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# **Pest and disease incursions: risks, threats and management in Papua New Guinea**

**Papers presented at the 2nd Papua New Guinea Plant Protection  
Conference, Kokopo, East New Britain Province, 8–10 November 2004**

*Editor: T.V. Price*



**Australian Government**  
**Australian Centre for  
International Agricultural Research**



Australian Centre for International Agricultural Research  
Canberra  
2006

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# Foreword

Papua New Guinea is an important partner country for ACIAR, reflecting the long-term relationship between Australia and PNG.

Village-based agriculture supports between 70 and 80 per cent of the PNG population with domestic trading of fresh produce a very important source of cash incomes. Crop protection is vital to sustain this sector.

The papers in this publication include reports from several projects supported by ACIAR. One project, which is active in PNG, Indonesia and Australia, has mapped sugarcane pests and diseases. Research has now extended to survey key areas to build up a comprehensive picture of pest and disease distribution.

In other projects, pests of horticulture crops, the *Oribius* weevil and the red-banded mango caterpillar, are being studied. The damage caused by the weevil has been determined, and treatments to exclude adult weevils from some plants have resulted in price premiums of up to 200 per cent.

The use of biocontrol agents against the invasive weed *Chromolaena* continues to gain momentum in PNG. A new control agent — a stem galling fly — has been widely released and is established at many sites.

ACIAR is pleased to be able to publish this series of papers, which were presented at the 2nd Papua New Guinea Plant Protection Conference. I congratulate the researchers working on plant protection in Papua New Guinea on forming their association and wish them well in the future.



Peter Core  
Director  
Australian Centre for International  
Agricultural Research



# Contents

Foreword	3
Preface	9
Opening remarks by the Governor of East New Britain Province	11
<b>Keynote address</b>	
Plant pest incursions: risks, threats and management <i>G.V. Maynard</i>	14
<b>Crop pests and management</b>	
Incidence of pathogens naturally associated with taro beetles in sugarcane fields in Papua New Guinea <i>A.N. Simbiken</i>	20
Pest species of <i>Oribius</i> Marshall (Coleoptera: Curculionidae: Otorhynchinae) and their host plants in PNG [Abstract only] <i>M.M. Ero, T. Clarke, P. Wesis and B. Niangu</i>	24
Incidence and distribution of <i>Bactrocera papayae</i> Drew and Hancock (Diptera: Tephritidae) in coffee plantations in the Eastern Highlands and Madang provinces of Papua New Guinea <i>A.N. Simbiken</i>	25
The use of sticky traps to study seasonal dispersal activity of the sweet potato weevil, <i>Cylas formicarius</i> (Fabricius), in Papua New Guinea <i>S.A. Sar</i>	31
Conservation of sugarcane germplasm: survey of Papua New Guinea, Indonesia and northern Australia <i>L.S. Kuniata, R.C. Magarey and G. Rauka</i>	36
Efficacy of neem extract, Delfin <sup>®</sup> (Bt) and Orthene <sup>®</sup> on lepidopterous insect pests in cabbages in the Gazelle Peninsula of Papua New Guinea <i>P. Mwayawa</i>	43
Evaluation of four insecticides as part of an integrated pest management strategy for diamondback moth, <i>Plutella xylostella</i> L., in the highlands of Papua New Guinea <i>J. Wemin and P. Wesis</i>	49

Breeding sites of major coconut beetle pest *Scapanes australis* Boisd. (Coleoptera: Scarabaeoidea, Dynastinae) in East New Britain, Papua New Guinea  
*P. Gende, T. Kakul, S. Laup and S. Embupa* 57

Farmer perceptions of coffee pests in Boana district, Morobe Province, Papua New Guinea  
*A.N. Simbiken* 60

## **Diseases and management**

Evaluation of fungicides against potato late blight disease (*Phytophthora infestans*) on susceptible and tolerant potato varieties  
*S. Hariki* 66

Preliminary assessment of two inorganic copper-based fungicides against late blight (*Phytophthora infestans*) in the tropical highlands of Papua New Guinea  
*D. Minemba and P. Sovo* 71

Improvement of compost mounding systems [Abstract only]  
*R. Raatsch* 75

Development of management strategies for ratoon stunting disease in sugarcane at Ramu Sugar, Papua New Guinea  
*L.S. Kuniata, G. Rauka and R.C. Magarey* 76

The role of tissue culture in the revival of the potato industry in Papua New Guinea  
*V. Mero* 82

A preliminary study of interrupting the epidemiological cycle of *Phytophthora palmivora*: integrated disease management of cocoa in relation to cocoa cropping cycle  
*Y. Namaliu, J.T. Vano and J.K. Konam* 84

Evaluation of disease on new planting materials developed at the Cocoa and Coconut Institute with potential for release to farmers in Papua New Guinea  
*A.B. Kamuso and J.K. Konam* 89

Disease performance of international cocoa clones at the Cocoa and Coconut Institute, Papua New Guinea  
*A.B. Kamuso, R. Wennani, J. Saul and J.K. Konam* 94

Diversity of *Phytophthora palmivora* on cocoa in Papua New Guinea  
*J. Saul* 100

## **Keynote address**

Plant protection in the 21st century: new developments, trends and training requirements  
*T.V. Price* 106

## **Weeds and management**

The importance of proper weed control in young hybrid cocoa (*Theobroma cacao* L.) in Papua New Guinea  
*D.S. Yinil, M.S. Powell and H. Tangbil* 110

Status and management of invasive weed <i>Chromolaena odorata</i> in Papua New Guinea [Abstract only] <i>I. Bofeng</i>	118
Control of <i>Monstera</i> species in cocoa: a preliminary investigation using various herbicide mixtures <i>D.S. Yinil</i>	119
<b>Pest incursions and quarantine</b>	
Invasive weeds: impacts, prevention, detection and responses <i>W. Orapa</i>	124
An overview of pest incursions in Papua New Guinea over the past 20 years <i>R. Masamdu</i>	132
Spread of citrus huanglongbing (greening) disease following incursion into Papua New Guinea [Abstract only] <i>R. Davis, T. Gunua, M. Kame, D.I. Tenakana and T. Ruabete</i>	136
Impact of some food-crop disease outbreaks in Papua New Guinea <i>P. Kokoa</i>	137
The PNG pest list database and its uses in quarantine surveillance and pest management <i>R. Masamdu</i>	144
Coconut inflorescence borer, <i>Synneschodes papuana</i> (Lepidoptera: Brachodidae), an important new pest of coconut in Papua New Guinea <i>T. Kakul, M. Aloysius and K. Samai</i>	146
Overview of internal plant quarantine and the challenges in Papua New Guinea <i>R. Masamdu</i>	151
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 <i>J.F. Grimshaw, B.M. Waterhouse and M.P. Weinert</i>	154
Red-banded mango caterpillar, <i>Deanolis sublimbalis</i> Snellen (Lepidoptera: Pyralidae: Odontinae), in Papua New Guinea <i>D. Tenakanai, F. Dori and K. Kurika</i>	161
Some aspects of banana entomology in northern Australia: what can we apply to Papua New Guinea? [Abstract only] <i>B. Pinese</i>	166
<b>Pest and disease identification</b>	
EntomID-PNG — a digital image database of the insects of Papua New Guinea <i>M. Wiemers and M. Ero</i>	168



Use of molecular markers in managing plant pests and diseases: a PNG perspective <i>B. Nass-Komolong and M. Maino</i>	174
The distribution of oryctes baculovirus in different species of Scarabaeidae on New Britain Island, Papua New Guinea <i>A. Schuhbeck and J. Bokosou</i>	184
A comparison of <i>Colletotrichum</i> species associated with berry diseases of <i>Coffea arabica</i> L. <i>M.K. Kenny, V.J. Galea, P.T. Scott and T.V. Price</i>	194

# Preface

The first Papua New Guinea (PNG) Plant/Crop Protection Conference was held at Lae in February 2002, and was sponsored by the Australian Contribution to the National Agricultural Research Service (ACNARS) and organised by the National Agricultural Research Institute (NARI).

At the suggestion of Professor Terry Price from the University of Vudal, it was decided at this meeting that a PNG Plant Protection Association be formed. An interim committee was duly elected by those participants present, with Roy Masamdu as the President and Professor Price as advisor, and it was decided that the next conference would be held in East New Britain in 2004.

There are currently very few scientists engaged in plant protection in PNG and these have to service five million people in the country; they work, often in isolation, under extremely varying conditions. The aim of the Plant Protection Association and Conference is to bring together all those working in this area, whether they be in national or provincial organisations or departments, corporations, non-government organisations or universities, to share their knowledge and experience of solving plant protection problems in PNG and neighbouring Pacific countries.

The members of the organising committee for the 2nd PNG Plant Protection Conference were:

Dr John Konam (Chairman), Cocoa and Coconut Institute, Tavilo  
Professor Terry Price (advisor), University of Vudal  
Mr Peter Mwayawa, University of Vudal  
Mr David Yinil, Cocoa and Coconut Institute, Tavilo  
Mr Paul Gende, Cocoa and Coconut Institute, Tavilo  
Mr Pere Kokoa, NARI Lowlands Agricultural Experimental Station (LAES), Keravat  
Mr Helmut Ludewig, NARI, LAES, Keravat  
Ms Amanda Mararuai, NARI, LAES, Keravat  
Dr Lastus Kuniata, Ramu Sugar Ltd  
Mr Roy Masamdu, National Agricultural Quarantine and Inspection Authority.

The organising committee wishes to thank the following international and local sponsors for their financial and other help in making this conference possible.

Secretariat of the Pacific Community  
Australian Centre for International Agricultural Research  
Department of Agriculture, Fisheries and Forestry, Canberra, Australia  
PNG Cocoa and Coconut Research Institute  
Ramu Sugar Ltd

National Agricultural Quarantine and Inspection Authority  
National Agricultural Research Institute  
Australian Contribution to the National Agricultural Research Service  
University of Vudal.

Dr John Konam  
Chairman, Organising Committee  
2nd PNG Plant Protection Conference

## Opening remarks by the Governor of East New Britain Province

I am indeed happy to accept your invitation and to have the honour in officially opening this very valuable conference today.

I am informed that this conference organised by the PNG Crop Protection Association is the second after the formation of this very important association. In fact, as I went through your notes I am alarmed at the lack of interest by government agencies or even from the NGO sector, especially when we view the serious ramifications it would cause if we were not careful about the risks and threats posed by the pests and diseases we are faced with.

I am very encouraged by the strong stand being taken by the members of this association in making sure that another forum can eventuate to get together and discuss ways and means and specifically the management aspects of the future of crop protection in PNG. No doubt this conference will also become an avenue for high-level discussions also keeping in mind that we are facing national emergencies, particularly in light of the new pests and diseases entering PNG. Let me say here that PNG is not immune to such threats, however, and we must be alert at all times and your conference is a step in the right direction.

Discussions to develop a system to combat new incursions and the importance of sharing ideas and information for the common good of PNG food and cash crops is also of paramount importance.

Ladies and gentlemen, what you are gathered here to deliberate on is strategic planning. When you talk about combat, we are talking about going to war. This looming war must be taken head on, and I am proud to say that although we do not have the manpower to combat this war, with fewer than fifty crop protection personnel in our country and you may be an unrecognised group of scientists in PNG, you are silently contributing highly to the well being of every Papua New Guinean.

I am further encouraged by your determination in carrying out your duties for the good of the nation. You are indeed the unsung heroes of our nation. I say this because with very few personnel you have been successful in bringing under control the coffee rust disease that threatened the loss of coffee revenue to the tune of over K100 million to PNG annually, the successful protection of Ramu Sugar which generates over K80 Million to PNG, the success in careful introduction of insects as biological control agents, especially for the massive weed *Salvinia molesta* that almost clogged the waterways and lakes in the Sepik River system. The success of this operation saved the lives of over 100,000 people in the Sepik river basin. The growing potato industry, valued at over K20 million, faces the threat of total collapse because there are no plant pathologists engaged in the potato pathology work. The oil palm industry benefited significantly from pollination work by its entomologists which resulted in the increase

of oil palm production, and I mention too the work carried out in the cocoa industry in reducing the effects of black pod disease. Continuous research work has also resulted in the release of the improved cocoa planting materials in 2003. Currently, the coconut industry would also be on the verge of collapsing especially from the giant rhinoceros beetle and the black palm beetle if not for you.

Bananas, part of the staple diet in East New Britain and the third-most important food crop, is under threat from banana blood disease now spreading rapidly in the Indonesian province of Irian Jaya. If protection strategies are not mapped out, this disease has the potential to wipe out our bananas.

Having said the above, ladies and gentlemen, and while the bulk of the responsibility rests with the commissioned statutory bodies such as NARI, CIC, CCI, OPRA, Ramu Sugar Ltd, FPDC and NAQIA, I take into account that this conference is the step in the right direction, but mainly as a central forum to promote the mission of the association.

The theme of this conference — *Pest and disease incursions: risks, threats and management* — is very important and reflects that deep in our hearts something is very wrong and if we do not act now, it will be too late. The risks and threats are very real.

For these, I am strongly calling on the national government to provide funding and to encourage more of our young people to join the army of crop protectionists in defending our livelihoods and our future generations.

I would be very interested to hear from you on whatever resolutions passed and further urge the division of primary industry within the provincial administration to become more proactive and responsive to the risks and threats that are posed by the immensity of this natural disaster that is waiting to happen. I further urge your association to work closely with the division in the sharing of ideas and information so that we become partners in the fight against the risks and threats we are facing now.

With pleasure I now officially declare your conference open.



Hon. Leo J. Dion, QPM, MP  
Governor  
East New Britain Province  
Vunapope  
8 November 2004

# **Keynote address**

# Plant pest incursions: risks, threats and management

G.V. Maynard<sup>1</sup>

## *Abstract*

People who look after crop plants and notice that something different is happening are often the first to detect incursions. Incursions of plant pests occur via several pathways; some of these are human assisted, while others are not. In most cases, it is very difficult to trace the exact source of entry of a plant pest. It is presumed, however, that in the majority of cases they have entered on their host or as hitchhikers on non-host material. It is these human-mediated incursion pathways that can be looked at, and ways of managing the risks can be considered. The risks include pathways and the pests of concern. Human-mediated pathways are those that involve the deliberate movement of plant material, and movements of people and non-plant commodities. The pests include insects, plant pathogens, plants (as weeds) and insects as vectors of plant diseases. Threats usually arise from lack of management of the risks; that is, limited control of the movement of material, people and goods into and within the country (particularly from infected areas); the amount of time taken to react to a detection of a pest; and the importation of infected/infested goods.

Incursions are generally unpredictable, but if some thought is given to what actions are possible if an incursion does occur, then actions can happen quickly. The more quickly things happen, the more likely containment or eradication can be achieved. Actions can be simple—such as having a system where people inform others of where things are happening and using that information, or they can involve much more extensive and intensive communication and control methods. In summary, plant protection is everybody's business.

## **Introduction**

Plant pests arise from several sources. Sources include the introduction of pests from outside an area, and indigenous organisms becoming pests. Plant pest incursions can occur by natural spread or can be mediated by humans. Incursions that are human-mediated can often be mitigated by various means, whereas it is much more difficult to control incursions that occur via natural spread. Any attempt to mitigate and control incursions across international borders requires the development and imple-

mentation of robust phytosanitary incursion management measures (Maynard et al. 2004).

Phytosanitary or quarantine measures need to be appropriate to the level of risk, as well as the capacity for implementation. It is impossible for any government or organisation to fully implement broad-scale quarantine measures or surveillance without wider community support. One of the most useful and practical tools is engagement of the community—local as well as global—and management options range from simple to complex. The implementation of phytosanitary or quarantine measures involves everyone, including plant protection workers and researchers. As a first step, researchers and plant protection officers need to start to practice some simple processes themselves. They need, for example, to go

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<sup>1</sup> Office of the Chief Plant Protection Officer, Department of Agriculture, Fisheries and Forestry, Canberra, Australia. Email: <Glynn.Maynard@daff.gov.au>.

through some simple processes when leaving an area, particularly locations or sites with weeds, diseases or pests. These include ensuring that their clothing and shoes are not carrying weed seeds or other organisms; nor should they move fruit from one area to another unless it has been disinfested.

## **Risks: pathway or pest**

While the risk of incursion depends on many factors, the focus of this paper are human-mediated incursions. Two of the main issues that should be considered when attempting to mitigate or prevent incursions are the pathways and the pests.<sup>2</sup> A pathway is any means that allows the entry or spread of a pest (FAO 2002). Pathways include movement of goods, containers, hosts, people and vehicles via air, land and sea.

Land borders are amongst the most difficult pathways to control, particularly if they are sparsely populated and permit free movement of people and goods between areas. Land borders may be either international or regional, with goods passing from an area of higher pest prevalence to an area of lower pest prevalence. Sea borders are often somewhat easier to control than land borders. The effectiveness of the sea acting as a barrier to incursions depends on the distance between ports and the pests involved, as well as the type and frequency of vessels and what processing they are subject to at their destination. Air transport, on the other hand, provides rapid movement of goods and people over long distances because of the shorter transit time. Survival of organisms that might have died on trips of longer duration, is significantly higher. This is particularly true where long distance international travel is involved. Highly perishable goods can be sent from one location to another; this provides a pathway for organisms that do not survive well when separated from their hosts.

In the absence of phytosanitary controls, there is a high likelihood of introducing plant pests through movement of plants and plant commodities. Pests can be hitchhikers on non-host products, as contaminants of packaging, in containers or on people. The movement of containers and people provides a significant pathway for weed seeds. Seeds can be attached to humans, goods or containers, such that not only the

weeds themselves could be transported but also associated insects or agents of plant diseases (pathogens). Decontamination processes should thus be considered before materials leave an area of high weed prevalence.

## **Threats**

Highly motile organisms (wind-borne or flying) pose a significant risk of incursion across land or narrow sea borders. In addition, there is an increased risk of pests entering and establishing when there are limited phytosanitary (quarantine) controls on the movement of material internationally or inter-regionally. Other threats include delays in reacting to the detection of an incursion; food from aircraft and ship galleys; and importing large quantities of low-grade goods without strict controls. Slow reaction to the detection of a pest lessens the possibility that it can be contained or eradicated. The import of low-quality goods often means that there is a high likelihood of infestation due to the lower level of investment in disinfestation procedures before shipping. Since aircraft and ship galleys provide an ideal breeding ground for many plant pests, significant care should be taken in disposal of waste material from these sources. A further source of potential incursions from ships is the potted amenity plants that are frequently carried. Ships and ocean liners often have plants as part of the décor; they provide a potential means of transport for plant pests, and hence pose a possible pathway for an incursion.

## **Management**

It is easier to take measures (both logistically and biologically) to prevent the entry of pests than it is to attempt to contain or eradicate them once they have arrived. Management options range from relatively simple to complex. The options include public awareness, pest risk analyses, control of goods movement and contingency planning, through to treatments to control organisms such as inspection on arrival, biological control or chemical controls. One of the simplest management measures is to make people aware of the problems that they could cause by the uncontrolled movement of plant material or soil. Public awareness is one of the most powerful tools of limiting entry and spread of plant pests. It can take place by word of mouth, by passing on basic information at the village level and helping them

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<sup>2</sup> Pest is any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products (FAO 2002).



understand the message. The method used needs to be appropriate to the target audience. Before starting any public awareness campaign there is a need to have a clear understanding of the message—only one at a time—that is to be transmitted. The message should be kept simple, so that it can be readily understood.

Under the International Plant Protection Convention (IPPC) countries can undertake pest risk analyses on which to base decisions if an incursion should occur, and what measures will be required. When undertaking import risk analyses, decisions are based on a combination of the biological attributes of the organisms, the possible pathways of entry and the level of protection that the country desires. Biological attributes that can be considered include risk of entry and establishment; host range; life cycle stages (size/detectability); dispersal method; persistence off host; reproductive capacity and method; the type of damage that the organism will cause; and capacity to carry other pests and diseases. Other issues used in the decision-making process are trade implications and the impact on the environment (agricultural and natural).

Government agencies will usually undertake the control of bulk goods and containers entering via airports and seaports. The measures taken on arrival can range from mandatory fumigation treatment, to inspection of goods, containers or vessels, through to no action. The level of intervention depends on the risk potential of the goods. Similarly, control measures applied to the movement of goods within a country are frequently the responsibility of government agencies.

Good planning before a pest incursion can considerably minimise delays and increase the ability to prevent further spread of the pest. Planning issues comprise factors such as consideration of who needs to know about an incursion; who speaks to whom; who does what; what resources (human, financial, equipment) are likely to be needed and who is going to provide them.

When an incursion does occur, one of the most critical resources is the capacity to be able to competently and efficiently diagnose a pest. The key to decision-making is the accurate and competent identification of the organism of concern. The need to use diagnostic skills is sporadic and unpredictable. However, there are data that can be used to focus some of the developmental resources; for example, interception data from material inspected at airports and seaports indicate that there are seven orders of insects

(Coleoptera, Psocoptera, Thysanoptera, Hymenoptera, Lepidoptera, Hemiptera and Diptera) that are most commonly encountered at international borders. Currently, there is a critical need to enhance diagnostic capacity worldwide; in all countries, skilled diagnosticians are not being replaced as existing personnel retire. It takes many years of experience to develop competent high-level diagnostic skills and a significant amount of diagnostic work is currently being carried out by specialists who are either retired or close to retirement.

Surveillance and monitoring are other significant components of managing incursions. Pests that are intercepted by quarantine provide general monitoring information on the organisms that are moving across international borders, and hence are useful to determine if there is a change in the health status of any particular commodity. Surveillance and monitoring can be done as a continuous process or for a specific incident (such as an incursion). However, it is difficult to decide how to undertake long-term surveillance, as general surveillance tools, when run continuously, generate enormous amounts of data that are almost impossible to process. Surveillance can include gathering information from, for example, people who grow food in village gardens and thus will be able to report on changes that have occurred in their area. If this information is consistently compiled it should provide an indication of what is happening.

After an incursion has occurred, focused surveillance and monitoring within the affected and surrounding areas are part of the management procedure, as are treatments to control the spread of a pest or to attempt its eradication. Treatments options include inspection of goods, use of chemicals—natural or synthetic (sprays, baits, pheromones), destruction of hosts in a defined area and biological control. Biological control, when established, can be very widely beneficial, but it needs to be undertaken with consideration of potential off-target impacts. The benefits of established biological control include the following: it is self-perpetuating; the costs and technology required for its maintenance are typically low; it provides continuous control, with populations of the control agents fluctuating as the pest populations fluctuate; it frequently yields a wide-area benefit; it is often non-commercial in application. Nevertheless, before committing to a biological control program some of the issues that need to be considered are: the cost it will take to establish the agent; the capacity to undertake research to determine what will the off-target impacts

(including on indigenous flora and fauna) and if they are acceptable; and the capacity to contain the organisms under quarantine conditions before release. One needs to get the right agent for the right place, as the consequences can be very significant if the wrong agent is released.

## Conclusion

Plant protection is not the responsibility of regulators alone. It needs the broad engagement of the community for it to be successful. Enlisting the help of villagers and other communities at a very simple level can have a big impact on how people move material. Regulators and researchers need to adhere to simple plant-health processes themselves because if they do not bother to

undertake basic plant health practices then there is little hope that others in the community will follow the requirements the regulators try to impose. In the end, plant protection is everybody's business. The production of food affects everybody, from regulators and researchers, to people who grow food.

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# **Crop pests and management**

# Incidence of pathogens naturally associated with taro beetles in sugarcane fields in Papua New Guinea

A.N. Simbiken<sup>1</sup>

## Abstract

*Bacillus popilliae* Dutky type A1 and *Vavraia* spp. were found in *Papuana woodlarkiana* (Coleoptera, Scarabaeidae) collected from sugarcane fields of Ramu Sugar Estate Ltd. Between May 1996 and March 1997, 5003 *P. woodlarkiana* insects, mainly third-instar (2816) and second-instar (1717) larvae, were collected. Of the total collection, *B. popilliae* was the most common pathogen found (69%), particularly in third instars (11%), compared to second (4%) and first instars (1%). *Vavraia* was less widespread and almost exclusively found in third instars (3%) and most of these infected larvae also carried *B. popilliae* (2%). Five fields had a high population density of 20–30 insects/m<sup>2</sup> and three a low density (4–6/m<sup>2</sup>). *B. popilliae* infections were significantly positively correlated ( $r = 0.91$ ) to mortality and negatively correlated ( $r = 0.89$ ) with the density. At low population densities, a high and stable percentage of the insects died (43–55%) and were infected with *B. popilliae* (30–38%). In the locations with high insect density, 0.7–4.2% *B. popilliae* infection was observed. The data indicated that in locations with low population density a (temporary) stable situation had been reached with constant high mortality due to *B. popilliae*. This could be a major factor in the control of *P. woodlarkiana* in sugarcane fields of Ramu Sugar.

## Introduction

Taro beetles (*Papuana* spp., Coleoptera; Scarabaeidae) are major pests of taro (*Colocasia esculenta* Schott.) and other aroids and banana crops in Fiji, Kiribati, Papua New Guinea (PNG), Solomon Islands and Vanuatu (Waterhouse 1997). These crops are major staple foods in the South Pacific. Eight species of the long-lived adult *Papuana* tunnel into corms and can cause complete crop loss. Larvae cause no damage as they are found in a range of soil habitats usually outside of the gardens (Waterhouse and Norris 1986; Thistleton et al. 1995).

There are no environmentally acceptable insecticides effective against taro beetles and until recently the fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hypomycetes) was the

only entomopathogenic microbe associated with *Papuana* spp. (Shaw 1984; Prior 1986).

The regional European Union/Secretariat of the Pacific Community (EU/SPC) Taro Beetle Project was set up to find environmentally acceptable measures for taro beetle control with emphasis on biological control and particularly entomopathogens.

Several pathogens have been found and tested (Theunis et al. 1996) including nematodes (Theunis 1998), isolates of *Metarhizium anisopliae* (Theunis and Aloali'i 1998) and *Bacillus popilliae* (Bacillaceae) (Theunis and Aloali'i 1999). The latter two have played a major role in biological control of scarabs all over the world. *Bacillus popilliae* has been reported from at least 29 scarabs, mostly Melolonthinae and Rutelinae, and is known for its species specificity. The finding of a *B. popilliae* isolate naturally associated with taro beetles was therefore very important in the search for potential biological control agents.

This study reports the incidence of a *B. popilliae* type A1 and of a protozoan *Vavraia* sp. in *Papuana*

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*woodlarkiana* Montrouzier in sugarcane fields of Ramu Sugar Estate, PNG.

## Materials and methods

Fields at Ramu Sugar Estate (5°50'S, 145°55'E), Morobe Province, PNG were sampled to collect taro beetle larvae for laboratory cultures between May 1996 and March 1997. One or more trips was made to Ramu Sugar Estate every month except during December 1996 and January 1997. Fields examined for larvae usually had ratoon crops and trash present. Various life-stages of *P. woodlarkiana* were collected by digging 5–10 cm below the soil surface under the roots of sugarcane plants and under sugarcane trash. A total of 34 samples was collected in 9 sub-fields of 8 major fields, which were separated by river or road. The number of samples in each field was unequal, and in some fields several visits were made in a month.

At sampling, dead or diseased insects were placed in glass tubes (25 × 100 mm). Live insects were placed in plastic boxes (25 × 25 × 10 cm) with soil collected about 12–15 m from the sample site; adults and immature insect were kept separately. Each box contained 5–10 insects of similar sizes (or instar stage). Samples were transported to the laboratory and live insects were then transferred into sterilised rearing medium mixture of sawdust/cow dung. Pieces of taro corms were added to boxes containing adult beetles. Insects were checked for infection from pathogens twice weekly for 2 weeks. Haemolymph, cadaver and mid gut samples from killed insects were prepared on slides and examined for pathogens under a bright field and light microscope at 400–1000× magnification.

Data for major fields in the same location (separated by river or road) were combined and tested for correlation between the proportion of insects affected by pathogens and total number of insects (both dead and alive).

## Results

From May 1996 to March 1997, 5098 *P. woodlarkiana* insects of varying life stages were collected from a total area of 241 m<sup>2</sup> (Table 1). Third-instar larvae represented 55.2% of the total collection. *B. popilliae* was the most common pathogen and was found in 68.8% of the total collection infected with pathogens. *Bacillus popilliae* was found in 11% of third-instar larvae, 4% of second instars, 1% of first instars and 1% of adults. Mixed infections of *B. popilliae* and the protozoan *Vavraia* sp. were observed in only 2% of third-instar larvae. *Metarhizium anisopliae* accounted for 3.2% of the total collection and was generally low in larval stages (3%) but more common in adults (12%). *Vavraia* tended to infect only the larval stages (1% of total collection).

High population densities (20–30 insects/m<sup>2</sup>) were recorded in five locations — ES, CS, FN, AN and AS — while relatively low population densities (<10 insects/m<sup>2</sup>) were recorded at locations BS, JS and DS (Figure 1). *Bacillus popilliae* infections were positively correlated with mortality ( $r = 0.91$ ,  $p < 0.05$ ) (Figure 1) and negatively correlated with population density ( $r = -0.89$ ). At low population densities, a high and stable percentage (43–55%) of the insects died and these were infected with *B. popilliae* (30–38%) (Figure 1). In locations with low insect density, there was a low and stable number of dead (1.8–3 insects) and infected insects with *B. popilliae* (1.5–1.8

**Table 1.** Numbers of insects collected in the field and laboratory and percentage infected with fungal, bacterial or protozoan pathogens

	Number of insects collected				
	Adults	Third instar	Second instar	First instar	Total
Total insects collected	86	2816	1717	470	5098
Insect density (no./ m <sup>2</sup> )	0.4	12.0	7.0	2.0	21.4
Infected with pathogens (%)	12.8	17.4	7.4	1.1	12.4
<i>Metarhizium anisopliae</i> infections(%)	11.6	3.3	3.3	0	3.2
<i>Bacillus popilliae</i> infections (%) <sup>c</sup>	1.1	11.2	4.0	1.1	7.6
<i>B. popilliae</i> + <i>Vavraia</i> sp. infections (%)	0	1.6	0	0	0.9
<i>Vavraia</i> sp. infections (%)	0	1.2	0.1	0	0.7
Mortality from unknown causes (%)	13.9	14.6	13.8	27.5	15.5

insects), compared with the densely populated areas which had a variable number of dead (3.9–9.5) and infected insects with *B. popilliae* (0.7–4.2) (Figure 1).

## Discussion

In September 1994, milky larvae were found in a collection from Ramu Sugar Estate (Theunis et al. 1997). The causal agent was *B. popilliae* type A1 (Milner et al. 1980; Milner 1981).

The results indicate that type A1 is quite virulent to *P. woodlarkiana*. The infection percentages recorded are comparable with those found by Boucias et al. (1986) — 16–30% in *Ligyris subtropicus*, depending on the season.

*Vavraia* sp. was found in a few *P. woodlarkiana* third-instar larva collected at Ramu Sugar Estate in 1996 (Theunis and Aloali'i 1999). This is the first record of this pathogen in *Papuana* species.

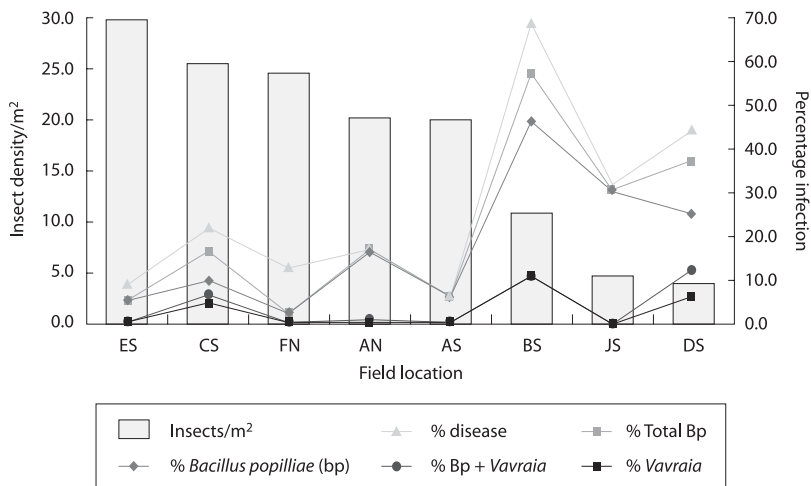
At the end of 1996 and in early 1997, more larvae with *Vavraia* sp. were found, often as mixtures with *B. popilliae* (Table 1). Highly infected larvae had a characteristic mottled appearance (clusters of spores), especially near the posterior end. Most of the fat body had disappeared and spores were found throughout the body.

*Metarhizium anisopliae* have been reported previously from *P. woodlarkiana* (Shaw 1984; Prior 1986; Theunis et al. 1996; Theunis and Aloali'i 1999). The discovery of *B. popilliae* and *Vavraia* sp. in *Papuana* species, especially the natural association of *B. popilliae*, known for its specificity to *P. woodlarkiana*, was of major importance in the search for a possible biocontrol agent for the taro beetle.

The potential of *Vavraia* sp. as a biological control agent is not known. From our field data we suspect that *Vavraia* sp. infections were facilitated by the presence of *B. popilliae* and that *Vavraia* sp. by itself would infect only a small percentage of the population. The effect of combined feeding/application of the two pathogens is yet to be elucidated.

