Keynote address

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Plant protection in the 21st century: new developments, trends and training requirements

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Abstract

The 20th century was marked by the increased use of pesticides to manage pests; the boom and bust cycles of genetic resistance to microbial pests, resistance to pesticides and the development of new tools such as integrated pest management and biotechnology to manage and identify pests.

This paper presents an overview of recent developments and trends in the plant protection industry, government and other organisations in relation to future management of pests and diseases, the role of genetically modified crops, networking, and the future training requirements for plant protection officers, especially in Papua New Guinea.

Introduction

Ever since man first learned to grow crops he has also known that insects may deplete the sap of plants, disease-producing pathogens may invade their tissues, rodents may consume the plants, birds may eat the fruit and weeds through competition may crowd-out crop plants. The losses to agricultural and crop production can be quite substantial, averaging around 30% but reaching 100% in some cases.

Crop losses due to weeds

More recent figures in Australia, for 2001–2002, have identified the crop losses due to weeds in agriculture. Losses to producers are 80%, while losses to consumers are 20%. The cost of weed control is A$3442m and losses total A$3927m (crops accounting for $1518m and livestock A$2409m) (CRC Weeds 2004). This is a good example of the need to quantify the losses due to pests in order to substantiate arguments for management of such pests.

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Pest management in the 20th century

Pest management in the 20th century had the following features:

1. Synthetic organic pesticides with protectant and systemic activity were developed, replacing the older inorganic pesticides.
2. The 'boom and bust cycles' that followed when plants were bred with race-specific resistance to microbial pests (vertical, single genes) as opposed to using race non-specific or horizontal resistance using multigenes. Thus, single gene resistance was followed by a breakdown in resistance and the whole cyclical process of developing new plants with resistance to the pest organisms had to continue.
3. Development of resistance to the synthetic pesticides following large-scale usage, especially with compounds with single targeted modes of action. This led to the search for newer compounds and so another 'boom and bust cycle' until the strategy was altered by using cocktail mixtures of pesticides with different modes of action to overcome development of resistance.
4. In the late 1980s, consumer concerns on pesticide residues in food led to a return to organic farming

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practices with little or no pesticide inputs and the creation of an image of 'Clean and green produce' to overcome consumers concerns. Alongside this was the development of integrated pest management (IPM) strategies which incorporated a range of measures to control pests whilst substantially reducing pesticide inputs and costs.

5. The change to offer protection to commercial plant breeders by allowing patenting via plant variety rights legislation also resulted in use of biotechnology to produce and market virus tested plants, genetically modified (GM) plants resistant to pests, and rapid methods of identifying pests using serology, DNA and PCR technology.

In the late twentieth century, there was increasing awareness of biosecurity and conservation and the potential effects of terrorism using microbes for uses such as biological warfare and increasing crop losses. In addition, more attention was given to the side-effects of pesticides in the environment, and the effects of global warming on increasing pest populations.

Current trends

Pesticide industry

The pesticide industry has been marked by continuous mergers and takeovers so that there are now substantially fewer but bigger international agricultural chemical companies. Due to the high costs of registration of new products, there is more emphasis on marketing than product development, and the main targets are high-value crops grown around the world, rather than minor crops.

Newer pesticides often have greater specificity and fewer broad-spectrum compounds are now available. Some compounds are also becoming generic as patent rights expire whilst older pesticides have been withdrawn from the market due to mammalian toxicity, and other safety and environmental issues including biodegradability. Agrochemical companies have diversified their interests and invested heavily in commercialising GM plants containing specific genes for pesticide resistance or to key pests such as cotton bollworm, due to patent rights protection.

GM crops

With the release of GM Bt-cotton and maize resistant to lepidopteran pests, strategies now include refuges to inhibit development of resistance. Farmers are now spraying less pesticides but former secondary pests are now becoming primary pests due to ecological shifts. GM vegetables are available but public opinion is against the use of GM, especially on food crops such as potatoes, due to labelling requirements.

Insecticides will still need to be applied for control of thrips and whiteflies.

In the UK, farm-scale evaluation (FSEs) of GM herbicide-tolerant crops has been introduced. This is the first time that a new agricultural technology has been subject to large, field-scale environmental impact assessment before adoption.

FSEs are a unique case in which biodiversity considerations are given as much weight as agronomic benefits in decisions about crop cultivation. It seems likely that such assessments will become a more regular feature of future approval procedures.

A key question still to be answered is 'What are the long-term effects of using GM crops?'

IPM adoption rates

It was found in Malawi that smallholder farmers were more concerned with matters other than adopting procedures to manage insects. IPM strategies are more likely to be accepted by smallholder farmers if they are clearly linked to technologies which raise cash incomes. IPM farmer field schools impact evaluation is a highly complex exercise because of methodological obstacles, including a need to improve study design, increase scope and rigor of results, and emphasise development impacts.

Future training requirements

Plant protection requires inputs from many disciplines. It requires teams of specialists and technicians and good teamwork. There are now specialist laboratories that are able to provide identification services using both low- and high-technology tools, but who will pay for these services? Western developed nations have a user-pays system which is not affordable or desirable in developing countries such as Papua New Guinea (PNG).

There are currently too few trained people in PNG. How can we increase the numbers? One possibility is by making more use of networking (nationally, regionally and globally).

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There is also a need to update skills via further training in areas such as global policies, crop loss quantification, pest risk analysis, pest databases, use of molecular techniques in rapid identification of pests, scouting and sampling techniques, and population ecology and epidemiology.

University courses in plant protection in PNG are not meeting current and likely future needs (schools, undergraduate and postgraduate level). As agricultural systems change and intensify, the management of pests also changes and researchers, scientists and teachers need to be alert to keep ahead of the evolving pattern, in order to minimise crop losses due to pests.

Some comments from a senior plant protection scientist with Bayer, USA are worth repeating here:

> 'Working 12 hour days, 6 days a week is normal.'
>
> 'Being a research scientist in industry is hard, dirty and very under-appreciated'.
>
> 'In this job a research scientist must also be a technician.'
>
> 'Plant protection is not a glamorous profession.'

Such circumstances apply equally to plant protection scientists in PNG and there is a need to educate the country's politicians on the importance of these professionals to the country's agricultural production.

Conclusions

Famines and locust plagues and loss of food security continue to threaten many developing nations of the world. Combating the current and future threats of pest damage in the 21st century, be they from farm, urban or terrorism related incursions will ensure that there will be a need for plant protection education at all community levels and trained personnel engaged in the war to minimise pest attack and crop damage.

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Weeds and management

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The importance of proper weed control in young hybrid cocoa (*Theobroma cacao* L.) in Papua New Guinea

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Abstract

The effect of weed-control intervals on early flowering and yield was monitored in two experiments on young hybrid cocoa in the Gazelle Peninsula of East New Britain. The first experiment, in August 1989 to May1994, compared blanket spray, combined strip spray with slash, and slash methods of weed control. The second experiment was conducted between January1996 and December 2002 on modified SG2 hybrids and hybrid clones under smallholder growing conditions.

The results indicated that the type of weed control used and its frequency of application had no significant effect on stem girth, tree height and tree loss to pest attack in the short term, but the cumulative long-term effects were significant as trees start to flower and produce pods. The use of herbicide sprays and one-monthly slashing greatly minimised the negative effects of weeds.

Monthly slashing and application of blanket sprays at intervals of 1, 2 and 3 months resulted in over 60% of trees flowering early and yields between 1.6 and about 2.0 t/ha in the third year's production. Slashing after 2 months or more, combined with inconsistent weeding intervals, resulted in large tree losses to pest attack, delayed early flowering and resulted in low cocoa yields. Less than 15% of these trees had flowered by 21 months after planting and they gave yields of less than 0.4 t/ha in the third year's production. Slashing for weed control during the establishment phase should preferably be done at 1–1.5 month intervals. Herbicide sprays should preferably be applied every 2–3 months.

Introduction

The Cocoa and Coconut Institute of Papua New Guinea (formerly the Papua New Guinea Cocoa and Coconut Research Institute) released SG2 hybrids in 1986. These hybrids were further separated in 1994 into SG2 small and SG2 big hybrids, and two polyclonal hybrid cocoa clones were released in March 2003. The hybrids can yield up to 2.0 t/ha/year when properly managed.

Proper weed control during the establishment phase is the most important agronomic practice before cocoa reaches the bearing stage. Bonaparte (1979a,b) reported that failure to control weeds, after planting can lead to poor tree development and reduced early yields.

In Papua New Guinea (PNG), weeds are commonly controlled by manual ring weeding, slashing and spraying with herbicides. Regular weed control must be done during the first 18 months after planting or until such time the cocoa leaf canopy is able to suppress regrowth of weeds. Smallholder cocoa farmers in Solomon Islands use similar methods of weed control for young cocoa (Linton 1984). There is, however, little information available on the type,

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frequency and the effects of the different methods of weed control.

We report here on two experiments on weed control during the establishment phase of cocoa. The results highlight the importance of frequent and consistent weed control in young hybrid cocoa, whether the plantings be hybrid seedlings or hybrid clones.

Materials and methods

The first experiment, conducted between August 1989 and May 1994, compared manual slashing, blanket spraying and combined strip spray and slash methods on mixed SG2 hybrids at the Cocoa and Coconut Institute (CCI). The combined strip spray with slash method consists of slashing along the cocoa rows and spraying of strips between rows

The second experiment, conducted between January 1996 and December 2002, compared the performance of modified SG2 hybrids with hybrid cocoa clones on representative smallholder cocoa farms in the Gazelle Peninsula of East New Britain. Cocoa farmers in PNG are not used to growing clones

Experiment 1

The site for the first experiment was in CCI's Tavilo plantation, Kerevat with commercially mixed Trinitario × Amazonian SG2 hybrids. Trees were planted in February1989 at 4.0 m square spacing, giving a plant density of 625 trees/ha. From planting until August 1989 weeds were controlled by manual ring weeding and slashing.

The design consisted of a randomised block with eight treatments and four replicates. The treatments formed an incomplete factorial of three weed-control methods (combined strip spray with slash, blanket spray, and slash) and three weeding intervals (1, 2 and 3 months). The strip spray/slash and blanket spray methods were applied at intervals of 1, 2 or 3 months, while the slashing method was applied every 1 or 2 months. Each plot contained 20 trees (4 × 5) with single guard rows between plots.

The herbicide used was either paraquat (Gramoxone) or ametrymne at 90 mL with 10 mL of spread sticker in each 15 L tank. Flow rate was 900 mL/minute, swath width 1.2 m and walking speed 0.5 m per second, giving an application rate of 3.0 L/ha. Slashing was done with grass knives.

Experiment 2

The second experiment was conducted on nine sites (Kareba, Vunapalading, New Masawa, Karavia, Bitavavar, Makurapau, Kadaulung I, Kadaulung II and Sigut) on the Gazelle Peninsula. The cocoa types used were SG2 small (SG2-S) hybrids and SG2 big (SG2-B) hybrids, and small hybrid clones (HC1-S), intermediate hybrid clones (HC1-I) and big hybrid clones (HC1-B), each in 0.25 ha plots at each site. The big clones were planted at a density of 625 trees/ha, intermediate at 714 trees/ha and small at 1000 trees/ha.

After planting, farmers were advised on the best management practices to apply. Monitoring was done monthly after planting, to obtain information on the management practices used. Based on these observations, weeding intervals were classified into two categories. Category 1 was good to average weed control, where weed control was relatively consistent at intervals of 1.5–2.5 months. Category 2 was poor weed control, where weeds were controlled at intervals of more than 2.5 months and inconsistently. Four sites were ranked as category 1 and five as category 2. The five types of cocoa planting material tested were thus subjected to two types of weeding intervals. During analysis, the intermediate hybrid clone variety was omitted, because it was not recommended for planting commercially. The fifth site in category 2 was excluded from analysis because its results were largely influenced by the soil type and shade management. This then allowed the experiment to be analysed as a 4 × 2 factorial, giving a total of eight treatment combinations with four replications. The eight treatments are the four cocoa types: SG2 small hybrids, SG2 big hybrids, small hybrid clones and big hybrid clones subjected to two types of weed control—relatively good to average and poor—under smallholder growing conditions.

Data collection

The number of trees lost to pest attack, number of trees that flowered and measurements of stem girth and jorquette height were recorded. After pod production, pods were harvested at two-week intervals for both experiments, and pod production and wet bean weights were recorded. The numbers of trees lost through pest attack, and the numbers that came into production were transformed into percentages, and the wet bean weights converted to dry weights before statistical analysis (ANOVA).

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Results

Experiment 1

Early flowering

There were no statistically significant differences between treatments for stem girth and jorquette height, 18 months after treatments commenced (Table 1).

The monthly blanket spray and combined monthly strip spray with slash weed control treatments resulted in a percentage of trees flowering by 18 months after planting significantly higher ($p < 0.05$) than all the other treatments. These two methods of weed control resulted in over 70% of trees coming into early flowering. They were followed by two-monthly blanket spray, one-monthly slashing, and combined two-monthly strip spraying with slashing. Flowering was lowest in the two-monthly slash treatment

The percentages of trees lost to insect pest attack were not statistically significant (Table 2). On average, for all cocoa types, the proportions of trees lost as a result of weed control were about 3% for relatively good weed control and 7.6% for poor weed control.

The average to relatively good weed control under smallholder growing conditions significantly ($p < 0.05$) increased the percentage of cocoa trees that came into flowering by 21 months after planting. The highest was 51.8% from the small hybrid clones, followed by big hybrid clones with 40.1%, then the SG2 small and SG2 big hybrids with about 30.0%. Weed control after every two and a half months or more significantly delayed the number of trees coming into flowering. Under conditions of poor weed control, over 80% of the cocoa trees had not flowered by 21 months after planting.

Table 1. Effects of weed control methods and their frequency of application on average cocoa tree girth, height and percentage of trees flowering by 18 months after planting in experiment 1

Treatments	Girth (cm)	Height to jorquette (cm)	Percentage of trees flowering
1. Strip, spray and slash every month	18.6	133.3	76.2 a
2. Monthly blanket spray	16.4	112.9	77.5a
3. Slash every month	19.0	144.2	66.2b
4. Strip, spray and slash every 2 months	13.5	113.9	63.7b
5. Blanket spray every 2 months	18.1	151.0	68.7b
6. Slash every 2 months	16.5	150.0	48.7d
7. Strip, spray and slash every 3 months	15.8	142.2	51.2cd
8. Blanket spray every 3 months	15.6	139.8	56.2c
LSD (5%)	NS	NS	7.0
CV (%)	16.2	18.7	25.1

Note: Values followed by the same letter are not statistically different. NS = not significant.

Table 2. Trees lost to insect pest damage and percentage of trees flowering 21 months after planting under smallholder growing conditions in experiment 2

Cocoa type	Percentage of trees lost to pest attack		Percentage of trees that flowering within 21 months	
	Relatively good weed control	Poor weed control	Relatively good weed control	Poor weed control
1. Small hybrid clones	0.8	10.8	51.8	12.4
2. Big hybrid clones	1.7	7.7	40.1	4.2
3. SG2 – small hybrids	5.6	4.3	31.3	13.9
4. SG2 – big hybrids	3.8	7.6	29.9	1.9
LSD (5%)	NS		33.1	

Notes: Relatively good to average weed control means weed control applied relatively consistently at between 1.5 and 2.5 month intervals under smallholder growing conditions. Poor weed control means inconsistent weed control intervals of more than 2.5 months under smallholder growing conditions.
NS = not significant.

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Effects on early yield

Pod production. In the first two years of production, good pod production from trees receiving monthly treatments was significantly ($p < 0.05$) higher than the combined two and three-monthly treatments (Table 3). For all weeding frequencies during the first two years of production, the number of good pods from the blanket spray method was always higher than the number from strip spray/slash and slash methods.

Pod yield in the third year of production was significantly higher for blanket sprays applied at 1–2 month intervals (Table 3). Two-monthly slash and combined three-monthly strip spray with slash methods produced a significantly ($p < 0.05$) lower number of good pods than all the other weed control treatments.

The total pod production by the end of three years production was highest from the two-monthly blanket spray. The lowest total pod yields were from the two-monthly slash, combined three-monthly strip spray with slash and three-monthly blanket spray treatments.

Bean yield. Dry bean yield in the first and second years of production was highest (2430 kg/ha) from the monthly blanket spray treatment. The lowest was from the combined three-monthly strip spray with slash and two-monthly slash treatments, which produced about 1500 kg/ha (Table 4).

A significant effect ($p < 0.05$) on dry bean yield was obtained in the third year of production. Yields of over 2 t/ha were obtained for monthly blanket spray, monthly slash and two-monthly blanket spray treatments. The highest yield (nearly 2.3 t/ha) was obtained from the two-monthly blanket spray, but this was not significantly different to the monthly blanket spray and monthly slash treatments. The yield from the monthly slash method was not significantly different from the three-monthly blanket spray, but was significantly higher than combined monthly strip spray with slash, two-monthly slash and combined three-monthly strip spray with slash in the third year of production. The lowest yield was recorded for combined three-monthly strip spray with slash, which yielded about 1.5 t/ha. This was not significantly different from the combined monthly strip spray with slash, combined two-monthly strip spray with slash, two-monthly slash and three-monthly blanket spray treatments.

Total dry bean yield from the two-monthly blanket spray treatment was significantly ($p < 0.05$) higher than from two-monthly slashing, combined three-monthly strip spraying with slashing and three-monthly blanket spraying. It yielded a total of 4.7 t/ha, which was also the highest yield. The total yield from the two-monthly blanket spray treatment at the end of three years of production was, however, not significantly different from combined monthly strip spraying with slashing, monthly blanket spraying, monthly slashing and combined two-monthly strip spraying with slashing. The lowest total yield (3.06 t/ha) was recorded for the three-monthly strip spray/slash treatment. This was not significantly different from combined two-monthly strip spraying with

Table 3. Effects of blanket spray, strip spray/slash and slash weed control methods and their frequencies of application on cocoa pod production (pods/tree)

Treatments	Number of good pods		
	Years 1 and 2	Year 3	Total
1. Strip spray and slash every month	92.0 abcd*	68.3 cd	160.3 bcd
2. Blanket spray every month	105.5 a	85.4 abc	190.9 abc
3. Slash every month	103.7 abc	93.5 ab	197.2 ab
4. Strip spray and slash every 2 months	78.8 de	70.0 cd	148.8 d
5. Blanket spray every 2 months	103.2 abc	100.7 a	203.9 a
6. Slash every 2 months	74.2 d	68.3 c	142.5 d
7. Strip spray and slash every 3 months	63.5 e	63.0 d	126.5 d
8. Blanket spray every 3 months	82.9 abcde	69.0 c	151.9 d
LSD $p < 0.05$	22.7	18.3	38.6
CV (%)	17.5	16.1	15.9

* Values followed by the same letter are not significantly different.

slashing, and three-monthly blanket spraying. Amongst the slash treatments, monthly slashing gave significantly ($p < 0.05$) higher total dry bean yields than two-monthly slashing.

Amongst the frequencies of weed control, the highest average total yield was from the monthly application, with almost 4.3 t/ha, followed by two-monthly weed control with 4.1 t/ha and three-monthly application with about 3.4 t/ha.

Relatively good to average weed control means weed control was applied relatively consistently at 1.5–2.5 month intervals under smallholder growing conditions.

Poor weed control means inconsistent weed control intervals of more than 2.5 months under smallholder growing conditions.

Small hybrid clones subjected to relatively good weed control produced significantly ($p < 0.05$) higher dry bean yields than all the other cocoa types by two years after planting (Table 5).

Relatively good to average weed control in smallholder farms resulted in significantly higher dry bean yields from small hybrids and small hybrid clones than from all cocoa types subjected to poor weed control. The yields from the small trees, however, were not significantly different to those from large trees

Table 4. Effects on early dry bean yields of blanket spray, strip spray/slash and slash methods of weed control at various frequencies of application

Treatments	Dry bean yield (kg/ha)		
	Years 1 and 2	Year 3	Total
1. Strip spray and slash every month	2162	1560 cd*	3722 abc
2. Blanket spray every month	2633	2008 abc	4641ab
3. Slash every month	2518	2108 ab	4626 ab
4. Strip spray and slash every 2 months	2096	1808 bcd	3904 abc
5. Blanket spray every 2 months	2430	2295 a	4725 a
6. Slash every 2 months	2003	1566 cd	3569 c
7. Strip spray and slash every 3 months	1562	1498 d	3060 c
8. Blanket spray every 3 months	2034	1658 bcd	3692 bc
LSD $p < 0.05$	NS	469	1024
CV (%)	20.4	17.6	17.4

* Values followed by the same letter are not significantly different.

Table 5. The effects of good versus poor weed control on average dry bean yields (kg/ha) of hybrid clones and SG2 hybrids on smallholder farms in East New Britain

Types of cocoa and weed control	Average dry bean yields (kg/ha/year) 2 years after planting	Average dry bean yields (kg/ha/year) 2–3 years after planting
1. Small hybrid clones with relatively good to average weed control	48.7 a*	1161.7 a
2. Big hybrid clones with relatively good to average weed control	5.4 b	636.0 ab
3. SG2 small hybrid clones with relatively good to average weed control	5.9 b	1254.5 a
4. SG2 big hybrids with relatively good weed control	4.9 b	826.9 ab
5. Small hybrid clones with poor weed control	5.2 b	407.2 b
6. Big hybrid clones with poor weed control	1.4 b	206.9 b
7. SG2 small hybrids with poor weed control	6.2 b	291.6 b
8. SG2 big hybrids with poor weed control	1.4 b	206.2 b
LSD (5%)	34.5	664.1

* Values followed by the same letter are not significantly different.

under relatively good to average weed management. The highest recorded yield was nearly 1.3 t/ha from SG2 small hybrids, followed by small hybrid clones with about 1.2 t/ha and big SG2 hybrids with 0.8 t/ha. Cocoa subjected to poor weed control produced about 400 kg/ha/year or less. The lowest yields were recorded from the big trees, which produced about 200 kg/ha/year. The small trees under poor weed control produced between 290 and 407 kg/ha/year.

Discussion

Effects on pest damage

Young cocoa after planting is highly susceptible to competition for light, water and nutrients. Weeds, apart from competing with the cocoa, harbour insect pests and pathogens which, in turn, attack cocoa trees. The major pest problems in young cocoa in PNG are grey weevil, longicorn and rhyparid beetles, cocao webworm (*Pansepta*), mealybugs, caterpillars and thrips. Insect pest damage was not assessed in experiment 1, but observations during application of weed control treatments showed no major insect pest damage. The minimal damage was due to consistent weed control. In experiment 2, monthly monitoring of management practices applied to the trees after planting revealed great variability in general block management. Although no significant infestation was recorded, damage was always slightly higher for the plots with poor weed control than for plots with relatively good to average weed control. This observation showed up in the numbers of trees lost to pest damage, which were relatively higher for poor weed control than for relatively good to average weed control. The average loss was about 3% for the relatively good to average weed control and 7.6% for the poor weed control (Table 2). During the first 21 months after planting in smallholder farms (experiment 2) damage from grey weevils, longicorn beetles, caterpillars and rhyparids was common, with longicorns the main cause of losses.

The entomology section of CCI did no begin assessing the extent of insect pest damage until two years after planting. During the period of assessment (2000–2003), the type and extent of damage was very localised for some insect pests (data not shown). Along the North Coast Baining of East New Britain, the main insect pest attack in New Masawa was from *Pansepta* and in Vunapalading was the cocoa weevil *Pantorhytes*. In Karavia (a site next to Vulcan and between Kokopo and Rabaul), damage by mealybugs to some trees was quite significant in some plots. Significant short-term effects of weed infestation on tree losses were therefore not observed.

Effects on vegetative growth, flowering and yield

There were no significant differences in vegetative measurements because cocoa is a perennial tree crop whose vegetative growth is normally slow. The effects of level of weed control, however, become significant as trees start to flower and produce pods. The trends in flowering and yield responses were due to cumulative effects over time resulting from weed infestation, as a consequence of the type of weed control practised, and its frequency and consistency of application. The data show that more frequent weed control significantly ($p < 0.05$) increased the percentage of trees flowering and resulted in higher numbers of pods and dry bean yields than the less frequent weed control.

Amongst the two manual slashing treatments, monthly slashing was always better than two-monthly slashing. For blanket spraying, the one- and two-monthly herbicide sprays were always better than the three-monthly blanket sprays, except for pod yield in the first and second years of production and total dry bean yields by three years after planting, when differences were not significant amongst the blanket spray treatments. This indicates that blanket spraying can be done at intervals of 2–3 months. The combined strip spraying with slashing applied at one- and two-monthly intervals, resulted in similar trends in percentage of trees flowering, pod yields and dry bean yields.

Although the patterns of flowering and yields were similar in both experiments, their magnitudes were very different. The use of herbicide sprays instead of slashing-only in experiment 1 enhanced early pod production and dry bean yields. Significant effects of dry bean yields in experiment 1 became evident at three years after planting. Under smallholder conditions, where the method of weed control was variable and very inconsistent, the effects were very severe. The percentage of trees that came into early flowering, and early yields, were significantly reduced compared with those in experiment 1. The small hybrid clones subjected to relatively good to average weed control under smallholder growing conditions resulted in only about 50% of trees coming into flow-

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ering. This was about the same as two- and three-monthly blanket spraying and combined strip spraying with slashing and two-monthly slashing by 21 months after planting in the on-station experiment. The non-significant differences in yields of the two small and big cocoa types (hybrids and clones) indicate that clones can perform as well as the hybrids under smallholder growing conditions in PNG. The results also indicate that consistent and frequent weed control, preferably every 1–1.5 months using slashing and 2–3 months weed control intervals when herbicide sprays are used, minimised the competition for light, water and nutrients and damage due to insect pests.

The low yields from the two-monthly slash, combined strip spray with slash and types of weed control under smallholder growing conditions were due to regrowth of weeds after slashing, whereas blanket spraying was expected to kill most weeds. Similar effects on young tea plants were shown for two-monthly spraying intervals using glyphosate at 1.2 or 3.0 kg/ha. This treatment gave a higher percentage of weed control than paraquat or hand weeding (Magambo and Kilavuka 1975). In the present study, although paraquat was used, the blanket spray method required that all weeds in the cocoa plots be sprayed. The period after which regrowth of weeds to levels likely to suppress the growth of cocoa is also longer following blanket spraying than for combined strip spraying with slash or slash methods. In the combined strip spray/slash and slash methods, the remains of actively growing plants continue to grow to compete with the young cocoa. Where manual slashing only is used, combined with less frequent weeding interval, the competition effect is much greater. Other studies on weed control in cocoa have also shown that chemical weed control benefits growth and yield more than does manual weed control (Snoeck 1978; Lima et al. 1983; Purusotman et al. 1988).

Effects on potential yields and income

The hybrid cocoa-planting materials released by CCI PNG, have yield potential of greater than 2.0 t/ha. Dry cocoa bean yields of over 1.0 t/ha/year is still considered a very good yield under smallholder conditions. Dry bean yields in experiment 1 ranged from about 1.5 t/ha/year to over 2.0 t/ha/year. This was a result of consistent weed control. In experiment 2, inconsistent weed control prevented high early yields. Dry bean yields in the first two years after planting were 3–5 times less than under consistent weed control. In the third year of production, the highest dry bean yield was about 1.2 t/ha following average to relatively good weed control under smallholder growing conditions. The very inconsistent weed control at more than two-monthly intervals produced dry bean yields of about 400 kg/ha or less. The national average cocoa production in PNG is about 0.3–0.4 t/ha/year. In monetary terms, 2 t would be worth about K8400 based on the average cocoa price in 2004 at K4200/t. The 1.2 t/ha/year from the relatively good to average weed control is worth about K5040/ha/year and the 0.4 t/ha/year from poor weed control K1680/ha/year. The poor weed control under smallholder growing conditions thus amounts to a loss of K3360/ha/year compared with the return from relatively good to average weed control. These values are not net incomes, because the net income would differ for the different types of weed control and frequency of application. They are included to give some indication on potential income that can result from the different strategies of weed control. The results show that poor weed control in cocoa during the establishment phase does play a big part in contributing to low cocoa production in PNG. The message is clear that weeds, if not properly controlled using the most suitable method, can result in increased pest infestation, competition for nutrients, and reduced early flowering and longer-term yields. These results are in agreement with those reported by Bonaparte (1979a,b).

Conclusions

The following conclusions can be dawn from the results of this work:

1. The method of weed control and its frequency of application in young cocoa after planting can, in the long term, significantly affect early flowering and early cocoa yields.
2. Herbicide spraying at intervals of up to two months combined with monthly manual slashing significantly promotes higher early flowering and yields. Consistent and frequent weed control, preferably every 1–1.5 months using slashing is recommended. Weed-control intervals of 2–3 months can be tolerated.
3. Inconsistent and infrequent weed control, particularly using slashing at intervals greater than 2.5 months, can significantly delay early flowering and reduce cocoa yields by

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approximately 15%. This practice of weed control therefore should be avoided for all young hybrid cocoa plantings in PNG.

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Status and management of invasive weed *Chromolaena odorata* in Papua New Guinea

I. Bofeng[1]

Abstract

The invasive weed *Chromolaena odorata* (L.) R.M. King & H. Robinson (Asteraceae) is regarded as one of the world's worst tropical weeds. Chromolaena interferes with human activities, affects crop yields, increases costs in agricultural production, invades and suppresses growth of pastures in grazing land, and transforms the ecosystem structure and species composition. In Papua New Guinea, the weed is present in Sandaun, East Sepik, Madang, Morobe, Kassam Pass in Eastern Highlands, Oro, Milne Bay, East and West New Britain, New Ireland, Manus and Bougainville. The impact of chromolaena in these provinces is varied. Serious infestations have been observed on fallow lands, cultivated land and smallholder cocoa, coconut and vanilla blocks, along roadsides reducing traffic visibility, on newly established oil palm plantations and reforestation areas, and in pastures. Further spread of chromolaena is expected through the movements of people, machinery and produce. The National Agricultural Research Institute is pursuing a biological control program funded by ACIAR to control the spread and infestation of chromolaena. Two biological control agents, the moth *Pareuchaetes pseudoinsulata* and the gallfly *Cecidochares connexa,* were introduced from Guam and the Philippines in 1998 and 2001, respectively. *Pareuchaetes pseudoinsulata* and *C. connexa* were released in the provinces affected by chromolaena and the assessment of the impact of the biological control agents on the weed is continuing.

