Development, Knoxfield, Australia.

Fruit resistance to disease (Project PHT/1997/094). Phase 1 approved, start date not yet set. This project will continue research to develop novel and improved controls for anthracnose and stem end rot diseases in tropical fruit (PHT/1988/044, PHT/1993/013). This project will monitor antifungals in fruit as they are subjected to a variety of elicitor treatments (heat, microbial challenges; UV, antioxidants), and assess the potential

for utilising or enhancing cultivar resistance by classic and molecular techniques. **Commissioned Organisation:** The University of Queensland (D. Irving). **Collaborators:** University of Peridenya, Sri Lanka; Queensland Horticulture Institute, Department of Primary Industries, Australia.

2) Postharvest systems improvement (PHT Outcome 2 & 4)

In store drying of grain in China (Project PHT/1994/037). Active 1 January 1997 to 31 December 2000. This project aims to design safe grain-drying systems that overcome the problems caused when wet maize and rice are stored in north eastern and subtropical southern China. Project staff will assemble existing data on economics, weather and grain handling. The data will form the context for computer models of the effects of various drying systems on grain quality and efficiency of grain handling. On the basis of model results, project staff plan to design, recommend and test specific drying systems with technical and economic characteristics to suit the various grain-handling depots. Finally, they will assess the systems at commercial scale and educate all users, by extension and training. **Commissioned Organisation:** University of New South Wales (R. Driscoll and G. Srzednicki). **Collaborators:** Ministry of Internal Trade (MIT), China.

Low cost disinfestation systems for fruit (Project PHT/1993/877). Active 1 January 1998 to 31 December 2000. This project will investigate alternative (low cost) heat systems to the currently available expensive heat treatments for the disinfestation of fruit. It will reduce the complexity of the technology and lead to major reductions in cost, opening up the treatment to use at the farm level. Outcomes of the project will be:

- prototype equipment built, tested and demonstrated in Australia, Thailand and Vietnam;
- modified conditions for the disinfestation heat treatment of the priority crops in Thailand and Australia, which will allow treatment to be undertaken using simple low cost equipment;
- disinfestation research work commenced in Vietnam;
- development of facilities and expertise in Vietnam for undertaking horticultural quarantine treatments, and postharvest storage research.

Commissioned organisation: Queensland Horticulture Institute, Department of Primary Industries (R. Jordan). **Collaborators:** Department of Agriculture, Thailand; Department of Plant Protection, Quarantine Section, Vietnam (disinfestation treatments) and the Research Institute for Fruit and Vegetables, Vietnam (fruit quality responses).

Cocoa fermentation, drying and genotype product quality assessment (Project PHT/1995/136). Active 1 January 1998 to 31 December, 2000. This project is one of a suite of ACIAR–PNG projects proposed for funding by the Australian Agency for International Development (AusAID). The project will evaluate the effects of minibox (250 kg) fermentations and solar drying technology and cocoa genotype on cocoa quality in PNG. The fermentation boxes will allow growers to prepare smaller volumes of harvested cocoa beans for fermentation and drying. The scale of the technology is well suited to household and village/family group cocoa production volumes. Complementary activities on cocoa product evaluation will be undertaken in Australia (Hollywood et al. 1997). **Commissioned Organisation:** Queensland Department of Primary Industries, Centre for Food Technology (N. Hollywood). **Collaborators:** University of New South Wales, Department of Food Science and Technology; Papua New Guinea Cocoa and Coconut Research Institute, Papua New Guinea.

Computer-aided learning as a tool to improve training standards in grain storage management in ASEAN countries (Small Project PHT/1997/131). Active (1 January 1998 to 31 December 1999). The major objective of the small project and the complementary AusAID (APEC) funding is to demonstrate the benefits of employing Computer-Assisted Learning (CAL) tools in the education of grain-storage personnel. A training course will utilise the suite of bilingual (English/Bahasa), multimedia Computer-Assisted Learning (CAL) tools, called 'Pest Management Workbench', that were developed in ACIAR project PHT/1993/021 in order to facilitate the rational and sustainable management of pests in grain storages in Indonesia, Vietnam and the Philippines. An important secondary objective of the small project/AusAID activity is to assist collaborating institute personnel in identifying and developing the mechanisms by which this new approach can be integrated into the training infrastructures of their organisations. **Commissioned Organisation:** CSIRO Division of Entomology, Canberra (B. Longstaff). **Collaborators:** Badan Urusan Logistik (BULOG), Indonesia; BIOTROP, Indonesia, Postharvest Technology Institute, Ho Chi Minh City, Vietnam; Bureau of Postharvest Research and Extension (BPHRE), Philippines.

Market based analysis of constraints to banana industry development in Indonesia and Australia (PHT/1997/161). Start date not yet set. This small project will develop a participatory process to

identify the major constraints to the competitive performance of a horticultural industry in Indonesia and Australia to:

- assist in industry development, and to
- more effectively direct R&D to priority areas with the greatest potential for improving producer profitability.

The project will test the feasibility of using the concepts of product market performance and supply chain management (management of the product from planting to consumption) both as drivers of industry development and to guide the contribution of R&D to industry development. **Commissioned Organisation:** Queensland Horticultural Institute, Department of Primary Industries, Nambour (P.J. Hofman). **Collaborators:** Ministry for Food and Horticulture, Indonesia; University of Queensland, Australia.

3) Improvement of pest & disease control (PHT Outcome 2)

Phosphine resistance in stored grain (Project PHT/1994/015). Active 1 January 1997 to 31 December, 1999. This project aims to develop and implement management practices that will delay further development of phosphine resistance stored grain pests in China, Australia and India. **Commissioned Organisation:** Farming Systems Institute, Department of Primary Industries (G. Daglish). **Collaborators:** Ministry of Internal Trade, Beijing; Chengdu Grain Storage Research Institute, China; Zhengzhou Grains College, China; Guangdong Institute of Cereal Science Research, China; Central Food Technology Research Institute, Mysore, India.

