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CONTENTS

THE DISTRIBUTION OF *AMBLYPELTA* Stål SPECIES (HEMIPTERA: COREIDAE) IN PAPUA NEW GUINEA

Mark M. Ero[1]

ABSTRACT

Amblypelta *is a genus of twenty known described species, eight of which occur in Papua New Guinea. As true bugs they have piercing and sucking mouthparts and, in this country, four species are important pests of horticultural crops. Using literature sources and label data from museum specimens, distribution records for* Amblypelta *species within Papua New Guinea were collated. The genus is widely distributed in the lower altitudes of the mainland and parts of the New Guinea islands. It is absent from the highland provinces. The greatest species diversity occurs in Central, Milne Bay and Oro provinces with a high degree of species overlap in these areas.* Amblypelta *from the North Coast provinces and Bougainville province are distinct from the remaining Papua New Guinea fauna. Absence of* Amblypelta *records from Western, West New Britain and Manus provinces may be a result of lack of collection from these areas, rather than a true absence, and further collection in these provinces is warranted.*

Keywords: Amblypelta, *Papua New Guinea, National Agricultural Insect Collection,distribution, locality*

INTRODUCTION

Amblypelta (Order: Hemiptera, Family: Coreidae) is a genus of twenty described species, of which seven are represented by subspecies (Ero 2002a). Many species within the genus are serious pests of a range of crops across multiple plant families. The insects are sap feeders with piercing and sucking mouthparts enabling them to pierce fruits and soft stems.

According to Ghauri (1984), Smith (1984) and Ero (2002b), the *Amblypelta* species are widely distributed throughout parts of the Australasian region (Solomon Islands, Vanuatu, New Caledonia, Australia, Papua New Guinea and Indonesia). Eight species of *Amblypelta* species (*A. ardleyi* Brown, *A. bukhanii* Ghauri, *A. madagana* Brown and Ghauri, *A. theobromae* Brown, *A. cocophaga cocophaga* China, *A. costalis szentivanyi* Brown, *A. gallegonis bougainvillensis* Brown and *A. lutescens papuensis* Brown) occur in Papua New Guinea (PNG) (Ero 2002b). *Amblypelta cocophaga cocophaga* is a subspecies of *A. cocophaga malaitensis* Brown which occurs in the Solomon Islands; *A. costalis szentivanyi* is a subspecies of *A. costalis costalis* Van Duzee and *A. costalis renellensis* Brown that occur on Bellona Island and Rennel Island respectively; *A. gallegonis bougainvillensis* is a subspecies of *A. gallegonis gallegonis* Lever that occurs in the Solomon Islands and *A. lutescens papuensis* is a subspecies of *A. lutescens* Distant that occurs in Australia and Indonesia (Ghauri 1984).

The general distribution of *Amblypelta* species in PNG, is considered to cover the lower altitudes of the mainland and parts of the outer islands (Smith 1984, Ero 2002b). However, precise information on the distribution of the PNG *Amblypelta* species is scattered across the literature and further, some species are poorly documented. This paper gives collated distribution records from the literature and collection records from specimens deposited at the National Agricultural Insect Collection (NAIC), Port Moresby. This forms a comprehensive distribution record for *Amblypelta* species in PNG and establishes occurrence records for the pest species.

MATERIALS AND METHODS

The collection data for all identified specimens of *Amblypelta* deposited in the National Agricultural Insect Collection (NAIC) at Kila Kila, Port Moresby, were collated. Regional collections (Forest Research Institute Insect Collection and National Agricultural Research Institute station collections) were searched but no identified *Amblypelta*

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specimens were found. Relevant literature sources were also scanned for records.

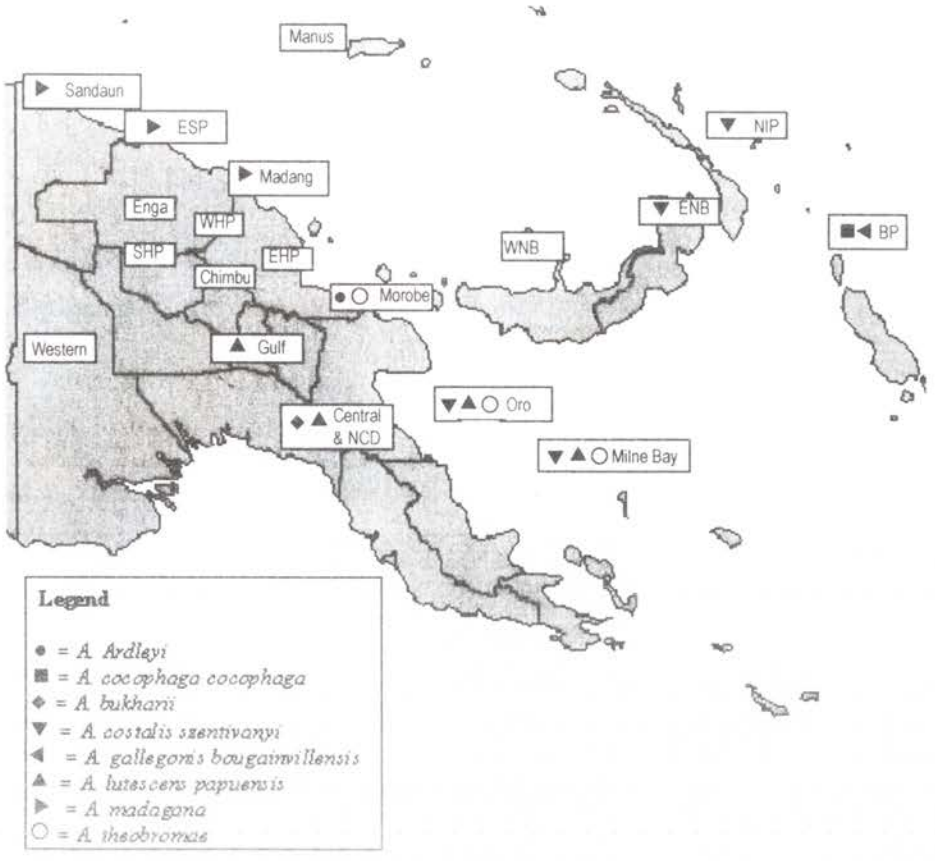
RESULTS

In PNG, the distribution of *Amblypelta* is restricted to the lower altitudes of the mainland and the adjacent islands (Table1, Figure1). *Amblypelta gallegonis bougainvillensis* and *A. cocophaga cocophaga* are restricted to the island of Bougainville, although the latter species also occurs in the neighbouring Solomon Islands from where the type species was described (Brown 1958). Other apparently restricted species include *A. bukharii*, which is known only from the type locality in the Central Province, and *A. ardleyi*, only collected from Morobe Province. *Amblypelta madagana* is restricted to the northern part of the mainland (Madang, East Sepik and Sandaun provinces), while *A. lutescens papuensis* only occurs in the southern region (Central, Gulf, Oro and Milne Bay provinces

Table 1. **Locality records of *Amblypelta* species in Papua New Guinea [Sources: Brown 1958; Szent-Ivany & Catley 1960; Smith 1984; NAIC label data]**

A.ardleyi [Morobe Province: Bubia (6°45'S 146°58'E), Lae (6°44'S 147°00'E)]	*A. gallegonis bougainvillensis* [Bougainville Province: Numa Numa (5°52'S 155°16'E), Kieta (6°13'S 155°38'E), Buin (6°50'S 155°44'E), Boku (6°34'S 155°21'E), Sevele, Kokorei, Kokure, Mosigeta (6°32'S 155°19'E), Simba, Torokina (6°14'S 155°03'E), Siara]
A. cocophaga cocophaga [Bougainville Province: Gagani (5°14'S 154°37'E), Sohano (5°26'S 154°40'E), Tanaboia, Numa Numa (5°52'S 155°16'E), Aroa]	*A.lutescens papuensis* [Oro Province: Mt. Lamington (8°56'S 148°10'E); Milne Bay Province: Samarai (10°37'S 150°40'E); Central Province: Bisianumu (8°43'S 147°25'E), Brown River (9°15'S 147°05'E), Baubauguina (10°07'S 148°44'E), Itikinumu (9°25'S 147°31'E), Maraboi, Ninoa (9°28'S 147°28'E), Lolorua (8°57'S 146°57'E), Subitana (9°25'S 147°32'E), Veimauri (9°02'S 147°03'E), Laloki (9°24'S 147°18'E), Kanosia (8°59'S 146°58'E), Sogeri (9°25'S 147°25'E), Doa (8°57'S 146°58'E), Tapini (8°22'S 146°59'E), Hula (10°05'S 147°43'E); National Capital District: Boroko (9°28'S 147°12'E), Konedobu (9°28'S 147°09'E); Gulf Province: Petoi (7°57'S 145°45'E), Karaita, Kerema (7°58'S 145°46'E), Murua (7°55'S 145°50'E)]
A. bukharii [Central Province: Brown River (9°15'S 147°05'E)]	*A. madagana* [East Sepik Province: Magafin; Sandaun Province: Aitape (3°08'S 142°21'E); Madang Province: Bogia (3°08'S 142°21'E), Bogadiiim (5°26'S 145°44'E)]
A. costalis szentivanyi [East New Britain Province: Kerevat (4°20'S 152°04'E); Milne Bay Province: Normanby Island (10°00'S 151°00'E), Oro Province: Isaveni (8°45'S 148°08'E), Kokoda (8°54'S 147°47'E), Mt. Lamington (8°56'S 148°10'E), Sangara (8°49'S 148°14'E); New Ireland Province: Gilingili (4°28'S 152°40'E)]	*A. theobromae* [Morobe Province: Simbang (6°35'S 147°50'E), Kunakumen, Leiwomba, Finschafen (6°27'S 147°47'E), Simbang (6°35'S 147°50'E), Lae (6°44'S 147°00'E), Gabensis (6°43'S 146°46'E), Bubia (6°45'S 146°58'E), Melambi River; Oro Province: Mt. Lamington (8°56'S 148°10'E), Azerita, Sangara (8°49'S 148°14'E), Mamoo, Kokoda (8°54'S 147°47'E), Lego, Sairope (8°55'S 148°02'E), Sairorota, Kepara (8°56'S 147°47'E), Epa (8°40'S 148°05'E), Opi (8°19'S 148°12'E), Seiha, Ioma (8°22'S 147°50'E); Milne Bay: Naura (10°19'S 150°15'E), Normanby Island (10°00'S 151°00'E); Central Province: Brown River (9°15'S 147°05'E); National Capital District: Konedobu (9°28'S 147°09'E)]

Figure 1. **Distribution of *Amblypelta* species in Papua New Guinea.**



and the National Capital District). *Amblypelta costalis szentivanyi* occurs both on the mainland (Oro Province) and the adjacent islands (New Ireland Province, East New Britain Province and the Normanby Island of the Milne Bay Province). In contrast to other species, *A. theobromae* has an extensive range covering Morobe, Central, Oro and Milne Bay provinces and the National Capital District. Smith (1984) recorded the species also in Madang Province but the basis for this record is unknown and further collections are warranted.

DISCUSSION

Ero (2002b) presents the horticultural crop host list of *Amblypleta* species in PNG and the economic importance of the pest. The most common pest species are *A. lutescens papuensis*, *A. theobromae*, *A. cocophaga cocophaga* and *A. gallegonis bougainvillensis*. The other species produce some degree of damage to the crops they attack but their level of economic importance is not yet established. Based on the present work, it can now be recorded that most of the pest species occur in the Central, Gulf, Milne Bay, Oro, Morobe and Bougainville provinces.

The richest *Amblypelta* fauna clearly occurs in the neighbouring Central, Oro, Milne Bay and Morobe provinces, which share many species (Figure 1). The north coast fauna and those of Bougainville are distinct from the rest of PNG. The apparent lack of species from Western, West New Britain and Manus provinces needs to be confirmed by further collecting to determine if there is a real absence or if this is an artifact of lack of sampling. The lack of records for the highland provinces is much more likely to represent a true absence of the genus at higher altitudes, rather than the result of a lack of sampling.

CONCLUSION

Further sampling is needed in Manus, West New Britain and Western provinces to determine if *Amblypelta* is genuinely absence from these areas.

Additional sampling is needed in the regions where the species have been recorded to determine the altitudinal distribution range of the genus.

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PROSPECTS OF BIOLOGICAL CONTROL OF THE COCOA MIRID, *SAHLBERGELLA SINGULARIS* Sahl (HETEROPTERA) IN GHANA: FIELD SURVEYS FOR ENTOMOPATHOGENS AND LABORATORY BIOASSAYS WITH *BEAUVERIA BASSIANA* ISOLATES.

Joseph B Ackonor*, Isaac Y Opoku, George Oduor**, Ignas Godonou** and Abraham Nkansah.

ABSTRACT

Distantiella theobroma (Dist) *is the most important species of mirids attacking cocoa in Ghana. Its control has mainly relied on the application of synthetic insecticides. Because of the hazards, cost and the resultant low adoption rate of research recommendations associated with such chemicals, the Cocoa Research Institute of Ghana continues to search for more cost effective and environmentally benign alternative means of managing the mirid menace in Ghana. Surveys were conducted for two mirid seasons; in all the six cocoa growing regions of Ghana for pathogens of S. singularis and species of Fusarium, Aspergillus, Nomuraea and Ascheinrsonia were isolated. Then followed four bioassays with five isolates of Beauvaria bassiana to assess their biological effect on S. singularis. The results indicated great potentials for the fungus as a biological control agent. Isolate I97 1035 was the most promising, followed by 197 1036, IMI 335249, I00 1183 and 197 1037. Details of the results are discussed.*

Keywords: *Cocoa, mirids,* Sahlbergella singularis, Beauvaria bassiana, *isolates.*

INTRODUCTION

Mirids (Capsids) (Heteroptera) are undoubtedly the most important insect pests of cocoa in Ghana and *Sahlbergella singularis* (Heteroptera) is considered the most prevalent of the four main species. For several decades, control of the bug and other cocoa mirids has mainly relied on the application of synthetic insecticides. Hazards associated with the use of such chemicals for pest control are known worldwide. In addition, chemical insecticides, applicators and other relevant inputs for their effective utilization have become increasingly costly for the average cocoa farmer in Ghana, resulting in low adoption rate of recommendations made by the Cocoa Research Institute of Ghana (CRIG) for chemical control of the pest (Padi 1991). CRIG, therefore, continues to search for more efficient, cost effective and environmentally benign alternative means of managing the mirid menace on Ghana's cocoa farms.

One possible alternative is the use of fungi that are pathogenic to insects, and such fungi are extremely important in microbial control of insect pests (Roberts and Humber 1981). Virtually all the insect orders are susceptible to fungal diseases, and this may be useful particularly for the control of sucking insects such as mirids (Roberts and Humber 1981). Among the fungi that have been exploited worldwide for insect pest control are *Metarhizium anisopliae* and *Beauvaria bassiana* (Ferron 1981), (Prior 1988), (Feng *et al.* 1994), (Oduor *et al.* 2000), (Godonou *et al.* 2000). Conidia of *B bassiana*, for instance, have been used to reduce populations of stored products pests of cereals by over 60 % by spraying onto the surface of bags in which these products were stored (Pham *et al.* 1995). (Adane *et al.* 1996) also reported that an isolate of *B bassiana* caused 88% mortality of *S zeamais* within 8 days and the pathogen was used successfully against the potato Colorado beetle, *Leptinotarsa decemlineata* in North America and Eastern Europe (Ferron 1978). In China, *B bassiana* has been used for the control of the European corn borer, *Ostrinia nubilalis* (Hussey and Tinsley 1981).

The humid nature of the cocoa ecosystem is believed to be conducive for rapid growth and sporulation of fungi, hence constituting a contributory factor to the menace of the cocoa black pod disease in Ghana (Dakwa 1973). It is possible, therefore, that such humid conditions would enhance the spread of fungal pathogens, such as

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B bassiana, of cocoa insect pests. *B bassiana* is also known to store well in a refrigerator at 5°C - 8°C without loss of viability and sporulation ability, whereas such temperatures will normally eliminate many other tropical fungi (Godonou 1999). (Lim *et al.* 1989) excluded *B bassiana* from the list of pathogens that are dangerous to humans and other mammals, but it has been cautioned that chronic exposure to high densities of fungal conidia could lead to allergic sensitization (Roberts and Humber 1981).

The use of *B bassiana* as a biological agent in the integrated control of mirids requires attention and this paper reports initial efforts at CRIG to include the fungus in the control of the pest, hopefully to minimize the use of synthetic insecticides.

MATERIALS AND METHODS

The project started with a search for pathogens of cocoa mirids in all the six cocoa growing regions of Ghana in August-January 2001/2002 and repeated a year later. Live, moribund and cadavers of cocoa mirids, as well as cadavers of other insects, were brushed into sterilized plastic vials and taken to the laboratory. They were incubated on moist filter paper placed in Petri dishes, observed for fungal growth and the causative fungi identified. Then followed four bioassays conducted with the following isolates of *B. bassiana* to determine their efficacies on *S singularis*: IMI 335249, I97 1035, I97 1036 and I97 1037 received from CABI Bio-Science in the UK, and I00 1183 originating from Ghana.

Experiment 1

All the five isolates were tested in the first bioassay. They were cultured on Potato Dextrose Agar (PDA) in 9cm diameter Petri dishes, and conidia extracted after 4-weeks with sterile distilled water (SDW) containing 0.05% Tween 80. The conidia concentration in SDW was determined for each isolate and adjusted to 10^7 spores ml^{-1}. SDW alone was used as the control. Filter papers with 12.5cm diameter were moistened with spore suspensions of *B bassiana* and placed in sterilized Petri dishes. To allow aeration, each dish was covered with a lid having a 2.5cm square hole in the center and sealed with a piece of plastic mesh. Five individuals of *S ingularis* comprising 4th - 5th instar nymphs and adults were released onto each treated filter paper. Each treatment was replicated five times. The insects were denied food for 24hrs in order to stimulate them to search for food by crawling all over the treated filter papers, thereby contaminating their bodies with the spores. They were transferred onto food (cocoa chupons) after the 24 hours and observed every other day for 15 days at 25 to 26°C. The following data were collected: (a) % mortality and (b) % of dead insects with external growth of *Beauvaria*. Mean % mortalities were corrected for natural mortality, using Abbotts' formula. Abbott.

Experiment 2

Only three isolates, IMI 335249, I97 1035 and I00 1183, were tested in the second experiment because of insufficient numbers of the mirid. The methodology and experimental conditions were largely the same as described above, except for the following modifications: The spore concentration of each test isolate was adjusted to 4 x 10 (Pham *et al.* 1995) ml^{-1} and *S singularis* was fed on fruits of *Desplastsia dewevrei* (Tiliales: Tiliaceae), reported to be more suitable for rearing the bug (Padi and Sarfo 2002). Ten insects were placed in each of four Petri dishes (replicates) lined with spore-contaminated filter paper, left undisturbed for four hours and thereafter transferred into plastic cages containing *D dewevrei* fruits. Mean % mortalities were recorded after 5, 10 and 15 days, as well as the number of dead insects with fungal growth and level of sporulation.

Experiment 3

In the 3rd bioassay, four isolates, IMI 335249, I97 1036, I97 1037 and I00 1183 were tested. Two milliliters of 4 x 10 (Pham *et al.* 1995) ml^{-1} concentration of each isolate were used to contaminate each of three filter papers (replicates). Three filter papers were treated with SDW plus 0.05% Tween 80 to serve as the control. Twenty *S singularis* were brushed into each bowl and left to crawl for one hour on the filter paper before being fed with a mixture of chupons and unripe cocoa pods.

Experiment 4

The final bioassay tested all the five isolates. Three batches (replicates) of four cocoa chupons each were contaminated (sprayed) with one of the five *B bassiana* formulations prepared as described above and placed in three different plastic bowls lined with untreated filter papers. The control treatment comprised chupons treated with SDW plus 0.05% Tween 80 only. Twenty *S singularis* were introduced onto the chupons in each plastic bowl.

RESULTS

During the two-season search for pathogens, *Fusarium* sp, *Aspergillus* sp, *Nomuraea* sp and *Ascheinrsonia* sp, as well as species of some unidentified fungi were isolated from capsids. *Cordyceps* sp and *Entomophaga* sp were isolated from ants (Hymenoptera: Formicidae) and *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae), respectively. It was not possible to recognize several other insects with fungal growth as they were completely covered by the fungal tissue. In any case, none of the fungi was bio-assayed since they were not amenable to exploitation in bio-control (Oduor, Pers comm)

In the 1st bioassay, isolate I97 1035 recorded the highest mirid mortality (71.4%) after 5 days, followed by I97 1036 (60%), IMI 335249 (48.6%), I00 1183 (31.4%) and I97 1037 (20%) (Table 1)

By the end of the 10th day, mortality levels had risen for all the isolates and isolate I97 1036 had already caused 100% mortality, while each of the other four had recorded 86.5% mortality. Three of the isolates caused 100% mortality after 15 days while two, including I00 1183 originating from Ghana, recorded 87.5% mortality.