Under laboratory and field conditions in PNG, *B. popilliae* infected *P. woodlarkiana* second and third-larval instars and adults (Simbiken, unpublished data) as daily temperatures (15–30°C) are relatively high throughout the year. Application of spores to the larval habitats, however, may be difficult as the beetles prefer certain breeding sites (under logs, grasses etc.) (Thistleton et al. 1995) and the areas to be treated are vast and often inaccessible.

*Bacillus popilliae* can persist. In Kiribati, for example, under very low larval densities infected



**Figure 1.** Density and percentage of insects infected solely with *Bacillus popilliae*, *Vavraia* spp. or mixtures of *B. popilliae* and *Vavraia* spp. at Ramu Sugar Estate, Papua New Guinea, A–J = field locations, N, S = northerly or southerly direction).

third instars were found one year after inoculation of a banana pit with  $2 \times 10^{10}$  *B. popilliae* spores/m<sup>2</sup> (Theunis and Teuriaria 1998). This persistence can result in high concentrations in the soil. Application of inoculum to known or artificial breeding sites could therefore control part of the taro beetle population.

The results indicated that in locations with low population density a (temporarily) steady state had been reached with constant high mortality caused by *B. popilliae*. This suggests the insects are living in an environment saturated with the pathogen. The mortality/infection rates at the locations with high density appeared to vary according to the point of interaction between beetle and pathogen populations.

*Bacillus popilliae* infection, which accounted for about 80% of variability in mortality, is therefore a potential mortality factor for taro beetles in sugarcane plantations.

## Acknowledgment

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# Pest species of *Oribius* Marshall (Coleoptera: Curculionidae: Otorhynchinae) and their host plants in PNG

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## *Abstract*

The weevil genus *Oribius* is a very diverse group with more species remaining to be confirmed to the genus. It is widely distributed throughout the island of New Guinea, the Torres Strait islands and the northern tip of Queensland, Australia. In Papua New Guinea (PNG), the genus is widely distributed throughout the mainland, with most species showing localised distribution patterns. *Oribius* weevils are polyphagous feeders of a wide range of plants, and some species are serious pests of horticultural crops in the areas where they occur. This study was conducted to identify the pest species of *Oribius* in PNG. The pest species survey was carried out in the highlands, and in the Morobe and the Central provinces. Seven species were identified as pests of a wide range of crops in the areas where the survey was conducted. The study establishes a preliminary listing of pest species of *Oribius* present in PNG and their host plant records.

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# Incidence and distribution of *Bactrocera papayae* Drew and Hancock (Diptera: Tephritidae) in coffee plantations in the Eastern Highlands and Madang provinces of Papua New Guinea

A.N. Simbiken<sup>1</sup>

## Abstract

The incidence and distribution of *Bactrocera papayae* Drew and Hancock (Diptera: Tephritidae) in coffee plantations at Aiyura, Eastern Highlands Province and several sites in Madang Province of Papua New Guinea (PNG) were monitored between 1998 and 2003 using methyl-eugenol lures and host-collection surveys. Field samples of *Coffea arabica*, *C. canephora*, *Carica papaya* and *Psidium guajava* were incubated in the laboratory and assessed for emergence of fruit flies. No adult flies were detected from any of the host fruits, but *Drosophila* sp. was reared from ripe and overripe *C. arabica*. Relatively low breeding populations of *B. curvifera*, *B. umbrosa*, *B. fulvicauda*, *B. musae* and *B. papayae* were recorded in Madang Province between July and September 2002 using the methyl-eugenol lures. Although *B. papayae* was not detected breeding on coffee in this study, its potential presence in the Eastern Highlands and Madang provinces poses a concern to the PNG coffee industry.

## Introduction

Papaya fruit fly, *Bactrocera papayae* Drew and Hancock (Diptera: Tephritidae), is a polyphagous pest species and the most virulent of all tropical fruit-fly species. It was first recorded in Papua New Guinea (PNG) in 1992 (Tenakanai 1997). It has spread rapidly and is now found in the Madang, East Sepik, Gulf, Oro and the Highlands provinces (Leblanc et al. 2001). *Bactrocera papayae* has been recorded on 209 plant species, including coffee, in the Asia-Pacific region (Sar et al. 2001). Although it has been recorded in the PNG Highland regions where Arabica coffee (*Coffea arabica* L.) predominates, it was only recently (April 2002) detected on Robusta coffee

(*C. canephora* Pierre) at Omuru, Madang (S. Sar, pers. comm.). However, there are currently insufficient data available to establish a comprehensive host list and infestation level for PNG. Coffee has been recorded as a host plant for *B. papayae* in Australia, Malaysia and Indonesia (Drew 1997; Fay et al. 1997). *Ceratitidis capitata* (Wiedemann) and *B. dorsalis* (Hendel), tephritid species related to *B. papayae*, were recorded as pests of coffee in Hawaii (Vargas et al. 1995). In Sao Paulo, Brazil, species recorded on seven varieties of coffee were *C. capitata* (75.6%), *Anastrepha* spp. (*fraterculus* group) (7.4%) and 17.0% Lonchaeidae family species (17.0%) (Raga et al. 1996). In the Venezuelan Andes, the fruit fly, *Anastrepha fraterculus* Wied., has been recognised as a pest of peach (*Prunus persicae* L.), Japanese nispero (*Eriobotrya japonica* L.), orange (*Citrus* sp.), coffee (*C. arabica* L.), cas (*Psidium* sp.) and blackberry

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(*Rubus glaucus* Benth) (Briceno 1996) crops in the warmer belt between 800 and 2000 m.

The biology of *B. papayae* on coffee is not fully known. It may oviposit on green cherry until ripening, after which larvae hatch and penetrate into the soft coffee pulp, as has been observed on coffee infected with *C. capitata* (Abasa 1972). At least one larva may occur per cherry, depending on cherry size. Tropical fruit fly development time from larvae to adult is 14–18 days, as observed in carambola (*Averrhoa carambola* L.), cashew (*Anacardium occidentale* L.), pawpaw (*Carica papaya* L.), pomelo (*Citrus maxima* Merr.), mango (*Mangifera indica* L.) and guava (*Psidium guajava* L.) (Leblanc et al. 1998, 2001). Larvae complete three morphological changes before pupating in the soil. Damage to coffee pulp and green bean is uncommon, though *C. capitata* causes damage to bean tissue and the bean embryo, resulting in defective coffee liquor flavours (Gibson 1970). Losses on coffee are commonly associated with premature cherry fall due to feeding by larvae beneath the skin.

Nevertheless, the hypothesis that coffee is an alternative host to one or several tropical species of fruit fly has not been fully tested. The host list of *C. papaya* is incomplete, although it is virulent and destructive of all tropical species (Leblanc et al. 2001). Development of *B. papayae* on coffee under favourable ecological conditions would have potential economic and social impact on the PNG coffee industry. Premature cherry fall and liquor taint could affect export quality and volume.

The seasonal distribution and abundance of papaya fruit fly within coffee plantations in PNG between coffee seasons is not fully known. This paper gives the results of field monitoring of fruit flies in the Eastern Highlands and Madang provinces between 1998 and 2003.

## Methods

Two studies were conducted to monitor the incidence and spread of papaya fruit fly at Aiyura and Madang between 1998 and 2003. The first study, conducted in 1998, included three separate observations and sampling of coffee cherries at Aiyura, Eastern Highlands Province at the Coffee Research Institute plantation and at the factory gate. The second study was conducted at Aiyura and Omuru, Aironis, Dylup and Siar in Madang between July 2002 and April 2003.

## 1998 study

Cherry samples were randomly collected on 9 July 1998 from 17 coffee plots at the Coffee Research Institute farm, Aiyura, Eastern Highlands Province. Records from each plot were pooled into three main plots, 252C+, A6+ and A7+, based on the proximity and/or location of the plots. Cherry samples were pooled and sorted into three categories: ripe, overripe and broken endosperm. Green berries were also collected as a control. Two hundred cherries of each category from each of the three main plots were placed in plastic lunch boxes 18 × 15 × 10 cm and covered with screen netting. Boxes were incubated at room temperature for 21 days. Emerging adult flies were collected with a pooter and examined using a dissecting microscope.

In a separate study, a further sample of 30 cherries selected from each category as well as underripe and fallen cherries were dissected longitudinally with a scalpel blade. The cherries were examined under a magnifying glass for the presence of fruit fly larvae.

Data on quality (underripe, fully ripe, overripe and light (weight)) of cherries harvested by farmhands were obtained from the factory gate.

## 2002–2003 study

### Cherry collection

Cherry samples, categorised as underripe, ripe or overripe, were collected between July 2002 and April 2003. Fruit fly samples in and around Omuru, Madang Province were collected in modified Steiner traps using a methyl eugenol lure. Each lure consisted of 3 mL of a mixture containing 80% methyl eugenol as attractant and 20% malathion (50% active ingredient) insecticide.

*Coffea canephora* samples at Omuru were collected from four coffee plots between July 2002 and April 2003. In each plot, 20 trees were randomly selected. Two laterals midway between the top and lower branches were selected on each tree. Ripe and underripe cherries were collected from these branches. Three sampling trips were made to Omuru, Dylup, Siar and Aironis sites during the study. Other potential host fruits of *C. papaya* and *P. guajava* located around the perimeter of coffee plots were also sampled accordingly.

The coffee cherries and host fruits were handled and incubated as described by Leblanc et al. (2001). Within 24 hours of collection, cherries and fruits were transported to a research centre at Lae, Morobe

Province and placed on wire mesh in boxes containing untreated fine sawdust. Emerging larvae fell onto and burrowed into the sawdust, prior to pupation. Sawdust was sieved after 21 days to check for fruit fly puparia. Adult flies were killed by incubating at 4°C for 60 minutes.

*Coffea arabica* samples were collected from six blocks at Aiyura in August 2002. Coffee berries in three categories of maturity were collected: ripe, overripe fallen and mature green. Coffee from six Arabica varieties—Arusha, Blue Mountain, Bourbon, Mundo Nova, Catimor and Caturra—were pooled into each category before incubation. Rearing and observation was carried out at the fruit fly laboratory, Sir Alkan Tololo Research Station (formerly Bubia Agricultural Research Station), Lae.

#### Lure traps

Three traps containing methyl eugenol lure were placed 300 m apart in the centre of three Robusta plots at Omuru, denoted as BRP 211, BRP 215 and BRP 214. A single lure trap was installed at each of two villages—Dylup and Aironis—which were 3 km apart. Flies were collected from the traps fortnightly, placed in paper envelopes and stored at 4°C before being sent to the National Agricultural Quarantine and Inspection Authority for identification to species level.

## Results

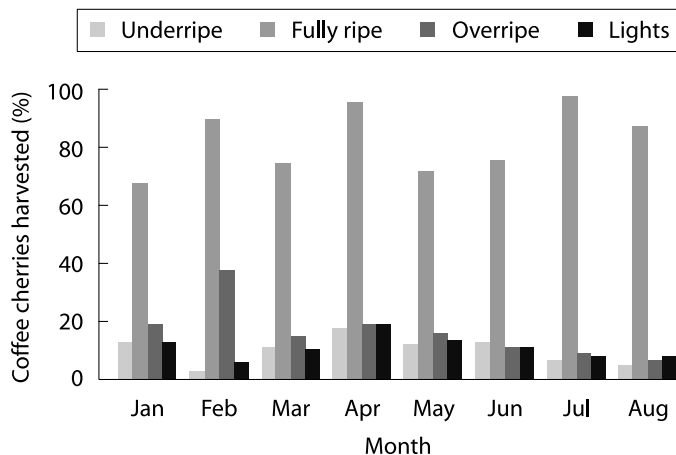
### Fruit fly surveillance in Aiyura during 1998

Fruit flies that emerged from the ripe, overripe and broken endosperm were all *Drosophila* sp. (*Drosophilidae*). *Drosophila* were recorded from only the 252C+ and A6+ coffee blocks. Populations were relatively low, with only two and three insects recovered from the overripe and broken endosperm categories, respectively.

Throughout the 1998 coffee season, more than 80% of total cherries harvested between January and August were fully ripe (Figure 1). These were of good quality and normally contributed to high quality green bean and taste. About 20% of cherries were from the underripe, overripe and light categories. Broken endosperm was typical in the field samples of overripe cherries.

### Fruit fly surveillance in 2002 and 2003

No fruit flies emerged from surveyed Arabica and Robusta coffee, cherries, pawpaw and guava (Table 1). Although 32 fly puparia were obtained from guava from Omuru, there were no adult emergences from them.



**Figure 1.** Quality of coffee cherries harvested from the Coffee Research Station farm at Aiyura, Eastern Highlands Province, Papua New Guinea between January and August 1998

There were low breeding populations of *B. curvifera*, *B. umbrosa*, *B. fulvicauda*, *B. musae*, and *B. papayae*. These were captured by methyl eugenol lure traps in Omuru and Aironis (Table 2). *Bactrocera musae* was most prevalent in August ( $58.4 \pm 30.1$ ) and September ( $22.8 \pm 19.4$ ). Significant numbers of *B. curvifera* ( $25.4 \pm 8.6$ ) and *B. umbrosa* ( $39.6 \pm 29.2$ ) were recorded in August. The number of *B. papaya* remained constant in August ( $26.2 \pm 11.7$ ) and September ( $22.8 \pm 19.6$ ). *Bactrocera curvifera* was the predominant fruit fly recorded at Aironis.

## Discussion

The presence of *B. fulvicauda* reflects the closeness of trapping sites to major forests. This fly usually breeds on forest fruits (Drew 1989) and causes less

significant damage than *B. papayae*. *Bactrocera musae*, *B. umbrosa* and *B. papayae*, which are major pest species in PNG (Sar et al. 2001).

The low numbers of adult fruit flies in August and September reflect variable population levels each year depending on fruiting seasons and climate. At Omuru, coffee maturity and harvesting peaks between April and June, and relatively low cherry numbers are found on trees after July. The guava and pawpaw fruit seasons in Omuru follow a similar fruiting phenology to Robusta coffee. This is dependent on rainfall patterns in the area, with the onset of rain in October through to May, and relatively low rainfall between June and September. Vueti et al. (1997) found that the temporal abundance of fruit flies was correlated to host fruiting season at sites in Fiji, Tonga and the Cook Islands, where larger numbers of fruit flies detected coincided with

**Table 1.** *Bactrocera papayae* (papaya fruit fly) host survey in Aiyura, Eastern Highlands Province and Omuru, Siar, Dylup and Aironis in Madang Province, Papua New Guinea, July 2002–April 2003

Location	Date collected	Plant species	Stage of maturity	Berry weight (g)	Fruit number	No. of fruit fly puparia	<i>Bactrocera papayae</i> detected
Aiyura	13 Aug	Arabica coffee	Ripe	1348	872	0	0
			Ripe fallen	794	630	0	0
			Mature green	1861	1591	0	0
Wau	22 Aug	Robusta coffee	Ripe	1820	735	0	0
			Mature green	1020	587	0	0
Omuru	22 Aug	"	Ripe	3270	3329	0	0
			Mature green	2050	2635	0	0
Omuru	23 Sept	"	Ripe	2500	3325	0	0
			Mature green	2030	2891	0	0
Dylup	23 Sept	"	Ripe	2100	2813	0	0
			Mature green	700	890	0	0
Siar	23 Sept	"	Ripe	600	382	0	0
			Mature green	300	273	0	0
Aironis	23 Sept	"	Ripe	500	386	0	0
			Mature green	350	234	0	0
Omuru	23 Apr	"	Ripe	6400	4535	0	0
			Mature green	7100	4550	0	0
Dylup	23 Apr	"	Ripe	1400	1000	0	0
			Mature green	1450	1300	0	0
Omuru	23 Sept	Guava	Mature green	400	3	0	0
	23 Apr	"	Ripe	1500	20	0	0
	23 Sept	Pawpaw	Ripe	1750	4	0	0
	23 Apr	"	Ripe	3000	4	0	0
Aironis	23 Sept	Pawpaw	Ripe	1990	3	0	0

the fruiting season. The peak abundance of *B. frauenfeldi* (Schiner) and *B. cucurbitae* Coquillett on *A. carambola* in coastal areas of PNG followed a similar trend, coinciding with the rainfall season between October and February (Sar et al. 2001). There was therefore presumably very low breeding activity after the major coffee season at Omuru. The early detection of papaya fruit fly in April 2002 (S. Sar, pers. comm.) was in opportunistic coffee samples at the beginning of the Robusta fruiting season. In April 2003, there were no observations of emergence of fruit flies from Robusta samples. The low number of adult fruit flies detected in lure traps in August and September suggests that there was generally an off-season for all host fruits in the area.

*Bactrocera papayae* is capable of using coffee as a breeding site. Its presence in a wide range of fruits and flowers is testimony to its extreme virulence compared with other species in the family Tephritidae (Fay et al. 1997; Leblanc et al. 2001). Although it has caused enormous economic and social impacts in Australia since 1993 (Drew 1997; Allwood and Leblanc 1997), it is too early to determine whether it would have the same impact on coffee production in PNG. However, PNG's inability to export fruits and vegetables because of fruit flies is a contentious issue, and quarantine problems have affected the economy of the country. *Ceratitidis capitata* (Mediterranean fruit fly) causes significant loss to production and quality of coffee (Gibson 1970). Similar losses in coffee in PNG could be attributed to *B. papayae*, but its impact on coffee has not been fully elucidated. Failure to detect fruit flies in the current study indicates that

*B. papayae* and other species are yet to establish themselves on coffee. The presence of *C. capitata* in Hawaii and New Zealand is of concern to the coffee industries in those countries.

Overripe cherries will fall if they are not harvested quickly, and were usually the source of secondary infection and damage.

Methyl eugenol and cuelure traps have the potential to lure several species of fruit flies from a distance of 500 m. Although cuelure was not used in the study, the insects caught in the methyl-eugenol traps were presumably from outside of coffee plants, as indicated by the presence of *B. fulvicauda*, a forest-dwelling species. The presence of *B. musae*, *B. umbrosa* and *B. papayae* suggests that if cuelure were used, trapping of *B. frauenfeldi*, *B. bryoniae*, *B. moluccensis*, *B. neohumilis* and *B. trivialis* pest species would be possible. These pest species are polyphagous and cause moderate to very high damage to primary hosts and several secondary hosts (Sar et al. 2001; Leblanc et al. 2001). Although they may not use some parts of coffee, for example, flowers and cherries, for breeding, there are few quantitative data to elucidate their potential impact on coffee. Coffee is an introduced crop and much of its interaction with the environment and the flora and fauna in the country is unknown. In its natural environment, coffee evolved with forest trees. The presence of other fruit fly species in the coffee ecosystem is of considerable concern to the coffee industry. A study on the biology, temporal and spatial abundance of pestiferous fruit flies in the coffee ecosystem is required to validate detection and losses on coffee.

**Table 2.** Numbers of *Bactrocera* fruit fly species collected using methyl eugenol lure traps at Omuru and Aironis, Madang Province, Papua New Guinea, July–September 2002

Date	Location	<i>Bactrocera curvifera</i>	<i>B. umbrosa</i>	<i>B. fulvicauda</i>	<i>B. musae</i>	<i>B. papayae</i>
15 Aug	Omuru resident	15	15	10	41	8 (8.9%)
15 Aug	BRP 215	28	42	8	46	29 (19.2%)
15 Aug	BRP 214	19	25	6	45	39 (29.1%)
15 Aug	BRP 215	28	31	8	48	32 (21.8%)
15 Aug	BRP 211	37	85	13	112	23 (8.5%)
30 Aug	Aironis	168	54	21	30	28 (9.3%)
4 Sept	BRP 214	3	2	3	17	6 (19.4%)
4 Sept	BRP 215	2	9	3	25	2 (4.9%)
4 Sept	Omuru resident	5	1	0	1	3 (30%)
4 Sept	BRP 211	6	15	1	48	8 (10.3%)

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# The use of sticky traps to study seasonal dispersal activity of the sweet potato weevil, *Cylas formicarius* (Fabricius), in Papua New Guinea

S.A. Sar<sup>1</sup>

## Abstract

The ecology and flight behaviour of the sweet potato weevil, *Cylas formicarius*, in Papua New Guinea was investigated. Flight activity monitored using sticky traps was strongly associated with prevailing weather conditions. Higher numbers of adults were trapped during the wet season. Adults showed reduced flight activity during the dry season. A high proportion of adults trapped came from a northwesterly direction. A high male-to-female ratio was observed from the trap data.

## Introduction

The sweet potato weevil *Cylas formicarius* (Fabricius) (Coleoptera: Curculionidae) is a cosmopolitan pest and is the most important constraint on sweet potato production. The seasonal occurrence of outbreaks of the sweet potato weevil has long been recognised (Floyd 1942; Cockerham et al. 1954; Smee 1965; Kimber 1972, Sutherland 1986). In Papua New Guinea (PNG) the intensity of infestation varies with the seasons. The seasonality of this pest has been reported only through accounts of weevil damage at harvest. There are very few studies on the seasonality and dispersal of this pest by flight. Although the weevil has been reported to fly (Deen 1940; Eddy 1940; Diaz Sanchez 1980), dispersal was thought to be through infested planting material (Trehan and Bagal 1957; O'Connor 1966; Jayaramaiah 1975). Other aspects of dispersal of the sweet potato weevil are poorly understood. This study investigated the role of flight in weevil dispersal.

## Methods

The experimental work was conducted at Laloki Research Station (147°14'E., 9°23'S., altitude 30 m), approximately 25 km from Port Moresby.

The sites consisted of grass regrowth after a fallow period of 2–3 years following previous trials, growing on a well-drained alluvial clay loam soil with a pH of 6.0.

## Experimental design

Entomological investigations were carried out mainly in the sweet potato trial areas in conjunction with agronomic trials at Laloki Research Station. Six sweet potato fields were used during 1983 and 1984. The first five trials were designed as lattice experiments, with a total of 49 varieties as treatments, replicated twice. Each plot contained three ridges 1.4 m apart and 4.5 m long. Sweet potato tips 0.4 m long were planted in each planting position 0.3 m apart along the ridges. The sixth experiment was designed as a factorial experiment — three varieties by three cultural methods by two harvest dates. Sweet potato tips were planted 0.2 m apart on ridges 2.2 m long

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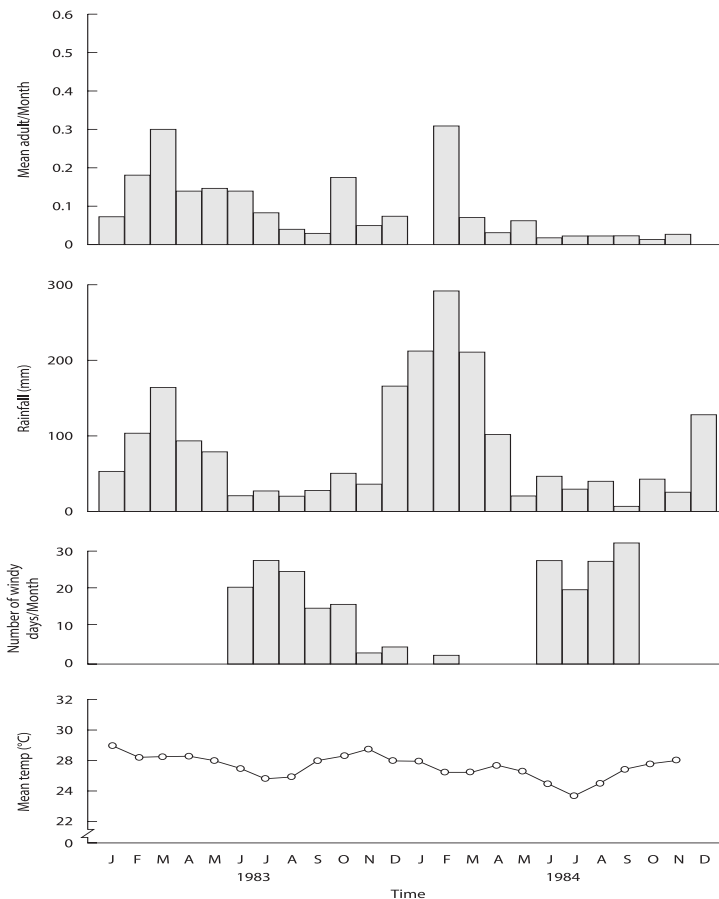


and 1.1 m apart. Natural rainfall was supplemented with water pumped from Laloki River. The plots were hand-weeded until the ground was completely covered by vines. There was no separation between plots within the blocks since vines tended to overrun plot boundaries.

### Sticky traps

The traps consisted of empty vegetable or fruit cans. One end of the can was removed, leaving a cylinder 23 cm long by 7.5 cm in diameter, open at one end. Forty-nine 1.2 m long wooden posts were used to support the cans, with one post being positioned in each plot. One end of the can was inserted over and nailed onto a post. The end of the post was buried to

a depth of 20 cm. On the top end of each trap, marks were made to delimit surfaces corresponding to the four quadrants of the compass, before Tanglefoot® ‘stickum’ (The Tanglefoot Company, Grand Rapids, Michigan, USA) was applied. The traps were set up soon after planting to get a series of zero counts before weevils first appeared in the crop. Traps were examined weekly or fortnightly during the growing season and all weevils removed were sexed. Dirt and other insects caught on the stickum were removed while counting weevils. The stickum was also replaced periodically. Counts of adults on sticky traps were made weekly for 86 weeks on the six crops. The seasonal abundance of the weevils was estimated from the weekly sticky trap data.



**Figure 1.** Seasonal distribution (mean/month, 1983–84) of adult *Cylas formicarius* collected on sticky traps at Laloki Research Station, Papua New Guinea and concurrent weather variables

**Table 1.** The numbers of adult *Cyclas formicarius* weevils collected from 49 sticky traps showing distribution at each direction and sex in 1983 (excluding dates with no captures)

Date of sampling	Number of adults	Direction				Sex	
		N	S	E	W	male	female
17 Jan	5	3	0	2	0	–	–
21 Jan	1	1	0	0	0	–	–
31 Jan	3	2	1	0	0	–	–
14 Feb	14	5	0	1	8	11	3
21 Feb	8	1	0	6	1	8	0
28 Feb	6	4	0	0	2	5	1
09 Mar	22	7	9	4	0	22	0
17 Mar	21	12	3	3	0	20	1
21 Mar	9	5	1	3	0	9	0
28 Mar	5	2	0	0	3	5	0
05 Apr	14	10	0	3	1	14	0
11 Apr	3	2	0	1	0	3	0
18 Apr	6	5	0	0	1	6	0
25 Apr	3	1	1	0	1	3	0
02 May	9	1	1	2	5	8	1
09 May	8	3	2	2	1	7	1
16 May	3	0	3	0	0	3	0
23 May	5	4	0	0	1	4	1
30 May	8	3	3	0	2	8	0
06 Jun	6	1	0	2	3	4	2
13 Jun	10	2	3	4	1	7	3
27 Jun	3	2	0	1	0	0	0
04 Jul	9	3	0	0	6	9	0
18 Jul	5	1	0	0	4	5	0
01 Aug	1	1	0	0	0	1	0
08 Aug	2	2	0	0	0	2	0
15 Aug	1	0	0	1	0	1	0
29 Aug	1	0	0	0	1	1	0
10 Oct	4	0	0	4	0	4	0
17 Oct	17	9	3	5	0	11	0
04 Oct	4	0	2	2	0	4	0
31 Oct	7	1	5	0	1	7	0
07 Nov	1	0	0	1	0	1	1
14 Nov	3	1	0	1	1	2	1
21 Nov	4	2	1	0	1	3	0
28 Nov	5	0	1	0	4	5	3
05 Dec	3	1		0	1	0	2
12 Dec	3	1	0		2	0	
Total	242	98	39	48	51	203	20

**Table 2.** Correlation coefficients between numbers of adult *Cylas formicarius* weevils caught in sticky traps and rain, humidity, temperature and wind in 1983 and 1984, showing standard errors and significance levels for each variable

Weather variable	No. of adults trapped	Correlation coefficient ( <i>r</i> )	Standard error	Level of significance ( <i>p</i> )
Rain	81	0.699	0.179	< 0.001
Humidity	81	0.069	0.113	> 0.1
Temperature	81	0.370	0.655	< 0.001
Wind	81	-0.281	0.192	< 0.05

Weather data were obtained from the National Weather Office at Jackson's Airport which is approximately 7 km from the station. Average daily temperature, humidity, rainfall and wind speed and direction were also determined. Data were analysed statistically using the BMDP statistical package (Dixon 1983) and methods from Snedecor and Cochran (1967).

## Results

Seasonal changes in trap catches of adults over the entire trapping period at each site are illustrated in Figure 1.

The number of weevils caught was variable and was relatively consistent between fields and years. The major peaks occurred between February and March, with numbers declining by July.

These maximum peaks appeared suddenly and were of relatively short duration, usually falling off as rapidly and abruptly as they started. On average over the two-year period, 50% of the weevils were caught within 14 days, with 54% in 1983 and 90% in 1984.

No flying adults were detected during the dry season and the numbers trapped remained at extremely low levels throughout the rest of the year.

### Seasonal flight patterns

Catches were compared through a goodness-of-fit test of the weevils caught on the north, south, east and west sectors of the traps. This indicated a significant difference ( $\chi^2 = 26.8$ ,  $p < 0.01$ , 3 d.f.) when considering data from all the fields in 1983 (Table 1). There appears to be a difference in the number of weevils flying up or downwind. Sixty-three per cent of the adults were collected on the northwestern side of the traps, and prevailing winds were from that direction during peak dispersal of this weevil. On about half the census dates, weevils were caught primarily on sides facing prevailing winds. During the period of most adult flights, wind speeds were usually low and temperatures were high relative to those in the dry season.

Catches of adults were examined for correlation with rainfall, temperature, wind and humidity using multiple regressions (Table 2). There were large standard errors for each flight variable analysed, indicating considerable variability among adult counts in the crops.

Over the two years, the numbers of adults caught per week were significantly positively correlated

with prevailing rainfall and temperature, and negatively correlated with the number of windy days during the preceding week (Figure 1 and Table 2).

The seasonal pattern of rainfall was typical for Papuan lowland areas. A relatively dry period with mean monthly rainfall of 80 mm occurred from May to October. By contrast, rainfall averaged 200 mm/month from November to April. Over 90% of the adults collected during the study period were trapped during the 34 weeks associated with higher rainfall. In both years, very few adults were trapped during the 13 weeks that had no rainfall.

## Discussion

The rapid increase or decline in number of flying adults, as determined through the seasonal studies, showed a clear correlation between catches of *C. formicarius* and rainfall. Peak dispersal took place before or soon after periods of heavy rain. The subsequent rapid decline in weevil captures could not be attributed to the population at large, because the weevil population increases during the dry season and is abundant enough that if weevils were flying they would likely be trapped.

During the wet season, most evenings were warm and calm. Temperatures were lower in the dry season because of the strong southeasterly winds that blew well into the night. The warm, calm evenings during the wet season provide optimal conditions for high weevil dispersal, while low temperatures and strong winds in the evenings possibly played an important role in suppressing any flight during the dry season.

There was no relationship between weevil catch and humidity, although broader tolerance has been shown by many insects towards relative humidity in the field (DeLong 1932; Williams 1940; Underhill 1940; Romney 1945).

The results of this study tend to confirm the seasonal differences in timing of sweet potato weevil activity reported by Diaz Sanchez (1980) from Cuba. However, there was a difference in the number of flight peaks. Diaz Sanchez (1980) reported two peaks per year while only one peak in weevil flights was observed at Laloki. The differences in activity between these two sites may be due to differences in rainfall patterns. There was a period of low rainfall for 2 months between heavy rainfalls during the wet seasons of 1977 and 1978 in Cuba while the heaviest rainfall at Laloki fell within a 3-month period with no gaps in between (Figure 1).

Most adults trapped came from northerly and northwesterly directions. In the wet seasons, wind directions are generally from the northwest and weevils may have flown downwind. At lower wind speeds, wind direction is more variable, especially during evenings in the wet season. These conditions are also likely to result in weevils being trapped from all directions.

Although the total number of weevils caught in the traps exhibited an annual fluctuation, there was usually an excess of males over females. There was no change in the proportion of each sex throughout the year. Similar findings were made by Diaz Sanchez (1980) with light traps in Cuba. Weevils emerging in the laboratory from exposed tubers have a sex ratio of 1:1 (Mullen 1981), indicating that the higher frequency of males in the traps was not due to dominance in number of males among emerging weevils. The high ratio of male to female adults trapped may be attributed to males dispersing more freely than females. In the wet season, males take to flight more readily, to search for females, so they may leave sweet potato fields while females remain feeding and laying eggs.

Fluctuations in the number of weevils caught on the traps do not necessarily reflect changes in population numbers. A small catch does not always mean a small population of weevils in a field, only that a few adults were flying. However, it is believed that these data give a good index of the population levels of *Cylas* adults in flight.

The use of sticky traps has demonstrated the flight behaviour of the sweet potato weevil, with peak dispersal activities associated with high rainfall and warm evenings, while dry, windy and cool night-time conditions suppress weevil dispersal. The effect of weather factors associated with weevil dispersal should be taken into account when developing sweet potato weevil management strategies. Further studies to assess strategic interventions using integrated crop management systems through the use of pheromones to monitor populations and to disrupt mating and dispersal, the use of overhead irrigation in the evenings, physical barriers and trap crops for commercial sweet potato production during dry seasons are warranted.

