BIOSECURITY RESEARCH IN PNG: 2015–2017

By

RC MAGAREY[1], KS BRAITHWAITE[2], LS KUNIATA[3], NP THOMPSON[2], K KOROWI[3], PR SAMSON[2], L TOM[3], N SALLAM[4], L DERBY[5]

[1]*SRA, Tully.* [2]*SRA, Indooroopilly.* [3]*RAIL, Gusap, PNG. Formerly SRA, Meringa.* [5]*SRA, Meringa*

rmagarey@sugarresearch.com.au

KEYWORDS: PNG, Downy Mildew, Moth Borers, Ramu Stunt, Resistance, Diagnostics.

Abstract

PAPUA NEW GUINEA is the centre of diversity for several species in the genus *Saccharum*, including *S. officinarum*, selections of which constituted the first commercial sugarcane varieties in Australia. Apart from providing germplasm for commercial sugarcane production world-wide, PNG is also home to pests and diseases that pose a unique and serious threat to commercial sugarcane production in Australia. These include members of the noctuid moth borer group, an oomycete causing downy mildew and the viral disease, Ramu stunt. Australian scientists have been working alongside PNG counterparts to develop management strategies that will assist with pest and disease management in PNG and enable effective preparation for a possible incursion into Australia. Over the past three years, significant outputs from the research have included a much better understanding of causal agents, specific diagnostic tests, an understanding of pest and disease distribution and faster methods for varietal resistance screening.

Introduction

Effective quarantine procedures have kept some of the worst sugarcane pests and diseases out of Australia, leading to more efficient commercial sugarcane production, reduced input costs for farmers, better utilisation of genetic resources and fewer pest and disease epidemics within the Australian industry. Financial crises associated with major epidemics have thus been minimised. However, there remains an ongoing threat from exotic pests and diseases, including sugarcane streak mosaic virus in Indonesia, phytoplasma diseases (for example, white leaf in SE Asia) and a range of other moth borers present in almost every other sugarcane industry

Some of the most imminent threats are located in PNG, to the near-north of Australia. Some of these have devastated the sole PNG commercial sugarcane estate located at Gusap, Ramu Valley, Madang Province.

With this in mind, Australian scientists have worked together with their PNG counterparts to improve IPM management strategies and so prepare the Australian sugarcane industry for a possible incursion (Kuniata *et al.*, 2010a, b; Magarey *et al.*, 2012). In all, eight years of research was undertaken in several projects addressing high-priority pest and disease issues. In this paper we consider the research that has been undertaken over the last three years.

PNG pests and diseases

Sesamia grisescens (Ramu stalk borer)

Sesamia grisescens is the most important moth borer species in commercial crops in PNG (Korowi *et al.*, 2011; Samson *et al.*, 2017). Significant research on *S. grisescens* has been undertaken in the past (Samson *et al.*, 2017) and only a brief description of the borer is included here. The small adult moth lays numerous eggs beneath the leaf sheath of healthy leaves; these hatch as first instar larvae that feed underneath the leaf sheath and then migrate into the apex of the

cane plant. Second instar larvae migrate from the original stalk to surrounding uninfested stalks and burrow into the top of the shoot destroying sugar storage tissue. Samson *et al.*, (2017) have reported on the presence of resistance among a group of Australian commercial varieties.

***Scirpophaga excerptalis* (top shoot borer)**

S. excerptalis has potentially devastating effects on commercial crops. The borer is also present in other parts of SE Asia causing significant yield losses. *S. excerptalis* also lays its eggs on the young leaves; the first instar larvae then feed on leaf blade tissue before penetrating the leaf mid-rib and tunnelling into the shoot apex. The larvae kill the meristem, leading to shoot tip death, reduced yields and, at times, crop devastation. Korowi *et al.* (2011), Korowi and Samson (2013) and Samson *et al.* (2017) also reported field varietal resistance to *S. excerptalis*. Knowledge gaps include a satisfactory rapid resistance screen.

***Chilo terrenellus* (Chilo stem borer)**

C. terrenellus is a borer of generally lesser significance in PNG, but can never-the-less cause significant crop losses. The adult moth deposits eggs in the unfolded spindle leaves, with the young larvae tunnelling into the semi-mature and mature internodes. Field resistance screening trials suggest there is a low level of resistance to this borer among PNG and Australian varieties (Korowi and Samson, 2013; Samson *et al.*, 2017).

Downy mildew (DM, caused by *Peronosclerospora* spp)

DM was once an endemic Australian disease but has since been eradicated, with the last observation in a commercial crop in 1957 (Leu and Egan, 1989). DM is now restricted to the western rim of the Pacific and Fiji (Leu and Egan, 1989; Suma and Magarey, 2000). The pathogen spreads through its conidial (asexual) spore stage, infecting buds in standing cane or very young cane plants. The organism is systemic and quickly invades the stalk tissues, leading to leaf striping, leaf splitting, poor stalk growth and poor ratooning.

Pesticides (for example metalaxyl) are known to eliminate the disease when applied to either planting material or soil at an appropriate time (James 1982; Malein 1993), but varietal resistance is the most sustainable and cheapest option. Knowledge gaps include the extent of pathogen variation, (Magarey *et al.*, 2012), specific diagnostic assays, variation in disease resistance caused by pathogen variation and a rapid resistance screening technique.