Results of the 2nd bioassay (Table 2). Five days after the experiment was set-up, isolate I97 1035 had recorded the highest mortality (52%), followed by I00 1183 (44%) and IMI 335249 (40%). But by the 10th day IMI 335249 had caused the highest mortality (80%) followed by I97 1035 (68%) and I00 1183 (64%). Mortality levels remained unchanged thereafter. Isolate I00 1183 sporulated most profusely on the dead insects (Plate 1), followed by I97 1036 (Plate 2).

Results for 3rd and 4th experiments were equally promising. The % mortality level in the former was highest among *S singularis* treated with isolate I97 1037, followed by I00 1183, I97 1036 and then IMI 335249. Similar results were obtained in the 4th experiment, the highest % mortality being, once

Table 1. **Percent *D theobroma mortalities* 5, 10 and 15 days after exposure to isolates of *B bassiana*.**

B bassiana isolates	Corrected % mortalities		
	5 days	10 days	15 days
I97-1037	20.0	86.5	100.0
I97-1036	60.0	100.0	100.0
I97-1035	71.4	86.5	87.5
I00-1183	31.4	86.5	87.5
IMI 335249	48.6	86.5	100.0

Table 2. **Percent *D theobroma* mortalities and sporulation 5, 10 and 15 days after exposure to isolates of *B bassiana*.**

B bassiana isolates	Number of *D theobroma* treated	% Mortalities at day:			% producing spores
		5	10	15	
IMI33 5249	10	40.0	80.0	80.0	20.0
I97-1035	10	52.0	68.0	68.0	68.0
I00-1183	10	44.0	64.0	64.0	12.0

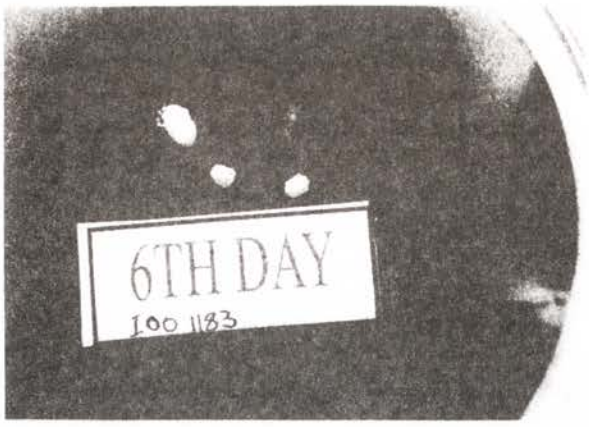

Plate 1. **Extent of sporulation by *B. bassiana* isolate I00 1183 on *S. singularis* six days after treatment.**

Plate 2. **Extent of sporulation by *B. bassiana* isolate I97 1036 on *S. singularis* six days after treatment.**

again, recorded on *S singularis* contaminated with isolate I97 1037 followed by I00 1183. However, *S singularis* treated with IMI 335249 had greater mortality than those treated with isolates I97 1035 and I97 1036.

DISCUSSION

The control of cocoa mirids in Ghana is mainly by the use of chemical insecticides such as Confidor, Cocostar and Carbamult. Although these are effective, the risk of pest resistance, residual toxicity, environmental concerns and the high cost of treatments make it imperative to look for alternative products such as natural enemies, including pathogens. The present study has demonstrated that *B bassiana* is a potential biological agent for the control of *S singularis*, and probably other cocoa mirids. The 20 to 71.4% mortality rates in the first experiment, and the 40 to 52% in the second, 5days after treatment, together with even higher mortalities after 10 and 15 days in the experiments clearly attest to the effectiveness of the isolates, particularly isolate I97 1035.

The mortality rates in experiment 1 were significantly higher than that in experiment 2. This disparity is difficult to explain, but it may be due to differences in the viability and, therefore, the persistence of *B bassiana* conidia in the two formulations (Godonou *et al.* 2000). Another possible cause for the disparity was the different food sources for the two experiments; i e, cocoa chupons for the 1st experiment and fruits of *D dewevrei* for the second. In any case, the results point clearly to the great potentials of *B bassiana* as bio-control agent for *S singularis* on cocoa.

The exact mode of mirid infection by *Beauvaria* is unknown and studies in this area will be useful. However, it is apparent in this study that infection of *S singularis* occurred through the mouthparts, the abdominal segments and the antennae as indicated by fungal growth and sporulation patterns.

Profuse sporulation of the isolates on the dead mirids was clearly evident in the 2nd bioassay. Thus, all the dead insects were completely covered with the fungal spores 10days after spraying. This suggests the possibility of healthy (uninfected) individuals being infected in a field situation through such profuse sporulation on infected (dead) individuals. *B bassiana* is known to be non-fastidious, growing and sporulating on a wide variety of media. Lim *et al.* (1989). In this work, all the test isolates grew and sporulated well at room temperature, i e 25 - 26 ° C. Although further investigations are required in these areas, it is apparent from the data that the cocoa ecosystem in Ghana will support the growth and sporulation of *B bassiana* and thereby enhance its persistence in the target environment. This will ultimately keep the *S singularis* population down without necessarily re-applying the control agent (Godonou *et al.* 2000).

CONCLUSIONS

The present study has demonstrated clearly the potentials for *B bassiana* isolates in the management of *S singularis* numbers on cocoa. Ultimately, microbial control could form an important

component of an IPM strategy against the mirid on cocoa. Implementation will, however, require in depth research in areas of mass-production, formulation and delivery. Further tests on human safety, virulence to target pests, safety of non-target fauna and persistence, among others, will also have to be thoroughly investigated.

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CURRENT ISSUES AND INITIATIVES IN THE CONSERVATION AND MANAGEMENT OF SHEEP GENETIC RESOURCES[1]

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ABSTRACT

Sheep are less prone to genetic erosion than many other major domestic animal species. A brief overview of the status of sheep genetic resources indicates 1,313 living breeds according to the FAO database with 20 percent at risk and a further 30 percent of unknown status. There are problems with breed definition which should be widened to include populations defined by geography or social system. It is also necessary to have clearer guidelines as to what breeds should be included by countries in the database. It is hoped that the current State of the World's Animal Genetic Resources reporting initiatives will help clarify the situation. While it is desirable to describe the sheep genome and look for Quantitative Trait Loci and their associated markers, the current interest is in readily available packages of genes as defined by breeds. There is likely to remain a satisfactory reservoir of breeds to draw on to construct new designer sheep. This is happening in response to changing requirements for products or production systems but these remain unpredictable. In many situations the conservation of sheep genetic resources is related to the conservation of pastoral or social systems and if or when these change, the sheep will need to change with them. Rational management and utilization of sheep genetic resources requires on-going genetic improvement within breeds. Sensible communal agreement on breeding objectives will enable the owners of breeds to avoid unnecessary genetic erosion and maintain breed utility at the highest possible level.

Keywords: *Sheep, Genetic Resources, Conservation and Management.*

STATUS OF SHEEP GENETIC RESOURCES

Many authors have commented on the trends in commercial livestock production to concentrate on a few highly selected breeds or hybrids (eg. Hall 1996). The examples usually given are the black and white (Holstein-Friesian) dairy cattle, the Landrace and Large White pigs and hybrid chickens. This has resulted in the marginalisation of large numbers of local breeds or landraces and genetic erosion. This tendency has been less dramatic with sheep because of less pressure towards specialization and intensification. Sheep are suited to a wide range of marginal environments and to nomadic or transhumance systems. They are kept to produce a diversity of products from within a single flock. They have a long history of domestication, a world-wide geographic spread and utility for smallholder, family husbandry. While there has been widespread dissemination and use for crossbreeding in developing countries of exotic fine-wool sheep, highly productive in their own environments, sheep (and goats) have retained a greater genetic diversity than the other major mammalian domestic species. The proportion of breeds at risk out of the total existing recorded breeds, according to the FAO-UN Domestic Animal Diversity Information System database (Scherf 2000), is 18 percent for goats, 20 percent for sheep, 24 percent for cattle and 33 percent for pigs.

Table 1 gives the numbers of sheep and breeds, and breed risk status, by region as classified by FAO. The Asia-Pacific region has only 18 percent of the world's breeds in spite of having 39 percent of the sheep. Of the Asia-Pacific breeds, only nine percent are classified as at risk with a further 29 percent of unknown status. Table 2 gives the numbers of breeds and breeds at risk for the countries of Asia and Pacific region. This grouping is not the same as the FAO Asia-Pacific grouping. It is clear where most of the genetic diversity can be found. However, the number of listed breeds alone does not give a clear indication of the existing genetic diversity in any one country or region because more breeds are present than are listed in the database. For example, I have counted a total of 51 breeds as actually present in New Zealand (NZ) of which only

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Table 1. **Numbers of sheep, breeds and risk status by region**

Region	Sheep		Breeds		Critical	Crit. Maint.	Endangered	Endang. Maint.	Total at risk	Unknown Status
	No. '000	%	No.	%						
Africa	127,440	12.0	147	11.2	10		9		19	53
Asia/Pacific	408,098	38.5	233	17.7	5		15	1	21	68
Europe	185,035	17.4	629	47.9	36	6	108	42	192	115
LatinA./Carib.	89,372	8.4	42	3.2	1		3		4	13
Near East	242,770	22.9	201	15.3			5		5	114
N. America	7,891	0.7	61	4.6	10		16		26	28
Total	1,060,606		1313		62	6	156	43	267	391

Source: Scherf (2000)

Table 2. **Breeds in the FAO database by country – Asia and Pacific**

	Breeds	Breeds at risk		Breeds	Breeds at risk
Afganistan	12	1	Myanmar	1	
Australia	34	6	Nepal	6	
Bangladesh	1		New Zealand	21	5
China	40		Pakistan	39	5
India	60	1	Papua New Guinea	1	
Indonesia	8	3	Philippines	1	
Iran	26		Sri Lanka	1	
Kazakhstan	17		Tajikistan	10	1
Kyrgyzstan	10	1	Turkmenistan	8	
Laos	1	1	Uzbekistan	8	
Malaysia	6		Vietnam	1	
Mongolia	19				

Source: Scherf (2000)

21 (41 percent) are in the database. Also there is less breed differentiation in Asia compared to Europe or North America because of historical mobility of people and sheep, and lack of breed societies, formal recording and organized breed improvement.

Many of the breeds listed as at risk are rescued feral populations, relatively recent synthetics or country specific sub-populations representing a more widely dispersed multi-country genotype. The five breeds at risk in NZ are all derived from isolated populations of Merino or Merino x Longwool sheep that have been feral for some 150 years. Four of the Australian breeds are two or three breed composites, one is a Border Leicester derivative and one a maintained early Merino type. Marco Polo's sheep is described as a variety of the Argali (*Ovis ammon polii*) and is listed for Pakistan, Afghanistan, Kyrgyzstan and Tajikistan. The other four Pakistani breeds are two or three breed composites as is the Laotian breed. The Indian breed is described as a variety of the Urial. One of the Indonesian breeds is a recently (1994) imported crossbred and there is no information on the other two breeds.

BREED DEFINITION

It seems that for NZ, and similarly for Australia, breeds included in the database are those breeds actually formed in NZ as composites, by crossing or from special selection, plus the rescued ferals, the NZ Romney and the Australian Merino. Not included are all the British and more recent introductions. It is clear that the Romney and the

Merino, and similarly the various Merinos in Australia, are different now from their European ancestors and are rightly listed as distinct. However, the assumption has been made that other breeds, even those of long-standing residence such as the Southdown and Cheviot, are not sufficiently different from their British ancestors to merit stand-alone status. Some of the minor breeds in NZ, if they were actually distinct, would fall into the at risk classes, eg. Shropshire (about 260 ewes), Dorset Horn (380 ewes), Ryeland (315 ewes) and Wiltshire Horn (270 ewes). Similarly for Papua New Guinea (PNG) I submitted the listed data on the PNG Priangan but did not submit anything on the newly formed PNG Highlands Halfbred nor on the Corriedale and Perendale breeds that, together with the Priangan, went into its formation.

From these considerations it would appear that there is a need for clearer guidelines as to what breeds or breed types should be listed in the FAO database. Better guidelines may also be needed for the assessment of the relative importance of the breeds at risk. The main problem areas would appear to be as follows:

1. Breeds having the same name, origins and purposes, but resident in different countries, not necessarily in clearly different ecosystems.
2. Breeds which are probably essentially of the same genotype but with listing in different country lists. Perhaps some cross-referencing is required here.
3. Composites or stabilised derivatives from crossbreeding – at what stage do these warrant separate new breed status and can they be considered as at risk if relatively recent in origin?
4. Exotic breeds – at what stage do breeds imported and then isolated, whether recently or long ago and subjected to more or less intense natural or artificial selection, become different from their source populations?
5. Rescued feral breeds – how do we assess and put a value on 100-200 years of natural selection in isolation, usually in a harsh environment?
6. Isolated populations, perhaps lacking apparent phenotypic uniformity but shepherded by people with common purposes in a definable ecosystem – do such populations warrant the status of distinct landrace or breed? There is clearly a need in such cases for better production characterization and evaluation.

It is to be hoped that the current State of the World's Animal Genetic Resources reporting initiatives will help in clarifying these issues and improving our knowledge of the world's sheep genetic resources.

GENETIC RESOURCE MANAGEMENT

There are four components to the process of management of a nation's sheep genetic resources – documentation, evaluation, conservation and utilization. Documentation is being undertaken as discussed above. Evaluation is more difficult but at least the first step of adequate production characterization must be done as a matter of urgency. It is less urgent to compare breeds in a common environment unless there are pressures to introduce, cross or change breeds. The evaluation process should attempt to determine the reasons for superior performance or fitness if these are indeed present. Breed differentiation has often taken place within an apparently homogeneous environment and we have to try to understand why this has happened. The differences may be more superficial than fundamental to production. However, if it proves possible to identify superior local breeds then the possibility opens up to use these in the development of synthetics rather than looking to import exotics (Turner 1991). It is absolutely essential both for evaluation and subsequently for formulation of breeding objectives to get the best possible understanding of all facets of the environment with their constraints and opportunities and the reasons, both biological and environmental, for the low productivity so often observed or perceived as present in local sheep flocks.

Effective utilization is the incentive for conservation. There has been some interesting debate on the necessity or justification for the conservation of breeds as such in order to ensure sufficient genetic variation for conceivable utilization in the future. Barker (1997) has put the conventional case for conservation which is that breeds have unique sets of genes and present unique genotypes. Franklin (1997) however argues that there is little evidence that breeds contain sets of unique genes that might be needed for the future. Most important genetic differences are polygenic in origin and genetic variation for quantitative traits is renewed each generation at a staggering rate and hence the ongoing response to selection. The argument is that there is ample variation in commercial populations and the major landraces in developing countries which is being constantly renewed and is available

to meet any future requirements. There is therefore no need to keep a wide range of obscure breeds for insurance purposes. Clearly many of the minor breeds at risk, conscientiously conserved by a range of voluntary organizations, by small or hobby farmers, in parks and zoos for heritage and educational reasons or in remaining feral populations, will never be needed for production by the wider farming community. As Barker (1997) points out, the breeds at risk have presumably been tested over time and found wanting or unable to adapt to changing circumstances. However, how is it possible to know what will be needed and how quickly it may be necessary to access traits?

GENETIC IMPROVEMENT

Useful genes at single loci have been found and used. These include the polled gene, the carpet wool genes as in the Drysdale, the fecundity genes (Booroola and Inverdale) and the recent Callipyge gene. The search for Quantitative Trait Loci goes on (Crawford 2001) with the possibility of Marker Assisted Selection (MAS) to increase rates of genetic progress or introgress desired alleles into existing breeds (Piper 1999). It is likely that the major advantage of MAS will come with traits that are hard to measure such as disease resistance and meat quality. Gene insertion to produce transgenic sheep for enhanced production, while of limited success so far, may yet prove possible to improve existing breeds (Rexroad 1995). None of this, therefore, diminishes the utility of having available a range of breeds with identifiable qualities for immediate use, generally through the creation of synthetics, to meet changing environmental challenges and market opportunities.

Utilisation of genetic resources or genetic improvement in sheep will most likely continue to involve within breed selection and the formation of composites. Systematic crossbreeding is not usually an option because of the problems of maintaining the different components in situations where the utility of the sheep is multi-purpose and such systems are rare. Even the simple use of terminal sires across base ewes, as in NZ, requires an effective production and marketing structure for the sires. The development of the Landcorp Lamb Supreme terminal sires in NZ (Nicoll *et al.* 1997) is an example of how the development of a new composite can be combined with systematic crossbreeding in the favorable circumstances of very large flocks under unitary control and established markets.

Banks (1997) has argued that little thought has been given as to how to optimize the portfolio of genetic variation in the form of breeds or types of breed necessary to enable continuing improvement and flexibility. He suggests that the development of composites, while apparently widening the choice for farmers, is an attempt at rationalization and there is likely to be, at least for the Australian sheep industry, an ongoing tendency for one breed or composite to dominate each sector of the industry. He raises a series of questions about how best to choose and manage the genetic variability to minimize costs (actual costs and lost production) and maximize potential benefits (current use and flexibility for change). There would appear to have been little progress over the last five years in answering these questions. No doubt eventually the measurement and analysis of genetic distance through microsatellite polymorphism (Crawford and Littlejohn 1998) and the FAO initiatives under the project for the measurement of domestic animal diversity (MoDAD) may help to eliminate some breeds, at least from public investment, but yet it will remain necessary to identify and maintain reservoirs of distinctive breeds with defined traits that may prove useful and easily accessed. However, breeds in their places of origin or major use are always locally adapted so their utility needs to be adequately tested for any new circumstances. Clarke and Banks (1995) have documented the developments in breed introduction and development in Australia and NZ over the last 20 years. These have come from new introductions, synthetics or new strains resulting from selection. Issues that arise here are industry acceptance, levels of investment, competition, adequate genetic information systems and the dangers of overlooking minor traits.

New technology appears to have opened up new freedoms for the safer movement of breeding stock across what were quarantine barriers. This has resulted in the re-examination of the utility of European breeds in NZ and African breeds for the dry tropics of Australia. Breeds showing particular promise in NZ are the Finn, East Friesian and Texel. These are being used along with existing breeds for example to develop composites for new market opportunities in wool (Growbulk sheep – Clarke *et al.* 1999) and sheep milk (Newman and Stieffel 1999). Market forces favouring lamb over wool have also resulted in serious efforts to find the best ways to use Finn and East Friesian sheep to raise reproduction rates (Garrick *et al.* 2000). The Australians are now experimenting with the use of Damara, Dorper and South African Meat Merino sheep in west Queensland (Kleeman *et al.* 2000). The breaking of quarantine barriers however

remains expensive as the Pacific countries strive to maintain their enviable epizootic disease free status. The Fijians have managed a breakthrough in developing a wool free composite from Barbados Blackbelly, Wiltshire and Corriedale sheep.

SHEEP BREEDING AND FARMING SYSTEMS

The general trend in livestock production as a response to increased demand for animal products has been towards intensification and industrialization of production with uniformity of breeding objectives. With sheep there is a clear differentiation of systems. On the one hand there are continuing efforts to make use of the fibre digestion abilities of small ruminants in sustainable intensive systems as seen in the traditional cut-and-carry systems in Asia (eg Java) or being developed as components of sugar cane production (Preston and Murgueitio 1992/3). On the other hand there is a reverse trend as sheep are increasingly marginalized into less productive environments by increased arable cropping, plantation forestry, irrigated agriculture, intensive cattle (dairy) production and horticultural techniques or intercropping excluding sheep from orchards and plantations. These trends have clear implications for breed utility assessment and very careful definition of breeding objectives for divergent requirements. An extension of the "easy care" concept, as discussed by Scobie *et al.* (1997), assumes greater and greater importance as sheep production is expected to increase but in low cost, sustainable systems. Pressures to pay more attention to extensive systems come from concerns about the value of maintaining traditional social systems and concerns over human and animal health, animal welfare and protection of the environment. However, as social or pastoral systems change, the sheep will need to change with them.

The main options for sheep breeding would therefore appear to be the development of new composites using existing breeds and selection for genetic improvement within breeds. Both options put value on the qualities, well defined or not, of the existing breeds. Putting value on a genetic resource through intellectual property rights may give a needed impetus to conservation and increased within breed local improvement programmes. The concept of Farmers' Rights is being developed for plant genetic resource management (Kambuou 2000) and is the subject of current debate. Rights may be invoked to assist farmers to take pride in their breeding achievements, or even recognize that there are achievements, and seek adequate compensation when selling breeding stock. It is not in anyone's interest, however, to seek commercial exclusivity, as recognized by Australian Merino breeders when they lifted a ban on export of breeding stock, but pre-agreement on access may benefit everyone.