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# Conservation of sugarcane germplasm: survey of Papua New Guinea, Indonesia and northern Australia

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## Abstract

New Guinea is considered as a centre of diversity for the genus *Saccharum* with *S. officinarum* (noble cane), *S. edule* (edible pitpit), *S. robustum* and *S. spontaneum* present in Papua New Guinea (PNG). Both exotic and native pests and diseases may erode this important germplasm for future plant improvement programs locally and worldwide. Identifying these pest and disease problems, and assessing the extent of their distribution in the centre of diversity and their impact on the germplasm, are essential for the development of management strategies to minimise their impact. Surveys were conducted in PNG, northern Australia and eastern Indonesia during 2001–2003. Ramu stunt disease was widespread in PNG, while suspected symptoms of the disease and the insect vectors were observed in Indonesia. Fiji disease was also widespread in PNG, while an unknown sugarcane mosaic was observed in *S. edule* at Keravat. The moth borers, *Chilo* spp. and *Scirpophaga* spp., were observed in both PNG and Indonesia, while *Sesamia griseocens*, a serious borer pest, was found mainly in PNG. Sugarcane smut was not present in PNG. *Eumetopina flavipes*, the leafhopper vector of Ramu stunt disease, was recorded at Bamaga (mainland Australia), signalling the need an eradication program, to prevent the Ramu stunt disease affecting the Australian sugar industry.

## Introduction

The island of New Guinea (including West Papua, Indonesia) and its major offshore islands are considered the centre of diversity for the genus *Saccharum*. The modern sugarcane hybrids were derived through interspecific hybridisation. Many diverse cultivars of cultivated and wild canes are found all over the island. A number of unique insect pests and diseases have been reported in Papua New Guinea (PNG) and these have proved to be major constraints to the country's only sugar industry, at Gusap in Madang Province. Given the strategic location of PNG (New

Guinea island), many of these pests and diseases have the potential to spread to the sugar industry in Queensland, Australia. It is therefore essential that the distribution of these pests be determined so that management strategies can be developed for these potential incursions. Similarly, the spread of potential pests and diseases (e.g. sugarcane smut) eastward from Indonesia can also have economic implications for the PNG sugar industry and the erosion of valuable germplasm.

In the late 1990s, a three-year project was funded by the Australian Centre for International Agricultural Research (ACIAR). The aims of the project were:

- to survey each country to determine the distribution of major pests and diseases in the region

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- to research key aspects of these pests and diseases to ensure there is adequate understanding of control practices
- to train quarantine staff in PNG, Indonesia and Australia to minimise the risk of pest or disease spread
- to extend information about the importance of sugarcane quarantine to the general public in each country.

This paper reports on the distribution of important pests and diseases. The results from the research components for key pests and diseases have been reported by Magarey et al. (2002).

## Materials and methods

### Papua New Guinea

Magarey et al. (2002) reported on the survey of PNG made in May–June 2001. A charter flight was organised and a team of scientists from Indonesia, PNG and Australia spent 14 days inspecting chewing canes (*Saccharum officinarum* L.), pitpit (*S. edule* Hassk.), *S. robustum* L., *S. spontaneum* L. and commercial canes around PNG. New pest and disease records were made, for which some identifications are still being finalised.

### Northern Australia

A 15-day survey was made of the northern Australian coastline from Cairns to Broome between 23 May and 6 June 2002. Two BSES scientists—a pathologist and an entomologist—participated. Australian Quarantine and Inspection Service (AQIS) personnel assisted, especially in the Northern Territory. Nineteen sites/places were visited during that survey. At each stop, local residents assisted BSES scientists in locating sugarcane in gardens or backyards.

#### Sample assay

Collections of sugarcane leaf samples and insects associated with any of the four sugarcane species were made and samples prepared for further identifications. The exact location of specimens collected was determined using GPS instrumentation. Care was taken to avoid quarantine risks associated with transfer of the observed pests or diseases. Leaf samples collected for viral pathogen identification were prepared as detailed by Magarey et al. (2002). Briefly, leaves were cut into small sections (2 × 2

mm) and dried over anhydrous CaCl<sub>2</sub>. The sealed containers with the leaf pieces were then sent to the David North Plant Research Centre (DNPRC) for assay. Leaf samples for fungal pathogen identification were pressed between sheets of newspaper until dry and placed in the herbarium of the Department of Primary Industries and Fisheries at Indooroopilly, Queensland.

### Indonesia

A survey of 15 days duration was made in the islands immediately east of Java, including Bali, Lombok, Sumbawa, Sumba and Flores. A charter flight was engaged in Cairns with both boat and bus transport combining to convey the team of five scientists from Australia, PNG and Indonesia around the islands. There were two BSES staff (entomologist and plant pathologist), two scientists from the Indonesian Sugar Research Institute (entomologist and plant pathologist) and one researcher from PNG (entomologist) in this survey.

#### Sample assays

Sample preparation was similar to that undertaken in the northern Australia survey except leaf pieces were not cut as small (around 6 × 1 cm) before drying over anhydrous CaCl<sub>2</sub>. Appropriate quarantine treatments were applied on entry to Australia. Samples were also assayed for viruses at the DNPRC, and leaf samples were preserved for fungal pathogen identification.

### Cape York Peninsula and Torres Strait

A 15-day survey was made in June 2003 to cover places on the Cape York Peninsula and in the Torres Strait. Two BSES staff (plant pathologist and entomologist), one scientist from Indonesian Sugar Research Institute (plant pathologist) and scientists from PNG (entomologist and plant pathologist) took part in this survey. Details of this survey have been reported by Magarey et al (2004).

Procedures for the sample assays were similar to those already discussed above for the other sites.

### Training

There is a need for updating of the skills and knowledge of all people involved in sugarcane quarantine and pest and disease control in the region with the latest sugarcane research and quarantine findings. The PNG survey highlighted the need to raise the

profile of sugarcane quarantine in the region. This was tackled in the following ways.

#### *Training scientists*

Scientists from PNG (Ramu Sugar) and Indonesia were flown to Australia to learn about progress in disease control in Australia. Scientists visited the DNPRC, the BSES Pathology Farm at Woodford and the Tully BSES Pathology Laboratory. Here they saw molecular methods for pathogen detection in quarantine, general quarantine procedures, a range of Australian diseases, and control methods and resistance screening techniques. This will help ensure the best methods for pest and disease control are used beyond Australian shores.

#### *Training quarantine staff*

Project staff attended (Torres Strait) or organised (PNG) two workshops for quarantine staff from remote parts of Australia and PNG.

*Torres Strait.* In early November 2002, a training workshop for AQIS staff located in the Torres Strait was held on Thursday Island. A BSES pathologist participated in the meeting and outlined the diseases of greatest threat to the Australian industry, the need for quarantine in the Torres Strait and provided a pest and disease manual prepared especially for the Torres Strait AQIS staff. It is hoped this will raise the profile of sugarcane quarantine in the region.

*Papua New Guinea.* During 18–21 November 2002, National Agricultural Quarantine Inspection Authority (NAQIA) staff from across PNG assembled at Ramu Sugar, Gusap for training in PNG sugarcane pests and diseases and in quarantine procedures. A manual on PNG sugarcane pests and diseases was also prepared and distributed. In addition, posters outlining the risks involved in carrying sugarcane pests and diseases around PNG were produced in English, Pidgin and Motu—the most commonly used languages in the country. These will be displayed at quarantine posts around the country and at ports of entry. This will help to raise public awareness in PNG of the importance of the crop.

## **Results**

### **Papua New Guinea**

The sites visited included the south coast (Daru Island, Morehead), western border area (Tabubil, Vanimo), north coast/northeast islands (Wewak, Manus Island, New Britain, Ramu Sugar), eastern

coast (Popondetta, Alotau) and Port Moresby (Sorgeri).

The following were the main findings of the PNG survey.

Fiji leaf gall disease was widespread, but at Alotau purple rather than the usual yellow–green galls were seen on pitpit (*S. edule*).

Ramu stunt disease, unique to PNG: symptoms similar to Ramu stunt were seen right around PNG (although this needs molecular assay confirmation). A broad distribution suggests the disease could be closer to Australia in areas where the vector (*Eumetopina*) is found.

Symptoms (somewhat atypical) of sugarcane mosaic disease were seen on New Britain. The failure to detect sugarcane mosaic virus through molecular assay suggests other, unknown forms of mosaic are present in the region. This has high importance for both the PNG and Australian cropping industries.

Commercial varieties of hybrid sugarcane from Ramu Sugar, Gusap had been spread to a number of far-flung locations around PNG, including New Ireland, Manus Island and Alotau and these were displacing chewing canes (*S. officinarum*). The loss of germplasm is accompanied by the increased likelihood of the spread of pests and diseases around the country and consequent additional germplasm loss.

Quarantine procedures were not applied rigorously within PNG: some PNG government department personnel had transferred sugarcane around the country with minimal quarantine precautions. As a result, pitpit (*S. edule*) from the highlands was found on New Britain with mosaic-type symptoms.

There was a lack of public awareness about the importance of sugarcane and sugarcane quarantine. At quarantine stations, posters were displayed on a number of tropical crops but none on sugarcane

Orange rust, which caused large losses to the 2000 crop in Queensland, was observed in almost every garden in PNG.

Leaf diseases observed during the survey included veneer blotch, zonate leaf spot, target spot and eye spot, but the causative agents of some other leaf symptoms were not identified.

Chlorotic streak disease caused by an as yet unknown agent was widely found during this survey, although it had previously been seen in only a very few places in PNG.

*Eumetopina* and *Perkinsiella* leafhopper species were observed throughout PNG. These insects are important vectors of Ramu stunt and Fiji disease,

respectively. Ramu stunt symptoms were also seen in a few places, especially associated with heavy *Eumetopina* infestations.

Woolly aphids were also found in most locations visited. In some places, infestations were severe, resulting in poor cane growth in village plots.

*Sesamia grisescens* stalk borer was collected at Popondetta while *Chilo terenellus* and weevil borer (*Rhabdoscelus obscurus*) were widespread.

At least three species of lophops (Hemiptera: Lophopidae) were encountered in the PNG survey. It has been observed at Ramu Sugar that a larger species is associated with Ramu scorch.

## Northern Australia

Sites inspected were in the Gulf country right across to Broome in Western Australia:

- Queensland: Normanton, Karumba, Burketown, Mornington Island
- Northern Territory: Yirrkala, Nhulunbuy, Groote Eylandt, Gapuwiyak, Galiwinku, Ramingining, Maningrida, Darwin, Daly River
- Western Australia: Kununurra, Wyndham, Kalumburu, Derby, Broome.

Sugarcane was found at most locations visited across northern Australia, but the amount at each centre was generally small, with few pests and diseases found. This was no doubt due to the small numbers of stools (making it difficult for the build-up of pests and diseases) and the relative isolation of the sugarcane. Some of the healthiest sugarcane canopies observed were seen on this survey. There was no evidence that smut had spread from Kununurra to other locations, even in Western Australia. Further inspections are needed around Darwin and Adelaide River, as there was not enough time to thoroughly inspect all sugarcane at these locations during the survey. Three *Saccharum* species were found on the survey (*S. officinarum*, *S. spontaneum* and *S. edule*) with hybrid (commercial) sugarcane the most commonly detected.

The following were the main findings of the survey of northern Australia.

There was a relative lack of pest species. Only a few types of insects (mainly scales, mites and mealybugs) were collected during the survey. Some termites were also found.

A lophops species thought to be *Lophops saccharicida* (Kirkaldy) (recorded from Queensland) was found around Darwin; this is a different species to the

one causing Ramu scorch in PNG. Some scorching symptoms were seen on infested cane in Darwin.

Planthoppers (*Perkinsiella* spp.) were found in only two locations, Daly River and Kununurra, where cane was growing in extensive stands. Stem borers were absent. Smut was found only at Kununurra, where it was previously known to occur.

Specimens were collected for molecular assay to determine the distribution of pathogens such as sugarcane badna virus (SCBV), yellow leaf virus and the phytoplasma associated with yellow leaf syndrome (YLS).

There were no signs of other diseases (including the major Australian diseases of quarantine significance; e.g. orange rust, brown (common) rust, yellow spot, Fiji leaf gall and chlorotic streak and major exotic diseases) during the survey.

Hybrid cane was dominant across the north. Only three sites with chewing cane (*S. officinarum*) were seen, in contrast to PNG where almost all sites supported these canes.

Pitpit (*S. edule*) was found at one site.

*Saccharum spontaneum* was first recognised on the Daly River in the 1940s and a special trip was organised to make collections from there. Further monitoring of the site should occur.

The low abundance of sugarcane was a feature of the survey, though it was found in most places. The small number of plants at each site (except Kununurra, lower Daly River and Darwin) reduces the risk of the establishment of exotic pests or diseases and, as a result, the area (except for Kununurra) is at low risk of pest or disease incursion.

As with the PNG survey, there was little publicity in remote towns and communities about sugarcane quarantine; this survey helped to rectify this across the north.

On the survey, we heard that a significant quantity of sugarcane was being used for fattening cattle at Adelaide River. This remains an inspection priority.

## Indonesia

Sites visited included the following: Sumba (Rae Ele Radjah), Flores (Cornelius Diaz), Sumbawa (Ardiansyah and Hariyono), Lombok (Al Ngateman) and Bali (Wharyoka).

Two major diseases—smut and leaf scald—were found on the islands east of Java; their spread from Java was caused by the transfer of commercial sugarcane varieties without adequate quarantine. This is a



cause for concern, since further spread east could see diseases such as smut come closer to Australia and to the centre of origin of *S. officinarum* in PNG. Orange rust was present on all islands and in a small commercial plantation on the island of Sumbawa, where it was causing significant damage to the commercial hybrid varieties. Orange rust was found on *S. spontaneum* and *S. arundinaceus* as well as *S. officinarum*. Species of planthopper including *Eumetopina* spp., the vector of Ramu stunt disease were also found on some islands. *S. officinarum* was widely grown on all islands. *S. spontaneum* was present on all islands but with variable abundance. Only one small plant was observed on the island of Sumba and just a few clumps on Sumbawa. On Flores, *S. spontaneum* was very common and large thickets were present at a number of locations. *Saccharum spontaneum* was common on Lombok and Bali. *Saccharum robustum* was not seen on any of the islands visited. A few plants of *Saccharum (Erianthus) arundinaceus* (Retz.) were observed on Sumba and Lombok.

Planthoppers (*Perkinsiella* and *Eumetopina* spp.) were widespread and are significant since they are vectors for Ramu stunt and Fiji leaf gall diseases. Quarantine measures to prevent the spread of these pathogens into these areas should be a high priority for the Indonesian sugar industry.

Pink mealybugs (*Saccharicoccus* spp.) and white woolly aphids (*Ceratovacuna lanigera* Zehnt.) were also widespread.

Several borers belonging to the genus *Chilo* were collected in this survey. Weevil borers (*Rhabdocellus* sp.?) were collected only in Sumba and Flores islands. The top shoot borer, *Scripophaga* sp., was also collected in a number of sites.

Smut and leaf-scald diseases were found only on the island of Sumbawa, but this was in commercial hybrid sugarcane that had been brought east from Java. Movement of the diseases (particularly smut) east is an important finding as it brings them one step closer to PNG and eastern Australia.

Fiji leaf gall and Ramu stunt were not found during this survey, even though the vectors for these diseases were present. Symptoms resembling the early stages of Ramu stunt were associated with high populations of *Eumetopina*, but the occurrence of the disease cannot be confirmed until a specific assay is developed. Downy mildew was not found during this survey.

Observations and subsequent molecular assays suggested more than one cause for sugarcane mosaic

symptoms seen during this survey. Recent research has found sugarcane streak mosaic widely distributed in Asia and the disease could also be in Indonesia. The presence of unknown types of mosaic in Indonesia and PNG is a cause for concern and needs further research. Preventing further types of mosaic virus spread should be a high priority for the Australian and PNG sugar industries.

Orange rust (*Puccinia kuehnii*) was found on all islands and was infecting sugarcane hybrids, *S. officinarum*, *S. spontaneum* and *Erianthus* sp.

## Cape York Peninsula and Torres Strait

Sites visited included the following islands and communities:

- Torres Strait (Mabuiag, Badu, Boigu, Dauan, Saibai, Yorke, Mer, Erub, Horn and Thursday islands)
- Cape York (Seisia, New Mapoon, Bamaga, Umagico, Injinoo, Weipa, Napranum, Aurukun, Lockhart River, Coen, Pormpuraaw and Kowanyama).

The main findings of the survey were as follows.

There was limited pest incidence, particularly on Cape York Peninsula. In the Torres Strait, mealybugs, scale insects, planthoppers, aphids, lophops, mites, white flies, *Phaenacantha*, a cicadellid and a bud moth were taken from sugarcane. There was evidence of *Chilo* stem borer activity at two locations in areas further south than in previous findings, though no individuals were seen.

A significant observation was the sighting of *Eumetopina flavipes*, the planthopper vector of Ramu stunt, on most Torres Strait islands as well as on the mainland at Bamaga (where it was found in two gardens).

Lophop individuals were found on some islands. It is not yet known how these might relate to the cause of Ramu scorch. A *Perkinsiella* species was found on *Rottboellia* (itch-grass) on Mer Island. It is not yet known if this species occurs on both *Rottboellia* and *Saccharum*. A *Perkinsiella* species was found feeding on sugarcane on Thursday Island.

Only a few diseases were observed during the survey. Chlorotic streak was seen on Badu Island, and is known from Dauan Island on a previous survey. Ring spot and brown stripe, minor leaf diseases, were seen at Bamaga. Smut was absent; assays for ratoon stunting disease were negative. Fiji leaf

gall, brown rust, orange rust and yellow spot were absent.

Some symptoms resembling Ramu streak were seen, especially in the northern Torres Strait islands. It is difficult to confirm the identity of the agent causing the symptoms since there is no assay for Ramu streak. Further research into the disease and this finding is needed.

Hybrid cane was common in the Torres Strait, though chewing cane (*S. officinarum*) predominated. The hybrid material came from sugarcane crops in the Cairns–Innisfail region and was taken north across the quarantine boundaries implemented by the state government. This is a cause for concern, as pests and diseases unique to Australian mainland cropping industries may be moving north toward PNG.

Chewing cane (*S. officinarum*) is not unusual in the Torres Strait area, being the traditional sweetener used there. Only a small amount of chewing cane was found on Cape York.

Pitpit (*S. edule*, *S. spontaneum*, *S. robustum*) were not found during the survey.

Torres Strait or Pacific Islanders tended to have diverse gardens including sugarcane, whereas Aboriginal people had few gardens and only occasionally grew sugarcane.

There is very little sugarcane between Bamaga and Cairns on the Cape York Peninsula. This was a very significant finding of the survey, as the low incidence of sugarcane provides a natural barrier to the spread of pests and diseases from the Torres Strait islands south.

## Training

Training of quarantine staff from Australia and PNG was very well received, with participants commenting they learnt a considerable amount about sugarcane and its pests and diseases. This will provide quarantine officers with greater knowledge as they approach their duties in remote locations, including a better knowledge of the types of inspections needed when greeting people at ports of entry. Manuals and posters will also provide ongoing background information for their work, and contact with sugarcane pathologists and entomologists will enable greater networking in the future.

## Discussion

Papua New Guinea and parts of Indonesia constitute the centre of diversity for *Saccharum officinarum*, and *S. robustum* and other *Saccharum* species provide an important resource for both Indigenous peoples and sugarcane breeders around the world. Other species of *Saccharum* are present in the region (*S. spontaneum* and *S. edule*) and some of these also have a role in indigenous culture and in commercial breeding programs. *Saccharum* species therefore remain a valuable resource.

The region is home to many sugarcane pests and diseases and a number are unique to Indonesia, PNG or Australia. Only limited surveys have been conducted in the region to determine their distribution. The advent of molecular technologies promises a greatly improved ability to detect viral and phytoplasma pathogens; additional surveys could therefore redefine the distribution of disease-causing agents. There are a number of previously known pests and diseases requiring further research, either for reasons related to biosecurity or the development of better control measures. ACIAR funding for this project provided a unique opportunity to investigate strategic issues for the benefit of Indigenous peoples and cane growers around the world.

The distribution data from these surveys will prove invaluable in developing strategies for incursion management. However, there are still some critical areas—eastern Indonesia, parts of PNG, areas around Darwin and Arnhem Land—that could not be surveyed in the current project due to security concerns or lack of time.

Dispersion of hybrid material is leading to disease spread and the loss of *S. officinarum* germplasm (PNG). People passing through Ramu Sugar took ‘souvenir’ canes to plant back in their villages without realising that they may be taking diseases and pests that could affect the chewing cane and the rich *Saccharum* germplasm. There is a lack of sugarcane quarantine legislation or implementation in PNG and parts of Indonesia and Australia.

Some specimens (e.g. Ramu stunt and Ramu streak) could not be diagnosed because of the lack of a specific molecular assay.

The widespread occurrence of the *Eumetopina planthopper* suggests the major disease Ramu stunt could be more broadly distributed than previously known. Smut and leaf scald disease have spread east from Java toward Australia and PNG. The incidence

of unknown forms of sugarcane mosaic disease in Indonesia and PNG are a cause for concern in cropping industries in those countries and Australia.

A low incidence of sugarcane across northern Australia generally and between Mossman and the tip of Cape York implies a reduced risk of pest or disease spread through these regions

Survey work undertaken so far has enabled a far better understanding of the location of potential threats to the Australian sugar industry and where the greatest attention to quarantine and control will be required. The widespread finding of *Eumetopina* spp. in PNG and in Indonesia raises the possibility of Ramu stunt being close to northern Australia; the finding of smut east of Java suggests the disease is moving closer to the centre of origin of *S. officinarum* (PNG) and to eastern Australia. It is critical that the spread of major exotic pests and diseases closer to Australia be restricted if the Australian industry is to remain free of them. There are still many pest and disease identifications to be completed and these will be used to update pest and disease listings for the three countries.

Training of quarantine personnel and the provision of extension and publicity material to these people will help ensure quarantine procedures are properly applied in places of greatest concern to Australia, such as in the Torres Strait and PNG. There is a critical need for increasing the awareness of quarantine in eastern Indonesia, particularly in the islands east of Java. There have been reports of Indonesian companies taking large quantities of commercial cane east of Java without any form of quarantine so that new commercial estates can be rapidly established. The risk of spread of smut and ratoon stunting disease will be greatly magnified if continued transfer occurs, and strategies to prevent this spread must be implemented. Further publicity of the devastating conse-

quences of such transfer is needed, along with the training of Indonesian quarantine staff in this region. It is probably not possible to do this within the scope of the current project and there is a need to obtain follow-up funding to put an adequate strategy in place. The Australian industry should be involved in this work if the spread of major Indonesian pests and diseases to PNG and Australia is to be prevented.

Continued project activities will include a survey of the Torres Strait and Cape York, and further research into resistance screening techniques for smut and *Sesamia* stalk borer. The identification of the causal agent for Ramu stunt continues to be a high priority. There remains much to be done in the South-east Asian region, and we hope that the Australian sugar industry will provide leadership in sugarcane pest and disease quarantine and control in the whole of Southeast Asia.

## Acknowledgment

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# Efficacy of neem extract, Delfin<sup>®</sup> (Bt) and Orthene<sup>®</sup> on lepidopterous insect pests in cabbages in the Gazelle Peninsula of Papua New Guinea

P. Mwayawa<sup>1</sup>

## Abstract

A field trial was conducted at the University of Vudal, East New Britain Province, Papua New Guinea to assess the efficacy of recommended rates of three insecticides: neem (azadirachtin) extract, Delfin<sup>®</sup> (*Bacillus thuringiensis*, Bt) and Orthene<sup>®</sup> for the control of diamond-back moth (*Plutella xylostella*) and other common lepidopterous insects on head cabbages, cultivar Green Crown Cross hybrid. The crop was monitored weekly for pests before and after spraying. The crop was harvested 84 days after field transplanting. The highest marketable mean yield (80 t/ha) was obtained from plots sprayed with Delfin<sup>®</sup> (Bt), followed by neem extract (71 t/ha). Orthene<sup>®</sup> produced more unmarketable heads (47%) than the control. Neem extract was considered preferable to Delfin<sup>®</sup> (Bt) in relation to pesticide resistance development by insect pests and cost benefits.

## Introduction

English cabbage (*Brassica oleracea* cv. *capitata* L.) was recently introduced into Papua New Guinea (PNG) as a commercial vegetable crop. Production and consumption of English cabbage is rapidly increasing due to its perception as a 'prestige crop'. Unfortunately, cropping of cabbage is threatened by some important Lepidoptera pests (Waterhouse and Norris 1993), including the diamond-back moth (DBM) (*Plutella xylostella* L.) which has rapidly developed resistance to the pesticides used. Forster (1994) reported that cropping of brassicas in some areas in East New Britain was being abandoned due to losses from this insect. Mwayawa (1995, 2000) reported 40% yield losses due to DBM.

The aim of the field trial reported here was to assess the efficacy of three commonly used pesticides, namely neem extract, Delfin<sup>®</sup> (Bt) and

Orthene<sup>®</sup> for controlling DBM and other lepidopterous insect pests of head cabbages.

## Materials and methods

Seeds of head cabbage cv. Green Crown Cross F1, (Tokita Seed Company, Japan) were sown in plastic seedling trays containing sterilised soil medium, raised in a nursery for 4 weeks and hardened for 5 days before field transplanting on 10 April 2001 at six-leaf growth stage.

Plots were initially ploughed and seedbeds constructed manually. They were hand-weeded regularly for 6 weeks for weed control and fine tith for transplanting.

A randomised complete block design (4 treatments × 4 replicates) was used in the experiment. The treatments were: 1, control (sprayed with water only); 2, neem extract (PDP) at 500 mL/ha (2.5% azadirachtin concentration); 3, Delfin<sup>®</sup> (Bt) (bio-agent) containing *Bacillus thuringiensis* spores at

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500 g/ha; and 4, Orthene® (organophosphate insecticide) at 400 mL/ha.

Plots were 5 × 7 m with a 1.0 m buffer between blocks and treatments. Plants were spaced 60 cm within the row and 50 cm between the rows with a population of 100 plants per plot and a plant density of 33,300 plants/ha. Border plants were excluded from the assessment.

All normal crop-husbandry practices were carried out except spraying for fungal diseases.

The crop was hand-weeded three times during the development stages up until complete canopy formation.

A Solo 15 L capacity knapsack sprayer was used to apply the treatments.

Nem oil was imported from Australia. Both Delfin® (Bt) and Orthene® liquid (EC) were obtained locally. The three pesticides were mixed with 20 mL Bond (sticker) before spraying the trial plots.

There were five spray applications. The first spray was applied 22 days after transplanting (DAT) and the last spray 63 DAT and 21 days before harvest.

The first spray was scheduled at a threshold of 1–2 lepidopterous larvae/3 plants. Subsequent sprays were applied when a threshold level of 1 larva/3–5 plants was reached. Plants were monitored for pests twice per week.

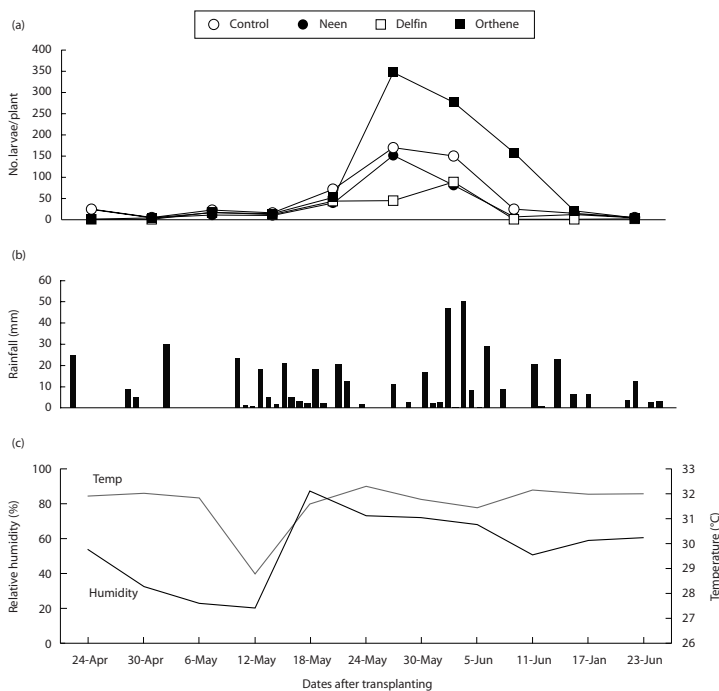
Insect pests were counted before and after each spray application.

The crop was harvested 84 DAT. Pest counts before and after pesticide application and marketable head weight per plot were analysed statistically (ANOVA) using the Minitab 13 statistical package.

Marketable heads were those showing good quality with little or no damage from insect pests.

## Results

The insects with high population densities which were most frequently intercepted were: DBM larvae (Figure 1); cabbage cluster caterpillar (CCC), *Crocidolomia pavonana (binatalis)* Zeller (Figure 2); cluster caterpillar (CC), *Spodoptera litura* (F.)



**Figure 1.** Population dynamics of (a) diamond-back moth (DBM) *Plutella xylostella* and the effects of (b) rainfall and (c) relative humidity and temperature before and after pesticide application. Arrows indicate spraying periods.

(Figure 3); and cabbage centre grub (CCG), *Hellula hydralis* Guenée (Figure 4).

Heavy rainfall (1277 mm) occurred during the crop growth period (April–July 2001) and rain occurred almost daily between 13 and 20 May, accompanied by an increase in insect populations.

There were no significant differences ( $p < 0.05$  %) between insect populations.

Delfin® treatment followed by neem gave significantly ( $p < 0.05$ ) higher marketable yields than the Orthene® and control treatments (Table 1).

## Discussion

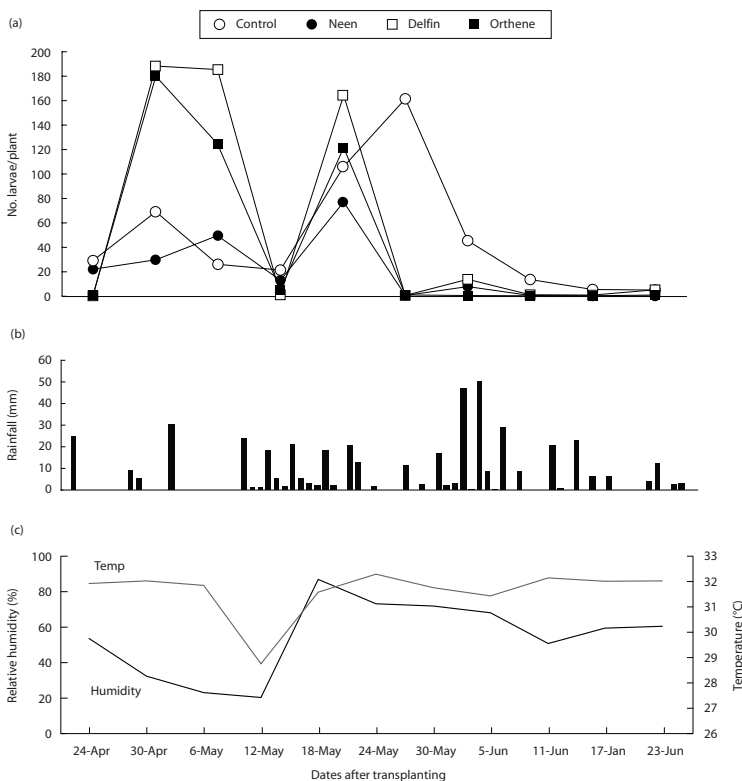
Overall, Delfin® (Bt) treatment resulted in less damage and improved yield, followed by neem treatment. Delfin® (Bt) produced the best control of diamond-back moth, cabbage cluster caterpillar and

related insect pests when compared with the untreated plots.

**Table 1.** Effect of insecticides on marketable yield of cabbage (t/ha)

Treatment	Mean yield (t/ha)
Delfin®	80.42
Neem	70.89
Orthene®	47.02
Control	47.62
LSD <sub>0.05</sub>	35.0
LSD <sub>0.01</sub>	21.4

Delfin® (Bt) is registered for the control of many important Lepidoptera caterpillar pests. It contains a microbial toxin from *Bacillus thuringiensis* (Bt). The commercial formulations contain a mixture of dried spores and cause stomach poisoning when ingested by



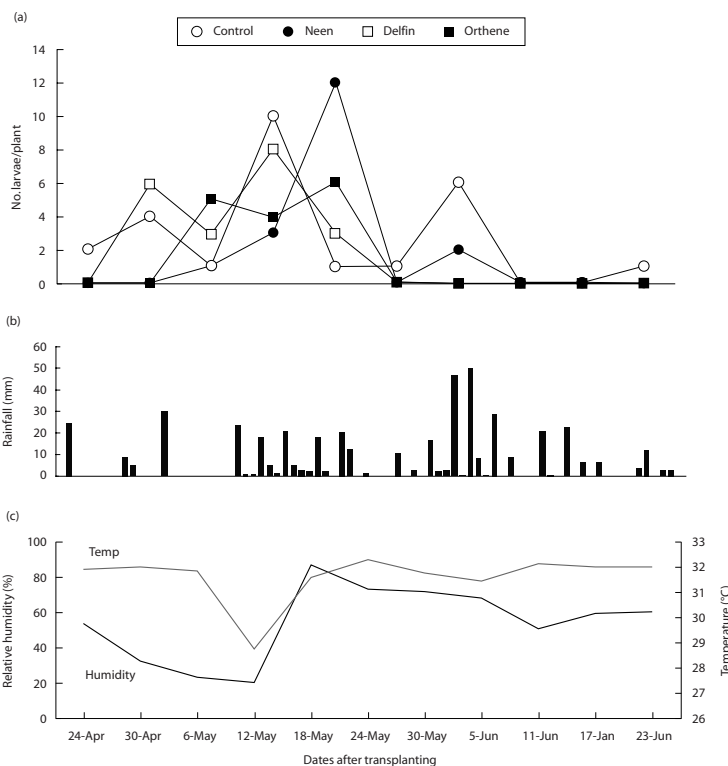
**Figure 2.** Population dynamics of (a) *Crocidolomia binatalis* and the effects of (b) rainfall and (c) relative humidity and temperature before and after pesticide application. Arrows indicate spraying periods.

chewing caterpillars. Delfin® (Bt) is resistant to adverse environmental conditions thus allowing effective insect pest control (Burges and Jones 1998). However, intensive applications have resulted in the development of Bt-resistant insect populations, but more slowly than with many chemical pesticides (Copping 1998). The product should therefore be alternated with other insecticides in order to reduce resistance.