Ramu stunt

Initial aetiology research in the late 1990s attributed the disease to a phytoplasma (Cronje *et al.*, 1999; Suma and Jones, 2000). Recent research and several lines of evidence suggest otherwise (Braithwaite *et al.*, 2012; Magarey *et al.*, 2012; Braithwaite *et al.*, 2014), with evidence that the disease is caused by a unique tenuivirus.

Ramu stunt almost brought financial ruin to Ramu Sugar when the major commercial variety, Ragnar, rapidly succumbed to the disease in 1985–86. Research in the 1990s suggested that the Delphacid planthopper, *Eumetopina flavipes*, is a vector of the disease (Kuniata *et al.*, 1994) and that varietal resistance exists in commercial germplasm (Kuniata *et al.*, 2010b). Knowledge gaps with Ramu stunt include proof of pathogen identity and pathogen variation around PNG.

Methods

***Scirpophaga excerptalis* (top shoot borer) rapid resistance screen**

Short-term resistance screening is likely to work best under controlled conditions with precise infestation levels. For this reason a short-term shadehouse test was devised and the first *Scirpophaga excerptalis* pot trial was established on 30 March 2016. The aims were to: i. assess the tolerance/resistance of six varieties to *Scirpophaga excerptalis*, and ii. confirm that larval dispersal to adjacent plants, after hand placement on selected stalks, provides for a reliable resistance assessment.

The trial consisted of 16 replicate clusters, with each cluster consisting of a central pot surrounded by six peripheral pots. The standard variety (RQ117) was planted in the central pot and in one peripheral pot, and the remaining five peripheral pots were planted with Q219$^{\phi}$, Q235$^{\phi}$, Q231$^{\phi}$, Q208$^{\phi}$ and Q135. All peripheral pots were distributed randomly around the central pot (Figure 1). Five stalks were maintained in each pot (Figure 2).

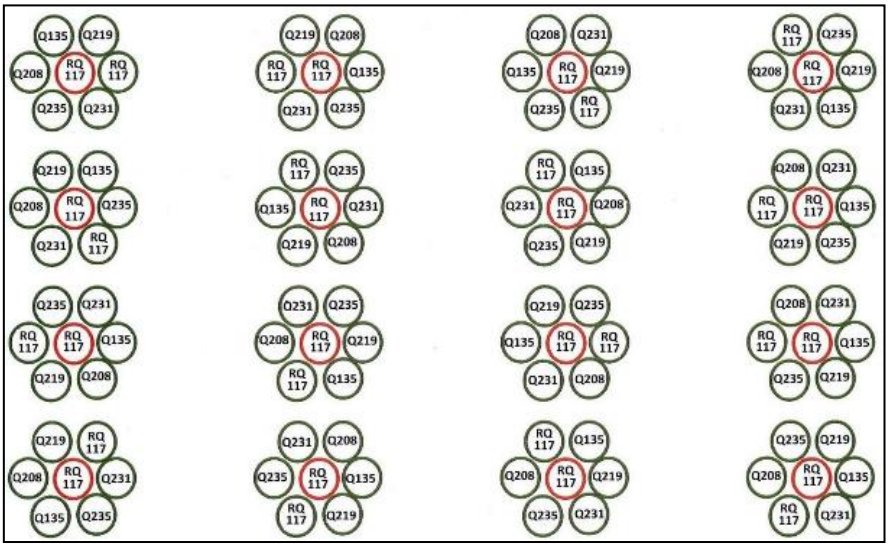

Fig. 1—Lay-out of the *Scirpophaga* pot trial in the white house at Ramu.

Fig. 2—*Scirpophaga* pot trial in the white house at Ramu (June 2016).

Plants in all central pots were infested 6–22 August 2016, by stapling two top borer egg batches onto the underside of two randomly chosen leaves. Plants were given an extra dose of phosphorous and iron fertilisers when significant signs of chlorosis were noticed in several pots. The symptoms subsequently disappeared.

The trial was destructively sampled on 10 October 2016, when plants were about six months old. Number of dead hearts, tunnel length (cm) and weights (g) of the recovered immature stages were recorded for all infested stalks, in all replicates. Data were analysed by a biometrician from the Department of Agriculture and Fisheries (QDAF). Additional analyses were required to account for infestations by other arthropods.

Replicates where the central pot had no measure of infestation were deleted. A generalised linear mixed model (GLMM) was fitted to the dead heart bernoulli (0, 1) data and the logit function was applied as the canonical link for this model. Varieties were fitted as fixed effects, to test for differences among the six varieties. Three factors were fitted as random terms to index the RCBD of the experiment and the sampling of stalks within pots. One factor indexed replicate blocks, one indexed pots within replicate blocks and one indexed the observational units of stalks within pots.

To fit these data to the GLMM, we assumed the responses from the individual stalk to be independently and identically distributed. Because of this assumption, the scale parameter was fixed to be 1.

Larval weight (g) and tunnel length (cm) can only be measured on stalks where a dead heart is recorded. When the stalk did not result in a dead heart the response was recorded as zero. In this experiment, this resulted in a data set with many zeros. A two-stage approach was used to analyse these data.

The first stage analysed the dead heart (0, 1) data using the GLMM method described for the dead heart variable above. The second stage modelled the dead heart non-zero data as a continuous variable using a linear mixed model (LMM) where variety was fitted as a fixed effect and replicate block, pots within replicate blocks and stalks within pots were fitted as random effects. The two model outcomes were then combined to provide an overall severity measure (larval weight and tunnel length) adjusted for the incidence of the infestation.