BREEDING OBJECTIVES AND PLANS

Barker (1997) has pointed out the problem that planned genetic change could cause loss of adaptability to future environmental challenges or changes to production systems. This emphasizes the need to understand the systems as they exist and realistically anticipate likely changes. The unique qualities of different breeds need study for the more we know about the physiological reasons for trait expression for traits associated with adaptability, the more clearly can we define our breeding objectives and the less likely we are to lose qualities through oversight or conflicting objectives. Qualities heretofore unappreciated in so-called unimproved sheep will be recognized and sought. Examples are the breed variations found for helminth resistance in Africa (Baker 1996) which should likewise be sought in Asia. In any case it is essential that selection be done within the marginal environments so often associated with the pastoral or social systems using sheep.

In considering breeding objectives it is necessary to appreciate that we are dealing with whole animals in systems. There have been trade-offs in traits as animals have been subjected to both farmer directed and natural selection and care must be taken not to lose some attributes while selecting for others. The traditional sheep farmer's main aim is optimal use of feed and other resources rather than production per animal although, as now recognized in NZ, there is a need to balance production per hectare against production per animal. Hence it is necessary to consider the following questions:

1. What should the animals look like?
2. What products do we expect them to produce?
3. What production output should they be able to sustain?
4. What sorts of environment are they likely to have to thrive in?
5. What variety of production systems is present now or likely to develop?

We can then set breeding objectives and develop sensible breeding plans. However, note the problem

highlighted by Ponzoni (1999) of an increasing complexity of breeding objectives, in his case for the Australian Merino, with a multiplicity of traits and increasing sophistication of the markets. The need for professional advice and access to adequate genetic evaluation schemes increases. That most of us are working at a more elementary level does not relieve us of the obligation to rigorously define objectives.

It seems that little attention has been given to the needs for really good techniques for genetic improvement by selection in systems with small, scattered, individually owned flocks, even if agreement could be reached on objectives, since Turner (1991) drew attention to this need over 10 years ago. Only sensible, communal cooperation will enable progress with the avoidance of unnecessary genetic erosion or loss of adaptation. There are good examples of cooperative schemes, notably the open nucleus group breeding schemes as developed in NZ and the Scandanavian schemes involving the rotation of rams identified as superior by field recording and testing as in the ram circles in Norway. Factors aiding the success of the Scandanavian schemes include a long tradition of cooperation and an absence of commercial competition in the sale of elite breeding stock. All schemes require recording but this may not need to be complicated nor expensive. Effectiveness will be increased if there are cooperatives to enlarge effective flock sizes and non-seasonal breeding to enable continuous ram rotation.

SUMMARY AND CONCLUSIONS

There is a wealth of breeds and genetic resources in sheep worldwide to satisfy the requirements of pastoralists and farmers to adapt to changing circumstances and meet future needs. On-going genetic improvement will require very clear definition of breeding objectives; the development of new composites and the utilization of new technologies for gene manipulation. At the same time, the array of existing breeds or breeding populations must be maintained and adapted to changing social and production systems. Cooperation will be essential for the implementation of sheep genetic improvement plans.

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INTEGRATION OF INSECTICIDES IN THE MANAGEMENT OF *SESAMIA GRISESCENS* WARREN (LEPIDOPTERA : NOCTUIDAE) IN SUGARCANE AT RAMU, PAPUA NEW GUINEA

Lastus S. Kuniata

ABSTRACT

The larva of the noctuid moth borer, Sesamia grisescens *Warren is a serious pest of sugarcane and has become one of the major constraints to sugar production at Ramu Sugar plantation, Papua New Guinea. Recent developments in the management of* S. grisescens *using insecticides are discussed in this paper particularly, the use of a pheromone trapping/monitoring system to schedule insecticide spraying. It has been found that spraying carried out during the moth flight period is effective in controlling young larvae before they bore into the stems, thus minimizing damage to sugarcane. Strategies to minimize the use of insecticides and the management of potential insecticides resistance are also discussed.*

Keywords: *sugarcane*, Sesamia grisescens, *integrated pest management, permethrin.*

INTRODUCTION

There are several species of sugarcane stem borers native to New Guinea. Those that are pests of sugarcane at Ramu Sugar plantation are a weevil borer *Rhabdoscelus obscurus* Biosduval and the moth borers *Sesamia grisescens* Warren, (Lepidoptera : Noctuidae), *Chilo terenellus* Pag. (Lep. : Pyralidae) and *Scirpophaga exceptalis* Walker (Lep. : Pyralidae) (Kuniata *et al* 2001). Among these stem borers, *S. grisescens* is the most serious with potential for causing crop losses of up to 31 tonnes cane per ha, valued at more than K11 mill per annum (Kuniata 1998). In addition to these crop losses, Ramu Sugar Limited now spends up to US$350,000 annually for the control of *S. grisescens* on its 9,200ha sugar plantation. The weevil borer is a secondary pest and has a strong association with damage from the moth borers especially that from *S. grisescens* (Kuniata & Sweet 1994). The control of *S. grisescens* larvae reduces the number of bored cane and thus weevil borer damage. Both *C. terenellus* and *S. exceptalis* may be serious pests at times but crop losses are usually significantly lower than those observed for *S. grisescens*.

Details of an integrated pest management (IPM) strategy for *S. grisescens* was discussed by Kuniata (1999) and this has been implemented on the Ramu Sugar plantation with significant success. This IPM strategy involves aspects of cultural control, use of natural enemies and insecticides against *S. grisescens*. In this paper, recent developments in the use of insecticides and their integration in this IPM strategy for the management of this stem borer are discussed. Strategies implemented to delay possible development of insecticide resistance are also discussed.

Biology and Ecology

The noctuid moth, *Sesamia grisescens* Warren, is native to New Guinea (Holloway 1989) and has become a serious pest of sugarcane at Ramu Sugar plantation in Papua New Guinea causing sugar losses as high as 18%. Studies of the biology of *S. grisescens* showed that the moth is largely confined to sugarcane and other *Saccharum* spp. with large diameter stalks (Young & Kuniata 1992). Female moths oviposit behind the green leaf sheaths of sugarcane. The young larvae feed gregariously on the leaf sheaths for 2-3 days before boring into the stalk, 8-15cm below the meristem (growing point) region [Young & Kuniata 1992]. Bored stalks are usually killed within 2 weeks and extensive rotting of damaged stalks occurs as a result of larval feeding and the invasion of saprophytic fungi. In the fourth to fifth instar stage, the larvae disperse to infest nearby un-bored stalks, boring large entry holes. In some cultivars, these stalks produce dead-hearts (dead spindles) with extensive production of side shoots.

The pest completes its whole life cycle on sugarcane. Populations have been highly synchronized with a generation time of 60-70 days and 5 ½ generations annually [Young & Kuniata

Ramu Sugar Ltd., P O Box 2183, Lae, Papua New Guinea, Email: lkuniata@ramusugar.com.pg

1992]. The population is at its highest in April-May, inflicting the greatest damage to the crop.

Pest Monitoring

Successful implementation of IPM strategies often requires an effective and efficient monitoring system of the pest population. This can be simple as; counting of pests/damaged cane, to sophisticated techniques, such as the use of pest modelling. The usefulness of these systems are that, they provide a predictive role based on historical records of population trends and can give an early warning that pest populations/damage could become economically important if they reach certain population thresholds.

From 1986 up until 2000, a destructive sampling method was used to monitor the pest and the damage caused. The method involves taking 200 stalks, sampled at random in a block; the stalks are split open and various life stages of *S. grisescens* and damage recorded. These are then used to direct releases of parasites and insecticide spraying. The cost of this sampling technique was estimated at about US$2 per tonne of sugar or US$90,000 per annum which is about 3% of potential crop loss (US$2.75 million) [Kuniata 1999] .

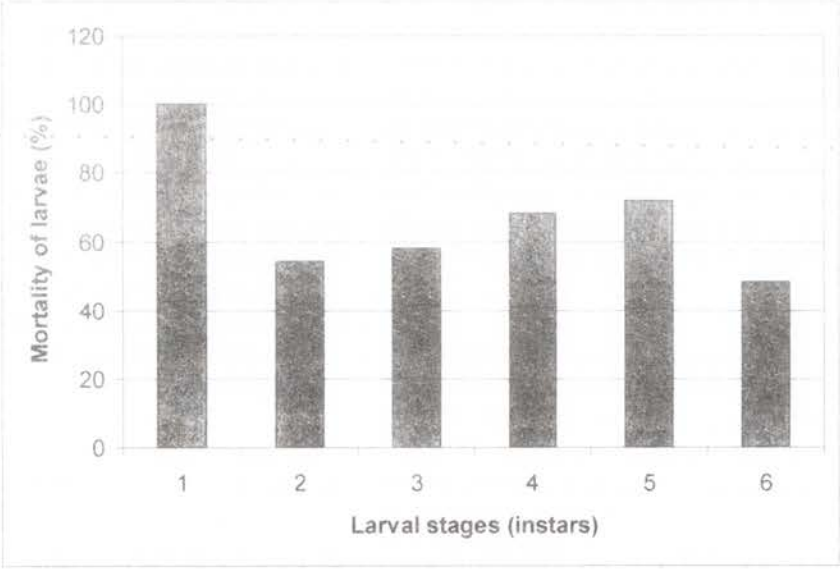
A pheromone has been identified for *S. grisescens* (Whittle *et al.* 1995) and this has been artificially synthesized and used to monitor moth numbers in the field. Recently, an economic threshold of 2 moths per trap per night was designated as the basis for scheduling insecticide spraying. The estimated cost of this monitoring technique was about US$25,000 per annum which is <1% of the value of potential crop losses. However, the increased effectiveness (reliability) and efficiency compared with destructive sampling have resulted in lower pest numbers and damage observed in the 2001 crop and the 2002 crop[(K. Korowi, unpublished data)] Insecticides were sprayed when the moths were in flight, in an attempt to have residues already on the plants when the eggs hatch. Up to 100% mortality in hatching eggs/young larvae was observed in the field (K. Korowi, unpublished data) thus preventing subsequent damage to the stems of sugarcane. Using this approach, and by alternating between insecticide groups, excellent control has been obtained in the 2001/2002 season.

Insecticide Screening

A number of products have been evaluated both in bioassays (in the laboratory) and in small plot trials in the field. Those that performed well in field trials were further evaluated in semi-commercial trials using a spray plane and custom-built spray rig. A product is used commercially after at least 18 months following the evaluation process.

It was generally observed that mortality of *S. grisescens* larvae was highest when the larvae are still feeding on the leaf sheaths, with mortalities increasing with dose rates (Figure 1). Once the larvae have bored into the stalks, insecticides have a limited effect on the borers, especially in the

Figure 1. **Mortality of *S. grisescens* larvae due to insecticide spraying.**



These data were averaged from weekly samples (40-80 blocks) taken over February –May 1997.

second and third instar stages which are normally found inside the stalks. The larvae start migrating from primary infested stalks to damage other (secondary) stalks at the 4th instar stage. This process causes the larvae to come into contact with insecticide contaminated surfaces, giving slightly higher mortalities but still lower than those obtained with 1st instar larvae.

Other field observations have shown that larvae found in the upper sections of a bored stalk, and above the exit/entry hole, are less affected by insecticides (Figure 2). However, all the larvae found at the entry/exit hole and those lower down are readily killed, indicating the re-distribution of the insecticide on the plant with rainfall. As such spraying done before rains are received, especially critical with spray plane, appear to cause high mortalities in larvae of *S. grisescens*.

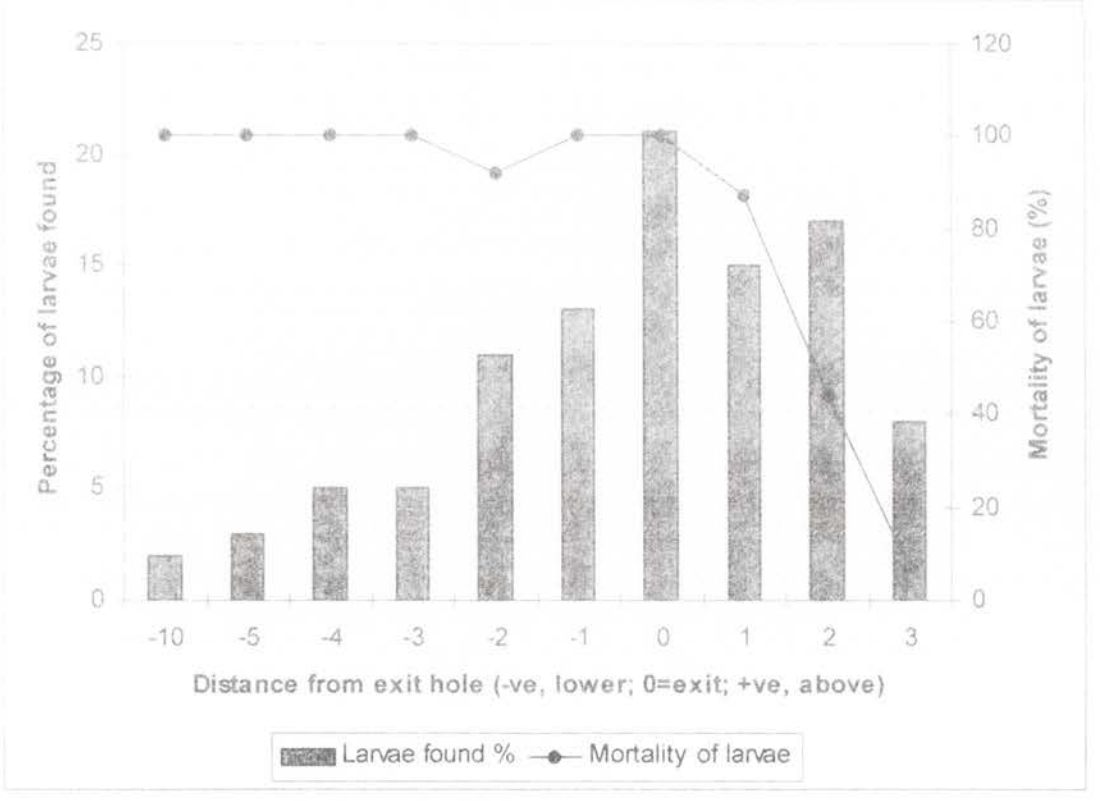
Commercial Spraying

The total area under sugarcane at Ramu is about 8,500 ha, with up to 16,000 ha sprayed for the control of *S. grisescens* annually. Data from initial whole field size trials (n=20) indicated highly significant increases in cane yields were achieved due to reduced infestations and damage in sprayed compared to unsprayed cane (Table 1). In other field trials, an increase of up to 200% in cane yield and up to 150% in sugar yields has been observed (Kuniata 1999).

Where no insecticide spraying was carried out, 5 ½ generations of *S. grisescens* were observed annually (Figure 3(a)), with larvae numbers highest during March-April. Although the peaks in larvae numbers were still seen in years where insecticides were used, these were generally lower and flatter. Similar trends were observed for damage associated with *S. grisescens* (Figure 3(b)) where damage levels were much flatter compared to unsprayed cane.

From 1997 to 2000, a threshold of 16 larvae per 200 stalks was used to select fields for insecticide spraying, while parasites are released at lower levels of attack. As a result, some damage would have already occurred before spraying was carried out. In 2001/2002, the system was changed slightly, using a threshold based on moth numbers from pheromone traps. Starting in July 2001, a threshold of 2 moths/ pheromone trap per night was used and insecticide spraying took place when the moths

Figure 2. **Distribution of *Sesamia* larvae in relation to entry/exit holes on the sugar cane stalks. As an indication of re-distribution of insecticides on the plant following spraying.**



The distance from exit hole is in centimetres.

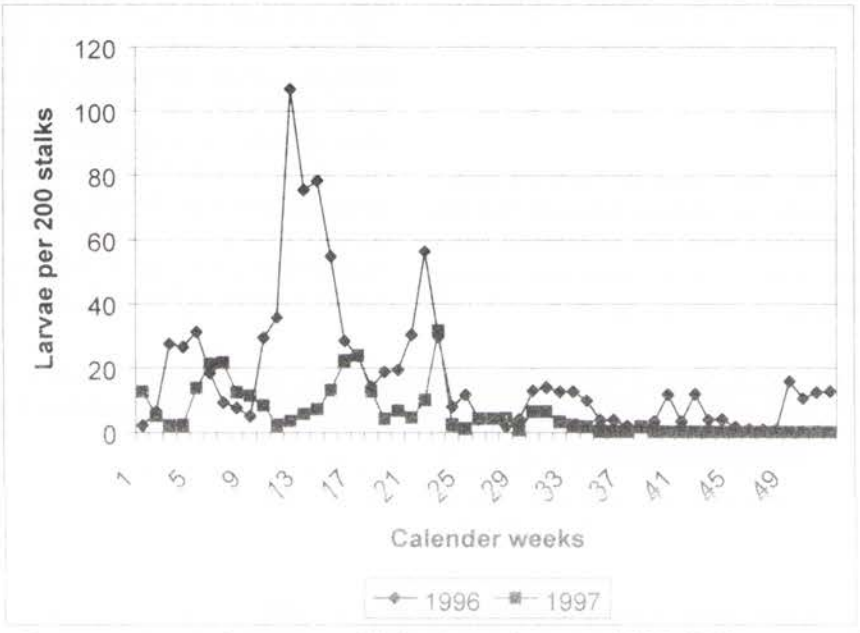
Table 1. **Summary of *S. grisescens* conrol in semi-commercial trials sprayed with lambda-cyhalothrin (Karate 2.5EC) in the 1996 season**

	Sesamia per 100 stalks			Cane Yield(t ha^{-1})
	Larvae	Pupae	Bored stalks	
Sprayed	11.3	0.3	10.7	75.0
Unsprayed	62.4	12.2	66.0	47.7
t-test	2.97 p<0.01	2.18 p<0.05	4.98 p<0.001	7.20 P<0.001

Number of fields used = 20.

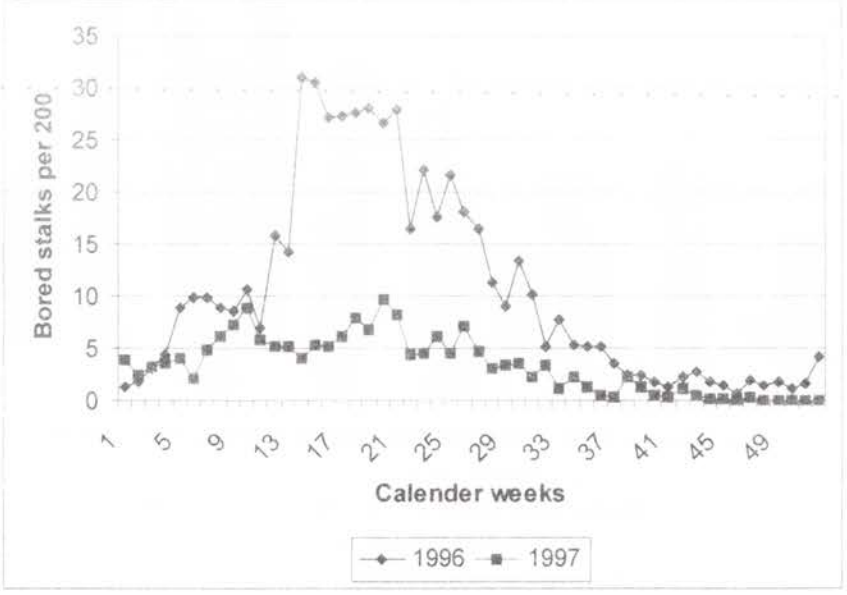
Figure 3. **Summary of *S. grisescens* larvae numbers and bored stalks observed in 1996 unsprayed compared to those**

a) Larvae



Counts were made on a weekly basis and covered 200-300ha per week.