In this field trial, neem treatment gave better yields than the controls. The lack of complete control shown by neem may be due to abiotic factors such as sunlight, humidity and rainfall. Azadirachtin, neem's active ingredient, loses its chemical effectiveness rapidly once exposed to direct radiation (Schmutter 1990; Saucke 2000). Neem was applied in the morning, when it was cool, rather than in late afternoon. Azadirachtin produces repellency, is anti-feedant and anti-ovipositional, disrupts growth and fecundity, and reduces insect fitness. Although it pro-

duces these effects, the chemical is not acutely toxic and does not kill insects immediately (Hassan 1986).

In this trial, a sticker (Bond) was added to neem, which may have some supplementary effect and add variability to the mode of action. Secondly, it could be argued that the monitoring of crop and spraying when the pest population reaches an economic threshold may not be the most effective way to use neem (Joshi and Sharma 1973; Krishnaiah et al. 1998). This is because azadirachtin works both as a protectant and antifeedant. Hussey (1991) suggested that as neem will protect the crop in this way it might be more beneficial to spray it every few days. This would give the crop regular protection and therefore prevent the insect pests from coming in. Hussey (1991) further stated that if, on the other hand, the crop was sprayed when the insects reached the economic threshold, the olfactory effect would be partly lost. When the insect has become established in the



**Figure 3.** Population dynamics of (a) *Spodoptera litura* and the effects of (b) rainfall and (c) relative humidity and temperature before and after pesticide application. Arrows indicate spraying periods.

crop, many larvae will be produced; these are immobile and therefore will not move from the crop in response to olfactory cues.

Orthene® was ineffective in controlling DBM and it required three spray applications to reduce the population of *H. hydralis*. DBM has developed resistance to organophosphates in many parts of the world. Forster (1994) reported that the DBM population in East New Britain, PNG has developed resistance to Orthene®. It is proposed that, in order for growers to manage DBM, biodegradable pesticides and biocontrol should be part of an integrated pest management (IPM) approach (Schubeck and Bokosou 1998). Dent (1992) stated that Orthene® compounds are non-persistent and hence less of a threat to the environment than organochlorines, but they should nevertheless be handled with care (Hill and Waller 1992).

In conclusion, despite Delfin® (Bt) giving the best control in reducing pest populations and increasing

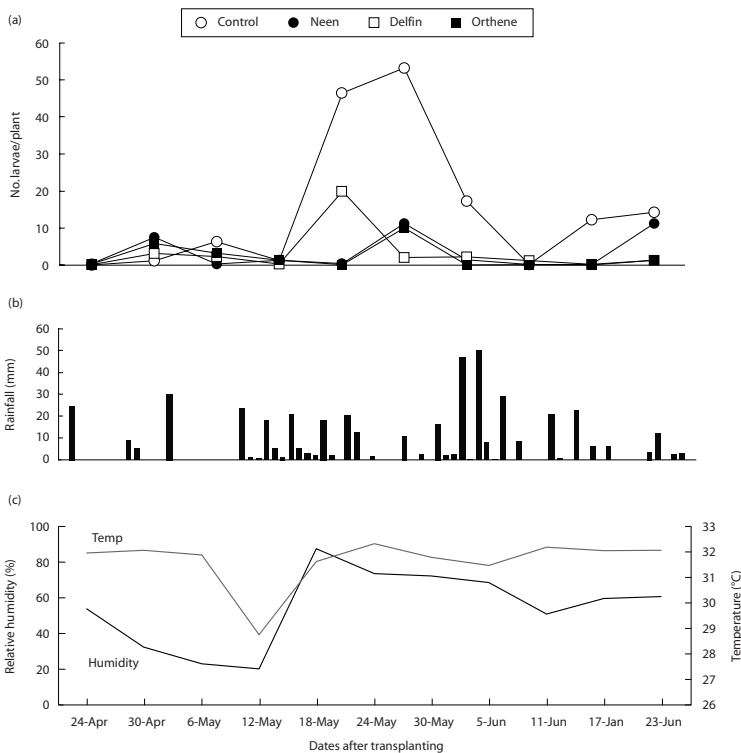
cabbage yields, neem was considered to be more effective and economical for long-term control by farmers. Further research is required to evaluate the various formulations and recommended rates of Delfin® (Bt) and neem, as well as their economic potential and application in IPM systems in PNG.

## Acknowledgment

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**Figure 4.** Population dynamics of (a) *Helulla undalis* and the effects of (b) rainfall and (c) relative humidity and temperature before and after pesticide application. Arrows indicate spraying periods.



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# Evaluation of four insecticides as part of an integrated pest management strategy for diamondback moth, *Plutella xylostella* L., in the highlands of Papua New Guinea

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## Abstract

Selective agropesticides, cultural practices, plant resistance and biological control of insect pests on crops are components of integrated pest management (IPM). Two insect growth regulators (IGRs) (spinosad, indoxacarb) and two commonly used broad-spectrum insecticides (lambda-cyhalothrin and pirimiphos-methyl permethrin) were evaluated to determine compatibility in the cabbage IPM for controlling diamondback moth (DBM) in the highlands. The initial DBM population was reduced by 76% by spinosad (Success<sup>®</sup>), 73% by pirimiphos-methyl permethrin (Target<sup>®</sup>), 48% by indoxacarb (Steward<sup>®</sup>) and 33% by lambda-cyhalothrin (Karate<sup>®</sup>). Average DBM counts for the entire spray period were in the range 0–2 per 10 plants in all chemical + frequency treatments, except lambda-cyhalothrin fortnightly treatment. The two IGRs were shown to be compatible in the cabbage IPM because they were effective against major lepidopterous pests of cabbages including DBM. The IGRs were also relatively safe to the introduced DBM parasite *Diadegma semiclausum* and other predators.

## Introduction

Diamondback moth (DBM) *Plutella xylostella* L. (Lepidoptera: Plutellidae) is the most important pest of brassicas and has been the subject of extensive research and development (including integrated pest management (IPM)) around the world for more than four decades (Verkerk and Wright 1996). In Papua New Guinea (PNG), DBM is a major pest of brassica crops, causing deterioration in both eating and marketing quality of the produce (Thistleton 1987; Saucke 1995). The distribution of DBM in PNG is as widespread as the host crops (Thistleton 1987).

General pest management practices among the majority of brassica farmers to keep DBM and other brassica pests under control have been based on cultural practices and the use of broad-spectrum insecticides such as pyrethroids (e.g. Karate<sup>®</sup>) and organophosphates (e.g. Orthene<sup>®</sup>) (Wemin and Pus, unpublished data). However, farmers have not been adequately informed of the adverse consequences that are associated with continuous application of insecticides. These include pest resistance and deleterious effects on beneficial insects and the ecosystem. There are numerous reports of DBM resistance to many chemical groups including organophosphates, pyrethroids, carbamates, chlorfluzuron, diflubenzuron and hexflumnon (Talekar 1990). Amano and Haseeb (2001) also reported a drastic decline in insect fauna on pesticide-treated crops.

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Therefore, it is essential that farmers are aware of alternative selective insecticides for use in an integrated pest management (IPM) approach, in order to overcome the undesirable chemical effects.

In order to promote a sound IPM, Heisswolf and Brown (1996) reported that more information was needed to improve insecticide selection within the IPM system, so that the most compatible insecticide is identified for a particular situation. The use of selective pesticides has already been considered as a prerequisite in IPM for any crop production. Without evaluating pesticide effectiveness on the pest and on the beneficial insect species, the selectivity and the hazards associated with pesticides in pest management cannot be determined (Amano and Haseeb 2001).

The discovery and development of new pesticides is becoming very difficult and expensive (Miyata et al. 1986). The alternative groups of insecticides that are available to overcome the resistance problem are insect growth regulators (IGRs) such as Success<sup>®</sup> and Steward<sup>®</sup>, *Bacillus thuringiensis* (Bt) toxin and insecticides with new modes of action (Cheng 1986). These considerations are very important in the selection and application of insecticides in an IPM strategy.

Some selective insecticides have already been recommended as part of the IPM strategy in the highlands of PNG. These include Bt (Delfin<sup>®</sup>, Thuricide<sup>®</sup>, Biobit<sup>®</sup>), an IGR (Atabron<sup>®</sup>) and neem (Saucke 1995). However, the withdrawal of Atabron<sup>®</sup> from PNG has left a gap. Hence, this study was undertaken to evaluate the compatibility of the IGRs Success<sup>®</sup> and Steward<sup>®</sup> under highlands conditions for use in the cabbage IPM package against DBM.

## Materials and methods

### Experimental design and analyses

The experiment was conducted in a randomised block design with nine treatments and four replicates (Table 1).

The data were analysed to test:

- the relative efficacies of chemical treatments versus the control
- the effects of the four chemicals and the two application frequencies and the interaction between these two factors.

All insect counts except those of the aphid *Myzus persicae* (Sulzer) were transformed to square root ( $x + 0.5$ ) before analyses. All means quoted are in back-transformed original 'counts per 10 plants' units.

### Site preparations and planting

The trial was conducted at an elevation of 1630 m (6°21'S. and 145°51'E.). The average annual rainfall is 2074 mm. The annual average maximum temperature is 22.6°C (17–24°C) while the average minimum temperature is 14°C (12–15°C).

The experimental site had been planted with sweet potato (*Ipomoea batatas* (L.)) for more than 5 years before the trial. The soil was of loam type with good drainage and was moderately acid (pH 5.0). The slope was less than 10° to the north.

The land was disc-ploughed and disc-harrowed before planting. Individual plot size was 7.5 m<sup>2</sup> with seven rows and seven plants within each row. The between-row spacing was 50 cm and the within-row spacing 40 cm giving a density of 50,000 plants/ha. There were 25 experimental plants in each plot surrounded by 24 guard plants. Adjacent replicates were separated by 2 m spacing and every plot was separated

**Table 1.** Details of chemical treatments tested against brassica insect pests

Treatment	Chemical	Application frequency
1	No insecticides (control)	
2	Spinosad (Success <sup>®</sup> )	Weekly
3	Spinosad (Success <sup>®</sup> )	Fortnightly
4	Lambda-cyhalothrin (Karate <sup>®</sup> )	Weekly
5	Lambda-cyhalothrin (Karate <sup>®</sup> )	Fortnightly
6	Primiphos-methyl + permethrin (Target <sup>®</sup> )	Weekly
7	Primiphos-methyl + permethrin (Target <sup>®</sup> )	Fortnightly
8	Indoxacarb (Steward <sup>®</sup> )	Weekly
9	Indoxacarb (Steward <sup>®</sup> )	Fortnightly

rated by 1 m spacing. Organic fertiliser (untreated wood chips + chicken manure) was applied at a rate of 16 t/ha, 7 days before planting.

The cabbage cultivar used in this study was Green Coronet. Seedlings were transplanted 42 days after sowing. During the first 3 weeks in the field, 7.2% of the seedlings were replaced due to losses caused by cutworms (*Agrotis ipsilon* (Hufnagel)) and a fungal disease suspected to be blackleg (*Phoma lingam*).

### Field management

Hand-weeding was done 4 and 8 weeks after transplanting. A side dressing of NPK (12:12:17 + 2 MgO) fertiliser at 120 kg/ha was applied in week 8.

The insecticide treatments were applied weekly or fortnightly for 7 weeks using CP15 knapsack sprayers (Table 2). The control was not sprayed.

### Field observations and assessments

The plants were observed daily in the nursery and field for insect pests and disease infestations. Ten plants were randomly selected from the 25 experimental plants for observations and insect counts. Field observation included assessment of the effect of insecticides on insect populations during plant growth, and assessments of insect damage to marketable heads at harvesting. The field assessments included observations and insect counts 1 day before and 2 days after spraying. The numbers of DBM adults caterpillars, pupae and other brassica pests such as *Crociodolomia pavonana* (F.) (cabbage cluster caterpillar), *Spodoptera litura* (F.) (armyworm), *Hellula undalis* (F.) (cabbage centre grub), *Helicoverpa armigera* (Hubner) (corn earworm), *Agrotis ipsilon* (cutworm), *Chrysodeixis eriosoma* (Doubleday) (semilooper), *Oribius* spp., *Myzus persicae* (green peach aphid), vegetable bugs and grasshoppers/locusts as well as beneficial insects such as the introduced parasitic wasp *Diadegma semiclausum* (Hellén), Coccinellidae (ladybird beetles), Arachnida (spiders) and Syrphidae (hoverflies) were recorded.

After harvest the yield and quality of the each head was assessed and scored as follows:

- 1 = no damage.
- 2 = fewer than 5 holes on outer leaf.
- 3 = 5–10 holes on outer leaf but still marketable.
- 4 = 10 and 20 holes, unmarketable, but edible by farmers after removing the damaged leaves.
- 5 = more than 20 holes or head extensively damaged and unsuitable for consumption.

## Results and discussion

### Diamondback moth (caterpillars) assessment

Mean initial counts were taken before the first spray application on week 0. Mean counts were 4 in the control. Treatment counts declined to less than 1 by week 2 to most of them to 0 by week 3 (Figure 1).

There were significant differences in counts between treatments in pre-treatment assessment (week 0). Hence, in analysis of week 1–7 data, the week 0 counts were used as a covariate. Tables 3 and 4 show these covariate analyses of post-treatment counts. In each of the analyses, the ‘control’ average was significantly higher than the average of the ‘rest’ (i.e. all the 8 chemical × frequency treatments). However, there were no significant differences ‘within the rest’ for any analysis. The plots sprayed fortnightly with Karate® were the only ones on which DBM caterpillars were found in weeks 4–7.

Analysis of average counts over weeks 1 and 2 showed that the control population (3.4) was significantly higher ( $p < 0.01$ ) than the ‘rest’ (average 0.8).

**Table 2.** Dosage rates and frequency of application of insecticide treatments

Trade name	Active ingredient	Rate	Sticker (3 mL/L)	Spray interval (days)	Withholding period (days)
Success®	120 g/L spinosad	1 mL/L (400 mL/250 L/ha)	+	7–14	3
Target®	95 g/L pirimiphos methyl + 5 g/L permethrin (emulsifiable concentrate)	5 mL/L	+	7–14	14
Steward®	150 g/L indoxacarb	0.5 mL/L	–	10–14	3
Karate®	25 g/L lambda-cyhalothrin (ec)	1 mL/L (500 mL/500 L/ha)	+	4–7	14

There were also significant differences ( $p < 0.01$ ) between the chemical treatments (Table 4). The ‘frequency’ main effect was almost significant ( $p = 0.07$ ), with a weekly mean (across four chemicals) of 0.6 compared with a fortnightly mean of 0.9.

For average counts over weeks 3–7, the control population (3.1) was significantly higher ( $p < 0.01$ ) than the insecticides treatments (0.1). There were no significant differences within the treatments. The ‘frequency’ effect was not significant (weekly 0.0, fortnightly 0.2).

**Table 3.** Effect of insecticide treatment on mean population<sup>a</sup> of diamondback moth over a 7-week period

Week	1	2	3	4	5	6	7
Control	3.0	5.0	2.0	2.0	4.7	4.9	2.9
Sprayed	1.2	0.4	0.2	0.1	0.1	0.1	0.1
Test <sup>b</sup>	*	**	**	**	**	**	**

<sup>a</sup> Counts per 10 plants

<sup>b</sup> Test between ‘control’ and ‘others’: \*  $p < 0.05$ , \*\*  $p < 0.01$ .

**Table 4.** Effect of weekly or fortnightly insecticide treatment on mean population of diamondback moth

Chemical	Average population of weeks 1 and 2
Success <sup>®</sup>	0.4 a*
Karate <sup>®</sup>	1.3 b
Target <sup>®</sup>	0.4 a
Steward <sup>®</sup>	1.1 b

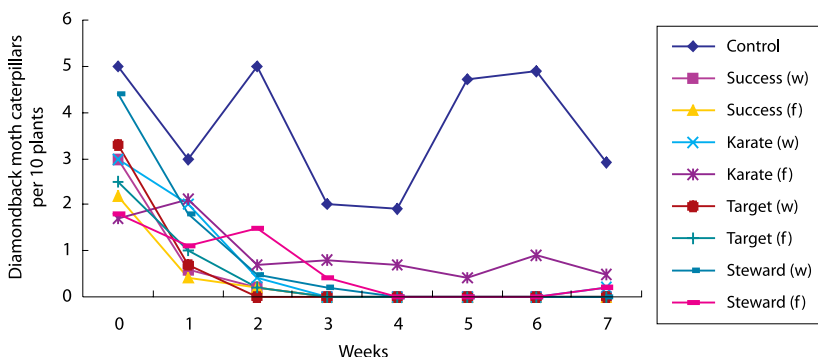
\* Means followed by a similar letter do not differ significantly ( $p > 0.05$ )

‘Averaging’ counts across several consecutive weeks (1 and 2, 3–7) reduced some of the natural variability in DBM counts, and increased statistical precision in comparing the pesticide and frequency treatments.

### Beneficial insects assessment

Analysis of counts of *Diadegma semiclausum* parasitic wasps indicated there was a significant interaction between chemical and frequency. The interaction means are shown in Table 5. There were very few *Diadegma* in the weekly application of Karate<sup>®</sup> and Target<sup>®</sup>. The *Diadegma* count in the control plots (7.7) was significantly higher ( $p < 0.01$ ) than the count in the insecticide-treated plots. More than 80% of DBM cocoons collected from untreated plots were parasitised by *Diadegma*. Analysis of counts of other beneficial insects indicated no significant interaction or frequency effect, so only chemical main effects are covered in Table 6. Though spider counts were low in Karate<sup>®</sup> and Target<sup>®</sup> treated plots, spiders were present on all chemical treatments. Counts of ladybird beetles were significantly higher in Success<sup>®</sup> than the rest of the treatments. There were significant effects ( $p < 0.05$ ) of chemical treatments for counts of Coccinellidae (ladybird beetles), Arachnida (spiders) and Syrphidae (hoverflies). The counts in Karate<sup>®</sup> and Target<sup>®</sup> treated plots were lower than Success<sup>®</sup> and Steward<sup>®</sup>. Success<sup>®</sup> treated plots had lower *Diadegma* counts but higher counts of other species.

Figure 2 compares beneficial insect counts following treatment with IGRs or broad-spectrum insecticides. Counts of beneficial insects on plots treated with IGRs



**Figure 1.** Counts of diamondback moth caterpillars on 10 cabbage plants over 7 weeks of insecticide application (w = weekly; f = fortnightly)

were more than 50% of the total insect population whereas they were less than 10% on plots treated with broad-spectrum insecticides.

**Table 5.** Effect of insecticides sprays on mean populations of the parasitic wasp *Diadegma semiclausum*

Treatment	Mean no. of <i>Diadegma</i> / 10 plants
Control	7.7 a
Success <sup>®</sup> (weekly)	0.4 b
Success <sup>®</sup> (fortnightly)	0.2 b
Karate <sup>®</sup> (w)	0.2 b
Karate <sup>®</sup> (f)	0.6 b
Target <sup>®</sup> (w)	0 b
Target <sup>®</sup> (f)	0.6 b
Steward <sup>®</sup> (w)	1.5 b
Steward <sup>®</sup> (f)	1.4 b

Note: Means followed by a similar letter do not differ significantly ( $p > 0.05$ )

**Table 6.** Effect of insecticides sprays on mean populations of beneficial insects

Treatment	Ladybird beetles/10 plants	Spiders/ 10 plants	Hoverflies/ 10 plants
Control	0.4 a *	7.6 a	5.7 a
Success <sup>®</sup>	1.5 a	1.3 c	2.5 b
Karate <sup>®</sup>	0 b	0.8 c	0.2 c
Target <sup>®</sup>	0 b	1.1 c	0 c
Steward <sup>®</sup>	0.2 b	3.6 b	5.3 a

\* Means in same column the followed by a similar letter do not differ significantly ( $p > 0.05$ ).

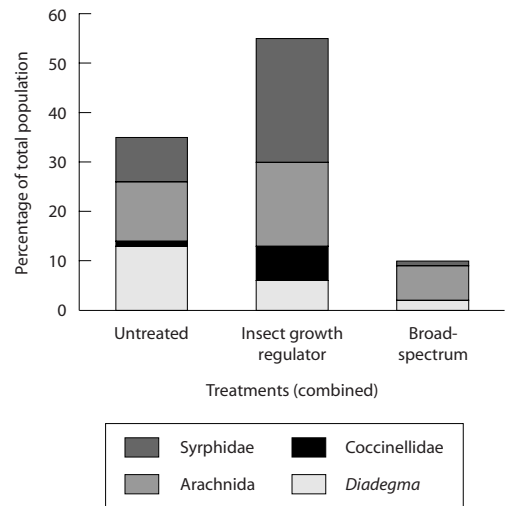
### Relationships between crop damage and DBM caterpillar counts

Correlations between counts of DBM (weeks 1–7) and final damage to the harvested cabbage heads were estimated. The highest correlations were of percentage of ‘no damage’ heads with DBM counts at weeks 2 ( $-0.55^{**}$ ) and 7 ( $-0.69^{**}$ ). However, because the weekly counts of DBM were themselves often highly correlated, it is not possible to accurately determine when the DBM caterpillars were doing the most damage.

### Yield assessment

Table 7 and Figure 3 show the average yields resulting from each treatment. There were no significant differences in yield between the eight chemical ×

frequency treatments (main effect or interaction), but the yield of the control treatment was significantly lower than the chemical treatments ( $p < 0.05$ ). The total heads harvested per plot depended on the survival of seedlings and ranged from 14 to 25. Because no compensation occurred for missing heads, this was used as a covariate in the analysis of yield.



**Figure 2.** Beneficial insect counts of different groups following insecticide treatments, expressed as percentages of the total population of beneficials

### Lethal effects on DBM populations

Brassicas are vulnerable to attack by a complex of insect pests with DBM as a core pest (Saucke 1995). Satisfactory control of DBM using insecticides is always a difficult task because the pest has the ability to develop resistance very quickly (Cheng 1986). Cheng (1986) further confirmed a high level of DBM resistance to synthetic pyrethroids such as Karate<sup>®</sup> and Target<sup>®</sup>. It is premature to discuss DBM resistance to insecticides evaluated in this study, but further research should be done under similar conditions to check this hypothesis.

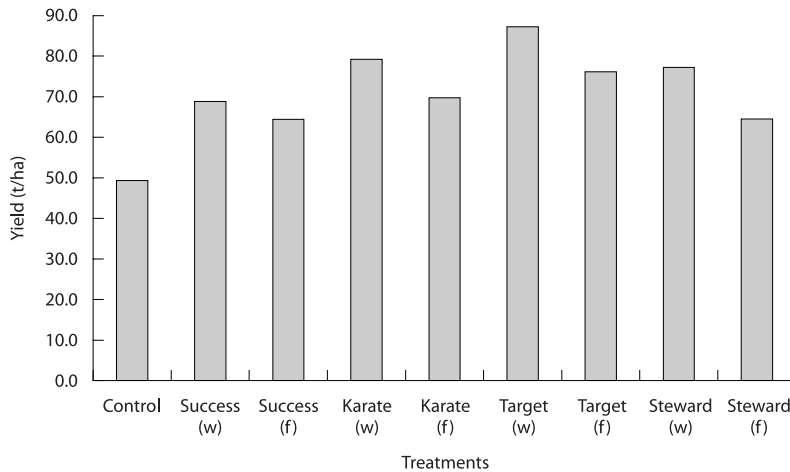
The observations and results from this study showed that lethal effects of the four insecticides tested on DBM were detectable within 3 days of initial application. The initial reduction of the DBM population observed using the different insecticides was Success<sup>®</sup> 76%, Target<sup>®</sup> 73%, Steward<sup>®</sup> 48% and Karate<sup>®</sup> 33%. Populations of DBM in all chem-

ical treatments remained between 0 and 2 counts per 10 plants for the spray period. Counts of DBM on plots sprayed fortnightly with Karate<sup>®</sup> fluctuated during the spray period but this could not be confirmed as being due to resistance.

Whether reduced DBM populations in weeks 3 and 4 (Figure 1) were due to chemical application or the effects of weather cannot be determined. There was high rainfall during the 2 months before the trial (October–November 2001), and again during the insect assessment period (weeks 2–7) (Figure 4). It is possible that wet weather affected the fecundity rate and consequently led to a reduced DBM population in the untreated plots in weeks 3 and 4 (compare Figures 1 and 4).

Since natural enemies (both indigenous and introduced) play a key role in suppressing pests such as

DBM, it is necessary to be mindful of the selection and use of insecticides that are available on the market. The use of broad-spectrum insecticides was one of the factors contributing to the disruption and absence of effective natural enemies, particularly parasitoids which keep DBM in check (Talekar 1993). Schuhbeck and Bokosou (2001) compared untreated cabbage plants with those regularly sprayed with broad-spectrum insecticides and found a decline in numbers of general predators (spiders and ants). The harmful effects of Karate<sup>®</sup> and Target<sup>®</sup> (broad-spectrum insecticides) on beneficial insects (natural enemies) became quite evident in our study also. Though some species of spiders had some tolerance to the four chemicals, the counts for *Dia-degma*, hoverflies and ladybird beetles were very low in Karate<sup>®</sup> and Target<sup>®</sup> treated plots, indicating a



**Figure 3.** Average yield of marketable cabbages (grades 1, 2 and 3)

**Table 7.** Effect of insecticide treatments on yield of marketable heads

Treatment	No. of heads	Yield* (total heads/plot)	Percentage of total heads	Percentage of total yield
Control	17.0 a	27.7 a	81.8 a	82.7 a
Success <sup>®</sup> (weekly)	21.5 b	35.5 b	96.9 b	96.4 b
Success <sup>®</sup> (fortnightly)	23.0 b	31.6 b	98.9 b	98.8 b
Karate <sup>®</sup> (w)	22.0 b	40.8 b	100.0 b	100.0 b
Karate <sup>®</sup> (f)	20.5 b	38.2 b	99.0 b	99.1 b
Target <sup>®</sup> (w)	24.2 b	39.7 b	99.0 b	98.8 b
Target <sup>®</sup> (f)	21.5 b	35.6 b	90.3 b	91.8 b
Steward <sup>®</sup> (w)	23.2 b	37.0 b	100.0 b	100.0 b
Steward <sup>®</sup> (f)	21.5 b	33.3 b	97.4 b	98.8 b

Column values followed by the same letter are not significantly different.

high level of toxic effects. *Diadegma semiclausum*, an introduced parasitic wasp, was most affected by weekly applications of Target<sup>®</sup>. Results (Table 5; Figure 2) showed that both Success<sup>®</sup> and Steward<sup>®</sup> were effective against DBM and relatively kind to the beneficial insects observed, with Steward<sup>®</sup> being least harmful. The abundance of natural enemies in the untreated plots with greater than 80% parasitism<sup>2</sup> of DBM by *Diadegma* was an indication of a natural balance between pest and beneficials in the crop ecosystem.

### Insecticide effect on cabbage quality

Generally, 90–100% of the cabbages from the insecticide treatments were free of serious insect damage and marketable (Table 7; Figure 3). Weekly applications of Karate<sup>®</sup> produced 100% grade 1 cabbages, and weekly applications of Steward<sup>®</sup> produced 100% grades 1–3. Success<sup>®</sup> applied fortnightly produced 98% marketable grades and Target<sup>®</sup> applied weekly produced 96% marketable grades. However, there was no significant difference between the two frequencies of chemical application and the marketable grades resulting.

High-grade cabbage heads and abundance of beneficial insects were indications of compatible pest management strategy. Though chemically treated

cabbages produced higher marketable yields than untreated cabbages (Figure 3), natural predators and parasites also contributed to reducing pest damage on cabbages. Hence, the selection and use of suitable insecticides has been shown to be an important component of success in producing crops that are free of insect damage.

The unmarketable and unfilled heads were mainly the result of insect damage. A fungal disease suspected to be blackleg caused seedling deaths during the early stages of growth. This also contributed to the total crop loss. Higher total crop losses (>30%) were observed in untreated cabbages.<sup>3</sup>

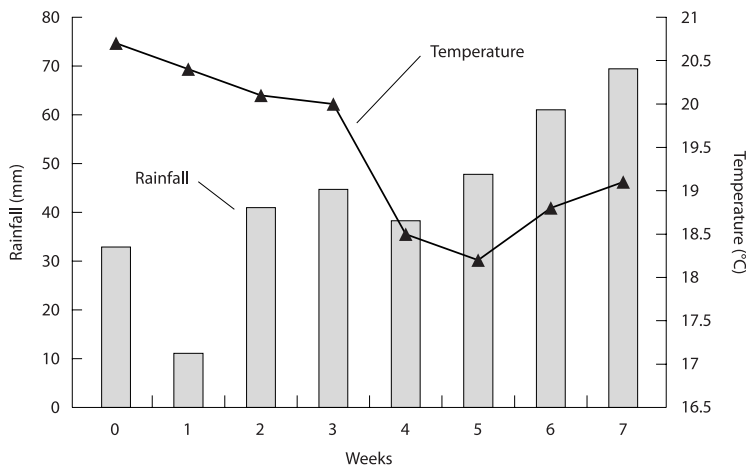
### Conclusions and recommendations

The two IGRs applied were shown to be effective against DBM, regardless of spraying weekly or fortnightly. The IGRs were also shown to be relatively safe to most beneficial insects, including *Diadegma semiclausum*.

The commonly used broad-spectrum insecticides (Karate<sup>®</sup> and Target<sup>®</sup>) were also effective on DBM when they were applied weekly but their use was accompanied by significant reductions in the beneficial insect population. Target<sup>®</sup> was more harmful to beneficial insects than was Karate<sup>®</sup>.

<sup>2</sup> Percentage parasitism = total number of *Diadegma*/total DBM cocoons × 100

<sup>3</sup> Total crop losses (%) = number of plants dying/total number of plants planted × 100.



**Figure 4.** Rainfall and temperature (°C) at the trial site over the 7 weeks of insecticide application



The two IGR have been shown to be highly compatible in the cabbage IPM strategy, in which natural enemies play a very important role in suppressing pest populations. Therefore, Steward® and Success® are recommended for use in the cabbage IPM package in the PNG highlands. Farmers are recommended to alternate these insecticides at fortnightly intervals with *Bacillus thuringiensis* (Bt) products, strictly adhering to manufacturers' recommended doses. It is strongly recommended that Success® and Steward® (IGRs) be made available on the market in PNG for use in the IPM strategy. The increased rainfall and wet weather before and during the trial could have affected fecundity rate, resulting in lower than normal DBM counts. Hence, it is recommended that further evaluation of insecticides (IGRs) be carried out during the dry season to check the findings in this study.

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# Breeding sites of major coconut beetle pest *Scapanes australis* Boisd. (Coleoptera: Scarabaeoidea, Dynastinae) in East New Britain, Papua New Guinea

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## Abstract

An intensive field survey for breeding sites of *Scapanes australis* was conducted on the Gazelle Peninsula, East New Britain Province, Papua New Guinea (PNG) in 2000–2002 on both large-scale commercial plantations and smallholder coconut plots. *Gliricidia sepium* is prominently used as a temporary shade tree for cocoa where cocoa and coconut are intercropped, a farming system widely adopted by farmers in PNG. Population monitoring using olfactory trapping systems indicated that there are still large reservoirs of unexploited breeding sites including *Gliricidia* stumps sustaining considerable populations of *Scapanes australis grossepunctatus*.

## Introduction

The Melanesian rhinoceros beetle, *Scapanes australis* Boisd. (Col: Scarabaeoidea), is one of the major coconut beetle pests of Papua New Guinea (PNG). It is endemic to PNG, Solomon Islands and Indonesia (Irian Jaya) (Prior et al. 2000). Four subspecies are recognised (Endrôdi 1957): *S. australis australis* Boisd. occurs on the mainland west of Huon Gulf (including Karkar Island) and through West Irian; *S. australis brevicornis* Sternberg occurs on the mainland east of Huon Gulf and Ferguson Island; *S. australis grossepunctatus* Sternberg occurs on the Bismarck Archipelago; and *S. australis solomonensis* Sternberg occurs on Bougainville and the Solomon Islands.

There are few records of investigations on the breeding and larval development sites of *S. australis*. Lepesme et al. (1947) recorded *Scapanes* larvae inside the rotten trunks of different species of trees (Beaudoin-Ollivier et al. 2001), while Bedford (1976) observed most *S. australis* larvae under rotting logs at the soil interface and in the base of a decayed sago palm stump (*Metroxylon sagu* Rottb.), and *S. australis grossepunctatus* under rotting cocoa pods.