This follows the conditional model method of Fletcher *et al*. (2005), where the *overall severity = the incidence of dead hearts × the severity conditioned on the responses from the dead heart.*

In addition to the two-stage approach, a separate analysis method was applied to the two severity measures (larval weight, g; and tunnel length, cm). This method simultaneously modeled the zero and non-zero data to a negative binomial distribution using a log link in a GLMM framework. Variety was fitted as a fixed effect and replicate block, pot within replicate block and stalk within pot were included as random terms. All data were analysed using ASReml-R (Butler *et al.,* 2009) using R software. All significance tests are provided at the 5% level.

Downy mildew (DM) rapid resistance screening

Previous research (Kuniata *et al.*, 2010a) has shown that Australian germplasm has a significant component of DM susceptibility in clones and varieties with around 50% being susceptible. This is a far greater proportion than in material from some other breeding programs (Kuniata *et al.*, 2010a).

Published research from south-east Asia showed that *Peronosclerospora* oospores may lead to DM expression in inoculated cane (Matsumoto 1961; Matsumoto *et al.*, 1961). Abundant oospores are produced in shredded leaf material; DM causes the leaf blade to become a tangled array of fibrous material with abundant oospores attached. Such material was collected, cut into pieces and used as an inoculum source (termed ‘leaf shred’).

Research conducted at Ramu has shown this material can infect single-eye setts, when the inoculum is mixed into soils used to fill the pots (Magarey *et al.*, 2012). The research reported here aimed to improve our knowledge of test reliability, as well as the ability to discriminate resistant from susceptible varieties. Several experiments were conducted over the course of the project, using DM leaf shred material as the inoculum source.

General method: The variety most prone to leaf shredding is Q136 (leaf shred is not seen in all varieties) and so affected leaves in this variety were sought as inoculum for all experiments. Shredded leaves were collected, taken to the pathology laboratory where the leaf mid-ribs were removed and the leaf blade material cut into sections 5 mm or less. Fresh weight of the material was recorded.

Inoculation: Forestry nursery soil (sandy loam) was obtained, weighed and specific quantities of the leaf-shred material mixed into the nursery soil to create specific inoculum levels. Mixing was thorough in order to ensure an even inoculum distribution.

Single-eye, disease-free setts of selected varieties were obtained and placed 3–5 cm below the soil surface, (in close contact with the infested soil), to provide opportunity for oospore infection.

Small polythene bags or peat pots were used to contain the soil (Figure 3) with the pots placed in the shade at ambient temperature immediately after planting. After 1–2 weeks the pots were shifted to a nursery bench to facilitate further cane growth.

Fig. 3—Close up view of a soil inoculated with the leaf shred material.

No insecticides were applied during the course of these experiments; however initial fertilising and regular watering were essential. DM incidence was the critical disease information recorded. This was assessed by examining leaves for the typical systemic leaf streaks that characterise DM. Multiple test plants were included in each replicate.

Experiment 1: DM infection using leaf shred material (January–March 2016)

The first experiment focused on confirming that leaf shred material can lead to DM-diseased plants. In September 2015, leaf-shred was collected from a commercial crop of Q136 by Ramu staff. The leaf material was cut into small pieces, dried and securely stored in the laboratory. With other oomycete species, oospores provide a long-term survival structure and so the long-term viability of stored material was considered likely. The experiment was initiated in January 2016; fresh leaf shred material was located at the time in a commercial crop of Q136. Leaf shred was added to, and mixed in with, the pot soil and disease levels compared between stored and fresh inoculum material.

In mid-January 2016, planting material was sourced from a project field trial site (Estate block B North). The site supported a number of Australian commercial varieties of known DM resistance. A susceptible variety ($Q221^{(\phi)}$) was found in reasonable quantity and condition, with sufficient healthy stalks to cut the required quantities of single-eye setts.

Treatments: the following treatments were established: i. control – no DM inoculum; ii. DM – stored leaf shred material, and iii. DM – fresh leaf shred material. A completely randomised design was employed with 20 plants per treatment.

Experiment 2: Identifying variation in disease expression associated with resistance.

The reaction of varieties in the pot screening technique needs to reproduce the reaction of varieties in commercial crops, so three varieties of differing resistance were included in this experiment.

Planting material was sourced from the isolated Leron quarantine plot, thus eliminating any risk of prior disease infection. Three varieties were used – two susceptible and one intermediate-resistant. These were sourced from Leron on 21 December 2016; the cane was collected in stalk lengths and cut into single-eye setts at Gusap.

After soil infestation with the required leaf shred material, the peat pots were one-third-filled with *Peronosclerospora* (DM) infested soil, and single-eye setts of each variety placed on top. The bags were then filled with the remaining infested soil and placed alongside (but not immediately adjacent) to the uninfested plants, in a shaded position. Inoculum was added at 13.2 g/pot, equivalent to 0.066 g material/g soil. A sample of the inoculum was selected and viewed under a microscope. Abundant oospores were attached to the split leaves.

The applied treatments were: i. MQ239$^{\Phi}$ resistant, uninoculated, ii. MQ239$^{\Phi}$ resistant, DM leaf shred, iii. Q221$^{\Phi}$ susceptible, uninoculated, iv. Q221$^{\Phi}$ susceptible, inoculated, v. Q200$^{\Phi}$ susceptible, uninoculated, and vi. Q200$^{\Phi}$ susceptible, inoculated. There were 10 replicates of each treatment. Test plants were monitored weekly both for germination and DM symptom expression. Inspections continued for approximately five weeks, until no further disease expression was noted.