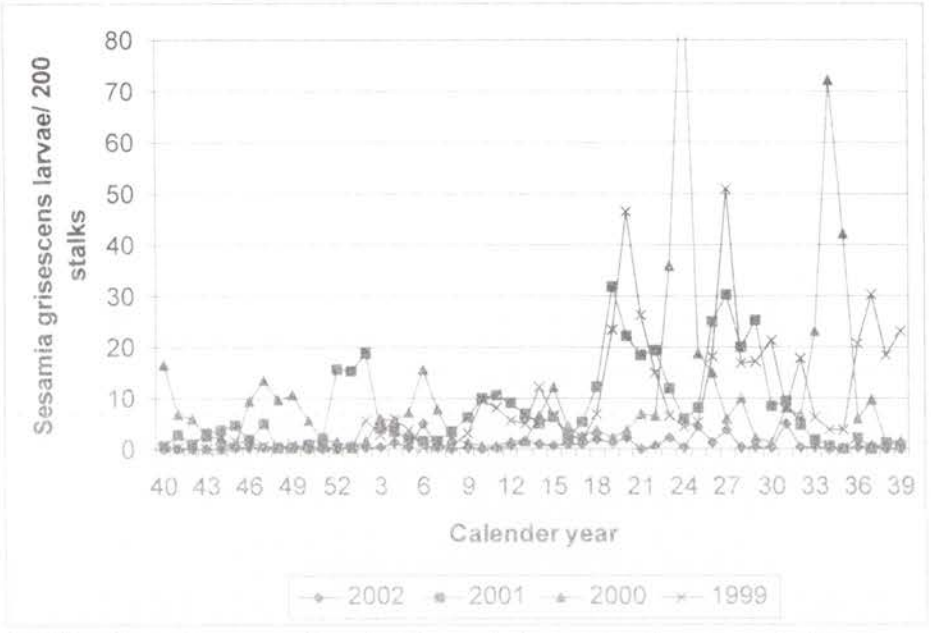
b) Bored stalks



were active. It is possible that some moths would have been killed during flights but the main objective is to ensure the insecticide is already on the plants, especially in the leaf sheaths, before the eggs hatch and before young larvae bore into the stems. Up to 100% mortality in eggs/ young larvae was observed in the field. As a result of this, *S. grisescens* populations and borer damage declined towards the latter part of 2001 and this continued to remain low during the 2002 season (Figure 4).

The other issues that need to be considered (Figure 5) are; insecticide resistance management (IRM), application techniques to be used, and cost of the product. Insecticide resistance has been observed in many tropical insect pests and it is important that management of this potential problem is taken into consideration when implementing a spraying program. A similar approach to that developed for cotton in Australia for *Helicoverpa armigera* Hubner (Lep. : Noctuidae) (Forester *et al.* 1993) is used for *S. grisescens* on Ramu Sugar plantation. This strategy involves alternating between groups of

Figure 4. **Summary of (a) larvae and (b) bored stalks observed in 1999- 2002**



In 2002 moth counts were used to schedule insecticide spraying where previously this was done based on counts made from split cane.

b) Bored stalks

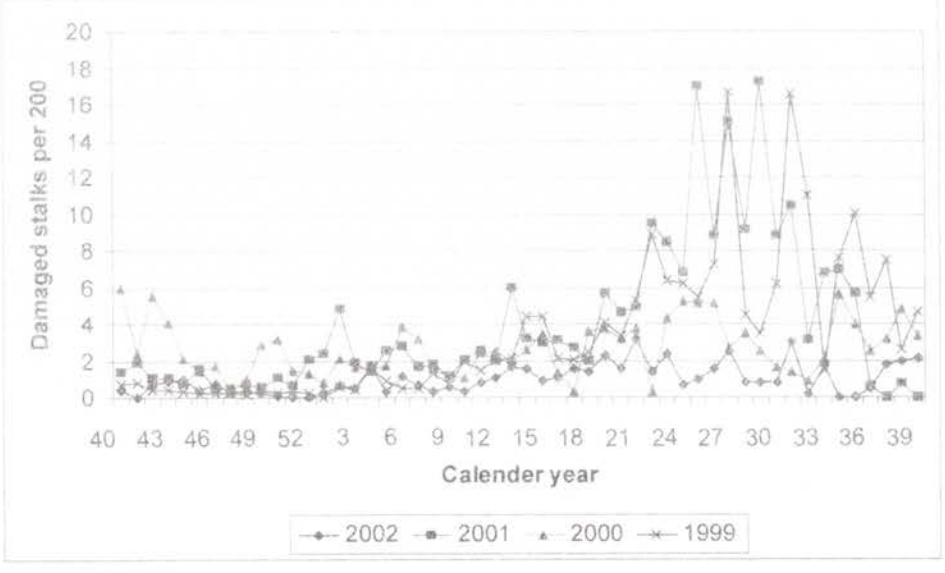
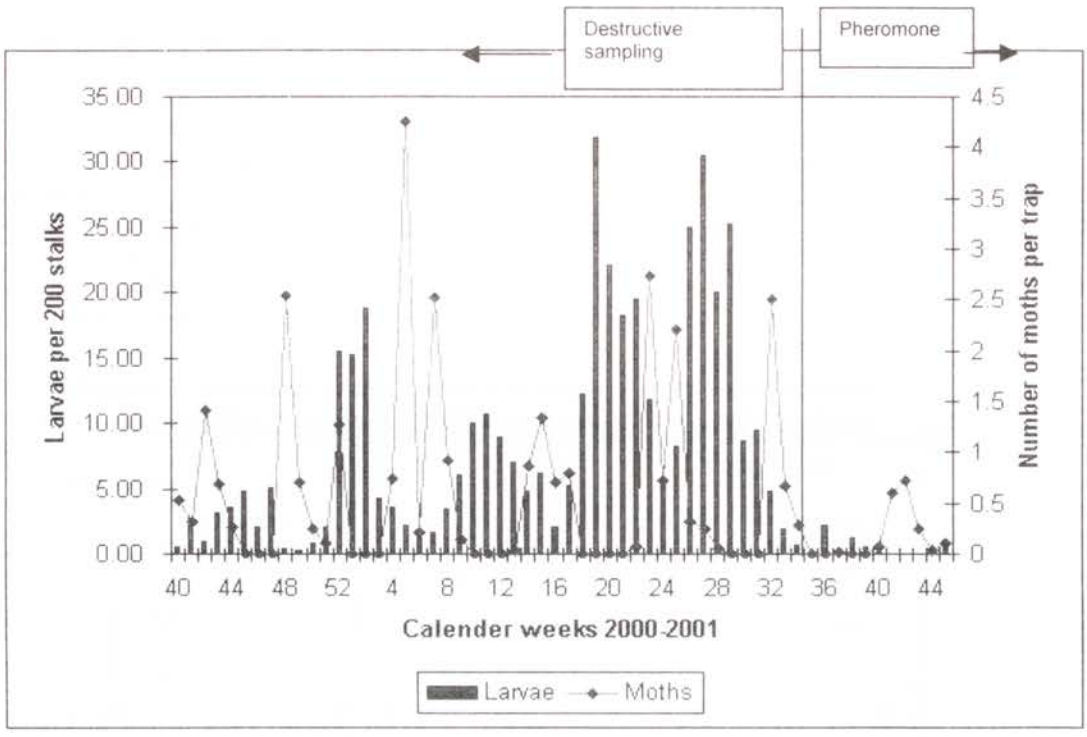


Figure 5. **Monitoring of moths using pheromone and larvae counts from split cane.**



Insecticides used for Sesamia spraying in 2000/2001 season				
Mimic	Permethrin/ Karate	Acephate	Mimic	Mospilan

insecticides in an attempt to delay the development of insecticide resistance (Figure 5). Terbufenozide (Mimic ®) is an insect growth regulator (IGR) specific for moths and it is used during July to October. It is an expensive product but is used on a small area of young cane (<2000 ha). The synthetic pyrethroids (permethrin/ lambdacyhalothrin) have a knock-down effect on the pest, a limited residue life in the field and are relatively cheaper than the IGR. These are used between December and April, effectively using the spray plane when large areas need to be covered in a limited time. Acephate, an organo-phosphate (OP) insecticide, did not work well in 2001 and therefore was withdrawn from commercial use. A new product, Mospilan ® (acetamiprid) has been introduced towards the end of 2001 and has performed quite well. This product affects the Ach receptor of the insects' central nervous system as an antagonist of Ach, the opposite action to OPs. Again, this is another expensive product but with a smaller area treated, it may fit in to the November – December period. So far no incidence of insecticide resistance has been reported in this system for *S. grisescens*.

Another aspect that has been incorporated in the spraying strategy is the consideration of insecticide residues. The pyrethroids have a with-holding period of up to 7 days and are therefore used in the January-March period. Harvesting starts in April. After this period selective spraying using permethrin/ lambda-cyhalothrin especially in younger cane is carried out. The other products (usually with extended with-holding periods) are not used in the crop to be harvested that year but only in younger crops that are usually planted/harvested in February-May.

To spray the large treatment area in December – April, a spray plane is used. Since in some blocks, the crop would be too tall and the use of ground rigs would cause more damage to the crop. Liquid formulations of permethrin/ lambda-cyhalothrin are used as opposed to mixing of wet-able powders/ granular formulations, which may result in long down-times during mixing and loading of the plane. The custom-built ground spray rigs are used between May and November in applying

terbufenozide and acetamiprid both granular formulations.

Monitoring of *S. grisescens* moths using a synthetic pheromone (Whittle *et al* 1995) in conjunction with larvae numbers determined from splitting cane began in 1999. During this time, it was observed that peaks of moths were immediately followed by larvae peaks (Figure 5), suggesting the possibilities for using the moth counts to schedule insecticide spraying. At the same time a threshold of 2 moths per trap per block was determined and this was used to trigger any insecticide spraying. Therefore, in October 2001, insecticide spraying was carried out in all the blocks showing >2 moths. In recovery surveys, it was found that up to 100% of larvae mainly 1st –2nd instars were killing while still in the leaf sheaths (before boring in to the stalks). This approach continued in to 2002 season and proved successful in reducing *S. grisescens* damage in the 2002 crop.

'Road to Reduced Insecticide Reliance'

Integrated pest management (IPM) utilizes all suitable techniques and methods in as compatible a manner as possible to maintain pest populations below a threshold causing economic injury. This represents a change from the philosophy of pest control by eradication to the management of entire pest populations, not just localized ones (Dent 1991). Emphasis is placed on the use of a combination of methods aimed at providing suitable, long-term, control with the minimum of harmful side effects. Development of IPM depends partly on a good understanding of the biology and life history of the target pest. All such information is then integrated in a range of cultural, insecticidal and bio-ecological controls so that potential pests remain at sub-economic levels.

The Crop Production system used at Ramu Sugar plantation has already been described (Kuniata 1999 & 2000). An interaction matrix was used to identify production factors that have a significant effect on S. grisescens populations. The most significant includes; varietal resistance, time of planting/ratooning, and the use of natural enemies. High risk sites have been identified on the plantation and resistant varieties are used in these areas. These sites are usually along river-banks and it is important that spray drift is reduced as a result of less spraying frequency (less drift to non-target sites).

More than 60% of the 1800 ha of cane to be planted each year is planted during March-June thus presenting a semi-mature crop (less attractive to S. grisescens) when populations start to increase in February to March the following year (Kuniata & Sweet 1994). Cane planted/ratooned from September to November will be highly susceptible to borer damage but this area is smaller and can be easily sprayed for borer control, thus reducing insecticide usage further.

A small parasite rearing facility has been established and is routinely producing Pediobius furvus Gahan (Hymemoptera : Eulopidae) and Cotesia flavipes Cameron (Hym. : Braconidae) parasites for field release. All the fields showing a threshold lower than 14 larvae per 200 stalks are used for parasite releases. It has been shown that parasite releases made 10-14 days following insecticide spraying appear not to be severely affected especially following the use of pyrethroid insecticides.

The implementation of the strategies described above has resulted in a reduction of the area sprayed from more than 19,400 ha in 1997 to about 2,560 ha in 2002 (Table 2). A useful assessment for effective use of insecticides is the calculation of ratios between sugar production and total active ingredients used. Total active ingredients used since 1997 have shown a declining trend from >4,000 kg a.i. to about 700 kg a.i. in 2002 (Figure 6). As efficient pest monitoring methods are used, combined with appropriate insecticides and application methods, the ratio between sugar production and active ingredients used should continue to increase.

CONCLUSION

Insecticides will continue to be used in IPM systems especially in mono-cropping situations. They provide important pest management tools, which need to be used sensibly because of the potential problems they can cause to the agro-ecosystem, to plantation workers and to the environment. Understanding the ecology and biology of *S. grisescens* and careful selection and phasing of insecticides for the control of this pest has had a significant impact on sugar production at Ramu Sugar plantation. The strategies implemented in late 2001 have resulted in the lowest *S. grisescens* levels ever seen. This made a significant contribution towards sugar yields in the 2002 season.

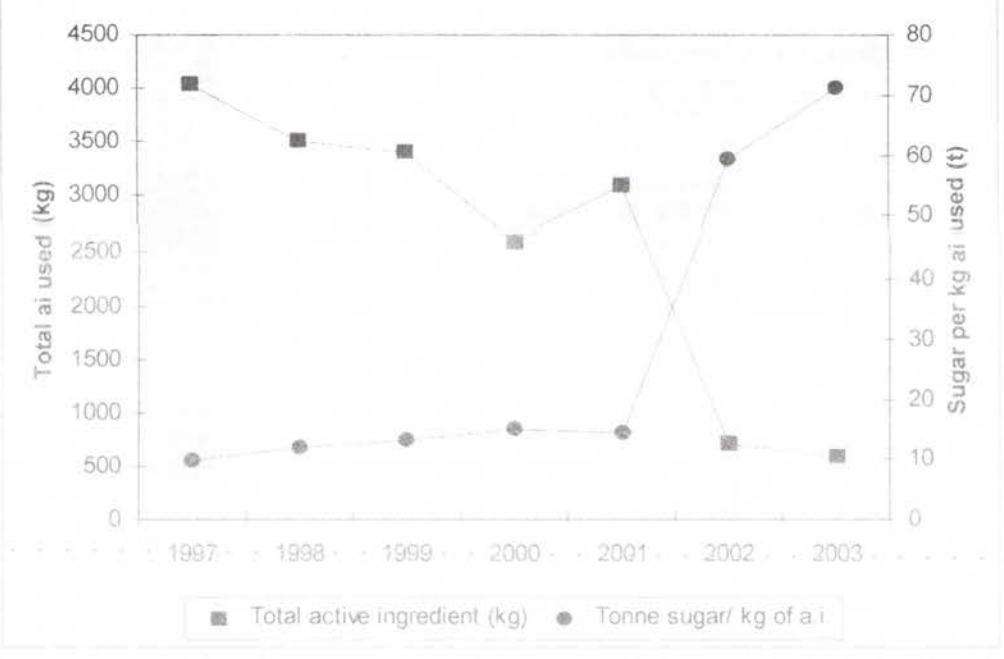
A reduction in active ingredients used in the last five years has indicated that positive trends in effective use of insecticides are being achieved. At

Table 2. **Summary of total area sprayed and active ingredients used for the control of *S. grisescens* at Ramu Sugar plantation. 1997 - 2002.**

Parameters	1997	1998	1999	2000	2001	2002
Total area sprayed (ha)	19,409	17,432	17,407	12,834	14,191	2556
Insecticides used (kg ai)						
- Permethrin	3,853	3,282	3,046	1,601	2,045	534
- - lambdacyhalothrin	78	79	72	4	1	0
- monocrotophos	29	0	0	0	0	0
- acephate	50	117	210	346	77	0
- terbufenozide	4	9	55	635	837	105
- aetamiprid	0	0	0	7	124	70
- others*	14	12	22	0	0	0
Total active ingredients (kg)	4,028	3,498	3,405	2,592	3,084	709

* others – malathion/chlorphyrifos / carbaryl.

Figure 6. **Summary of total active ingredients of all insecticides used for the control of *S. grisescens* in sugarcane at Ramu Sugar plantation.**



the same time, there has not been a case of resurgence of secondary pests since the implementation of this strategy in 1997.

ACKNOWLEDGEMENTS

Professor G.A. Norton (CPITT, University of Queensland) and Dr Peter G. Allsopp (BSES Ltd, Australia) provided useful comments on the original drafts of this paper. Financial assistance was provided by Ramu Sugar Limited for most of the field trials and AusAID for my PhD studies. Most of the data in this paper were derived from my PhD thesis (Kuniata 1999).

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PHYTOPHAGOUS INSECTS ON BROADACRE SUGARCANE IN PAPUA NEW GUINEA

L.S. Kuniata [1*], K.J. Chandler [2], H. Nagaraja [3], G.R. Young [4]

ABSTRACT

Phytophagous insects associated with sugarcane at the first plantation established in Papua New Guinea (PNG) at Ramu Sugar L td, Gusap are listed, with notes on pest status. Almost all are native to PNG and most do not cause significant loss. . Noctuid and pyralid caterpillars and a weevil larva, which bore in sugarcane stems, are the most damaging. Root-feeding cicadids and a white grub also frequently reduce yield. Two plant-hoppers vector are potentially devastating disease organisms, including one that was previously unknown. These insects and three disease organisms place major constraints on production. In the pas,t it was suggested that PNG species that co-evolved with sugarcane - considered to have originated in PNG from the ancestral form Saccharum robustum - *would severely damage commercial plantations. Species present now that large scale production is established seem representative of the families that adapt to sugarcane in the rest of the world. None seem dependent on sugarcane, despite prolonged opportunity for associations to evolve. The adaptive nature of these fauna suggests to us that priority pest lists based on species infesting sugarcane elsewhere are misleading. Perhaps the size of family groupings of pests, and not only from sugarcane, is a more relevant determinant of quarantine risk.*

Keywords: *pests, endemicity, evolution, pest-risk analysis*

INTRODUCTION

Papua New Guinea (PNG) is the centre of diversification of the genus *Saccharum* and the origin of the ancestral forms *S. robustum* and *S. spontaneum* and the cultivated forms *S. officinarum* and *S. edule* (Brandes, 1956). Since 1983, Ramu Sugar Ltd (RSL) has developed the first large-scale sugarcane plantation in PNG. Over 9,200 ha is under cultivation to imported intra-specific *S. officinarum* hybrid sugarcane in the Ramu valley (5°50'S, 145°E). Wild *Saccharum* and close relatives are prolific in the region especially along the Ramu River.

Pemberton and Williams (1969) discussed the origins of sugarcane-pest insects, and noted that "there would seem to be no authentic case of an insect limited either to cultivated sugarcane or to the genus *Saccharum*, although in some environments alternative host-plants seem to be absent." They concluded, particularly for continents and large islands that, "sugarcane insects are generally local insects that have adopted sugarcane as a host consequent to its cultivation". Strong *et al.* (1976) found, from the recorded history of sugarcane introductions around the world and from regional lists of pest species, that "the most obvious bio-geographic pattern among the insects of sugarcane is high endemicity. This pattern is true for regions within Oceania and Asia, where there are wild species of *Saccharum*, and for regions where there is no wild *Saccharum*".

We consider the composition and pest potential of the insect fauna in a modern, commercial sugarcane-production environment, at the source of origin of the crop, whereas previous lists of insect fauna on sugarcane in PNG (Szent-Ivany & Ardley (1962); Bourke 1968; Bourke *et al.* 1973) were from collections in small domestic gardens and in wild canes.

We consider the fauna relative to hypotheses on the origin of sugarcane pests generated by Pemberton and Williams (1969) and Strong (1976), and whether previously unrecognized pest groups have become prominent in this commercial environment. The relevance of lists of priority pests determined through pest-risk analysis (e.g. FitzGibbon *et al.*, 1998) is considered relative to these data.

MATERIALS AND METHODS

During field surveys conducted between 1982 to 1989 in commercial cane at Ramu Sugar, Gusap, Madang Province and also in numerous wild cane stands and village gardens around the sugar plantation and PNG.

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[2] Bureau Sugar Experiment Station, Gordonvale 4865, Aust.
[3] 233 8th Main Rd, Malleswaram, Bangalore 560055 India.
[4] Department of Primary Industry and Fisheries, Darwin, 0810, Australia.

Insects were collected from sugarcane stalks, leaves, and rhizosphere, and effects on the plants noted. Immature stages were reared to adults. Pupal exuviae of Lepidoptera and Diptera were usually preserved with the adults. Adults were preserved in 80% ethanol, or pinned, or were cleared, fixed, stained, and slide-mounted.

Specimens were identified at Natural History Museum, London, CABIBioscience, London; Bureau of Sugar Experiment Stations, Bundaberg, Australia; Institut voor Taxonomische Zoologie Zoologische Museum, Universiteit van Amsterdam, Amsterdam; University of California, Riverside, USA; Queensland Department of Primary Industries, Indooroopilly, Australia; Department of Agriculture and Livestock , Port Moresby, PNG. Voucher specimens are retained at RSL.

RESULTS AND DISCUSSION

Insect fauna on sugarcane. Insects damaging sugarcane at or near RSL; plus known disease vectors, and species from RSL not causing obvious damage but with pest status for the family in other countries are listed in Table 1. Species not recorded at RSL, but seen damaging domestic sugarcane plants elsewhere in PNG are listed by Bourke (1968) and Bourke *et al.* (1973).