Management of Phytophthora diseases of durian (Project PHT/1995/134). Active 1 July 1997 to 30 June 2001. This project will develop strategies to control fruit rot, patch canker and dieback of durian (*Durio zibethinus* M.) caused by *Phytophthora palmivora*). The primary objective will be to improve orchard sustainability and the consistency of fruit supplies and quality in Vietnam, Thailand and Australia, by developing an integrated program of orchard and postharvest management of *P. palmivora.* Key elements of the strategy will be definition of the biological interactions involved and the development of integrated control strategies based on biological and environmental variables, and include the application of phosphonate derivatives by trunk injection.

See: http://www.botany.unimelb.edu.au/labs/mycology/durianWeb/index.html

Commissioned Organisation: University of Melbourne, School of Botany (D. Guest). **Collaborators:** Kasetsart University, Thailand; Southern Fruit Research Institute, Vietnam, Northern Territory Department of Primary Industry and Fisheries and the Queensland Department of Primary Industries.

Postharvest handling and disease control in melons (Small project PHT/1996/152). Active 1 July 1997 to 30 June 1999. This small project will document the harvest, handling and marketing of melons in Western China and assess the feasibility of using novel 'defence eliciting' treatments for postharvest disease control. Novel strategies are urgently required to replace benomyl, withdrawn from postharvest use in Australia at 31/12/1996. The aims will be approached by the following means:

1. Documentation of postharvest melon handling systems and the major causative agents responsible for postharvest melon loss in China;
2. Investigation of the factors affecting the resistance of melons to the diseases. These include a) test of resistant cultivars, b) evaluation of systemic inducing resistance (SIR) agents and c) understanding of environmental factors on the expression of host resistance;
3. Preliminary development of biocontrol methods for control of postharvest melon diseases.

Commissioned Organisation: The University of Sydney (Y Huang). **Collaborators:** China Agricultural University; Ganshu Melon Research Institute; General Seed Management Station of Xinjiang; Sichuan Pomological Institute, China; and the Sydney Postharvest Laboratory.

Phytophthora in Southeast Asia (PHT/1996/193) Phase 4 start date not yet set. This small project will assess the range and severity of diseases caused by *Phytophthora* spp. in Southeast Asia and make some preliminary assessment of variability. Options for future research complementary to project PHT/1995/134 will be developed. **Commissioned Organisation:** The University of Queensland (A. Drenth). **Collaborators:** Several research institutes in ASEAN countries.

Enhancing the efficacy of phosphine fumigation (PHT/1998/137) Phase 1 approved, start date not yet set. This project will develop recommendations for national fumigation standards for phosphine to improve fumigation practice in China, Australia and Vietnam. The project will devise technical innovations to enhance the efficacy of phosphine, develop management strategies to control emerging pests (especially psocids), and characterise phosphine resistance in new strains of major pests. The project will build on research collaborations between Australia and China under PHT/1999/015 *Phosphine resistance in stored grain*, and extend involvement

to Vietnam. **Commissioned Organisation:** Farming Systems Institute, Department of Primary Industries (P. Collins). **Collaborators:** State Administration of Grain Reserves, Beijing; Chengdu Grain Storage Research Institute; Zhengzhou Grains College; Guangdong Institute of Cereal Science Research, China; Ministry of Agriculture Research and Development, Vietnam.

4) Reduction of contaminant risks or environmental impact (PHT Outcomes 3 & 5)

Reducing mycotoxin and pesticide contamination in grain, fruit and vegetables in Vietnam (Project PHT/1996/004). Phase 2 approved, start date not yet set. In this project, a range of the commodities produced in Viet Nam will be sampled and the incidence and severity of contamination by mycotoxins (esp. aflatoxins, fumonisins and alternaria toxins) and pesticides will be quantified. Sampling protocols and field-laboratory immunoassays for total mycotoxins and pesticides will be developed and Vietnamese researchers at provincial laboratories will be trained in their use. Following the initial survey, a network for the ongoing monitoring of mycotoxin and pesticide contamination in agricultural produce (food and feed) and fermented foods in regional laboratories will be strengthened. **Commissioned Organisation:** University of Sydney, Australia (I. Kennedy). **Collaborators:** Postharvest Technology Institute, Food and Commodities Control Center; Pasteur Institute, Ho Chi Minh City; University of Agriculture and Forestry, Vietnam; CSIRO Division of Plant Industry; Australian Wheat Board (AWB) Research Pty Ltd, Werribee, Australia.

Replacements for methyl bromide in timber for quarantine fumigation (Project PHT/1994/006) Phase 2 in preparation, start date not set. This project will seek replacements for methyl bromide for the commercial fumigation of timber, with the selection made from among: sulfuryl fluoride, phosphine, hydrogen sulphide, carbonyl sulphide, methyl ithiocyanate and carbon bisulphide for control of timber pests. Rapid methods to measure fumigant sorbtion and penetration through timber will be tested, and the methodology verified on tropical hardwoods using methyl bromide and oregon timber as reference standards. In addition, the potential for thermal disinfestation during kilning will be investigated. **Commissioned Organisation:** CSIRO Division of Entomology, Stored Grain Research Laboratory. **Collaborators:** Forest Research Institute of Malaysia, Malaysia; Papua New Guinea Forest Research Institute, Papua New Guinea.