Peronosclerospora (DM) pathogen variation

Research focused on survey material gathered primarily during the project period (2015–2017). Leaves expressing DM symptoms were collected from a range of *Saccharum* species, including commercial sugarcane and a close relative (*S. officinarum*, *S. robustum*) and other host species (including *Miscanthus*, corn and sorghum) during a survey of Ramu and surrounding areas in the Eastern Highlands/Morobe Province (Table 1).

Dr Roger Shivas (taxonomist) and Dr Malcolm Ryley (experienced with DM in cereals) were participants on the survey and provided advice with regard to sample preparation and species identification. In addition, all samples from the survey were sent to the Queensland Herbarium for storage and analysis.

Table 1—Specimens collected during survey of Ramu plantation (samples BRIP65983-BRIP65995 and BRIP66004-66005); Eastern Highlands (BRIP65996-66003) and Ramu valley to Madang Province (BRIP66006-66011). The SRA accession and BRIP codes are shown.

BRIP	Host	ColCollectionDate	SRA Accession	LocPreciseLocation
65983	Sugarcane	1/11/2016	A1621-01A	FN 306
65984	Sugarcane	1/11/2016	A1621-02A	FN 305
65985	Sugarcane	1/11/2016	A1621-03A	BN 101
65986	Sugarcane	1/11/2016	A1621-04A	BN 401
65987	Sugarcane	1/11/2016	A1621-05A	Ramu office prop plot
65988	Sugarcane	1/11/2016	A1621-06A	Ramu office
65989	Maize	1/11/2016	A1621-07A	E South Corn plot
65990	Sorghum	1/11/2016	A1621-08A	GN
65991	Sugarcane	1/11/2016	A1621-09A	BN 101
65992	Sugarcane	1/11/2016	A1621-10A	BN 401
65993	Maize	1/11/2016	A1621-11A	E South Corn plot
65994	Maize	1/11/2016	A1621-12A	E South Corn plot
65995	Sugarcane	1/11/2016	A1621-13A	BN 401
65996	Coix sp.	2/11/2016	A1621-14A	O'ekara
65997	Maize	2/11/2016	A1621-15A	O'ekara
65998	Sugarcane	2/11/2016	A1621 16A	O'ekara
65999	Maize	2/11/2016	A1621-17A	O'ekara
66000	Maize	2/11/2016	A1621-18A	O'ekara
66001	*S.robustum*	2/11/2016	A1621-19A	Norekora
66002	Miscanthus	2/11/2016	A1621 20A	Between Yonki and Kainantu
66003	Miscanthus	2/11/2016	A1621-20C	Between Yonki and Kainantu
66004	Sugarcane	3/11/2016	A1621-21A	BS 104
66005	Sugarcane	3/11/2016	A1621-22A	Asas
66006	Wild sorghum growing within clump of *S robustum*	3/11/2016	A1621-23A	River site from 2009
66007	*S.robustum*	3/11/2016	A1621-24A	Walium bridge
66008	Maize	3/11/2016	A1621-25A	Popoeta
66009	*S.robustum*	3/11/2016	A1621-26A	Digicell tower on road towards Madang
66010	*S.robustum*	3/11/2016	A1621-27A	River site from 2009
66011	*S.robustum*	3/11/2016	A1621-28A	Walium bridge

Microscopic analysis

Table 2 shows the size characteristics for oospores and conidia for described species of *Peronosclerospora* spp. infecting *Saccharum* and other grasses. Light microscopy was used to examine oospores in leaf shredding survey samples.

Table 2—Distinguishing features of *Peronosclerospora* species, as defined in Sivanesan and Waller, 1986.

Species	Distribution	Host	Symptom	Conidia	Oospores
P. sacchari	Australia*, Fiji, India, Indonesia, Japan, PNG, Philippines, Taiwan, Thailand	*Saccharum, Zea*	Leaf streaking and shredding	25–55 x 15–25 µm	50 µm with wall 3.5–5 µM thick
P. philippinensis	India, Philippines	*Saccharum, Euchlaena, Sorghum, Zea*	Leaf streaking	27–39 x 17–21 µm	15.5–22.5 x 2–4 µm
P. miscanthi	Fiji, Philippines, Taiwan, possibly PNG	*Saccharum, Miscanthus*	Leaf shredding	37.0–48.5 x 14–30 µm	32.5–56.5 µm diameter
P. spontanei	Thailand, Philippines	*Saccharum, Zea, Euchlaena, Miscanthus*	Leaf streaking	39–45 x 15–17 µm	Similar to *P. sacchari*

Molecular analyses

Specimens collected during a 2016 survey were submitted to the Queensland Plant Pathology Herbarium and sequenced. Molecular analyses were undertaken using Cox2-F and Cox2-RC4 primers (Choi *et al.*, 2015). All DNA analysis was undertaken using the Geneious Version 11.0.2 (http:www.geneious.com, Kearse *et al.*, 2012)

Alignment of the sequences with *Peronosclerospora* voucher specimens published in GenBank was undertaken using the ClustalX algorithm using default parameters. The Cox1 primers are in the Cox-1 and Cox1-2 spacer region, and are able to pick up the more subtle differences between the grass-infecting *Peronosclerospora*.

Ramu stunt

To assess Ramu stunt incidence and potential variation around PNG, diseased material was accessed from specific surveys (Braithwaite *et al.*, 2014). A stunt survey was conducted during this project; archived material was also accessed from previous surveys conducted in 2001 and in the period 2009–2012.