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Control of *Monstera* species in cocoa: a preliminary investigation using various herbicide mixtures

D.S. Yinil[1]

Abstract

Seven herbicide mixtures were tested in a preliminary investigation to determine their effectiveness in controlling *Monstera* species in cocoa plantings. Two combinations that controlled *Monstera* species were glyphosate with Li–700 and Ally with Li–700.

Introduction

Monstera spp. (cheese plant) are becoming a hard to control weeds in many cocoa, coconut and oil palm blocks and plantations in Papua New Guinea (PNG). The presence of *Monstera* creates conditions suitable for pests, particularly longicorn beetles and termites, to attack cocoa trees. When *Monstera* grows up the cocoa trees, it can reduce the bearing surface area as it winds around the flower cushions. Flowers and cherelles can also be damaged when workers attempt to remove *Monstera* from the cocoa stems using bush knives, grass knives or by hand. Following slashing, *Monstera* grows back within 2 weeks. The commonly used herbicide mixtures for weed control in cocoa only enhances its establishment, as other weeds are killed but not *Monstera*. Only burns to the leaves are made but new shoots develop a few weeks after spraying, even using glyphosate, a systematic herbicide. While the long-term effects of this weed have not been studied, work elsewhere on weed control in cocoa, coconut and oil palm has shown that soil nutrients, tree growth and yield over the long term can be significantly affected by it (Bonaparte 1979a,b; Iremiren 1986; Romney 1988). There are no reported studies on the control of *Monstera*.

Monstera leaves and stems have a greasy or waxy surface. The commonly used herbicide mixtures for weed control in cocoa therefore run-off fairly quickly. Furthermore, the herbicides used are probably not applied at high enough concentrations to cause any significant injury to the plant.

A preliminary investigation, testing the effect on *Monstera* of seven different herbicide mixtures was carried out in June 1996. The seven herbicide combinations tested were glyphosate with Li-700 surfactant, glyphosate with Chemwet wetting agent, Gramoxone with Li-700, 2,4-D with spread sticker, Banvel with spread sticker, Ally with Li-700 and MSMA with spread sticker.

The objective of this preliminary investigation was to determine if any of these herbicide mixtures were able to control *Monstera*

Materials and methods

The tests were conducted at the Tavilo Plantation of the Cocoa and Coconut Institute of Papua New Guinea. The seven herbicide mixtures, each in a 15 L knapsack spray tank, were as follows:

1. glyphosate at 320 mL with Li-700 as surfactant at 80 mL
2. glyphosate at 320 mL with Chemwet as a spread sticker at 12 mL
3. Gramoxone at 96 mL with Li-700 as surfactant at 80 mL
4. 2,4-D at 320 mL with spread sticker at 12 mL
5. Banvel at 60 mL with spread sticker at 12 mL and Li-700 at 80 mL
6. Ally 6.0 g with Li-700 at 80 mL
7. MSMA at 440 mL with spread sticker at 12 mL and Li-700 at 80 mL.

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The 15 L knapsack spray was fitted with a blue polijet nozzle, which gives a flow rate of 1.6 L/min and a swath width of 1.5 m. The walking speed was about 45 m/minute and the nozzle was held at around knee height during spraying. An area of about 0.05 ha was therefore sprayed for each herbicide mixture.

There was no randomisation or replication of treatments in this preliminary experiment. Observations on the extent of damage caused were made 1 and 4 weeks after the treatments were applied.

Results

Details of observations made 1 and 4 weeks after treatment application are shown in Tables 1 and 2.

Discussion and conclusions

The present preliminary investigation has shown that glyphosate + Li-700 and Ally + Li-700 herbicide mixtures can control *Monstera* spp. The effect on *Monstera* and other weed species was observed a week after spraying with glyphosate, whereas the effect of Ally was not seen until the second week after spraying. By the fourth week, all the weeds, including *Monstera*, were completely killed.

Glyphosate is normally mixed with Chemwet when spraying in cocoa blocks. This mixture had no major effect on *Monstera*. Both glyphosate and Ally are systemic herbicides, whereas the other five are non-systemic. The combination of the two systemic

Table 1. Effects of various herbicide mixtures on *Monstera* sp. 1 week after application

Herbicide mixtures	**Observations**
1. Glyphosate + Li-700 surfactant	Leaves of *Monstera* and all other weeds present starting to turn yellow.
2. Glyphosate + Chemwet spread sticker	No visible effect on *Monstera* or other weed species present.
3. Gramoxone + Li-700 surfactant	Burns on stems and young leaves of *Monstera*. Leaves of grass, *Makenia* sp., members of Emphophis and Compositae family and *Centrosema* spp. burnt.
4. 2,4-D + spread sticker	Similar to 3
5. Banval + spread sticker + Li-700	Similar to 3
6. Ally + Li – 700	No visible effect on *Monstera* or any of the weed species present.
7. MSMA + with spread sticker + Li-700	Burns on young leaves and stems of *Monstera,* but less severe than for Gramoxone + Li-700. The mixture, however, greatly affected grass species, *Makenia*, members of the Emphophis and Compositae family and *Centrosema* spp.

Table 2. Effects of various herbicide mixtures on *Monstera* sp. 4 weeks after application

Treatments	Observations
1. Glyphosate + Li-700	Over 80% of small *Monstera* killed. Leaves have turned completely yellow and stems have dried up. Larger plants have some leaves turning yellow and parts of stems also starting to rot and dry out. All other weed species present killed. This effect was not observed previously.
2. Glyphosate + Chemwet spread sticker	Leaves of *Monstera* turning yellow but no signs of dead plants. The mixture controlled other weed species were present.
3. Gramoxone + Li – 700	New shoots of *Monstera* starting to grow back. Growth of *Makenia*, members of the Emphophis and Compositae family and *Centrosema* spp. still suppressed.
4. 2,4-D + spread sticker	Only minor burns to new leaves and stems of *Monstera* compared with the Gramoxone treatment. *Makenia*, members of the Emphophis family and Compositae families, *Centrosema* spp and other weed species present effectively controlled.
5. Banvel + spread sticker + Li-700 at 80 mL	No effect on *Monstera*. All other weed species named above killed.
6. Ally + Li-700	Same effect as for glyphosate with Li-700; over 80% of the small *Monstera* killed. Leaves of larger plants turning yellow and stems drying out. All other weed species killed.
7. MSMA + spread sticker + Li-700	Burning to young leaves and stems of *Monstera,* but degree of injury less than that caused by treatment 3. Other weed species named above controlled.

herbicides with Li -700 enhanced their effectiveness in controlling *Monstera*. Addition of Li -700 to Gramoxone killed other weed species but not *Monstera*. This study has shown that *Monstera* cannot be killed by non-systemic herbicides or by a systemic herbicide mixture used for general weed control in cocoa.

Li-700 is an adjuvant which enhances the performance of a herbicide, especially systemic herbicides, in controlling hard-to-kill weeds. The dosage used equates to an application rate of 1.6 L/ha.

The glyphosate mixture in most common current use for general weed control in cocoa is 90 mL/15 L knapsack and 110 mL in 20 L knapsack spray, giving an application rate of 1.8 L/ha. The recommended application rate is, however, 3 L/ha (150 mL/15 L and 200 m/20 L). The rate used in the preliminary testing was 320 mL/15 L (6.4 L/ha). This is nearly four times the current application rate, and twice the recommended application rate, and may be too expensive. Further investigation to determine more economical application rates is required.

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Pest incursions and quarantine

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Invasive weeds: impacts, prevention, detection and responses

W. Orapa[1]

Abstract

Increased global trade and movement of people and commodities between countries or between different geographical regions have accelerated the risks associated with invasive weeds and other organisms. Weeds are among the most important but often least-recognised category of all invasive species. Even in the presence of legislative mechanisms prohibiting the movement of species, many potential weed species cross international and geographical boundaries. Too often the impacts of weeds are not noticed early because they do not exhibit their invasiveness for some time after arrival, failing to raise concern for years. The difficulty we currently face is identifying 'sleeper' weed threats before they become troublesome. The best predictor is to assess a particular plant's ability to become invasive elsewhere in a similar environment. In Papua New Guinea (PNG), as well as the Pacific region generally, there are already well-known cases of invasive weeds affecting agriculture, rural livelihoods, human health and the environment. Yet, many other species notorious elsewhere are absent from the country. Maintaining a vigilant quarantine service and awareness of such threats is vital to combatting these future threats. The development of an effective national invasive weed strategy to meet existing and potential weed threats is lacking in PNG. This paper assembles current knowledge of the occurrence of some invasive weeds in PNG and their potential economic, social and environmental costs.

Introduction

Increased globalisation and international trade, and the subsequent movement of biological material across international and biogeographical borders, poses an ever-increasing threat of invasive species problems. Globalisation and an ever-increasing dependence on trade by all countries is providing enormous benefits, but these also present enormous new challenges, the movement (intentionally or accidentally) of invasive alien species such as weeds being among the most notable. Invasive weeds can cause a wide range of agricultural, social and environmental problems. These may include increased costs of production or loss of income, actual loss of a crop harvest or livestock loss through displacement of useful pasture species or direct death, and harbouring of harmful pests or diseases that can affect crops or human health. There is the ever-increasing risk of pesticides usage and even the development of herbicide resistance in agriculture. Introduced weeds also have an impact on community life, such as by affecting health and access to recreational areas. Aquatic weeds can cause flooding in wetland systems, resulting in loss of property, while weeds which die back seasonally can promote intense bushfires in dryland areas, leading to permanent changes in ecosystems, the appearance of new landscapes and, more importantly, the loss of biodiversity. Aggressive invasive weeds can modify habitats permanently, which can be profound because endemic flora and fauna can be lost altogether. This can also indirectly affect agriculture because tools such as integrated pest management in production areas are reliant on the abundance of high numbers of predators and functioning ecosystems to control crop pests. The impact of invasive weeds on agriculture, natural ecosystems and their associated biodiversity can be very serious for small islands, for which the environment is generally less complicated and biodiversity lower biodiversity than for larger landmasses.

The causes of introduced weeds becoming invasive are numerous. The most important factor is the

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absence of restraining natural factors, such as highly evolved herbivores or competitors that keep the weeds under check in their native range. Other factors that promote invasive behaviour in plants include human-induced modifications to ecosystems that favour the introduced species. Habitat modifications may be in the form of land-use practices resulting from expansion of settlements, logging, frequent burning and agricultural activities. On a global or regional scale, changes in climatic conditions — such as increasing average temperatures — are likely to promote introduced weeds by favouring expansion of the altitudinal or latitudinal range of a species.

In PNG, the general lack of information and awareness on existing or potentially invasive weeds continues to result in the relegation of invasive weed problems and their management to low-priority status in agricultural research and development. Effort, time and money often get spent on tackling less important pest or disease problems. This paper briefly discusses the economic, social and environmental costs of invasive weed species.

Impacts

Socioeconomic costs

There are very close linkages with social problems when economic gains are affected by weed invasions. Invasive weeds can cause direct income losses as a result of their negative impact on primary production (agriculture, horticulture, grazing, forestry and fisheries). Indirectly, invasive weeds can reduce income through their effects on other human activities such as tourism and recreation, and their impacts on biodiversity reduction and infrastructure. It is difficult to estimate such losses in most cases.

There is very little information available on the direct costs associated with invasive weed infestations in PNG or the region, except in commercial plantation agriculture or grazing operations where some information on control costs may be locally available. Reliable information on losses as a direct result of weed infestation in farming systems is non-existent. However, its common knowledge that managing an invasive weed will cost money, time and energy (mechanical or labour) that could be better used in increasing the value of production. A typical case could be in the Gudsup and Ramu valley areas where the costs of control of two invasive *Sida* species on grazing properties following the El Niño of 1997 cost Ramu Sugar Ltd thousands of kina (K). In mid-1997 (pre 1997 drought) Ramu Sugar Ltd spent between K60,000 and K80,000 on spray chemicals for sida weed and had three tractors dedicated to slashing it all year round at an approximate total annual cost of about K136,000. Ramu Sugar Ltd had about 45,000 cattle, so the per capita cost of sida control per head was therefore K4.80 (Kuniata, pers. comm.). Extrapolation to PNG's total herd of 150,000 would give a potential control expenditure of K720,000 per year.

In Tonga, squash farmers spend about 25% of their time combatting two major weed invaders: *Commelina benghalensis* L. and *Cyperus rotundus* L. Although the average return on labour input is estimated at T$23 per labour hour for farmers, this would be higher if the competing weeds and crop diseases were controlled effectively (S. Halavatau, pers. comm.).

Invasive weeds can directly impinge on people's personal income by reducing returns from sources such as agriculture, tourism and forestry. The recent invasion of the Sepik River wetlands by aquatic weeds such as salvinia (*Salvinia molesta* D.S. Mitch.) and water hyacinth (*Eichhornia crassipes* (Mart.) Solms) contributed to the loss of income of many villagers. Such invasive weeds limited access to backwater villagers by tourists, and caused serious disruptions to boat traffic for access to local markets for produce such as artifacts, vegetables, fish and rubber. The costs of the occurrence of exotic weeds at damaging levels are difficult to estimate. Economic costs associated with such weed invasions included the greater use of boat fuel and the costs of servicing outboard motor engines. Between January 1993 and December 1998, over A$1.5m was spent trying to reduce the damaging impact of water hyacinth (M. Julien and W. Orapa, unpublished data).

The water hyacinth invasion caused much social hardship and directly affected food security, health and the daily lives of people dependent on open waterways in the Sepik River system. An extreme consequence was the death at Tambali Village in the Lower Sepik in 1996 of a villager trapped in the middle of large mats of water hyacinth. The deaths of several others in the Grass Country area were also linked to water hyacinth clogging barats (canals), preventing access to health services at Angoram or Wewak. Had eradication of this noxious weed been successful in the 1960s in the Wau–Bulolo area such human costs 30 years later would have been avoided.

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Another invasive weed for which management efforts are currently underway in PNG and several other countries in the Asia–Pacific region is the scrambling shrub chromolaena (*Chromolaena odorata* (L.) R.M. King & H. Rob.) (Orapa et al. 2002; Bofeng, these proceedings). Biological control efforts against chromolaena in PNG had cost well over A$450,000 by the end of the project in mid 2005. The economic costs of this invasive weed for PNG are difficult to predict but the impact of its physical presence is already being felt by villagers, farmers and graziers, and in agroforestry enterprises in affected areas. Troublesome weeds like chromolaena are already increasing the costs of production of crops such as cocoa and coconuts, but information on economic losses or costs of control are difficult to assess due to limitations in capacity. In Australia, the cost of an eradication program against chromolaena in the Tully region of North Queensland since 1996 has already exceeded A$6m. If the weed is not eradicated, it is predicted that losses due to its invasion will cost the Australian economy well over A$100m.

In PNG, common invasive weeds such as elephant grass (*Pennisetum purpureum* Schumach) and Johnson grass (*Sorghum halepense* (L.) Pers.) invade arable land and crops such as coffee, cocoa, coconuts and fruit trees, causing loss of income from poor yields. This affects food security for many rural households. Several other introduced weed species, including *Piper aduncum* (L.), are already increasingly affecting rural incomes and food security by direct competition and indirectly by reducing the value of farm or grazing lands. Loss of traditional or native flora and fauna used as food, building materials (e.g. loss of *Imperata cylindrica* (L.) Beauv. grasslands as a result of *P. purpureum* and *P. aduncum* invasions), medicinal or for other cultural purposes can have significant effects on village communities. Reduced diversity of plant or animal resources caused by the occurrence of invasive plant species can indirectly threaten social status, incomes and food security, at levels from rural communities to national. Some weeds can promote bushfires (e.g. chromolaena) or flooding (e.g. water hyacinth) and cause damage to infrastructure and loss of lives and property. In parts of the Markham Valley and south-eastern New Ireland traditional villages are constantly under threat of bushfires fuelled by chromolaena. Chromolaena also causes problems elsewhere in the region. In East Timor, it makes up over 60% of all vegetation and has many social impacts including the loss of imperata grasslands, constant threat of annual fires and loss of useful pastures for grazing (McWilliam 2000).

Some invasive weeds, simply by their presence, have the potential to directly threaten the health of people. Parthenium weed (*Parthenium hysterophorus* L.), for example, is a health hazard in India and Australia because its pollen grains cause dermatological and respiratory diseases. Indirectly, invasive weeds can contribute to health problems through an increase in disease-causing organisms or vectors due to the presence of weed infestations. Water weeds may reduce flow and increase stagnation, which could favour mosquito populations and result in a higher incidences of malaria in tropical areas. Stagnation of water might lead to a general increase in the prevalence of water-borne diseases.

Impacts on biodiversity and the environment

Loss of biological diversity due to the invasiveness of introduced species such as exotic weeds is a serious concern. Globally, it is now widely accepted that invasive species are second only to habitat destruction (from development, logging and extreme climatic conditions) in their impacts on biodiversity. They have been recognised by the Convention of Biological Diversity of the United Nations as a serious threat.

The ways in which non-native species affect indigenous species are varied and may be profound when they include non-reversible changes to habitats or whole ecosystems. Small islands are particularly vulnerable to invasive species incursions because of their long history of isolation coupled with their simple ecosystems and relatively high rates of endemicity. Invasions by introduced weeds can have greater consequences for small islands than for larger islands or continents.

There are few data available to demonstrate the significance of exotic weed incursions on indigenous biodiversity or the environment in PNG or the Pacific islands. In the absence of information, we can only speculate on their impacts. The survival of the endemic Queen Alexandria Butterfly in Oro Province, for example, is likely to be threatened by both forest clearance and encroachment by invasive introduced weeds such giant mimosa (*Mimosa pigra* L.), chromolaena and elephant grass. At the foothills of the Surrawaget Range of Morobe Province, invasion by *P. aduncum* and chromolaena following shifting

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cultivation or forest burning after the 1997 El Niño event appears to be delaying forest regeneration. Seed-bank studies at two disturbed forest sites in Morobe Province (Rogers and Hartemink 2000) support this observation. They found that seeds of *P. aduncum* and *C. odorata* were more abundant than native-plant seeds. In New Ireland, invasion of disturbed forests by a combination of chromolaena and African tulip (*Spathodea campanulata* P. Beauv.) threaten biodiversity and the recovery of native forests following logging and shifting cultivation. On Misima Island, efforts to restore the mine landscape have been significantly affected by the invasion of chromolaena, which dominated and choked planted trees (T. Zeringa, pers. comm.).

In other Pacific countries and territories, the fragile ecosystems are being threatened by the occurrence and spread of a few major invasive weeds, including *Merremia peltata* (L.) (Vanuatu, Samoa), mile-a-minute weed (*Mikania micrantha* Kunth.) (Samoa following cyclones which open up canopies), *S. campanulata* (Fiji), and miconia (*Miconia calvescens* DC) (French Polynesia and Hawaii). In New Caledonia, invasive weeds (and ungulates) now threaten the survival of 233 highly endemic sclerophyll forest plants (De Garine-Wichatitsky and Spaggiari 2006).

Other environmental problems associated with invasion by exotic weeds have been observed in PNG. Aquatic weeds generally cause loss of water and eventual drying up of shallow ponds as a result of higher evapotranspiration rates, disruptions to free flow of creeks and increased sedimentation. Invasion of sewage-treatment ponds by *E. crassipes* in Port Moresby during the mid 1990s impaired the oxygenation process used to treat raw sewage, causing partially treated sewage to be released into the Waigani Lakes. In serious infestations, entry of sunlight into water can be reduced, causing anoxic conditions that could kill plankton and fish.

Prevention, detection and responses

Prevention

Potentially invasive plant species can arrive at a country's border by various pathways. The most common route of accidental introduction is when viable seeds come as unwanted companions on other goods, machinery or on the shoes or other possessions of travellers. Accidental introductions can be minimised if only clean, uncontaminated cargo, vessels, machinery, clothing etc. are allowed entry and proper checks are made at ports of arrival. Many troublesome weeds have small, lightweight and highly evolved seeds that are hard to detect. Accidental introductions of plants will continue to increase due to globalisation and increased trade.

Intentional imports of potentially invasive species can be either illegal (smuggled) or legal (imported with permits). Smuggled imports can be a problem and are difficult to detect even in the presence of stringent border protection systems. The legally imported plants are usually introduced for agriculture, pasture improvement, ornamentals, forestry or other uses. The most critical action for an island country such as PNG is to prevent new weeds from arriving at its shores. This can be achieved by putting in place good border-control procedures, including pre-import screening and approval systems, preferably assisted by an appropriately designed weed risk assessment system based on widely agreed protocols, such as those of the International Plant Protection Convention. These protocols allow for exporting countries to export only prescribed products and detail the procedures that must be applied by both exporters and importers to prevent unwanted hitchhikers.

A challenge for PNG is to increase and maintain its biosecurity and quarantine capacity to protect and sustain its agriculture, biodiversity and the way of life of its people by minimising impacts of new potential invasive species. This can be done by having properly trained and motivated quarantine personnel, supported by good sources of information, capable of making informed decisions to prevent intentional introduction of potential weeds. In addition, increased compliance of quarantine regulations can be achieved through increased awareness among the general public, particularly those groups most likely to be involved in the introduction (accidental or intentional) of potentially harmful plants.

Detection

Most current weed problems were not recognised until well after the invading species had become well established and begun to interfere with human interests. Ideally, it would be better to detect an invasive weed before it establishes and spreads from a point of introduction. However, the difficulty is predicting which introduced plant will become a future weed. From the 1950s to the 1970s, biologists researching

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the invasions of species, particularly weeds, looked for traits that would make a species invasive or weedy. Biological traits like growth rate and size and number of seeds were seen as crucial traits for a species to become weedy. In the 1980s and 1990s, it was recognised that this technique did not work and variably gave many false positives or negatives. Some plants predicted to be invasive weeds did not, while others thought to be benign have become problems (Wittenberg and Cock 2001). Risk assessment for non-indigenous plant species is still largely guesswork.

Plant species intentionally introduced for various purposes can became serious economic or environmental disasters. Screening is essential, and it is now acceptable and recommended practice to conduct intensive pest risk analysis before importation of a species of questionable biological and ecological characteristics. Today, with increasing availability of information on weed problems faced in other parts of the world, the most reliable predictor of a particular plant's ability to become invasive in a given environment is to know whether the species has proven to be a serious weed elsewhere. Not all introduced plants will become invasive weeds and many introductions have brought significant economic and food security benefits. If a plant species is from a region with similar climatic and ecological features as those present in PNG this should raise concern. Then, as noted above, it will be useful to find out if the plant species in question has caused problems elsewhere in the world.

Detection of potentially new invasive weeds can be achieved through various strategies such as the establishment of an invasive weeds network, improved institutional capacities in invasive species detection and management, and strengthening international linkages (Orapa 2001). At a minimum, the services of a few experienced and motivated people who have a keen interest in invasive species and know about weed problems in other countries, should be engaged. The early detection of, and response to, an outbreak of parthenium weed (*P. hysterophorus*) in Lae in 2000 (SPC 2003) was possible because it was found there by a person familiar with the problem the weed was causing in Australia. Similarly, concern about the invasiveness of chromolaena (*C. odorata*) in PNG was raised by quarantine botanists who knew the problems it caused elsewhere (Waterhouse 1992)

Eradication

On detection of a potentially invasive weed, a decision has to be made as to whether it needs to be eradicated or controlled. Such decisions are usually based on factors including knowledge of the weed, feasibility of approach, level of concern and stakeholder support.

Ideally, it is in the country's long-term interests for any introduced plants exhibiting, or likely to exhibit, invasive behaviour to be eradicated upon discovery. Eradication is often difficult to achieve, however, as a whole range of factors compound a seemingly simple decision. The first step is for the identity of the species to be confirmed by an expert. If sufficient information exists to indicate that the plant species has caused problems elsewhere in similar environments, every effort should be made to eliminate it. If, by the time it is detected, a species is already naturalised widely over large and difficult areas, decisions have to be made as to whether it is worth financing a management program. There may be other, more pressing agricultural or environmental problems that directly affect food security facing the country. In such circumstances, a reasonable action would be to continue to monitor the weed's behaviour and spread, declaring it a 'notifiable noxious plant' or a 'noxious plant' using existing legislation and working to increase public awareness on the dangers of spreading it. This should contribute towards containing the weed in one area and preventing further intentional spread. An attempt was made to eradicate water hyacinth from the Wau–Bulolo areas in PNG in the 1960s. Despite repeated treatments, this effort failed to prevent further spread to other parts of the country because the general public was either not fully aware of its potential impacts or the bans were not policed properly. Experience over the past 40 years is that declared weeds eventually get spread well away from their initial outbreak areas (Mitchell 1979; Laup 1987; Julien and Orapa 1999).

An eradication effort which began in 2002 is slowly under way in and around Lae, Morobe Province to eradicate parthenium weed. At first, officers from the National Agricultural Quarantine and Inspection Authority (NAQIA) and the National Agriculture Research Institute (NARI) were involved in treating two initial outbreak sites and conducting surveys with support from the Secretariat of the Pacific Community's Plant Protection Service. This ad hoc arrangement between the two agencies did not

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result in rapid eradication of the weed. A delimiting survey in November 2002 found that the weed was localised at two outbreak sites; a third site was found in October 2004. Following the hiring in September 2004 of two full-time staff to undertake the work, this species is likely to become the first invasive weed successfully eradicated from PNG (R. Masamdu and W. Orapa, pers. comm.).

Management/control

Once invasive weeds are widespread and beyond the reach of eradication, management or control using appropriate techniques are the only approaches available. These vary with species, locations, land use and other factors. It is foolish to attempt eradication of an invasive weed from a certain location when it is already widespread. Infestations of a relatively widespread invasive weed in cropping areas can be controlled successfully, but such efforts are often temporary because regular build-up of seedbanks in natural areas can be the source of reinvasion of controlled areas.

In PNG, attempts at management of invasive weeds are ad hoc, driven by the notoriety of a few troublesome invasive weeds that have emerged in recent years. While a national invasive weed strategy has yet to be developed for PNG, control strategies against a number of invasive weeds (Table 1) have been attempted after some of these species became troublesome. For most targeted weeds, biological control has been used to achieve area-wide control and has mostly been successful. Table 1 indicates those invasive weeds already successfully controlled using a combination of biological control and other techniques, including information resulting from public awareness programs.

Existing and potential weeds

In the absence of recent survey data for the whole country, it is difficult to make a full list of invasive weeds occurring in PNG or to identify those weeds that might be invasive if they established here. A comprehensive weed survey would provide reliable information on the importance of weeds that are already common in the country. We know only of the existence of a few common invasive weeds. Table 2 lists weeds according to their perceived levels of importance. There are several species of invasive weeds that are already 'widespread' and these include a number of species that are currently 'restricted' in distribution but are known to be spreading.

Table 1. Invasive weeds under management or being targeted for eradication in Papua New Guinea

Under biological control	Control or eradication efforts in progress	Possibilities for eradication	Fortutious biological control
Chromolaena odorata (Asteraceae): biological control undertaken in 11 provinces *Eichhornia crassipes* (Pontederiaceae) *Lantana camara* (Verbenaceae) *Mimosa diplotricha* (Mimosaceae) *Pistia stratiotes* (Araceae) *Salvinia molesta* (Salviniaceae) *Sida acuta* (Malvaceae) *Sida rhombifolia* (Malvaceae) *Tribulus cistoides* (Zygophyllaceae)	*Parthenium hysterophorus* (Asteraceae): eradication from Lae, Morobe	*Mimosa pigra*: from Danip, Madang. *Piper aduncum*: single tree seen in mainland New Ireland in September 2004.	*Leucaena leucocephala* (Mimosaceae): the bug *Heteropsylla cubana* (Homoptera: Psyllidae) is controlling leucaena in many parts of PNG, having spread fortuitously into the country, to the dislike of pastoralists who consider leucaena as a useful fodder.

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Table 2. Invasive weeds that are widespread, restricted and spreading, potential 'sleeper' weeds, or are absent from Papua New Guinea[a]

Widespread and important	Restricted, locally important or spreading	Potential or 'sleeper'	'Absent' or unknown from PNG
Alternathera bettzickiana (Amaranthaceae)	*Adenanthera pavonina* (Fabaceae)	*Argemone mexicana* (Papaveraceae)	*Acacia nilotica* (Fabaceae)
Cyperus rotundus (Cyperaceae)	*Acacia farnesiana* (Fabaceae)	*Azolla filicoides* (Azollaceae)	*Ageratina adenophora* (Asteraceae)
Hyptis capitata (Lamiaceae)	*Chromolaena odorata* (Asteraceae)	*Azadirachta indica* (Meliaceae)	*Ageratina riparia* (Asteraceae)
Melinis minutiflora (Poaceae)	*Clerodendrum chinensis* (Verbenaceae)	*Clerodendrum quadriloculare* (Verbenaceae)	*Alternanthera philoxeroides* (Amaranthaceae)
Mikania micrantha (Asteraceae)	*Jatropha gossypifolia* (Euphorbiaceae)	*Clidermia hirta* (Melastomataceae)	*Austroeupatorium inulaefolium* (Asteraceae)
Mimosa pudica (Fabaceae)	*Merremia peltata* (Convolvulaceae) native	*Croton hirtus* (Euphorbiaceae)	*Cryptostegia grandiflora* (Asclepiadacaeae)
Muntingia calabura (Muntingiaceae)	*Mimosa pigra* (Fabaceae)	*Dissotis rotundifolia* (Melastomataceae)	*Macfadyena unguis-cati* (Bignoniaceae)
Panicum maximum (Poaceae)	*Monstera deliciosa* (Araceae)	*Hydrilla verticillata* (Hydrocharitaceae)	*Miconia calvescens* (Melastomataceae)
Pennisetum purpureum (Poaceae)	*Rhynchelytrum repens* (Poaceae)	*Limnocharis flava* (Limnocharitaceae)	*Parkinsonia aculeata* (Fabaceae)
Piper aduncum (Piperaceae)	*Pennisetum polystachyon* (Poaceae)	*Mucuna pruriens* (Fabaceae)	*Pontederia* spp. (Pontederiaceae)
Rottboelia cochinchinensis (Poaceae)	*Samanea saman* (Fabaceae)	*Salix* sp. (Salicaceae)	*Solanum mauritianum* (Solanaceae)
Sorghum halepense (Poaceae)	*Sphagneticola triloba* (Asteraceae)	*Tecoma stans* (Bignoniaceae)	
Spathodea campanulata (Bignoniaceae)	*Thunbergia laurifolia* (Acanthaceae)		
Sphaerostephanus unitus (Thelypteridaceae) native	*Tithonia diversifolia* (Asteraceae)		
Stachytarpheta spp. (Verbenaceae)	*Syngodium angustatum* (Araceae)		
	Xanthium strumarium (Asteraceae)		

[a] This list is incomplete and not in any order of importance.