Reducing aflatoxin in peanuts using biocontrol and agronomic management strategies in Indonesia and Australia (PHT/1997/017). Start date not yet set. This project will improve management systems for reducing aflatoxin contamination in peanuts in Indonesia and Australia. It will:

- Survey Indonesian peanuts at various stages in the food delivery chain (farm to retailer) for incidence of *Aspergillus flavus* and aflatoxin, in order to assess the magnitude of the pre- and postharvest aflatoxin problem.
- Develop, evaluate and adapt the biocontrol approach for aflatoxin control, using competitive non-toxigenic strains of *A. flavus* applied to soil, in Indonesian and Australian farming systems.
- Develop and implement integrated packages of agronomic management and varietal options that minimises late season kernel moisture stress and hence aflatoxin risk in Indonesian and Australian cropping systems.
- Use a crop modelling approach to define the probability of aflatoxin risk for peanuts grown in various Indonesian regions/cropping systems, and hence assist in defining peanut research objectives (e.g., optimum maturity varieties in peanut breeding programs).

Commissioned Organisation: Farming Systems Institute, Department of Primary Industries, Brisbane (J. Wright). **Collaborators:** Research Institute for Legume and Tuber crops, Malang; Gadja Madah University; BIOTROP, Indonesia; Food Science Australia, Sydney Australia.

Pesticide risk reduction strategies for sustainable pest management (Small project PHT/1998/059). Start date not yet set. In this small project, strategies for pesticide risk reduction will be developed and tested. The current policies, research and development needs and regulatory infrastructure for minimising pesticide residue risks in food and the environment in the Philippines will be documented. A consultative process modelled on that used to develop Australia's National Strategy for the Management of Agricultural and Veterinary Chemicals will be used, with involvement of government agencies, scientists, producers, pesticide industry personnel and produce marketers. The consultations will be used to define improvements in policy, regulatory infrastructure, training and research and development plans to improve implementation of strategies for minimising pesticide levels in produce and the environment. High-risk production systems and environmental hazards for priority attention will also be identified, to ensure the strategies developed will be adequate. Options for the development of a

National Strategy for the Philippines will be explored using two commodity case-studies — potatoes and leafy vegetables. In Australia, procedures for implementing the National Strategy will be developed using potatoes and leafy vegetables as case studies. **Commissioned Organisation:** Agriculture, Forestry and Fisheries Australia. **Collaborators:** Agencies the Philippines; Institute for Horticultural Development, Knoxfield Victoria.

Postharvest Technology in All ACIAR Programs

ACIAR's postharvest technology research has traditionally been concentrated on crops. A key strategy for increasing investment in postharvest technology research has been to identify relevant opportunities and priorities in other program areas (Table 4). In close consultation with partner countries, several current and future research topics have been identified.

These include:

- Production of particle-board using acacia and eucalypt pulp in the Philippines (Forestry Program, FST/1995/000) (Outcome 1)[1].
- Management of rodent pests in Southeast Asia and Vietnam (Animal Sciences 1, AS1/1994/020 and AS1/1996/079) (Outcome 2).
- Management of tannery waste in India (Land and Water 1 Program, LWR1/1993/022) (Outcome 3).
- Wool processing and treatment of scouring effluent, with China and India (Animal Sciences 1 Program, AS1/1997/049; AS1/1997/070) (Outcome 3).
- Studies on the nutrient content of some Pacific Island Foods (Crop Sciences 2, CS2/1993/006) (Outcome 5).
- Constraints to production and consumption of nutritious foods in Fiji, a joint feasibility study by the ANRE, PHT and Crops Science Programs (Outcome 5).

Priority topics for collaborative research funding are listed:

Crop Products

- Controlling deterioration, disease and defects by genetic modification of fruit, vegetables and grain.
- Phosphonate injection technology and other novel treatments for disease control or storage life extension in fruit and vegetables.
- Postharvest technology for cocoa, coffee and other smallholder cash crops.
- Low-cost systems for fruit fly control and disinfestation.
- Pest and product quality management during drying, storing and marketing.
- Replacement treatments for methyl bromide and sulphur dioxide fumigation.
- Improving dietary intake of nutrients and reducing intake of toxins and digestion inhibitors.
- Technology for detection and remediation of produce contamination.
- Policy and regulatory interventions to monitor and control food contaminants.

Agricultural and Natural Resource Economics

- Developing recommendations for policy and institutional reforms to facilitate international trade and harmonise food safety and quarantine regulations.
- Assure food and nutrition security, promote postharvest quality maintenance and loss reduction, and encourage consumer confidence.
- Promote gender equity and occupational health and safety.

Animal Products

- Feed quality enhancement and contamination control.
- First stage processing to improve wool quality and utilise livestock by-products.
- Species confirmation tests.
- Meat quality enhancement.
- Reducing adverse environmental impacts of processing facilities.

Fish Products

- Reduction of contaminants in fish products.
- Technology that preserves the texture and flavour of aquatic produce and extends shelf life.

Forestry

- Synchronisation of harvest rate of forest trees with processing and kilning capacities.
- Improving the yield of useable timber and extending timber product life.
- Developing policies, monitoring and handling systems and technologies in forests, mills and timber processing facilities and timber structures which minimise environmental pollution.

Land and Water Management.

- Minimising offsite pollutants from postharvest enterprises.
- Productive use of crop residues.

1. Outcomes of the ACIAR Postharvest Strategic Plan.

Table 4. The relative priority of resource commitment to research strategies across commodity group or ACIAR program area, with *** indicating high emphasis, ** indicating moderate emphasis, and * indicating minor emphasis to the strategy.