Specimens from garden canes (*S. officinarum, S edule*), wild canes (*S. spontaneum, S. robustum*) or weeds showing suspect stunt symptoms were sourced and screened with the diagnostic test. If positive, the entire 1.2 kb RNA 6 genome fragment (based on the naming system of Mollov *et al.*, 2016) of the sample was sequenced for phylogenetic comparisons.

Results

Scirpophaga excerptalis rapid resistance screen:

The effect of variety on percentage of dead heart (Table 3) was not significant (p=0.056). However, since the test is close to significance, an unprotected least significance difference (lsd) comparison is provided to indicate the trend in the separation of the means (Table 4).

Table 3—Number of dead heads present across the 11 replicates by 5 stalks (total n=55).

Variety	Q135	Q208$^{\phi}$	Q219$^{\phi}$	Q231$^{\phi}$	Q235$^{\phi}$	RQ117
Number of dead hearts	1	3	16	6	12	12

Results from the analysis of larval weight and tunnel length conditional on the dead heart indicated a significant effect of variety (p=0.022) and (p=0.017) respectively.

Analysed means and standard errors are provided in Table 4 with letters indicating varieties that are significantly different. This showed Q135 had a lower percentage of dead hearts compared with Q235$^{\phi}$, Q219$^{\phi}$ and the standard RQ117. No varieties showed significantly lower severity measurements than RQ117. Q235$^{\phi}$ had significantly higher severity measurements than RQ117.

Table 4—Analysed means and standard errors from GLMM analysis of dead hearts (back-transformed) and LMM analysis of larval weight and tunnel length. Letters indicate treatments that are significantly different at the 5% level.

	Larval weight (g) (LMM)		Larval weight (g) (LMM)		Tunnel length (cm) (LMM)		Overall severity (Two-stage result)	
Variety	Back transformed mean (%)	Letters	Mean	Letters	Mean	Letters	Larval (g)	Tunnel (cm)
Q135	1.7	c	0.25	ab	28.43	abc	0.004	0.48
Q208$^{\phi}$	5	bc	0.15	abc	28.31	ab	0.007	1.40
Q219$^{\phi}$	27	a	0.16	ab	26.33	b	0.043	7.11
Q231$^{\phi}$	8.4	abc	0.07	c	26.16	abc	0.006	2.19
Q235$^{\phi}$	18	ab	0.19	a	32.7	a	0.034	5.88
RQ117	19.8	ab	0.12	bc	20.86	c	0.024	4.14

The overall severity results are the severity predictions adjusted for the incidence of dead hearts, following the method of Fletcher *et al.* (2005). For instance, Q135 was heavily down weighted because it had a low incidence of dead hearts (therefore less overall severity damage). Trial sampling revealed a lack of infestation in several replicates, with five visible clusters of infestation.

This was unexpected since active dispersal and establishment of neonate *S. excerptalis* larvae has been demonstrated in previous trials. Lack of infestation made it difficult to interpret the results.

Overall, there was a noticeable effect of variety on dead heart symptoms, tunnel length and larval weights, with Q135 being the least affected and Q219$^{\phi}$ being the most affected varieties. This is consistent with results obtained during previous Ramu research. Another variety that demonstrated low damage levels was Q208$^{\phi}$, while Q235$^{\phi}$ appeared to be more susceptible to *S. excerptalis* damage.

Downy mildew rapid resistance screen

Experiment 1: Test plants were monitored over an extensive time period, but no DM was observed in any test plants.

Experiment 2: There was good symptom expression in plants where soils had been inoculated with leaf shred material. Substantial disease development occurred after 5–6 weeks growth of the test plants (Figure 4).

Downy mildew pathogen variation

A number of DM specimens were collected from various *Saccharum* spp specimens during the DM survey (Figure 5).

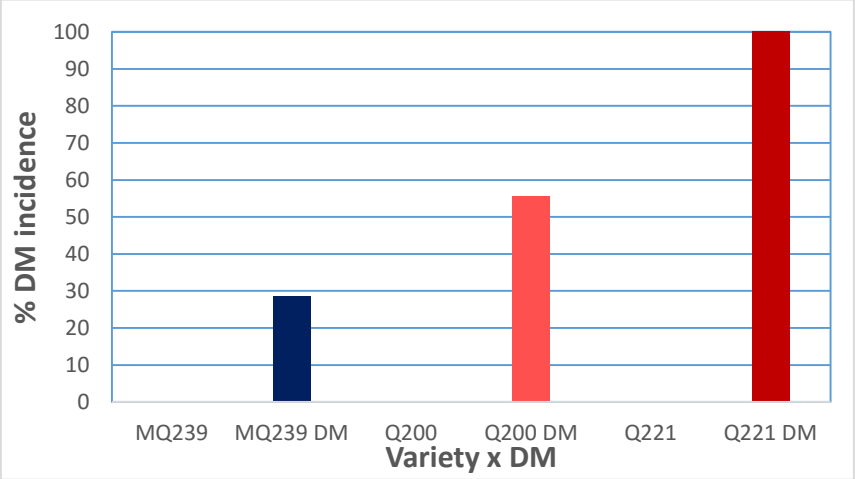

Fig. 4—DM incidence in varieties inoculated with leaf shred material (vs uninoculated) five weeks after trial establishment at Gusap, PNG.

Fig. 5—Dr Mal Ryley and Dr Roger Shivas collecting leaf shredding on *Saccharum robustum* from the Eastern Highlands province.

