RESEARCH INTO EXOTIC DISEASE AND PEST THREATS TO SACCHARUM GERMPLASM IN AUSTRALIA AND NEIGHBOURING COUNTRIES

By

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Abstract

A FIVE-YEAR project was funded by ACIAR to gain further information on the exotic diseases and pests that threaten the Australian sugar industry and *Saccharum* germplasm in Indonesia and Papua New Guinea. In this paper, we examine the results from surveys and research into specific pests and diseases, and we explore the most important threats that face the Australian sugar industry. Some of the major outcomes from surveys include spread of several major diseases east from Java into more eastern islands in the archipelago, the widespread distribution of symptoms similar to Ramu stunt around PNG, the lack of major pests and diseases in northern Australia, and the first record of some minor pests within the region. Major research outcomes include the development of protocols for screening sugarcane varieties for resistance to sesamia stem borer, a preliminary test for screening sugarcane for Ramu stunt disease, much better information on sugarcane mosaic diseases present across the region, and improvements in the sugarcane smut screening technique used in Indonesia. Training outcomes were significant with quarantine officers in northern Australia and Papua New Guinea being instructed in the recognition of important sugarcane pests and diseases. Individual scientists from Indonesia and Papua New Guinea also benefited from training in Australia.

Introduction

Agricultural projects in developing countries are funded by the Australian Centre for International Agricultural Research (ACIAR), an Australian Federal Government aid institution. In 2000, funding was provided by ACIAR for work in Papua New Guinea, Indonesia and northern Australia to assist with the conservation of *Saccharum* germplasm.

Papua New Guinea and eastern Indonesia (Papua) constitute the centre of diversity for several *Saccharum* species, including *S. officinarum*, *S. robustum* and *S. edule*. This germplasm is not only important for traditional purposes within the region, but is a potentially valuable source of genes for improved yield and sugar content in commercial sugarcane varieties. There are a number of threats to this germplasm, not least of which are exotic pests and diseases. With the rapid increase in travel by indigenous people, tourists and others, the risk of an exotic pest or disease incursion is increasing. Already, several sugarcane diseases have been introduced into PNG, including leaf scald (*Xanthomonas albilineans*) and more recently ratoon stunting disease (RSD – *Leifsonia xyli* s.sp. *xyli*). These pose a direct threat to germplasm; indeed leaf scald has already led to the loss of *Saccharum* germplasm in the Ramu Valley.

This paper reports on project activities undertaken during 2000–2006, and draws together the results from research, surveys and training activities.

Materials and methods

Surveys

Four major surveys were conducted across the region. In addition, four minor surveys followed up on issues identified during the first four surveys. Scientists with specialist expertise from sugarcane institutions in Australia, Indonesia and Papua New Guinea participated to provide the best possible identification of encountered pests and diseases – specimens were subsequently sent to other experts for identification. Generally, light planes were chartered to increase the efficiency of the survey operation, to minimise deterioration of collected samples and to allow the necessary preservation materials to be carried by the scientists – strict controls on commercial flights prohibit the carrying of some volatile preservatives. This approach worked very well; surveys usually lasted between 10 days and two weeks. Many specimens were collected, collection details recorded and photographs of specimens taken – with care taken to apply appropriate quarantine precautions. Separate pest and disease databases were created to ensure collection details are freely available to others later. Details of each survey are provided in Table 1.

Table 1—Details of pest and disease surveys conducted during the course of the ACIAR-funded project work (2000–2006).

Survey	Timing	Places visited	Scientists
Papua New Guinea	June 2001	Border communities around western, northern, north-eastern islands, eastern border areas and Pt Moresby	Lastus Kuniata, Sidney Suma (PNG); Pak Irawan (Indonesia); Peter Allsopp and Rob Magarey (Australia)
Eastern Indonesia	June 2002	Islands immediately east of Java	Pak Lilik Putri and Pak Djoko Pramano (Indonesia); Lastus Kuniata (PNG); Keith Chandler and Barry Croft (Australia).
Northern Australia	June 2002	Communities between the Gulf country to Broome in Western Australia	Peter Samson and Rob Magarey (Australia)
Cape York Peninsula/ Torres Strait	June 2003	Many of the Cape York Peninsula communities and a number of Torres Strait Islands	Lastus Kuniata and Gou Rauka (PNG), Pak Irawan (Indonesia); Peter Samson and Rob Magarey (Australia)
East New Britain	November 2004	Kerevat, East New Britain	Gou Rauka, Lastus Kuniata (PNG); Rob Magarey (Australia)
Java	May 2005	Eastern Java sugar producing districts	Anthony James and Rob Magarey (Australia)
Bamaga	June 2005	Bamaga, New Mapoon, Seisia and Umagico	Kylie Anderson, Sallam Mohamed and Rob Magarey (Australia)
Darwin	April 2006	Darwin gardens and several surrounding townships	Peter Samson and Rob Magarey (Australia)

Research

Resistance to sesamia stemborer

Sesamia stemborer is the most important pest species at Ramu Sugar, PNG, and would be a very serious pest of sugarcane if it was introduced to Australia (FitzGibbon *et al.,* 1999). Varietal resistance offers an important control should an incursion of the pest occur. Varieties displaying a range of reactions to infestation by *Sesamia grisescens* were selected and grown in plots in experiments at Ramu with and without insecticide sprays. Damage and yield responses were related to infestation parameters to determine a suitable indicator of insect resistance. Ratings calculated from damage parameters give an indication of plant tolerance while larval/pupal numbers give an assessment of the breeding potential of *S. grisescens* in that particular variety. Small plots were planted into the field, pesticides that control sesamia were applied to one half of all plots, and a range of plant and damage parameters recorded during crop growth (Magarey *et al.*, 2002).