	Crop products	Animal products	Forest products	Fishery products	Agricultural & Natural Resource Economics	Land and Water impacts
Outcome 1. High quality, robust produce suited to market requirements						
1.1 *Through strategic (or basic?) research and genetic modification, improve or modify produce quality, utility and resistance to decay or deterioration.*	***					
1.2 *Consider and optimise the impact of production environments and technologies on postharvest quality & safety, and product suitability.*	**	*	**	**		*
1.3 *Develop postharvest technologies that conserve and promote the vigour and viability of seed and planting material.*	*		*			
Outcome 2. Postharvest technology systems and packaging which reduce losses, minimise costs and optimise produce suitability and quality.						
2.1 *Through modelling, systems analysis and technology development, introduce affordable harvesting, drying, handling, packaging and storage systems to conserve the suitability and quality of produce and improve temporal and energy efficiencies and effective pest management in the postharvest system.*	***		**	**		
2.2 *Devise effective Quality Assurance (QA) protocols or improvements to work practices which improve (i) personnel performance and the operating effectiveness of postharvest systems and (ii) uptake of appropriate technology at the farmer and household level, to reduce losses and improve returns.*	**	*	*			
2.3 *Devise first stage processing treatments or technologies which improve and optimise product suitability.*	*	***	**			
Outcome 3. Improved environmental safeguards in postharvest systems.						
3.1 *Develop technologies and systems to detect, eliminate or reprocess pollutants arising as by-products or waste from postharvest treatment or processing facilities.*		*	*			*
3.2 *Develop monitoring and remediation systems which minimise the risk of agrochemicals or environmental pollutants contaminating produce during production, transport and processing.*	***	***		*		*

Table 4 (con't)

	Commodity group or ACIAR Program area					
	Crop products	Animal products	Forest products	Fisheries products	Agricultural & Natural Resource Economics	Land and Water impacts
Outcome 4. Enhanced food security, trade and market access delivering improved returns to producers, traders and processors.						
4.1 *Develop and test novel treatments, technologies or management systems to assure product quality, satisfy regulatory requirements or reduce produce losses.*	***	**	**			
4.2 *Develop and apply technologies and systems to (a) monitor or minimise risks of product contamination by pesticides, mycotoxins or microorganisms and (b) confirm the identity of species or varieties.*	**	*	*			
4.3 *Devise and promote trade and regulatory policies which promote consumer confidence and facilitate market improvements*	**	*			***	
Outcome 5. Improved health and nutrition of consumers and livestock.						
5.1 *Investigate the socio-economic, institutional and technical constraints to the marketing and consumption of nutritious food.*	*				*	
5.2 *Establish and promote effective monitoring and remediation systems to eliminate mycotoxins pesticides, pathogens and toxic principles from food and feed.*	**	**				
Outcome 6. Increased postharvest research capability in the National Agricultural Research Systems (NARS) and international agencies.						
6.1 *Promote dialogue within CGIAR of postharvest research imperatives.*	*		*		*	
6.2 *Develop opportunities for interagency collaboration and complementarity through the Group for Assistance on Systems relating to Grains After harvest (GASGA), a research collaboration group involving ACIAR, NRI, CIRAD, FAO and GTZ.*	***				*	
6.3 *Develop complementary projects between NARS and international agencies which improve the postharvest impact of their research.*	*	*	*	**	*	

Conclusions

This document has briefly outlined the rationale of ACIAR's framework for research and development on postharvest technology, and the scope of the current suite of projects. ACIAR's strategic emphasis on postharvest R&D during 1997–2000 will help producers and marketers to achieve greater efficiencies and reduced losses in postharvest systems in Australia and its partner countries. Exploiting opportunities for co-funding and synergistic collaborations are important mechanisms by which the 'quantum leap' of effort can be made. ACIAR welcomes comments and suggestions on priorities for future research and collaboration that would be of regional significance and which would match the national priorities of partner countries.

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Appendix 1

ACIAR supported initiatives in mycotoxin research

Fungi and mycotoxins in Asian food and feed stuffs (PHT/1988/006). Completed project (1988–1991).

This project studied the distribution, prevalence and importance of postharvest spoilage, primarily in cereals, but also in other durable food and feedstuffs in Indonesia, Thailand and the Philippines. The scientists sought to enumerate, isolate and identify to species level fungi growing in specific commodities of both high and low grade, to determine which mycotoxins each fungus could produce and to assess each one's significance as a spoilage fungus or mycotoxin-producer, according to its relative prevalence in samples. Having thus identified specific mycotoxins, they also assayed samples of each major commodity throughout its postharvest history to determine its mycotoxin status.

The project team established a computer database covering significant fungi, including the incidence of particular species, the mycotoxins produced and major factors influencing mycotoxin production by particular species. Work also commenced on the development of computer-assisted keys to explore techniques for simplified identification of important toxin-producers and sought simple techniques for monitoring quality. Training programs for Asian microbiologists and chemists on the standard methods was undertaken to ensure comparable assessments of mycological and mycotoxin quality throughout the region.

As its secondary aims, the project assessed where appropriate, the impact of improved handling, drying and storage procedures on the incidence of both fungi and mycotoxins, and solutions to any identifiable problems. More fundamental studies were undertaken to investigate the influence of temperature and water activity on growth or toxin production by significant fungi.

Commissioned Organisation: CSIRO Division of Food Science, (Pitt, J.), **Collaborators:**

Australian Wheat Board, D. Webeley, Research Institute for Veterinary Science, Sjamsul Bahari; Gajah Mada University, Z. Noor; SEAMEO BIOTROP, O. Dharmaputra, Indonesia; National Postharvest Institute for Research and Extension, S. Andales, Philippines; Division of Plant Pathology and Microbiology, Department of Agriculture, Prawat Tanboon-Ek, Thailand.

Occurrence and distribution of *Aspergillus flavus* and aflatoxins in Asian peanuts (PHT/1991/004). Completed project (1991–1994).

This project sought to establish when *Aspergillus flavus* invaded Thai peanut plants and nuts in order

to gauge whether competitive inhibition of aflatoxin production was feasible. The rationale for the project was based on established data that aflatoxin contamination was a major economic and health problem in Southeast Asian foods and feeds, with levels of aflatoxin in retail peanuts ranging up to 550 µg/kg and in maize up to more than 4000 mg/kg (compared to an Australian limit of 15 mg/kg). In addition, preliminary research by CSIRO had shown that *A. flavus*, the major source of aflatoxins, invaded peanut plants and developing nuts prior to harvest. Subsequent work established that it was feasible to control toxigenic *A. flavus* by competitive inhibition with non-toxigenic strains of the same species under field conditions in Australian growing areas.