From: Price, T.V., ed., 2006. Pest and disease incursions: risks, threats and management in Papua New Guinea. Canberra, ACIAR Technical Reports No. 62.

Many others are 'sleeper' weeds with the potential to become troublesome in the future. The threats posed by 'sleeper' weeds can be predicted only if these have become invasive in other parts of the world with climates and environments similar to those present in PNG.

Making a list of potential weeds 'absent' from PNG would be a cumbersome job, as numerous species occur outside which can enter PNG's borders. Table 2 lists only a few species that are known to be in neighbouring countries but not known or reported from PNG.

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An overview of pest incursions in Papua New Guinea over the past 20 years

R. Masamdu[1]

Abstract

This paper reviews exotic pest incursions into Papua New Guinea (PNG) over the past 20 years, the pathways these incursions have taken and how PNG quarantine and other responsible agencies responded. The responses varied according to the economic status of the pest and the availability of pest management and containment measures. For many pests, adaptive research on appropriate management measures was required in order to successfully manage the pests. Eradication has not been achieved. Emergency responses to some of these pests, e.g. the coffee leaf rust (*Hemeleia vastatrix*), proved to be expensive and eradication was impossible.

Introduction

Papua New Guinea (PNG) has diverse agricultural systems and biodiversity due to its wide range of environments, from atoll islands to mountains over 4000 m high. PNG has some of the wettest areas in the world, e.g. Kandrian and Kikori receive over 6000 mm of rain annually, while some areas in the central province receive less than 1200 mm (Hanson et al. 2001). Vegetation ranges from tropical forests to dry savannah grasslands, and huge swamplands to moss forests of the highlands.

This unique biodiversity and the country's agricultural production systems, which provide for 87% of the rural population's food security, cash income and cultural and social security, have been threatened by exotic pests and diseases and other invasive pests of environmental significance.

This paper provides examples of recent invasions of exotic pests that have contributed to changes in people's lives through loss of production, social and cultural values, and impact on food security and biodiversity. All government and private-sector institutions, non-government organisations, the farming community and the general public need to work together to minimise risks of introduction and spread of exotic and endemic pests and diseases, and to manage pests.

Previous pest incursions

Papua New Guinea recorded over 20 incursions of pests, weeds and diseases incursions during 1980–2003 (Table 1), a rate of one new incursion per year. These incursions were the results of either intentional or unintentional introductions. A few of the organisms arrived without human assistance.

Exotic weeds

Eight exotic weed species were recorded in PNG over the past 20 years (Table 1), most of them threats to biodiversity, agricultural production systems and fishing. The effect of weeds on the lives of ordinary Papua New Guinean's was not realised until *Salvinia molesta* D.S. Mitch. started clogging up the Sepik River system, especially the oxbow lagoons. The weed prevented villagers from fishing and getting access to schools and health services. This led to eventual

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migration of some villages to urban areas and new locations. Similarly, water hyacinth, *Eichhornia crassipes* (Mart.) Solms followed *S. molesta* into the same river system. Fortunately, both weeds are now under biological control, due mainly to programs funded by multilateral and bilateral donors. It was obvious from these two weed-control programs that PNG itself could not afford the cost of research and introduction of these agents (over 1 million kina) at the expense of other goods and services. The successful control of the terrestrial weeds *Mimosa diplotricha* C. Wright, *Sida rhombifolia* L. and probably *Chromolaena odorata* (L.) R.M. King & H. Rob. using biological control has demonstrated that PNG has the ability to manage serious weeds, but requires international and national collaborative support.

Exotic insects

Six exotic insect were recorded over this period: spiralling white fly *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae); banana skipper butterfly *Erionata thrax* (L.) (Lepidoptera: Hisperidae); papaya fruit fly *Bactrocera papayae* Drew & Hancock (Diptera: Tephritidae); citrus psyllid *Diaphorina citri* Kuway. (Hemiptera: Psyllidae); the leucaena psyllid *Heteropsylla cubana* Crawford (Hemiptera: Psyllidae); and the small fire ant *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae). All except one insect arrived without human assistance, three via PNG's border with Indonesia, and three (one human assisted) via the Pacific. Two species, *A. dispersus* and *E. thrax*, have had biocontrol agents introduced with help from the Secretariat of the Pacific Community (SPC) and the Australian Centre for International Agricultural Research (ACIAR), respectively.

Exotic diseases

Four exotic diseases have been recorded: coffee rust (*Hemileia vastatrix)*, potato late blight (*Phytophthora infestans*), huanglongbing (*Lactobacter asiaticum*) and fusarium wilt of banana (*Fusarium oxysporum* f. sp. *cubense*). The economic damage caused by the first two diseases costs many millions of kina. Similarly, research and extension to contain these two diseases in the respective industries cost the government and industries more than 20 million kina. Changes in farmers' attitudes and commitment to management practices are required to obtain and maintain high yields. Of the two other diseases, fusarium wilt is spreading faster because of the movement of banana planting material to other areas,

Table 1. Pest incursions into Papua New Guinea, 1980–2003

#	Species	Common name	Class	Control
1	*Salvinia molesta*	Salvinia	Weed	Biological
2	*Eichhornia crassipes*	Water hyacinth	Weed	Biological
3	*Mimosa diploctrica*	Giant mimosa	Weed	Biological
4	*Mimosa pigra*	Woody mimosa	Weed	Cultural, chemical
5	*Chromolaena odorata*	Siam weed	Weed	Biological
6	*Parthenium*	Parthenium	Weed	Chemical
7	*Limnocaris flaves*	Limnocaris	Weed	None
8	*Antigonon leptopu*	Chain of hearts	Weed	Cultural
9	*Mikania micrantha*	Mile-a-minute	Weed	Biological, cultural
10	*Aleurodicus dispersus*	Spiralling whitefly	Insect	Biological
11	*Erionata thrax*	Banana skipper butterfly	Insect	Biological
12	*Bactrocera papayae*	Papaya fruit fly	Insect	Cultural
13	*Diaphorina citri*	Citrus psyllid	Insect	Cultural, quarantine
14	*Wasmannia auropunctata*	Small fire ant	Insect	Cultural, quarantine
15	*Hemileia vastatrix*	Coffee rust	Fungus	Chemical, cultural
16	*Phytophthora infestans*	Potato late blight	Fungus	Chemical, cultural
17	*Lactobacter asiaticum*	Huanglongbing (citrus greening disease)	Bacterium	Cultural, quarantine
18	*Fusarium oxysporum*	Fusarium wilt of banana	Fungus	Cultural
19	*Pomacea canaliculata*	Golden apple snail	Mollusc	Cultural
20	*Heteropsylla cubana*	Leuceana psyllid	Insect	

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but surveys would be required to confirm the latest distribution in the country.

Other invasive plant pests

Molluscs

The golden apple snail was intentionally introduced for eating and escaped into the drains in Port Moresby and Lae. Since then the Port Moresby infestation has been eradicated, but the Lae population is slowly spreading into the surrounding waterways, drainage systems and paddy fields. The snail is difficult to control and prevention of its spread to other waterways will be achieved only through public awareness and action.

Rodents

Six pest species of rodents are recorded in PNG (M. Wamala, pers. comm.). While little is known on their history in the country, they are likely to have been introduced before the 1980s. They are known to affect oil palm, coconuts, stored grain in warehouses and seed stores, and field crops such as maize and rice.

Pathways of incursions

There are three main pathways of introduction: natural incursions, unintentional importation and intentional importation. Examples of natural incursions include the spiralling whitefly, papaya fruit fly, the banana skipper butterfly, coffee rust and the leuceana psyllid. The unintentional importations include huanglongbing, potato late blight, siam weed, *M. diaplotrica,* parthenium weed and *W. auropunctata*. Intentional introductions include *S. molesta*, water hyacinth, golden apple snail and the giant African snail (*Achatina fullica*).

The unintentional introductions were through machinery, goods and products introduced illegally or unsupervised, while intentional introductions were all illegal, without the prior approval and knowledge of quarantine or other authorities.

Minimising pathways of incursions

The identification of incursion pathways of many pests and diseases is important so that appropriate actions and efforts are made to minimise the risk of introduction of new pests through these pathways. It is clear that quarantine, as the first line of defence against these exotic organisms, must be strengthened to improve inspection and public awareness. Education of the travelling public, importers and traders will help to minimise incursions. Closer collaboration is required between industry, research institu-

Table 2. Pathways of introduction of pests into PNG

Pest	Mode of introduction	Pathway
Salvinia	Intentional	Aquarium
Water hyacinth	Intentional	Aquarium
Giant mimosa	Unintentional	Machinery
Woody mimosa	Unintentional	Machinery
Siam weed	Unintentional	Machinery
Parthenium	Unintentional	Machinery
Limnocaris	Unintentional	?Aquarium
Chain of hearts	Intentional	Floriculture
Mile-a-minute	Unintentional	Machinery
Spiralling whitefly	Natural	Jet stream air currents
Banana skipper butterfly	Natural	Dispersal by air
Papaya fruit fly	Natural	Dispersal by air
Citrus psyllid	Unintentional	Plant materials
Small fire ant	Unintentional	Plant materials, personal effects
Coffee rust	Unintentional	Plant material
Potato late blight	Unintentional	Plant material
Huanglongbing	Unintentional	Plant material
Fusarium wilt of banana	Unintentional	Plant material
Golden apple snail	Intentional	With personal effects
Leuceana psyllid	Natural	Jet stream air currents

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tions and National Agricultural Quarantine and Inspection Authority (NAQIA).

Discussion and conclusions

NAQIA already has a regular pest and disease surveillance program at the PNG–Indonesia border region. It also has a commodity inspection and certification system which inspects goods landing in PNG before their release. The internal quarantine inspection and certification for plant products is a much more difficult proposition. Inadequate resources, large numbers of ports of origin and destinations, and wider modes of transport including bush tracking and local motorised dinghies and dugout canoes make it difficult. NAQIA currently relies on public awareness, adherence, understanding and support from the public and industry to minimise the introduction of endemic pests and diseases into new areas.

Emergency response plans for all exotic pests are needed, and availability of funds is important for large-scale eradication and/or to prevent spread of incursions. The coffee rust eradication program cost approximately K10 million. The program did not have a emergency response plan and hence the eradication was costly. Similarly, no emergency response plan was available for late blight of potato, so the industry stakeholders had to devise a rapid plan of response.

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Spread of citrus huanglongbing (greening) disease following incursion into Papua New Guinea[1]

R. Davis[2], T. Gunua[3], M. Kame[3], D.I. Tenakana[3] and T. Ruabete[2]

Abstract

In much of Asia, huanglongbing (HLB, previously known as greening), a disease caused by the bacterium '*Candidatus* Liberibacter asiaticus' and vectored by the Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) is the most damaging disease of citrus. HLB spread widely through Southeast Asia last century, eventually reaching the island of New Guinea when it was confirmed by polymerase chain reaction (PCR) testing in the Indonesian province of Papua in 1999. By late 2002, the disease (detected by PCR) and the psyllids were discovered in and near the border town of Vanimo in the Sandaun Province of Papua New Guinea (PNG). An immediate follow-up survey identified features of the outbreak, which implied that eradication by killing psyllids with insecticides and destroying trees was not a feasible option. Instead, a response plan focused on minimising further spread within PNG. This was an intensive campaign of quarantine containment in the Vanimo region and public awareness throughout PNG. A second delimiting survey undertaken one year later indicated that long distance movement of the disease and its vector had not occurred. Of a total of 115 trees indexed by PCR, 4 were HLB-positive in 2002 compared with 11 in 2003. The second survey found evidence for limited HLB disease cluster expansion and further independent introduction of infected planting material. Vector dispersal in the Vanimo region was also found to be restricted and patchy. It appears that movement of the disease in this remote semi-urban environment is considerably slower than what has been observed in intensive Asian orchard production situations.

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Impact of some food-crop disease outbreaks in Papua New Guinea

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Abstract

Many plant pathogens or agents cause disease outbreaks or epidemics when they spread from their endemic areas of origins into new regions. These outbreaks often result in disasters, particularly to cultivated crops which farmers rely on for food and other purposes. This paper presents some examples of food crop disease outbreaks that occurred in Papua New Guinea after the Second World War and their impact on farmers who cultivated these crops.

Introduction

There is a great diversity in the way plant diseases occur and progress. Certain pathogens appear in a sporadic or isolated manner, whilst others occur and spread over a wide geographic area, with disastrous consequences for humans. A disease outbreak becomes an epidemic when the causal agent causes mass infections due to favourable conditions and spreads rapidly to other areas away from its area of origin.

Several disease outbreaks or epidemics have occurred in Papua New Guinea (PNG) since the end of the Second World War. All the outbreaks were different from each other in the way they occurred and spread, and their impact on the host crop and the farmers that cultivate them for food and cash income. Only two of the possible seven disease outbreaks that occurred during this period had obvious but disastrous effects, while the occurrence of the others appeared to be largely unnoticed by the farmers though they could have significant quarantine risks.

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This paper presents an overview of six food-crop disease outbreaks to see how they occurred and their impact on subsistence farmers. In addition, the paper briefly highlights what has been done to reduce the impact of the diseases in terms of disease management and internal quarantine.

Taro leaf blight

The disease

Taro leaf blight, caused by the fungus *Phytophthora colocasiae*, is one of the three major diseases affecting cultivated taro in coastal areas of PNG. The disease was first reported in PNG in the 1940s (Connell 1978). It is believed that taro leaf blight was first introduced into the country through Bougainville during the Second World War or through Indonesia's Irian Jaya province. The first reported outbreak of the disease on Bougainville resulted in its spread to the other parts of the New Guinea islands and eventually onto the northern mainland. The second major epidemic originated on Normanby Island in Milne Bay in the early 1980s, then spread to other areas of Milne Bay, eventually reached the taro-growing areas of South Fly in the Western Province. The disease is now endemic and widespread. It can be commonly

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seen on both cultivated and wild taro from sea level up to mid-altitude areas of all the provinces in the country.

Symptoms

The initial symptom of the disease is the appearance of small, dark lesions or spots on both sides of the leaf but water-soaked on the underside. Infection usually starts in the centre and along the leaf margins where retention of water droplets is greater. At first the spots are circular but become irregular as they enlarge or coalesce with other spots. Depending on climatic conditions and the variety, the spots are often zoned and surrounded by a yellow halo or border. Infected leaves can collapse within 5–10 days depending on the severity of the infection, size of the leaf blade and the location of the initial site of infection. Otherwise, the infected tissues become necrotic, dry and fall off, leaving shot-holes. In severe infections, petioles and fruit-covers develop symptoms.

Infection and spread

Under favourable weather conditions, spores initiate infection on leaves. Secondary infection occurs on the same leaf as spores are splashed or moved around by rain-splash, wind and dew. Infection and spread of the pathogen is favoured by temperatures in the range 25–28˚C and relative humidity of 65% during the day, while 20–22˚C and 100% humidity favours disease spread in the morning. Long-distance dispersal occurs through infected planting material and corms. Corms become infested with spores while in the field and initiate rots of corms in storage. Wild taro and other species of aroids have been found to be hosts of the pathogen in Papua New Guinea.

Effect of the disease

The most obvious effect of the disease is the damage caused to the functional leaves. Any fully opened leaf is prone to attack by the fungus. Work in PNG and elsewhere shows that the reduction in corm weight is related to leaf loss due to infection. Yield loss of up to 50% has been reported under climatic conditions favourable to the disease (Jackson 1977; Cox and Kasimani 1988).

The post Second World War epidemics that started from Bouganiville and Normanby Island caused serious losses and resulted in loss of taro varieties and a general decline in taro cultivation (Connell 1978). The epidemics were very devastating because there was no resistance or tolerance in all or most varieties grown by farmers. According to reports and observations, farmers in certain parts of Central Province began growing taro again only after about 10 years.

Control

Cultural methods of control include removing and burning infected leaves, crop rotation, use of wider spacing, mixed cropping and making new gardens away from other taro gardens. Generally, these are not effective and are not practised by subsistence farmers. However, farmers may consider using these practices to reduce the incidence of the disease under commercial production systems.

Several fungicides can control the disease. Work carried out in PNG has shown that 0.3% Ridomil plus 72WP is effective against the disease, and spraying should concentrate within the period 2–5 months after planting. Use of chemicals is not recommended to subsistence farmers. Commercial farmers who wish to use fungicides against the disease must contact plant pathologists at Keravat or Bubia for further advice.

Taro leaf blight has a short history in PNG. The first outbreaks of the disease were very devastating and farmers lost many of their taro varieties. Because of the high genetic diversity of taro in PNG, the situation was not as bad as that experienced by farmers in Western Samoa after the 1993 epidemic.

Research carried out at the Lowlands Agricultural Experimental Station (LAES) in the 1960s showed taro varieties had no field resistance to the disease. Taro varieties with good resistance to taro leaf blight were first identified from the national taro germplasm at Bubia Agricultural Research Centre in 1992. The varieties were originally collected from the Gazelle area in East New Britain Province. They were initially used for the current taro breeding work at Bubia, which started in 1992.

Use of taro varieties with field resistance or tolerance is the most suitable method to combat taro leaf blight in subsistence food gardens. It is very simple and the safest method that farmers can use.

A breeding program was initiated in 1992 at the Bubia Agricultural Research Station, with a broad aim of developing resistant taro varieties to control or manage taro leaf blight in PNG. By 2001, three varieties were released which are highly resistant or tol-

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erant to taro leaf blight but also are high yielding and have good eating quality. These varieties are now being distributed to farmers from National Agricultural Research Institute (NARI) research stations at Bubia and Keravat. Subsistence farmers must be advised to grow the varieties together with other taro varieties they have in their gardens. Observations in food gardens in the Gazelle area of East New Britain at present seem to indicate that at least two varieties with high resistance to TLB are present in farmers' fields. Farmers must be encouraged to grow more of these varieties with their preferred varieties to manage taro leaf blight.

Potato late blight

The disease

Potato late blight is caused by the fungus *Phytophthora infestans*. It is the most destructive disease of potato where it is grown as both a commercial and subsistence crop. The disease was first noted in PNG on potato crops in the Sirunki area of Enga Province in January 2003. and also in the Mt Hagen and Tomba areas of the Western Highlands. Within three months the disease had spread to all five Highlands provinces. The disease was also reported during a survey carried out in the Telefomin and Oksapmin areas of Sandaun and it is possible that the disease may have entered PNG as early as mid 2002. The occurrence of potato late blight in PNG was confirmed by NARI and National Agricultural Quarantine and Inspection Authority (NAQIA) plant pathologists in February 2003 (P. Kokoa, unpublished data).

Symptoms

Symptoms of late blight vary widely depending on factors such as moisture, temperature, light intensity, cultivar and age of plants. Lesions on leaves usually first appear as small, light to dark green irregularly shaped spots which rapidly expand. As lesions age, the centres become necrotic, turning brown to black. Larger lesions on some potato cultivars are often bordered by a light green halo. Under moist conditions, profuse sporulation occurs, especially on the undersides of leaves. Presence of white mycelial growth containing numerous spores on a lesion is often used to distinguish foliar late blight infections from other diseases.

Lesions can also occur on petioles and stems, often killing entire leaves or branches of affected plants. Terminal leaves on newly lesioned stems often roll upward and margins turn red to purple, symptoms which can be mistaken for those of other diseases such as leaf roll virus or Rhizoctonia stem canker. Old stem lesions on dead plants can easily be mistaken for advanced symptoms of black leg caused by *Erwinia* spp. Foliar symptoms of early blight (*Alternaria solani*) can be confused with late blight infection.

Late blight also affects potato tubers. Susceptibility of tubers can differ from that of foliage. Tuber lesions are irregular in shape, brown to purplish in colour, and are slightly depressed. Internally, lesions are dry, tan in color, without distinct margins, and are usually confined to the outer 1–2 cm of the tuber flesh. Tuber lesions are readily invaded by secondary pathogens such as *Fusarium oxysporum* that cause decay and often makes tuber diagnosis difficult. Tuber symptoms caused by *A. solani*, *Erwinia* spp. and other diseases can often be confused with tuber symptoms of late blight.

Infection and spread

Phytophthora infestans reproduces both asexually and sexually. Depending on environmental conditions, asexual regeneration time can be very rapid and the entire cycle repeated in 5–7 days. In addition, a single sporangium can undergo indirect germination and produce 6–12 motile zoospores. These spores are mainly responsible for the new infections, which may take place through any part of the epidermis of leaves and stems, either through stomata or the intact cells. Foliar infections may occur through either the upper or lower surfaces of the leaf, but the undersides seem to be more susceptible. The sporangia are thin-walled and may retain their viability for 10–14 days only.

Tuber infection following an attack of late blight is by means of sporangia or zoospores, which are produced on the diseased foliage and are subsequently washed or swim into the soil. The general infection of the tubers can take place through lenticels, or cracks in the periderm. Contact of tubers with blighted foliage at harvest is also responsible for infections. Healthy tubers can become infected from blighted ones in the soil or storage if sufficient moisture is available.

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Effect of the disease

Since the first outbreak of potato late blight over 150 years ago, this disease has remained a primary problem in potato production areas throughout the world. The impact of potato late blight in PNG has been disastrous. The PNG potato industry, with an annual production of 18,000 tonnes valued at 10–15 million kina, was almost destroyed within a short period. Before the epidemic many farmers grew susceptible potato varieties for food and cash income. Smallholder subsistence production is now restricted to farmers who can afford to buy seeds, fertilisers and equipment for chemical control. The potato seed production and distribution scheme in the highlands was also significantly affected. Most of the potatoes for consumption or seed planting material are now imported and, as a result, the retail price has increased dramatically.

Potato production is also confronted by a new threat; the widespread occurrence of the A2 race of the pathogen, which can lead to sexual recombination and a breakdown in resistance to strains of the fungus.

Clubroot of brassicas

The disease

Clubroot of brassicas or crucifers is caused by *Plasmodiophora brassicae*. The disease is present worldwide wherever brassicas such as cabbage, broccoli, cauliflower and related plants are cultivated.

Symptoms

Plants affected by clubroot show yellowing of leaves and reduced plant growth (Agrios 1978). Wilting of leaves occurs, particularly on sunny days, because the uptake of water through the root system has been greatly disrupted. The characteristic symptoms of the disease are seen on the roots and the below-ground part of the stem. Infection by the pathogen causes the main and feed roots to become enlarged, distorted and form spindle-like or club-shaped swellings. Older and larger clubbed roots will become necrotic and decompose due to infection caused by other organisms in the soil. The symptoms on the roots are similar to root-knot nematode infections on tomato and aibika plants, except there the swellings are not club-shaped. Also, brassicas are highly resistant or tolerant to root-knot nematode infection

Infection and spread

The can survive for many years as resting spores in infected crop debris or in the soil.

The disease can be readily transmitted to non-infested soil through infected seedlings or mature plant roots, and by soil-contaminated farm machinery or tools.

Effect of the disease

Clubroot is a major disease problem that can significantly affect production of cabbages and other brassicas in certain parts of the PNG Highlands where the disease is present. It is impossible to grow cabbages in soil heavily infested with spores of *P. brassicae*. The Kabiufa SDA farm, a few kilometres west of Goroka in Eastern Highlands Province, used to supply cabbage, broccoli and cauliflower to retail stores in major towns in PNG until a clubroot outbreak in May 1987. The disease has been reported to have spread to neighbouring villages in the Asaro Valley where control of the disease would be impossible under subsistence production conditions.

Control

The disease is difficult to control because the resting spores can survive in the soil for long periods. Crop rotation and bush fallow are the best options in areas where the disease is already present. Farmer awareness is another disease-management strategy. There is no information available on whether or not any of the varieties of cabbage, broccoli and cauliflower currently available in PNG are resistant or tolerant to the race of the pathogen that occurs here.

Fusarium wilt of banana

The disease

Fusarium wilt, or Panama disease, is one of the most destructive diseases in the recorded history of agriculture. It is caused by the fungus *Fusarium oxysporum* f. sp. *cubense*. The fungus is a soil-inhibiting organism and attacks the xylem of susceptible cultivars through the roots, causing wilt and death of the whole plant.

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There are four races of *Fusarium oxysporum* f. sp. *cubense* (FOC), three of which are primary pathogens of banana. Race 1 is found in most banana-growing regions and pathogenic to many cultivars. Race 2 is pathogenic to cooking banana and Race 3 attacks *Heliconia*. Race 4 was described in recent years and seems to be a major threat to banana cultivars such as Cavendish in the tropics. Race 1 of the fungus has been reported from parts of Western Province and Sandaun Province, PNG, detected by border surveys conducted by Australia and PNG (Davis 2004).

Symptoms

Any aged plant is attacked by the Fusarium wilt pathogen (Ploetz and Pegg 1999). The fungus is a wilt-causing pathogen that enters the stem through the water-conducting vessels of the roots. The first external symptoms of the disease are progressive yellowing and browning of the older leaves at the margins towards the midrib, and longitudinal splitting of the lower part of the outer leaf sheaths on the stem. The affected leaves collapse and hang down as a skirt around the stem. Eventually the whole plant or stand is killed. The plant nevertheless still produces healthy looking suckers, but these will also become infected later on. The most characteristic symptom of the disease is reddish to dark brown discoloration of the water-conducting tissues of the pseudostem near ground level when a diseased stem is sliced with a knife.

Infection and spread

FOC is a common soil inhabitant, found in almost all parts of the world. The pathogenic strain that specifically infects bananas gains entry to the corms (rhizomes) and the pseudostem through the roots. The fungus grows and spreads up the stem through the water-conducting tissues. When the plant dies and rots, the fungus makes its way back into the soil as chlamydospores (another form of spore). The chlamydospores are resting structures of the fungus that persist in soil for many years. These are the main source of inoculum to initiate new infections.

The disease is most commonly spread in infected planting materials (rhizomes). The fungus can also be introduced in soil on farm implements and machinery and in running water.

Effect of the disease

Fusarium wilt is ranked as one of the most important crop plant diseases. The disease threatened to wipe out the banana production industry in Central America and the Caribbean in the 1940s and 1950s. It was then that the disease was recognised as a major threat to commercial production of export bananas. At that time, Race 1 of the pathogen destroyed plantation banana varieties like Gros Michel. Resistant Cavendish cultivars were introduced into the tropics during the 1960s but they were reported to succumb to a new race (Race 4) in South Africa in the 1970s. This race is now present in Australia and Irian Jaya, and threatens the diverse banana germplasm in PNG.

Control

Control of the disease is very difficult because the fungus persists in the soil as chlamydopores for many years and the disease can be very easily spread on infected planting material. Control of the disease should be based on preventing its spread within a province or to other provinces in PNG through cultural and quarantine measures. Quarantine authorities must take a more vigorous approach to inform people, particularly in Western Province, about the disease and its threat to PNG's diverse native banana varieties.

Sweet potato stem and leaf blight

The disease

Stem and leaf blight is a new disease of sweet potato in the highlands of PNG. The disease was first recorded from a subsistence food garden in the Nebilyer Valley of the Western Highlands Province in early 1987 (Kokoa 1991, 2002). A fungus, *Alternaria alternata*, was identified as the causal agent of stem and leaf blight on sweet potato in PNG. Other species, namely *Alternaria solani*, *Alternaria tenuissima* and *Alternaria bataticola*, have also been recorded as causing stem and leaf blight of sweet potato in few other countries. Symptoms of stem and leaf blight on sweet potato were first recorded from Ethiopia, probably before 1988.

Stem and leaf blight at present is confined to the highlands of PNG. Disease surveys carried out in the late 1980s showed that the disease was present in isolated areas of Western Highlands, Southern High-

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lands and Simbu provinces. The disease was recorded at Aiyura Research Station in early 1992.

Symptoms

The early or initial symptom of the disease in the field is the appearance of small, black, oval or circular lesions about 1 mm in diameter on the stems and petioles. The lesions become irregular as they enlarge or when they coalesce. Under favourable weather conditions, the lesions continue to enlarge and completely girdle the stem and petiole. Under stress conditions, severe infections eventually result in the death of the whole terminal shoot or individual leaves. The lesions are initially superficial and became depressed as they increase in size. An individual lesion on the stem may enlarge to 5 cm in length.

Affected leaves initially show general yellowing and eventually the whole leaf blade or lamina dries up. Infection on the lower surface of the leaf can also lead to uneven chlorosis of the leaf blade. Occasionally, death of leaves on one side of the stem above the lesion is observed. This occurs when a lesion does not completely girdle the stem, especially with varieties that have thicker stems. Shoot or tip dieback is another symptom associated with the disease. It is uncommon in wet weather except on varieties with thinner stems and petioles. Dieback usually more common in dry weather, when the lesions completely girdle stems and petioles and become bleached and cracked.