Morphology – oospores

All oospores measured ~50 µm, regardless of their collection location or host species. This suggests that there is no microscopic evidence for the presence of *P. philippinensis* in PNG; the oospores of this species are smaller. Morphological observations did not enable differentiation of other *Peronosclerospora* species, since oospores of many others are around 50 µm (Figure 6).

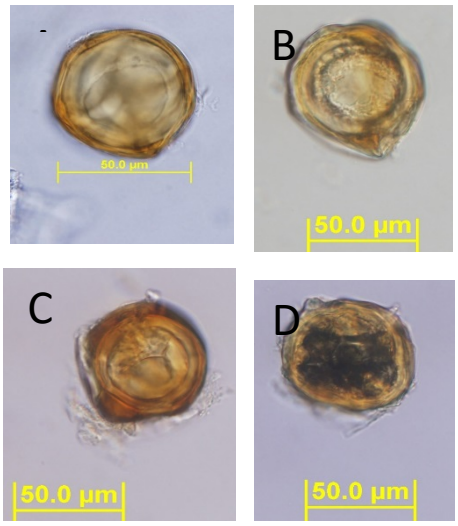

Fig. 6—Oospores from specimens collected during project surveys. A: from Q136, Gusap estate; B: from *Miscanthus,* Eastern highlands; C: from *S. robustum*, Eastern highlands; D: from *S robustum*, Madang Province/Lowlands.

Morphology – conidia/conidiophores

Examination of the conidiophores also was not conclusive, as species variation in these structures was not clearly visible in the specimens examined. It should be noted, that only specimens that produce down can be examined for conidia, so this fact limited morphological studies in a number of wild host specimens.

Molecular studies

Data from molecular studies on the collected specimens suggest the following:

i. *Miscanthus highlands DM*: that the species causing leaf splitting in this host is not *P. miscanthi* and is likely to be a new species of *Peronosclerospora*. The status of the described *P. miscanthi* is not clear – whether it is present in PNG or not. The undescribed species is clearly different to other *Peronosclerospora* specimens collected on the survey;

ii. *Saccharum robustum DM*: the species causing leaf splitting in specimens collected on the lowlands in *S. robustum* is also likely to be an undescribed species;

iii. *DM in commercial cane and other hosts, Ramu Valley*: the molecular data suggest that in addition to the other two species, there is also a *Peronosclerospora* species complex present in the Ramu Valley – an, as yet, undefined group of closely aligned species causing DM in commercial sugarcane and related species.

It is likely that *Peronosclerospora sacchari* is one species within this species group; there is likely to be at least one other as yet undescribed species within this species complex. Further research is needed to clarify the taxonomy/biology of *Peronosclerospora* species within PNG. Molecular data and morphological studies suggest it is unlikely that *P. philippinensis* is present in PNG.

There appear to be at least two species causing DM on commercial crops at Gusap: one causing leaf shredding in Q136 and one (or more) causing the down symptoms. DNA sequence analysis strongly suggests that these are distinct species. Q136 displays leaf shredding symptoms, which indicates that the DM pathogen infecting this variety may have originated on this particular germplasm.

Ramu stunt pathogen variation

Survey data suggest the disease is present in other canes away from the Ramu commercial estate but that Ramu stunt is not common. Ramu stunt infected noble canes have been detected in the Ramu Valley and Alotau region, but not in the Goroka or Madang regions. Variation was detected between isolates with a link between location and host (molecular data are not reported here). Isolates from noble canes in gardens in the Ramu Valley were slightly different from the isolates from commercial canes on the Ramu estate, while isolates from Alotau in the south of PNG were found to be genetically very different from the isolates collected from Gusap. No Ramu stunt has been detected so far in *S. edule*, blady grass, elephant grass, guinea grass or itch grass.

Discussion

BSES/SRA has had a long association with the PNG sugarcane industry (more than 35 years). Over this time there has been very significant progress in understanding the nature and effects of the different PNG pests and diseases. Ramu staff have conducted important research, particularly on the major pests present in PNG.

Research on *Sesamia grisescens*, *Chilo terrenellus* and *Scirpophaga excerptalis* has included work on the etiology of the pests, management strategies including targeted insecticide applications, the use of pheromones to determine the timing of adult moth flights and research on resistance of commercial varieties (Kuniata 2017).

Recent research has built on this foundation, addressing significant areas where knowledge was lacking. The aim has been to develop better-targeted IPM applications (addressing key aspects related to the causal agents), and/or biosecurity issues related to the Australia sugarcane industry.

Rapid moth borer resistance screening research has provided some confidence that the larval dispersal technique is a sound method for assessing resistance. However, strict measures are needed to ensure the viability of the eggs used to infest plants and that the potted plants remain free of any other arthropods that may hinder borer establishment – the latter may be quite important. Moth borers pose the most serious pest threat to the Australian sugarcane industry; representative pests cause very serious losses and major issues for sugarcane industries around the world.

Previous research (Samson *et al.*, 2017) focused on assessing the resistance of Australian varieties in field trials. Significant resistance was found in Australian and overseas varieties to both *S. grisescens* and *Scirpophaga excerptalis*, with lesser resistance evident to *Chilo terrenellus*. In an effort to develop faster, more efficient screening, the short-term shadehouse technique was trialed with *S. excerptalis*; the method shows promise, but further investigation is necessary if it is to become a routine screening test.