Project research in Thailand confirmed that a significant amount of invasion by *A. flavus* occurs prior to harvest while glasshouse and field studies in Australia indicated that the use of non-toxigenic strains had potential for the control of aflatoxin contamination, with reduction in aflatoxin up to 80% in some plots. However, further research remained necessary in order to develop reliable implementation strategies.

Commissioned Organisation: CSIRO Division of Food Science, (Pitt, J.). **Collaborator:** Division of Plant Pathology and Microbiology, Department of Agriculture, Prawat Tanboon-Ek, Thailand.

ACIAR Economic Evaluation Unit appraisal of PHT 1988/006 and 1991/104.

This study by Lubulwa and Davis (1996) calculated the potential benefits of two ACIAR projects on fungi and aflatoxins while acknowledging that the projects were not designed to develop technologies for the control of aflatoxin contamination in maize and peanuts. The information the two projects generated could be catalytic in the development of technologies applicable at the farm level or in the postharvest sector to reduce or even eliminate aflatoxin in maize and peanuts and aflatoxicosis in the human population and in livestock in Indonesia, Philippines and Thailand.

Lubulwa and Davis (1996) estimated that a technology that eliminated aflatoxins in maize and peanuts could generate up to A$755 million of discounted benefits over a 30-year time horizon and an associated internal rate of return of 66%.

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Lubulwa, G. and Davis, J. 1996. Completed-project economic assessment of two ACIAR projects on fungi and aflatoxins: A discussion of methodology issues and some estimates of potential benefits. In: Highley, E. and Johnson, G.I. ed. ACIAR Technical Reports, 37: 66–111. *This paper includes a bibliography of scientific publications arising from PHT 1988/006 and 1991/004 (110–111).*

Applications of in-store drying in the grain industry in Southeast Asia (Project PHT/1990/008). Completed project (1993–1996).

This project extended two-stage grain-drying strategies for cereal grains in the humid tropics from large-scale, mainly government operations to the small-scale private sector (mainly traders, millers and farmer cooperatives) (Srzednicki 1996; Champ et al. 1996). Some evaluation (ergosterol assays) was made of the utility of grain drying technologies in reducing fungal contamination.

Commissioned Organisation: University of New South Wales (R. Driscoll and G. Srzednicki). **Collaborators:** National Postharvest Institute for Research and Extension, Philippines; King Mongku's Institute of Technology, Thailand; University of Agriculture and Forestry, Vietnam.

Development and application of simple test kits for pesticide residues in plant-derived foods (Project PHT/1993/009). Completed project 1993–1998.

This project developed and introduced a range of simple, affordable tests using specific antibodies and enzyme–pesticide complexes for individual pesticides that already cause concern in the collaborating countries (India and Australia) (Skerritt 1997; Karanth 1998).

Project PHT/1993/009 developed assays for a number of key residues in Australia, applied them to food matrices in both countries to demonstrate that the methods can be performed reliably in developing country conditions. With these objectives achieved, the next step was to establish the scientific independence of the Indian work. This was facilitated in an 18 month extension of PHT/1993/009 (1997–1998), through the involvement of another laboratory in India (Osmania University) with expertise in development of immunoassays for related small toxins, such as mycotoxins. A major aim of the project extension was establishment of expertise in all areas of immunoassay for agrochemical residues within India, including chemistry for hapten synthesis, hapten-protein conjugation, antibody production and purification, ELISA development and troubleshooting, production and quality control of prototype kits. Project work also continued on the dissemination of immunoassay methods for agrochemical residues to other food analytical laboratories in India, through a network of eight analytical laboratories.

Commissioned Organisation: CSIRO Division of Plant Industry (J. Skerritt). **Collaborators:** University of Sydney; Central Food Technological Research Institute, Mysore (Dr N.G.K. Karanth); Osmania University, Hyderabad (Dr B. Sashidhar Rao), India.

Publications

The Australian Mycotoxin Newsletter

During 1989–1990, agreement was reached between ACIAR and CSIRO on joint publication of the Australian Mycotoxin Data Centre Newsletter as *The Australian Mycotoxin Newsletter* as an insert in the *ACIAR Postharvest Newsletter. The Australian Mycotoxin Newsletter* consists of a comprehensive abstract service for world mycotoxin literature. The newsletter is compiled and edited by J.C. Eyles, A. Hocking, and J.I. Pitt, Food Science Australia, Sydney. The first quarterly issue appeared in March 1990. Recent back issues can be viewed at http://www.aciar.gov.au/aciarptp/myconews.htm.

Mycotoxins or pesticide contaminants

ACIAR Proceedings No. 36. Fungi Mycotoxins in Stored Products. Champ, B.R., et al. ed.

Contamination of stored food and feedstuffs by fungi and the mycotoxins they produce poses a serious health risk for humans and livestock and causes economic losses worldwide. A conference held in Bangkok, Thailand, in April 1991 examined the problem from a global perspective. These Proceedings present an overview of the problem, detailed taxonomy of the spoiled fungi, two sections on the mycotoxins, and two sections on ways to manage the fungal and mycotoxin contaminants. There are also 26 poster papers dealing with case studies in varying situations, and the publication is drawn together with recommendations for future research and strategies to improve product quality throughout the processing-handling-marketing system.

ACIAR Technical Report No. 37. Mycotoxin Contamination in Grains. Highley, E. and Johnson, G.I. ed. 1996 145 p.

Contains papers on mycotoxin contamination presented at the 17th ASEAN Technical Seminar on grain postharvest technology, Lumut, Malaysia, 25–27 July 1995.

ACIAR Proceedings No. 85. Seeking Agricultural Produce Free of Pesticide Residues. Kennedy, I.R., Skerritt, J.H., Johnson, G.I. and Highley, E. ed. 1998 405 p.