Infection and spread

The fungus persists or lives as mycelium in infected crop debris in or on the surface of the soil. Infection of stems and petioles occurs under favourable weather conditions when spores germinate and enter healthy tissues either directly or through wounds. The fungus does not infect tubers. Disease surveys carried out in 1988 and 1989 showed that the disease was readily spread to parts of Western Highland Province and Simbu Province through infected planting material from Kuk Agricultural Research Station (KARS).

Effect of the disease

Stem and leaf blight of sweet potato is a relatively new disease in PNG and other countries where it has been reported and there is no information on the effect of the disease on yield. However, observations made during disease surveys in the late 1980s indicated that the extent of stem and petiole infection during favourable climatic conditions was quite extensive. The severe effect of the disease on the growth of vines is seen during periods of dry weather when lesions, particularly on petioles, become bleached and cracked leading to development of dieback symptoms.

Control

There is no information on cultural or chemical control of leaf spots and leaf and stem blight of sweet potato caused by *Alternaria* species. However, varieties that are resistant to *Alternaria* been reported in other countries. Limited work carried out at KARS indicated that 41 of the 600 varieties in the germplasm were susceptible to the disease. It is therefore likely that a large number of varieties resistant or tolerant to the disease may already exist in farmers' fields.

Peanut witches' broom disease

The disease

Witches' broom disease of peanuts is present in China, India, Indonesia, Japan, Taiwan, the USA and a few other countries. The disease was first reported in PNG in February 2004 (P. Kokoa, unpublished data). Symptoms of the disease were first reported from an experimental site at LAES. Examination of specimens sent to the UK confirmed that the symptoms were due to a phytoplasma (Jones, unpublished data).

Symptoms

Infected plants show a proliferation of axillary shoots, general leaf chlorosis and stunting. Pegs of infected plants tend to curl upward but this symptom was not observed until about a month later. By then, affected plants were in very poor state; many leaves had became necrotic and fallen off. There were hardly any pods on severely affected plants.

Infection and spread

Disease assessment carried out at LAES in February 2004 showed that 20 of the 23 varieties had symptoms of witches' broom. Peanut gardens or

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blocks in Kokopo and Gazelle were surveyed shortly after the disease was reported at LAES, to find out if the disease was also present in village gardens. Experimental plots at other NARI research stations were also surveyed for the disease. It was tentatively concluded that the witches' broom outbreak was confined to LAES. It is believed that witches' broom is transmitted by insect vectors and through seed. All plants with disease symptoms were destroyed and, to prevent the spread of the disease to other areas, no seeds were distributed to farmers.

Effect of the disease

The disease incidence at LAES ranged between 0.2% and 0.3%. However, the effect of the disease on each plant seemed to be very serious because all or most affected plants would have died without producing pods.

Control

Quarantine and the roguing of diseased plants are the only control methods available.

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The PNG pest list database and its uses in quarantine surveillance and pest management

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Abstract

The Papua New Guinea (PNG) pest list database is a tool created to foster decision-making and exchange of information in quarantine, pest-management research, pest management and trade-related issues. This paper outlines the uses of this database and suggests how sharing of vital information between research and development organisations in PNG in the pest list database would enhance the plant protection activities between organisations and agencies in the country.

Introduction

The island of New Guinea has a unique flora and fauna, with perhaps the highest biodiversity in the world. Most of its inhabitants, as hunters and gatherers, have depended on agriculture, fishing and forest products for food and shelter. This unique biodiversity is now threatened by various human development activities.

Papua New Guinea (PNG) has long had a pest list database, but fragmented and in various forms; e.g. the Department of Agriculture and Livestock (DAL) annual insect-pest survey records and plant-disease survey records. All agricultural crop, quarantine and livestock research was implemented by one department, namely DAL.

The restructuring of the departments and the formation of a number of commodity research and development institutions led to further fragmentation of the information. The National Agricultural Quarantine and Inspection Agency (NAQIA) and the National Agricultural Research Institute (NARI) supported by the Plant Protection Service of the Secretariat of the Pacific Community (SPC) therefore took the initiative to establish a formal national pest list database that would help decision-making in quarantine surveillance, pest-management research and development of pest-management measures and trade. Continued surveillance and update of such records are important for the application of global trade protocols.

This paper presents an outline of the pest list database and how PNG institutions can benefit from use of the information in it. It highlights the need for collaborative efforts between institutions, agencies and the private sector.

The PNG pest list database (PLD)

The PLD is an information system that records pest occurrences within a country and provides reports on these occurrences. The most important record is the list of all pests that have been found on any particular crop. Such lists are needed by exporting countries for establishment of trade agreements and are required as part of the International Plant Protection Convention (IPPC) (Article 7i). Similarly, the list of hosts for any given pest is required for import risk analysis.

The system can provide other reports such as a list of all weeds found in a country, and supporting pub-

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lications, provided the necessary data has been entered into the system. It can also be used by quarantine services to record pest interceptions at ports and airports.

The database has agriculture pests records from DAL insect-pest records, plant-disease survey records and weed-survey records, as well as individual pest records on crops such as sugarcane, coffee and cocoa. Contributions from national institutions such as universities and from the private sector would help to make further essential information available.

The database is also a quarantine tool to facilitate trade between countries. It is a requirement of the IPPC article 4/2 that:

> The responsibilities of an official national plant protection organization shall include the following: the surveillance of growing plants, including both cultivated and wild flora, particularly with the object of reporting the occurrence of pests.

Therefore, surveillance for new pests, spread of endemic pests and establishment of new pests, and interception records, are being maintained and updated. In pest management, it is a useful tool for information on pest distribution, host plants and crop damage.

Discussion

The database is an important information tool and all organisations involved in plant protection education, extension, quarantine, research and production should have input into the database and the output made available to them. NAQIA is the official national IPPC plant protection organisation in PNG. It will continue to liaise with partner institutions to make this database a useful plant protection tool. Farmers need to market their produce and, particularly to tap export markets, need our collaborative assistance. It is recommended that further training be carried in PNG to include staff in institutions that were not able to attend two training courses held previously.

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Coconut inflorescence borer, *Synneschodes papuana* (Lepidoptera: Brachodidae), an important new pest of coconut in Papua New Guinea

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Abstract

Coconut palm occupies a total land area of 260,000 ha in Papua New Guinea and is cultivated for cash and food. In 2000, *Synneschodes papuana*, the coconut inflorescence borer, was reported in the northern region of the country. This paper describes the insect, symptoms caused, nature of damage, and yield and monetary losses.

Introduction

Coconut palm (*Cocos nucifera* L.) is the most popular oil crop in Papua New Guinea (PNG), providing cash income for an estimated 250,000 households; as food it is consumed by over three million people (Anon. 1990). Nut production is declining due to aging coconut palms and lack of replanting, hindered by endemic insect pests. In the main coconut producing areas of the country's Islands region, the beetle pests *Scapanes australis* Boisduval (Coleoptera: Scarabaeidea), *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidea), and the black palm weevil *Rhynchophorus bilineatus* Montrouzier (Coleoptera: Curculionidae) continue to cause economic loss. In the northern region of the PNG mainland, a new pest of coconut, the inflorescence borer, is an economically important pest.

Insects that feed on coconut inflorescences are very important as they directly destroy the flowers, resulting in fewer nuts per inflorescence. The pest status of such feeders has changed over the years and insects that were previously insignificant pests have now become pests of economic importance. One such insect is the coconut inflorescence borer (CIB), *Synneschodes papuana* (Lepidoptera: Brachodidae). This insect was recorded on Manam Island in Madang in 1913 and recognised as a pest of coconut in 2000. Other inflorescence feeders of economic importance reported in the past included the coconut spathe moth (SPM), *Tirathaba rufivena* (Walker) (Lepidoptera: Pyralidae) (Smee 1965; Perry 1980). The larvae of this pest bore into both male and female flowers (buttons). The larvae and adults of the coconut spathe bug *Axiagastus cambelli* Distant (Hemiptera: Pentatomidae) (Smee 1965; Perry 1980) feed on the spathe and immature flowers, causing button nut fall, whereas *Amblypelta* sp. (Hemiptera: Coreidae) (Smee 1965; Perry 1980) larvae and adults feed on inflorescences and immature fruit, causing button shed.

Stewart Research Station (SRS) is the centre for coconut research in PNG. Yield of nuts from young producing coconut palms on the station is low. One problem is the coconut inflorescence borer, *S. papuana*. This paper reports on studies of its pest status and economic impact.

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Materials and methods

Symptoms

The two important pests of mature coconut palm inflorescence are SPM and CIB. The symptoms of damage caused by these two insects are difficult to differentiate, as the CIB symptoms at ground level and close range have yet to be described and compared with SPM and the natural physiological wilting of developing fruit.

Description of the insect pest

As CIB has not been previously described and classified, the characteristics of the insect, the type of damage it causes and the life stages of economic importance were investigated.

Assessment of yield loss (nut production)

Yield loss in terms of nut production per palm in two trial plots was assessed at SRS during 2002 and 2003. The first was a coconut-based farming system trial in which cocoa (*Theobroma cacao*), abiu (*Potouria sopata*), vanilla (*Vanilla fragrans*) and pepper (*Piper nigrum*) were inter-planted as sub-plots with coconut planted at four different densities. All coconuts in the trial plots were assessed for pest incidence and severity of infestation. All inflorescences in the crown of each palm were visually checked for borer infestation. Samples of inflorescence were also removed and checked for the presence of larvae and pupae. All nuts on all inflorescences of the sample palms were counted. The coconut palms were 5 years old and were about 5 m high from palm base to the crown.

The second trial consisted of the coconut breeding progeny trials numbers 702, 703 and 704. The sample palms were systematically selected (one palm/progeny/replicate) from a complete randomised trial design with 5 replicates and 15 palms per plot per progeny. The palms were 9, 8 and 7 years old, respectively, and data collection was identical to that in the first trial.

Results

Symptoms of damage

Infested inflorescences were easily observed from the palm base because their colour changed to dark brown (damaged) (Figure 1). Economic loss is caused by the larval stage of the CIB. The spikelets bored into by the larvae of this insect first turn brown, then black once dead. As the feeding (tunnelling) progresses towards the base of the spikelet adjoining the rachis, the portion of the rachis above the entry point (tunnel) gradually dies, turning black from an original brown/yellow/red or green. The developing fruits on the damaged inflorescences die and become blackened and can remain attached. Dead and blackened fruits on healthy inflorescences result from physiological causes and not caused CIB. Developing fruits damaged by SPM would have fallen off soon after death and the late stages of SPM complete their life cycle in fruit on the ground. Partially damaged inflorescences (> 6 months) would normally carry a small number of fruit towards the basal end of the inflorescence rachis. Dead and dying inflorescences that have blackened remain on the palm and can be spotted easily.

Figure 1. Typical symptoms on a coconut palm inflorescence of damage by *Synneschodes papuana* larvae

Description of adult

The moth of *S. papuana* was described from a specimen collected on Manam Island off the north coast of Madang Province in 1913, but there was no record of this insect as a pest of coconut palms.

The adult moth (Figure 2a) is about 11 mm long and 4 mm wide. Its wings measure about 20 mm from tip to the tip when fully opened. The head and ventral part of the thorax, and the basal parts of the thoracic appendages, are creamy yellow. There are two pairs of wings which are blackish brown. Each forewing contains a yellow quarter-circle, which at rest forms a thick semicircle. The undersides of the wings contain creamy yellow spots. Antennae are filiform.

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The pupae (Figure 2b) are brown, 12 mm long and 3 mm wide. The larvae (Figure 2c) are creamy and, when fully grown, are about 22 mm long and 2 mm wide. They then shorten and widen in preparation for pupation. The larvae have three pairs of short, thin legs on the thorax and four pairs of short, round and fleshy legs on the abdomen. The dorsal ends of the nine body segments point outwards to form leg-like appendages, probably an adaptation for burrowing in tunnels.

The number of larval stages is not known. The following account is based on feeding galleries and head capsules observed. The eggs are probably laid on the tips of spikelets on newly unfolded inflorescences after male flowers have fallen off. The newly emerged larvae begin to feed on the surface of the spikelets then, after moulting, they bore into the spikelet. The second instar larvae then begin to feed on the epidermal layer of the spikelets. After another moulting, the third-stage larvae then bore into the centre of the spikelet and burrow their way towards the spikelet axil. The portion of the spikelet above the damaged region eventually dies. The larvae then continue to burrow towards the axil of the spikelet and into the adjoining rachis. The portion of the inflorescence above the excavated region dies. Large larvae have been seen migrating to neighbouring lower inflorescences on the same palm and attacking them.

Yield loss

The results from the survey are shown in Tables 1–4. Generally, almost 100% of the palms sampled were infested by CIB. A high number of inflorescences per palm were also infested. As the insect burrows its way down the rachis, more spikelets were destroyed, resulting in the abscission of further immature fruit.

The range of nut loss recorded in the farming system trial was 20–41% in 2002 and 12–20% in 2003.

Mean fruit number per damaged inflorescence varied from 5.6 on young (1–6 months) and 2.3 on older inflorescences (7–12 months).

Economic importance

Three main products can be sold at the farm gate: copra, nuts (both mature and tender) and coconut oil, as well as other minor products from shells and husks. Table 1 shows the market value of each product and the number of nuts required to produce a tonne of copra or 1 L of virgin coconut oil. Selling 5000–6000 dry nuts at the farm gate will raise K750–1200. A tonne of copra will fetch about K600, while virgin oil produced from the same number of nuts will fetch K1733–2080 at the local market (Madang) price of K5.20 per litre. An average of 3456 nuts/ha (28% of

Table 1. Comparison of prices of different coconut products sold

Product	Average price/nut (toea)	A tonne of copra equivalent (kina)
Dry nut	20	1000–1200
Tender nut	30	1650
Copra	15	600
Coconut oil – virgin	30	1733–2080
Coconut oil – small scale	15	1000
Coir product	Yet to be calculated	
Activated carbon	Yet to be calculated	

Figure 2. *Synneschodes papuana*: (a) adult, (b) pupa and (c) larvae

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total nuts produced) was lost due to pest damage, amounting to a monetary loss of K691/ha.

Discussion

The symptoms of infestation and economic importance of *T. rufivena* and CIB differ. *Tirathaba rufivena* attacks mostly male flowers and button nuts, whereas CIB are internal feeders; the young larvae feed inside the spikelet, then bore into the endodermis of the spikelet and rachis. Death and blackening of part of the spikelet and inflorescence are typical symptoms of CIB infestation. *Tirathaba rufivena* larvae are very active and move quickly when disturbed; they are external feeders and webbing on male flowers and button nuts is common. They complete their life cycle on fallen button nuts.

The economic losses caused by CIB can be estimated easily, because of obvious symptoms; that is, dead or dying inflorescences and dead fruit. Losses caused by *T. rufivena*, by contrast, can be difficult to assess, because the damage symptoms can easily be confused with physiological effects in which the inflorescence remains alive with some or no nuts.

The CIB is a small moth which completes its life cycle on the coconut inflorescence. The adult has not been observed in the coconut crown, including on inflorescences, but it has been seen flying on the vegetation at the base of palms in mid afternoon and at dusk. They are not attracted to light. Late larval stages of CIB have been observed moving from damaged inflorescences to healthy ones on the same palm, suggesting that a larva can destroy more than one inflorescence.

At SRS, at least 12% (range 12–41%) of nut production is lost as a direct result of the insect pest described here. Damage by these insects poses a bigger than previously thought threat to the coconut production at SRS and perhaps the neighbouring palm groves. Most palms in the sampled plots showed 100% infestation. The number of inflorescences damaged was also high, ranging from 2 to 7, with an overall mean score of 4.1 per palm. The number of nuts per infested inflorescence was also low (range 0–7) with an overall mean of 3.4 nuts per inflorescence.

A high percentage of nuts is lost in coconut breeding progeny trials. Palm age and palm height does not appear to influence *S. papuana* infestation (Table 2), although there are some indications that the level of infestation falls with increased height of the coconut palm. The results also indicate that plant density does not influence the level of pest infestation (Tables 3 and 4). Nut losses generally increased with increasing coconut densities.

Normally in coconut, one inflorescence matures per month. The damage caused by *S. papuana* usually results in the death of all or part of an inflorescence, resulting in the complete or partial loss of nuts from that bunch.

The insect attacks and usually kills the spikelet and inflorescence above the feeding site, resulting in death of immature fruit. The dead part of the inflorescence sheds nuts, resulting in a smaller number of nuts.

Insect-infested palms utilise assimilates produced, but this does not translate into economic gain. A low number of inflorescences infested can be tolerated, as assimilates can be re-directed to support more nuts on a new inflorescence.

High nut-set is sometimes observed on palms that have undergone strong disturbance in their production in response to accumulation of surplus assimilates (Foale 1993).

Table 2. Fruit losses recorded in the coconut breeding progeny trials during 2003

Mean infestation and nut lost	Progeny trials			
	ID 702	ID 703	ID704	Average
Number of palms sampled	80	80	27	
Palms infested (%)	100.0	100.0	88.9	
Mean inflorescence damaged per palm	5.6	5.6	4.1	5.1
Mean nut per damaged inflorescence	3.6	3.5	4.3	3.8
Mean nut/damaged inflorescence (1–6 months)	5.2	5.3	6.2	5.6
Mean nut/damaged inflorescence (7–12 months)	2.0	1.9	3.1	2.3
Estimated nut loss/ha	4136.6	3860.9	2370.3	3456
Losses (PNGK @ 20 toea/nut)	827.32	772.18	474.06	691.2
Percentage loss (based on 12-monthly data)	33.7	32.2	18.3	28.1

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Table 3. The infestation and losses of immature fruits caused by *Synneschodes papuana* during 2003 in the coconut agronomy fertiliser trial 801, plots 703, 704 (160 palms/ha)

Replicate	Sample plots (6 palms/plot)	Palms infested (%)	Mean inflorescence damage/palm	Mean nut/damaged inflorescence
1	7	92.7	2.3	7.5
2	9	100.0	3.0	4.6
3	11	97.0	2.8	4.9

The percentage of palms infested is based on the number of palms flowering.
Nut loss/ha = $(\mu - ob) \times d \times n$
where μ = mean nut number per healthy inflorescence per palm per year (on SRS it is 6.25). An average of 12 inflorescences mature in a year producing 75 nuts per year.
ob = mean nut number per damaged inflorescence
d = mean number of damage inflorescence per palm
n = number of palms per hectare.

Table 4. Pest infestation and nut yield loss in 2003 and 2002, in coconut-based farming system trial 810, plots 041, 042, 051, 052

Treatment	Number of palms infested	Palms infested (%)	Mean damaged inflorescence/ palm	Mean nut/ damaged inflorescence	Total nut loss/ha	Losses (PNGK @ 20toea/nut)	Nut loss/ha (%)
2003							
1	9.0	100.0	4.7	3.6	495.0	99.00	16.3
2	16.0	100.0	4.4	3.9	980.9	196.18	20.0
3	23.7	98.6	3.5	3.6	949.4	189.88	12.2
4	30.0	100.0	4.5	3.1	1722.4	344.48	18.9
2002							
1	8.5	100.0	6.1	1.2	1203	240.60	41.1
2	14.5	100.0	3.5	1.8	1074	214.80	20.8
3	23.0	100.0	5.7	1.0	3072	614.40	39.4
4	28.0	100.0	4.7	1.3	3024	604.80	31.0

In insect-infested palms, assimilates produced are lost through shedding of nuts, death of inflorescences and maintenance of inflorescences without nuts. In cases where most inflorescences are dead, resources may be redirected to non-productive parts of the palm, such as vegetative production, or stored in the trunk.

The extent of economic loss depends on the type of coconut product the farmer sells, from low-value copra to virgin coconut oil and other high-value products. The loss could be even higher in locations where the average annual yield is greater than 100 nuts per palm per year. On Stewart Research Station, the average yield is about 70 nuts per palm per year.

Synneschodes papuana is a very important pest of coconut, and biological and ecological studies on it are continuing.

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Overview of internal plant quarantine and the challenges in Papua New Guinea

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Abstract

Although legislation on internal plant quarantine in Papua New Guinea existed before and soon after independence, for a number of reasons it is currently not enforced strictly. These include the transfer of extension service functions to provincial governments and gradual decline in funding support and logistical capability of the National Agricultural Quarantine and Inspection Service, whose duties include inspection and certification of movement of plant material between provinces. There are also many ports and modes of travel and an increasing frequency of movement of goods and services through minor ports. Also, people disregard authorities and take whatever plant and animal material they can fit into their baggage and cargo when they travel.

Introduction

Internal quarantine in Papua New Guinea (PNG) is ineffective and this had led to the intentional and unintentional introduction and spread of pests into new regions and provinces. There are internal quarantine restrictions in place for some plants, but it is very difficult to monitor the movement of people and the types of plant products they are carrying, and the risks for introduction into new areas remain high. The use of motorised dinghies to travel between islands from any point of departure, villagers walking with planting material for days through bush tracks, and the presentation and sharing of food items as gifts in traditional ceremonies all make monitoring difficult. The travelling public also does not often use the available public transport system and existing infrastructure to travel between regions.

The National Agricultural Quarantine and Inspection Agency (NAQIA) is a state agency that enforces quarantine regulations, but it is not adequately resourced and hence it is capable of enforcing internal quarantine only to key target pests of commercial importance. The public often fails to notice these pests at first and therefore reports to NAQIA and other agriculture agencies usually come well after the pest has become established.

This paper describes previous and current quarantine issues and makes suggestions for improvements.

Pre-independence quarantine perspectives

The Territory of Papua and New Guinea (TPNG) was governed by the Australian colonial administration up to 1975. The country was divided into districts, subdistricts and patrol posts. A district was the equivalent of a province. While a subdistrict was equivalent to an electorate, subdistricts were not based on electorates but on tribal, local language and geographic attributes.

All goods imported into TPNG came via Australia and New Zealand. No product was directly shipped into TPNG from other countries.

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The Department of Agriculture, Stock and Fisheries (DASF) was responsible for implementing programs in environment, conservation, agriculture, livestock, forestry and fisheries and was administratively a single department. All district rural development officers (DRDOs) and the rural development officers (RDOs) had the a role as quarantine officer and were a presence in all administrative districts and subdistricts of TPNG. All DRDO and RDOs effectively policed internal quarantine. All boats leaving New Britain to sail to mainland New Guinea and Papua, for example, were checked for giant African snail by DRDOs and RDOs, as it was a gazetted quarantine pest. This procedure remained until 1978 when the provincial government system was introduced.

Quarantine perspectives 1975–1996

The quarantine regulations were enforced by DRDOs and RDOs up until the formation of provincial governments. The inception of provincial governments disrupted quarantine activities and a new structure had to be built up, as quarantine remained a function of the national Department of Agriculture and Livestock (NDAL). Provincial extensions officers were no longer directly under NDAL and hence their duties were slightly changed to suit provincial and district development needs and priorities. The quarantine service, still under NDAL, was reorganised and quarantine officers were recruited and placed at major ports of entry to facilitate trade as well as conduct surveillance and inspection. After independence there were no major renovations or replacement of laboratory equipment.

Quarantine perspectives 1996–2004

Agricultural quarantine was one of the technical divisions of NDAL that was restructured to improve productivity and output, as under the old NDAL structure, quarantine was not given adequate funding and support. In 1996, NAQIA was established under the NAQIA Act. In 1998, however, the national government failed to fund NAQIA and thus fees were introduced to meet operating costs. The fees are modest but inadequate government funding allocations in subsequent years made it difficult for NAQIA to expand and increase its operational and technical capability.

NAQIA acquired from NDAL poor and run-down infrastructure such as buildings and laboratory equipment and inadequate staff resources. All entomologists, for example, were transferred to the National Agricultural Research Institute (NARI). The laboratory buildings and some equipment still need repair and/or replacement.

NAQIA is now challenged with the task of providing an effective, quarantine surveillance and inspection service to cater for the wide range of products being traded, and cope with faster modes of travel and increased economic activity, all of which increase the likelihood of new incursions of exotic pests and the spread of endemic pests to new localities within the country. PNG is a member of the World Trade Organization (WTO) and must meet the requirements for crop pest lists, be able to deal with new products for both import and export, and seek to harmonise its regulations with international quarantine protocols. All this, in addition to keeping tag on developments in internal quarantine, matters of biodiversity, biosecurity, food safety, trade facilitation, and prevention of pest incursions, has been a challenge to NAQIA.

The mass migration of people from villages into towns, seeking employment, has helped the spread of endemic pests and diseases into new regions. Papua New Guineans often like to take their favourite local varieties of plants — food crops, fruits, nuts and ornamentals — into new areas where they are settling. The giant African snail was initially only on New Britain, but has now found its way into other provinces. The spread of the banana leaf roller, banana fruit fly and *Chromolaena odorata* are other examples of pests being spread, either intentionally or unintentionally. It is therefore reasonable to say that there was previously little public awareness of the risks of spread of plant pests and diseases. Some specific public awareness programs, such as those for water hyacinth and *Salvinia molesta*, were nevertheless very effective and people were aware of the consequences of the occurrence and spread of these plants. Their spread was thus restricted. Targeted pest awareness is better understood by the public than more general information.

Discussion

Many endemic and introduced pests are often not widely distributed and have a restricted range because of the isolation of regions due to poor trans-

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port infrastructure. However, faster transport systems and increased migration into new areas and between locations, provinces and towns have increased the likelihood of pest spread.

Internal quarantine awareness requires adequate planning, and the involvement of many state and private agencies to help NAQIA in extension and increasing the awareness of the public. There are now many vessels and aircraft travelling to many ports. There must be dialogue between NAQIA and shipping companies and agents, transport companies, civil aviation, express delivery service providers, airline workers and management, and traders in agricultural commodities.

The provincial government system has disrupted the previous quarantine procedures that were considered effective and NAQIA relies on understanding and cooperation from various agencies to implement internal quarantine. The movements of plant and plant products are gazetted and notices are issued to all stakeholders to ensure plants and plant products are not moved out of a locality without a quarantine check. This system remains the best way to restrict the spread of pests and diseases.

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The value of early detection and internal quarantine boundaries in the management of incursions: some examples in plant protection from northern Australia and Papua New Guinea

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Abstract

Surveillance under the auspices of the Northern Australia Quarantine Strategy has led to the early detection of a number of new plant pests, plant diseases and weeds in northern Australia and the western border regions of Papua New Guinea (Western and Sandaun provinces). In many cases, these detections have led to the implementation of eradication, containment or other strategies (e.g. local quarantine of the affected area or host, biological control efforts) to manage these incursions. Examples discussed here are the annual eradication effort in the Torres Strait against exotic fruit flies, the introduction of biocontrol agents against spiralling whitefly (*Aleurodicus dispersus*) in northern Australia and against chromolaena (*Chromolaena odorata*) in Papua New Guinea and the role of internal quarantine boundaries within Australia to limit the movement of plant pests and diseases. The detections of citrus psyllid (*Diaphorina citri*) and huanglongbing, a serious citrus disease, around Vanimo in Sandaun Province in 2002, and the aquatic weed limnocharis (*Limnocharis flava*) in Kiunga Western Province in 2003, represent serious threats to people's lives, productivity and the environment in those areas. Possibilities for management of these are discussed. They could include one or more of the abovementioned methods, or intensive extension and education of the local producers to reduce the impacts.

Introduction

The Northern Australia Quarantine Strategy (NAQS) was established, within the Australian Quarantine and Inspection Service (AQIS), in 1989 to meet the unique quarantine risk presented to Australia's northern shores. The large-scale movement of people along the Indonesian archipelago, particularly to Papua (formerly Irian Jaya) on the island of New Guinea, was changing the pest, weed and disease spectrum present on that land mass. The gap between Australia and Papua New Guinea (PNG) is narrow in places (only 150 km between Cape York and the shores of the Western Province). The Torres Strait islands lie scattered across this gap. Traditional movement between islands of the Torres Strait Protected Zone and certain coastal villages of the Western Province is permitted under the terms of the Torres Strait Treaty established in 1978.

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Australia's northern shoreline is vast and carries a low population. It was expected that if some new, alien pest were to arrive in this part of the country it could be many years before its discovery, by which time it would be too late to take control or eradication action, and other options would be limited.

The NAQS program combines three aspects of quarantine:

1. Scientific teams undertake surveillance and monitoring in northern Australia and (in collaboration with the respective governments) Timor L-este, Indonesia and Papua New Guinea.
2. Operational staff regulate the movement of goods carried by travellers (including those moving within the terms of the Torres Strait Treaty).
3. There is a strong focus on public awareness to promote quarantine throughout communities in northern Australia.

Each of these plays a significant role in the maintenance of a quarantine border in this area.

The scientific teams carry out domestic surveys within northern Australia, along the coastal region between Broome in the west and Cairns in the east, including the Torres Strait islands. Memoranda of understanding between PNG and Australia, Timor L-este and Australia, and Indonesia and Australia facilitate collaborative surveillance work in PNG and Papua. The first NAQS surveys in PNG were of the coastal regions of the Western Province adjacent to islands of the Torres Strait, and included an extension component to inform the residents of the quarantine restrictions which apply when travelling into Torres Strait on 'treaty' visits. At the request of the PNG Government, later surveys were extended to include sites within the Western and Sandaun provinces, which lie close to the border with Indonesia. The first survey along this route occurred in 1992. There is usually one NAQS survey within PNG every year.

The program also provides opportunities for PNG staff to accompany survey teams within Australia. Gapi Kula (1997), Marjorie Kame (2002) and Tony Gunua (2004) from the PNG National Agricultural Quarantine and Inspection Authority (NAQIA) have participated in plant health surveys in the Torres Strait. Ilagi Puana, Nime Kapo and Tom Malaisa have accompanied animal health surveys within Torres Strait and Western Australia. Timor L-este and Papuan staff have also accompanied NAQS staff on surveys in the Northern Territory of Australia and Torres Strait.

Surveys along the northern Australian coastline are timed to match the level of assessed incursion risk and range from visits once per year to once every five years. Covering the vast expanse of the northern shoreline of Australia (and all the inhabited islands of Torres Strait) keeps the survey staff well occupied.