Status. Our estimates of the severity of damage to sugarcane at RSL by various species are summarized in Table 1. Insects that damage internal tissue and / or growing points of semi-mature culms (stalks) are the most severe constraints. Sesamia grisescens, Chilo terenellus, and Rhabdoscelus obscurus, together reduce crop and sugar yield by 15 20% (Kuniata & Sweet 1991). The whitegrub Lepidiota reuleauxi (Kuniata & Young 1992), and nymphs of a cicadid Baeturia papuensis (Kuniata & Nagaraja 1992) together affect 15 - 30% of the production area annually. Both damage roots and stems underground, and cane and sugar yield are reduced, and severely infested plants fail to ratoon (re-generate following harvest).

Scirpophaga excerptalis which destroys meristems of moderately grown stems causing profuse side-shooting, is occasionally a severe pest. Pyralid shoot-borers and shoot flies (Pont, 1988) that infest and kill the meristem of young shoots are minor pests. Secondary shoots usually compensate for loss of large primary shoots, but damage kills very small primary shoots.

Stem-sucking mealy-bug and scale insects can be moderately damaging. Populations are normally relatively low, but large populations do develop, particularly where plants are severely moisture-stressed. Enormous populations of woolly aphids, *Ceratovacuna lanigera*, which frequently blacken the leaf canopy with 'sooty mould', usually decline rapidly under the influence of natural control, and cause little yield loss.

Two *Lophops* spp. are common, and we suspect cause the condition "Ramu leaf-scorch" (Waller *et al.* 1987), although this does not appear to cause crop loss. Several froghoppers (Aphrophoridae and Cercopidae) cause minor leaf-blights, but do not reduce yield. The plant-hopper *Perkinsiella vitiensis* vectors the viral agent of Fiji disease, which is a constraint at RSL. Also *Eumetopina* spp. planthoppers vector "Ramu stunt" disease (Waller, *et al.* 1987; Kuniata *et al.* 1994), a major constraint on yield.

Armyworms occasionally defoliate plants but the ability of sugarcane to compensate by prolific leaf production, combined with the effect of parasitic insects and insect diseases that reduce their populations, usually nullifies any effect on yield.

Natural enemies appear to maintain reasonable control of some of the potential 'pest' species, and attempts to accentuate the effectiveness of some of these agents are part of the integrated management plan at RSL (Kuniata *et al.* 2001).

Relativity to previous records and theories of pest origin: All the pest species appear to be endemic to PNG and all of the major or intermediate pests were identified in preliminary surveys before commercial production commenced (Szent-Ivany & Ardley 1962); Bourke 1968; Bourke *et al.* 1973). However, Kumar (2001) recently listed up to 155 species of insects found on sugarcane in PNG. A high proportion of species found in Bubia/ Lae and in the Markham valley were similar to those collected at RSL suggesting that these pest species were already here when commercial sugar production began. Recognition of *S. grisescens* as "the most important sugarcane pest in PNG" (Szent-Ivany & Ardley 1962) proved so for RSL though, it would be mistaken to assume this relativity is entirely attributable to the pest. Past cultural practices contributed significantly to the severity of losses to this pest, until a range of cultural tactics were researched and integrated into a management package (Kuniata 1999; Kuniata *et al.* 2001). The integration of insecticide spraying, time of planting, variety resistance and augmentative releases of parasites has resulted in *S. grisescens* attaining a lesser pest status. Likewise, some of the currently 'less-severe' pest species may have much greater impact in the absence of effective management plans.

For example, the cicadid B papuensis was a minor pest before the use of carbofuran in 1988 for the control of S. grisescens. However, cicadids became a severe problem in 1989-1991 following this insecticide, especially in areas with histories of carbofuran use. The withdrawal of carbofuran and the cultural methods used resulted in this pest re-assuming a minor pest status in sugarcane [Kuniata & Nagaraja 1992].

The pest fauna at RSL conforms to Pemberton and Williams' (1969) and Strong *et al's* (1976) hypotheses on endemicity. There are no families at RSL from which species have adopted sugarcane, that are not represented on sugarcane in other parts of PNG or the world. This conforms with findings by Strong *et al* (1976), that numbers of insect species utilizing a host plant do not increase with the time of association even for this region where the > 3,000 years association between sugarcane and the insect fauna is far older than any suggested by Strong *et al* (1976).

Relativity of pest records for pest-risk analysis and quarantine purposes: Eight species feeding on sugarcane at RSL are present in four or more widely dispersed sugarcane regions (Box, 1953); *R. obscurus* (weevil-borer), *S. sacchari* (mealy-bug), *N. bergii* (white-fly), *A. tegalensis* (scale), *C. lanigera* (wooly aphid), *M. loreyi* (armyworm), *S. inferens* (shoot-borer), *C. infuscatellus* (shoot-borer). The cryptic habits of the weevil, mealy-bug, white-fly, scale, and possibly

Table 1. **Organisms damaging or feeding on sugarcane at RSL plantation,**

ORDER / family	Name	Status	Association with sugarcane
COLEOPTERA			
Ceranbycidae	*Prosoplus* sp		Larvae boring in semi-dry stalks, Sogeri (HN[2])
Chrysomelidae	*Rhyparida coriacea* Jacoby		Larvae burrowing in shoot tissue
Curculionidae	*Rhabdoscelus obscurus* (Boisduval)	***	Larvae bore stem internode tissue
Elateridae	unidentified		Larvae tunnel in roots
Scarabaeidae	*Lepidiota reuleauxi* Brenske	**	Larvae feed on roots, loss of yield and ratooning ability
	Papuana woodlarkiana (Montrouzier)	*	Adults eat root mass and burrow into underground stem
Scolytidae	*Xyleborus perforans* Wollaston		Adults and larvae boring base of stems, Bubia (Kimoto, et al., 1984)
	Xyleborus sp		Adults and larvae boring base of stems, Sausi (HN & LK)
Tenebrionidae	*Casnonidia* sp		Feeding inside shoot-tissue
DIPTERA			
Muscidae	*Atherigona orientalis* Schiner		Larvae bore into and kill young shoots
	A. ramu Pont	*	Larvae bore into and kill young shoots (Pont 1988)
HEMIPTERA			
Aleyrodidae	*Neomaskellia bergii* (Signoret)		Colonies on leaves, all plant stages
Aphididae	*Ceratovacuna lanigera* (Zehntner)	*	Colonies on leaves, semi-mature plants, blackened by sooty-mould
	Longiunguis sacchari (Zehntner)		Infestation on leaves (Bourke, *et al.* 1973)
Margarodidae	*Promargarodes australis* Jakubski		Encysted nymphs on roots
Pseudococcidae	*Saccharicoccus sacchari* (Cockerell)	*	Infest stems at nodes, behind leaf bases
Diaspididae	*Aulacaspis tegalensis* (Zehntner)	*	Encrustations of scales on mature stalks
Cicadidae	*Baeturia papuensis* De Boer	***	Nymphs feed on roots; shoots die, or plants fail to ratoon after harvest
	B. vitiaka Blote	*	Nymphs feed on roots; shoots die, or plants fail to ratoon after harvest
	Gymnotympana sp	*	Nymphs feed on roots; shoots die, or plants fail to ratoon after harvest
Aphrophoridae	*Clovia* sp		On leaf, semi-mature plants. May cause leaf-blight symptoms??
Cercopidae	*unidentified*		On stems & leaves of mature plants. May cause leaf-blight symptoms
Delphacidae	*Eumetopina flavipes* Muir	***	Vectors phytoplasma causing Ramu Stunt disease, a severe constraint.
	Eumetopina sp		
	Perkinsiella vitiensis Kirkaldy	**	On leaves and stems. Vectors viral agent of Fiji disease, a major constraint.
Lophophidae	*Lophops* spp #1		Feeds and oviposits on leaves; probable cause of "Ramu leaf-scorch" symptoms
	Lophops spp. #2		Feeds and oviposits on leaves, probable cause of "Ramu leaf-scorch" symptoms
Colobathristidae	*Phaenacantha* sp		Adults & nymphs feed on leaves, all plant stages. Purpling discolouration
ISOPTERA			
Termitidae	*Microtermes* sp		Hollowing underground and above-ground stems
LEPIDOPTERA			
Hesperiidae	*Arrhenes oschiplus* Plotz		Larvae rolling & cutting leaves
Elachistidae	*Elachista solena* (Bradley)		Larvae mining in leaf (midrib / lamina??)
Noctuidae	*Agrotis interjectionis* (Guenee)		Larvae eat leaf lamina
	Agrotis sp		Larvae eat leaf lamina
	Hydrilloides sp		Larvae eat leaf lamina
	Mythimna loreyi (Duponchel)	*	Larvae eat lamina, young plant stages
	Sesamia grisescens (Warren)	***	Larvae tunnel unopened leaf spindle, meristem, and stems of young and semi-mature stems
	S. inferens (Walker)	*	Larvae bore stem , meristem and unopened leaf spindle of young plant stage
	Spodoptera exempta (Walker)	*	Larvae eat leaf lamina , all stages
Pyralidae	*Bleszynskia malacelloides* (Bleszinski)		Larvae bore stems, young plant stage
	Chilo infuscatellus (Snellen)	*	Larvae bore stems, young plant stage

Status[1] ;occassional and slight pest status unless otherwise stated; * minor; ** intermediate; *** severe.

the aphid would have allowed those species to be transported with growing sugarcane plants, or with a range of other plants such as palms, particularly to isolated islands. However records from Asia, India and Indonesia, particularly for the moths and aphid, could be due to natural dispersal.

Our data suggest three remarks pertinent to quarantine and risk assessment. Firstly, it is obvious that quarantine is necessary to prevent species known to be adapted to sugarcane spreading to new locations, particularly where the local predators or parasites may be unable to maintain control. Six of the RSL species are potential quarantine risks, having already been spread by man. Secondly, the other species recorded at RSL are probably no more of a quarantine risk (FitzGibbon *et al*, 1999) than numerous other insects never before or only occasionally recorded on sugarcane. The generality of sugarcane pests suggests that any species able to adapt to sugarcane is a potential risk. This leads to a third comment, that perhaps the real value in pest lists is to indicate the *families* most likely to contain species that could constitute a risk in a foreign environment.

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MANAGEMENT STRATEGIES FOR RATOON STUNTING DISEASE IN SUGARCANE AT RAMU SUGAR

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ABSTRACT

Ratoon stunting disease (RSD) of sugarcane is caused by the bacterium, Leifsonia xyli *Davis and it is a major problem in many sugar industries worldwide. Recently, this disease was detected in commercial crops at Ramu Sugar. Data from estate wide survey indicated up to 84% of the samples taken were tested positive to RSD. In 2004, crop losses are estimated to be at least 15%, this is worth up to K14.2million. Management strategies have been developed and these would take up to 5 years to be fully implemented. This program would cost up to K0.5million in the first year and in subsequent years, this will be around K300-400,000 per year.*

Keywords: *ratoon stunting disease,* Leifsonia xyli, *Sacharum, sugarcane, disease management*

INTRODUCTION

New Guinea is the centre of origin for several *Saccharum* species, including the 'original' sugarcanes belonging to *S. officinarum* (noble cane), the domesticated vegetable sugarcane 'pit pit', *S. edule* and the 'wild' canes belonging to *S. robustum* and *S. spontaneum*. Extensive stands of wild canes grow along rivers and roadsides as well as domesticated chewing and vegetable canes in village gardens. Up in the Highlands of PNG, both *Miscanthus floridulus* and *Erianthus* sp which are related to *Sacharum* are common. Pests and diseases of sugarcane are common and these present a high disease pressure resulting from the widespread occurrence of *Saccharum* species.

Commercial hybrid varieties were introduced into PNG since 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 the Madang Province. Over 8,500 ha is under sugarcane to produce around 500,000 tonnes cane to make 48-50,000 tonnes of sugar. About 2.5 million litres of ethanol is also produced mainly for export [Ramu Sugar Ltd unpublished Annual Reports].

With the monoculture of hybrid sugarcane over an extensive area, the 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. These varieties are pre-fixed 'PN'.

Endemic diseases have had significant impact on commercial production at Ramu. In 1985-86, the then unknown endemic disease, Ramu stunt, severely affected the widely grown variety Ragnar, causing heavy yield losses and near collapse of the sugar industry in PNG (Eastwood 1990). Downy mildew, caused by *Peronosclerospora sacchari*, has caused on-going yield losses and led to the discard of a number of high yielding varieties. Since production first began, leaf scald (*Xanthomonas albilineans*) and ratoon stunting disease (*Leifsonia xyli s.sp. xyli*) have appeared and are also affecting sugarcane production. This paper gives details of ratoon stunting disease (RSD) epidemic at Ramu Sugar and its impact on sugar production. Implementation of management strategies for RSD have started and these are also discussed here.

RATOON STUNTING DISEASE

Causal organism

The causal organism of RSD is a small, slender, usually bent or Y-shaped bacterium called *Leifsonia*

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xyli subsp. *xyli* Davis *et al* which can be grown on complex artificial media.

Distribution

The disease occurs in all cane-growing areas of the world including Australia. The disease was recently detected in commercial cane at Ramu Sugar, Papua New Guinea. The disease is spread mainly by the use of diseased planting material and subsequently by cutting implements such as; mechanical harvesters, bush knives and stool splitters. Volunteers from the previous crop may harbour the disease and may cause scattered distribution in the newly planted crops. Harvesters also may spread the disease throughout the ratoon crops.

Symptoms and identification

The external symptoms of RSD are a general stunting and un-thriftiness in diseased plants, similar to that caused by poor cultural practices such as, inadequate moisture, poor soils, fertilizer 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 through yellow, orange, pink, red and 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 dis-coloured 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 3mm 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 dis-coloured 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 dis-coloured 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 stalks.

Apparently healthy cane can also show discoloured vascular bundles closely resembling those of ratoon stunting and some varieties will show no nodal symptoms even when they are diseased. Thus, a positive identification of the disease can be made by examining a vascular extract with an electron or phase-contrast microscope for the presence of the small bacteria which cause the disease.

The standard procedure for examination for bacteria with the phase-contrast microscope involves:

(i) Collect the vascular extract by exerting a positive air pressure to one end of a piece of stalk and collect by sucking up the extract with a Pasteur pipette. Stalk pieces should be from the base of the stalk and pipes in the stalk must be plugged with plasticine or a pencil.

(ii) Place as small drop of the extract on a clean slide and cover with a coverslip.

(iii) Place a drop of microscope immersion oil on the coverslip and examine for RSD bacteria at 1000x magnification or greater with phase-contrast illumination.

The RSD bacteria are thin rods (0.25-0.5 x 1-4 *m*m) which are often bent and occasionally Y shaped (Davis and Bailey 2000). The detection of RSD in a field depends on the number of stalks examined and the sensitivity of the diagnostic technique. Slicing stalks by experienced people can be quite accurate; however, varieties which show no nodal markings or false positive markings cannot be accurately diagnosed by this technique. Phase-contrast microscopy is currently the most sensitive technique for routine diagnosis. The greater the number of stalks examined by phase contrast microscopy the greater the probability of detecting the disease in a field that is not 100% diseased. This probability of detecting the disease can be increased by selecting the largest stalk in poorly grown stools (possibly poorly grown because of RSD), volunteer cane or canes showing possible nodal markings. At least 10-20 stalks need to be examined to have a reasonable chance of detecting RSD in a field with 10% diseased stools.

Transmission

Diseased planting material is an important means of spreading RSD. The base-cutter and the spray of juice from the chopper box and extractor fan of harvesters can spread the disease to cut stubble. They can carry it into clean blocks if not adequately sterilised beforehand, and they increase the amount of infection in blocks that already have some disease.

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

The disease is readily transmitted artificially by dipping the freshly cut ends of setts into 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 any natural means.

Economic importance

The effects of ratoon stunting are a general reduction in yield, the extent of which depends on the variety 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 not usual (Davis and Bailey 2000).

Control

RSD infected planting material can be effectively controlled by the treatment of stalks at 50°C for three hours. Clean seed plots are a good way of providing disease-free planting material but extra care is needed to ensure the clean seed plot never becomes infected. The sterilization of all cutting implements which are likely to infect healthy planting material, or carry infection into healthy fields 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 (Davis and Bailey 2000). In harvesters the base-cutter, throat, chopper box, extractor fans and toppers should all be sterilized. This is important when using a harvester for cutting billet plants. Once a field is infected the prevention of spread within that field is virtually impossible. Tolerant varieties can play some part in reducing losses, but they are only a few.

THE SITUATION AT RAMU SUGAR

Monitoring

A limited number of samples were taken from cane in village gardens and the commercial cane at Ramu Sugar but the results of the diagnosis were negative. The record of the presence of the RSD in PNG by Davis and Bailey (2000) could not be proven. It was only in 2002 that selective sampling was carried out that RSD was detected in commercial cane at Ramu (Ramu Sugar Limited, internal reports). Out of the 78 samples tested for RSD in South Africa, 40% of these tested positive. This was the first record of RSD in PNG. Further samples were taken in 2003 and the samples were split and got tested by BSES in Australia and South Africa. The results received for these samples from both laboratories confirmed the presence of the causal organism.

A comprehensive sampling program was carried out covering over 700 samples taken from commercial cane on the sugar estate and 120 samples from wild and village garden canes. The results from these samples showed up to 84% of the samples tested positive to RSD (Ramu Sugar Limited, internal reports). Apart from the commercial cane on the sugar estate, up to 20% of the wild and village garden canes sampled also tested positive (Table 1). The concentration of RSD was generally high in the commercial cane most likely effectively spread by mechanical harvesting and other agricultural equipment used. In the village and wild canes, the concentration of the bacteria was generally low. The spread of the disease in the wild and village garden canes may be through planting of infected material or use of contaminated bush knives.

Effect on 2004 crop

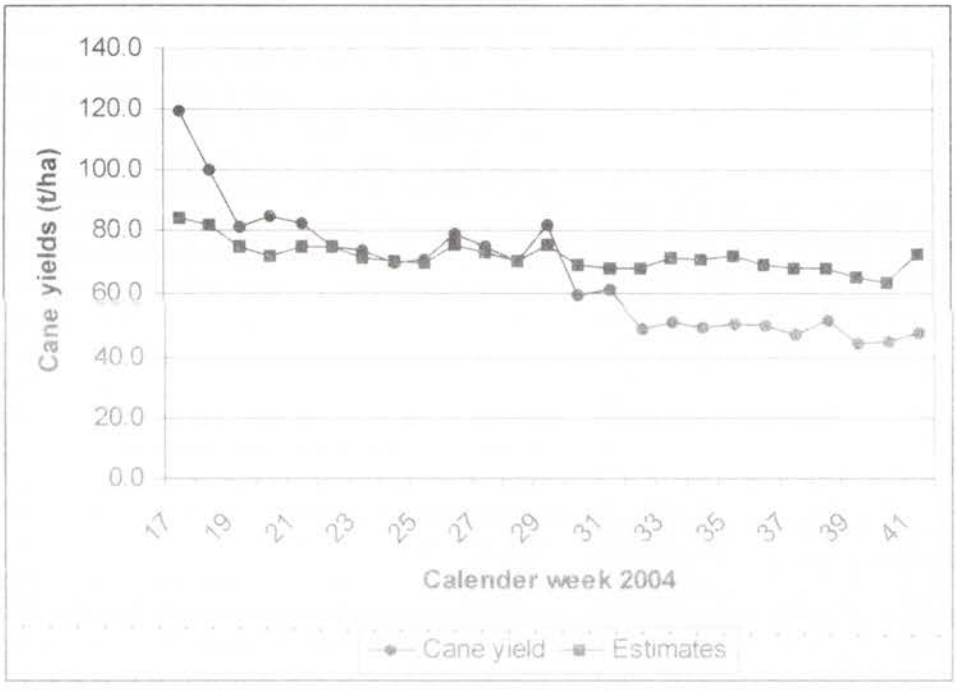
The 2004 crop started very well with the cane yields in the first 2 months of harvest (late April-June) giving over 17% higher yields than the estimates (Figure 1). As the dry season progress the cane yields rapidly declined and this was lower than the estimates for July through to the last week of harvest. By the end of the harvest, most of the blocks harvested were yielding less than 50 tonnes cane per ha [36% below the estimates]. This reduction in cane yield represented more than 72,000 tonnes of cane (15%), which is equivalent to 7,100 tonnes of sugar [valued over K14.2 million]. The cost of

***Table 1*. Summary of RSD infections in commercial, wild and village garden canes from the 2004survey.**

Location	Nil	Low	moderate	high	RSD positive %
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 wildcane (4)	2	2			50
Sausi/Kesowai gardencanes (3)	1	2			67
Kainatu wild cane (15)	12	1	1	1	20
Kainatu village garden canes (12)	9	3			25
Watarias-Lae wild cane (23)	20	2	1		13
Watarias-Lae garden cane (25)	18	7			28

Nil, tested negative; low, moderate and high means 1, 2-3, 4-5 wells positive, respectively.
Numbers in brackets show total number of samples taken.