Beaudoin-Ollivier et al. (2001) comprehensively described the larval development sites of *S. australis australis* and *S. australis grossepunctatus*. On the mainland, particularly Karkar Island, *S. australis australis* immature stages were common in wildfowl (*Megapodius freycinei* Abbotti) nests associated with breadfruit (*Artocarpus altilis* (Parkinson)) tree roots. On the island regions of the Bismarck Archipelago, particularly the Gazelle Peninsula (East New Britain), *S. australis grossepunctatus* was common in rotting *Gliricidia sepium* (Jacq.) Steud. stumps. This is of concern, because *G. sepium* is widely used as a shade tree in cocoa and coconut intercropping systems.

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## Methods and materials

An intensive field search for breeding sites of *S. australis* was conducted on both large commercial plantations and smallholder coconut plots on the Gazelle Peninsula, East New Britain between 2001 and 2002.

### Commercial plantations

Two large commercial plantations (Tavilo Plantation, owned by the PNG Cocoa and Coconut Institute, and Gunanur Plantation owned by Coconut Products Limited) were searched for breeding sites, by inspection of rotting cocoa and coconut stumps and heaps of rotten cocoa pods. *Gliricidia sepium* stumps and other unidentified bush tree stumps were dug up. Fallen, rotted logs in the vicinity of the plantations were searched thoroughly at the log–soil interface.

### Smallholder coconut plots

Three smallholder coconut plots at Napapar II, Putanagororoi and Vunapalading were also inspected. High-yielding hybrid coconut seedlings were distributed and planted on these sites before the search. Napapar II and Putanagororoi had some old tall coconuts and *G. sepium* scattered within adjacent cocoa blocks and bushes. Vunapalading is a recently cleared secondary forest area.

All suspected breeding sites were searched, while rotten *G. sepium* stumps and other unidentified tree stumps were dug up and inspected.

Other tree species within and at the periphery of the smallholder coconut blocks were randomly sampled. Twenty trees were searched for each suspected breeding site.

All coleopteran larvae collected were directly identified in the field using a simplified key developed by Beaudoin-Ollivier et al. (2000). Associated scarabaeoid larvae about which there was uncertainty were taken to the laboratory for confirmation using a key devised by Bedford (1974). The sample sizes for each suspected breeding sites varied between locations, so 20 suspected breeding sites were randomly selected for analysis.

## Results

### Tavilo plantation

At Tavilo Plantation, the population of *S. australis grossepunctatus* larvae per rotten *G. sepium* stump on two coconut blocks was higher than the popula-

tions of other, associated scarabaeoid larvae (mainly elephant beetle, *Xylotrupes gideon* L., with some larvae from the Cetonidae and Lucanidae families). Fewer *Scapanes* were found on cocoa and other hosts (Table 1).

### Gunanur plantation

*Scapanes* larvae were present in all the suspected breeding sites examined (Table 1). However, *Gliricidia* had the highest mean number of larvae per stump (2.1).

### Smallholder coconut plots

The mean number of *S. australis* larvae was higher for *G. sepium* stumps for each of smallholder coconut blocks (Table 1) with the maximum at Vunapalading, a recent resettlement area. Other suspected breeding sites within the sampled area had low incidences of *Scapanes* larvae.

## Discussion

Previous work has shown that *S. australis* had a wide range of breeding sites (Lepesme et al. 1947; Bedford 1976; Beaudoin Ollivier et al. 2001).

Our results from Tavilo Plantation and on several smallholder coconut blocks on the Gazelle Peninsula, East New Britain in 2000–2002 have confirmed the results of Beaudoin-Ollivier et al. (2001) that *Scapanes* larval populations occur under rotting *G. sepium* stumps.

The observations by Beaudoin-Ollivier et al. (2001) and the results from our work clearly demonstrated a shift of breeding sites from forest areas to farms and plantations where coconut and cocoa are intercropped, with *G. sepium* used as temporary shade tree for cocoa.

Records from the Department of Agriculture and Livestock indicated that *G. sepium* was introduced into PNG in the 1950s (from Sri Lanka in 1955 and from the National Botanical Gardens, Trinidad by the Department of Forestry in 1959). It thus appears that *S. australis* has gradually established its breeding sites in dead *G. sepium* stumps over the past 20 years as *Gliricidia* became widely promoted, distributed and established as a shade tree for cocoa as more land was cleared for farming. *Scapanes australis* therefore now poses a threat to the rehabilitation and/or replanting of coconuts in East New Britain, PNG.

**Table 1.** Mean numbers of larvae of *Scapanes australis*, *Xylotrupes gideon* and other scarabaeoids recorded in surveys of breeding sites on *Gliricidia sepium* stumps and other hosts in commercial and smallholder intercropped coconut plantations in Gazelle Peninsula, East New Britain from 2000 to 2002

Plant	Location/date	Mean no. of larvae/stump or site		
		<i>Scapanes</i>	<i>Xylotrupes</i>	Other
<i>Gliricidia</i>	Tavi#10, July 2001	2.3	0.8	0.2
	Tav#9, Oct 2000	0.5	0.2	0
	Tav#12, 2002	0.6	0.1	0
	Tav#11	0.3	0.1	0
	Tav#13, May 2001	1.2	0	0
	Tav#6, May 2001	0.6	0	0.1
	Guanur,	2.1	0.1	0
	Vunapalading, May2001	0.2	0.3	0.2
	North Coast, May 2001	0.6	0	0
	Napapar II, June 2001	0.3	0.1	0
Cocoa	Tavilo1	0	0	0
	Tavilo2	0	0	0
	Tavilo3	0	0	0
	Tavilo4	0.1	0	0
	Guanur	0.2	0.1	0
	Vunapalading, May 2001	0.1	0	0.1
	North Coast, May 2001	0.1	0.1	0
Others	Napapar II, June 2001	0.1	0	0
	Tavilo1	0	0	0
	Tavilo2	0	0.1	0.1
	Tavilo3	0.6	0	0
	Tavilo4	0.2	0.3	0.1
	Guanur	1	0.2	0.1
	Vunapalading, May 2001	0	0.1	0
	North Coast, May 2001	0.1	0	0.1
Coconut	Napapar II, June 2001	0.3	0	0.2
	Guanur	0.2	0	0.6
	North Coast, May 2001	0.1	0	0.3

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# Farmer perceptions of coffee pests in Boana district, Morobe Province, Papua New Guinea

A.N. Simbiken<sup>1</sup>

## Abstract

The adoption of technologies for the management of pests and diseases on coffee by rural farmers in Papua New Guinea is rare and very erratic. Coffee leaf rust and coffee green scale are major pests seriously affecting coffee production. A farmer survey on integrated pest management of these pests was carried out in Boana, Morobe Province in 2001. Less than 30% of the 45 farmers surveyed were able to recognise green scale insect as a pest on coffee, attributing the scale problem to a resurgence of ants after the 1997 drought. Coffee centre borer (*Zeuzera coffea* (Lepidoptera: Cossidae)) and coffee ring borer (*Meroleptus cinctor* (Coleoptera: Curculionidae)) featured prominently in the survey. Sooty mould was patchy in the smallholder gardens. Ants present in the coffee plots were predominantly species of *Anoplolepis*, but *Pheidole*, *Oecophylla*, *Technomyrmex* and *Iridomyrmex* spp. were also present. *Oecophylla* spp. posed considerable hindrance during harvesting. Coffee leaf rust (*Hemileia vastatrix*), pink disease (*Corticium salmonicolor*) and thread blight (*Corticium koleroga*) diseases were present. Farmers were unable to differentiate between other coffee diseases and coffee leaf rust. Very little chemical control was used against pest, diseases and weeds. Coffee pruning was carried out to improve access to gardens for harvesting and weeding. Most farmers were reluctant to recycle the coffee after the productive years, but the trees were maintained on multiple stems for sustainability of yield and income. Past extension efforts appear to have yielded no improvement in coffee pest and disease management in smallholder gardens located in remote sites.

## Introduction

An understanding of farmers' perceptions of pests and their existing pest control methods can assist in developing effective integrated pest management (IPM) strategies. IPM promotes conservation of the natural enemies of pests (predators and parasitoids) and growing of pest tolerant varieties. It emphasises hygienic cultivation, and recommends mechanical control and the use of chemicals as a last resort (Dent 1991). Successful rice IPM technologies in Thailand reduced pesticide use by 60–79% in a single year, and increased rice yield by about 10%, with a financial benefit of up to US\$56/ha (Abdus et al. 1991). The current world trend is to accelerate agricultural production by reduced reliance on pesticides, and

growers are encouraged to adopt IPM techniques. Crop production and pest control in Papua New Guinea (PNG) already relies heavily on the use of chemicals, biological control agents and use of pest resistant varieties. There is potential, however, to develop IPM programs for agricultural crops, based on a better understanding of the biology of pests, population dynamics and economic damage levels, and the use of combinations of cultural, physical and chemical controls. So far, farmer adoption of IPM techniques is rare, which may reflect limited success to date from implementation of coffee IPM strategies in the country.

Smallholder coffee farming systems in the highlands have been described by Carrad (1982) and Bourke (1985), and coffee management in low input/high output systems by Harding et al. (1986). Fertilisers, pesticides and fungicides are almost never applied and systematic pruning schedules are never

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followed. Plots are manually weeded occasionally. Yields vary considerably between growers and in response to price fluctuations (Harding et al. 1986) but are usually less than 0.5 t green bean/ha. Pest and disease incidence vary widely from region to region and depend on coffee management input. Coffee leaf rust (CLR, *Hemelea vastatrix* Berk. and Broome) and coffee green scale (*Coccus celatus* De Lotto and *C. viridis* Green) can severely reduce production.

Following an outbreak of CLR disease in 1986, considerable extension efforts were made by the Coffee Industry Corporation Ltd (formerly the Coffee Development Agency) to improve the management of smallholder coffee. This included dissemination of information not only on control of CLR, but also on plant rehabilitation, fence construction, drainage improvement, shade control, pruning, weed control and application of fertilisers. Farmers still lacked knowledge on disease epidemiology and costs of damage and control, however. Information on the impact of green scale insect pest on coffee has not been actively extended to farmers.

Farmers have been resistant to change and to adopt new production technologies, possibly partly because they feel they cannot afford to. This has contributed to the low uptake of pest and disease technologies extended to farmers in rural, remote areas. This paper describes coffee pests, pest perceptions and management practices of smallholder coffee farmers in the Boana district, Morobe Province, PNG.

## Methods

Field days were convened at Boana (900 m a.s.l.) on 13–15 March 2001 to train local farmers on IPM of green scale. Coffee at Boana is planted in small blocks of 100–3000 trees.

General knowledge on coffee husbandry and analysis of green scale was evaluated by interviewing 45 smallholder coffee farmers from four census divisions of Nawab district and from five council divisions in the Erap and Wain circuits. The interviews were conducted by scientists from the Coffee Research Institute.

Site visits to assess smallholder coffee husbandry practices and to provide site advice on appropriate methods were conducted on the following day. Pest and disease data were also collected.

## Results

### General coffee husbandry

Some 80% of farmers owned between 500 and 1500 coffee trees and the rest 1600–3000 trees. Coffee is planted on moderate to very steep slopes, along river banks and narrow fertile valleys. The area receives, on average, 300 mm rainfall per month and more than 3000 mm annually.

Arusha, Mundo Nova, Blue Mountain and Catimor coffee varieties, either alone or mixed, were planted in each plot. Blue Mountain was introduced in the early 1950s and some stands of early plantings were maintained as multiple stems by 40% of the farmers. Catimor, introduced in 1995, was found in 60% of gardens, planted between Blue Mountain.

Coffee was grown under medium to heavy shade. The main shade species were *Leucaena* and dwarf *Albizia*. Weed problems were minimal. Most farmers lacked knowledge on coffee pruning practices and the importance of shade. Pruning was performed predominantly to access coffee gardens for harvesting or weeding. Almost all the farmers were willing to rehabilitate their coffee upon site supervision by extension officers. They maintained their coffee on multiple stems to sustain yield and income annually. However, a relatively high number of farmers did not see any link between pruning and pest and disease control.

Narrow-leaf annuals and perennials were the major weeds encountered. The majority of farmers weeded manually each month. A few farmers used herbicides for weed control. Fencing and drainage of coffee gardens were uncommon.

### Pests and diseases

The majority of farmers correctly identified symptoms of CLR on their coffee, but lacked knowledge on sampling methods to assess the severity of damage. Nevertheless, severely infected trees were removed from the garden, and debilitated and weak branches were pruned.

Pink leaf and thread blight diseases were present, but farmers had little knowledge of the symptoms and associated these with dieback of coffee trees. Generally, farmers pruned and discarded affected twigs and trees at the garden edges.

Coffee ring borer (*Meroleptus cinctor* (Coleoptera: Curculionidae)) and coffee centre borer (*Zeuzebra coffea* Nietner (Lepidoptera: Cossidae)) insects received considerable attention from the farmers but

green scale was of little concern, though severely affected trees were removed.

### Yield pattern

Farmers did not relate coffee flowering and yield to weather patterns. An unusual weather pattern was reported for the 1997 drought period. Most coffee survived the drought and in 1998 a bountiful crop was harvested. Annual green bean yields were 0.1–0.4 t/ha.

### Green scale

The majority of farmers lacked knowledge of green scale and how to control it. They reported sooty mould and ant incidences in their gardens but failed to relate sooty mould and ants to green scale. All farmers reported patchy distribution of sooty mould in coffee gardens, but expressed concern about a number of ant species. Five ant species in the genera *Pheidole*, *Oecophylla*, *Anoplolepis*, *Technomymex* and *Iridomyrmex* were present in the coffee plots. *Anoplolepis* sp. was the most abundant ant. *Oecophylla* sp. causes considerable hindrance during harvesting.

Chemical spraying of sooty mould, ants and green scale was uncommon, but affected trees were removed by pruning.

Several coffee gardens were planted at 2 m × 1.5 m spacing under *Leucaena*. Dwarf *Albizia* were planted closer to the garden edge. Coffee planted without shade showed nutrient deficiency with yellowing of leaves, fewer leaves and higher numbers of black cherry beans on heavily bearing branches.

There was a low incidence (4.2%) of green scale prevalent on unshaded coffee. Sooty mould infestation was patchy and minimal. The incidence of ants (18%) was low. A number of predatory ladybirds (9.7%) were present.

There was significantly positive correlation ( $p < 0.05$ ,  $r^2 = 21\%$ ) between ants and green scale on coffee under shade. The correlation between ladybird predators and green scale incidence was not significant.

Soil cover of mulch and coffee litter under shaded coffee was 38.9%. Under this soil cover, the ant population is expected to be low. An edge effect on green scale was observed on the east and west of shaded coffee.

Low numbers of leafhoppers (family Cicadellidae) were recorded in coffee trees on the garden edge. An unidentified white fungus was recorded on several insects.

## Discussion

Coffee centre borer, coffee ring borer and ants were major concerns raised during the interviews of smallholder coffee farmers. Coffee leaf rust was also mentioned by a number of farmers. Green scale was not perceived as a problem because farmers did not know about this insect. The relatively low level of green scale incidence, and the relationship between ladybird beetles and green scale in spite of the presence of ants, indicates distinct food web interactions in the coffee-growing ecosystem. There were relatively low incidences of coffee centre borer, coffee ring borer, sooty mould, coffee leaf rust, pink disease and thread blight in smallholder plots.

The results suggest that the green scale population is stable in smallholder coffee gardens under medium to heavy shade, which provides an environment suitable for the natural enemies of green scale.

The condition of coffee trees from the sample did not correlate with the response from farmers to ants, although five species of ants were recorded. An upsurge of a fast-moving ant resembling *Anoplolepis* sp. was said by farmers to interfere with coffee growing and other gardening activities. The drought of 1997 had a profound impact on the environment, providing suitable breeding sites for ants.

Weed, pests and diseases were not well managed. Most farmers could not relate weather patterns to pest and diseases, weeds and even pruning of coffee. However, irregular pruning of suckers and vegetative growth were carried out and, in a few gardens, pegging of lung branches to the ground for cherry harvesting was observed. Farmers had little knowledge of pruning for pest and disease control. This was also reflected in very low understanding of shade management and yield. Most farmers could not correlate shade management and maintenance pruning each coffee season. Shade management and maintenance pruning were essential for promoting flowering and thus maintaining yield for the next season.

There was evidence that Catimor coffee was widely grown in the area. Of all the farmers interviewed 80% of them were Catimor growers. Farmers described Catimor as bearing both in and out of season while Blue Mountain produces seasonally. All farmers claimed Catimor to be higher yielding than Blue Mountain and most could not wait two years to harvest their coffee. As Catimor was widely grown and is resistant to CLR, farmers were largely

unconcerned about this disease. Severely affected trees were usually pruned.

Farmers generally had only a vague knowledge of coffee pests and diseases. Their diagnosis of pests and diseases is traditional and derived from general symptoms that occur on related crops. Unusual dieback of coffee, drooping and wilting foliage were common descriptions in their answers to questions about the symptoms and diagnostic features of pests and diseases. The positive correlation between ants and sooty mould is comparable to the positive correlation between green scale and ants since both responses are directly associated with green scale. Sooty mould develops from and ants are attracted to the honeydew secreted from green scale (Apety 1998). Nearly all the farmers did not carry out chemical control. They were ignorant of the types, use and safety of chemicals. Chemical weeding was carried during the smallholder pest management project's five-year lifespan. However, few farmers showed concern about chemical contamination of water. Further use of agricultural chemical would pose a considerable danger to smallholder farmers at Boana unless they received further training.

Farmer perceptions of pest and disease problems on coffee and their control vary from region to region. Coffee production is higher in the highlands region than in coastal areas and extension and information dissemination on pests and diseases has, in the past, favoured regions with high production statistics. This has partly contributed to lack of awareness on pests and diseases in Boana area.

## Conclusion

Farmer awareness of pests and diseases and their control seem to be sporadic. The smallholder practice of intercropping coffee and shade trees has significant impact on green scale control. The incidence of coffee leaf rust and other diseases was relatively low. Under these circumstances, the use of pesticides is not recommended. This has been the practice in the area. Natural enemies including ladybird beetles will deliver good control if disruption to their activity is minimised. Green scale outbreak is likely to escalate quickly in the area if suitable conditions permit since

there is a serious lack of knowledge about this pest among farmers.

It is apparent that farmers generally had scant knowledge of coffee agronomy including soil and plant nutrition, pests and diseases, and processing. The situation is similar in other smallholder coffee growing areas (Matei, pers. comm.). Farmers need training in IPM. The farmer participatory approach is an important option to undertake if the current method of information transfer has not delivered benefits. This approach also promotes farmer participation in research and extension essential for the development and implementation of IPM.

## Acknowledgement

I am grateful to scientists and technical officers who worked with farmers to complete the questionnaires. Thanks are also due to the Small Support Services Facility in Morobe for funding the coffee IPM field day at Boana, Morobe Province and facilitating the Coffee Industry Corporation's involvement.

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# **Diseases and management**

# Evaluation of fungicides against potato late blight disease (*Phytophthora infestans*) on susceptible and tolerant potato varieties

S. Hariki<sup>1</sup>

## Abstract

Seven fungicides were tested against potato late blight disease on Sebago (tolerant) and Sequoia (susceptible) potato varieties. The fungicides tested included four copper-based compounds (copper oxychloride, copper Nordox, Kocide and Champion) and three based on chlorothalonil (Banis, Echo and Barrack). All chlorothalonil products were found to be more effective than the copper products in controlling the disease on both varieties.

## Introduction

Late blight disease, caused by *Phytophthora infestans*, is a serious disease of potato (*Solanum tuberosum* L.) that can reduce the potato yields (Harrison 1992; Pitt and Wicks 2003; www.montcalm.org). In the USA, potato late blight (PLB) disease was not described until it caused reduction in yield and losses of stored tubers in 1843 (Harrison 1992). In western Europe in 1845 the disease caused the Irish potato famine. Bordeaux mixture—made up of copper sulfate, hydrated lime and water—was initially used to control the disease (Montcalm County Community Center 1999).

Major economic losses caused by the disease were reported from the main potato-growing areas of Papua New Guinea (PNG) in January and February 2003 by Pitt and Wicks (2003), who confirmed the presence of the disease in the country. Sequoia has been the dominant variety planted by people growing potatoes at altitudes between 1800 and 2700 m in PNG. This variety was severely affected by late blight

disease. The disease was new to the country and potato farmers were unaware of control measures.

The aim of this study was to evaluate the efficacy of seven protectant fungicides against PLB disease under PNG conditions, and the yield response from its use. The fungicides included four copper-base compounds (copper oxychloride, copper Nordox, Kocide and Champion) and three based on chlorothalonil (Banis, Echo and Barrack), all available locally. The fungicides were tested in Sequoia (susceptible) and Sebago (tolerant) varieties of potatoes.

## Materials and methods

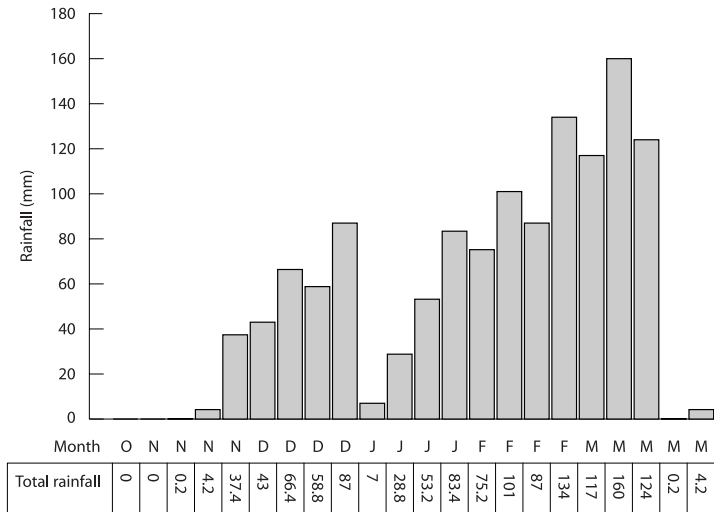
The experiment was laid out in a split plot design. There were seven levels of treatments in the main plots, with the two varieties Sequoia and Sebago as the subplot treatments. Each treatment was replicated four times. The subplot size was 32 m<sup>2</sup> and the total experimental area 1792 m<sup>2</sup>.

There were four rows with 25 tubers per subplot planted 10 cm deep. Plant-to-plant spacing was 40 cm and rows were 80 cm apart. The fertiliser used was potato mix (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O:MgO:B 10:25:12:2.5:0.3) applied at 1200 kg/ha.

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The spray program commenced immediately after the crop emerged. Spraying was done every 5–7 days with 15 L knapsack sprayers under high-pressure (3 bars/15 psi) with hollow cone nozzles. The fungi-

cides were mixed and applied at the rates as shown in Table 1. A wetting agent (Chemwet 600 (a.i. 60% non-ionic ethoxylates, Chemica Ltd) was added to the spray mixture at the rate of 5 mL/L.



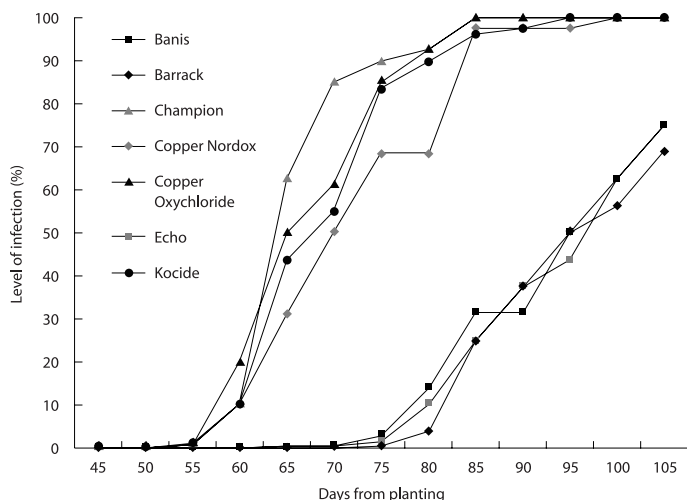
**Figure 1.** Weekly rainfall data collected at the National Agricultural Research Institute research station, Tambul, Papua New Guinea

**Table 1.** Fungicide mixing rates

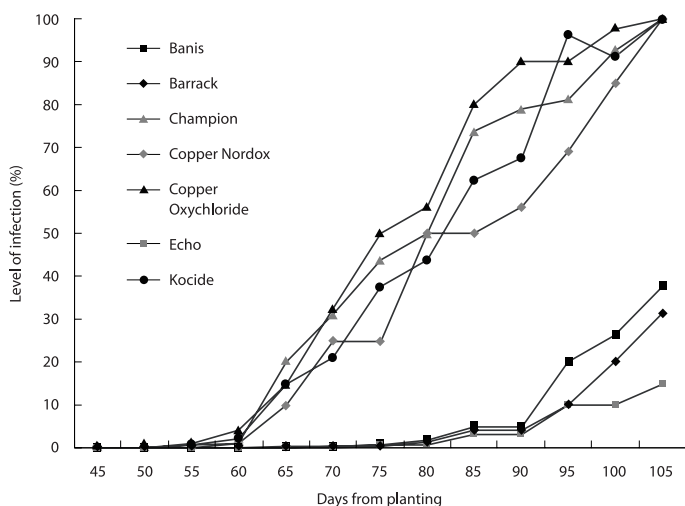
Fungicide	Active ingredient	Recommended application rate	Tank mixture/15 L
Copper Nordox	Copper	3 kg/ha	45 g
Copper oxychloride	Copper	3 kg/ha	150 g
Kocide	Copper	2.2 kg/ha	30 g
Champion	Copper	2.5 kg/ha	40 g
Banis 400	Chlorothalonil	4.5 L/ha	45 mL
Echo 720	Chlorothalonil	1.7 L/ha	25 mL
Barrack 720	Chlorothalonil	1.8 L/ha	25 mL

**Table 2.** International Potato Centre modified rating system for blight

Rating (%)	Plant condition
0.01	2 to 5 leaves per 10 plants affected, about 5 large lesions per quadrant
0.1	5 to 10 infected leaflets/plant OR about 2 affected leaves/plant
1.0	General light infection, about 20 lesions/plant OR 10 leaves affected; 1 in 20 leaves affected severely
5.0	About 100 lesions/plant, 1 in 10 leaflet affected (up to 50 leaves affected)
25	Nearly every leaflet infected but plants retain normal form; plants may smell of blight, field looks green although every plant is affected.
50	Every plant is affected and about 50% of the leaf area is destroyed; field appears green flecked with brown.
75	About 75% of the leaf area destroyed; field appears neither predominantly green nor brown.
95	Only a few leaves on plants, but stem are green.
100	All leaves dead; stems dead or dying.



**Figure 2.** Effect of fungicides on incidence of potato late blight disease on Sebago potatoes.



**Figure 3.** Effect of fungicides on incidence of potato late blight disease on Sequoia potatoes.

Plants were sprayed to run-off and on both sides of the leaves and the stems. The spray volume applied to each subplot was measured.

The level of disease in each subplot was assessed weekly using the International Potato Centre modified blight rating system (Table 2). Disease assessment was made on all plants in each subplot. The categories of tubers harvested were: marketable tubers (>100 g), seed size tubers (20–99 g), mini-tubers (<20 g) and infected tubers. Rainfall data (Figure 1) were obtained

from the weather station at the National Agricultural Research Institute’s Tambul station. The data were analysed using Genstat.

## Results

The rate of emergence of Sequoia was very poor (40.2%) during the trial. There was a high incidence of *Erwinia* and *Rhizoctonia* rots.

No or very little disease infection was observed in the first 6–7 weeks (50–55 days; Figures 2 and 3). After 55 days, disease increased exponentially to 100% after 85 days on Sebago potatoes treated with copper-based fungicides (Figure 2). The disease increased slowly on Sequoia potatoes, reaching a maximum after 105 days (Figure 3).

Infection was first recorded on cv. Sebago treated with chlorothalonil-based fungicides after 55–60 days, then increased slowly to 75–80 days, after which the disease increased exponentially.

The yields (Tables 3 and 4) were lower than expected. There were significant differences between the copper and chlorothalonil fungicides but not between the individual formulations of copper or chlorothalonil.

## Discussion

The low plant density of Sequoia was due to wet conditions experienced at the time of planting in mid-November and the high incidence of *Erwinia* and *Rhizoctonia* throughout the trial period, which also affected yield (Stevenson et al. 2001).

The onset and build-up of disease was delayed on both varieties by about 20 days on potatoes treated with chlorothalonil compounds. Slower disease onset and a lower rate of disease increase on potatoes with chlorothalonil treatments implies that there is a potential to obtain higher marketable yields.

In conclusion, the chlorothalonil compounds gave better disease control. Further evaluation of these chemicals on the potato varieties is warranted to ensure a practical economical analysis of their use.

**Table 3.** Yield of Sebago tubers following treatment with various fungicides

Fungicide type	Marketable tubers (> 100 g) (kg/ha)	Seed tubers (20–99 g) (kg/ha)	Mini-tubers (<20 g) (kg/ha)	Damaged tubers (kg/ha)	Total (kg/ha)		Total yield (kg/ha)
					Undamaged	Damaged	
Copper-based compounds							
Copper Nordox	2910	5.0	2.8	4.5	10.8	4.5	15.3
Copper oxychloride	4190	6.9	5.4	2.6	16.5	2.6	19.0
Kocide	3270	4.2	3.8	2.9	11.3	2.9	14.1
Champion	3770	4.6	4.6	4.1	12.9	4.1	17.0
Chlorothalonil-based compounds							
Banis	4110	3.6	2.8	13.8	10.5	13.8	24.3
Echo	2220	3.5	2.7	14.9	8.4	14.9	23.3
Barrack	3440	3.7	3.7	12.2	10.9	12.2	23.1

**Table 4.** Yield of Sequoia potato tubers following treatment with various fungicides

Fungicide type	Marketable tubers (>100 g) (t/ha)	Seed tubers (20–99 g) (t/ha)	Mini-tubers >20 g) (t/ha)	Total (t/ha)		Total yield (t/ha)
				Undamaged	damaged	
Copper-based compounds						
Copper Nordox	0.7	0.8	0.6	2.1	1.6	3.7
Copper oxychloride	0.4	1.1	0.7	2.2	1.1	3.3
Kocide	2.2	2.6	0.5	5.2	1.2	6.4
Champion	0.8	1.0	0.7	2.5	1.7	4.1
Chlorothalonil-based compounds						
Banis	1.1	0.6	0.5	2.2	6.8	9.0
Echo	2.0	0.8	0.6	3.4	5.4	8.8
Barrack	1.9	0.9	0.5	3.4	8.8	12.1

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# Preliminary assessment of two inorganic copper-based fungicides against late blight (*Phytophthora infestans*) in the tropical highlands of Papua New Guinea

D. Minemba<sup>1</sup> and P. Sovo<sup>2</sup>

## Abstract

Copper Nordox and Kocide were evaluated on sequoia potatoes in a farmer's garden to test their effectiveness against the potato late blight disease (*Phytophthora infestans*) under highland environmental conditions near Kagamuga in Mount Hagen, Papua New Guinea. Disease infection in treated plots remained below 1% in the first 5 weeks, while infection in untreated plots reached 100% infection by the 5th week. A yield of 6.4 t/ha was obtained after both fungicide treatments.

## Introduction

Copper Nordox<sup>®</sup> (cuprous oxide) and Kocide<sup>®</sup> (cupric hydroxide) are inorganic copper fungicides used as protectants for control of a range of fungal diseases. They were initially used in Papua New Guinea (PNG) for control of coffee leaf rust disease (*Hemileia vastatrix* Berk. and Br.). These fungicides are also used to control late blight disease *Phytophthora infestans* (Mont) de Bary in potatoes.

The two fungicides act by killing spores and their germlings on the plant surface before infection occurs (Drenth 2001), and are effective only when they are applied frequently and coverage of the foliage is complete. Thus, at each application, protectant needs to be applied ensuring that new foliage is adequately protected and that fungicide residues

that have been lost due to weathering are replaced. The efficacy of fungicides used in the management of late blight on potato crop is affected by the timing of the initial fungicide application, the interval between fungicide applications, the rate of fungicide applied and the extent of fungicide coverage throughout the plant canopy.

In PNG, *P. infestans* was first recorded in the Sirunki Valley of Enga Province in mid January 2003. Within a few months it had spread to all potato-growing areas in the highlands. The introduction of *P. infestans* has severely affected the potato industry in PNG, an industry that is worth about 15 million PNG kina per year (Pitt and Wicks 2003).

The objective of the trial reported here was to evaluate the effectiveness of Nordox<sup>®</sup> and Kocide<sup>®</sup> copper fungicides against late blight in the potato crop grown under highlands environment conditions in PNG.

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## Materials and methods

### Trial site

The trial was conducted in newly planted potato plots of cultivar Sequoia in a farmer's field. The field was located about 2 km west of Kagamuga airport in Mt Hagen. Two equal-sized plots, each of area 160 m<sup>2</sup>, were selected from 30-odd plots. Each plot was equally divided into treated and control subplots. The plants were at the 2–3-week-old stage and were free of disease symptoms. Plants were spaced 40 cm apart within rows and 65 cm between rows.

The treatments could not be replicated as all the other plots were infected.