The NAQS role is largely restricted to detection of new incursions, while management of incursions is the responsibility of State and Territory agencies. NAQS surveys have resulted in the early detection of a number of significant insects, weeds and plant diseases. The responses to these detections have covered a range of options, some of which are examined below.

Australia has a strong quarantine boundary and is known around the world for its strict regulations and management of the movement of goods and people into the country. Aside from the international quarantine barrier managed by AQIS, there are a number of internal and regional quarantine zones within Australia. State agencies, for example, manage State quarantine boundaries between Tasmania and the mainland and between Western Australia and the rest of the country.

Even within the eastern states there are quarantine boundaries that relate to specific pests or planting material (as well as movement of stock such as horses and cattle). The 'Tri-State fruit fly exclusion zone' lies in the southeast and includes parts of South Australia, Victoria and New South Wales. This is a major fruit-producing area and movement restrictions keep the area free of fruit flies. Road blocks are maintained and there is a monitoring grid of traps within the zone, as well as an extensive public awareness campaign which encourages residents to send in any fruit that is possibly infested. Anyone flying to Melbourne from Queensland will be asked to drop their fruit into an 'amnesty bin' within the airport.

Within Queensland there are State-managed restrictions on the movement of sugarcane and banana planting material between various designated districts, and restrictions on moving a wide range of plants south along Cape York Peninsula. These regulations are designed to restrict the movement of pests and diseases (both existing and potential) between the zones.

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Some case studies in management of incursions

1. Exotic fruit flies in Torres Strait

Melon fly (*Bactrocera cucurbitae* Coquillett) was recorded from the Western Province of PNG before the start of NAQS surveys in 1989. The collaborative survey between NAQS and the PNG Department of Agriculture and Livestock (NDAL) personnel detected the presence of Asian papaya fruit fly (*Bactrocera papayae* Drew & Hancock) in the Western Province of PNG in 1992. Australian quarantine already had a series of fruit-fly monitoring traps on selected islands of the Torres Strait (Morschel 1983), but these PNG detections, particularly that of Asian papaya fruit fly (PFF), prompted an increase in the number of traps on the islands closest to PNG. In March 1993, PFF was detected on two islands in the Torres Strait. At the time there was no established plan of action. A first response was to set-out additional traps on more islands and a total of 200 Steiner traps (baited with methyl eugenol) were placed on 50 islands. Within 10 days of establishing these traps, PFF was found to be present on a further three islands. Continued trapping did not detect any more affected sites. If these detections had been on the Australian mainland, then another immediate response would have been to establish a quarantine zone around the affected area. However, the islands of Torres Strait had already been divided into separate quarantine zones in 1985, along with the establishment of the Torres Strait Treaty between PNG and Australia. All of the affected islands lay within the Torres Strait Protected Zone which covers Australian territory from the coast of PNG south to the 10°28' parallel. The few islands between there and Cape York are in the Special Quarantine Zone and include the islands clustered around Thursday Island and others close to Cape York.

It was decided to suppress the fly numbers on the three islands which lay closest to PNG, on the assumption that reinfestation was inevitable, and attempt eradication on the other two islands. Bait spraying, using a mixture of yeast and insecticide to attract and kill fruit flies, began on all five islands immediately. There was a delay of several months before delivery of the male annihilation component on Stephen and Darnley islands. During that time, drop tests were carried out with lengths of soaked caulking cotton, to determine the length that would quickly drop away from the helicopter and then entangle in vegetation, and carry the required dose per hectare for effective treatment. The eradication was effective, but expensive, costing $A160,000 (or $202,550 in 2004 terms) (Sabine et al. 1994). The incursion of exotic fruit flies soon proved to be a regular event, occurring with every northwest monsoon after 1993. To keep costs down, the methods for dealing with these incursions were gradually refined.

There is now an established plan of action for dealing with these incursions which details prescribed responses for given events. The plan is revised after each season and modified where necessary. The work is funded under an agreement with the Primary Industries Standing Committee (PISC). Where the events fall outside the guidelines, the Technical Advisory Panel, a group of six scientists and AQIS operational staff, meet (usually by teleconference) to decide what action should be taken. Funding is guaranteed by the terms of the PISC agreement. Eradication of a similar incursion to that experienced in 1993 occurred in 1997 and was effected at a cost of $A44,745 (or $53,247 in 2004 terms; about a quarter of the cost of the original incursion).

In August 1995, PFF was detected at a site near Cairns. Following the initial detection, a large number of traps were established in a wide area around Cairns. The fly was found to occur at several sites remote from Cairns (up to 80 km away), but there was no evidence of it between these sites. It was assumed that the pest had been distributed by local fruit-sellers carrying their produce to weekend markets at these remote sites, rather than by natural spread of the fly. A full economic assessment was made before deciding to attempt eradication. It was anticipated that this would take around five years to accomplish. The methods that had been refined by previous seasons in the Torres Strait were used here. Bait spray was applied weekly and soaking caneite blocks with the methyl eugenol mixture and nailing them to trees and posts throughout the affected area achieved male annihilation. In addition, feral guava trees, chilli plants and coffee plants were removed. Eradication was accomplished in three years at a cost of $34 million (Cantrell et al. 2002).

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2. Spiralling whitefly

Spiralling whitefly (*Aleurodicus disperses* Russell) was first reported in PNG in 1987 (Waterhouse and Norris 1989). In 1990 it was well established in Port Moresby and in coastal communities of the Western Province (Grimshaw 1990). In February 1991 it was detected on Boigu Island in Torres Strait (Grimshaw 1991) and recorded from a range of hosts in the commercial, native and weedy flora. An application to import the micro-wasp parasitoid (*Encarsa* sp. nr *haitiensis:* Aphelinidae) directly into the population of whitefly on Boigu Island was made. Permission was granted by AQIS, and two batches were imported (in April and September 1992). The wasps were sourced from cultures managed by the Secretariat of the Pacific Community in Fiji. The numbers imported were small and the release conditions were less than ideal. However, two years later the parasitoids had established and the residents were once more able to grow chillies and other Solanaceae. The whitefly gradually spread through the islands of Torres Strait. Further specimens of the parasitoid were sourced from Fiji in 1993 and1994 when the whitefly arrived on Thursday Island (Cantrell 1997). The AQIS insectary and laboratory on Thursday Island allowed for a much more controlled release and establishment of the wasp at this location, but parasitoids from the now established population on Boigu were also introduced to Thursday Island (Lambkin 2004). The whitefly gradually spread south through major population centres, appearing at Seisia near the tip of Cape York Peninsula in March 1995 and in Cairns in March 1998. It is apparent from the distribution pattern of the whitefly that its spread is human assisted. Parasitoids drawn from the mixed culture on Thursday Island have been used as the source population to introduce into new whitefly infestations on mainland Australia.

In spite of strong quarantine regulation it was not possible to restrict the whitefly to the Torres Strait Protected Zone (where it first appeared). Nevertheless, the early introduction of the parasitoid probably reduced its rate of spread and certainly reduced its impact.

Similar success has since been achieved in PNG using the same parasitoid for control of spiralling whitefly.

3. *Chromolaena odorata* (chromolaena, Siam weed)

Chromolaena odorata (L.) King and Robinson (Asteraceae), widely regarded as one of the world's worst tropical weeds, was known to occur in East New Britain, PNG before 1970 (Henty and Pritchard 1973; cited in Bofeng et al. 2004) and its presence elsewhere was suspected. However, it was first officially recorded on mainland PNG in June 1992, during a NAQS survey of the border region of Sandaun Province (Waterhouse 1992, 2003a). PNG and Australian authorities were immediately notified of its presence there and further infestations were subsequently found in other provinces.

PNG authorities successfully sought to join an ACIAR-funded biological control program that was already underway in Indonesia and the Philippines. Two agents, the arctiid moth *Pareuchaetes pseudoinsulata* Rego Barros and the stem-galling tephritid fly *Cecidochares connexa* (Macquart), have been introduced as part of this program (Orapa et al. 2002; Bofeng et al. 2004). A further two species, the leaf miner *Calycomyza eupatorivora* (Agromyzidae) and the stem-boring weevil *Lixus aemulus* Faust (Curculionidae) are expected to be brought into quarantine in PNG soon. Bofeng et al. (2004) report that *P. pseudoinsulata* has not established at all the sites where it has been released (10 of 35 sites), but that *C. connexa* has established readily at more than half of the sites where it has been released, with no apparent evidence of parasitism (Bofeng et al. 2004). During the most recent NAQS survey to the Vanimo and Bewani districts of Sandaun Province, it was observed that, although *C. connexa* had only been released in Sandaun Province in 2002, it seemed to be spreading rapidly and already appeared to be having an impact on chromolaena there (Waterhouse, pers. comm., October 2004).

Some cases in need of solutions

1. Citrus psyllid and huanglongbing

Huanglongbing (HLB), previously called citrus greening, is caused by two species of uncultivable, phloem-limited bacteria. The disease is vectored over short distances by psyllids. Candidatus '*Liberibacter asiaticus'* (the Asian form of the disease) is vectored by the Asian citrus psyllid (*Diaphorina citri* Kuway.) and Candidatus '*Liberibacter africanus'* (the African

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form of the disease) is vectored by the African citrus psyllid (*Trioza erytreae* (Del Guercio)), but spread over greater distances is by the movement of diseased planting material. HLB is not known to be seed borne. The pathogen causes a slow decline of citrus trees, and disease symptoms are the same as those of nutrient deficiencies, in particular zinc and/or manganese. Citrus varieties vary in their susceptibility to the disease but there are no known treatments for it.

NAQS surveys in Papua detected the presence of Asian citrus psyllid in the Jayapura area in 1992. The Asian form of HLB was confirmed from the same area by a NAQS survey in 1999 (Davis et al. 2000b). Both the psyllid and the disease were detected in Vanimo in August 2002 (Weinert et al. 2004) and the psyllid was present at Wutung, with no evidence of the disease. The disease seemed limited to a few trees in Vanimo and it was hoped that eradication could be achieved. A delimiting survey in November 2002 by the Secretariat of the Pacific Community and NAQIA staff (Davis et al. 2006) determined that there were other sites close to Vanimo where both the insect and the disease were well-established and the planned eradication attempt was abandoned in favour of attempting to limit the spread to other parts of PNG by quarantining the area.

However, regulatory staff are few and the routes out of the area are many. Any limitation of the spread will be heavily reliant on public compliance, which will require a large extension effort. Further concerted and continual extension effort in this area are required to carry information on local management of the disease, as well as efforts to restrict the movement of diseased planting material and the citrus psyllid. Unless HLB and the psyllid can be restricted to Sandaun Province, citrus production in all areas of PNG will be under threat.

2. Banana wilt diseases

Fusarium wilt of banana is a severe disease of banana that kills the banana plant usually before fruit production. The disease is caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (FOC). There are many strains of FOC, which have generally been grouped by their ability to cause disease on different banana cultivars, and are referred to as races 1,2 and 4. A more accurate laboratory technique divides the pathogen into vegetative compatibility groups (VCG). Several FOC VCGs have been found in PNG to date: VCG 0126 has been found three times on cooking bananas (genotype ABB) (Shivas and Philemon 1996; Davis et al. 2000a) and two new VCGs recorded on two different banana cultivars were recently determined (S. Bentley, pers. comm., 2004). Of greater concern to PNG, are strains classified in the VCG1213/16 group, erroneously called 'Tropical Race 4'. This strain of the pathogen can attack a much wider range of banana types and has devastated Cavendish banana production in Indonesia and Malaysia. The disease has been found in three locations in Papua (Davis et al. 2000b) with the closest location Merauke. The disease is present in Australia, but under quarantine containment near Darwin in the Northern Territory. It is spread via infected planting material and as a contaminant in soil attached to other commodities, such as sweet potato or yams.

Blood disease is a lethal bacterial disease of bananas. The disease is spread in infected planting material and soil but can also be transmitted from flower to flower by insects. The disease is particularly common on ABB varieties with dehiscent bracts but other varieties are also affected. The disease was first reported in the early 1900s from the Indonesian island of Sulawesi where it forced the abandonment of newly established dessert banana plantations (Eden-Green 1994). In parts of Indonesia, blood disease is spreading at rates of more than 25 km per year (Eden-Green 1994). In 1999, the disease was found at Timika in Papua during a NAQS survey. Unless appropriate quarantine measures are enacted and enforced, both the VCG 1213/16 strain of FOC and blood disease pose serious risks to banana production and biodiversity on the island of New Guinea, a centre of diversity for bananas. Due to the dependence on banana, a starchy staple for many people in PNG, this also represents a serious threat to food security.

3. *Detection of* **Limnocharis flava** *(limnocharis, yellow burr-head) in the Kiunga area*

Limnocharis flava (L.) Buchenau (Alismataceae or Limnocharitaceae) is an aquatic or semi-aquatic herb that forms clumps up to 1 m tall. It reproduces both vegetatively and by seed. A native of Central and South America, it has become invasive in humid tropical localities in southern and southeastern Asia, where it was probably originally introduced as an ornamental species. Here it has become a serious weed of paddy rice, irrigation canals and wetlands.

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Soerjani et al. (1987) reported that *L. flava* had not yet been recorded from Papua. However, NAQS surveys in 1997 and 1999 found it to be widely naturalised in Papua, and sometimes in cultivation as an edible green vegetable (Waterhouse 2003a).

Limnocharis flava has been a focus of NAQS weed surveys since 1990. Several small infestations were discovered in Far North Queensland in 2001 and are currently the target of eradication efforts (Waterhouse 2003a). Knowledge of its presence in the Jayapura and Merauke districts of Papua, gained from NAQS surveys, led to the suspicion that it might turn up in border regions of PNG. Several small, wild populations were found in the Kiunga district (Western Province) during a NAQS survey in 2003 (Waterhouse 2003b). Voucher specimens were submitted to the PNG National Herbarium at Lae and verified at the Queensland Herbarium. To date, limnocharis has not been detected in Sandaun Province, but anecdotal reports when residents are shown leaflets suggest that it may be present there.

Limnocharis poses a major threat to the permanent and seasonal wetlands of major river systems in PNG. Unlike the superficially similar floating aquatic weed, water hyacinth, limnocharis plants root in the muddy substrate and thus are less likely to completely occlude deep bodies of water. Limnocharis grows as a perennial species in regions with humid climates, but behaves as an annual species in regions where there is a long and pronounced dry season. It is thus ideally suited to invade extensive areas of seasonal wetland in the Fly and Sepik River systems. At Kiunga, the small, known infestations already occur in tributaries of the Fly River. Anecdotal reports received during the NAQS survey in 2003 suggest that it is also established in the Alice (Ok Tedi) River which flows into the Fly.

Further surveillance to establish the current extent of *Limnocharis flava* and assessment of its potential threat to PNG arerequired. Unfortunately, it does not appear to have a history of successful biological control efforts elsewhere. The eradication efforts in Australia are mostly reliant on hand-pulling of small infestations in suburban areas. The Queensland Department of Natural Resources and Mines has also undertaken preliminary investigations into chemical control (P. Wilkinson, pers. comm. 2003). However, these methods are not well suited to potentially extensive areas in remote locations.

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Red-banded mango caterpillar, *Deanolis sublimbalis* Snellen (Lepidoptera: Pyralidae: Odontinae), in Papua New Guinea

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Abstract

Deanolis sublimbalis Snellen (red-banded mango caterpillar) affects 20–40% of fruit in Papua New Guinea (PNG) where mangoes are a significant part of the diet. Control is difficult. Laboratory trials and field observations were therefore conducted to investigate its behaviour and biology in PNG and formulate cultural or other control methods to reduce the damage it causes.

Introduction

Deanolis sublimbalis Snellen (Lepidoptera: Pyralidae: Odontinae), the red-banded mango caterpillar (RBMC), is a serious pest of mango, *Mangifera indica* L. (Anacardiaceae), where the fruit is grown in Southeast Asia and Papua New Guinea (PNG). RBMC attacks most cultivated varieties of mango in PNG and has been recorded from *Mangifera minor* Blume, a wild fibrous mango found in the Central Province of PNG. Additional hosts for RBMC (Waterhouse 1998) include *Mangifera odorata* Griffith from Indonesia, and *Bouea burmanica* Griffith (Anacardiaceae, maprang or marian plum) in Thailand. The genus *Mangifera* contains many species, with at least 27 species known to have edible fruit and it is therefore quite possible that further wild hosts will be found.

RBMC was first recorded on the Australian mainland in October 2001. In 2000–2001, the Australian mango industry produced approximately 45,555 t of fruit valued at $A100m (R. Williams, pers. comm.). Control of RBMC is fairly difficult but in some countries four sprays of cyfluthrin or deltamethrin commencing at 60 days after fruit induction are applied (Waterhouse 1998).

In PNG, 20–40% of fruit is damaged. Damage occurs in young developing fruit of varying sizes. Fruit as small as 10 mm in diameter are commonly attacked in PNG. The damage is caused by the first larval instar that bores into the fruit immediately after hatching. The early larval instars damage the flesh of the mango, whereas the later larval instars are generally found in the seed. Although the feeding is internal, the damage to mango fruit is conspicuous. Sap oozes from the larval entry point, accumulates on the apex or the drip point of the fruit and darkens. The damage caused by the larvae to the fruit makes it vulnerable to diseases and provides an entry point for secondary insect invaders such as the mango fruit fly, *Bactrocera frauenfeldi* (Schiner), *B. papayae* sp. n

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and ferment flies, *Drosophila* spp., which cause rapid deterioration of fruit.

This paper reports on biological studies conducted on *D. sublimbalis* at Laloki Agriculture Research Station, PNG. The station is in Central Province, 30 km from Port Moresby (9°22'S; 147°15'E). The study site consisted of seven varieties of *M. indica* introduced from Queensland, Australia, as well as two local PNG varieties. Mango has two flowering seasons in Central Province, the first June–August and second November–January. There is synchronised flushing and fruiting of all mango varieties during these periods. This study began during first flower flushes of the period June–August and was repeated in the following season, October–January, when eggs were discovered and laboratory egg-laying was improved.

The study included life history investigations that were made possible by the discovery of egg-laying sites in the field and subsequent modification of laboratory rearing techniques that led to laboratory oviposition. Studies were made of fecundity, larval behaviour, the survival of first-instar larvae immediately after eclosion and the longevity of adults in captivity. RBMC damage assessment studies gave initial estimates of 30–40% damage, but this will be further investigated in subsequent research that is planned as a result of these initial studies.

Materials and methods

Life history data were recorded for 62 caterpillars, from egg-laying through to adult emergence.

Five mango varieties used were: three introduced varieties (‘Kensington Pride’, ‘Glen’ and ‘Banana Calo’) and two local varieties (‘Carrot’ and ‘Local Mango’). All varieties of mango were in full bearing, and thus provided fresh developing fruit as a larval food source and egg-laying sites for adults. The study site consisted of two orchards with 5 trees in one location and 20 trees in another location about 750 m apart. The understorey of both orchards was mowed regularly to reduce weeds and high grasses. The introduced varieties were all 10-year-old, grafted trees and had well-maintained canopies. The average height of the trees was 5 m. The local varieties were grown from seed, about the same age as the grafted mangoes, but were 10 m high. Because of their height, the canopies were not maintained.

Field-damaged fruit were cut open and semi-mature larvae were extracted from the seed then placed in plastic specimen tubes (100 mm × 50 mm diameter) containing sawdust from untreated timber and fresh blocks of mango kernel (20 mm × 10 mm) as food supplement for the larvae. The larval tubes had lids with 2.5 mm openings with 1 mm^2 nylon mesh glued on for ventilation. The larvae were monitored daily. Blunt tip forceps were used to handle them to ensure minimal damage. The mango kernal was replenished daily until the pre-pupal stage was reached.

Adults, as they emerged, were transferred into breeding cylinders (203 mm diameter × 304 mm high) consisting of clear plastic with a round aluminium pan on the bottom and top. A ratio of 1:3 females:males was placed in the cylinders. Adults were sexed by careful compression of the posterior end of the abdomen. Female abdomens are tapered posteriorly, whereas males have a rounded end.

A solution of sugar, honey and water soaked into 20 mm square blocks of florists foam was attached to the lid and sides of the breeding cylinders as a food source for the adults. Torula yeast was added to the sugar/honey solution as a protein source for maturation of adults. The following day, fruit was introduced for egg-laying. Unripe fruit, 10 mm to 50 mm diameter, still attached to peduncles was introduced into the breeding cylinder and attached by paper clips to its lid. The fruit was examined daily with a 10× hand lens until eggs were observed on the fruit, following which the fruit were examined at 50× under a stereo-dissecting microscope.

Mated individual females were introduced into separate breeding cylinders for fecundity and longevity studies and provided with food and fresh fruit for egg deposition. One fruit at a time was introduced. When oviposition commenced, fresh fruit was supplied daily and the previous day’s fruit was removed. The egg lay from each fruit was recorded daily.

The studies were conducted under laboratory conditions in which the ambient temperature ranged from a minimum of 25°C to a maximum of 35°C. There was no airconditioning in the rearing room and natural ventilation occurred through insect screens covering the walls of the building. Lighting was provided by a series of 36 watt fluorescent tubes during daytime. The lights were switched off at night.

Egg-laying sites on living mango trees were located after meticulously searching through the fruit panicles. Developing ‘Kensington Pride’ fruit of 10 mm in diameter with conspicuous indications of

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RBMC attacks were examined, along with undamaged fruit on the same panicle.

Results

Life-cycle studies

The field-laid eggs were ovoid (0.45 × 0.7 mm) and milky white when laid, but gradually changed colour to crimson in 2–3 days. In the field, this colour index was important for differentiating between newly laid and older eggs. Subsequently, the eggs sometimes became stained with foreign matter including exudates from the fruit stalk and appeared reddish brown.

The orientation of field-laid eggs was similar to that observed for the laboratory-laid eggs. When eggs were laid singly or in twos they were regular in shape and size. However, when laid in clusters the eggs were often irregular in shape and size; some conforming to the shape of crevices in which they were laid. A maximum of 15 eggs has been recorded in a cluster, Even though the eggs were irregular in shape the emerging larvae do not appear to be deformed. The eggs had a waxy covering that became stained with dirt and other debris, making them difficult to locate. Eggs were usually laid on the peduncle or the base of the developing fruit. The eggs were inserted underneath the sepals, or amongst dried debris around the sepals, or pealed crusts of the peduncle to which the sticky eggs adhere. At times the female embedded the eggs along the crevice of brown crusts that formed on the skin of the fruit. The eggs that were laid within crust crevices remained exposed. If the fruit skin was clean and free of crusts, the female oviposited only on the peduncle, up to a centimetre or so along the fruit stalk. More than one cluster of eggs was found on the peduncle of one fruit.

Egg and larval development

The incubation period of the eggs ranged from 8 to 12 days, with a mean of 9.8 days. The day before hatching, the eggs appeared watery and transparent. The larvae of a cluster hatched as a group, proceeded individually downward to the apex, then returned to about one-third to halfway from the apex by means of silken thread. They then clustered at a site generally on the sides of the fruit and collectively bored into the rind and flesh. Up to 11 larvae per fruit were recorded, but they dispersed in search of fresh fruit as the food source ran out.

Green mangoes produce a large amount of very viscous exudates when injured. Similarly, the early instars of RBMCs induce large amounts of exudates whilst feeding on the flesh of the young mangoes. It was therefore not possible to follow the early instars in this set of experiments. First-instar larvae, which are about 1 mm long, survive the exudates induced by the initial penetration of the larva into the fruit by protruding a pair of posterior spiracles through the surface of the ooze. The posterior spiracles are the only visible appendage apparent. The larva then remains static till the flow of exudates diminishes, before boring further into the fruit.

The period from larval eclosion to pre-pupal stage was 11–21 days (mean 16.3 days). The pre-pupal

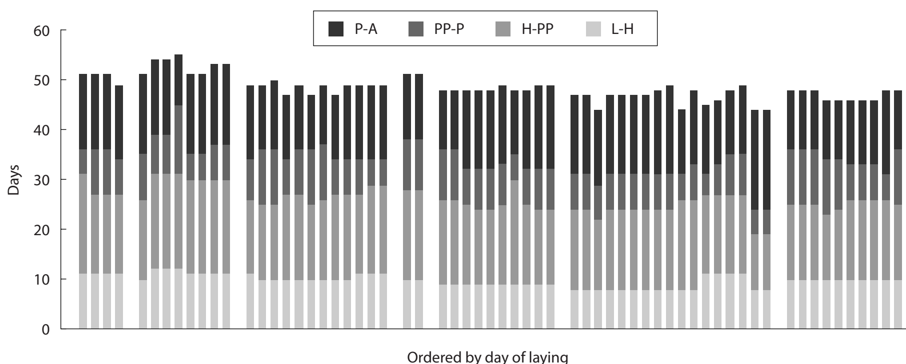

Figure 1. Life cycle of the red-banded mango caterpillar (*Deanolis sublimbalis* Snellen) from hatch to adult. L–H = lay–hatch; H–PP = hatch–pre-pupae; PP–P = pre-pupae–pupae; P–A = pupae–adult

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stage commenced when the late-stage larvae stopped feeding and changed colour from the conspicuous red and white band to a pale bluish-green colour with pinkish stripes. The period between pre-pupal and pupal stage was 4–14 days (mean 7.8 days) for the summer generation and from pupa to adult 5–20 days (mean 14.2 days). (RBMC overwinter as pre-pupae.) The average period from egg-laying to adult was 41–55 days (mean 48.1 days) (Figure 1). The variety of mango on which the larvae were laid did not seem to affect the interval from egg-laying to adult eclosion (Figure 2).

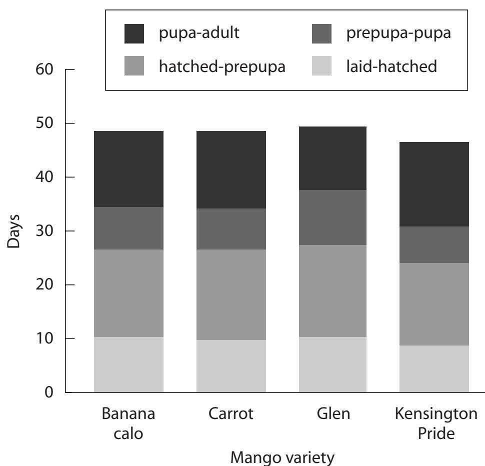

Figure 2. Life cycle of the red-banded mango caterpillar (*Deanolis sublimbalis* Snellen) on different mango varieties

When the pre-pupal stage was reached the larvae stopped feeding and displayed minimal mobility when a stimulus was applied. The larvae spun their cocoons incorporating sawdust with their own silk, forming a capsule in which they pupated. The cocoons adhered to any hard surface within the tube. Pupae are pale brown and gradually turn dark brown as they age. Adult *D. sublimbalis* are nocturnal and mate and lay eggs only in the dark.

This study was not repeated in subsequent mango seasons, but it was conducted coincidentally with the life cycle studies and the availability of laboratory-reared adults from field-collected *D. sumlimbalis* caterpillars. Currently, there are plans to enhance and build on the study over 3 years and thus six mango flowering seasons.

Initial fecundity and female longevity studies were undertaken with four moths. It appears that early-season moths lay a large number of eggs, whereas later in the flowering season this is substantially reduced (Figure 3). The data, however, are insufficient to draw firm conclusions. It appears that the adult females lived for 3–9 days.

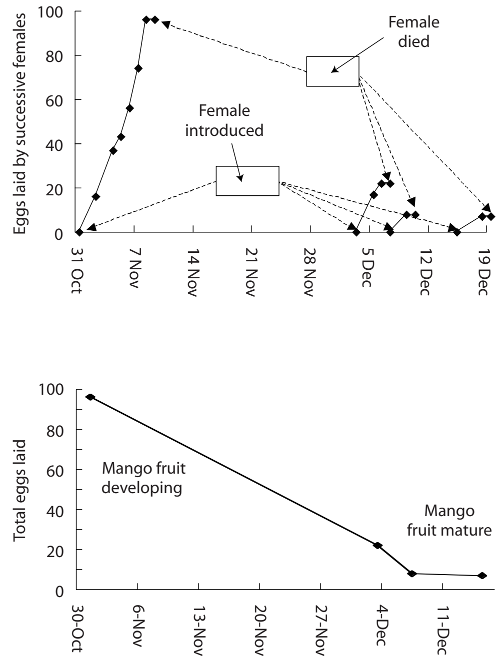

Figure 3. Fecundity of the female red-banded mango caterpillar (*Deanolis sublimbalis* Snellen) with time of fruit development and maturation

A further observation on the adult moths was that they are not attracted to light, which means that light-trapping is not useful for monitoring.

Discussion

This initial study set the groundwork for a future study to elucidate further details of the biology of the insect, with particular emphasis on discovery of aspects of the biology that may lead to cultural control methods.

The results indicate that *S. sublimbalis* is a bivoltine insect that lays its eggs on the peduncle of the panicles at the time of fruit-set. The larvae hatch fairly quickly, then collectively bore into the small fruit. This group behaviour may be an adaptation to assist in overcoming the plant defence of producing

large quantities of sticky exudates. So far, the development of the larvae internally in the fruit has been difficult to follow. Pupation apparently occurs outside the fruit, with the insect attaching itself to a hard surface to spin its cocoon, and the adults are short-lived. Fenner (1987) described the morphology of the larval and adult stages.

This small moth is a significant pest of mango in PNG. Mangoes comprise a significant part of people's diet when they are in season. This pest is difficult to control, partly due to a lack of understanding of the biology of the insect and inability to monitor it. As the moth does not respond to light-trapping, further work is needed to assess other monitoring tools, such as physical inspection of trees etc. to find over-wintering pre-pupae, or the development of pheromone traps. Red-banded mango caterpillar causes primary damage. It is probable that if this insect can be controlled, vulnerability to secondary organisms such as diseases and fruit flies would also be reduced.

Acknowledgments

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Some aspects of banana entomology in northern Australia: what can we apply to Papua New Guinea?