***Figure 1*:** Summary of actual cane yields observed during 2004 crop compared to the crop estimates (budget).



harvesting a low yielding crop, fertilizers and pesticides used could not be estimated here but these costs are additional to the value of crop losses from RSD.

The effect of RSD on cane yields is severe during a dry year and this proved to be the case in 2004. Rainfall received 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 yields. Re-growth from cane harvested was greatly affected leading to shoot death with lower yields anticipated in the future crop.

STRATEGIES FOR RSD MANAGEMENT

The effective control method of RSD is the use of disease-free seed cane at planting, usually obtained from use of hot water treated cane. Cane used for planting is placed in a hot water bath at 50°C for three hours can lead to 98% control of RSD bacteria

in the planting material. Repeat treatment in the next round of planting (double treatment) could produce almost 100% RSD-free seed cane.

A hot water treatment (WHT) facility was built in 2004 at a cost of K150,000. This facility has been established and is now in operation. As this is a new problem, more that K350,000 will be spent this year to purchase various laboratory equipment and other operating costs/ consumables to get the control program started. The cost of the program will be K300-400,000 annually, an expenditure previously not necessary.

One of the most important factors in the control of ratoon stunting disease is the education of growers and harvesting contractors to adhere strictly to the recommendations for disease control. If these procedures are followed the disease can be kept under control with potential crop losses minimized.

The main challenge after the introduction of disease-free seed cane in to production, will be to minimize the spread of the disease in to clean cane blocks. This will require constant awareness of the potential risks to production and getting all crop production staff to follow strict hygiene procedures now being implemented. Sterilizing of all harvesting and fertilizer application equipment, bush knives and minimizing volunteer cane in fallow blocks will be very critical in reducing the spread of the disease.

Monitoring of disease levels on the estate will greatly facilitate the management of RSD. A laboratory has been established and personnel trained in the diagnosis of the RSD bacteria. An ELISA equipment has been purchased with funding from the Australian Centre for International Agricultural Research project on sugarcane and this will be used for rapid testing of RSD. Linkages between the 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 K14.2 million. The management of the RSD will be very important economic consideration and the company has invested up to K0.5 million to set up HWT plant and develop strategies for the control of this disease. Use of disease-free planting material and keeping clean sugarcane will be very critical to minimize its impact on sugar production and maximizing potential profits. Monitoring disease levels in the 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.

The selection of resistant varieties will provide long term solutions but this is an expensive process. The low concentration of RSD in village gardens and wild canes suggests that these canes may be tolerant to the disease. Recently, Omarjee *et al* (2004) found a number of bacteria from the genus, *Burkholderia* extracted from PNG village gardens that has proved successful in inhibiting the growth of *Clavibacter michiganensis*, a close relative to RSD. It is possible that this bacterium is providing some form of suppression on RSD in village and wild canes in PNG. It may be possible that RSD got in to the commercial cane through contaminated bush knives (commonly used by local workers) and rapidly spread through the commercial cane by mechanical harvesting.

CONCLUSION

The impact of RSD in the 2004 crop was severe with crop losses estimated to be 14% valued at more than K14million. This loss is already significant for a small sugar industry. The cost of implementing management strategies will also be significant. Given the ease in the transmission of the disease through contaminated machinery and implements, it will be necessary to educate all workers about the importance of RSD and its management strategies adopted.

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EFFECT OF PROGRESSIVE BI-MONTHLY WEEDING ON THE YIELD OF YAM (*Dioscorea Esculenta*) AT SARAMANDI, EAST SEPIK PROVINCE

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ABSTRACT

*Lesser yam (*Dioscorea esculenta *var Mangilmu) was planted in a weeding trial in which weeding duration was 0, 2, 4, 6, 8 and 10 months after planting (MAP), allowing weed competition in five out of six treatments. Total yield increased from 4.2 t/ha with no weeding to 7.7 t/ha with weeding for four MAP. It then slightly decreased between weeding for four and eight MAP to 6.4 t/ha and yielded the highest at 9.3 t/ha under weeding for 10 MAP. Total yields with weeding for zero and two MAP were significantly (P<0.05) lower than with 10 MAP, and yield with weeding for four MAP was also significantly higher than with no weeding. Total tuber number only for 0 weeding (11470/ha) was significantly lower than 15760/ha from weeding for 10 MAP. Tuber number and yield proportion of large tubers increased with increased duration of weeding. Weeding for four and two MAP gave the highest estimated net incomes per hectare of K13, 457 and K12,518 after subtracting the weeding cost.*

Keywords: *weeding, competition, yield, yam*

INTRODUCTION

In many parts of Papua New Guinea (PNG) where *Dioscorea esculenta* is grown as the main food specie, gardens are hand-weeded regularly and even kept weed-free for the entire crop duration. Similar practices are followed for the successful cultivation of all other species of yam. Weed management has been a major factor apart from the decline in soil fertility for the traditional shifting cultivation.

Common weed control practices include two to three times of major hand weeding starting from one or two months and ending about four to six months after planting while allowing the yam vine canopies to provide some ground cover. For D alata, with non-pre-sprouted setts and without mulch 3-5 rounds of weeding would be needed (Pido and Pepino 1987). Weeding has been reported to take up between 20 to 30 % of the total man-days devoted to yam production (Degras 1993, Lyonga and Ayuk-Takem 1979). Although chemical weed control is an option this study will focus on hand weeding and other cultural techniques that contribute to weed control as PNG farmers are not yet producing yam commercially.

Unchecked weed competition can lead to different levels of yield reduction. Hahn (1984) reported 43% yield reduction with Unamma and Akobundu (1989) reporting 76-79 % while Moody and Ezumah (1974) reported yield losses of 69-91% from yam crops in West Indies, Ivory Coast and Nigeria respectively.

From this range of yield reductions it appears that a number of factors including a critical period determine the extent of yield reduction.

Beale *et al.* (1985) reported for *D. rotundata* the critical period to be from week 8 to week 10 in Puerto Rico and from week four to week ten for *D. alata* in Costa Rica.

This trial was carried out with the aim to assess the yield loss due to weed competition and to explore ways for farmers to increase labor efficiency in weed control for yam crops.

MATERIALS AND METHODS

This study was conducted at Saramandi Research Station near Angoram in the East Sepik Province of PNG. The trial was the third crop of yam to be grown after the land was cleared from primary forest. The soil is clay based on fine limestone sediments with a local topography of low undulating hills. The area receives between 1500 and 2000 mm of rain and experiences a pronounced dry season from June to September. This trial was planted in December 1986 and harvested in October 1987. A local Maprik variety (Mangilmu) was planted at 444plants/ha (1.5 x 1.5 m) using a standard 2 m staking height. To

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plant each sett, a hole of 30-cm diameter and 30 cm deep was dug using a garden spade. The soil was broken up by hand, removing roots, sticks and stones and was returned to fill up the hole. The sett tuber was placed in the middle of the covered hole and soil from the rim of the hole was dug up, pulverized by hand and used to build a mound about 20-30 cm high over the sett. One treatment consisted of no weeding, four treatments were hand weeded for two, four, six and eight months after planting (MAP) and then allowed to experience weed competition up to harvest. The final treatment was weeded for 10 MAP up to harvest. The randomized complete block design was used with five replications.

The plot size was 67.5 m^2, including a guard row around each plot. The net plot area was 27 m^2. Stakes were cut from the adjacent forest and erected one month after planting allowing one plant per stake. Weeding by hand was carried out monthly. At harvest the tubers were separated into five 250 g interval size or weight categories; Yield 1: < 250g, Yield 2: 250-500g, Yield 3: 501-750g, Yield 4: 751-1000g and Yield 5: >1000g. Tubers in each category were counted and weighed for each plot. Tuber numbers in each yield category were calculated to thousands of tubers per heactare and are referred to as Tuber number 1-5 of the corresponding yield categories. The data were processed using the Analysis of variance (ANOVA).

Using 2005 weed management information at NARI Laloki receipts, from sales of tubers 250g or heavier and weeding costs were estimated and net income estimates for each treatment were derived. Information used were 196.2 person days to weed 1 ha, K9.00 per person day casual hire and K3.00 per kg for yam sales in Port Moresby.

RESULTS

The tuber yields from this trial were quite low and could be due to it being the third crop of yam after land was cleared from forest. The total yield increased with increased duration of weeding and results from the weed-free plot were significantly higher than yields from plots that were unweeded and weeded only for two MAP. The next highest total yield was obtained from weeding for four MAP but this was not significantly different from weeding for two, six and eight MAP (Table 2).

Weeding for 6 and 8 MAP (Table 2), yielded less total tubers progressively. Yield loss from unchecked weed competition amounted to 55 percent while yield reduction at weeding up to four MAP was only 17 percent. Weeding up to four MAP gave the highest estimated net income of K13, 457.00 followed by weeding up to two MAP and no weeding respectively (Table 2). As total yield increased, the yield proportion of large tubers also increased as shown in Figure 1.

Similarly the total number of tubers ('000/ha) increased with duration of weeding (Table 2). However, only the unweeded treatment yielded significantly lower total tuber numbers than the weed-free treatment of weeding for ten MAP.

Figure 2 and figure 3 show that tuber numbers and yield distribution increased in favor of large tubers with increased weeding duration.

DISCUSSION

The general relationship between weeding duration and increased yield reaffirms the importance of

Table 1. Six weeding treatments that were applied to the trial.

Treatment No.	Duration of weeding from planting (MAP) (Planted December 1986)	Duration of weed competition in months to harvest (MTH) (Harvested October 1987)
1	0	10
2	2	8
3	4	6
4	6	4
5	8	2
6 (Control)	10	0

Table 2. **Effect of weed competition on total yield, total number of tubers and estimated net income (K) in *D. esculenta***

Treatment	Total tuber yield (t/ha)	Total tuber numbers ('000/ha)	Estimated net income (K) after taking out weeding cost
6	9.3a	15.8a	7, 999
3	7.7ac	13.6ab	13, 457
4	7.1abc	11.7ab	8, 695
5	6.4abc	12.4ab	2,794
2	5.9bc	12.4ab	12, 518
1	4.2b	11.5b	10, 020
LSD (0.05)	3.0	3.7	NA
	* **Treatment means followed by the same letter, are not significantly different at the 5% LSD level.**		

Figure 1. **Effect of weed competition on *D. esculenta* tuber yield components**

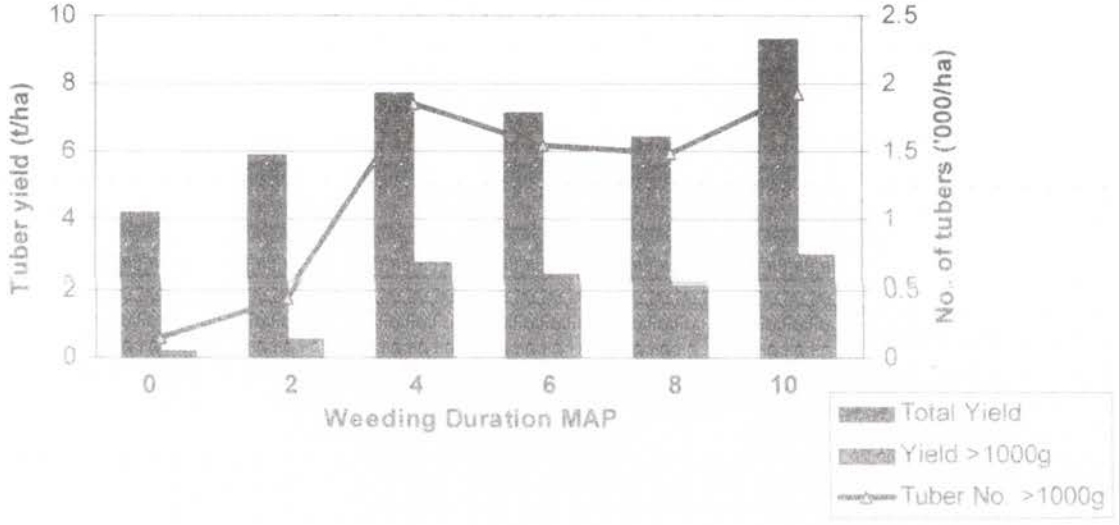


Figure 2. **Effect of weed competition on yield partitioning into different tuber size in D. esculenta**

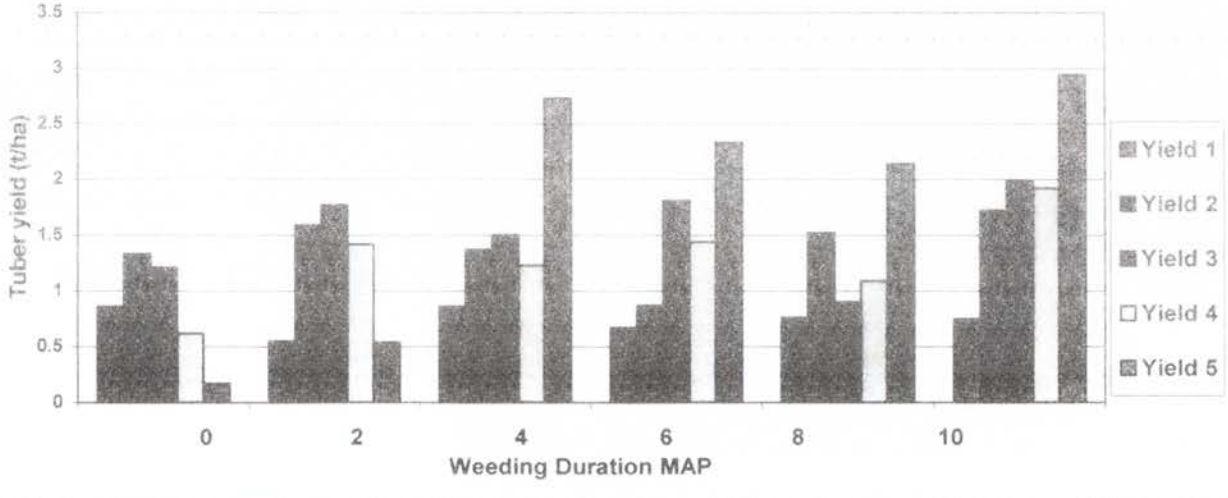
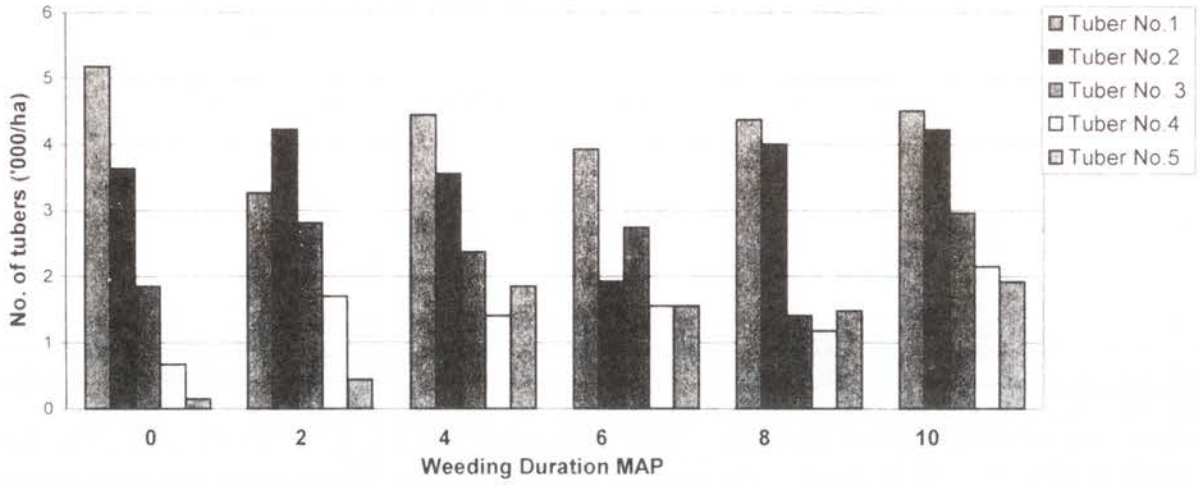


Figure 3. **Effect of weed competition on number of tubers in different size categories in *D. esculenta***



weed control in a yam crop. Yam total yields were low compared to figures reported by Quin (1985) 18t/ha and by King and Risimeri (1992) 63.3 t/ha at the same plant population. A contributing factor could be that Southern Oscillation Index in 1987 was one of the 7 strong El Nino Southern Oscillation (ENSO) events between 1877 and 1988 (Kapal *et al.* 2003).

While weed control by clean-weeding on a monthly basis produced the highest yields this trial has raised the issues of cost-benefit of weeding and the identification of the critical stage in the phenology of *Dioscorea esculenta* after which the severity of yield reduction from weed competition is less. Highest estimated net income after subtracting the weeding cost was obtained from weeding up to four MAP followed by weeding up to two MAP and even the unweeded treatment was higher that the others. The data presented here is in agreement with Onochie (1973) whose work suggested that yield is most affected by weed competition between the fourth and sixteenth weeks.

There appears to be a second factor interacting to reduce yield as continued weeding up to six and 8 MAP resulted in lower yield than 4 MAP. As 1987 experienced a strong dry season it appears that treatment 3 benefited from a mulching effect by the herbaceous weed cover going into the tuber bulking and crop maturity phase. Weed mulch can be beneficial as discussed by Moody and Ezumah (1974).

Appropriate planting hole arrangements and staking techniques that would prove more economical can better achieve mulching and shading effects.

Ndegwe *et al.* (1990) supports this suggestion with their findings that; staking six plants onto one stake achieved the highest net cash returns per hectare. Using the "Pyramid and "A Frame" staking techniques, observations at Bubia and Laloki show that; after full canopy establishment the yam crops create ample self-generated shading to reduce weed establishment and vigor. Further research is required to refine cultural practice combinations to formulate economical weed control options.

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VOLATILE CHEMICAL CONSTITUENTS OF PATCHOULI (*POGOSTEMON CABLIN* (BLANCO) BENTH.: LABIATAE) FROM THREE LOCALITIES IN PAPUA NEW GUINEA

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ABSTRACT

Fresh aerial parts of patchouli (Pogostemon cablin) *were obtained from Rabaul, Port Moresby and Inawabui in the Mekeo area of the Central Province. The volatile oil constituents were extracted by exhaustive hydrodistillation where each of the patchouli samples afforded oil yields of about 0.1 percent. Detailed chemical investigations of the oils indicated patchouli alcohol to be the main constituent in the oils from the three localities. It was noted that the patchouli alcohol composition in the cultivar from Rabaul, Port Moresby and Mekeo were 71.8, 71.7 and 43.7 percent compositions respectively, suggesting that the patchouli oil from PNG can attract a ready market on the basis of its high patchouli alcohol contents in the patchouli oil as opposed to between 30 – 40 percent composition from other patchouli oil producing countries.*

Keywords. Pogostemon cablin, *Labiatae, Patchouli, Essential oil composition, Patchouli alcohol, Sesquiterpene.*

INTRODUCTION

Patchouli oil is obtained from *Pogostemon cablin* (Blanco) Benth., a member of the plant family Labiatae. It is a predominantly tropical herbaceous and perennial plant species with wide distribution in most South East Asian nations where it has also been documented as having many significant uses in traditional medicine and agriculture (Guo 2001). The leaves and stem contain a yellowish and viscous oil that has a unique and intense camphoraceous odour with many useful applications, hence high market value in the perfumery industry.

On the basis of its traditional uses as alternative medicine, agricultural pest control agent and applications in the perfume industry, detailed phytochemical studies were pursued to identify the chemical constituents responsible for the perceived activities. In one such study, the acetone extracts of the leaves were found to contain sesquiterpene hydroperoxide, which showed significant trypanocidal activities (Kiuchi *et al.* 2004). Another study also identified the cytotoxic Licochalcone A, Ombuin and 5,7-dihydroxy-3',4'-dimethoxyflavanone as the main chemical compounds from the aerial parts (Park *et al.*, 1998). The hexane extracts were also noted to contain patchouli alcohol, pogostol, stigmast-4-en-3-one, retusin and pachypodol, which showed antiemetic activities (Yang *et al.* 1999).

The study of the chemical components within the essential oil extracts were found to be made up of patchouli alcohol, delta-guaiene, alpha-guaiene, seychellene, alpha-patchoulene, aciphyllene, trans-caryophyllene (Feng *et al.* 1999; Zhao *et al.* 2005; Guan *et al.* 1994). Luo and co-workers (1999) investigated the patchouli oil from the Gaoyao County, China and noted the stem to contain high pogostone content while the leaves contained high patchouli alcohol contents. While the chemical components were noted to be the same from different regions studied, the compositions were found to vary between regions as influenced by various environmental factors (Singh *et al.* 2002; Yan *et al.* 2002). Furthermore, the oil composition from different cultivating locations and different harvesting times were obviously different (Luo *et al.* 2002), suggesting that detailed chemical study was required to establish the reasons for such differences. Such studies revealed that there were two main chemotypes in patchouli; one being the pogostone-type and the other being the patchouliol-type on the basis of the molecular evidences (Liu *et al.* 2002). The pogostone-type contained rich oxygenated components especially pogostone in the volatile oil while the patchouliol-type contain rich patchouliol, delta-guaiene, alpha-guaiene and other non-oxygenated components (Luo *et al.* 2003).