### Fungicide application

The fungicides were applied at high volumes (1000 L/ha) at a rate 3 kg/ha (3 g/L) of Copper Nordox and 2.2 kg/ha (2.2 g/L) of Kocide. Spraying was done at 7-day intervals for 8 weeks using a knapsack sprayer (CP15) with hollow cone nozzles. Chemwett wetting agent was added at a rate of 2 mL/L. Sprays were applied to run-off stage. The remaining fungicide mixture was recorded after each spray application.

### Disease assessment

Potato blight was assessed using the International Potato Centre (CIP) modified blight rating system, as shown in Table 1.

The assessments were made before the first application of fungicides and every 7 days thereafter for 8 weeks. Disease was assessed on 10 plants randomly

selected using a W-shaped transect in treated and untreated plots.

### Other assessments

A portable rain gauge was set up at the trial site to record the rainfall during the trial period. The trial was harvested after 97 days and tubers were grouped into marketable tubers, seed-size tubers, mini tubers and infected tubers before weighing.

## Results

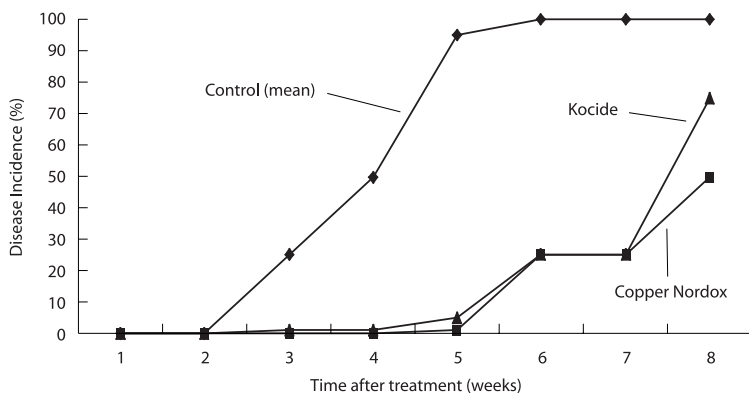
Figure 1 shows that disease incidence in the untreated plots began to increase after the second week of spray application to treated plots, and reached 100% infection after 21 days. In the treated plots, the disease was controlled until after the fifth week of application when the level of infection started to increase. The potato plants were more than 21 days old when the spray program started. In the fifth week of spray application, the potato plant were 56 days old. This is the period when potato plants start to flower with full foliage cover. The spray program ceased after the eighth week.

The weather was dry during the trial period, with below-average weekly rainfall for the Kagamuga area (Figure 2)

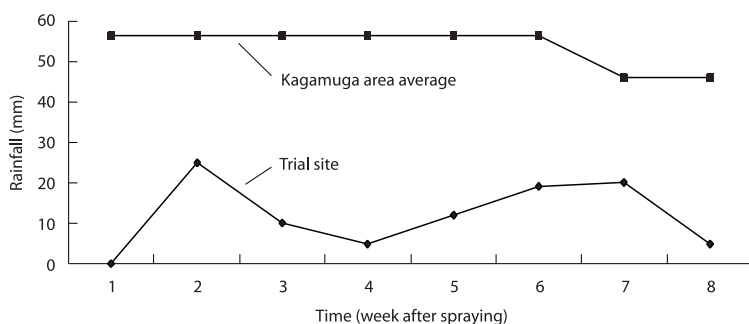
The total yield produced in the fungicide treated plots was 6.4 t/ha (Table 2), while yield in the untreated plots was less than 1 t/ha. There was no difference in yield between the two fungicides. Plots treated with Copper Nordox produced more marketable tubers than Kocide-treated plots, the latter producing more seed tubers.

**Table 1.** International Potato Centre modified rating system for blight

Rating (%)	Plant condition
0.01	2 to 5 leaves per 10 plants affected, about 5 large lesions per quadrant
0.1	5 to 10 infected leaflets/plant OR about 2 affected leaves/plant
1.0	General light infection, about 20 lesions/plant OR 10 leaves affected; 1 in 20 leaves affected severely
5.0	About 100 lesions/plant, 1 in 10 leaflet affected (up to 50 leaves affected)
25	Nearly every leaflet infected but plants retain normal form; plants may smell of blight, field looks green although every plant is affected.
50	Every plant is affected and about 50% of the leaf area is destroyed; field appears green flecked with brown.
75	About 75% of the leaf area destroyed; field appears neither predominantly green nor brown.
95	Only a few leaves on plants, but stem are green.
100	All leaves dead; stems dead or dying.



**Figure 1.** Incidence of late blight on fungicide-treated and untreated plots



**Figure 2.** Rainfall recorded at the trial site (2 April–14 May 2003) compared with average weekly rainfall at Kagamuga

## Discussion

The results indicated that both Copper Nordox and Kocide controlled late blight in the first 5 weeks under the trial conditions. The failure to control late blight after this period is probably due to inadequate coverage allowing infection to occur. Gudmested (quoted in Drenth (2001)) indicated that microclimates favourable for development and infection of *P. infestans* are most likely to occur in the lower plant canopies where

deposition of fungicides is most difficult. Success in managing late blight disease will therefore largely depend on the timing of initial fungicide application, the application frequencies, fungicide application rates and the extent of fungicide coverage throughout the plant canopy. The total yield of treated plots was below the average yield of 18–34 t/ha for Sequoia, the common variety grown in PNG (Wiles 2000) but better than the untreated controls. This may have been due to poor disease control after week 5.

**Table 2.** Yields in fungicide treated and untreated plots of sequoia potatoes

Treatment	Total yield (t/ha)	Marketable tubers (t/ha)	Seed tubers (t/ha)	Mini-tubers (t/ha)
Copper Nordox	6.4	2.7	3.0	0.5
Kocide	6.4	1.6	4.1	0.5
Unsprayed control	0.5	0.0	0.3	0.2

## Recommendations

The preliminary results suggest that effective late blight management under highlands environment in PNG may be achieved by the implementing the following procedure:

1. Application of copper-based fungicides or any other protective fungicides should commence immediately after emergence.
2. Subsequent fungicide applications must be at intervals of 5 days for wet areas and 7 days for dry areas.
3. Under PNG conditions the recommended application rates are 3 kg/ha for Copper Nordox and 2.2 kg/ha for Kocide.
4. When applying protective fungicides all surfaces of the foliage must be thoroughly covered. The fungicide residues must not only cover individual leaflets and leaves, but also be present at a high enough concentration to be effective.

Further work including other fungicides that have curative and systemic activity in properly replicated trials is required to substantiate these findings.

## Acknowledgments

We thank Mr Martin Gunther (ACNARS project) for invaluable support and advice, Dr Geoff Wiles for sourcing funds, the staff of the Fresh Produce Development Company and the National Agricultural Research Institute in Hagen, and Mr John Konts for allowing us to use his garden for this trial.

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# Improvement of compost mounding systems

R. Raatsch<sup>1</sup>

## *Abstract*

Wide parts of the Papua New Guinea highlands are threatened by frost. The last heavy frost occurred in 1997 and destroyed a large part of the food base of the population in these areas. Consequently, a program to improve food security and farmers' incomes for high altitude areas of Papua New Guinea began in 2000, funded by the European Union. Improvement of traditional compost mounding systems, is one of the objectives under points 7.1 and 7.2 of the main program. This paper highlights the studies to be undertaken in this project.

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# Development of management strategies for ratoon stunting disease in sugarcane at Ramu Sugar, Papua New Guinea

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## Abstract

Ratoon stunting disease (RSD) of sugarcane is caused by the bacterium *Leifsonia xyli* s.sp. *xyli*, and is a major problem in many sugar industries worldwide. This pathogen was recently detected in commercial crops at Ramu Sugar, Papua New Guinea (PNG). Data from an estate-wide survey indicated 84% of the samples taken tested positive to RSD. In 2004, crop losses were estimated to be at least 15%, with a monetary value of 14.2 million PNG kina (K). Management strategies for RSD have been developed and these will take 5 years to be fully implemented. The program will cost K0.5m in the first year, and around K0.3–0.4m in subsequent years

## Introduction

New Guinea is the centre of diversity for several *Saccharum* species, including the ‘original’ sugarcanes belonging to *Saccharum officinarum* L. (noble cane), the domesticated vegetable sugarcane *S. edule* Hassk. (‘pit pit’), and the ‘wild’ cane *S. robustum* E.W. Brandes & Jeswiet ex Grassl. Extensive stands of wild canes grow along rivers and roadsides, and domesticated chewing and vegetable canes are grown in village gardens. High pest and disease pressures on the Ramu Sugar estate result from their build-up on the widely occurring *Saccharum* species growing close by.

Commercial hybrid varieties were introduced into Papua New Guinea (PNG) during 1960–1980, with a view to establishing a PNG sugar industry. The planting of commercial fields began in 1980 in the

Ramu Valley, with the first commercial harvests made in 1982. Ramu Sugar Limited operates the commercial estate located at Gusap in Madang Province. Over 8500 ha of sugarcane crops produce around 500,000 t of cane to make 48,000–50,000 t of sugar. About 2.5 million litres of ethanol are also produced, mainly for export.

With the monoculture of hybrid sugarcane over an extensive area, the Ramu Sugar estate has predictably had problems with outbreaks of endemic pests and diseases. The import of sugarcane from other cane-growing countries has also led to the introduction of several major ‘exotic’ diseases. In the early 1990s, Ramu Sugar initiated a breeding program to develop local varieties with a higher level of resistance to diseases endemic in the area.

Endemic diseases have had a significant impact on commercial production at Ramu. In 1985–86, the then unknown disease, Ramu stunt, severely affected the widely grown variety Ragnar, causing heavy yield losses and the near collapse of the PNG sugar industry (Eastwood 1990). Downy mildew, caused by *Peronosclerospora sacchari*, has caused ongoing yield losses and led to the discard of a number of

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high-yielding varieties. Since production first began, leaf scald (caused by *Xanthomonas albilineans*) and ratoon stunting disease (RSD; *Leifsonia (Clavibacter) xyli* s.sp. *xyli* (Davis et al.) Evtushenko have appeared and are also affecting sugarcane production. This paper gives details of the RSD epidemic at Ramu Sugar, its impact on sugar production and the management strategies that have been implemented.

## Ratoon stunting disease

### Causal organism

The causal organism is a small, slender bacterium *Leifsonia xyli* s.sp. *xyli* that can be grown only on a complex artificial medium.

### Distribution of the disease

The disease occurs in all cane-growing areas of the world and until recently was absent from PNG. The disease was recently detected in commercial crops at Ramu Sugar, PNG. RSD is initially spread by the use of diseased planting material and subsequently by cutting implements such as mechanical harvesters, bush knives and anything else that leads to the transfer of infected vascular extract to the cut surface of healthy cane stalks or leaves. Volunteers from the previous crop may harbour the disease and lead to a scattering of diseased stools in newly planted crops. Mechanical harvesters then spread the disease through ratoon crops.

### Symptoms and identification

The only external symptoms are a general stunting and unthriftiness in diseased plants, similar to those caused by poor cultural practices, inadequate moisture, poor soils, lack of fertiliser or other stresses. Variation in growth between healthy and diseased stools in partly diseased fields, or between badly stunted and less-affected stools where there is 100% infection, frequently gives a characteristic irregular 'up and down' appearance to infected crops. Two types of internal stalk symptoms may be found associated with the disease; one is a discolouration (which varies from yellow, orange, pink, red to reddish brown) of individual vascular bundles in the nodes of mature cane, and the other a general pink colour or 'pink blush' throughout the nodes of very young cane.

The discoloured vascular bundles can be seen at the base of the nodal tissue when a reasonably mature diseased stalk is sliced longitudinally with a sharp knife. They are first seen just below the rind as small dots. As slices are made more deeply into the stalk, they appear in the shape of dots, commas and various forms of straight or bent lines up to 3 mm long, depending on the angle at which the vascular bundles are cut. In transverse sections made at about the level of the wax band, the discoloured bundles are seen as small spots throughout the node with streaks in the leaf traces radiating from near the centre of the stem. For a diagnosis to be reasonably reliable, the discoloured vascular bundles should occur throughout the node and in virtually all the fully developed nodes of a stalk. Symptoms are generally better developed at the base of mature stalks.

Healthy cane, especially in some varieties, can show discoloured vascular bundles closely resembling those of RSD, while other varieties show no nodal symptoms even when they are diseased. A positive identification of the disease can be made by examining a vascular extract under electron or phase-contrast microscopy (PCM) for the presence of the bacteria.

The standard procedure for examination of bacteria with PCM involves the following steps:

1. The vascular fluid is extracted by exerting positive air pressure to one end of a stalk piece, and collecting the fluid emanating from the other end with a pasteur pipette. Stalk pieces should be from the base of a stalk; pipes in the stalk must be plugged with plasticine or a pencil to maintain air pressure.
2. A small drop of the extract is placed on a clean slide and covered with a coverglass.
3. A drop of immersion oil is added to the coverglass, and the extract is examined for the RSD bacteria at 1000× magnification using phase-contrast optics.

The RSD bacteria are thin rods (0.25–0.5 × 1–4 µm), which often appear bent or occasionally Y-shaped because of the budding process.

The detection of RSD in a field depends on the number of stalks sampled and the sensitivity of the diagnostic technique. The stalk-slicing technique practised by experienced people can be quite accurate, but the presence of varieties that show no nodal markings when diseased, or the occurrence of false positive markings, makes accurate diagnosis with this method very difficult. Serological-based assays with detection sensitivity equal to that of PCM have

been developed, and some of these are used routinely to detect the pathogen in countries such as Australia. The greater the number of stalks examined by PCM, the greater the probability of detecting the disease in a field with less than 100% disease incidence. Detection can be improved by selecting the largest stalk in the weakest stools (probably poorly grown because of RSD), stalks of volunteer cane or canes showing nodal markings. At least 10–20 stalks need to be examined to have any chance of detecting RSD in a field with 10% diseased stools.

### **Disease transmission**

Diseased planting material from nurseries is an important means of spreading RSD. In diseased crops, the base-cutter and the spray of juice from the chopper box and extractor fan of harvesters can spread the disease to cut stubble, thus increasing disease levels in already infected crops. The harvester, if not adequately disinfected before entry, may also carry the disease into clean crops.

Harvesters used to cut plants for billet planters, plant cutting machines and cane knives can all readily transmit the RSD bacterium to healthy cane.

The disease is readily transmitted by dipping freshly cut ends of setts in juice extracted from diseased plants, or by applying this juice to the cut surface of a stalk decapitated above the growing point, as for leaf scald. The disease does not appear to spread readily in the field by natural means.

### **Economic importance**

The effects of RSD are a general reduction in yield, the extent of which depends on the variety planted and weather conditions. Losses can be very severe during droughts, but they can be reduced considerably by regular irrigation. Sugar content is usually not affected unless death occurs. The slow ratooning of infected crops, particularly during dry weather, allows weeds to become established. Some varieties may fail to ratoon with the disease, but this is unusual.

### **Control**

Infected planting material can be treated to eliminate RSD by dipping infected stalks for 3 hours in hot (50°C) water. Temperature control needs to be exact to ensure the pathogen is killed but the cane is not exposed to lethal temperatures. Disease-free seed

plots are a good way of providing commercial disease-free planting material but extra care is needed to ensure the disease-free plots never become infected. The sterilisation of all cutting implements used in the plots, or in commercial disease-free crops, is essential.

The recommended method for disinfecting machinery is to thoroughly clean off all dirt and organic material, spray with the recommended rate of benzalkonium chloride (cane knife steriliser) and leave for 5 minutes. In harvesters, the base-cutter, throat, chopper box, extractor fans and toppers should all be disinfested. This is important when using a harvester for cutting billet plants. Once infected, it is virtually impossible to prevent spread of disease within that field. There are few tolerant varieties. They may help to reduce losses, but they do not eliminate the pathogen.

## **Circumstances at Ramu Sugar**

### **Monitoring**

A limited number of samples had been selected in previous years from sugarcane growing in village gardens and commercial crops at Ramu Sugar, but the results of the diagnoses were negative, so the record of RSD in PNG (Davis and Bailey 2000) could not be confirmed. RSD was detected in commercial cane at Ramu (Ramu Sugar Limited, internal report) only after selective sampling of commercial crops in 2002. Of the 78 samples sent and tested in South Africa, 40% tested positive for RSD. This was the first record of RSD in PNG. Further samples were taken in 2003, and duplicate samples were tested in Australia (by BSES Ltd) and South Africa. RSD was confirmed by both laboratories (using both PCM and serological techniques).

A comprehensive sampling program was undertaken, with over 700 samples taken from commercial cane on the sugar estate, and 120 samples from wild and village garden canes. Of these, 84% were positive for RSD (Ramu Sugar Limited, internal report). Apart from the commercial cane on the sugar estate, up to 20% of the wild and village garden canes were also positive (Table 1). The concentration of RSD in vascular extracts was generally high in the commercial canes, and generally lower in village and wild canes. The spread of the disease in the wild and village garden canes may be due to planting infected material and through contaminated bush knives.

## Effect on the 2004 crop

The cane yields in the first two months of harvest (late April–June 2004) were 17% above the original estimates (Figure 1). Cane yields rapidly declined as the dry season progressed, falling below the original yield estimates for July through to the last week of harvest. At harvest end, most crops yielded less than 50 t/ha, 36% below that estimated. This reduction was equivalent to 72,000 t of cane (15% or 7100 t of sugar valued at more than K14.2m). Production inefficiencies associated with harvesting a low-yielding crop, such as increased harvesting costs and lack of growth responses to fertilisers and pesticides, could not be estimated, but these costs are additional to the value of crop losses directly resulting from RSD infection.

The effect of RSD on cane yields is more severe during a dry year and this appeared to be the case during 2004. Rainfall from June to September was 40% below the long-term average. Severe moisture stress was widespread in the crops, resulting in no growth and lower final crop yields. Regrowth in harvested crops was greatly reduced, in cases leading to shoot death; lower yields are also anticipated in the 2005 crop.

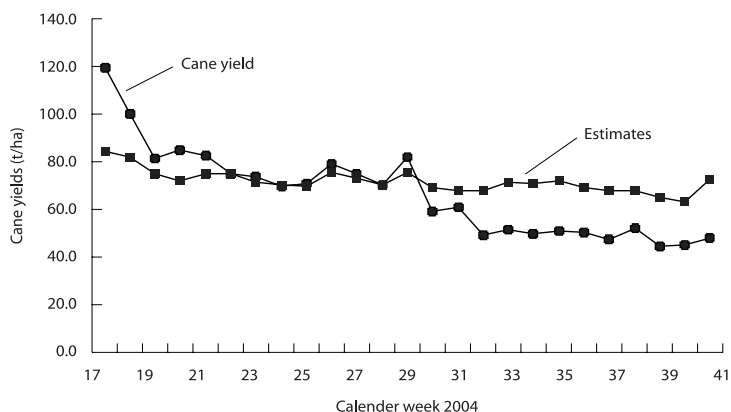
## Strategies for RSD management

Effective RSD control relies on planting hot-water treated (HWT) pathogen-free seed cane. Although most stalk pieces are rendered pathogen-free, up to 5% may remain infested when 100% diseased stalks

**Table 1.** Ratoon stunting disease (RSD) infections detected by ELISA assay in commercial, wild and village garden canes from the 2004 survey in and around the Ramu Sugar estate in Papua New Guinea

Location	Nil infection (0 wells positive)	Low (1 well positive)	Moderate (2–3 wells positive)	High (4–5 wells positive)	Percentage RSD infection
Commercial (692)	106	48	158	380	85
Ramu Estate wild cane (8)	8				0
Ramu Estate, backyard plots (31)	24		5	2	23
Sausi/Kesowai wild cane (4)	2	2			50
Sausi/Kesowai garden canes (3)	1	2			67
Kainantu wild cane (15)	12	1	1	1	20
Kainantu village garden canes (12)	9	3			25
Watarias–Lae wild cane (23)	20	2	1		13
Watarias–Lae garden cane (25)	18	7			28

Note: Numbers in brackets indicate the total numbers of samples taken.



**Figure 1.** Actual and estimated cane yields in the 2004 crop at Ramu Sugar, Papua New Guinea

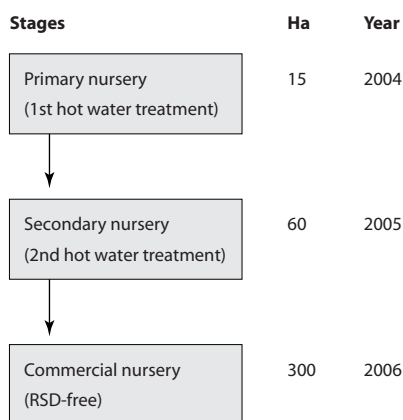


are treated. When mature stalks are produced in these initial HWT nursery plantings, a second HWT (double treatment) of stalk pieces is required to produce RSD-free seed cane.

A HWT facility costing K150,000 was built in 2004 and is currently in operation. As this is a new problem, with extra facilities required, an additional expenditure of over K350,000 is required this year for purchase of laboratory equipment, consumables and other operating costs. The cost of the program will entail new expenditure of K0.3–0.4m annually.

One of the most important issues associated with RSD control is educating growers and harvesting contractors to strictly adhere to disease-control recommendations. If procedures are followed, the disease can be kept under control and crop losses minimised.

Establishment of RSD-free seed-cane plots at Ramu Sugar commenced in October 2004. By the end of November 2004, about 15 ha will be planted with HWT cane (Figure 2). In April 2005, planting material from these plots will receive a second HWT to produce 60 ha of disease-free material. This disease-free cane will then be planted into 300 ha of commercial nurseries in 2006. In 2007, all commercial plantings (up to 1800 ha) will be under RSD-free seed cane. The process will continue in subsequent years as diseased crops are terminated and replanted. It is anticipated that up to 98% of crops will be diseased by the end of 2006. Disease levels will then decline following planting of disease-free cane in 2007, and by 2011 disease levels will fall below 5% of the cropped area.



**Figure 2.** Flow chart of hot-water treatment (HWT) to produce RSD-free seed cane for commercial planting

The main challenge for the Ramu Sugar control program is minimising disease spread into disease-free crops. This will require continual education of all staff on how the disease is transmitted, and strict adherence to hygiene procedures. The disinfecting of all harvesting and fertiliser application equipment, bush knives and minimising volunteer cane in fallow blocks will be critical for reducing disease spread.

Monitoring of disease incidence on the estate will greatly facilitate the management of RSD. A laboratory has been established and personnel trained in assays for the RSD bacteria. Mass screening for RSD will be possible using ELISA equipment that is to be purchased shortly with funds from an Australian Centre for International Agricultural Research (ACIAR) sugarcane project. Linkages between BSES Ltd (Australia) and the South African Sugarcane Research Institute laboratories will be maintained for collaborative research on this disease.

## Discussion

The RSD epidemic at Ramu Sugar has caused severe production losses valued at more than K14.2m. The company has invested up to K0.5m to set up a HWT plant and to implement strategies for disease control. Use of disease-free planting material and maintaining crops disease-free will be critical for minimising the impact of RSD on sugar production and for maximising potential profits. Monitoring disease levels in the commercial crops and seed cane will enable effective implementation of the control program. The establishment of a diagnostic laboratory at Ramu Sugar is essential to achieve this.

Resistant varieties provide a long-term control strategy but impose a reduced capacity to select commercial varieties with other desirable agronomic traits. The low concentration of RSD in village gardens and wild canes raises the possibility that these canes may have induced tolerance to the disease. Recently, Omarjee et al. (2004) found that a number of bacteria from the genus *Burkholderia*, extracted from PNG village gardens, inhibited the growth of *Clavibacter michiganensis* in culture; this species is a close relative of *L. xyli* s.sp. *xyli*. It is possible some of these bacteria are suppressing RSD in village and wild canes in PNG, but this needs to be confirmed. If *L. xyli* s.sp. *xyli* was native to PNG, the disease may have spread into commercial crops through contaminated bush knives (commonly used

by local workers) and then rapidly disseminated through the commercial cane by mechanical harvesting.

Further work is needed to show whether or not the disease is endemic.

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# The role of tissue culture in the revival of the potato industry in Papua New Guinea

V. Mero<sup>1</sup>

## Abstract

Potato late blight disease caused by *Phytophthora infestans* has virtually wiped out an industry valued at 10 million kina in the Papua New Guinea Highlands. Control methods, including fungicide sprays, have eliminated the role of subsistence farmers in the industry. Tissue culture can play an integral role in the revival and the sustainability of the potato industry.

## Introduction

Except for farmers living more than 2000 m above sea level, potato is not an important crop in the diet of Papua New Guinea (PNG) highlanders. There is, however, a great demand for potatoes in the lowlands. In 1998, about 2700 t of processing potatoes, 600 t of ware potatoes and 260 t of seed potatoes were imported. These were the amounts of potatoes that local producers could not supply to the market.

The potato industry reached 15,000 t per annum in production for urban consumption and another 600–900 t per annum for trade in seed potatoes alone. The farm-gate value of this industry reached an estimated 10 million kina (K) by 2003.

## Potato late blight

Potato late blight had a devastating affect on the potato industry. It was first detected in January 2003 in Sirunki, Enga. A survey conducted by the Fresh Produce Development Company (FPDC; Anon. 2003) revealed that most of the potato gardens in

Enga and the Western Highlands had been completely devastated by March of that year.

Evidence of the rapid movement of the disease is that, while the Eastern Highlands Province was reportedly disease-free in March 2003, it was infested by the end of April.

Since Sequoia, the main variety planted happened to be highly susceptible to potato late blight, the disease caused a serious shortfall in ware and seed potatoes. FPDC predicted that this would lead to local producers losing over K20m to overseas suppliers, due to the need to import seed and ware potatoes.

The cultural methods developed for controlling the disease involve intensive application of fungicides. Apart from the fact that cost of pesticides eliminates the subsistence farmer's role in the industry, there may be hidden costs in the use of pesticides. In Ecuador nearly all potato-farming families have known of, or experienced, some degree of pesticide poisoning. Poor personal hygiene and poor handling practices are largely responsible for this. Entire families were found to be at risk, not just the farmer who applied the pesticide.

The disease is caused by the fungus *Phytophthora infestans*. Although the identity of the strain present

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in PNG has yet to be determined, it is vital to take measures to prevent the introduction of other sexual mating types into PNG.

There are no resistant varieties of potato at present, and it is unlikely that a fully immune variety can be found. The introduction of a more resistant variety will probably involve a lag phase of 2–3 years, during which sufficient seed stocks would be multiplied nationally.

## Role of tissue culture

The vital role of tissue culture in this instance is not only for speedy multiplication, but also for the introduction of certified clean planting material with a known degree of resistance.

Tissue culture can reportedly be used to multiply virus-free plantlets by a factor of 3<sup>17</sup> annually. Furthermore, plantlets can be induced to produce micro-tubers in culture, which can be extracted and used to produce seed potatoes for the farmers. Some 1210 culture flasks incubated on a 10 m<sup>2</sup> bench can produce about 36,300 micro-tubers in 4 months.

The current importation of fresh potatoes for sale in supermarkets is a possible means of introduction of the Australian strain of *P. infestans* into PNG. Furthermore, the importation of what may be susceptible cultivars could exacerbate the situation if farmers were to grow the susceptible seeds. Not only would it be a loss to individual farmers, the susceptible plants could also act as hosts to the disease and a source of inoculum of the fungus to neighbouring gardens.

Although the potato late blight disease is now endemic in the highlands region, and it seems inevitable that it will spread to other regions, there are other diseases, such as those transmitted by viruses, that can and should be contained and controlled.

Thus, the role of tissue culture in this case is the prevention of the spread of other diseases within the country. Coupled with effective quarantine measures it can also prevent the introduction of *P. infestans* mating types from Australia or other countries while facilitating the importation of Australian varieties into PNG.

In addition, new cultivars can be held in storage in tissue culture until such time as their general release is approved. This would occur after their performance is evaluated in the field. Maintaining them in tissue culture can be seen as a local quarantine measure, as they remain uninfected by local pathogens. The report of Pitt and Wicks (2003) states that, without tissue culture, 'the development of locally selected cultivars will be severely constrained'.

Researchers at the National Agricultural Research Institute (NARI) High Altitude Program have selected four of the nine cultivars that were evaluated for resistance and suitability. They include Sebago, Knox, Kennebec and Spunta. Sebago is the cultivar most suitable for multiplication and distribution. There are about 36 varieties currently being maintained in the NARI Lowland Agricultural Experiment Station at Keravat tissue-culture laboratory for future evaluation. In this case, when a variety is determined to have superior qualities in resistance or other favourable characteristics, the changeover will be more easily facilitated via micropropagation than traditional methods.

## Conclusion

Tissue culture can be used to rapidly multiply planting stocks of potato and other important and/or commercial crops in times of disease epidemics or other events such as drought. The technique cuts down on the normal time lag between identification of an appropriate variety and its distribution. Apart from the obvious advantages of using verifiably clean material, tissue culture costs less than normal field multiplication and conservation methods.

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# A preliminary study of interrupting the epidemiological cycle of *Phytophthora palmivora*: integrated disease management of cocoa in relation to cocoa cropping cycle

Y. Namaliu, J.T. Vano and J.K. Konam<sup>1</sup>

## Abstract

Diseases are a serious constraint to cocoa production, and the synergistic effects of other factors such as insect pests, weeds, heavy shade and poor drainage often increase their incidence and severity. Applying appropriate management systems can minimise disease and pest problems. For significant control of a disease, an integrated approach may be necessary. Pod rot, stem canker, flower cushion, chupon and leaf blight, all caused by *Phytophthora palmivora* Butl., are the major causes of low cocoa (*Theobromae cacao* L.) production in Papua New Guinea (PNG), with annual losses estimated at 30–40%. The disease cycle of this pathogen is complex, with numerous sources and routes of dissemination of the causal agent. A study was conducted using epidemiological knowledge to demonstrate the benefits of adopting integrated disease management. Crop protection recommendations, applied in line with the crop phenological cycle, were implemented at T11A, Tavilo Plantation CCI on 10 clones each planted in 12 plots with 16 plants per clone. Preliminary results of a high-input treatment showed that a significant increase in profit is possible due to reduction in disease and a corresponding increase in yield. The inputs must also be timed to capture the opportunities presented by the environment in terms wet or sunny weather during the cropping cycle: shade and pruning of cocoa has to be timed to minimise disease pressure and maximise yield.

## Introduction

Pod rot, stem canker, flower cushion, chupon and leaf blight caused by *Phytophthora palmivora* Butl. are major constraints to cocoa (*Theobromae cacao* L.) production in Papua New Guinea (PNG). Losses due to this disease are estimated at 30–40% annually. Our recent investigation into the disease cycle, sources of primary inoculum (mycelia, zoospores, sporangia and chlamydospores) and dissemination of propagules, indicates that all sources of inoculum

are important. Soil is an important source of primary inoculum for *P. palmivora* disease epidemics on cocoa. Rain splash, aerosol droplets, termites and ants disseminate soil-borne primary inoculum. Rain splash was found to be insignificant above 75 cm from the ground under typical plantation conditions. Tent-building ants and termites were one of the most important vectors in PNG, contributing to 24% of primary pod infections. Old, infected cocoa pods (mummified pods) and stem cankers are also sources of primary inoculum, but strict sanitation, including their careful removal, failed to delay the onset of the wet-season epidemic of black pod, indicating that there are multiple sources of primary inoculum.

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Invertebrates were first proposed as vectors of *P. palmivora* in cocoa plantings as early as 1927, but experimental evidence in support of this did not appear for over 40 years. Flying invertebrates are known to play an important role in the transmission of plant pathogens, such as *Ophiostoma ulmi*, the cause of Dutch elm disease, which is disseminated by the European elm bark beetle, *Scolytus multistriatus* (Marsham). Recent studies at the Cocoa and Coconut Institute of Papua New Guinea indicate that flying scolytid and nitidulid beetles are vectors and they passively transmit viable inoculum in the frass they generate and also on their body parts. Ants, termites and other crawling insects were associated with the spread of the pathogen and their elimination from the field will reduce incidences of *Phytophthora* pod rot (PPR).

Vascular streak dieback (VSD) of cocoa nearly led to the collapse of the PNG cocoa industry in the 1960s (Keane and Prior 1992) and is now increasing in prominence in East New Britain. Pink disease is a major constraint to cocoa production in the main cocoa-producing provinces. This disease can cause whole trees to become unproductive if bearing parts of the branches are infected. The whole plant can also die when the main stem becomes infected. The disease is common on young 2–4-year-old trees. While *Phytophthora* pod rot is a major disease for East New Britain cocoa growers, pink disease is the major constraint in North Solomon and Northern Provinces. The factors affecting the epidemics of these diseases are identical; thus management of one disease will lead to the control of the others.

This work aims to demonstrate the benefits of adopting crop-protection recommendations focusing on interfering with the epidemiological cycle of *P. palmivora* by disrupting and limiting the activities of crawling and flying insect vectors. It focuses on integrating all the epidemiological knowledge into a single package and the timely application of management practices in line with the cropping cycle.

## Materials and methods

### Design

Two studies:

1. interfering with the epidemiological cycle of *Phytophthora palmivora*
2. integrated disease management (IDM) in relation to the cocoa cropping cycle

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

were conducted at block 10A, Tavilo Plantation on 10 clones planted at 16 trees per clone. The backgrounds of the 10 clones were known and they were ranked based on their susceptibility to black pod. Clones 1, 2 and 3 were known to be highly disease-resistant and have good yielding ability. They were released to growers in March 2003. Clones 8, 9 and 10 were known to be highly susceptible to black pod. The other four clones were known to be moderately susceptible and high yielding.