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Abstract

The banana industry in Australia differs from that of Papua New Guinea by being almost entirely driven by commercial factors and hence relies on production of blemish-free quality fruit to remain economically viable. Pesticides are still the main weapons used against the main bunch pests (banana scab moth *Nacleia octasema* and rust thrips *Chaetanaphorthris signipennis*) and the corm pest (banana weevil borer *Cosmopolites sordidus*). The use of persistent pesticides is banned in the Queensland industry.

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Pest and disease identification

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EntomID-PNG — a digital image database of the insects of Papua New Guinea

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Abstract

The EntomID-PNG project aims to compile a database of all Papua New Guinea (PNG) insect species. Such a database is deemed to be important first and foremost on a national and regional level to facilitate the identification of insects needed to meet conservation, crop protection and quarantine needs. As a first step to achieve this goal, a database of all taxa from the two most species-rich insect orders, the Lepidoptera and Coleoptera, which are kept in the national entomological collections of PNG and in the Australian National Insect Collection, will be completed through a newly established network of the major national entomological research institutes—National Agricultural Research Institute, Forest Research Institute, University of Papua New Guinea, PNG University of Technology and the New Guinea Binatang Research Centre (NGBRC)—and in an international collaboration with the German Development Service, the Commonwealth Scientific and Industrial Research Organisation (CSIRO Australia) and the Smithsonian Institution (Washington). The computer program BioLink (produced by CSIRO) will be used to create the database, which will include digital images of all taxa and geo-referenced locality data of all identified specimens which can then be used to produce distribution maps. Funding from the Global Biodiversity Information Facility (GBIF) is available to enable the rapid finalisation of this core database which will be integrated into the GBIF network and in this way made freely accessible on the Internet.

Background on the insect collections of Papua New Guinea

Papua New Guinea (PNG) is a developing country which had a very short colonial history of about 100 years and became independent some 30 years ago. The country's economy is still mainly dependent on its natural resources, either through subsistence farming or through logging and mining operations. Due to its long isolation from the outside world and its mountainous topography, the country's extensive tropical rainforests are still in relatively good condition and home to an extraordinary biodiversity of plants and animals, many of which are endemic to the island of New Guinea.

Before the Second World War, biodiversity research was almost entirely based on an expedition format, concentrating on the description of new taxa, and the type specimens were deposited in various overseas museums. An early entomological collection at Kerevat (near Rabaul) was destroyed during the war. After the war, several insect collections were set up in the country, the largest one being the National Insect Collection at Konedobu (National Capital District). It was based mainly on material collected in different parts of the country during the 1960s and 1970s, with an emphasis on pest species of agricultural importance. Currently, it includes about 200,000 specimens. However, the collection was on the brink of being destroyed as no curator was in charge for several years due to a shortage of financial

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and human resources at the Department of Agriculture and Livestock (DAL) during the 1990s. In the late 1990s, the collection was saved through the collective efforts of three organisations: the Australian Agency for International Development (AusAID) funded a new collection building at Kilakila (National Capital District); the German Development Service (DED) placed a development worker to train a national counterpart as curator; and the newly founded National Agricultural Research Institute (NARI) took over the responsibility for the three insect collections with an agricultural background, the other two being the regional collections at Kerevat (on the island of New Britain, with 12,000 specimens) and at Bubia Agricultural Station (near Lae in Morobe Province, 8000 specimens). All NARI collections together include about 220,000 insect specimens. The second-largest insect collection in the country is situated at the Forest Research Institute (FRI) in Lae, Morobe Province. It concentrates on forest insects, houses approximately 70,000 insect specimens and is kept in an excellent condition. Another large collection with about 60,000 insect specimens, most of them from Central Province, is kept at the University of Papua New Guinea (UPNG) in Waigani (National Capital District). Parts of this collection have suffered severely in recent years due to technical problems (airconditioning out of order), but currently it is well looked after and the curator is trying to rescue the collection. Some of the identifications have been made by visiting scientists from overseas universities.

The remaining national PNG collections are of only regional importance: the Bulolo Forestry University College (BFUC) has a collection of about 4000 forest insects from the Bulolo area (Morobe Province) and the Insect Farming and Trading Agency (IFTA) in Bulolo holds a representative collection of the more conspicuous insect species (especially butterflies and beetles). Both institutions belong to the PNG University of Technology (UNITECH) in Lae (Morobe Province) and the collections are kept in a reasonable condition, although no entomologist is currently in place to work in them. The privately funded Wau Ecology Institute (WEI) also has a representative regional collection of higher altitude species from the Mt Kaindi area (Morobe Province). This is currently at risk due to management problems and shortage of funding.

One of the main reasons why the national collections are divided between institutes in different regions is the lack of road links between the main centres of the country. While road links do now exist within Morobe Province, and from there into the Highlands, the national capital depends entirely on air transport to all other major towns. In 2004, the only bridge linking Bulolo and Wau with Lae and the Highlands was washed away, which resulted in a critical isolation of these two towns for several months.

Although the majority of insect specimens from PNG might still be found in overseas collections (including almost all primary type specimens, most of which are kept at the British Museum of Natural History in London), the national collections serve a vital role for the country as reference collections for biodiversity research, pest control and quarantine issues. This is especially due to the fact that a large number of specimens in the collections have been bred and thus include invaluable but largely unpublished information on their host plants. This information is rarely found in overseas collections and is thus of high scientific importance. Many specimens from the 1950s to 1980s were identified by the former International Institute of Entomology in London, as well as other international specialists, some of whom have worked on the PNG collections at times, and have also left behind a considerable number of type specimens. Most acquisitions from recent years have been identified by acknowledged entomologists, e.g. in cooperation with the Smithsonian Institution in Washington (NMNH). Within the Papuan biogeographic subregion (New Guinea and the surrounding Melanesian islands) no further major insect collections exist which could be included into the project. However, the Australian National Insect Collection in Canberra, which is the most important insect collection in the Australasian region, will be participating in the project.

Current digitisation status of the PNG insect collections

The creation of an insect collection database is not a new task in PNG. Already during the 1950s a pest information database was introduced by J.J.H. Szent-Ivany. It was based mainly on the specimens in the National Insect Collection. In 1996, a Microsoft® Access database for the national Entomology and Plant Pathology collections of PNG (named 'Collections') was specifically developed by Ian T. Riley, a consultant of the Department of Agriculture and

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Livestock, funded through AusAID's Commodities Assistance Program. From 1996 to 2000, however, there was no curator at the National Insect Collection at Kila Kila who could have carried out the task of computer-aided digitisation, and the first digitisation efforts were carried out at the collection of BFUC, where John Dobunaba (FRI) and Michael Schneider (DED) produced a pictorial catalogue of PNG's insects on CD-ROM (Dobunaba and Schneider 1999), which was sponsored by the DED and distributed free of charge.

At FRI, John Dobunaba has already digitised 14,000 specimens of the Coleoptera families Platypodidae and Scolytidae, which are economically important as timber borers. These two families have already been fully treated taxonomically and all records have been fully geo-referenced. The database is currently kept in an Excel spreadsheet.

In June 2002, Stefan Krull (DED) and Mark Ero (NARI) started a database of the insects in the former National Insect Collection, which is now called the National Agricultural Insect Collection (NAIC). The necessary equipment was sponsored by the Australian Contribution to the National Agricultural Research System (AusAID-ACNARS). At the end of 2003, all Lepidoptera (approximately 10,000 specimens) and a third of the Coleoptera (totalling 80,000 specimens) had been digitised, using the software tool LINNAEUS 2.1 (developed by ETI, Amsterdam), and digital images have been taken of all Lepidoptera and Coleoptera taxa as well as part of the Heteroptera and Diptera. After the end of his contract in August 2003, Stefan Krull was replaced by Martin Wiemers (DED) in November 2003. He identified BioLink (produced by CSIRO and provided free of charge) as a more appropriate program for the collection database and as the ideal basis for integrating all currently available datasets. The LINNAEUS Lepidoptera database of NAIC has already been successfully converted into BioLink.

In December 2003, the newly placed development worker, Helmut Ludewig (DED), started to digitise the insect collection at Keravat. A preliminary Excel database of all Lepidoptera and Coleoptera taxa in the collection has already been completed. No digitisation efforts have yet been undertaken at the Bubia and UPNG collections but paper records exist for parts of the collection.

The NGBRC keeps a Microsoft® Access database which currently includes about 40,000 specimen records (30,000 Lepidoptera and 10,000 Coleoptera) including host-plant information.

EntomID-PNG Project outline

Biodiversity research in PNG is currently severely hampered by the fact that keys allowing identification to species level are available only for a few insect groups, such as the butterflies (Parsons 1999) and the carabid beetles (Darlington 1952–1971), and no single collection within PNG can serve as a reference collection for all groups of insects or at least all species within one insect order. Within the Lepidoptera, for example, NAIC holds a good reference collection of butterflies, but the FRI collection has a much better coverage of moth species. Therefore, most material still needs to be shipped to various overseas museums for identification, which is a time-consuming and costly exercise. The development of a national database of PNG insects which includes digital images as an important means of identification thus gains importance in order to facilitate research and conservation measures in PNG, one example being the development of identification keys to insect groups of economic importance by the national agricultural, forestry and quarantine services. One such example is the online key to the forest insect pests of PNG, produced by Michael Schneider (Schneider 1999).

The need for networking among the national PNG collections to acquire such a database is realised by their curators, but the extremely limited core budgets do not allow this without additional funding. GBIF funding is therefore mainly sought to facilitate networking and to speed-up the digitisation process. This is especially urgent, since the support of DED ceased with its withdrawal from PNG by the end of 2005.

As a common database platform, the CSIRO software BioLink (Shattuck and Fitzsimmons 2004) has been identified as the ideal basis for integrating all currently available datasets. BioLink fulfils the Darwin Core Version 2 exchange standard and allows export of data into two different XML formats. It also includes a convenient import module for Excel spreadsheets and Access databases. The LINNAEUS Lepidoptera database of NAIC has already been successfully converted into BioLink via Excel. BioLink has the advantage that existing records can be corrected and upgraded easily. Most importantly, BioLink has electronic gazetteers, including PNG, which enable fast geo-referencing of records and the

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generation of distribution maps. A variety of reports (e.g. species lists of localities or provinces) can also be created. In October 2004, a workshop was organised by NAIC at UPNG to train the participants in the use of BioLink and to ensure a common database standard. The training was done by Dr Steve Shattuck, the developer of BioLink.

Within the envisaged project time frame of 18 months (August 2004–January 2006), it is planned to complete a geo-referenced database of the two largest insect orders, the Lepidoptera and Coleoptera, kept in the national insect collections, together with material held by CSIRO in Canberra.

These two insect orders were chosen for the following reasons:

- they are the two largest insect orders and include a high number of species of economic importance, e.g. in commercial trade (butterflies), or as pest species (e.g. weevils, timber borers, many moth species)
- the level of correct identification to the species level is relatively high compared with other insect groups and many families have previously been systematically treated by acknowledged experts
- the current team of curators and volunteers involved in the project consists of experts on different families of Lepidoptera or Coleoptera, but this unique potential can only be fully utilised if time-consuming tasks such as recording of label information and geo-referencing are carried out by technical staff
- previous digitisation efforts have already been concentrating on these orders, and it appears a priority to finalise these datasets in order to achieve a useful product for the user communities within a short period.

At the start of the project in August 2004, about 90,000 specimens had already been digitised. This represented more than 35% of the estimated total number of 240,000 Lepidoptera and Coleoptera records which are either specimens kept in the PNG national insect collections (about 220,000 specimens) or material which has been entered into the database in PNG but which is currently deposited overseas (about 20,000 specimens). The material also includes more than 1200 type specimens (mainly Coleoptera). With the help of GBIF funding, the complete database with 240,000 geo-referenced specimen records should be finished by the end of 2005, with an additional 10,000 specimens held by CSIRO also being accessed to the database. Currently, digital images of 5500 taxa of Lepidoptera and Coleoptera (present in NAIC, BFUC and NGBRC) have already been taken. It is estimated that digital imaging of additional taxa kept in the FRI and remaining PNG collections will double this figure. The records cover all provinces of PNG, including the dry and the wet lowlands, the highlands and all larger islands, and are thus representative of the Papuan biogeographic subregion. Although almost no material from the western half of the island of New Guinea (part of Indonesia) is present in the PNG national collections, only a relatively small proportion of described taxa is restricted to Irian Jaya (e.g. in butterflies: 139 (= 14.5%) of 959 species according to Parsons (1999).

The Australian National Insect Collection, which is very well curated and has an excellent level of identification, also holds some important type specimens (e.g. they are the second-most important depository of PNG butterfly holotypes after the British Museum of Natural History) and CSIRO agreed to make images available of those types and other taxa which are not present in the PNG national collections. This is a first big step towards the repatriation of data on Papua New Guinea's insects which are spread around the world and currently hardly accessible to PNG entomologists.

At NAIC, the database is nearing completion, but there is still the need for quality control (already done for two thirds of the Lepidoptera) and geo-referencing. NAIC will coordinate the digitisation process of the participating institutions and integrate the data into a joint database. FRI will concentrate on the completion of its database and provide NAIC with supplementary digital images of taxa which are missing from the NAIC collection, a part of which can already be taken from the pictorial catalogue of the Bulolo collection. The Kerevat collection will be digitised by Helmut Ludewig (DED), the Bubia collection by Adrian Schuhbeck (DED), the Bulolo collection by Michael Schneider (CIM) and John Dobunaba (FRI), and the UPNG collection by Tamari Mala. For the GBIF project, emphasis will be placed on Lepidoptera and Coleoptera but, if time permits, other insect orders will also be digitised. In the case of the Kerevat collection it is anticipated that the complete insect collection of 12,000 specimens will be digitised by the end of the GBIF-funded period. CSIRO has well established database-building procedures led by Steve Shattuck and is currently a GBIF data provider. However, only a limited

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amount of its extensive PNG holding has been accessed into a database so far. During this project approximately 10,000 additional specimens will be entered into the database and made available and specimens already in the database will be validated and their quality checked.

Although each institution will hold a database of its own collections, technical constraints currently do not permit most of them to provide direct online access. Neither NARI nor FRI have their own server and thus depend on one of the two universities (or expensive commercial Internet service providers) to host their databases. In the case of the smaller collections, it is also preferable that the data be first checked by the curators of NAIC or FRI to ensure their quality before being served to the online community. Therefore, it is currently planned to serve a joint database on a central server at UPNG, which agreed to set up a web service for EntomID-PNG. Steve Shattuck of CSIRO offered his assistance in the set-up procedures and CSIRO will also host a copy of the database. In the course of the project, the option of hosting the FRI database regionally on the UNITECH server in Lae might be reconsidered, although few advantages are seen in this option as UNITECH is outside Lae town and postal services between Lae and Port Moresby (for sending data on CD-ROM) are as efficient as those between Lae town and UNITECH. Email (and fax) communication services are reliable in Papua New Guinea and enable fast exchange of information and images in order to receive feedback from data users and implement corrections into the database. Procedures will be implemented to assure that corrections are always made to the master copy of the database.

The imaging of taxa will facilitate quality control in cooperation with partners at NMNH (Washington), BMNH (London), BBMH (Honolulu) and ANIC (Canberra). This includes a linkage to the joint project of NMNH and BMNH to image the wings and genitalia of the types of New Guinea Geometridae (over 2000 species) which has recently been funded. A further cooperation which will be strengthened through this project exists with the NGBRC in Madang (PNG). The NGBRC keeps a reference collection of more than 20,000 specimens (19,000 Lepidoptera and 1300 Coleoptera), and samples of the high number of specimens collected during its biodiversity studies are regularly deposited at NAIC (currently about 500 Lepidoptera and 500 Coleoptera specimens from the last two years cooperation). The NGBRC cooperation is currently the main source for new material in NAIC. This material is extremely valuable because it is very well set, all identifications have been verified overseas by specialists, and a large proportion of the material is reared from larvae (i.e. host plants are known). NGBRC keeps an up-to-date database of this material, including digital images, and has agreed to provide these data for inclusion into the national database. At the moment the NGBRC database consists of about 40,000 specimen records (30,000 Lepidoptera and 10,000 Coleoptera) and during the GBIF project this figure is estimated to increase by about 15%.

Due to the severe shortage of trained entomologists in PNG, the cooperation with NGBRC and the planned training of selected parataxonomists in the digitisation of collection data will significantly increase the number of trained personnel in the country who are able to continue the databases after the end of this project.

Most of the PNG material has been collected during the past 40 years and, partly for this reason, the precision of the records is generally very good, usually within 1–10 km. Geo-referencing encounters no major problems because only few place-name changes have occurred during this time. This task is facilitated enormously by the fact that the PNG electronic gazetteer is implemented in the BioLink program thus avoiding the need to type in the longitude and latitude data which would otherwise be a very error-prone operation. Instead, the coordinates are automatically recorded when the name of a locality is selected. (The list of localities can be restricted to the level of provinces and the location of a place name can be easily checked on a map to avoid mistakes.) The level of precision will be indicated by recording the maximum error distance in metres (coordinate precision in Darwin Core or coordinate error distance in meters in ABCD standard).

The digital image database will be made available through various routes:

- BioLink, in cooperation with CSIRO, will be used to make information available in GBIF-compliant format. CSIRO has also agreed to host the database as a mirror site until a permanent PNG-based site can be found.
- UPNG agreed to become a DiGIR provider and host the EntomID-PNG database on its server. The costs of setting up the server have been estimated and will be covered through GBIF funding. However, it is anticipated that the data will also be

kept on overseas servers (e.g. CSIRO, see above) because of their higher bandwidth and the higher reliability.
- Initiated by NAIC, PNG became a GBIF participant on 27 February 2004, which includes a commitment to establish national data nodes. It is anticipated that the EntomID-PNG database can serve as a test case for the establishment of a national data node at UPNG. The timeline and funding for the national node is currently being negotiated between DEC, NARI, FRI, UPNG and UNITECH.
- Because of the slow and often unreliable Internet connections in PNG, especially in rural areas, a CD will also be produced which will be distributed at a low price or free of charge with sponsorship from DED.

Due to the high biodiversity and number of endemic species in the Papuan biogeographic subregion the database will substantially increase our global knowledge on tropical ecosystems.

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Use of molecular markers in managing plant pests and diseases: a PNG perspective

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Abstract

Plant diseases are often the cause of reduced yields and deformed or poor-quality products. Because of the losses incurred by pests and diseases a lot of effort is put into developing management strategies that will prevent or minimise the effect of pests and diseases on crops. In recent years, molecular techniques have been increasingly used to help in solving plant-protection problems. Of these, molecular marker technology, especially DNA fingerprinting, has found widest application particularly in plant pest and disease diagnostics, genetic diversity studies of pest and pathogen populations and in plant breeding for marker-assisted selection for pest and disease resistance. This paper reviews what molecular markers are and how they are produced using the polymerase chain reaction. The application of molecular markers in plant protection in general is discussed, followed by consideration of their potential use in agricultural research in Papua New Guinea in particular.

Introduction

Pests and diseases are often the cause of reduced yields and deformed or poor-quality products. In Canada and the USA, it has been suggested that losses due to pests and diseases could be as high as 15–20% in crops such as wheat, cotton and rice. In India, some estimates put crop losses due to pests and diseases at up to 35% (Lucas et al. 1992). In Papua New Guinea (PNG), very few studies have been conducted to quantify the losses due to pests and diseases. Based on losses incurred by pest and diseases, there has been increasing emphasis on developing management strategies that will prevent or minimise the effect of pest and diseases on crops.

Prevention, combined with the ability to rapidly and accurately identify a harmful agent, is usually the first line of defence in pest and disease management, and many tools and methods have been developed to diagnose and identify specific pests and diseases. However, virus phytoplasma diseases are often difficult to diagnose because this requires specialised equipment and expertise. For other pests and diseases, diagnosis can involve a lengthy process of isolation, culturing, and application of taxonomic characteristics and this requires considerable taxonomic expertise.

Another important pest and disease management strategy is the deployment of crop varieties resistant to pests and diseases. Breeding of new crop varieties resistant to pests and diseases is an ongoing and often lengthy process using conventional plant breeding techniques. This results from changes in pest and pathogen population structure.

Recent advances in biotechnology techniques have allowed their use in crop production. These include micro-propagation of plant material (using tissue culture), use of transgenic plants (gene manipulation and transfer), utilisation of bio-pesticides, and the use of

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molecular markers (genetic markers) (FAO 2001) in (i) diagnosis and management of plant pests and diseases, (ii) studies on genetic diversity, and (iii) breeding of crop varieties resistant to pests and diseases.

In PNG, application of these techniques is constrained by limited availability of technical expertise and technology. This technique anchors on basic knowledge of heritable genetic information and, with adequate financial and technical support, it could become a common tool in effectively managing pests and diseases in PNG.

This paper gives an introduction tomolecular markers, techniques for generation of molecular markers and applications of these in pest and disease management in a PNG context.

Molecular markers

All genetic information of a living organism is stored in the genome, which is composed of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). In most plant viruses, DNA and RNA are composed of a sequence of nucleotides: guanine, cytosine, adenosine and thymine (replaced by uracil in RNA). The chemical structure of every organism's nucleic acid is the same, but the difference between organisms lies in the sequence of their nucleotides. That is, complementary bonding of adenine and thiamine (or uracil), and guanine and cytosine in an organism's genome (chromosome) is unique. Certain sequences of the DNA/RNA encode genes that provide the genetic information for certain functions or traits. such as yield potential or resistance to diseases in plants, or virulence in pathogens.

It is to this uniqueness in the genomic sequences of individuals that molecular marker techniques are applied in agriculture and other areas of biological sciences. Molecular markers (at DNA/RNA level) are one of a range of genetic markers. Others include morphological markers and biochemical markers (isozymes, proteins) (de Vienne 2003). Isozymes were one of the first molecular markers used (Burdon et al. 1982; Newton 1987; Newton and Caten 1991), but the main focus is now on DNA sequences as the source of informative polymorphisms (Michelmore and Hubert 1987; Weising et al. 1995). Molecular markers reveal neutral sites of variation at the protein or DNA sequence level. Nucleic acid derived markers serve as landmarks for specific regions of the DNA. Characteristics of the landmarks may vary between genotypes. The size of the DNA fragment (landmark) may vary between individuals. The landmark may be present in one individual but absent from another. Alternatively, the actual composition (DNA sequence) of the landmark may vary between individuals (McIntyre 2001).

Finally, there are different classes of molecular marker techniques (e.g. DNA fingerprinting) and the choice and application of these techniques depends on research targets and the organisms being researched.

Preparation of molecular markers

Methods for deriving information from DNA sequences can be broadly separated into hybridisation-based, non-polymerase chain reaction (PCR)-based techniques and PCR-based multi-locus profiling or DNA fingerprinting techniques, including DNA sequencing (Karp and Edwards 1997). Only PCR-based methods will be considered in this paper. The following section gives a brief overview of the principles of the PCR amplification protocol.

Principle of the PCR

The PCR machine is an artificial analogue for replication of the genetic information (DNA) in a biological cell, except that only targeted sequences are amplified. PCR multiplies a target DNA sequence with the aid of a DNA polymerase enzyme (e.g. Taq, isolated from the thermophilic bacterium *Thermus aquaticus* found in hot springs) (Saiki et al. 1988). The PCR process is shown in Figure 1 and Table 1.

PCR reaction mixtures usually contain appropriate proportions of double-stranded DNA with target sequences from the organism to be investigated, oligonucleotide primers (single-stranded lengths of nucleic acid that are complementary to those on either side of the target DNA sequence to be multiplied), deoxynucleotide triphosphates (building blocks for new lengths of DNA), the polymerase enzyme, a suitable buffer and distilled water.

The cycle is repeated between 25 and 40 times during which the quantity of DNA is multiplied at an exponential rate until one of the reaction components is exhausted or the polymerase is denatured.

The products of the PCR process are detected and analysed using electrophoresis which separates DNA fragments into groups of similar length that form bands in the gel. These bands can then be made visible using a suitable dye and identified by comparison with a standard control (molecular weight marker) run alongside (Ebbels 2003) (Figure 2a).

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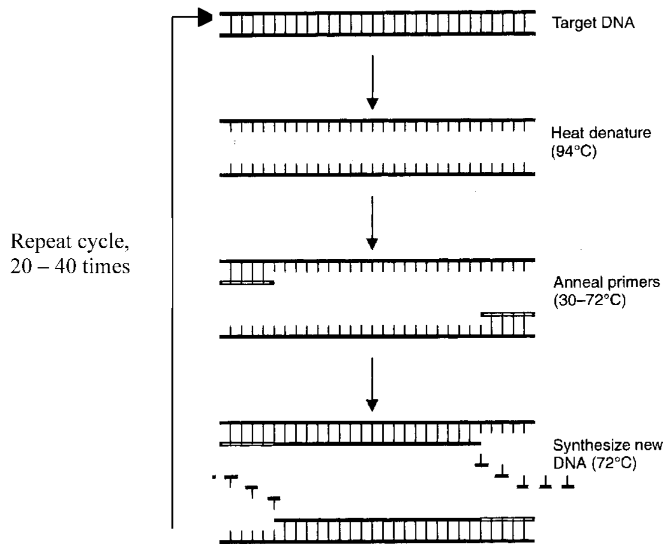

Figure 1. Principle of PCR amplification (Ebbels 2003). Repeat cycle, 20–40 times

Table 1. Different steps in PCR amplification

Step 1: Denaturation	Mixture is heated to 94°C. This denatures the double-stranded DNA, separating the two strands.
Step 2: Annealing	Mixture is cooled to a suitable temperature (between 30°C and 72°C), which will allow the oligonucleotide primers to anneal to the complementary sequences on each of the separated DNA strands.
Step 3: Extension	Mixture is heated to 72°C, at which temperature the polymerase promotes the assembly of new DNA stands with nucleotides complementary to those on the target sequence between the primers, thus resulting in two new lengths of double-stranded DNA.

DNA fingerprinting

DNA fingerprinting (DNA-Fp) is based on knowledge that every individual organism carries unique DNA sequences in their genome. DNA-Fp is a procedure that helps determine whether or not two or more samples being tested originate from the same organism.

Many DNA fingerprinting techniques have been developed. Some of the most common include random amplification of polymorphic DNA (RAPD) (Figure 2a; Williams et al. 1990), amplified fragment length polymorphisms (AFLP) (Figure 2b; Vos et al. 1995) and inter simple sequence repeat (ISSR) marker (Godwin et al. 1997). Techniques differ in the length and sequence of the primers, the stringency of the PCR conditions and the method of fragment separation and detection (Karp and Edwards 1997). An advantage of these methods is that prior information on DNA sequences is not required and only minute (nanogram) amounts of DNA are needed (Bridge and Arora 1998). Common to these techniques is that anonymous DNA sequences are amplified and the presence or absence of a particular amplification product is used for the evaluation of genetic diversity and relatedness. However, these methods do not provide information about the identity or sequence context of the amplified band. The resulting DNA fingerprint can be used to characterise or distinguish species or genotypes within a population (Weising et al. 1995).

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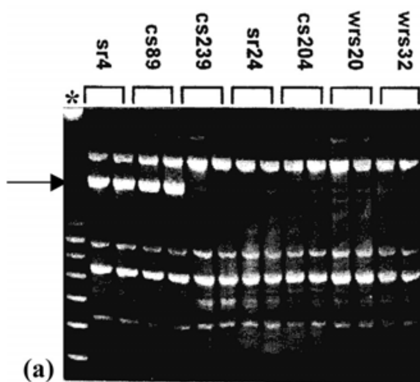

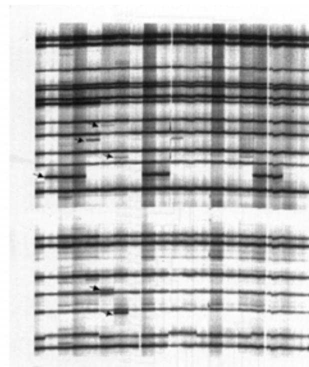

Figure 2. (a) DNA fingerprints using RAPD; *– lane with the molecular weight marker (b) DNA fingerprints using AFLP. Arrows indicate polymorphic bands, the presence or absence of which is used to determine genetic diversity**.**

Application of molecular markers

Molecular nucleic acid techniques using PCR are used in a wide range of agriculture-related research such as ecology, evolutionary biology, taxonomy, agronomy, breeding, pest and disease diagnostics and germplasm conservation.

Plant disease diagnostics

Diagnosis of a plant health problem refers to determining the cause of disease symptoms and the nature and identity of the causal organism. The identification involves the assignment of the organism to a particular taxon in an appropriate classification system (Ebbels 2003).

Some diseases can be identified from symptoms alone or after visual inspection, perhaps involving microscopy, to detect pathogen structures (Waller 2002). More often it is necessary to isolate or extract the pathogen from its host or substrate before the process of identification can start. There are a number of procedures, protocols and tests available for different kind of pests.

It is still often difficult to detect and identify a pathogen to the species level using morphology alone. *Phytophthora* species, or example, are identified after the formation of sporangiospores. After these are detected, the morphology of the spores, chlamydospores, hyphal swellings and other characters associated with the formation of oospores is examined. This requires considerable taxonomic expertise and training (Drenth and Irwin 2001).

Development of molecular marker technologies has opened up avenues for more specific and reliable detection of pathogen species. The following example illustrates one of the various approaches used in pathogen detection and identification using molecular markers.

Example: *Diagnostic test for* Phytophthora *species*

Recently, Drenth and Irwin (2001) developed a DNA-based diagnostic test for economically important *Phytophthora* species in Australia (Figure 3). The test can be used to detect and distinguish 27 different *Phytophthora* species.

For this test, primers were designed to amplify regions of genes encoding ribosomal RNA, which are conserved among different *Phytophthora* species. The result is a single band indicating the presence of a *Phytophthora* species in the sample. The next step is to digest the PCR product with a restriction enzyme (enzymes that cut strands of DNA at specific base sequences). Electrophoresis then reveals a species-specific banding pattern, which can be compared with a molecular key and the species identified.