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Morphological studies of the leaves of patchouli also indicated the leaves to contain external trichomes as well as specialized internal accumulatory cells where concentrations of the sesquiterpenes were noted to be exceptionally higher than other parts of the plant (Henderson *et al.* 1970). Further biosynthetic studies revealed high concentrations of the enzyme sesquiterpene cyclase (patchoulol synthase) in the leaves, which was postulated to be responsible for the conversion of the farnesyl pyrophosphate, through biosynthetic mechanisms, to the cyclic sesquiterpenes (alpha and beta-patchoulene, alpha-bulnesene and alpha-guaiene) found in patchouli oil (Munck and Croteau, 1990; Croteau *et al.* 1987). Another study of the leaf extracts revealed the presence of patchoulan-1,12-diol, which was also postulated to be the main precursor of nor-patchoulenol (Trifilieff 1980).

Interesting biological activities have been reported for the extracts of patchouli by various authors. The patchouli oil has been reported to show activity against three strains of methicillin-resistant *Staphyloccocus aures* (Edwards-Jones *et al.* 2004), cytotoxic (Park *et al.* 1998), bactericidal activity against *Campylobacter jejuni* and *Listeria monocytogenes* (Friedman *et al.* 2002), antibacterial and antifungal activities (Pattraik *et al.* 1996; Osawa *et al.* 1990), unusual tissue destructive effects on the exoskeletons of Formosan subterranean termites, *Coptotermes formosanus* Shiraki (Zhu *et al.* 2003), effective mosquito repellency effects (Trongtokit *et al.* 2005), showed promise as alternative preservative of perishable foods (Holley and Patel 2005), antiemetic activities (Yang *et al.* 1999) and trypanocidal activities (Kiuchi *et al.* 2004). Such interesting biological activities led to a number of synthetic studies to attempt their synthesis on a laboratory scale (Magee *et al.* 1995; Niwa *et al.* 1984; Niwa *et al.* 1987; Cory *et al.* 1990) including microbial assistance in the conversion of the substrates into the desired products (Arantes *et al.* 1999).

Currently, patchouli oil is one of the important natural essential oils because of its base and lasting aromatic character. The yellowish brown coloured viscous oil obtained from the leaves and stems have an intensely camphorous odour, a character that is utilized in the cosmetic industries to scent perfumes, flavour toothpastes and other health and self-care products. The global demand for patchouli oil has been noted to increase annually with Indonesia as the major producer of this oil (Robbins 1983; Tao 1983). The main buyers of the patchouli oil are the USA, Switzerland and France.

Preliminary studies on the chemical constituents in the PNG cultivar of patchouli (Wossa *et al.* 2004a) has shown the PNG oil to contain superior patchouli alcohol content at 70 percent as compared to the oil from Indonesia, Philippines, Malaysia, China and other South East Asian nations, which have patchouli alcohol compositions between 30 - 40 percent. In view of the economic potential in the cultivation and extraction of patchouli oil as an alternative agribusiness in PNG, we report herein the chemical constituents in the volatile oil extracts from patchouli from three localities and discuss these data in the light of the global market trends. This report is part of an ongoing study to document the chemical constituents in the essential oils obtained from the diversity of aromatic plants of PNG (Rali *et al.* 2003; Wossa *et al.* 2004a; 2004b; 2005).

MATERIALS AND METHODS

Samples of Patchouli were obtained from Rabaul, Port Moresby and the Mekeo area of the Central Province. The stem cuttings of the Rabaul cultivar was grown in a Port Moresby backyard garden to compare its oil yield and chemical composition with that of the samples from Rabaul. A native cultivar from the Mekeo area was also used in this study for comparative analysis of the chemical constituents.

The matured leaf samples of the patchouli were collected while fresh and the volatile oils obtained through exhaustive hydrodistillation. The distillates were extracted with diethyl ether and the ether removed under reduced pressure to afford yellowish brown-coloured pure oils. The oils were dried with anhydrous magnesium sulphate and stored at 4 degrees Celsius until further analysis. The analyses of the oils were done using a gas chromatograph coupled to a mass spectrometer (GC and GC/MS) and the individual components identified on the basis of their individual retention indices.

The analyses of the oil constituents were done as previously described (Wossa *et al.* 2005). The individual oil constituents were identified on the basis of their respective retention indices and confirmed by comparison with the mass spectral data of the authentic reference compounds or with the library of the published data (Adams 1995).

RESULTS AND DISCUSSION

The results of the GC and GC/MS analysis of the patchouli oil obtained from Rabaul, Port Moresby and Inawabui in Mekeo (Table 1) indicate that the Rabaul cultivar of patchouli has a higher patchouli

Table 1. **Volatile chemical constituents (% area) of *Pogostemon cablin* from three localities in PNG**

Chemical Constituents	Rabaul	Port Moresby	Mekeo
alpha-pinene	-	-	1.2
alpha-guaiene	7.5	4.6	7.8
seychellene	3.9	3.2	6.2
gamma-patchoulene	-	-	3.7
beta-patchoulene	-	-	1.8
alpha-patchoulene	1.7	1.5	1.4
delta-guaiene	9.9	6.5	9.5
pogostol	5.1	-	-
Patchouli alcohol	**71.8**	**71.7**	**43.7**
beta-caryophyllene	-	1.1	2.4
aciphyllene	-	1.2	-
beta-patchoulene	-	2.2	-
viridiflorol	-	1.4	-
Selina-3,7(11)-diene	-	-	5.1
Benzyl benzoate	-	-	1.7
$C_{15}H_{22}O$	-	6.6	0.7
$C_{15}H_{24}$	-	-	1.9
$C_{15}H_{26}O$	-	-	0.9

- = not detected.

alcohol content than the Mekeo cultivar with compositions at 71.8 and 43.7 percent compositions respectively. Such differences can be expected due to the various environmental and genetic factors that may be involved in the genesis of the different chemical constituents in the two cultivars of patchouli. Similarly, the Port Moresby cultivar was found to be superior in yield and composition of the patchouli alcohol than the Mekeo cultivar with compositions at 71.7 percent and 43.7 percent respectively.

On the other hand, the chemical constituents and composition of the Port Moresby cultivar were noted to be similar to that of the Rabaul cultivar. This result was as expected because the cuttings were from the Rabaul cultivar and were cultivated in the backyard garden in Port Moresby to see the possible effects of environmental factors on oil yield and chemical composition. The similar oil yield and chemical constituents and composition led us to infer that the environmental factors had minimal effect on the yield and composition of the patchouli oil. These results further suggest that the Rabaul cultivar has a higher patchouli alcohol content compared to that reported from other South East Asian cultivars. The comparison of the patchouli alcohol contents from the Mekeo cultivar with that of the other South East Asian cultivars can be categorized as being similar on the basis of the patchouli alcohol composition at around 40 percent, however they differ in the composition of the other constituents. This study, further shows that the three patchouli cultivars analyzed so far belong to the patchouliol-type with high patchouli alcohol contents and other non-oxygenated constituents.

On the basis of the chemical compositional data on patchouli oil presented, it can be realized that the patchouli alcohol content in the patchouli oil from Rabaul are higher compared to those reported from other patchouli oil producing countries in the region. This implies that patchouli oil from PNG can be readily accepted on the global patchouli oil market on the basis of the high patchouli alcohol contents. These results further suggest that patchouli can be encouraged and cultivated as a rural based agro industry in PNG. With the current global market for patchouli oil fluctuating between US$ 12 – 27 per kilogram, PNG could do well in introducing patchouli oil production as an alternative income earner in the country.

It is therefore recommended that further studies into the effects of the soil nutrients and chemistry, irrigation systems, age of crop at harvest, other important environmental as well as genetic factors be pursued to identify the optimum conditions for maximum yield of the patchouli alcohols with higher patchouli alcohol content from the patchouli cultivars of PNG. Such studies will pave the way for commercial production of patchouli oil in PNG as an alternative revenue earner.

CONCLUSION

This study has given new insight into the commercial potential for the patchouli oil production in PNG in terms of its oil yield and chemical constituents. The Rabaul cultivar of patchouli was found to contain higher patchouli alcohol content compared to the Mekeo cultivar while the patchouli alcohol content in the Mekeo cultivar were noted to be within the marketable range. It was also noted that the cultivation of the cuttings from the Rabaul cultivar in Port Moresby gave similar yield and composition, suggesting that the environmental factors had minimal effect on the oil yield and chemical composition.

In view of the commercial potential in the cultivation and extraction of the patchouli oil as an alternative revenue earner in the country, further studies into the soil nutrients and chemistry, the irrigations systems, age of plant at harvest, other environmental factors and genetic composition needed to be established to assist farmers in cultivating *Pogostemon cablin* for the extraction of its oil for the global patchouli oil markets.

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GLUCOSINOLATES - A LITERATURE REVIEW

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ABSTRACT

Glucosinolates are syntesised in dicotyledonous plants, especially the order of Capparales; families Resedaceae, Capparidaceae, Caricaceae, Euphorbiaceae, Gyrostemonaceae, Limnanthaceae, Moringgaceae, Salvadorraceae, Toviraceae, Tropaeolaceae and Cruciferae plants' genera Thilasp, Cochlearia, Sisymbrium, Sinapis, Raphanus, Descurainia, Stanleya and Brassica, which includes kale, rape, turnips and Swedes are fed to livestock. Roots and leaves of forage Brassica crops are rich in nutrients for finishing lambs and cattle. Glucosinolates and S-methyl cysteine sulphoxide (SMCO) concentrate in seeds and vegetative tissues. Effect of SMCO is aggravated by the presence of nitriles that deplete glutathione leading SMCO to cause haemolytic anaemia in ruminants. Toxic effects of glucosinolates on the production performance of animals include changes in productive characteristics such as growth rate, egg and milk production, general animal performance including reproduction and weight performance. Adaptive function of glucosinolates associate with protection against herbivore and also influences the degree of herbivory of phytophagous insects. Hhydrolysed products may have a role in pathogen resistance, especially anti-fungal and anti-microbial properties and may act as phago-stimulants in which certain Cruciferae could be stimulate and attract certain species of insects. Breakdown of glucosinolate following their absorption from the digestive tract of animal species need more research work to improve the isolation techniques and isotope labelling. Administration of pure compounds in laboratory animals has proved useful and clarified excretory routes of mercapturic acid derivatives of isothiocyanates in the urine.

Keywords: *Capparales, Cruciferae plants, Brassica, glucosinolates, S-methyl cysteine sulphoxide (SMCO), toxins, allyl isothiocyante, allyl cyanide, thioglucosidas, mercapturic acd, hydorlysis, metabolites, detoxification, in-vitro, in-vivo, zenobiotic, high performance liquid chromatography (HPLC) and enzyme-linked-immunosorbent assay (ELISA)*

INTRODUCTION

Glucosinolates occur in dicotyledonous plants and exist as thioglucosides or sulfur containing glycosides (Duncan and Milne 1989; Tiedink *et al* 1991; Clarke and Clarke 1975; Palmieri *et al* 1986). They are found among the order of Capparales, in the families of Resedaceae, Capparidaceae, Caricaceae, Euphorbiaceae, Gyrostemonaceae, Limnanthaceae, Moringgaceae, Salvadorraceae, Toviraceae, Tropaeolaceae and Cruciferae (Heaney and Fenwick 1980b; Larsen 1981). Cruciferae plants of the genera *Thilasp, Cochlearia, Sisymbrium, Sinapis, Raphanus, Descurainia, Stanleya and Brassica* have been found to contain glucosinolate toxins (Smith and Dacombe 1987). The genus *Brassica* includes kale, rape, turnips and Swedes, which are fed to livestock with potentially harmful consequences (Clarke and Clarke 1975). Forage *Brassica* crops, particularly the roots and leaves are valued as a rich source of nutrients for finishing lambs and cattle at a time of year when pasture is declining or unavailable (Duncan and Milne 1989; Tiedink *et al* 1991). Glucosinolates are well concentrated particularly in the seeds and the vegetative tissues (Heaney and Fenwick 1980a). Some of the R-groups are alkyl, alkenyl, aryl, indole groups, methyl, thiol and hydroxyl groups (Rodman 1978).

BIOSYNTHESIS AND DISTRIBUTION OF GLUCOSINOLATES

Glucosinolates are synthesised in the *Brassica* plants from amino acid precursors. For example, indole glucosinolates are produced from tryptophan while benzyl glucosinolate and hydroxybenzyl glucosinolate are produced from phenylalanine and tyrosine respectively (Underhill 1980). The distribution of amino transferase within the plant was found to be highly correlated with prop-2-enyl glucosinolate concentration in different parts of *Brassica carinata* (Duncan and Milne 1989) and in *Brassica juncea* glycosylation and sulphation steps in glucosinolate bioactivity. The activity of these

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enzymes correlates well with glucosinolate concentrations in the glucosinolate biosynthesis (Duncan and Milne 1989) and localised in the vacuoles of cells (Grob and Matile 1980a)

The toxins identified in *Brassica* species include the glucosinolates and S-methyl cysteine sulphoxide (SMCO), which are hydrolysed to dimethyl disulphide (Smith 1974). Smith and Szabo *et al.* (1977) noted that the effect of SMCO is aggravated by the presence of nitriles that deplete glutathione leading SMCO to cause haemolytic anaemia in ruminants.

The toxic effects of glucosinolates on the production performance of animals have been noted in the context of the feeding of glucosinolate-containing rapeseed to farm animals. The effects include changes in productive characteristics such as growth rate, egg and milk production (Bell *et al* 1971; Lo and Bell 1972; Wight *et al* 1987), general animal performance including reproduction and weight performance.

The concentrations of glucosinolates vary within the family Cruciferae plants, and ihe genus *Brassica*. The highest glucosinolate concentrations tend to occur in rapidly growing young *Brassica* plant parts, such as shoot, root tips and seeds (Palmieri *et al* 1986). This may be associated with a defence mechanism against damage by herbivores (Klingauf *et al.* 1972; Nault and Styer 1972; Greenhalgh and Mitchal 1976; Hardman and Ellis 1978). It is known that the toxic effects of glucosinolates include goiter, damage to liver and kidney tissues, and the toxic compounds responsible for these effects could be due to 5-vinyl-2-oxazolidinethione (5-OZT) (Elfvinging 1980) and other breakdown products.

All over the world man has been consuming significant amounts of glucosinolates, by eating large amount of cabbage, broccoli, cauliflower, brussels sprout, mustard or horseradish on regular basis (Albert 1987; Tiedink *et al.* 1991). Once these Crucifers are consumed, the glucosinolates are hydrolysed enzymatically, during the preparation for the table or within the stomach after ingestion. There is not much information on the effects of food processing on glucosinolates content and the breakdown products arising following cooking. However, Slominski and Campbell investigated that heat treatment including steaming and cooking resulted in substantial decomposition of indole glucosinolates with thiocyanate ion and indoleacetonitriles accounting for 50% and 30% respectively. Autolysis of indole glucosinolates in raw *Brassica* vegetables resulted in the production of little or no indoleacetonitrils but produced substantial thiocyanate ion and related compounds. The anti-carcinogenic properties of some indoles and isothiocyanates and other glucosinolates derived compounds reacting with nitriles are among the potential positive effects of glucosinolates (Duncan and Milne 1989). In the areas where *Brassica* plants contribute heavily to the cattle fodder, Heaney and Fenwick had reported that the ionic metabolite of thiocyanate ion can be transferred to humans through milk. They also considered that this may be partly responsible for the development of goiter carcinogen when human beings consume raw cabbage to prevent cancer development (Albert 1987).

FUNCTIONS OF GLUCOSINOLATES IN *BRASSICA* PLANTS AND METABOLIC FATE

The function of glucosinolates in *Brassica* plant metabolism is not entirely clear. Their rapid turnover within the plant tissues with the associated metabolic costs indicates an adaptive function, to protect against herbivore damage. This seems analogous to other secondary compounds in other plants (Klingauf *et al.* 1972; Nault and Styer 1972; Hardman and Ellis 1978). An important area of research is the study of the mechanism of protection against insect herbivory, in which the glucosinolate content of various plant species has been shown to affect larval development and pupation. This also influences the degree of herbivory of phytophagous insects (Klingauf *et al.* 1972). Therefore glucosinolate breakdown products may have a role in pathogen resistance, especially those that have been shown to have anti-fungal and anti-microbial properties (Duncan and Milne 1989). Glucosinolates may also act as phago-stimulants in which the certain Cruciferae could be stimulatory and attractive to certain species of insects (Nielsen *et al.* 1979).

The fate of specific glucosinolate breakdown products, following their absorption from the digestive tract of animal species has been less researched, particularly their degradation process. This may be attributed to analytical problems, particularly in determining the hydrolysed products in the digestive fluid. However, the possibility would be that, the isolation techniques and isotope labelling could be improved, thus enabling analysis to be more specific. The digestive fate of glucosinolates in poultry fed rapeseed have been researched extensively, especially the production of 5-OZT and nitrile hydrolysis from progoitrin (Smith and Campbell 1976). The recent studies of glucosinolate recovery in the faeces and urine samples of hens were in the range of 15 – 50 %,

while the un-recovered fraction may have undergone hydrolysis in the digestive tract (Slominski *et al.* 1987; Slominski and Campbell 1988). In hens, caecectomy and addition of antibiotics in the diets increased the hydrolysis by the hind-gut micro-organisms (Freig *et al.* 1987; Slominski and Campbell 1988). About 1 – 2% recovery of intact glucosinolates were seen in the faeces from rats following rapeseed feeding (Marangos and Hill 1974). Low concentrations of 5-OZT were determined in the gut contents of rats and there has been lots of research carried out on 5-OZT with its effect by various authors (Langer and Michajlovskij 1969; Peltola and Krusius 1971; Elfving 1980).

The detection of other hydrolytic products of glucosinolates in the digestive fluids of rats has been difficult (Lo and Hill 1971; VanEtten and Daxenbichler 1977). Administration of pure compounds in laboratory animals has proved useful and clarified excretory routes of mercapturic acid derivatives of isothiocyanates in the urine. However species differences exist in excreting the mercapturic acid (Brusewitz *et al.* 1977; Gorler *et al.* 1982).

GLUCOSINOLATE HYDROLYSIS, METABOLITES AND FACTORS AFFECTING HYDROLYSIS

Glucosinolates are normally associated in plants with the enzyme thioglucosidase (myrosinase). Hydrolysis takes place under the action of myrosinase (Heaney and Fenwick 1980b; Palmieri *et al.* 1986), which catalyses the cleavage of the thioglucoside bond of glucosinolates (Heaney and Fenwick 1980a; Duncan and Milne 1989; Tiedink *et al.* 1991). Once the cellular structure in the *Brassica* plant is disrupted, glucosinolates are broken down by myrosinase to various metabolites including free glucose (Heaney and Fenwick 1980a; Duncan and Milne 1989) and an aglucone intermediate. This is then degraded spontaneously to one of a number of toxic metabolites. The common metabolites normally produced from enzymic hydrolysis are the volatile isothiocyanates, thiocyanate ion and nitriles (Duncan and Milne 1989; Tiedink *et al.* 1991; Duncan and Milne 1992a) while the others are sulphate, hydroxynitriles and hydroxyepithionitriles However different metabolites are formed depending on the conditions during the hydrolysis. Likewise figure IIa and IIb may be seen as possible routes for cysteine conjugate of benzyl isothiocyanate and hydrolysis to form allyl mercaturic acids (AMA) via aglucone.