The Proceedings of an International Workshop held in Yogyakarta, Indonesia 17–19 February 1998. The workshop reviewed options for monitoring and minimising the contamination of agricultural produce and the environment by residues of pesticides while maintaining the benefits of pesticide use to agriculture. Some of the approaches may have relevance to the management of mycotoxin contamination and some papers concern mycotoxins.

Mycotoxins in Grain

GASGA Technical Leaflet No. 3. 1997 12 p.

A brochure which describes mycotoxins and strategies for managing them. Produced by the Group for Assistance on Systems relating to Grains after harvest (GASGA), of which ACIAR is a member. Published with the assistance of Technical Centre for Agricultural and Rural Co-operation (CTA), the Netherlands, in English French and Portuguese.

Appendix 2

Other Mycotoxin Initiatives

This document contains summaries prepared from file information on related activities.

Rotary 3-H project (France, USA and Ghana, Togo and Benin)

***Food quality control in Africa* (1999–2002)**

This project will educate the people of Ghana, Togo and Benin about the dangers of consuming diseased grain. The project will include multiple suggestions on how to store, sort, clean, prepare and utilise grain so that marketers and consumers have options for reducing mycotoxin risk. It will:

- Train food quality scientists in low-cost, sustainable monitoring methods and the monitoring of grain quality in urban markets.
- Test the feasibility of using amendments to turn aflatoxin contaminated grain into safe animal feed and

- Conduct feasibility studies for the creation of small-scale animal feed industries in West Africa to provide an alternative outlet or poor quality grain.

Co-sponsors: Rotary Club of Cotonou, Benin District 9100; Georgetown Texas USA District 5870 and Montpelier France District 1700. (ACIAR Contact, Mr G. Thiessen, MCP, Montpellier, France)

Reference Project proposal document prepared by project team, as supplied by Mr Gilbert Thiessen, MCP, France.

Survey on aflatoxins in Papua New Guinea peanuts

As part of a broader project with the PNG chemical laboratories, aflatoxin contamination in peanuts have been surveyed. Peanuts were purchased from local markets in several provinces and screened to assess the presence of Aflatoxins B_1, B_2 and G_1 at 5 and 20 ppb levels. Aflatoxins were present at levels between 5 ppb and 20 ppb.

Of the twenty provinces of PNG, five provinces, Morobe, Gulf, East New Britain, National Capital District, and Central were surveyed for aflatoxins. Samples from the Highlands and Momase regions are currently being collected and testing will continue when they are transported to the laboratory. It is expected the remaining provinces will be surveyed during 1998.

Funding: AusAID

FAO-IAEA Co-ordinated Research Program (CRP) on 'Evaluation of Methods of Analysis for Determining Mycotoxin Contamination of Food and Feed'.

The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, responding to the need to contribute to enhance the capability of food control laboratories in Member States, has established a FAO/IAEA Training and Reference Centre for Food and Pesticide Control in Vienna, Austria. One of the activities of the Centre from 1998 will emphasise training and standardising analytical methods for determining mycotoxin contamination of food and feed.

It is essential that the analytical capabilities of laboratories in developing countries are strengthened in order to enable them to effectively monitor the mycotoxin content of food and feed in trade in accordance with the Agreement on Application of Sanitary and Phytosanitary Measures (SPS) and the Agreement on Technical Barriers to Trade (TBT) being implemented by the World Trade Organisation (WTO).

To support this activity of the Centre the Joint FAO/IAEA Division is organising a new Co-ordinated Research Program (CRP) on 'Evaluation of Methods of Analysis for Determining Mycotoxin Contamination of Food and Feed'. This CRP plans to develop research on analytical methods commonly employed by different laboratories involved in export-import food control. Priorities will be given to methods used to detect and quantify mycotoxins in commodities, which represent trade problems and are the object of present or planned regulatory activities. Research emphasis should also be in time and cost efficient methods with good performance characteristics (validation and quality assurance parameters) which have a realistic opportunity of being implemented in developing countries.

Contact: Dr Maya Pineiro, Consultant, Food and Environmental Protection Section FAO-IAEA

Reference: (319-D6.01/21679 Circular from Dr Maya Pineiro, Consultant, Food and Environmental Protection Section FAO-IAEA 1998–08–13)

Monitoring mycotoxins in grain and food production systems for risk management in Vietnam

Part of

FAO-IAEA Co-ordinated Research Program on Evaluation of Methods of Analysis for Determining Mycotoxin Contamination of Food and Feed. This small project involving the University of Sydney, CSIRO and the Postharvest Technology Institute, Ho Chi Minh City, Vietnam will run in parallel with ACIAR project PHT/1996/004 to:

1. To conduct a baseline review and survey for mycotoxin contamination in Vietnam;
2. To schedule a training workshop in Vietnam (September 1999) to train Vietnamese personnel in the use of simple tests (ELISAs) for mycotoxins;
3. Train Vietnamese personnel in Australia in techniques related to development of ELISAs for aflatoxins and *Alternaria* toxins.

Commissioned Organisation: University of Sydney, Australia (I. Kennedy). **Collaborators:** Postharvest Technology Institute, Food and Commodities Control Center (L. Van To); CSIRO Division of Plant Industry (J. Skerritt).

(I. Kennedy, University of Sydney Grant application submitted to FAO-IAEA proposal, 1998)

Joint FAO/WHO/UNEP International Conference on mycotoxins Tunis, Tunisia, 3–6 March 1999

This third mycotoxin conference organised by the Food and Agriculture Organisation (FAO), the World Health Organisation (WHO) and the United Nations Environment Program (UNEP) a will consider new emerging mycotoxins, progress made in sampling, testing and monitoring programs as well as in decontamination procedures, the issuing of mycotoxin regulations by a number of countries, and the greater concern about the health effects of specific mycotoxins.