Diagnostics using DNA-based molecular markers can offer reliable and rapid detection of plant pathogens. Detection methods have been developed for a number of important plant pest and diseases including methods that identify the causal agent

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directly from plant tissue without the initial need to extract and purify DNA/RNA.

Plant disease management

There are a number of areas in disease management where molecular markers can be used directly or indirectly. Some of those areas include the study of genetic diversity of pathogen populations, plant breeding for resistance and the indexing of planting material.

Genetic diversity studies

DNA marker technology has been used extensively in studies establishing the genetic diversity of pathogen populations. Pathogen populations are constantly evolving. The major mechanisms that generate genetic variation in pest and disease populations are mutation, recombination and migration (Leung et al. 1993). In pathogens that predominantly reproduce asexually, variation would be created mostly through mutation or mechanisms specific to certain pathogen groups, e.g. parasexuality in fungi (Taylor et al. 1999). Creation of genetic variation is usually slow, and populations consist of discrete clonal lineages with only a few genetically distinct genotypes. On the other hand, in populations of sexually reproducing pathogens genetic diversity is usually very high (Leung et al. 1993). Ultimately, creation of genetic diversity enables individuals in the pathogen population to respond to changes in their environment. Disease management would involve the release of resistant crop varieties or the deployment of a new pesticide. Highly variable pathogen populations would be more effective than clonal populations in overcoming the resistance or developing resistance to pesticides. DNA fingerprinting techniques are used mostly to conduct genetic diversity studies and the following example shows a recent study relevant to PNG.

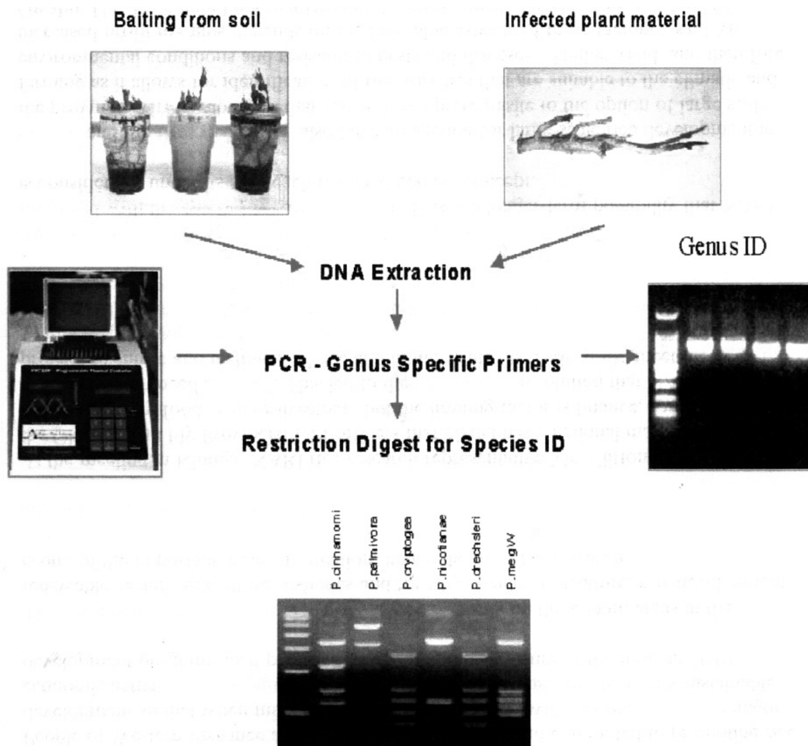

Figure 3. Schematic representation of PCR-based diagnostic assay for *Phytophthora* species (Drenth and Irwin 2001)

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Example: Isozyme and RAPD variation among *Phytophthora colocasiae* isolates

Ninety-four isolates of *P. colocasiae* from Southeast Asia and Oceania (including PNG) were characterised by Lebot et al. (2003) using isozyme and RAPD markers as part of the project 'Taro: evaluation and breeding for rainfed cropping systems in Southeast Asia and Oceania'. Their results showed that there was significant genetic variation among strains of *P. colocasiae* both between and within countries including PNG despite the fact that *P. colocasiae* was introduced into PNG as recently as the 1940s and only the mating type A2 is present (Ivancic and Lebot 2000). Lebot et al. (2003) believe that taro cultivars bred for resistance to leaf blight in one country may not be as resistant in other countries due to the presence of genetically different pathogen populations.

Germplasm maintenance and plant breeding

The use of crop varieties resistant to diseases or pests is generally viewed as one of the most cost-efficient and sustainable means of pest and disease management. However, breeding of new varieties is an ongoing process. Farmers may require different varieties in order to react to changes in the market such as consumer preferences, export requirements or the incursion of new pests and diseases or new races of a pathogen. Therefore, any breeding program relies on the availability of genetically diverse germplasm in order to respond to the demands caused by changes in the market or the environment.

Molecular markers can be used at different stages of germplasm conservation and plant breeding. Molecular marker assisted selection is one of the major applications of this procedure in plant breeding. Molecular markers are used to determine if desirable traits are present in one or other samples, accessions or populations of genetic resources. This is possible if a particular marker is part of the DNA (gene) of interest, or because it is closely genetically linked to the target gene or sequence (Ford-Lloyd 2001).

The national PNG taro germplasm collection of 859 accessions from 16 provinces provides an example of application of molecular markers. They were used to establish the genetic diversity of the taro population in PNG, as well as to assess material for entry into a 'core collection'.

As part of the TaroGen (Taro Genetic Resources: Conservation and Utilisation) project, Godwin et al. (2001) used simple sequence repeat (SSR) markers to analyse the genetic diversity of taro collections between and within Pacific Island countries.

SSRs, also known as microsatellites, are short, 2–8 nucleotide units, such as CA or AGC or GATA, which are repeated in tandem hundreds of times. They are widely dispersed throughout the genome of eukaryotes and display high levels of genetic variation based on differences in the number of tandem, repeating units at a locus (Vogel and Scolnik 1997; Godwin et al. 2001).

Godwin et al. (2001) first identified microsatellites in the taro genome and designed primers flanking the selected repeat motifs. PCR was then used to produce DNA fingerprints based on SSR markers. Using this technique, they established that generally there is not a high genetic diversity among taro accessions in the Pacific. PNG accessions showed the highest level of diversity among Pacific Island countries, while accessions from Vanuatu or Hawaii showed no variation. SSR markers, together with morphological descriptors, were then used to rationalise the national taro collection into a core collection of 83 varieties, which represent the greatest genetic diversity among accessions.

Two other studies using DNA fingerprinting to establish genetic diversity of taro were conducted as part of the TANSAO project (Taro Genetic Resources: Conservation and Utilisation), which included Southeast Asian taro accessions as well as PNG accessions. Noyer et al. (2003), using SSRs, and Lebot et al. (2004), using AFLP markers, identified two diverse gene pools of taro, one in Southeast Asia and one in PNG. Within each gene pool, genetic diversity is rather low but between both pools there is a considerable diversity and the recommendation is that any breeding program will need to utilise varieties from both pools in order to broaden the genetic base to be able to make improvements to any desired characteristic (Lebot et al., 2004).

Molecular markers are used increasingly in plant breeding to develop genetic linkage maps, which graphically represent the arrangement of a large number of markers along the chromosome. The distance between markers is expressed in centimorgans (cM) which represents the recombination rates between the markers (1 cM – 1% recombination) (Kumar 1999). Genetic linkage maps can be used to tag economically important traits with molecular markers. Certain traits in crops can be controlled by single 'major' genes, which are inherited in a Men-

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delian manner and whose allelic forms give qualitatively distinct phenotypes. They are either expressed or not (Jones et al. 1997). Many other traits, such as yield, quality, or resistance to several biotic and abiotic stresses, are controlled by a relatively large number of loci or genes, each of which makes a small positive or negative contribution to the final phenotype. They are observable in a segregating population as a more or less continuous variation in phenotype. Such loci are termed 'quantitative trait loci' (QTLs) (Jones et al. 1997; Kumar 1999).

A number of genes (major genes and QTLs) conditioning resistance to pathogens or pests as well as other important traits have been mapped with DNA markers in some of the major crops (Mohon et al. 1997; Kumar 1999). Mapping and tagging of agriculturally important genes forms the foundation for marker-assisted selection (MAS) in crop plants. It is based on the concept that the presence of a marker indicates the presence of a gene and the marker should co-segregate or be closely linked (1cM or less) with the desired trait (Jones et al. 1997; Mohon et al. 1997). In conventional breeding for disease and pest resistance, it is necessary either to select genotypes that are under natural pest/disease pressure or to artificially create this pressure. The problems encountered here include time and labour-consuming procedures, susceptible plants often escape attack and plants cannot be screened with several different pathogens or pests at the same time. MAS can significantly shorten the selection process and plants can be screened simultaneously for a number of different traits at the same time, which can be used to increase the durability of resistance in crop varieties by increasing genetic diversity of resistance genes and applying it in the form of cultivar diversification, cultivar mixtures, multilines and pyramiding of resistance genes (Jones et al. 1997; Mohon et al. 1997; Kumar 1999).

Anthracnose disease caused by the fungus *Colletotrichum gloeosporioides* results in severe losses in a number of yam species, but especially in water yam (*Dioscorea alata*) a species commonly planted in PNG. Scientists at the International Institute of Tropical Agriculture (IITA) have constructed a genetic linkage map of the *D. alata* genome using AFLP markers. Mignouna et al. (2002a) identified a RAPD marker closely linked to a single locus that contributes to anthracnose resistance in water yam. QTL mapping also revealed a marker that was associated with anthracnose resistance (Mignouna et al. 2002b). They plan to convert the RAPD marker into PCR-based sequence-characterised amplified regions (SCARs) that can be used to efficiently screen large numbers of plants for the presence of anthracnose resistance genes.

Pest and pathogen free-planting material

Molecular markers are especially useful for pests and pathogens that can occur as latent infections or where symptoms are not easily recognisable, such as plant viruses or phytoplasmas. Techniques used for indexing of planting material are similar to those developed for the diagnosis of plant pathogens.

In PNG, two regional projects, the TaroGen project and the SPYN (South Pacific Yam Network) project, also had components that developed serological and molecular technologies for the indexing of taro and yam planting material so that it is possible to exchange planting material within the region. Briefly, a serological-based diagnostic test has been developed using the recombinant protein against the core region of *Dasheen mosaic potyvirus* (DsMV) coat protein (Maino 2003). The antiserum is highly sensitive and can be used in an indirect-ELISA system to effectively detect DsMV in taro samples. The study also identified some DsMV generic primers and these can be used in the PCR system to index DsMV in taro to ascertain their virus-free condition.

Areas of research for application of molecular markers in PNG

1. There are a number of serious plant pests and diseases present in West Papua. Among them are the banana diseases including Panama wilt (*Fusarium oxysporum* f.sp. *cubense*, FOC, 'tropical' race 4) and blood disease (caused by blood disease bacterium, BDB). Both diseases have the potential to devastate banana production in PNG. A rapid DNA-based diagnostic test has been developed by the Cooperative Research Centre for Tropical Plant Protection in Australia that is specific for the detection of the 'tropical' race 4 strain of FOC and a test for the BDB is in development. PNG currently has a very low capacity in carrying out any diagnostic work on plant pest and pathogens. Most specimen have to be sent overseas for identification, which is a lengthy and costly exercise. Development of a capacity for diagnosis of pest and disease problems

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including the application of modern technologies such as molecular markers, enzyme-linked immunosorbent assay (ELISA) etc. is required to be able to better protect PNG agriculture from the incursion and establishment of potentially harmful agents.
2. Lebot et al. (2003) included only eight isolates of *P. colocasiae* from PNG. There is a need to expand studies on the genetic variability of the *P. colocasiae* population in PNG in order to better assess the effectiveness of newly bred taro varieties against different pathogen genotypes.
3. Southeast Asian taro accessions (31) recently received from the Regional Germplasm Centre (Suva, Fiji) will be evaluated and incorporated into the ongoing National Agricultural Research Institute (NARI) taro-breeding program. Molecular markers could assist in tracking the origin of new introductions of cultivars.
4. Molecular marker technology could also help characterise and rationalise ex situ germplasm collections held in PNG of important crops such as yams, bananas, sweet potato, aibika, coconuts and cocoa.
5. PNG does not currently have a yam breeding program, in which markers linked to anthracnose resistance could be utilised. However, PNG and other Pacific Island Ccountries have a rich diversity of yam germplasm, and molecular markers could be used to screen this collection to identify sources of resistance to yam anthracnose. This information could be exploited directly in recommending resistant varieties to farmers or the germplasm could be utilised in future breeding programs.
6. At the moment it may not be possible to develop molecular markers for certain traits in important crop plants in PNG. However, the application of markers developed elsewhere is a real possibility in any breeding program in the country.

Outlook for PNG

A forum in 2001 (FAO 2001) indicated that biotechnology, including molecular markers, has considerable potential to help overcome problems facing food and agriculture in developing countries. Pests and diseases are among the greatest threats to agriculture (Schaad et al. 2003) and molecular markers find application in many areas in managing these constraints. These techniques are already an integral part of agricultural research in many developed and developing countries. However, PNG lacks capacity and human resources, and utilisation of molecular markers in PNG agricultural research has been conducted mainly by overseas scientists and organisations.

PNG is the biggest country among the developing Pacific Island Countries, both in landmass and population. It has a high genetic diversity for many of the important crops plants but also has the highest number of pests and diseases among Pacific Island Countries. PNG should therefore play a leading role in developing disease management solutions for the Pacific Island region and agricultural research by including the utilisation of molecular marker based technologies.

The Biotechnology Centre of the University of Technology, Lae, in collaboration with NARI, now has the capacity to use some of the molecular marker technologies, especially in the field of pest and disease diagnosis and DNA fingerprinting. Establishment and maintenance of molecular laboratories and application of molecular technologies involves a considerable financial investment. Public funding for agricultural research in PNG is poor and any advance in the application of molecular technologies can come only through a cooperative and collaborative effort of all research and development (R&D) organisations in the country. At the same time, donor-funded projects that include components with molecular technology applications should aim at conducting at least part of the work in-country in order to build-up capacity and provide essential training for PNG scientists and technical officers.

It is hoped that this may lead to continued dialogue and discussions among R&D organisations and scientists on how we can advance in this area of research to help smallholder farmers in PNG and the other Pacific Island countries.

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The distribution of oryctes baculovirus in different species of Scarabaeidae on New Britain Island, Papua New Guinea

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Abstract

With the aim to develop an inexpensive control strategy for taro growers, this paper reports on efforts to establish whether oryctes baculovirus (OBV) is a parasite of *Papuana* spp. and other dynastid beetle species and whether infections occur naturally. Data from this study show that OBV is present in *Oryctes rhinoceros* (L.) in East New Britain. No other scarab species examined displayed cytological symptoms of OBV infection. Two species of taro beetle (*Papuana woodlarkiana* and *P. huebneri*) showed increased mortality and symptoms of OBV infection following artificial infection in the laboratory. The practical implications of these findings for a future biological control strategy of taro beetle are discussed.

Introduction

Oryctes baculovirus (OBV), earlier described as oryctes rhabdovirus, has been successfully used as a biological control agent in several South Pacific Island nations against the Asian rhinoceros beetle *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae). This introduced scarab species is a major pest of coconut palms. In Papua New Guinea (PNG), OBV has been successfully established in all provinces where *O. rhinoceros* occurs (Gorick 1980). Several other members of the same subfamily of scarab beetles (Dynastinae) are core pests of important agricultural crops. Most important in economic terms are the Melanesian rhinoceros beetle *Scapanes australis* Boisduval and the taro beetles *Papuana* spp. As a control agent, OBV was reported to be highly effective against *O. rhinoceros* in East New Britain (Gorick 1980). As OBV appears to be widespread wherever its host occurs, other closely related species could also have become naturally infected by the virus if they are hosts for OBV. An inexpensive control strategy for taro growers is required and this paper tries to establish whether OBV is a parasite of *Papuana* spp. and other dynastid species, and whether infections occur naturally. If *Papuana* proves to be a host to OBV, the further aim is to obtain baseline information on the feasibility of OBV as a biological agent to control taro beetle under the environmental conditions of New Britain.

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Materials and methods

Field collection

Most larvae and beetles of dynastid species were collected on the Gazelle Peninsula of New Britain Island, PNG. As a rule, larvae were collected only as

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later larval stages. Collection of different species was predominantly made on sites where Gorick (1980) reported the successful establishment of OBV: Vuvu, Malabunga, Malaguna, Raluana, Keravat, Vudal Beach, Malabunga and Ralawat. *Oryctes rhinoceros* larvae and adults were collected from killed coconut (*Cocos nucifera* (L.)) palms. Other scarab species were collected from a wide variety of ecological sites, including flowers, at artificial light, in various decaying materials, feeding on different host plants and in a wide variety of soils. All material from the Gazelle Peninsula was transported as live specimens in plastic containers, preferably in its natural substrate. Containers were placed in isolation boxes in order to avoid temperature stress en route.

Small numbers of taro beetle *Papuana* spp. and *Xylotrupes gideon* L. were collected from several coastal areas along the New Britain south coast: Wide Bay, Pomio station, Palmalmal, Amio, Gasmata and Kandrian as well as the New Britain north coast: Kimbe, Hoskins and Bialla. As these collections were made during field trips with limited stay at one area only few samples were derived from these areas when compared to the material from the Gazelle Peninsula. The material from areas other than the Gazelle Peninsula was killed using chloroform and fixed on site using formol. The material was transported in this form as fixed specimens for later microscopic examination.

Laboratory assessment

All specimens collected were screened immediately after collection. They were keyed to species using the keys developed by Thistleton and Masamdu (unpublished data), Beaudoin-Ollivier et al. (2000) and NARI Keravat reference collection. They were also screened for any abnormalities when compared with the reference collection specimen. Symptoms were described using Weiser and Briggs (1973), Gorick (1980) and Theunis (1997a,b,c). They were then processed further, as follows:

- Dead field specimens were either directly processed or immediately frozen. They are called ‘dead field specimens’ in this paper.
- Abnormal specimens found in the field were kept individually in plastic containers filled with heat-sterilised humid coconut wood dust (all palm rhinoceros beetle species) or soil/sawdust/compost mix (*Papuana* spp. and most other scarabs) as substrate. The substrate was regularly humidified. Different food was provided to adult specimens: e.g. pieces of taro for *Papuana* or sugarcane for other species. All specimens which died within 28 days were referred to as ‘specimen, diseased in field, died in lab’.
- Specimens that survived 28 days in the laboratory and still had symptoms were killed using chloroform, then sectioned and examined. They are referred to as ‘specimen diseased in field, survived in lab’. Specimens where symptoms disappeared within 28 days were referred to as ‘specimen diseased in field, survived in lab’. These specimens were either processed directly or frozen.
- Specimens with no obvious disease symptoms were kept separately in small groups for different sites, species and life-cycle states. They were checked daily for disease symptoms and death. Laboratory culturing was conducted as previously described. Dead specimens were either processed directly or were frozen. They are referred to as ‘diseased in lab, died in lab’.
- Diseased specimens were again separated and kept in the same way as diseased specimens in the field. Those that died were referred to as ‘diseased in lab and died in lab’.
- Specimens which were still alive after 30 days were considered ‘healthy specimens’.

These specimens were consequently used for further studies of cytopathology and infection biology.

Cytopathology

Material used for direct detection of symptoms and organisms was subjected to different cytopathological detection techniques. Larvae and beetles were dissected and the alimentary canal and fat tissue removed. Smears of the midgut epithelium and the fat body were produced. Formol (4% solution in water) was used as a primary fixative for air-dried smears. This was followed by secondary fixation with ethanol. The fixed smears were allowed to air-dry and then immediately dyed using the two following methods: giemsa stain (Boch and Supperer 1983) and haematoxylin (Weiser and Briggs 1973). This technique produces greater contrasts. Light microscopy was conducted using standard bright-field and dark-field illumination techniques as well as Abbe decentralised condenser contrast. As a standard procedure, all samples without clear OBV symptoms were considered negative. OBV infection was confirmed only when smears of at least

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one tissue of a specimen displayed the typical cytological alterations of the nuclei. Specimens were confirmed as healthy if all tissues not directly in contact with the gut lumen were free of micro-organisms and the insect cells did not display any cytological abnormalities. Specimens containing other micro-organisms were examined further to identify or confirm their state of parasitism. The data from these examinations are reported separately.

Sub-samples from collection rounds in 1996 and 1997 were submitted to the EU Pacific Regional Agricultural Programme – Project 5 to be used for the establishment of a polymerase chain reaction (PCR)-based detection system. The OBV DNA was successfully sub-cloned and PCR testing was subsequently done with the material from these sub-samples at AgResearch Lincoln, New Zealand. The data from these materials were reported separately by Jackson (1997).

Infection studies

To produce infection, suspensions of different materials were utilised. Before the infection studies, pre-trials were done with smaller numbers of specimens in order to identify whether there was any increased mortality. Pre-trials commenced using material directly derived from the field. Following increased mortality, *O. rhinoceros* displaying macroscopic symptoms of OBV were used as infection material for *Papuana* spp. trials for more in depth investigations.

Material used for these trials is listed in Table 2. Only material with clear macroscopic symptoms for OBV was considered. For infection studies, specimens were frozen immediately after death. Infective material was kept in airtight containers in deep-freeze storage (–20˚C), and was defrosted at the start of the experiment. Dates of collection, death and infection were all recorded for later reference. For each infection trial, one infected specimen was mashed up using a kitchen blender. A small proportion of the midgut was withheld to confirm microscopic OBV infection symptoms. The specimens to be infected were left swimming in this suspension. These target specimens from the infection experiment were kept in the same way as described with the field material. Any abnormal symptoms were recorded.

The following infection experiments were conducted:

- *O. rhinoceros* specimens showing OBV symptoms were used as a source of infection for adult and larvae of *Papuana huebneri* Fairmaire and *Papuana woodlarkiana* (Mont.)
- *P. huebneri and P. woodlarkiana* adults infected artificially in an earlier trial in the laboratory were used for infection of healthy *O. rhinoceros* adults.

Artificially infected specimens were inspected daily and the date of death recorded. Dead specimens were fixed as described earlier if not needed as infection material in a later trial. In this case, material was frozen immediately in the same way as described earlier.

Results

Collection of different species of Scarabaeidae

Almost all adults and larvae of *O. rhinoceros* were collected from dead coconut palms. Adults and larvae were mostly found together at the same site. *Oryctes rhinoceros* was not collected beyond the lowland areas of the Gazelle Peninsula of East New Britain. Typical damage symptoms to coconuts were not observed during the study (1997–2000) south of Rugen (Put Put) Harbour. All samples of *O. rhinoceros* were collected at altitudes below 600 m on lowland areas of the Gazelle

Adult taro beetles were mostly collected in the soil surrounding their main host plans; i.e. taro, *Colocasia esculenta* (L.) Schott; Chinese taro, *Xanthosoma sagittifolium* (L.) Schott; giant taro, *Alocasia macrorrhiza* (L.) Schott; swamp taro *Cyrtosperma merkusii* (Hassk.) Schott; and banana *Musa* × *paradisiaca* L. Larvae of *Papuana* spp. were only occasionally found near host plants of adult beetles but in a wide range of ecological situations. The site with the highest abundance of *Papuana* spp. larvae was the banks of the lower Keravat River. All taro beetle found were either *P. woodlarkiana* or *P. huebneri*. No other scarabs were found feeding on taro corms or banana rootstock.

Small numbers of *Scapanes australis* Boisduval and *Xylotrupes gideon* L. were collected on the Gazelle Peninsula, mostly from around banana and coconut stands.

Adults and larvae of various species of Dynastinae, Cetoniinae, Melolontinae and Lucanidae were found. Of these, only small numbers of specimens of each species were collected. In their larval form they were keyed to subfamily. Adult forms include the species

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Dermolepida uniforme Fairmaire, *Parastasia guttulata* Fairmaire, *P. inconstans* Fairmaire, *Poecilopharus bimaculata* Schurhoff and *Trichogromphus vicinus* Dechambre.

Numbers of specimens are listed. The cause of mortality was confirmed using both macroscopic and cytological symptoms. All other causes of death are separated from OBV. Specimens without obvious pathological symptoms are listed separately. All healthy specimens were alive after 4 weeks.

Cytological observations

Mortality and pathological assessments are summarised in Table 1. In *O. rhinoceros* specimens designated 'diseased in field and died in lab' and

Table 1. Survival of Scarabaeidae in the laboratory

Species and field symptoms	Pathological diagnosis		
Mortality per species	No cause of death	Oryctes baculovirus symptoms	Other cause of death
Oryctes rhinoceros, total	199	203	101
Dead in the field	0	79	58
Diseased in field, died in lab	0	112	34
Diseased in field, survived in lab	3	0	0
Diseased in lab, died in lab	0	12	9
Healthy specimen	196	0	0
Scapanes australis, total	54	0	3
Dead in field	0	0	3
Diseased in field, died in lab	0	0	0
Diseased in field, survived in lab	1	0	0
Diseased in lab, died in lab	0	0	0
Healthy specimen	53	0	0
Xylotrupes gideon, total	63	0	16
Dead in field	0	0	9
Diseased in field, died in lab	0	0	7
Diseased in field, survived in lab	0	0	0
Diseased in lab, died in lab	2	0	0
Healthy specimen	61	0	0
Papuana woodlarkiana, total	221	0	28
Dead in field	0	0	18
Diseased in field, died in lab	0	0	10
Diseased in field, survived in lab	3	0	0
Diseased in lab, died in lab	0	0	0
Healthy specimen	218	0	0
Papuana huebneri, total	258	0	34
Dead in field	0	0	15
Diseased in field, died in lab	0	0	18
Diseased in field, survived in lab	8	0	1
Diseased in lab, died in lab	0	0	0
Healthy specimen	250	0	0
Other scarabs, total	18	0	2
Dead in field	0	0	1
Diseased in field, died in lab	0	0	0
Diseased in field, survived in lab	0	0	0
Diseased in lab, died in lab	0	0	1
Healthy specimen	18	0	0
Grand total	811	203	184

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'diseased in lab and died in lab', cytological symptoms typical of OBV were regularly observed. Healthy specimens had small nuclei and contained patches of chromatin, which stained purple with giemsa stain. Virus-infected nuclei were hypertrophied and stained pink fairly homogeneously. In the nucleolus, ring-like structures were sometimes visible. Slides of infected specimens showed identical symptoms to the reference collection of Gorick.

Specimens with these typical OBV cytological symptoms nearly always showed distinct macroscopic symptoms. Most larvae with microscopic symptoms appeared translucent. Faeces were sometimes liquefied and a few specimens had a prolapsed rectum. Adult beetles have only internal symptoms. Healthy midguts are brown, thin and strong, whilst infected midguts are white, swollen and very fragile. In frozen specimens, the tissue often looks disintegrated. Macroscopic symptoms often alter following freezing. Preliminary diagnosis using macroscopic symptoms was therefore done before freezing.

At room temperature the symptoms of infected nuclei disappear quickly due to cell disintegration. Deep-freezing stops bacterial or autolytic decay, but ice crystal formation had undesirable effects on tissue integrity. Several specimens were therefore pre-fixed and stored in formol. In these cases it was advantageous to split the cuticle dorsally in order to aid penetration of fixative. Cytologic symptoms were enhanced through chemical fixation.

Dead specimens without macroscopic symptoms often had no cytologic OBV symptoms. Instead bacteria were found in tissues of these specimens. Overall there was no difference in symptoms between male and female specimens.

None of the other species collected showed cytological symptoms of OBV. Mortality in all other species was lower than in *O. rhinoceros*. Nearly all dead specimens of other species showed bacteria or other micro-organisms in the tissues.

Infection trials

Mortality in *Papuana* spp. was generally higher in specimens swimming in infective material than it was in the controls. Pre-testing confirmed that there was no difference in mortality between the two species of *Papuana*, or between male and females after swimming in suspensions containing OBV-infected tissue. Mortality rate was usually high. Most specimens died after 5 days. Pre-testing further confirmed that mortality in *Papuana* spp. was often low when obviously aged cadavers of *O. rhinoceros* were used. On the other hand, high mortality occurred when *O rhinoceros* specimens with obvious OBV symptoms were used. Only specimens with full pathological symptoms developed in the laboratory were therefore used in trials.

In taro beetles, macroscopic symptoms are not as easily observable as in *O. rhinoceros* due to the specimens and organs being smaller in *Papuana* spp. However, similar symptoms are still visible on larvae. Initial pre-trial results have shown that tissue decays faster. Hence, containers were checked daily for dead or moribund specimens (Table 2).

Cytological symptoms similar to OBV-infected cells of *O. rhinoceros* were observed in the midguts of male and female *P. woodlarkiana* and *P. huebneri*. The symptoms of cells of other tissues were not reliable for OBV assessment, as tissues were too damaged to see structural differences in nuclei clearly. Symptoms could be produced in obviously healthy *O. rhinoceros* adults using guts of both *Papuana* species. Mortality of *Papuana* spp. larvae is high but microscopic OBV symptom detection was impossible as tissues were decomposed by bacteria. This experiment therefore needs to be repeated.

Typical symptoms were observed in the target specimens only when fresh infective agents were used, and not from material derived from long-term frozen storage, from heated material, from material exposed to bright sunlight and weather, or from specimens left for more than two weeks at room temperature.