Both cocoa and *Gliricidia* trees were planted under old cocoa trees at 4 × 4 m square spacing in October 2000 at the same density. The cocoa trees were established under extremely high insect pest and disease pressure. In the block, factors such as the number of trees, genetic uniformity of material, number of and types of clones, and heterogeneity of environment, were taken into consideration. The cocoa trees were pruned lightly to achieve the desired tree form.

### Interfering with the epidemiological cycle of *Phytophthora palmivora*

The study on interfering with the epidemiological cycle of *Phytophthora palmivora* was conducted in three plots.

The following treatments were applied within each clone, four trees in each treatment, in three replicates.

- Treatment 1: Control, no treatment.
- Treatment 2: As an insect and pest barrier, grease was applied on the stem of the cocoa tree at about 30–40 cm above ground level.
- Treatment 3: Chlorpyrifos 500 EC and Orthene, both persistent insecticides, were applied at rates of 25 mL and 4.95 g, respectively, per 20 L knapsack spray. A wetting agent was used at 10 mL/20 L of water. To standardise the spraying, tree branches were sprayed from the ground level to the canopy until just before run-off.
- Treatment 4: A combination of treatments 2 and 3.

Spray treatments were re-applied at 4-weekly intervals and the grease bands were refurbished at 3-monthly intervals. The trial was monitored fortnightly, when the number of diseased and healthy ripe pods harvested were recorded. The first records were made on 4 September 2002.

### Integrated disease management (IDM) in relation to the cocoa cropping cycle

At the end of the epidemiological study, these plots were superimposed as a fourth treatment of IDM

packaging treatment. The IDM package comprised the original three treatments, plus one or other of four additional treatments (three replicates) applied in relation to the cropping cycle. The following are the four disease-control options applied as treatments. They are packaged as management options for the farmers.

- Treatment 1: Control; no sanitation, no chemicals and no fertilisers applied.
- Treatment 2: Minimum input; low-level sanitation, no chemicals and no fertilisers applied. Manual slashing and strip weeding, conventional sanitation operations and advanced pruning techniques. All inputs applied 2 months before peak flowering and cherelle development.
- Treatment 3: High-input management; high-level sanitation, chemicals and fertiliser applied. Manual and chemical weed control, insecticide/fungicide solution as paint to treat canker and longicorn beetle damage and advanced pruning technique. Urea and NPK (12.12.17 + 2.5MgO +0.3B) applied at the rate of 100 g and 240 g/tree/year, respectively.
- Treatment 4: Very high input. Same as treatment 3 plus incorporation of control of insect vectors and mirids.

The field preparation commenced in January and the designated IDM inputs were applied from April to May 2004. Pre-treatment assessment of the trees and yield and black pod data were used to allocate plots for the first three treatments. Treatment 4 was

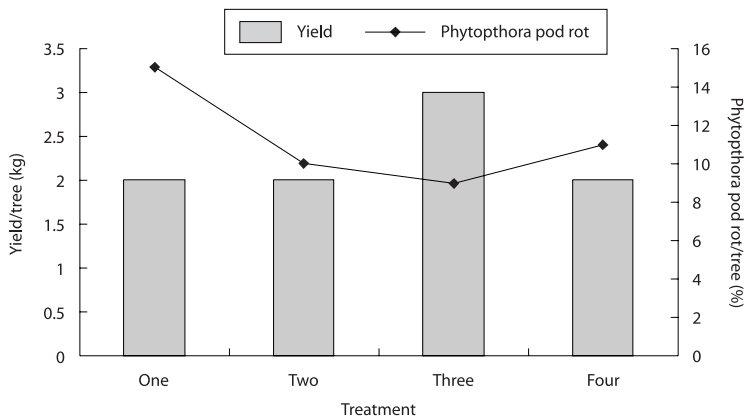
included later and inputs applied in the September–November period while the first three treatments plots were given their second round of IDM application.

## Results and discussion

Interfering with the *P. palmivora* epidemiological cycle significantly ( $p < 0.001$ ) reduced the incidence of pod rot disease. Low incidence was recorded where vectors were controlled, and higher numbers of ripe, healthy pods were obtained, particularly in treatment 3 (Figure 1).

When IDM was applied in relation to the cocoa cropping cycle, there was a significant ( $p < 0.001$ ) effect within the first 5 months after treatment application. Where no IDM was applied, a high incidence of disease was recorded (Figure 2). Reduction in incidence of black pod reflects the reduction in sources of inoculum, and the interference with vector activities thus lowering the disease pressure. There was a difference in mean healthy ripe pods ( $p > 0.01$ ). Trees in IDM-applied blocks had more ripe pods than trees in the control blocks. Analysis indicated a low level of interaction between treatments and clones, which may be an effect of the early stage of treatment.

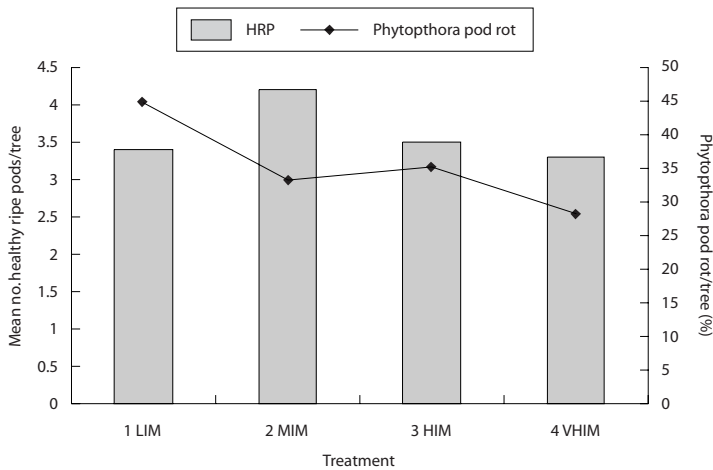
Some trees in the control blocks produced an average of 4.3 pods, which was higher than trees in the IDM-applied blocks. However, most of these pods were lost to black pod (Figure 2).



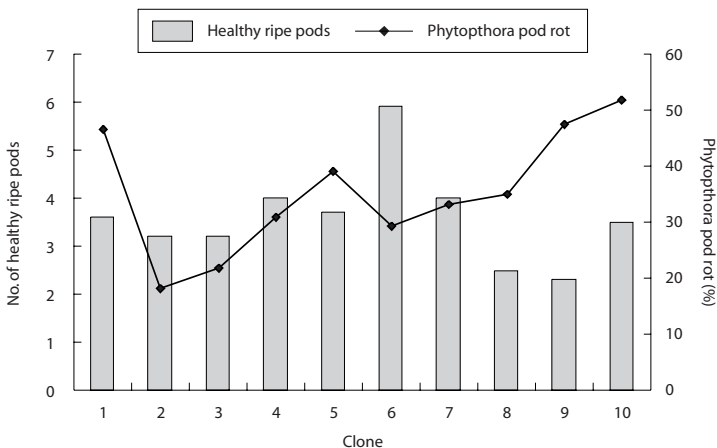
**Figure 1.** Mean yield and percentage *Phytophthora* pod rot (PPR)/tree/fortnight in trees where *Phytophthora palmivora* vectors were controlled

Despite low interaction between clones and treatment, there were significant ( $p < 0.001$ ) differences in performance of each clone. There were significant differences in percentage PPR and mean healthy pods (Figure 3). Clones 8, 9 and 10 are known to be very susceptible to PPR, and this study confirmed that. Clone 1 had a high disease incidence (46.7% PPR). Clone 6 produced the highest pod count per tree, a moderately low black pod count and the third-highest number of healthy pods.

Diseases are a serious constraint to cocoa production worldwide. Their impact can be amplified through the synergistic effects of many factors; e.g. insect pests and vectors, weeds, heavy shade, and poor field ventilation and drainage. The results of this study shows that the application of appropriate IDM packages aimed at interrupting the disease cycle can minimise losses to disease, as demonstrated by these two preliminary studies. Low disease pressure also increased yields.



**Figure 2.** Mean healthy ripe pods and percentage *Phytophthora* pod rot (PPR)/tree/fortnight for each treatment over a 5-month period ( $p > 0.001$ ). LIM = low inputs; MIN = minimum; H = high; VHI = very high inputs.



**Figure 3.** Percentage *Phytophthora* pod rot (PPR) and healthy ripe pods (HRP)/tree/month for 10 cocoa clones over 5 months



Packaging and utilisation of this technology will become a useful tool for PNG farmers to increase their production and profitability, and indicated the added value to the technology developed from research. Pruning of cocoa and shade trees has resulted in pods and stems being more exposed to sunlight, thus discouraging longicorn beetle attack. Weed control in IDM blocks also resulted in similar effects, as was the case for insect associated canker control with fungicide/insecticide mix which had a significant effect in reducing canker and bark damage by longicorns. This finding agrees with the previous report by Moxon (1992). The activities of ant vectors also were reduced through the application of the IDM package, which led to enhanced crop protection and tree health.

The sound IDM strategies to reduce crop losses and increase cocoa yield can be an effective method by reducing sources of inoculum, enhancing tree health and controlling vectors (Brown et al. 1997).

Farmer adoption of these IDM practices will enable them to become greatly involved in improving income.

## Acknowledgments

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# Evaluation of disease on new planting materials developed at the Cocoa and Coconut Institute with potential for release to farmers in Papua New Guinea

A.B. Kamuso and J.K. Konam<sup>1</sup>

## Abstract

Elite cocoa planting lines developed at the Cocoa and Coconut Institute of Papua New Guinea were tested under different management practices to determine their yield performances from a breeding and commercial perspective. This study assessed their performance when challenged by the three major diseases affecting cocoa production in Papua New Guinea: phytophthora pod rot and canker (*Phytophthora palmivora*), vascular streak dieback (*Oncobasidium theobromae*) and pink disease (*Corticium salmonicolor*). The cocoa lines (big, intermediate, small and very small) were planted at various sites under different management practices and under varying pressure of pests and diseases. The information obtained should ultimately be used to assist breeders to select materials and make informed decisions for the cocoa growers in Papua New Guinea. The reasons for initial low disease incidence are discussed. Vascular streak dieback was high in all blocks. The assessment criteria can be used to increase the amount of clones to be tested to help breeders and plantation owners to select clones that are relatively resistant to vascular streak dieback, canker and pink disease at each site.

## Introduction

The three major diseases affecting cocoa (*Theobroma cacao* L.) production in Papua New Guinea (PNG) in order of importance are: phytophthora pod rot (PPR) and canker caused by *Phytophthora palmivora* Butl.; vascular streak dieback (VSD) caused by *Oncobasidium theobromae* Talbot and Keane, and pink disease caused by *Corticium salmonicolor* (Berk. & Broome).

Losses to PPR are conservatively estimated at up to 40% (Konam 1999), but may be higher in areas with wetter periods. All parts of the plant are at risk,

but of most significant concern is fungal invasion of the pods at all stages of development (Sitapai 1989). When stems are infected, the result is defoliation, twig death, generally poor tree appearance and performance, and eventual death. Trees can die suddenly and over 2% of mature trees can be lost annually to canker (Turner 1967; Prior and Smith 1981). Attacks on flower cushions can reduce yield potential of the trees. Blights of seedlings, shoots, chupons and young leaves can severely reduce long-term production. Seedlings in the nurseries can also become diseased during wet weather, resulting in serious losses (Anon. 1992). Severe attacks of the roots can result in poor tree health, leading to lower productivity.

VSD led to the near collapse of the PNG cocoa industry in the 1960s. The 1960s VSD destruction led

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to the selection of VSD-resistant material from Trinitario and Upper Amazonian survivors. The Trinitario × Upper Amazonian crossing programs resulted in VSD-resistant poly-cross hybrids: SGI released in 1982 and SG2 in 1988. As a result, VSD became less significant and currently it is not regarded as a disease of major economic importance in PNG. However, VSD is still a commonly occurring and slowly developing disease in PNG and it is now increasing in prominence again in East New Britain Province.

Pink disease is a major constraint to production in major cocoa-producing provinces. This disease can cause the whole tree to become unproductive if the fruit-bearing parts of the branches are infected. At times, the whole plant can die when the main stem is infected. The disease is common on 3–4-year-old plants. PPR is a major concern in East New Britain, while pink disease is the primary constraint in North Solomon and Northern Province.

While an ‘accelerated breeding program’ is under way to shorten the length of the breeding cycle of cocoa, no proper pathological assessments have been done on VSD over the past 7 years to assess the performance of newly developed materials against the three major diseases.

The major constraint to cocoa production in PNG is crop losses due to pests and diseases. The development of planting materials through the various process of selection, screening, crossing, preliminary testing and advanced testing under a range of environments and conditions before they are finally released for commercial use is quite lengthy. In 2003, the Cocoa and Coconut Institute (CCI) of PNG released new clones for commercial use. Following this, the cocoa breeding section developed and tested new elite clones for future release. These clones were planted at different sites under different management practices and varying pressure of pests and diseases.

It is highly desirable to identify planting material suited to particular locations, as well as material that performs consistently in all locations and environments and is therefore suitable for general distribution. The aim of this study was to assess the disease resistance of four different sizes of clones planted at four different locations under different management approaches. The information obtained should ultimately be used to assist breeders to select materials and make informed decisions for the cocoa growers in PNG. The information can also highlight the level of disease under different management practices.

This paper reports the progress of disease assessment of elite clones with high potential for possible release for commercial use.

## Materials and methods

Disease assessment was carried out at four sites planted to big, intermediate, small and very small size clones (Table 1). At each site, 20 or more trees per clone were planted in one, two or three rows. Treatments at each site were not replicated and were randomly assigned.

The assessment at site one—Tokiala Plantation, New Mark Plantation, Gazelle area—commenced in the third quarter of December 2002 on trees planted in 1999 at 4 m × 4 m spacing for big clones, 3 m × 3 m for intermediate and at 4 m × 2.5 m for small and very small size clones.

The second and third sites were at Raulavat and Kevera plantation, CCI commercial blocks. The fourth site was at the National Agriculture Research Institute (NARI) block at the Lowlands Agricultural Experimental Station, Keravat. The assessments at Raulavat, Kevera and Keravat also commenced in December 2002 on trees planted in 1999 at similar spacings as for the Tokiala plantation.

## Disease assessment

Plants were assessed and scored for disease severity using a 1–7 scale, modified after Efron et al. (2002). Each tree in the rows was given a score of 1 (healthy plant) to 7 (very badly diseased plant). The diseases assessed were: canker, VSD and pink disease.

The scores were used to determine the severity index for each disease as follows:

$$\sum_{i=1}^7 ni \frac{i}{N}$$

where *i* refers to the disease severity score (1–7), *n* refers to the number of plants in each severity group (*i*) and *N* refers to the total number of plants per clone tested.

## Results and discussion

Although VSD, pink disease and canker affected the plants, the severity was low, presumably due to the young age of the planting materials. Canker infection

**Table 1.** Different sizes of the new elite cocoa clones, their codes and pedigrees. These materials have the potential to be released.

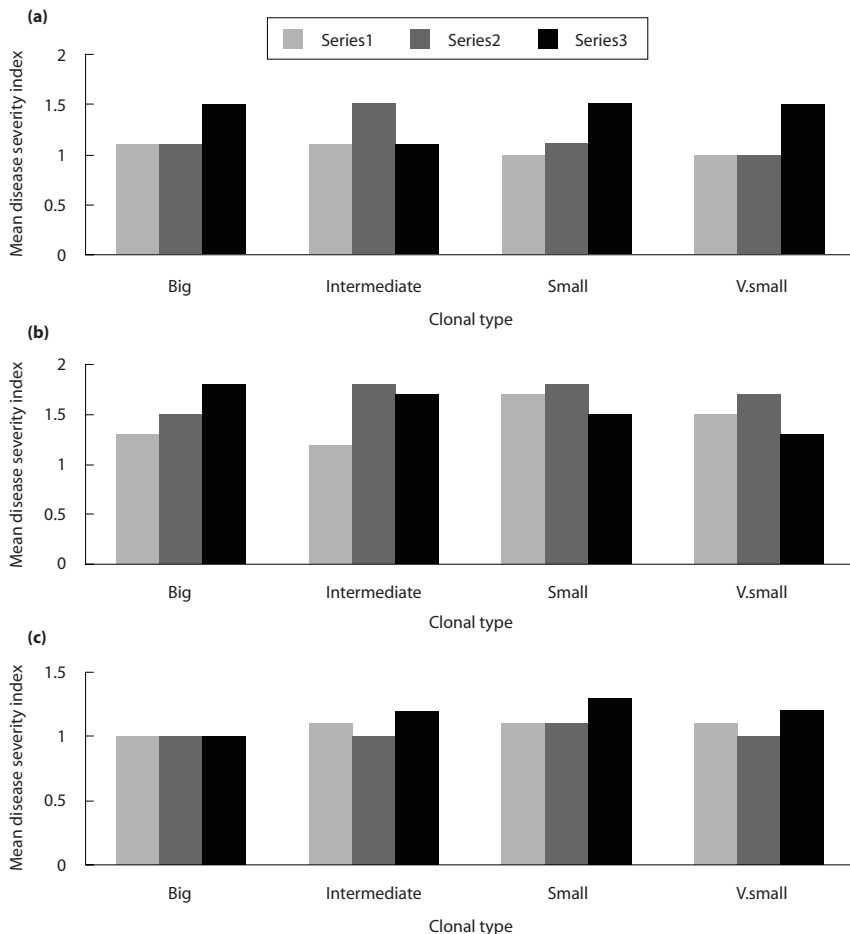
Clone	Exp. code	Pedigree
<b>Big</b>		
1	12-2-12	K82 × KEE 6
2	12-4-4	K82 × KEE 6
3	22-3-5	KA2-101 × KEE 6
4	35-4-5	KA2-106 × KEE 23
5	66-3-9	KA6-101 × KEE 42
6	72-3-14	K24-102 × KEE 6
7	77-4-6	K24-102 × KEE 37
8	92-4-6	KT140 × KEE 6
9	K-4	Unknown
TA 101 16-3/2		K82 × KEE 42
TA103 37-3/1		KA2-106 × KEE 43
<b>Intermediate</b>		
1	16-3-1	K82 × KEE 42
2	17-2-16	K82 × KEE43
3	21-4-8	KA2-101 × KEE5
4	24-2-8	KA2101 × KEE 22
5	24-4-5	KA2101 × KEE22
6	36-3-2	KA2106 × KEE 42
7	73-2-3	K24-102 × KEE 12
8	92-4-15	KT140 × KEE6
9	K-6	Unknown
10	K-9	Unknown
11		KA2-106 × KEE12
<b>Small</b>		
1	13-1-13	K82 × KEE 12
2	16-4-2	K82 × KEE42
3	17-4-10	K82 × KEE43
4	23-2-13	KA2101 × KEE 12
5	31-3-12	KA2106 × KEE 5
6	33-1-10	K24-102 × KEE 12
7	34-1-13	KA2 106 × KEE22
8	35-2-13	KA2-106 × KEE23
9	63-3-8	KA2101 × KEE 12
TA 202 17-14/4		K82 × KEE43
TA 305 73-14/1		K24-102 × KEE12
<b>Very small</b>		
1	11-2-10	K82 × KEE 5
2	13-2-2	K82 × KEE12
3	13-2-13	K82 × KEE12
4	13-3-2	K82 × KEE 12
5	15-4-7	K82 × KEE 23
6	17-3-6	K82 × KEE 43
7	23-3-3	KA2 101 × KEE12
8	37-2-10	KA2-106 × KEE43
9	33-1-13	KA2106 × KEE 12
10	73-3-8	K24-102 × KEE12
TA 302 17-7/4		K82 × KEE43

rates at all sites were relatively low but increased steadily with time, presumably due to tree bark being healthy and at maximum growth. There were also very low levels of *Phytophthora* inoculum pressure around the trees (Figure 1).

VSD disease severity at all sites was slightly higher than canker and pink disease index. VSD disease index increased with time, presumably due to build-up of inoculum sources within the tree. A similar trend was reported by Efron et al. (2002) for Trinitario and Amazonian crosses (TA) in Madang. The higher severity index was due to prolonged wet weather experienced at the beginning of 2004, followed by a very dry period between July and August.

These weather patterns were conducive for rapid fungal development and growth on the tree. The prolonged dry period assisted symptom expression. Even relatively resistant planting material was susceptible to VSD under this weather pattern. VSD infections can be minimised by using recommended management practices published by CCI in its information bulletins. Sanitary pruning practices can greatly help reduce conditions favourable for fungal establishment and development.

Pink disease occurred only in isolated patchy pockets at relatively low levels. This was probably due to environmental conditions unfavourable to it and resistance in the clonal materials.



**Figure 1.** Mean disease severity index of (a) *Phytophthora* canker, (b) vascular streak dieback and (c) pink disease of the different clones over the three assessment periods

Generally, all clones performed extremely well against all the major diseases at all sites, confirming the CCI Breeding Section's prognosis on the potential of these clones.

## Acknowledgments

We thank Y. Namaliu, R. Wennani, J.T. Vano and R. Labur from the Pathology Section for their help in assessing the clones, the Cocoa Breeding Section of CCI for allowing us to assess its materials and the CCI for permission to present this paper at the 2nd PNG Crop Protection Conference.

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# Disease performance of international cocoa clones at the Cocoa and Coconut Institute, Papua New Guinea

A.B. Kamuso, R. Wennani, J. Saul and J.K. Konam<sup>1</sup>

## Abstract

Nearly all crops when planted outside their centre-of-origin remain relatively free of pests and diseases. In Papua New Guinea, local strains that have become major pathogens of the introduced cocoa (*Theobromae cacao*) are, in order of importance: *Phytophthora palmivora*, causing phytophthora pod rot and canker, root and blight infections of seedlings, leaves and shoots; *Oncobasidium theobromae*, which causes vascular streak dieback (VSD) disease of cocoa; and *Corticium salmonicolor*, which causes pink disease of cocoa. The development of new planting material is a long, time-consuming process. One of the quickest methods of incorporating highly desirable characteristics is to use, in breeding programs, elite materials developed in other countries.

This paper reports results of disease assessment and artificial screening for resistance to *P. palmivora* of local and introduced elite clones. The work involved the cocoa breeding programs of 10 countries participating in an agreed CFC (Common Fund for Commodity) project in which each country exchanged materials.

The reasons why incidences of phytophthora canker and pink disease were minimal and why many clones of international origin were highly susceptible to VSD are discussed.

## Introduction

Almost all crops remain relatively free of pests and diseases when planted outside their centre-of-origin. Unlike coffee, which has its major co-evolved pathogen, *Hemileia vastatrix*, following coffee around the tropics (Keane 1992), cocoa is attacked by different pests and diseases wherever it is introduced. *Moniliophthora roreri*, a serious pathogen in Central and South America, caused losses estimated at 20–30% (Evans 1986); ‘witches’ broom’, a shoot proliferation caused by mushroom mycelial growth in the cells, caused decline in production in Central America. Cocoa encountered cocoa swollen shoot virus when introduced into West Africa and vascular

streak dieback (VSD) when introduced into South-east Asia. Fortunately, intercontinental spread of the major pathogens has been restricted by strong quarantine regulations.

In Papua New Guinea (PNG), the three major pathogens of cocoa (*Theobromae cacao* L.), an introduced crop, are, in order of importance: *Phytophthora palmivora* causing phytophthora pod rot and canker; *Oncobasidium theobromae* Talbot and Keane causing VSD disease; and *Corticium salmonicolor* Berk. & Broome responsible for pink disease.

Losses to phytophthora pod rot are conservatively estimated at 30–40% (Konam 1999) but can be higher in some areas and during wetter periods. All parts of the plant are susceptible. Of significant concern is fungal invasion of the pods at all stages of development (Sitapai 1989). When stems are infected, the result can be defoliation, twig death, generally poor tree appearance and eventual death.

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Trees can die suddenly and over 2% of mature trees can be lost annually to canker (Turner 1967; Prior and Smith 1981). Attacks on flower cushions can reduce yield potential of the trees. Blights of seedlings, shoots, chupons and young leaves can severely reduce long-term production. Seedlings and bud-dings in the nurseries can also become diseased during wet weather, resulting in serious losses (Anon. 1992). Severe attacks on the roots can result in poor tree health, resulting in lower productivity. There are numerous sources and routes of pathogen, resulting in a very complex disease epidemiology (Konam 1999).

VSD disease of cocoa, first identified and reported from PNG, nearly wiped out the PNG cocoa industry in the 1960s. VSD is a serious disease encountered by cocoa when the crop was introduced into PNG and Southeast Asian countries. Interestingly, its distribution in PNG is quite circumscribed. It is absent from the North Solomons, New Ireland, Manus, Milne Bay Islands and Bali Witu Islands, but is a debilitating disease in East New Britain, where it is increasing in prominence, and Madang. The losses in the 1960s resulted in the development of VSD-resistant material from Trinitario and Upper Amazonian survivors. The crosses resulted in VSD-resistant poly-cross hybrids: SG1 released in 1982 and SG2 in 1988.

Pink disease is a major constraint to cocoa production in the major cocoa-producing provinces. The disease can cause whole trees to become unproductive if the fruit-bearing parts of the branches are infected. At times, the whole plant can die when the main stem is infected. Pink disease can become prominent in new plantings and can become severe on young trees (18 months to 5 years old), and apparently linked to seasons and areas of high rainfall. Pink disease is the major constraint in North Solomons and Northern provinces.

The development of new plant material involves a lengthy process of selection, screening, crossing, and preliminary and advanced testing under different environmental conditions before they are finally released for commercial use. One of the fastest methods of developing new cocoa planting materials is to use elite materials developed in breeding programs in other countries. It is highly desirable that the materials selected overseas be suited to the importing country, perform consistently in all locations and environments, and are suitable for general distribution.

Ten cocoa-producing countries participated under a Common Fund for Commodity (CFC) project in

which pest-resistant materials of elite quality were exchanged. The Cocoa Breeding section at the Cocoa and Coconut Institute (CCI) of PNG was a participant and, in 1998, elite materials supplied by other participating countries were imported into the country.

The main aim of the assessment reported here was to screen against the major pests and diseases:

- 29 elite clones introduced into CCI under the CFC/ ICCO/IPGRI, 'Cocoa germplasm utilization and conservation' project
- 25 locally selected clones.

The information obtained would help plant breeders in further selection of cocoa clones with some degree of resistance to phytophthora canker, VSD and pink disease.

## Materials and methods

This trial was conducted in Blocks 9C and 10C at Tavilo in East New Britain Province, where the 29 international and 25 local cocoa clones were planted. The international clones were introduced into the quarantine station at Kilakila, Port Moresby in February 1998 from intermediary quarantine stations in Montpellier, France and Reading University, England. The origins of the clones and types and characteristics are shown in Table 1. Upon release from quarantine, they were budded at the cocoa breeding nursery at Tavilo in October 1998. Field planting was done in 1999 using a randomised complete block design with six replicates each containing eight trees at a density of 833 trees/ha planted at a spacing of 4 m × 3 m according to the standard procedures of the project.

## Disease assessment

Plants were scored for disease symptoms using a severity scale of 1 (healthy plant) to 5 (severely diseased plant) (Efron et al. 2002). Each tree in the rows was scored. The diseases assessed were canker, VSD and pink disease.

The severity index for each disease was calculated using the formula:

$$\sum_{i=1}^5 ni \frac{i}{N}$$

where  $i$  = disease severity (1–5),  $n$  = the number of plants in each severity group ( $i$ ) and  $N$  = the total number of plants per clone.



## Screening for resistance to *P. palmivora*

Pods were screened for resistance to *Phytophthora* using a modified method of Iwaro et al. (1997). Pods from international clones were also artificially inoculated and screened for resistance to phytophthora pod rot.

A concentration of 150,000–200,000 zoospores/mL produced from cocoa pods (KA2-101) was used as inoculum with an isolate collected from the field at CCI Block T11A, Tavilo. *Phytophthora* mycelium and sporangia were brushed off 10-day-old inoculated pods into a Petri dish containing sterile distilled water. *Phytophthora* mycelia, spores of other fungi and dirt were filtered using mesh cloth. The mesh with mycelia and sporangia was placed into chilled

sterile distilled water for up to 30–40 minutes for the zoospores to be produced. Zoospores were filtered and adjusted to the desired concentration using chilled, sterile distilled water.

A day before inoculation, test pods were collected and incubated overnight in inoculation trays before they were sprayed and inoculated using an atomiser sprayer. Inoculated pods were incubated at 25°C. Pod infections were assessed 3, 5 and 7 days after inoculation. Only 6 of the 10 visible ridges of the pods were assessed. Infection coverage was scored as 0 (no obvious lesion) to 100% (full area infected) and pod susceptibility was calculated as a ratio in comparison to a susceptible control pod (hybrid clone 73-14/1).

**Table 1.** Name, type and country of origin of the international and local cocoa clones tested

International clones				Local clones		
Clone	Name	Type <sup>a</sup>	Country	Clone	Name	Type
1	CATIE 1000	UA × Catongo	Costa Rica	32	K82	T
2	P7	UA	Brazil	33	KA2-101	T
3	ICS 1	T	Trinidad	34	KEE-12	UA
4	IFC 5	AM	Cameroon	35	KEE-23	UA
5	EET 59	C	Ecuador	36	KEE-43	UA
6	SNK 413	T	Cameroon	37	NAB-11	OT
7	T85/599	UA	Ghana	38	T-11	OT
8	PA 150	UA	Brazil	39	T-49	OT
9	PA 120	UA	Brazil	40	T-45	OT
10	MOCORONGO	UA	Brazil	41	K-72-153/4	OT
11	GU 225P	–	French Guiana	42	K-72-46/51	OT
12	VENC 4-4	–	Venezuela	43	K-72-7/6	OT
13	APA 4	UA	Colombia	44	K-78-3	OT
14	SNK 64	T or LA	Cameroon	45	L-14	OT
15	IMC 47	UA	Brazil	46	B-22	OT
16	MAN 15-2	LA-UA	Brazil	47	K-7	OT
17	LCT EEN 46	UA	Ecuador	48	66-3(36-8/3)	HC
18	AMAZ 15-15	UA	Brazil	49	21-4-8	HC
19	EQX 3360-3	–	Ecuador	50	17-3/1	HC
20	PA 107	UA	Brazil	51	36-3/1	HC
21	UF 676	T	Costa Rica	52	K-4	T ?
22	MXC 67	C	Mexico	53	K-6	T ?
23	SCA 6	UA	Brazil	54	K-9	T ?
24	BE 10	LA	Brazil			
25	SPEC 54-1	UA	Columbia			
26	SIAL 339	LA	Brazil			
27	SIC 5	–	Brazil			
28	IMC 105	UA	Brazil			
29	P 30	–	–			
30	ICS 95	T × c	Trinidad			
31	EET 308	R	Ecuador			

<sup>a</sup> UM – Upper Amazonian; LA – Lower Amazonian; T – Trinitario; AM – Amelonado; C – Criollo; R – Refractario; OT – Old Trinitario; HC – hybrid clones

**Table 2.** Disease severity index of *Phytophthora* canker (canker); vascular streak dieback (VSD); pink disease (PD) and phytophthora pod rot (PPR) ranking of 54 International clones held by Cocoa Breeding, Tavilo in December 2002 and October 2003

Clone		2002			2003			PPR ranking <sup>a</sup>
Number	Name	Canker	VSD	PD	Canker	VSD	PD	
4	IFC 5	1.0	1.0	1.0	1.0	1	1.0	1.0
19	EQX 3350-3	1.0	1.4	1.0	1.0	1.6	1.0	1.0
	73-14/1 <sup>b</sup>							1.0
3	ICS 1	1.2	3.3	1.0	1.3	3.7	1.0	0.9
5	EET 59	1.0	1.7	1.0	1.0	1.4	1.0	0.9
20	PA 107	1.0	1.3	1.1	1.0	1.1	1.1	0.9
21	UF 676	1.0	1.1	1.0	1.3	1	1.0	0.9
22	MXC 67	1.0	1.1	1.0	1.0	1	1.0	0.9
25	SPEC 54-1	1.0	1.6	1.1	1.0	2.1	1.1	0.9
30	ICS 95	1.0	1.2	1.1	1.1	1.2	1.1	0.9
12	VENC 4-4	1.0	1.0	1.0	1.0	1.2	1.0	0.8
15	IMC 47	1.0	1.0	1.1	1.0	1.4	1.1	0.8
28	IMC 105	1.0	1.8	1.0	1.0	2.4	1.0	0.8
39	T-49	1.0	1.0	1.0	1.0	1.5	1.0	0.8
1	CATIE 1000	1.0	1.2	1.0	1.0	1.7	1.0	0.7
11	GU 225P	1.0	1.2	1.1	1.0	1.7	1.1	0.7
27	SIC 5	1.0	1.1	1.0	1.2	1.2	1.0	0.7
37	NAB-11	1.0	1.1	1.1	1.0	1.2	1.1	0.7
40	T-45	1.0	1.0	1.0	1.0	1	1.0	0.7
41	K-72-153/4	1.0	1.1	1.0	1.0	1.5	1.0	0.7
48	66-3(36-8/3)	1.0	1.1	1.0	1.0	1.0	1.0	0.7
13	APA 4	1.1	2.2	1.2	1.0	4.7	1.2	0.6
17	LCTEEN 46	1.0	1.1	1.0	1.0	1.3	1.0	0.6
23	SCA 6	1.0	2.5	1.0	1.0	3.8	1.0	0.6
31	EET 308	1.0	1.0	1.0	1.0	1	1.0	0.6
32	K82	1.0	1.0	1.0	1.0	1.1	1.0	0.6
34	KEE 12	1.0	1.5	1.1	1.0	3.5	1.1	0.6
35	KEE 23	1.0	1.5	1.0	1.0	1.5	1.0	0.6
45	L-14	1.0	1.2	1.0	1.1	1.3	1.0	0.6
47	K-7	1.1	2.9	1.0	1.0	4.2	1.0	0.6
16	MAN-15-2	1.1	4.	1.0	1.0	6.3	1.0	0.5
33	KA2-101	1.0	2.3	1.0	1.2	3.2	1.0	0.5
36	KEE 43	1.0	1.4	1.0	1.0	1.1	1.0	0.5
44	K78-3	1.1	1.2	1.1	1.1	1.3	1.1	0.5
51	36-3/1	1.0	2.6	1.1	1.0	4.1	1.1	0.5
54	K-9	1.6	1.4	1.4	1.0	2	1.4	0.5
6	SNK 413	1.1	1.2	1.1	1.0	2.4	1.1	0.4
9	PA 120	1.0	1.4	1.0	1.0	1.3	1.0	0.4
14	SNK 64	1.0	1.6	1.1	1.0	2.2	1.1	0.4
18	AMAZ-15-15	1.2	3.5	1.0	1.0	4.9	1.0	0.4
46	B-22	1.0	1.0	1.0	1.1	1.3	1.0	0.4
52	K-4	1.0	1.9	1.0	1.0	3.4	1.0	0.4
53	K-6	1.0	1.0	1.0	1.0	1.3	1.0	0.4
8	PA 150	1.0	2.9	1.0	1.0	5.6	1.0	0.3
10	Mocorongo	1.0	1.0	1.1	1.0	1.1	1.1	0.3
24	BE 10	1.0	1.0	1.0	1.0	1.4	1.0	0.3
43	K-72-7/6	1.0	2.2	1.0	1.0	2.6	1.0	0.3
50	17-3/1	1.0	1.1	1.0	1.0	1.4	1.0	0.3
2	P7	1.0	1.3	1.1	1.0	2.4	1.1	0.2
7	T85/599	1.0	2.5	1.0	1.0	6.2	1.0	0.2
26	SIAL 339	1.0	3.0	1.1	1.0	5.6	1.1	0.2
29	P30	1.0	1.0	1.0	1.0	1	1.0	0.2
38	T-11	1.0	3.3	1.0	1.0	4.3	1.0	0.2
42	K-72-46/51	1.1	2.3	1.1	1.2	3.2	1.1	0.2
49	21-4-8	1.0	1.7	1.0	1.0	3.5	1.0	0.2

<sup>a</sup> Clones ranked nearer to 1 are highly susceptible and those ranked nearer to zero are relatively resistant.