Objectives

The objectives of the Conference are:

- to increase the awareness of policy makers about the health risks and potential economic impact of mycotoxin contamination of food products and animal feeds, including its impact on international trade of these commodities;
- to provide a forum for the exchange of current scientific and technical information related to mycotoxins;
- to promote the harmonisation of mycotoxin regulations and control procedures; and
- to make recommendations on strategies and programs for enhancing the prevention and control of mycotoxin contamination, thus ensuring the safety and wholesomeness of food supply.

The conference will produce a report, which will contain information on the current scientific knowledge concerning major mycotoxins and their impact on human health, the environment and the national economies of developing countries. It will give particular attention to mycotoxins of emerging concern. The Conference will also identify areas for future research and investigation, and make specific recommendations to governments, to the industry/trade, and to concerned international and national institutions on strategies and programs for enhancing the prevention and control of mycotoxin formation in the field, during storage, and throughout the distribution chain, the decontamination procedures, and the regulatory framework for mycotoxin control.

Reference – FAO Information Circular — Ezzeddine Boutrif e-mail: Ezzeddine.Boutrif@fao.org Tel. +39–06–5705 6156, Food and Nutrition Division, Food and Agriculture Organisation. Fax. +39–06–57054593)

AFTOXNEWS Newsletter of Asia Working Group on Groundnut Aflatoxin Management Issue 1 January 1999

This newsletter of the Asia working group on GN Aflatoxin Management (AFTOXNEWS) will be edited and circulated among concerned country members as an informal information exchange. AFTOXNEWS has also the role to excite the activities on Aflatoxin research and control in practice.

Editor-in-chief: *Prof. Dr PHAN LIEU, Technical Coordinator, Asia Working Group on Aflatoxin Management; Director, Oil Plant Institute of Vietnam, 171–175 Ham Nghi St., Dist. 1, Hochiminh City, Vietnam. Phone: (848) 8243527, Fax: (848) 8243528, Email: opi.vn@hcm.vnn.vn*

Collaboration and Investment Overview—the Growing Innovations Group

P.G. Crawley[1]

Structure

FOUR Growing Innovations companies have been incorporated:

- *Growing Innovations Limited* (GIL)
  This is an unlisted public company owned by Peter Crawley. It is involved in raising, deploying and managing seed and venture funds in spheres of $100 million to commercialise and exploit baskets of agri-science R&D.
- *Growing Innovations Ventures Pty Limited* (GIV)
  This is a wholly owned subsidiary of Growing Innovations Limited. Its purpose is to act as JV partner in research projects at the pre-incorporation stage
- *Growing Innovations Investment Holdings Pty Limited* (GIIH)
  This is owned by Peter Crawley. Its purpose is to hold an interest directly in each project company as an incentive for management to achieve commercial realisation for project companies.
- *Growing Innovations Management Pty Limited* (GIM)
  A wholly owned subsidiary of Growing Innovations Investment Holdings Pty Limited. It has been formed to provide all appropriate management activities and services for project companies under contract to Growing Innovations Limited and Growing Innovations Ventures Pty Limited.

The focus for the GIG is 'agribusiness' in its broadest sense, and includes products, systems and services related to the fields of agriculture, aquaculture, bio-genetics, botany, crops, entomology, environment, feeds, fisheries, food technology, forestry, horticulture, intensive livestock, natural resources, new animal industries, nutrition, plant, pharmacology and veterinary science.

[1]Growing Innovations Ltd, 2 Alana Ct, Chapel Hill, Qld 4069

Project Sources and Development

Projects will be drawn from: Government departments such as Primary Industries (DPI), Natural Resources (DNR), Environment (DE&H), Health (DH), Education (DE); CSIRO, Collaborative Research Centres (CRCs); universities, agricultural colleges and private research organisations. These are all recognised research organisations that are particularly highly regarded both in Australia and importantly overseas. Together, they will generate quality deal flow for the substantial investment from GIL.

Research Organisations Working with Growing Innovations

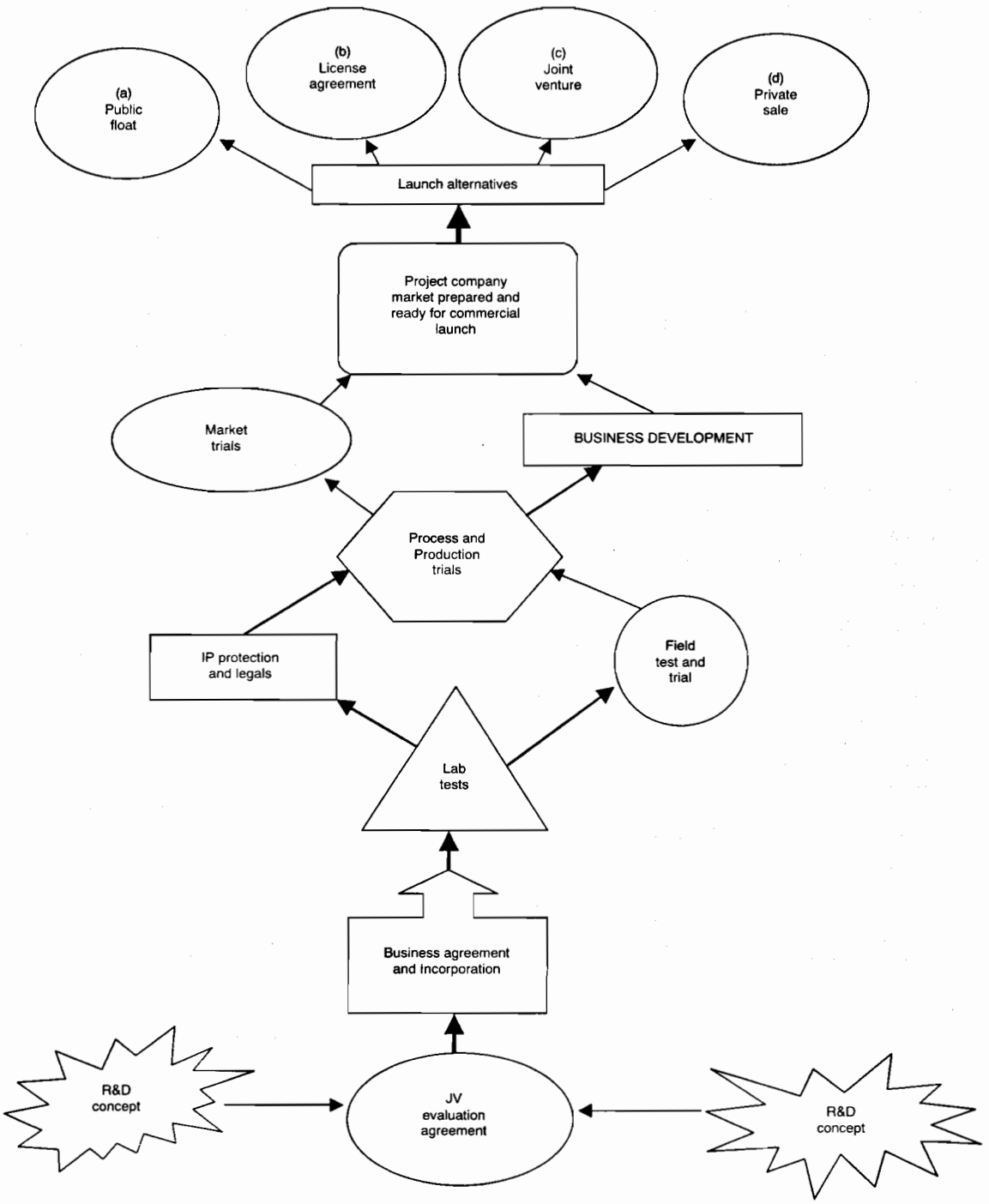
Growing Innovations Limited (GIL) has established agreements with a number of Australia's leading research and development institutions to invest in and commercialise their R&D out-put inventions and innovations.