The production of metabolites following glucosinolates hydrolysis is influenced by factors such as the presence of various protein cofactors, temperature, metallic ion concentrations and pH (Duncan and Milne 1989; Duncan and Milne 1993). These conditions may interact at complex ways to form toxic products such as aglucone, arising from hydrolysis. An aglucone is rearranged to form cyanoepithioalkane by protein co-factor at the expense of aliphatic nitrile. Temperature has an indirect effect associated with the denaturing of certain heat-labile factors involved in glucosinolates hydrolysis, such as epithiospecifier protein (Tookey 1973), however, Gil and MacLeod (1980) have identified that temperature has little effect on the proportions of glucosinolate hydrolysis. The presence of other compounds such as ferrous ions (Fe^{++} and Fe^{+++}) and copper ion (cu_2^{+}) also influences glucosinolate breakdown and consequently may alter their toxicity. Thiol compounds such as cysteine and glutathione increase the action of ferrous ions to favour nitrile production. Also ferrous ions and pH interactions increase the glucosinolate hydrolysis; low and high pH favour nitrile and isothiocyanate production respectively (VanEtten *et al.* 1966; Tookey and Wolff 1970; Uda *et al.* 1986). Glucosinolate hydrolysis is a complex process with the range of products formed depending on the factors discussed above. This of course, is influenced by the conditions in the digestive tract producing toxic products when plants are ingested by the animals. This can be seen with pH, which is low in the monogastric stomach, but higher in rumen of ruminants and this will influence glusosinolate hydrolysis (Duncan and Milne 1989). There has been some evidence that addition of mercaptoethanol to the hydrolysis medium influences glucosinolate hydrolysis; sinigrin is hydrolysed to epithioalkanes and allyl cyanide at the expense of isothiocyanate production. The mechanism is that the mercaptoethanol activates epithiospecifier protein (Duncan and Milne 1989).

Thiocyanate ion is a known myrosinase induced hydrolysed product of indole glucosinolates and may catalyze the nitrosation reaction (Fenwick *et al.* 1983). Indolyl glucosinolates yield indole compounds, such as indole-3-carbinol, indole-3-acetonitrile, di-indolylmethane and ascorbigen (Tiedink *et al.* 1991). Other compounds such as cyanoepithioalkanes and organic thiocyanate are produced from aliphatic nitriles. Also progoitrin, with its beta-position side chain hydroxy group forms less volatile oxazolidine-2-thiones and epithionitriles (Gil and MacLead 1980; Elfving 1980; Hassan *et al.* 1988; Tiedink *et al.* 1991).

Tiedink *et al.* (1991) investigated the association of sinigrin and some of the glucosinolates in forming N-nitroso compounds following nitrosation. The results showed positive response of hydrolysis occurring in the presence of myrosinase and acidic conditions. However the authors were not convinced of their work, because there was no correlation with the previous work of formation of direct mutagenic N-nitroso compounds in vegetable extracts. Thus, the chemical natures of the precursor of N-nitroso compounds in cruciferous vegetables and biologically active compounds needed further investigation. The activity of various enzymes is affected by the glucosinolate metabolites such as benzyl isothiocyanate, indole-3-carbinol and indole acetonitrile and they may then protect against potential carcinogens (Albert 1987; Duncan and Milne 1989).

In addition to enzymic breakdown, Tiedink *et al.* (1991) reported that glucosinolates undergo chemical hydrolysis although the reaction was found to be very slow. The low pH in the stomach could be responsible for the chemical hydrolysis. The ability of intact and hydrolysed glucosinolates, particularly glucobrassicin and 4-hydroxybrassicin to form N-nitroso compounds showed positive results.

ABSORPTION, STORAGE AND EXCRETION OF GLUCOSINOLATE PRODUCTS

There is no available information regarding the detection of the hydrolytic products of glucosinolates in the blood and tissues of ruminants and monogastrics fed with rapeseed. The detection of glucosinolates in their original form in the gut is also unknown, although post-absorption of the metabolic process of transforming xenobiotics of foreign compounds into excretable compounds is known (Kamrin 1988). Studies performed on laboratory animals, such as rats have proved useful in identifying the excretory routes of the metabolic products. For example, aliphatic isothiocyanates induce glutathione conjugation in the liver as the major excretory route. However, dogs have been found to excrete hippuric acid derivatives while guinea pigs and rabbits excrete cyclic mercapturic acid derivatives, and mice excrete other metabolites as well as mercapturic acid derivatives (Brusewitz *et al.* 1977; Gorler *et al.* 1982). The metabolic fate of glucosinolate-derived nitriles needs further research, though similar aliphatic nitriles have indicated of their likely mode of catabolism. Administration of nitriles increases the urinary thiocyanate ion excretion, due to free cyanide release via hydrolysis in the tissue and then the conventional thiocyanate (SCN) excretion. Early experiments conducted on nitrile toxicity in rodents suggested the compounds are potentially toxic (VanEtten *et al.* 1969; Nishie and Daxenbichler 1980) following the administration of acrylonitrile to rats and observed the excretion of urinary mercapturic acid derivatives. Other experiments suggest that a number of proposed catabolic routes for nitriles can be either direct or indirect conjugation to an epoxide intermediate.

GLUCOSINOLATE TOXICITY AND ITS EFFECTS

Glucosinolate toxicity may occur mostly on a chronic basis while the animals are grazing in paddocks continuously over along period of time. Chronic toxicity effects may occur if the animals graze on the *Brassica* forages or ingest feed such as rapeseed that contains glucosinolates (Kamrin 1988). However, little is known about the digestive fate of glucosinolates in ruminants *in-vivo* (Duncan and Milne 1992a).

Glucosinolate hydrolysis is considered important in identifying the effects of its toxicity. The rapeseed, which is a protein-rich meal contain glucosinolate and is normally fed to farm animals (Palmieri *et al.* 1986). The main production performance problem associated with feeding of rapeseed meal is the presence of glucosinolates, which not pleasant enough to taste due to the toxic effects. These toxic effects have been considered a danger to farm animals and the plant breeders have made lots of progress in breeding programmes to reduce the levels of glucosinolates and erucic acid, which reduces the oil quality in rapeseed (English *et al.* 1988). The effects of rapeseed meal when fed to farm animals show that the productive characteristics affect the growth, egg and milk production (McDonald *et al.* 1992).

The simplest techniques of assessing the toxicity of glucosinolates are by correlating with the amount of glucosinolates present in the diet. But the hydrolysis of glucosinolate is important in altering their toxicity so the processing of diet in various ways to favour the toxic metabolites may influence the resulting toxicity. For example, controlling the hydrolysis of glucosinolates in rapeseed meal fed to rats and chicken may influence the toxicity. Rapeseed meal, which also is rich in nitrile products and 5-vinyl-2-oxazolidinethione (5-OZT) (Duncan and Milne 1989; McDonald *et al.* 1992) causes reduction in live-weight gain. Canola seed which is high in fat and protein, but low in glucosinolates depressed the feed intake and weight gain in pigs and feed containing Canola seed was not utilized efficiently (Cromwell *et al.* 1989). Underlying effects

were kidney enlargement and increase in thyroid weight (Dierschke 1980; Albert 1987). This may have resulted from the presence of 5-vinyl oxazolidinethione (Palmieri *et al* 1986; Duncan and Milne 1989; Duncan and Milne 1992a) and following the hydrolysis of 2-hydroxy-3-butenyl glucosinolate. This may also cause liver damage. Liver haemorrhage may also relate to the presence of glucosinolates in the diet but there seems to be no correlation with the particular metabolites. The cellular damage occurs in the liver and the kidney tissues of rats following ingestion of nitriles possibly as a result of free cyanide release in liver and brain tissue (Nishie and Daxenbichler 1980; Willhite and Smith 1981). The presence of aliphatic nitriles as toxins may inhibit cytochrome oxidase activity and may form thiocyanate ions (Ahmad and Farooqui 1982). Thiocyanate ions may cause a direct goitrogenic effect (McDonald *et al.* 1992; Duncan and Milne 1992a). The metabolites formed from hydrolysis of glucobrassicin can inhibit the neoplastic effects of carcinogens (Tiedink *et al.* 1991).

Glucosinolates toxicity may involve glucosinolate extracts from glucosinolate free diets, which are fed to rats had problem of productive characteristics. Rats dosed with butenyl cynide prepared from rapeseed showed depressed live-weight gain (Sirvastava *et al.* 1975). Allyl isothiocynate (AITIC) and other isothiocynates are well documented electrophilic compounds, which are very reactive and this may underlie toxic nature due to molecular polarity. However, this evidence is based on a limited number of experiments. It was found that AITIC dosing increase plasma phospholid concentrations in rats (Muztar *et al.* 1979a) and this work was confirmed by Idris and Ahmad (1975), also both authors have found to reduced the plasma glucose and uric concentrations (Idris and Ahmad 1975; Muztar *et al.* 1979b). AITC dosed rats appear with hypothyroidism during carbohydrate metabolism, following the altered activity of liver succinic dehydrogenase and kidney xanthine oxidase (Ahmad *et al.* 1967). Rats dosed with also inactivated the antidiuretic hormone and addition of AITC on to the sulphydry group of tyrosine may reduce thyroid hormone synthesis. Phenyl isothiocyanate also affects the presence of iodine and plasma thyroxin (T_4). Other AITC reactions, which are important biologically, are unclear (Duncan and Milne 1989). In an *in-vitro* experiment, proton-potassium adenosine triphosphatase has been seen to be inhibited by the presence of AITC (Takeguchi *et al.* 1983). Similarly a number of *in-vitro* and *in-vivo* experiments demonstrated the effects on thyroid function (Muztar *et al.* 1979b). These effects showed that there were potential toxic actions of isothiocyanate as a result of sulphydryl groups. Benzyl isothiocyanate cleaved the disulphide bonds of important proteins, which led to toxic actions (Tang 1974).

In-vitro and *in-situ* trials carried out with sheep in Russia and had found that neutral detergent fibre disappeared much faster with high glucosinolate than with low glucosinolate containing substrates. The authors argued that the high glucosinolates in the forage did not interrupt the fermentation and was infact more degradable than low glucosinolate diet. In a growth trial, fresh forage with low and high glucosinolates fed to lambs, and it was found that there was an improved growth performance in high glucosinolate fed lambs than low glucosinolate fed lambs. This experiment suggested that ruminal digestive function may tolerate a high level of dietary glucosinolate (Pearce *et al.* 1989). However this level of glucosinolate may exist where digestive function is compromised without noticeable toxicity symptoms in the animals. Duncan and Milne (1992a), reported the growth of sheep fed with *Brassica* crops was low, though the crops were highly digestible. But the *Brassica* forage seed had an adverse effect on the animals and resulted in low voluntary forage intake, due to the presence of glucosinolate formaing toxic compounds (Duncan and Milne 1992a). Voluntary food intake (VFI) was depressed by the administration of allyl cyanide (ACN), but the blood gluthathione concentration and plasma urea were not affected, while the plasma creatinine varied in the concentration depending on the treatment (Duncan and Milne 1992a). The treatment also affected the concentration of plasma gamma-glutamyltrans-peptdase. The kidney cytochrome oxidase activity, a terminal enzyme involved in the electron transport chain, was not affected (Duncan and Milne 1992a).

In a subsequent experiment Duncan and Milne (1992a) investigated the rumen microbial degradation of ACN in sheep fed with chopped cabbage, *Brassica oleraccea va capitata* and dried grass pellets. Rumen fluid analysis suggested that in animals fed with grass pellets, the ACN was stable, in contrast to rapid decline in ACN concentration in animals fed with cabbage.

Indolyl glucosinolates may inhibit the neoplastic effects of carcinogens. Besides the anti-carcinogenic effects of indole-3-acetonitile, the compound is reported to be a precursor of N-nitroso compound, which have been identified from nitrile treated Chinese cabbage. Although indole-3-acetonitrile is reported to be anti-carcinogenic, the nitrosation seemed to initiate and promote tumour in rats, however the effects of such compounds in

man are not known (Tiedink *et al.* 1991). These authors also reported that indole compounds are mutagenic to bacteria, after the nitrite treatment and its rapid reaction endogenously.

The plants mentioned in the introduction can cause haematuria in dairy cattle, particularly by the poisonous AITC (Albert 1987), though not many animal deaths have been reported. Death may occur due to exhaustion, resulting from intensive irritation of the alimentary tract (Clarke and Clarke 1975).

Brassica species such as *Sisymbrium irio* present in United Kingdom (UK) do not seem to poison the chickens, pigs, cattle, sheep and horses (Clarke and Clarke 1975). However, cattle in Argentina have died of gastroenteritis after the consumption of *Sisymbrium irio*. In France and Western Australia, fatal poisoning in lambs and cattle have occurred because animals were allowed to grazed on the field covered with wild radish, in which the flowers were in advanced stage. In America, the adverse effect of ingesting *Descurainia pinata* had caused blindness and paralysis of tongue (Clarke and Clarke 1975). Also the inclusion of rapeseed meal in the diet of growing chickens and pigs have reduced or inhibited the growth rate and increased the weight of liver, kidney and thyroid gland (Wight *et al.* 1987).

The thyroid gland problem can be caused by the thiocyanate ion, which is a goitrogen causing hypothyroidism. Hypothyroidism occurs as a result of hyperplasia, prevents the thyroid gland from taking up the iodide ion and inhibits the iodination of tyrosine (McDonald *et al.* 1992), the precursor of thyroxin, to produce thyroid hormones. Most goitrogenic effects have been experimented on rabbits, but goitre is noticeable in human as "big neck" (Dierschke 1980; Robinson 1980; Albert 1987). The goitrogenic effect in lambs can be prevented by intramuscular injection of iodine (Clarke and Clarke 1975). Supplying of adequate iodine to animal diet which are exposed to some form of iodine deficiency may also prevent goitrogenic effect (McDonald *et al.* 1992). Despite the discovery of goitrogenic activity in cabbage and other plants of the *Brassica* family, the chemical agents responsible for this biological effect have not been pinpointed whether the presence of such an effects exists. It has been proved on rats and guinea pigs that cabbage consumption has a marked goitrogenic potency, through significant increase in the thyroid weight (Langer and Stolc 1965). However the total iodine content in the thyroid was not significantly altered, but the serum protein-bound iodine (PBI) was significantly depressed (Langer and Stolc 1965). Different authors have reported the effect on iodine uptake by the thyroid hormone (Langer and Michajlovskij 1969; Marangos and Hill 1974; Akiba and Matsumoto 1976; Elfving 1980). Attempts also have been made to counter-act the growth depressing effect by processing and the extraction of dietary supplements to reduce the glucosinolate levels.

The use of rapeseed meals, *Brassica napus* for pigs and poultry are restricted in the diet because of the presence of thiocyanate and 5-vinyloxazlidine-2-thione (McDonald *et al.* 1992). In Canada, careful selection of *Brassica campestris* has resulted in obtaining low contents of glucosinolates and erurcic acid, which causes heart lesions in experimental animals. The meals such as Canola produced from this careful selection technique are being used widely (English *et al.* 1988; McDonald *et al.* 1992). The toxicity of glucosinolates can be denatured by cooking or heating, which greatly reduces or inactivates the goitrogenic potency of the plant (Slominski and Campbell 1989; Dietz *et al.* 1991; McDonald *et al.* 1992). However, in autolysis of raw *Brassica* vegetables substantial quantities of thiocyanate ion and related compounds (indolemethanols) can be experienced. It may be said that the anticarcinogenic properties of *Brassica* vegetables depend on the method of preparation (Slominski and Campbell 1989).

Rats dosed with glucosinolates, such as allyl isothiocyanate have been noted to develop vesication and slow healing of ulcers, and the vapours are harmfull to lungs. Ingestion of large doses causes severe gastrointestinal inflamation and my result in circulatory collapse and death (Ahmad *et al.* 1967).

In stomach intubation of 1-cyano-3,4-epithiobutane (CEB), rats showed symptoms of strong vocalization (make noise) to touch, lost right reflex action, and muscle tone. Administration of CEB and 4-pentenenitrile by stomach intubation showed a significant retardation in weight gain (Dietz *et al.* 1991). These symptoms were further examined on histopathological assessment with a mild to moderate periacinar necrosis and congestion in liver. The kidney had tubular degeneration from terminal hypoxia, a result of CEB administration. This investigation confirmed the acute toxicity of epithionitriles. LD50 of 3-hydroxy-4-pentenenitrile administered by stomach intubation lead to a loss of right reflex and intermittent drooling seizers followed by death (Nishie and Daxenbichler 1980).

DETOXIFICATION

The importance of understanding the metabolic fate and potential excretary routes of glucosinolates when ingested through different diets would allow the understanding of the potential of the toxicity in the animal species. However, research in this area has been rather limited and the knowledge of the digestive fate of glucosinolates is very poor. The systemic fate of glucosinolate hydrolysis products show that the chemical nature had helped to provide metabolic routes and detoxification processes.

The metabolic fate of glucosinolate hydrolytic products following absorption are well known, however attempt to detect hydrolytic products of glucosinolates in the blood and tissues of animals fed on rapeseed have been fruitless.

Different species of animals have excreted different levels of mercapturic acids in urine based on the effective detoxification system in an animal (Gorler *et al.* 1982). Glutathione conjugation of isothiocyanates especially conjucgation of benzyl isothiocyante (Bruggemann *et al.* 1986) have been studied *in vitro* experiments and *in vivo* with rats fed on Brussels sprouts (Godlewski *et al* 1985) and cabbage (Stoewsand *et al.* 1986). While the metabloic fate of glucosinolates derived nitriles has the chemical nature of aliphatic nitriles such as acrylonitrile in catabolism had been known due to the nature of compound in the manufacture of plastics (Szabo *et al.* 1977; Langvardt *et al* 1980) and metabolism of similar unsaturated nitriles such as allyal cyanide (Willhite and Smith 1981). The nitrile metabolism experiments in urinary thiocyanate ion excretion has been continuously been studied, especially in the areas of conventional excretion of cyanide as SCN.

The complexity of xenobiotic metabolism has been highlighted following the consumption of the certain cruciferous vegetables, especially the beneficial effect of inhibiting the tumour formation (Wattenberg 1977). Similar situation of interactions in the metabolism of other xenobiotics may exist and have significant relationship to the overall effects of the anti-nutritive factors of the Cruciferae.

ANALYTICAL METHODS

A wide range of analytical methods are being applied to analyse glucosinolate metabolites as a means of screeing glucosinolates hydrolysis. These analytical procedures can quantify both the individual glucosinolates and the total concentration of glucosinolates. The determination of individual glucosinolate is exclusively performed by chromatographic methods and lots of improvements have been made to obtain high resolution and quantification over the years. Early methods included paper chromatography, which identified the thiourea derivatives of isothiocyanates in the chemo-taxonomic studies, but linked into quantitative analysis (Larsen 1981). The quantitative analysis of glucosinolates is performed by using gas liquid chromatography (GLC). Originally this method was used for separating hydrolysis products, after autolysis with exogenous myrosinase (Daxenbichler *et al.* 1970; Daxenbichler *et al.* 1977; Macleod *et al.* 1978). But now, this method is identified to suit the volatile nature of glucosinolate metabolites with high resolution during separation of indicidual glucosinolates (Grob Jr. and Matile 1980b).

The recent and current analyses are concentrated on the identification of parent glucosinolates, either by GLC or high performance liquid chromatography (HPLC). The GLC methods or techniques of glucosinolate separation are based on the derivatization with trimethsilane (TMS) to increase the volatile condition of the metabolites (Thies 1976), and detection level (Heaney and Fenwick 1980a; Heaney and Fenwick 1982). HPLC method is now increasingly used to identify the parent glucosinolates due to the method's simplicity and flexibility (Minchington *et al.* 1982; Spinks *et al.* 1984).

The total glucosinolates analysis is based on the detection of glucose level after the enzymatic breakdown by myrosinase (Joseffeson and Appelquvist 1968). This analytical method is suited to vegetative and seed material, it involves the retention of glucosinolates on an ion exchange resin, followed by washing and on-column hydrolysis (Heaney and Fenwick 1981, Heaney *et al.* 1988, VanEtten *et al.* 1974; VanEtten and Daxenbichler 1977). Another analytical method recently reported is the enzyme-linked-immunosorbent assay (ELISA), which screens a large number of samples very quickly for sinigrin levels, but is not appropriate for the determination of other glucosinolates or their metabolites (Hassan *et al.* 1988).

CONCLUSION

The physiological effects in animals consuming Cruciferae plants containing glucosinlates are influenced by many factors. These give rise to constraint effects on biosynthesis in the plant and to the efficiency of glucosinolate catabolism in the metabolic system of an animal. The studies have centred mainly on the aspects of glucosinolate

chemistry, particularly the enzymic hydrolysis and give rise to the identification of metabolites. Studies have not established the gross effect of glucosinolates in animals. The adaptative significance of the structural diversity of glucosinolates and their relative effects of different glucosinolates are not known at the moment.

This review is mainly on the potential effect of different glucosinolates, especially on the limited number of breakdown products in number of animal species which have been studied. Because of the importance of the physiological effects of glucosinolates in the animal, it is a significant area of research for the agriculture and livestock sector.

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kg - kilogram
t - tonne
l - litre
ml - millilitre
ha - hectare
mm - millimetre
cm - centimeter
M - metre
a.s.l. - above sea level
yr - year
wk - week
h - hour
min - minute
s - second
k - kina
n.a. - not applicable or not available
n.r. - not recorded
var - variance
s.d. - standard deviation
s.e.m. - standard error of difference
d.f. - degrees of freedom

Levels of significance

n.s - not significant
* - $0.01 \leq p < 0.05$
** - $0.001 \leq p < 0.01$
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