Discussion

Circumstances of natural OBV infection in **Oryctes rhinoceros** *and other species*

Twenty years after the distribution of OBV to various sites on the Gazelle Peninsula, typical viral symptoms were still detected in this study. This finding is confirmed by PCR testing done with an aliquot of two samples by Jackson (1997). Thus, OBV has been successfully established on the Gazelle Peninsula. However, OBV was not present in any of the other species examined. In addition, *O rhinoceros* was the only species which is restricted to only a part of the island, the Gazelle Peninsula. Therefore, cytological examinations reconfirm the virus for Vuvu, Keravat, Rapollo, Rabaul old airport, Matupit, Malaguna, Vudal Beach and Tokua. PCR

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testing by Jackson (1997) confirms the virus for all of the above sites except Tokua, Vudal beach and Keravat, but for Toleua in addition. Both detection methods produced highly conclusive results. It can therefore be stated that *O. rhinoceros* is distributed across the lowland areas of the Gazelle Peninsula north of the Baining Mountains wherever coconut palms are grown. In New Britain, the virus has spread across the entire area of distribution of *O. rhinoceros.*

This study and the results of Jackson (1997) showed OBV was absent in any other species and in any other area on New Britain or New Guinea islands The combination of both detection methods is preferable due to limitations in each method by itself. PCR is expensive and has limitations in handling large quantities of samples. For example, 1198 specimens were examined using cytology compared with 74 specimens in the PCR sub-sample. Cytology, on the other hand, is dependent on the tissue conditions. Our results clearly show the limitations of this method and hence the need for good laboratory procedures to obtain well-preserved tissues. Dead specimens from the field are not suitable for cytological analyses. On the other hand, PCR might detect OBV in more decayed samples. It may also detect sub-lethal infections. This has advantages and disadvantages. PCR establishes a better figure for general total mortality levels and thus efficiency of OBV. The cytological method on the other hand records recent fresh infections. These are the only samples that should be used for infection. Thus cytology is still the approach to manage a potential field release program while PCR is rather the back-up tool to monitor the efficiency of such a program.

Table 2. Infection[a] following treatment with different materials containing oryctes baculovirus (OBV)

Infective material	Target species (10 individuals in each treatment)	Treatment: no. dead/ no. with OBV	Control: no. dead/ no. with OBV
Oryctes rhinoceros, L3 larvae, diseased in lab, died in lab	*Papuana huebneri* adults	8/4	0/0
O. rhinoceros, L3 larvae, diseased in lab, died in lab	*Papuana woodlarkiana* adults	9/4	1/0
O. rhinoceros, adult, diseased in lab, died in lab	*P. huebneri* adults	8/5	0/0
O. rhinoceros, adult, diseased in lab, died in lab	*P. woodlarkiana* adults	7/5	0/0
O. rhinoceros, L3 larvae diseased in lab, died in lab	*Papuana* spp. larvae	9/0	3/0
O. rhinoceros, L3 larvae, diseased in lab, died in lab, 2 month frozen	*P. woodlarkiana* adults	6/5	0/0
O. rhinoceros, L3 larvae, diseased in lab, died in lab, 18 month frozen	*P. woodlarkiana* adults	2/0	0/0
Gut of *P. huebneri* adult, infected through *O. rhinoceros* L3 (diseased in lab, died in lab) in previous trial	O. rhinoceros	7/5	0/0
Gut of *P. woodlarkiana*, adult, female, infected by *O. rhinoceros* L3 (diseased in lab, died in lab) in previous trial	*O. rhinoceros*	7/4	0/0
O. rhinoceros, L3 larvae, diseased in lab, died in lab, 10 min boiled	*P. huebneri* adults	0/0	2/0
O. rhinoceros, L3 larvae, diseased in lab, died in lab, left at room temperature for 15 days before infection	*P. huebneri* adults	1/0	1/0
O. rhinoceros, L3 larvae, diseased in lab, died in lab, left 5 days exposed to sun and weather before infection	*P. huebneri* adults	2/0	0/0

[a] Listed are the number of treated and control specimens that died and those showing positive symptoms of OBV.

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OBV has been recorded in PNG only in *O. rhinoceros* so far. Distribution of the host and its virus seems to be restricted to the Gazelle Peninsula, New Ireland and Manus. This result can form the baseline of future work with OBV and potential transmission to other species. Any detection of OBV in future from a specimen other than the Asian rhinoceros beetle collected in the wild can be seen as a first transmission of OBV to a new host, either naturally or through a mass-release program.

Possibility of establishment of OBV in **Papuana** *spp.*

Although there is no record of any OBV infection of *Papuana* spp. in the wild, laboratory experiments show that both species are abundant in New Britain and can be infected in the laboratory. Symptoms can be reproduced in *O. rhinoceros* from an infected *Papuana* specimen. However, Koch's postulates still need to be proved, as certified virus-free target specimens were not available under the laboratory conditions at Keravat. There is, however, a very high consistency between cytological data and first PCR examinations in the natural infections of *O. rhinoceros*. This has yet to be proven for the artificially transmitted infections in the laboratory. Therefore, future studies will require parallel examination using the cytological and the PCR method.

With the cytological method, sub-lethal infections may not be detected. At the Lowland Agricultural Experiment Station, Gorick (unpublished data) conducted infection trials using lab-infected *O. rhinoceros* to infect *Scapanes australis*. Mortality appeared in first and second-instar larvae and symptoms recorded were similar to OBV in *O. rhinoceros* larvae. Adults and third-instar larvae showed no sign of infection by higher concentrations. Virus particles were detected using electron microscopy of tissues of adult *S. australis* by the unit of invertebrate virology at Oxford University, UK. This clearly indicates a need for more studies on the biology of OBV.

The detection of symptoms is more complicated in *Papuana* spp. Macroscopic symptoms are not clear. Bacteria enter the body tissue of *Papuana* spp. more rapidly than they do in *O. rhinoceros*. The reason for this is the smaller body size and the soil environment. Soil bacteria enter the beetles and larvae very quickly after death.

The level of infection in *Papuana* spp. may therefore actually be higher than detected by our trials. Hence, the results of our laboratory trials are a very promising. On this basis, future studies could build on the following conclusions.

1. OBV is a major candidate for biological control of taro beetle species. However, more data are needed on the biology of OBV in *Papuana* spp. as a host. Infections from *Papuana* to *Papuana* have yet to be done in all possible combinations to confirm intra-species and intra-genus infection. Data are needed as to whether all stages of *Papuana* spp. can be infected, and more natural modes of infection need to be tested. In laboratory and semi-field assessments, spontaneous transmission of the infection has yet to be proven.
2. Ability to infect is lost fairly quickly under field conditions such as UV radiation, heat and humidity as well as decay of tissue by other micro-organisms. Other ecological factors may also reduce infection rates. Only fresh, moribund larvae of *O. rhinoceros* should be used in an infection program. Repeated defrosting reduces the infective qualities of the material. Material intended for later use for infection needs to be deep frozen and stored for only a short period. Material for cytologic identification of OBV symptoms should be pre-fixed using formol.
3. Swimming beetles in a suspension containing virus is a successful method to infect taro beetles and *O. rhinoceros*. In mass-release programs the earlier described labour-intensive method of force feeding can therefore be replaced by the rapid infection method described in this paper.
4. The low stability of OBV in decaying material in the wild is probably the reason why the virus has not crossed host species borders. Adults and larvae of *O. rhinoceros* meet frequently in the confined space of stem tops of dead coconut palms. In this environment hardly any other scarab species is found. Further on, close contact is necessary and infection is oral. Within *O. rhinoceros* this occurs through mating and egg laying. This brings *O. rhinoceros* individuals into close contact with each other, but not with other species. That is most likely the reason why OBV did not cross the species border. The number of possible accidentally infected specimens in *Papuana* spp. is too low to generate a permanent infection pool. Permanent transmission of OBV into other species has to be initiated through a release program to generate a permanently infected sub-population. If laboratory and semi-field population tests prove

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potential transmission of infection within a population, then mass release should be seriously considered.

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A comparison of *Colletotrichum* species associated with berry diseases of *Coffea arabica* L.

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Abstract

Forty isolates of *Colletotrichum* species associated with coffee berry anthracnose in Papua New Guinea were characterised and identified on the basis of cultural, morphological and molecular characteristics. Of these, 29 isolates were identified as *C. gloeosporioides,* while the remaining 11 were identified as *C. acutatum*. None of the isolates had characteristics common to *C. kahawae.*

Introduction

Fungi of the genus *Colletotrichum* include some 900 species (Sutton 1992) and are most commonly associated with anthracnose symptoms in the respective host-plant tissues. Despite the large number of species only three, *C. kahawae* J. M. Waller & P.D. Bridge, sp. Nov. (formerly *C. coffeanum* Noack), *C. gloeosporioides* Penz, and *C. acutatum* Simmonds, have been isolated from coffee (Hindorf 1970). *Colletotrichum kahawae* attacks all stages of the crop from flowering to ripe berries can result in yield losses as high as 80% (Griffiths et al. 1971) and can lead to the abandonment of coffee growing as has happened in some parts of Africa (Turner 1992). This compares with *C. gloeosporioides* where the attack is restricted to the ripe berries only and yield losses can be up to 40% (Mignucci et al. 1985). *Colletotrichum acutatum* is also associated with ripe berry anthracnose but there is no evidence on the nature of its pathological or saprophytic association with berry anthracnose and resultant yield loss.

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Colletotrichum kahawae is a very serious threat to economic coffee production in all countries where the fungus has not been reported, including Papua New Guinea (PNG). Therefore, a general knowledge on the features characteristic of *C. kahawae* is useful in diagnosing the cause of berry diseases in PNG. The purpose of this paper is to present data obtained on *Colletotrichum* species isolated from coffee berry anthracnose in PNG and compare them with *C. kahawae* causing coffee berry disease (CBD) in African countries.

Materials and methods

Samples of diseased berries were collected from various localities in five provinces (Southern Highlands, Wabag, Western Highlands, Chimbu and Eastern Highlands) during May–July 2002. Forty sites were visited for sample collection and for each site a pure culture of a single spore isolate was obtained. This was used in the cultural, morphological and molecular studies. The isolates were code named based on provincial, electorate and sampling site codes (Table 1).

Cultural characteristics were studied on potato dextrose agar (PDA) amended with 0.02% streptomycin sulfate. Spore morphology was described

Table 1. *Colletotrichum* isolates derived from provincial, electoral and site codes

Isolate code	Province	Provincial code	Electorate	Electoral code	Sampling site	Site code
1001a 1001b 1001c 1001d	Southern Highlands (SHP)	1	Mendi	001	Mendi1 Mendi2 Mendi3 Mendi4	a b c d
1002a 1002b			Imbonggu	002	Kaugel1 Kaugel2	a b
2007a 2007b	Enga	2	Wabag	007	Wabag1 Wabag2	a b
2008a 2008b 2008c			Wapenamenda	008	Wapenamenda1 Wapenamenda2 Pausa	a b c
3012a 3012b	Western Highlands	3	North Wahgi	012	Numans Banz	a b
3013a 3013b 3013c	(WHP)		South Wahgi/ Angalimp	013	Panga Kudjip Minj	a b c
3014a 3014b 3014c			Tambul/ Nebilyer	014	Togoba1 Togoba2 Togoba3	a b c
3016a 3016b 3016c 3016d			Hagen	016	Keltiga1 Keltiga2 Dobel Mt Ambra	a b c d
3018a 3018b			Dei	018	Nunga Kinjibi	a b
4019a	Chimbu	4	Chuave	019	Chuave	a
4022a			Kerowagi	022	Kunabau	a
4023a 4023b 4023c			Kundiawa/ Gembol	023	Mindima Wandi Kundiawa	a b c
4024a			Sinasina/ Yonggamugl	024	Masual	a
5025a 5025b	Eastern Highlands	5	Daulo	025	Watabung Asaro	a b
5026a 5026b	(EHP)		Goroka	026	Kabiufa Kamaliki	a b
5027a			Henganofi	027	Kompri	a
5028a			Kainantu	028	Yonki	a
5030a 5030b 5030c			Obura/ Wonenara	030	Aiyura Kovuta Urara	a b c

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from specimens fixed and stained in lactophenol cotton blue. Each isolate was identified using Sutton's identification keys (Sutton 1980).

Molecular techniques involving the polymerase chain reaction (PCR) technology were used as an additional tool for isolate identification. The methodology used follows that of Manaut et al. (2001) with some modifications. Mycelium for DNA extraction was collected directly from cultures grown on PDA. Amplification of the internal transcribed spacer (ITS) regions between 18S and 28S including the 5.8S segment of the ribosomal deoxyribonucleic acid (rDNA) was carried out using the universal primers ITS1 and ITS4. The restriction enzymes *DpnII*, *HhaI*, *HinfI*, *TaqI* and *HpaII* were used for the analysis of restriction fragment length polymorphism (RFLP) of the ITS region.

In order to determine the rDNA sequence of the ITS region, the following procedure was followed. The PCR product of the ITS region was purified with Nucleospin Extract Kit and ligated into the pGEM-T easy vector following the manufacture's protocols. The ligation product was transferred into *Escherichia coli* component cells, strain 109. DNA plasmid was prepared from the transformed *E. coli* cells using the Ultra mini plasmid preparation kit following the manufacture's protocol. Sequencing reactions were primed on both strands of plasmid DNA using the SP6 and T7 promoter sequences. Sequencing of the plasmid DNA was done by the Australian Genome Research Facility at the University of Queensland. The sequence was manually aligned and blast searched on the database to determine sequence homology with the already sequenced ITS region of rDNA of *Colletotrichum* species.

Results

The cultural and morphological features have been described for all the 40 isolates of *Colletotrichum,* with only the features relevant for species identification summarised under the categories of (a) whole colony, (b) mycelium and (c) reproductive structures (Table 2). Species identification given in Table 2 is based on Sutton (1980).

Differences and similarities are evident among the 40 isolates in one or more of the features used to characterise each isolate. For example, variation in the colony growth rate ranged from 4.0–11.8 mm/day with 11 isolates (1001a, 1001b, 1001d, 1002b, 2007b, 2008a, 2008b, 3013a, 3016a, 3016c, and 3016d) characterised by relatively slower growth rates (<5 mm/day) compared with the rest of the isolates. Differences between the isolates in some of the other characteristics included colony colour (wool white to dirty white/grey), mycelial form (loose, compact) and elevation (low, moderate and high), and presence of sclerotial bodies. However, these differences were not as consistent as the growth rates within the slow and fast-growing groups of isolates.

The PCR product of the entire ITS region between 18S and 28S of the rDNA showed identical banding pattern for all the 40 isolates, with the size of the amplified products measuring just over 500 base pairs long, as illustrated by the six isolates (Figure 1). The six isolates represent five provinces and three conidial shapes. Except for restriction enzyme *DpnII*, none the restriction enzymes tested was able to separate the isolates into different RFLP groups. Digestion with *DpnII* separated the isolates into two different RFLP groups. as represented by isolates 1001a and 5030a (Figure 2). All the 11 slow-growing isolates fell under the RFLP group of 1001a, while the remaining 29 isolates fell under 5030a. Although isolates 3012a and 5030b differed in conidial shape and size, none of the restriction enzymes tested proved that these two isolates were genotypically different from the rest of the fast-growing isolates. On the contrary, they were grouped together with the rest of the fast-growing isolates by enzyme *DpnII*. Indeed, the mean conidia size of these two isolates falls within the size range of some fast-growing isolates, such as 1001c and 2007a.

DNA sequence analysis of the two RFLP groups showed that the RFLP group represented by isolate 1001a was 584 base pairs long, while those represented by 5030a were 574 base pairs long. By comparing the DNA sequence of the ITS regions of the two isolates with data on the database by using the BLAST (Altschul et al. 1997) search program, it was found that isolate 5030a was similar (e-value 0.0) to *C. gloeosporioides*, while isolate 1001a was similar to *C. acutatum*. There was 99% DNA sequence homology between isolate 5030a and *C. gloeosporioides* accession reference gi/31745580/gb/AY245021.1. Differences in the DNA sequence were detected at nucleotides 427 and 496. Similarly, the DNA sequence homology between isolate 1001a and *C. acutatum* accession reference gi/24459953/dbj/AB042301.1 was 99%, with differences at nucleotides 129, 448 and 515.

Table 2. Descriptions of some features of the colony, mycelium and reproductive structures of 40 isolates of *Colletotrichum* from Papua New Guinea and species identification

Isolate	Descriptive features										Species identification[k]
	Whole colony			Mycelium			Reproductive structures				
	Colony			Mycelial		Sclerotial bodies[f]	Acervuli[g]	Setae[h]	Conidium		
	colour[a]	growth[b]	margin[c]	form[d]	elevation[e]				size[I]	shape[j]	
1001a	1	4.4	2	1	2	0	1	0	11.4 × 3.3	1	a
1001b	2	4.6	1	2	1	0	1	0	15.1 × 2.3	1	a
1001c	1	11.6	2	1	1	1	2	1	16.2 × 4.2	2	g
1001d	3	4.6	2	1	2	0	1	0	13.2 × 2.8	1	a
1002a	3	10.7	2	1	2	1	2	1	14.6 × 4.2	2	g
1002b	3	4.6	2	1	2	0	1	0	14.0 × 2.1	1	a
2007a	1	9.8	2	1	1	1	2	1	15.9 × 5.4	2	g
2007b	3	4.5	2	1	1	0	1	0	11.6 × 3.5	1	a
2008a	2	4.1	2	1	2	0	1	0	15.0 × 4.2	1	a
2008b	1	4.3	1	1	1	0	1	0	14.4 × 2.4	1	a
2008c	1	11.2	2	1	1	0	2	1	15.2 x 3.9	2	g
3012a	5	10.0	1	1	3	1	2	1	19.5 × 4.6	3	g
3012b	5	9.9	1	1	3	1	2	1	16.4 × 5.4	2	g
3013a	1	4.0	2	1	2	0	2	1	15.9 × 4.9	2	a
3013b	1	10.9	1	1	2	0	2	1	16.0 × 4.8	2	g
3013c	4	11.1	1	2	1	0	0	1	14.8 × 5.2	2	g
3014a	3	10.4	1	1	3	1	2	1	14.7 × 4.7	2	g
3014b	3	11.4	1	1	3	1	2	1	14.6 × 4.2	2	g
3014c	3	10.8	1	1	3	1	2	1	15.4 × 4.8	2	g
3016a	1	5.0	1	1	2	0	1	0	12.9 × 4.3	1	a
3016b	1	9.7	2	1	2	1	2	1	15.9 × 3.2	2	g
3016c	2	4.8	2	2	1	0	1	0	11.6 × 2.2	1	a
3016d	2	5.0	2	2	1	0	1	0	13.5 × 2.4	1	a
3018a	1	10.1	2	1	2	0	2	1	13.1 × 3.9	2	g
3018b	1	10.6	1	1	2	0	2	1	15.2 × 4.7	2	g
4019a	2	10.2	1	1	2	0	2	1	16.0 × 4.1	2	g
4022a	4	10.1	1	2	1	1	2	1	15.6 × 5.1	2	g
4023a	4	10.3	1	2	1	1	2	1	15.9 × 4.7	2	g
4023b	4	10.8	1	2	1	0	2	1	15.0 × 5.2	2	g
4023c	1	9.8	2	1	2	0	2	1	14.2 × 4.0	2	g
4024a	3	11.2	1	1	2	1	2	1	15.1 × 4.1	2	g

Table 2. (cont'd) Descriptions of some features of the colony, mycelium and reproductive structures of 40 isolates of *Colletotrichum* from Papua New Guinea and species identification

Isolate	Descriptive features										Species identification[k]
	Whole colony			Mycelium			Reproductive structures				
	Colony			Mycelial		Sclerotial bodies[f]	Acervuli[g]	Setae[h]	Conidium		
	colour[a]	growth[b]	margin[c]	form[d]	elevation[e]				size[I]	shape[j]	
5025a	1	10.5	1	1	2	0	2	1	14.7 × 4.5	2	g
5025b	1	11.4	1	1	2	0	2	1	15.6 × 4.2	2	g
5026a	5	10.4	1	1	3	1	2	1	14.8 × 5.1	2	g
5026b	5	11.0	1	1	3	1	2	1	16.4 × 4.8	2	g
5027a	3	11.5	1	1	3	1	2	1	15.1 × 4.6	2	g
5028a	3	11.5	1	1	3	1	2	1	14.9 × 4.0	2	g
5030a	3	11.8	1	1	3	1	2	1	19.8 × 5.2	3	g
5030b	3	10.1	1	1	3	1	2	1	15.6 × 5.0	2	g
5030c	5	10.5	1	1	3	1	2	1	14.8 × 4.8	2	g

[a] Colony colour, 1 = white, 2 = grey, 3 = white to dirty white, 4 = dirty white, 5 = wool white.
[b] Colony growth, average growth per day given in mm.
[c] Colony margin, 1 = regular, 2 = irregular.
[d] Mycelial form, 1 = loose, 2 = compact
[e] Mycelial elevation, 1 = low, 2 = moderate, 3 = high.
[f] Sclerotial bodies, 0 = absent, 1 = present.
[g] Acervuli, 0 = rare, 1 = poor, 2 = abundant.
[h] Setae, 0 = absent, 1 = present.
[i] Conidia size, conidia dimensions given in micrometres.
[j] Conidia shape, 1 = cylindrical/fusiform, 2 = cylindrical/straight, 3 = cylindrical with one end tapering.
[k] Species identification, a = *C. acutatum*, g = *C. gloeosporioides.*

From: Price, T.V., ed., 2006. Pest and disease incursions: risks, threats and management in Papua New Guinea. Canberra, ACIAR Technical Reports No. 62.

Discussion

The species identification of the 40 isolates of *Colletotrichum* from PNG was made using both molecular techniques and conventional methods relying on cultural and morphological features. The cultural and morphological features described for 27 isolates fall within Sutton's (1980) species identification for *C. gloeosporioides*. The variation in cultural features observed in this group of isolates agrees with earlier studies (Hocking 1966; Gibbs 1969; Hindorf 1970; Hindorf and Muthappa 1974; Muthappa 1974; Waller et al. 1993) and is characteristic of *C. gloeosporioides*.

Two isolates, 3012a and 5030b, had conidia sizes outside the range observed by Hocking (1966), Hindorf (1970) and Hindorf and Muthappa (1974) for *C. gloeosporioides* isolated from coffee. It is not uncommon for *C. gloeosporioides* to produce conidia in the range observed in this study. The size range covered in Sutton (1980) is 9–24 × 3–4.5. Manaut et al. (2001) reported conidial dimensions ranging from

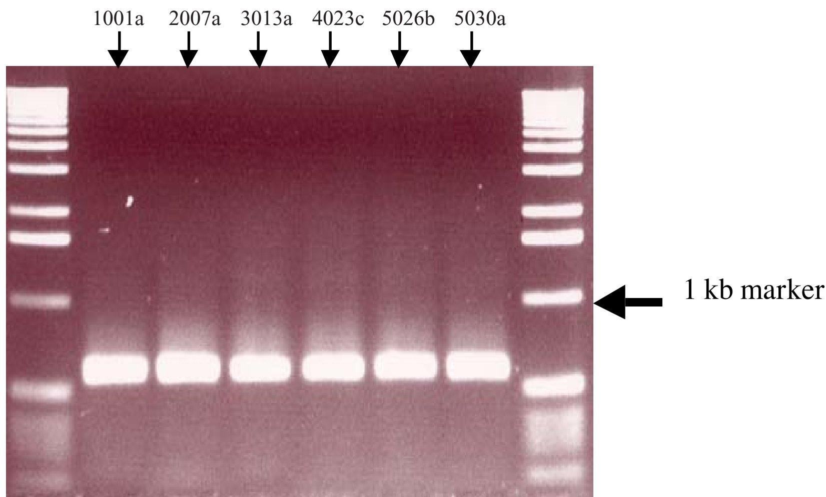

Figure 1. PCR amplification of ITS region of rDNA for *Colletotrichum* isolates 1001a, 2007a, 3013a, 4023c, 5026b and 5030a

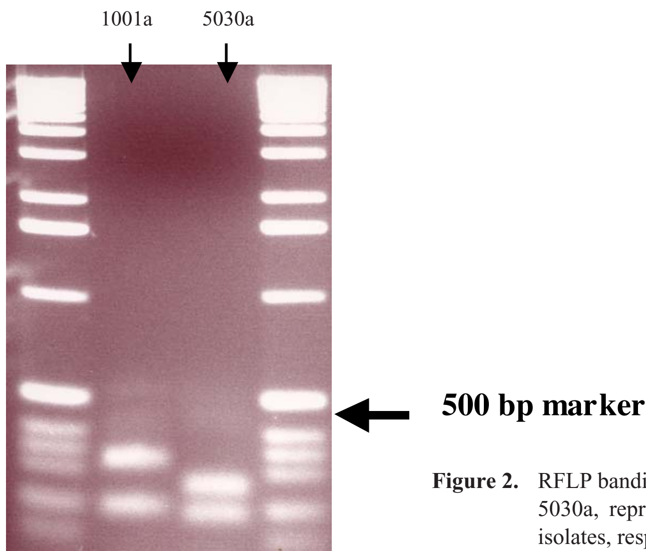

Figure 2. RFLP banding pattern for isolates 1001a and 5030a, representing slow and fast-growing isolates, respectively

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9.2–29.5 × 2.3–5.8 for *C. gloeosporioides* isolated from *Stylosanthes* spp., while Peres et al. (2002) made similar observations with size ranging from 9.6–20.6 × 3.4–8.2 of isolates from various fruits. Given such variations in conidial dimensions, this study identifies both isolates 3012a and 5030b as *C. gloeosporioides*.

Molecular characterisation based on PCR banding patterns for the ITS region of the rDNA confirmed that all the 40 isolates from PNG were *Colletotrichum* isolates since the ITS 1 and ITS 4 primers used were able to produce an identical banding pattern across all isolates. Similar banding patterns for various segments of the ITS region were reported for *Colletotrichum* isolates originating from coffee and other hosts (Mills et al. 1992; Buddie et al. 1999; Manaut et al. 2001; Abang et al. 2002; Peres et al. 2002). Furthermore, the size of the PCR product (500–600 bp) reported in this study is also similar to those reported in these earlier studies.

Given that three species of *Colletotrichum* have been found on coffee, the results of the PCR product analysis are not adequate to separate the three species, *C. kahawae*, *C. gloeosporioides* and *C. acutatum.* This is further complicated by the argument that *C. kahawae* should be considered as a subspecies of the *C. gloeosporioides* species group (Sreenivasaprasad et al. 1993). Hence, further characterisation by way of RFLP analysis revealed two distinct groups among the 40 isolates, of which DNA sequence analysis resulted in the identification of the PNG isolates as *C. gloeosporioides* and *C. acutatum*.

The 11 isolates that were identified as *C. acutatum* were difficult to diagnose initially on the basis of conidial morphology and size, and colony characters. Sutton (1992) pointed out that one of the problems that could arise in trying to identify *C. acutatum* and *C. gloeosporioides* lies in the fact that there exist strains of the latter species which are intermediate in conidial morphology and size and show variable colony characters. Because of this confusion, the individual worker may identify his/her strains as either *C. acutatum* or *C. gloeosporioides* depending on the criterion that is considered most important. *Colletotrichum acutatum* species identified in this study reflect the complexity of the problem discussed by Sutton (1992). While the DNA sequence analysis indicates the isolates as being closely related to *C. acutatum,* some of the cultural and morphological features easily fit the isolates into the broad category of *C. gloeosporioides*. However, in this study, the results of the DNA analysis have been accepted together with the consistency of slow growth rates and the absence of setae as characteristic features of *C. acutatum*. The study also accepts conidial dimensions from these isolates as features characteristic of *C. acutatum* on coffee in PNG although these do not fall within the range observed by Simmonds (1965) and Hindorf (1970).

Colletotrichum acutatum has been isolated from high altitude coffee (Hindorf 1970). This is also evident in this study, in which the species was found only in parts of the upper highlands, i.e. Southern Highlands, Enga and Western Highlands provinces. On the other hand, *C. gloeosporioides* was found in all the provinces and confirms itself as a species of common distribution.

While this study was able to reveal the presence of *C. gloeosporioides* and *C. acutatum,* there is no evidence to indicate the presence of the CBD pathogen, *C. kahawae*, in PNG. For comparison purposes the common cultural and morphological features of *C. kahawae* are as follows; conidia straight, cylindrical, measuring 12.5–19 × 4 µm formed from the mycelium, colonies dense to floccose, pale chocolate brown, sclerotia absent, setae usually absent (Sutton 1980). Waller et al. (1993) described the colony characteristics on 2% MEA as follows: *C. kahawae*, slow-growing (2–4 mm/day at 25˚C), profuse olivaceous to greenish dark grey mycelium, no acervular conidiomata produced, sporulation occurs from simple hyphae. *Colletotrichum gloeosporioides*, faster growing (3–6 mm/day at 25˚C), white to pale grey mycelium, sporulation from acervuli or simple hyphae. At the molecular level, Sreenivasaprasad et al. (1993) found some variations in the DNA sequence of the ITS region for *C. gloeosporioides* isolates, whereas for *C. kahawae* isolates there were no DNA sequence variations. None of the isolates from PNG resemble the descriptions of *C. kahawae*. Furthermore, field observations during sampling failed to identify infection of young green berries, which is characteristic of CBD. Infection of ripening green berries was quite common, however. Since the CBD pathogen can also infect ripening green berries, the widespread occurrence of it is often the cause of anxiety and confusion among coffee growers as to its true pathological cause. This study confirms that infections of ripening green berries in PNG are caused by *C. gloeosporioides,* either alone or in combination with *C. acutatum,* although the pathogenicity of the latter needs further investigation.

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Diagnosis of the cause of berry anthracnose in PNG in the future can be done easily and quickly by adopting the methodology followed in this study. The methodologies used for extracting DNA, amplifying the ITS region of the rDNA and determining RFLP banding patterns, and cloning for DNA sequencing are relatively simple and can be done in PNG using some of the existing basic research facilities, such as the UNITECH Biotechnology Centre. The only task that cannot be performed in PNG is DNA sequence determination and will thus rely on services provided by overseas laboratories. Analysis of DNA sequence appears to be the most relevant technique for separating *C. kahawae* from the other species, given that Sreenivasaprasad et al. (1993) reported 100% sequence homology among isolates of *C. kahawae*. Should the CBD pathogen enter the country it will be possible to identify it within 2–4 weeks from the time of sample collection.

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