Projects Investment Template

An investment template has been devised and is now being fine-tuned by Growing Innovations to allow it to proceed with a 'basket' of initial pilot projects. The template can be summarised as follows:

- A project is selected from those introduced to Growing Innovations (from the aforementioned R&D institutions) following initial diligence scrutiny and commercial evaluation.
- A short-form standard JV agreement is entered into by Growing Innovations Ventures Pty Ltd, and Growing Innovations Investment Holdings Pty Ltd with the research organisation and researcher to establish their respective equity interests in the project at concept stage and to provide a broad framework for future collaboration.
- The JV is incorporated subject to achievement of one or more pre-determined milestones. At this

Commercialisation Process Flow



stage, the investment held by Growing Innovations Ventures Pty Ltd is transferred to Growing Innovations Limited.
- Following incorporation, budgeted equity funding is made available to the Project Company through Growing Innovations Management Pty Ltd.
- The Project Company, if successful, will ultimately be sold or listed thereby enabling Growing Innovations Ltd to make a full or partial exit. In some cases, (where the Project Company generates platform IP and/or strong cash flow), Growing Innovations Ltd may decide not to sell down its investment, retaining IP control and licensing the technology to marketing, processing and/or manufacturing companies.

Investment and Exit Strategy

It is envisaged that Growing Innovations Limited will raise its investment funds from both institutional and private investors. In view of the long-term nature of R&D investment requirements, it is most likely the primary investors will be superannuation funds. There is a strong probability that government will take a strategic anchor investment in GIL. Growing Innovations Limited will almost certainly be registered as a PDF (pooled development fund) to secure income tax advantages not otherwise available to investors

Following the successful development of GIL fund 1, it is anticipated the same system and structure will be replicated in each state of Australia. It should be noted that as a result of interest already being shown from investment houses in Europe and the USA, a number of off-shore funds will also be established to take advantage of global IP distribution opportunities thrown up by this Growing Innovations program.

Some Obvious Benefits

- Decrease the level of dependency on government (taxpayer) funds.
- Decrease the level of levies placed on farmers, growers and processors.
- Decrease funding waiting times for researchers.
- Increase the level of funding to R&D in general.
- Ensure projects are commercially oriented with broad relevance and application to global markets.
- Accelerate the research process through focussed outcomes.
- Ensure projects are closely managed against pre-determined, and measurable, performance milestones.
- Increase the proportion of R&D projects which are commercially viable.
- Ensure there is no risk of liability for project participants – in particular research organisations.
- Motivate researchers with equity incentives and reverse the brain drain overseas.
- Generate a substantial portfolio of IP-rich businesses based in Australia and predominantly owned by Australians.
- Produce substantial financial returns for project participants (research organisation, researchers, investors and management alike).
- Increase the productivity and competitiveness of Australian agribusiness.
- Enhance school, college and tertiary level education systems through the provision of better facilities, equipment and prospects for student employment.
- Increase the skill levels of Australian farmers, growers and agribusiness participants.
- Create new and productive employment within Australia Increase the publics awareness of the self beneficial linkages be environment, agriculture, food technology, nutrition and health.
- Advance rapidly the use of technological and innovative products, systems and services in Australian agribusiness.
- Add considerable value to Australian primary resources, products, systems and services.
- Create opportunities for valuable cross-border collaborative ventures in research and development.
- Drive toward a sustainable self sufficient (self income generating) research and development system and place Australia at the leading edge in agri-science and business development, IP commercialisation and global service oriented exports.

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Aflatoxin workshop participants in Kingaroy. From left to right: Sudarsono, Greg Johnson, Sudjadi Sudjono, Ralf Dietzgen, D.V.R. Reddy, John Pitt, Wayne Bryden, Steve Lee, Nancy Keller, Brian Vernon, John Cullen, Agustina Rahmina, Graeme Wright, R.C.N. Rao, Endang Sutriswati Rahaya, Okky Dharmaputra.