Joint research with PNG scientists in this project did not touch on other aspects of an IPM strategy. It is highly unlikely that varietal resistance alone would provide an effective moth borer management strategy and other components will be needed. PNG research led by local scientists has identified a combination of management measures that will be needed if a moth borer incursion was to occur in Australia.

These include pheromone detection, strategic insecticide applications, biological controls (parasitoids) and several cultural controls. Some refinement of these measures to adapt the management to the Australian sugar industry will most likely be needed if yield loss is to be kept at sub-economic levels.

Research into the causal agents of Ramu stunt and downy mildew have clearly shown significant variation in both pathogens, particularly so with *Peronosclerospora* species.

Ramu stunt research not only confirmed that the disease is caused by a tenuivirus, (Braithwaite *et al.*, 2014) but that significant variation in the virus exists in cane from commercial crops at Gusap, Ramu Valley compared with Alotau in the Milne Bay region. This is significant in terms of pathogen detection as well as in potential consequences related to varietal susceptibility.

Pathogen variation may mean that some resistant varieties may show a significant level of susceptibility if a second variant of the pathogen was to become prevalent. The current project did not provide for time nor resources to test how pathogen variation will affect disease incidence or symptom expression; this remains a research need. Our knowledge of the pathogen has been significantly strengthened by the research reported here.

Downy mildew is caused by species of *Peronosclerospora*; previous texts (Leu and Egan, 1989; Suma and Magarey, 2000) state the species causing the sugarcane disease worldwide are *P. sacchari* (Pacific Rim; PNG) and *P. philippinensis* (Philippines).

There has never previously been a detailed molecular study of the variation in *Peronosclerospora* species in PNG before the current project began; research reported here has shown the situation to be much more complex and that there is very significant variation in *Peronosclerospora* species infecting *Saccharum* and *Miscanthus* species.

Discussions with Dr Marco Thines suggests that oospores are generally only produced on the primary/natural host, i.e. it is host-species specific (M Thines, pers comm). The non-primary host reaction is the formation of asexual conidiophores and conidia. This lends weight to the theory of multiple species being present in PNG, as leaf shredding was observed on a wide range of hosts, except for corn.

Further discussion with Dr Shivas, Dr Ryley and Dr Marco Thines suggested that the *Peronosclerospora* that infect graminicolus plants in PNG are not well described, and that molecular DNA sequence analysis is most likely the best way of determining the species relationships in PNG. It is envisaged that perhaps three new species will need describing and that *P. sacchari* (originally described from maize (*Zea mays*) may need re-describing.

The variation in *Peronosclerospora* clearly requires further taxonomic research as well as pathogenicity investigations to determine which species infect which hosts.

There will also be a need to determine how specific *Peronosclerospora* assays are in relation to these species and which species are the most important to target in relation to the Australian sugarcane industry.

DM field resistance screening trials take at least 18 months from planting to final disease assessments (Kuniata *et al.*, 2010a). Rapid resistance screening research suggests that a method for speeding this process could be developed from oospore inoculation of potting soil. The infesting of potting soils with leaf shred material (containing oospores) and the subsequent planting of single-eye setts led to high levels of disease in susceptible plants in some trials. The most susceptible variety (Q221$^{\phi}$) exhibited 100% infection.

This is important for any resistance screening technique – that susceptible varieties show high disease levels. The variety Q200$^{\phi}$ is also susceptible, but of lower susceptibility compared with Q221$^{\phi}$ (from personal observations); just over 50% DM incidence was recorded in this cane. MQ239$^{\phi}$ is more resistant than either of the other two varieties; <30% disease incidence was recorded in this variety. These results suggest the technique is promising; disease expression follows expectations judging from other data and field observations. The results are also consistent with previous research (Magarey *et al.*., 2012) where very high disease levels were recorded in the most susceptible variety and a moderate amount in canes of intermediate resistance.

In several other experiments, little infection developed. The reason for this requires further investigating; there remains some uncertainty regarding inoculum viability. Further research on *Peronosclerospora* taxa, coupled with a better understanding of the factors leading to leaf shredding (versus more typical leaf striping) symptoms would assist in the development of a routine screening technique. Currently, DM causes a significant loss of imported germplasm through varietal susceptibility and a rapid screening test would enable many more canes to be discarded early in the variety selection program – with better opportunity to select for good agronomic traits.

Knowledge gained from the research undertaken in PNG has been significant and extensive. Resistance data have been added to the SRA database SPIDNet for industry reference. Contingency plans have been updated with new information so that, if an incursion occurred, informed decisions on eradication/management can be made. Significant areas for future research include pathogen variation as it affects disease development and varietal resistance – for both DM and Ramu stunt.

Acknowledgements

The authors would like to thank a range of technical staff at Ramu, Tully and Indooroopilly for assistance with planting field trials, recording results and assisting with shadehouse experiments. This includes Judi Bull, Liz Wilson, Simeon, Tirua, Wamba, Valerencia and Bua. We also thank a number of collaborators including Dr Roger Shivas and Dr Malcolm Ryley. Funding for the Ramu research over an extended period has been provided by SRDC/BSES/SRA and this is gratefully acknowledged.

REFERENCES

Braithwaite KB, Croft BJ, Magarey RC (2007) Progress in identifying the cause of Ramu stunt disease of sugarcane. *Proceedings of the Australian Society of Sugar Cane Technologists*, **29**, 235–241.

Braithwaite KS, Kombukon R, Kuniata LS, Magarey RC (2012) Ramu stunt resistance screening and validation of the diagnostic test. *Proceedings of the Australian Society of Sugar Cane Technologists*, **34** (electronic format).