<sup>b</sup> Clone 73-14/1 is the *Phytophthora*-susceptible control used.

## Results and discussion

The average disease indexes and clone susceptibilities to phytophthora pod rot are shown in Table 2. Generally, all the clones had very low scores for canker and pink disease and are therefore relatively resistant to phytophthora canker and pink disease.

There was a strong association ( $r = 0.84$ ) between pink disease and canker; clones that were susceptible to phytophthora canker were also susceptible to pink disease. The reasons for this are not clear, although environmental factors, particularly wet weather, can predispose trees to both canker and pink disease attack (Anon. 1992). Pink disease is common in the Asian region (Konam and Waine 1994) while phytophthora diseases occur worldwide (Keane 1992). Further close monitoring is needed to confirm this relationship.

Many clones were badly attacked by VSD over time. International clones MAN-15-2 and AMAZ-15-15 showed high VSD in the first recording in December 2002 and the disease index increased even further a year later. In the second assessment, clones that had the highest levels of VSD in order of severity index were: MAN-15-2; T85/599; SIAL 339; PA 150; AMAZ-15-15; APA; T-11; K-7; 36-3/1; SCA 6; ICS 1; KEE 12 and 21-4-8. Of the 13 highly suscep-

tible clones above, only three—K-7; 36-3/1 and KEE 12—are of local origin. This presumably reflects the level of adaptation of the local materials, which have been selected for resistance to VSD. The introduced clones do not appear to have the genetic capabilities to withstand the PNG VSD and were therefore more susceptible.

Some clones highly susceptible to phytophthora pod rot were detected through the artificial pod-screening test (e.g. clones EQX 3350-3; IFC 5; ICS 95; SPEC 54-1; EET 59; PA 107; MXC 67; UF 676; ICS 1; VENC 4-4; T-49; IMC 105; IMC 47 and CATIE 1000). These are international clones, while most of the local selections in the trial were relatively resistant to phytophthora pod rot. The fact that their level of susceptibility to phytophthora pod rot ranked close to that of the local *Phytophthora* susceptible control material (73-14/1) could again be attributed to absence of genes and phenological features to withstand the local strain of *P. palmivora*. Keane (1992) discussed the potential problems that plant material can encounter once introduced outside its centre of origin. The susceptibility reported here could be attributed to such causes and suggests that clonal material for commercial use should be developed and selected for site-specific locations.

**Table 3.** Cocoa clones indexed for resistance and susceptibility to vascular streak disease in 2002 and 2003

2002				2003			
Highly resistant < 1.0	Resistant 1.1–1.2	Susceptible 2.5–4.0	Highly susceptible > 4.0	Highly resistant < 1.0	Resistant 1.1–1.2	Susceptible 2.5–4.0	Highly susceptible > 4.0
EET 308	B-22	KA2-101		EET 308	66-3(36-8/3)	K-72-7/6	36-3/1
IFC 5	T-49	K-72-46/51		IFC 5	KEE 43	K-72-46/51	K-7
K82	P30 <sup>a</sup>	T85/599		P30 <sup>a</sup>	K82	KA2-101	T-11
Mocorongo <sup>a</sup>	T-45	36-3/1		T-45	Mocorongo <sup>a</sup>	K-4	APA 4
VENC 4-4	IMC 47	K-7		UF 676	PA 107	21/04/2008	AMAZ-15-15
K-6	SIC 5	PA 150		MXC 67	SIC 5	KEE 12	PA 150
BE 10	UF 676	SIAL 339			VENC 4-4	ICS 1	SIAL 339
	LCTEEN 46	ICS 1			NAB-11	SCA 6	T85/599
	NAB-11	T-11			ICS 95		MAN-15-2
	(36-8/3)	AMAZ-15-15					
	MXC 67	MAN-15-2					
	17-3/1 <sup>a</sup>						
	K-72-153/4						
	ICS 95						
	K78-3						
	SNK 413						
	GU 225P						
	L-14						

<sup>a</sup> Clones with resistance to both phytophthora pod rot and vascular streak disease

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# Diversity of *Phytophthora palmivora* on cocoa in Papua New Guinea

J. Saul<sup>1</sup>

## Abstract

Phytophthora pod rot of cocoa in Papua New Guinea (PNG) and elsewhere is still of significant importance in spite of decades of research on disease-management strategies. It is now realised that pathogen populations need to be considered when developing disease-management strategies. Furthermore, laboratory isolates used in resistance studies were not representative of the pathogen populations in the field which are constantly changing to adapt to their environment. This paper aims to construct the population structure of *Phytophthora palmivora* in PNG in order to improve disease management.

## Introduction

Cocoa is of economic importance as a cash crop for chocolate and cocoa butter for many developing countries. Can increasing demands for these cocoa products be met? Over the past two decades, several fungal diseases have gained considerable importance and pose a serious threat to the supply of chocolate. In Papua New Guinea (PNG), the most important cocoa disease is Phytophthora pot rot caused by *Phytophthora palmivora* (Butl.) which results in about 40% crop losses annually (Saul 1993).

Numerous reports are available on research conducted worldwide on control measures. PNG studies include use of fungicides (McGregor, 1983, 1984; Jollands and Jollands 1989; Jollands et al. 1989; Holderness 1992; Anderson et al. 1989), cultural practices (Konam and Guest 2003) and screening for resistance in vivo and in vitro (Thrower 1960; Hicks 1975; Prior and Sitapai 1980; McGregor 1981; McGregor, unpublished data; Saul 1993; Efron et al. 1996; Efron and Blaha 1998; Saul Maora et al. 2003), but effective control is still a major problem.

Variation in *P. palmivora* was realised (Appiah et al. 1999) through artificial resistance screening tests (Saul 1993; PNG Cocoa and Cocoa Institute, unpublished data). Pathogen populations evolve in time and space in order to adapt to a changing environment, and laboratory isolates are therefore not representative of natural populations. Thus, surveys of isolates in the different cocoa-growing areas are required in order to obtain representative samples of natural populations in the field. The use of resistance genes in cocoa populations is essential, but its success depends on the knowledge of genetic composition of *P. palmivora* populations within the country.

## *Phytophthora palmivora*

*Phytophthora palmivora* is a diploid, heterothallic (out-breeding) fungus with two mating types, A1 and A2, that can reproduce asexually and sexually. In PNG, both mating types are present in Madang, Siassi and North Solomons Province (NSP) (Arentz 1986) whilst mating type A2 is present in all the cocoa-growing areas of the country. *Phytophthora palmivora* can survive in the soil, on mummified pods, in bark/stem cankers and on ant tents/runs. It can be dispersed short distances through rain splash

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(Gregory et al. 1984) and tent-building ants. Nitidulid and scolytid beetles are capable of dispersing inoculum between cocoa trees and between farms (Konam and Guest 2004) and through infested soil in nursery bags (Saul, personal observations).

Apart from cocoa, *P. palmivora*: also infects coconut (*Cocos nucifera* L.), betelnut (*Areca catechu* L.), breadfruit (*Artocarpus altilis* (Parkinson) Fosberg), durian (*Durio zibethinus* Murr.), and nutmeg (*Myristica fragrans* Houtt).

These hosts, especially coconut and betelnut, are of some importance, because they are also used as shade for cocoa in PNG. The significance of durian and nutmeg may increase with greater stress on growing alternative minor crops. Breadfruit is part of the native vegetation and is used as food by many Papua New Guineans, and therefore may be grown around cocoa plots.

Apart from pods, *P. palmivora* also infests the bark, chupons, and seedlings/buddings at the nursery stage and cherelles.

The following studies are proposed, to test if:

1. *P. palmivora* populations vary between different cocoa-growing regions in PNG
2. *P. palmivora* populations vary with time
3. *P. palmivora* populations from different hosts are different
4. there is variation in cocoa tissue specificity.

## Methods

A hierarchical experimental design is to be used.

### Variation between locations

*Phytophthora palmivora* isolates will be obtained from the following 12 cocoa-growing areas of the country: East New Britain; West New Britain; North Solomon; New Ireland; Manus; East Sepik; Madang; Karkar; Morobe; Oro; Milne Bay; Central.

Five cocoa farms will be surveyed at each location and isolations made from two diseased pods sampled from three trees per farm.

These isolates will be tested as follows:

- Genetic diversity of isolates between locations will be tested using molecular techniques — restriction fragment length polymorphisms (RFLP) and microsatellites.
- Morphological comparisons will be made by growing representative isolates and measuring sporangia shape and size, pedicel length and

culture appearance. Isolates will be paired to test whether oospores are formed (if two different mating types are present).

- Isolates will be tested for pathogenicity on standard cocoa clones, using cocoa leaves and pods  
Variances will be tested:
  - between locations
  - between farms within locations
  - between cocoa trees within farms within locations.

### Comparison of *Phytophthora* population in time

For logistic reasons, only two locations, Madang and East New Britain, will be used for comparison of isolates between different times.

There will be two sampling times (T1, big peak period; T2, minor peak period), two locations, five farms per location, three trees per farm, two pods per tree.

Isolates from the two sampling times in the two locations will be tested for genetic diversity, variation in morphology and pathogenicity, as described previously.

Variation will be tested:

- between times
- between times within locations
- between times within locations within farms
- between times with locations within farms within trees.

### Comparison of *Phytophthora* from different hosts

Ten isolates of *P. palmivora* will be collected from each of the following: cocoa, coconut, Areca palm, breadfruit, nutmeg and durian. These will be tested for genetic diversity and morphological differences as previously described, and for pathogenicity on leaves and fruits of the different hosts, using representative isolates by cross inoculation.

### Comparison between different host tissues

Ten isolates of *P. palmivora* will be obtained from each of the different host tissue samples; e.g. cocoa pods, cocoa bark, cocoa leaves and cherelles and seedlings, and tested as described previously for genetic diversity, variation in morphology and pathogenicity.

## Statistical analysis

The observed gene diversity (or genotype frequencies of each of the populations) will be tabulated and subjected to a chi-square homogeneity test. Significant deviations of observed from expected frequencies will lead to the rejection of the null hypothesis of no differentiation between sub-populations within the country.

## Conclusions

The results of this study will demonstrate whether:

1. different sub-populations of *P. palmivora* exist in the different locations in PNG
2. different sub-populations of *P. palmivora* exist in time
3. different sub-populations of *P. palmivora* infect different hosts
4. different sub-populations of *P. palmivora* infect different tissues on cocoa.

This information will help formulation of management policies such as:

- enforcement of strict quarantine regulations between different locations
- use of different cocoa planting materials for different locations
- use of different cocoa planting materials over time
- removal of alternative hosts
- enforcement of cultural practices; e.g. removal of mummified pods.

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# **Keynote address**

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

T.V. Price<sup>1</sup>

## *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

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).

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.

# **Weeds and management**

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

D.S. Yinil, M.S. Powell and H. Tangbil<sup>1</sup>

## 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 May 1994, compared blanket spray, combined strip spray with slash, and slash methods of weed control. The second experiment was conducted between January 1996 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 February 1989 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 ametrynne 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, Kadalung I, Kadalung 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).

## 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.



### 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 rhyarid beetles, cocoa 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 rhyarids was common, with longicorns the main cause of losses.

The entomology section of CCI did not 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-

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 drawn 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

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<sup>1</sup>

## *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.

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

D.S. Yinil<sup>1</sup>

## 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.

<sup>1</sup> Cocoa and Coconut Institute of Papua New Guinea, PO Box 1846, Rabaul, East New Britain Province, Papua New Guinea.

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 <i>Monstera</i> and all other weeds present starting to turn yellow.
2. Glyphosate + Chemwet spread sticker	No visible effect on <i>Monstera</i> or other weed species present.
3. Gramoxone + Li-700 surfactant	Burns on stems and young leaves of <i>Monstera</i> . Leaves of grass, <i>Makenia</i> sp., members of Emphophis and Compositae family and <i>Centrosema</i> 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 <i>Monstera</i> or any of the weed species present.
7. MSMA + with spread sticker + Li-700	Burns on young leaves and stems of <i>Monstera</i> , but less severe than for Gramoxone + Li-700. The mixture, however, greatly affected grass species, <i>Makenia</i> , members of the Emphophis and Compositae family and <i>Centrosema</i> 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 <i>Monstera</i> 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 <i>Monstera</i> turning yellow but no signs of dead plants. The mixture controlled other weed species were present.
3. Gramoxone + Li – 700	New shoots of <i>Monstera</i> starting to grow back. Growth of <i>Makenia</i> , members of the Emphophis and Compositae family and <i>Centrosema</i> spp. still suppressed.
4. 2,4-D + spread sticker	Only minor burns to new leaves and stems of <i>Monstera</i> compared with the Gramoxone treatment. <i>Makenia</i> , members of the Emphophis family and Compositae families, <i>Centrosema</i> spp and other weed species present effectively controlled.
5. Banvel + spread sticker + Li-700 at 80 mL	No effect on <i>Monstera</i> . All other weed species named above killed.
6. Ally + Li-700	Same effect as for glyphosate with Li-700; over 80% of the small <i>Monstera</i> 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 <i>Monstera</i> , 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 mL/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**

# Invasive weeds: impacts, prevention, detection and responses

W. Orapa<sup>1</sup>

## *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 'sleepers' 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 back-water 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.

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 endemism. 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 as 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

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, ves-

sels, 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

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 become 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



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	Fortuitous biological control
<i>Chromolaena odorata</i> (Asteraceae): biological control undertaken in 11 provinces <i>Eichhornia crassipes</i> (Pontederiaceae) <i>Lantana camara</i> (Verbenaceae) <i>Mimosa diplotricha</i> (Mimosaceae) <i>Pistia stratiotes</i> (Araceae) <i>Salvinia molesta</i> (Salviniaceae) <i>Sida acuta</i> (Malvaceae) <i>Sida rhombifolia</i> (Malvaceae) <i>Tribulus cistoides</i> (Zygophyllaceae)	<i>Parthenium hysterophorus</i> (Asteraceae): eradication from Lae, Morobe	<i>Mimosa pigra</i> : from Danip, Madang. <i>Piper aduncum</i> : single tree seen in mainland New Ireland in September 2004.	<i>Leucaena leucocephala</i> (Mimosaceae): the bug <i>Heteropsylla cubana</i> (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.

**Table 2.** Invasive weeds that are widespread, restricted and spreading, potential 'sleeper' weeds, or are absent from Papua New Guinea<sup>a</sup>

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

<sup>a</sup> This list is incomplete and not in any order of importance.

Many others are ‘sleepers’ weeds with the potential to become troublesome in the future. The threats posed by ‘sleepers’ 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<sup>1</sup>

## 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 (*Hemileia 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 biodi-

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

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. diplotrica*, 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

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 an 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<sup>1</sup>

R. Davis<sup>2</sup>, T. Gunua<sup>3</sup>, M. Kame<sup>3</sup>, D.I. Tenakana<sup>3</sup> and T. Ruabete<sup>2</sup>

## *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.

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 (Con-

nell 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-

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.

## 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.

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 chlamydospores 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-

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 germ-plasm 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 become 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

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

R. Masamdu<sup>1</sup>

## *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 Sec-

retariat 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.

## Acknowledgments

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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.

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

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

**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.

$$\text{Nut loss/ha} = (\mu - ob) \times d \times n$$

where  $\mu$  = 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

R. Masamdu<sup>1</sup>

## *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-



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 consid-

ered 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.

## **Acknowledgment**

I thank David Kanawi of NAQIA for briefing me on the quarantine protocols that applied before independence.

# 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 limnorcharis (*Limnorcharis 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.

## 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).

## 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 and 1994 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

form of the disease) is vectored by the African citrus psyllid (*Trioza erythrae* (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.

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 are required. 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<sup>2</sup> 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 kernel 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

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

## Results

### Life-cycle studies

The field-laid eggs were ovoid ( $0.45 \times 0.7$  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 peeled 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

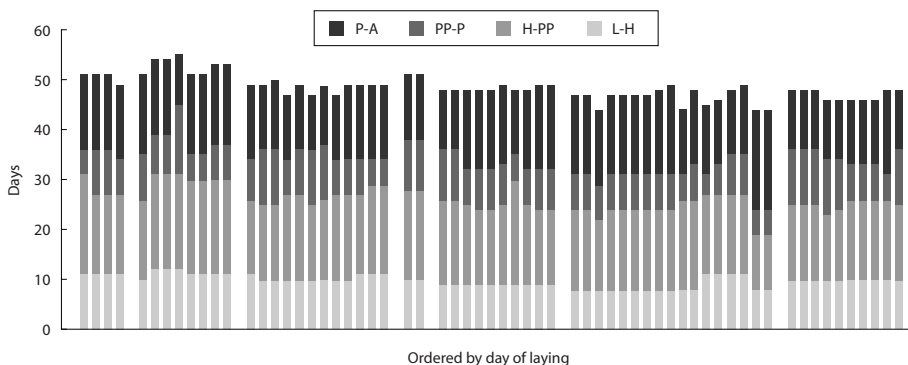
were laid within crust crevices remained exposed. If the fruit skin was clean and free of crusts, the female oviposited only on the peduncle, up to a centimetre or so along the fruit stalk. More than one cluster of eggs was found on the peduncle of one fruit.

### 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.

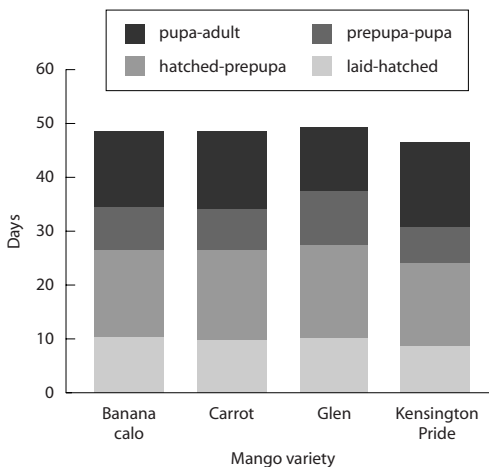
Green mangoes produce a large amount of very viscous exudates when injured. Similarly, the early instars of RBMCs induce large amounts of exudates whilst feeding on the flesh of the young mangoes. It was therefore not possible to follow the early instars in this set of experiments. First-instar larvae, 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

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). (RBMCO 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).



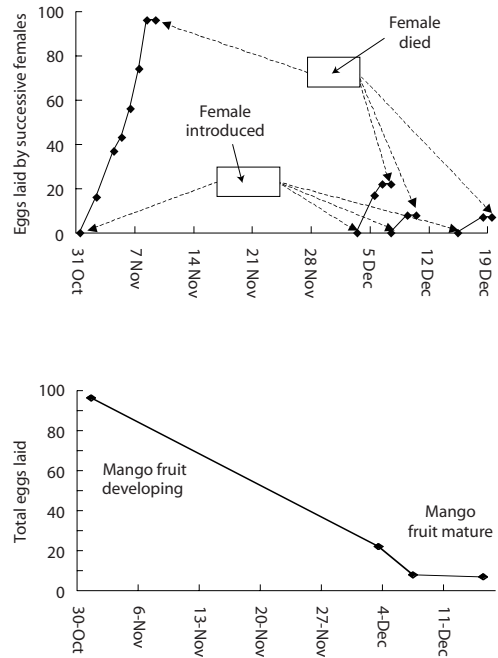
**Figure 2.** Life cycle of the red-banded mango caterpillar (*Deanolis sublimbalis* Snellen) on different mango varieties

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. sublimbalis* 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?

B. Pinese<sup>1</sup>

## *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**

# 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

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

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. Cur-

rently, 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

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

B. Nass-Komolong<sup>1</sup> and M. Maino<sup>2</sup>

## *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 to molecular 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.

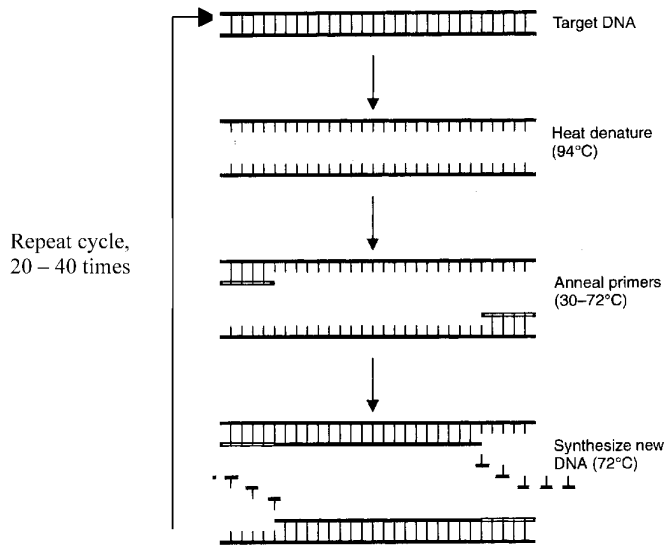
## Principle of the PCR

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).



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

**Table 1.** Different steps in PCR amplification

<b>Step 1: Denaturation</b>	Mixture is heated to 94°C. This denatures the double-stranded DNA, separating the two strands.
<b>Step 2: Annealing</b>	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.
<b>Step 3: Extension</b>	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.

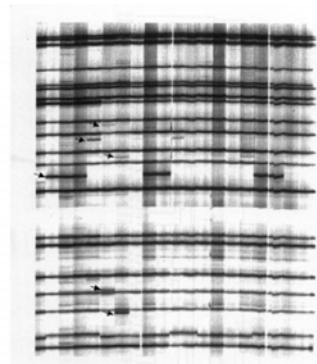
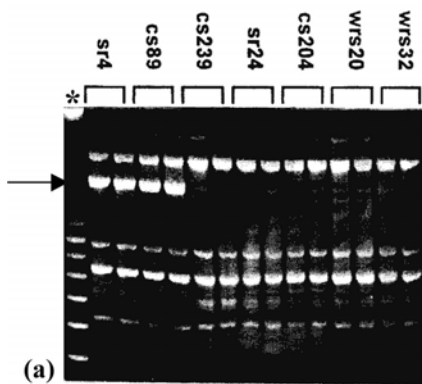
## DNA fingerprinting

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).





**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, for 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

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

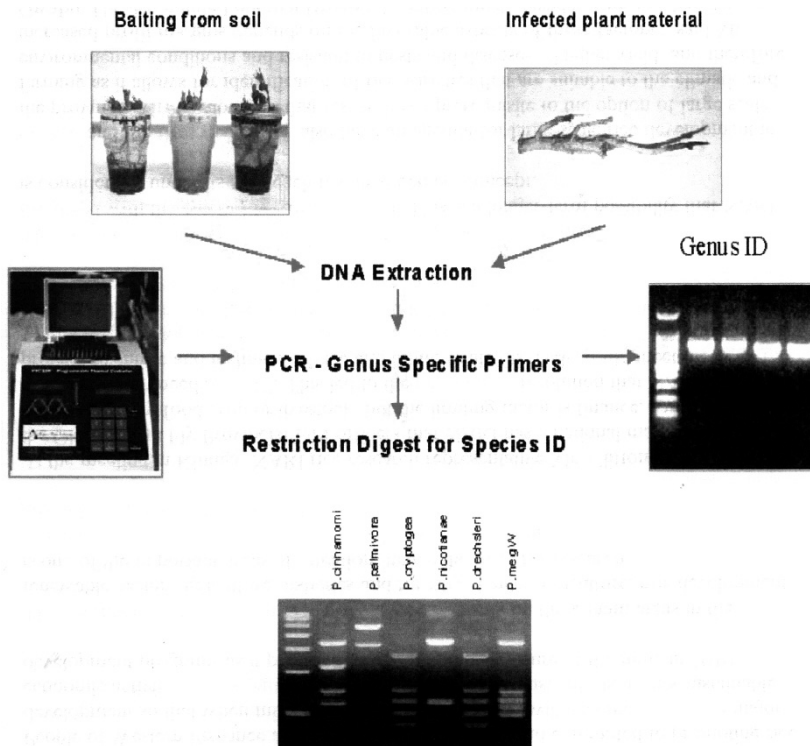
### Plant disease management

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)

**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 TANSO 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-

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).

Anthraxnose 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 specimens 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

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 Countries 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

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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.

## 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

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 ( $-20^{\circ}\text{C}$ ), 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 plants; 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*  $\times$  *paradisica* 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

*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		
	No cause of death	Oryctes baculovirus symptoms	Other cause of death
<i>Oryctes rhinoceros</i> , 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
<i>Scapanes australis</i> , 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
<i>Xylotrupes gideon</i> , 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
<i>Papuana woodlarkiana</i> , 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
<i>Papuana huebneri</i> , 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
<i>Other scarabs</i> , 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
<b>Grand total</b>	<b>811</b>	<b>203</b>	<b>184</b>

'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 con-

firmed 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

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<sup>a</sup> 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
<i>Oryctes rhinoceros</i> , L3 larvae, diseased in lab, died in lab	<i>Papuana huebneri</i> adults	8/4	0/0
<i>O. rhinoceros</i> , L3 larvae, diseased in lab, died in lab	<i>Papuana woodlarkiana</i> adults	9/4	1/0
<i>O. rhinoceros</i> , adult, diseased in lab, died in lab	<i>P. huebneri</i> adults	8/5	0/0
<i>O. rhinoceros</i> , adult, diseased in lab, died in lab	<i>P. woodlarkiana</i> adults	7/5	0/0
<i>O. rhinoceros</i> , L3 larvae diseased in lab, died in lab	<i>Papuana</i> spp. larvae	9/0	3/0
<i>O. rhinoceros</i> , L3 larvae, diseased in lab, died in lab, 2 month frozen	<i>P. woodlarkiana</i> adults	6/5	0/0
<i>O. rhinoceros</i> , L3 larvae, diseased in lab, died in lab, 18 month frozen	<i>P. woodlarkiana</i> adults	2/0	0/0
Gut of <i>P. huebneri</i> adult, infected through <i>O. rhinoceros</i> L3 (diseased in lab, died in lab) in previous trial	<i>O. rhinoceros</i>	7/5	0/0
Gut of <i>P. woodlarkiana</i> , adult, female, infected by <i>O. rhinoceros</i> L3 (diseased in lab, died in lab) in previous trial	<i>O. rhinoceros</i>	7/4	0/0
<i>O. rhinoceros</i> , L3 larvae, diseased in lab, died in lab, 10 min boiled	<i>P. huebneri</i> adults	0/0	2/0
<i>O. rhinoceros</i> , L3 larvae, diseased in lab, died in lab, left at room temperature for 15 days before infection	<i>P. huebneri</i> adults	1/0	1/0
<i>O. rhinoceros</i> , L3 larvae, diseased in lab, died in lab, left 5 days exposed to sun and weather before infection	<i>P. huebneri</i> adults	2/0	0/0

<sup>a</sup> Listed are the number of treated and control specimens that died and those showing positive symptoms of OBV.

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 microorganisms. 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

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.

*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

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**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

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

<sup>a</sup> Colony colour, 1 = white, 2 = grey, 3 = white to dirty white, 4 = dirty white, 5 = wool white.

<sup>b</sup> Colony growth, average growth per day given in mm.

<sup>c</sup> Colony margin, 1 = regular, 2 = irregular.

<sup>d</sup> Mycelial form, 1 = loose, 2 = compact

<sup>e</sup> Mycelial elevation, 1 = low, 2 = moderate, 3 = high.

<sup>f</sup> Sclerotial bodies, 0 = absent, 1 = present.

<sup>g</sup> Acervuli, 0 = rare, 1 = poor, 2 = abundant.

<sup>h</sup> Setae, 0 = absent, 1 = present.

<sup>i</sup> Conidia size, conidia dimensions given in micrometres.

<sup>j</sup> Conidia shape, 1 = cylindrical/fusiform, 2 = cylindrical/straight, 3 = cylindrical with one end tapering.

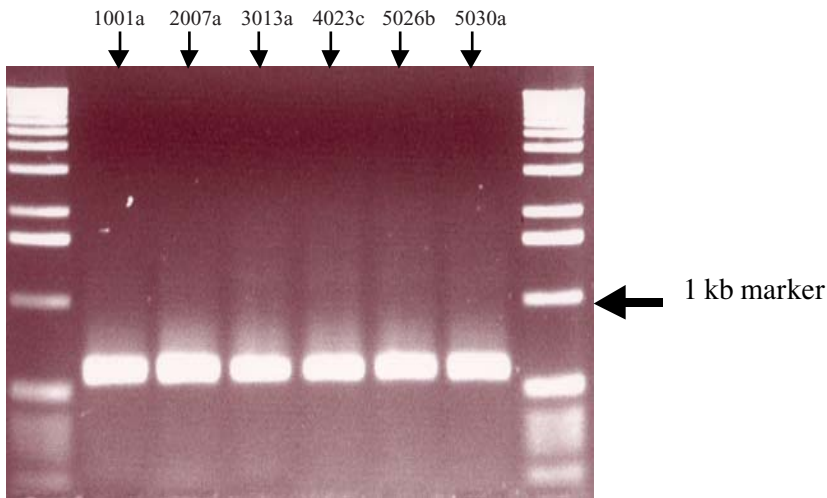
<sup>k</sup> Species identification, a = *C. acutatum*, g = *C. gloeosporioides*.

## Discussion

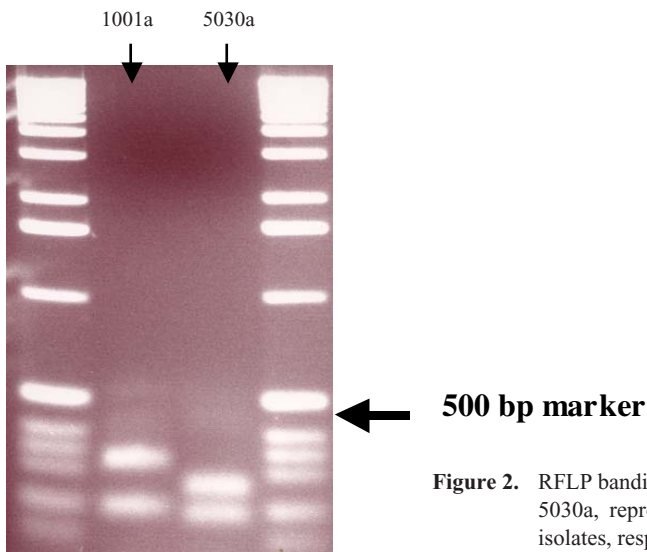
The species identification of the 40 isolates of *Colletotrichum* from PNG was made using both molecular techniques and conventional methods relying on cultural and morphological features. The cultural and morphological features described for 27 isolates fall within Sutton's (1980) species identification 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  $9\text{--}24 \times 3\text{--}4.5$ . 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

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.

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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