Braithwaite KS, Kuniata LS, Magarey RC (2014) The importance of disease surveys for understanding quarantine pathogens. *Proceedings of the Australian Society of Sugar Cane Technologists*, **36**, (electronic format).

Butler DG, Cullis BR, Gilmour AR, Gogel BJ (2009) ASReml-R reference manual, release 3. *Technical report, Queensland Department of Primary Industries.*

Choi Y J, Beakes G, Glockling S, Kruse J, Nam B, Nigrelli L, Ploch S, Shin HD, Shivas RG, Telle S, Voglmayr H, Thines M (2015) Towards a universal barcode of oomycetes – a comparison of the cox1 and cox2 loci. *Molecular Ecology Resources*, **15**(6)**,** 1275–1288

Cronje CPR, Bailey RA, Jones P, Suma S (1999) The phytoplasma associated with Ramu stunt disease of sugarcane is closely related to the white leaf phytoplasma group. *Plant Disease*, **83**, 588

Fletcher D, MacKenzie D, Villouta E (2005) Modelling skewed data with many zeros: A simple approach combining ordinary and logistic regression. *Environment Ecology Statistics*, **12,** 45.

James G (1982) A note on the efficacy of acylalanine for control of downy mildew. *Sugarcane Pathologists Newsletter,* **29**, 11–13

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organisation and analysis of sequence data. *Bioinformatics*, **28**(12)**,** 1647–1649.

Korowi K, Samson P, Kuniata L (2011) Screening for borer resistance among sugarcane varieties in Papua New Guinea: 2003–2008. *Proceedings of the Australian Society of Sugar Cane Technologists*, **33,** 9 pp.

Korowi K, Samson P (2013) Screening for borer resistance among sugarcane varieties in Papua New Guinea: 2010–2012. *Proceedings of the Australian Society of Sugar Cane Technologists*, **35,** 9 pp.

Kuniata LS (2017) Integration of insecticides in the management of *Sesamia grisescens* Waren (Lepidoptera: Noctuidae) in sugarcane at Ramu, Papua New Guinea. *Proceedings of the Australian Society of Sugar Cane Technologists*, **39,** pp.

Kuniata K, Magarey RC, Rauka GR, Suma S, Bull JI (2010a) Screening for downy mildew resistance at Ramu Agri-Industries, Gusap, PNG 1986–2008. *Proceedings of the Australian Society of Sugar Cane Technologists*, **32,** 301–311.

Kuniata K, Magarey RC, Rauka GR, Suma S, Bull JI (2010b) Screening for Ramu stunt resistance at Ramu Agri-Industries, Gusap, PNG 1986–2008. *Proceedings of the Australian Society of Sugar Cane Technologists*, **32,** 312–321.

Kuniata LS, Young GR, Pais E, Jones P, Nagaraja H (1994) Preliminary observations on *Eumetopina* sp. (Hemiptera: Delphacidae) as a vector of Ramu stunt disease of sugarcane in Papua New Guinea. *Journal of the Australian Entomological Society*, **33**, 185–186.

Leu LS, Egan BT (1989) Downy mildew. In 'Diseases of sugarcane: Major diseases'. (Eds C Ricaud, BT Egan, AG Jr Gillaspie, CG Hughes) pp. 107–121. (ISSCT, Amsterdam).

Magarey RC, Kuniata LS, Samson PR, Korowi K, Braithwaite KS, Thompson NP, Kombukon R, Bull JI (2012) Preparation to enhance Australia's biosecurity: Part 2-Resistance screening and pathogen research at Ramu Agri-Industries, PNG. *Proceedings of the Australian Society of Sugar Cane Technologists*, **34,** 12 pp.

Malein P (1993) Fungicidal control of Peronosclerospora sacchari (T. Miyake) Shirai and K. Hara in sugarcane in Papua New Guinea. *International Journal of Pest Management*, **39**, 325–327.

Matsumoto T (1961) Downy mildew of sugarcane in Taiwan. 1 Germination of oospores derived from shredded leaves of diseased sugarcane. *Report of the Taiwan Sugar Experiment Station*, **24**, 1–5.

Matsumoto T, Chen PC, Yang SM (1961) Downy mildew of sugarcane in Taiwan. III. Leaf splitting and infection by oospores. *Report of the Taiwan Sugar Experiment Station*, **25**, 95–110.

Mollov D, Maroon-Lango C, Kuniata L (2016) Detection by next generation sequencing of a multi-segmented viral genome from sugarcane associated with Ramu stunt disease. *Virus Genes*, **52**, 152–155.

Samson PR, Korowi K, Sallam N (2017) Resistance of sugarcane clones to moth and weevil borers in Papua New Guinea. *Crop Protection*, **96,** 14–21.

Sivanesan A, Waller JM (1986) *PERONOSCLEROSPORA* (Ito) Shirai and K. Hara. In 'Sugarcane diseases' pp 58–61. (CAB International).

Suma S, Jones P (2000) Ramu stunt. In 'A guide to sugarcane diseases'. (Eds P Rott, RA Bailey, JC Comstock, BJ Croft, AS Saumtally) pp. 226–230. (CIRAD and ISSCT).

Suma S, Magarey RC (2000) Downy mildew. In 'A guide to sugarcane diseases'. (Eds P Rott, RA Bailey, JC Comstock, BJ Croft, AS Saumtally) pp 90–95. (CIRAD and ISSCT).