Causal agent of Ramu stunt

Ramu stunt almost destroyed the commercial sugar industry of PNG in 1985–86. Previous limited studies have suggested a viral aetiology with transmission by the delphacid planthopper *Eumetopina flavipes* (Kuniata *et al.*, 1994). An assay for the disease is very important for confirming the incidence of the disease and for ensuring germplasm can be safely exchanged between countries. Previous reports had suggested that a phytoplasma was associated with this disease (Cronje *et al.*, 1999). Initial investigations centred on attempting to detect a phytoplasma in diseased tissue (sent under quarantine from Ramu Sugar, PNG). Later, attempts to detect a virus were made by extracting crude viral extracts, and examining tissue under an electron microscope (Braithwaite *et al.*, 2007). A range of other tests were also applied (Braithwaite *et al.*, 2007)

Sugarcane smut

Previous research with partial SRDC and QDPI&F funding enabled smut-resistance screening trials to be established on the island of Madura, on the north-eastern side of Java, Indonesia. This is a collaborative effort with the Indonesian Sugar Research Institute (ISRI). Research in this project aimed to improve spore storage so that trials could be inoculated with previously collected spores. Investigations addressed long-term survival of spores stored at low temperature (Magarey *et al.*, 2003).

In 2004, project funding also enabled Ramu-bred varieties to be passed through tissue culture quarantine facilities at CIRAD, France, and these will be forwarded on to the Indonesian Sugar Research Institute later in 2007 for smut screening.

Training

Torres Strait (AQIS staff)

A very significant part of the project work was the training of quarantine staff and scientists in each of the participating countries. Quarantine staff in the Torres Strait provide a key barrier for the Australian industry in preventing the movement of sugarcane, and key pests and diseases, from PNG to mainland Australia. A training workshop was held on Thursday Island in November 2002 with Quarantine Officers from many Torres Strait islands attending. A sugarcane pest and disease manual describing symptoms of the major pests and diseases of quarantine risk was prepared as a reference text.

PNG quarantine staff

The National Agriculture Quarantine Inspection Agency is the official quarantine agency in PNG. Personnel are located all around the country. These staff previously had very little information on sugarcane pests and diseases, even though sugarcane is a very common domestic commodity. A training workshop for some of these staff was held at Ramu Sugar in

November 2002. Posters written in English, Motu and Tok Pisin were given to participants to distribute within their local area. The first PNG sugarcane pest and disease manual was also written specifically for these staff.

Individual scientists

Ms Ari Kristini (pathologist, Indonesian Sugar Research Institute) was awarded funding through a John Allwright Fellowship to undertake a Masters degree at the University of Queensland. She spent 18 months in Brisbane.

Pak Irawan (pathologist from Indonesia) visited BSES laboratories in Brisbane, Woodford and Tully learning various techniques associated with disease management including molecular assays, resistance screening and issues related to leaf disease assessments.

Ms Gou Rauka (Ramu Sugar) visited BSES Brisbane and Tully learning skills in the RSD ELISA assay, resistance screening techniques and leaf disease issues.

These learning opportunities proved valuable for these scientists and, along with contact made during the surveys, contributed to much better networking of scientists within the region.

Results

Major surveys

Papua New Guinea

Ramu stunt-type symptoms were widespread. Other common diseases were chlorotic streak, orange rust, yellow spot, Fiji leaf gall and Ramu streak. Specimens of Fiji leaf gall with purple, instead of pale green, galls were found at Alotau; this is the first known report of purple Fiji leaf gall 'galls'. Downy mildew (*Peronosclerospora sacchari*) was only seen on the northern side of PNG, and even then only near Wewak, Popondetta and Alotau. Leaf splitting disease, also caused by a *Peronosclerospora* species, was common in *S. spontaneum* around Popondetta.

A number of minor uniquely PNG diseases were seen including veneer blotch and target spot. No sugarcane smut was observed at any location; ratoon stunting disease was not detected on this survey. Stemborers, *Eumetopina* and *Perkinsiella planthoppers* were common in places.

There seemed to be a low awareness of sugarcane quarantine in PNG; movement of hybrid cane to remote villages around the country has been occurring for some time. A number of villages had substituted hybrid cane for traditional *S. officinarum* clones and they referred to these canes as 'Ramu Sugar'.

Eastern Indonesia

Smut was seen east of Java on a commercial plantation on Sumbawa. This is the first report of the disease on the archipelago east of Java and highlights the spread of the disease towards West Papua and PNG. Records suggest the cane was planted in an experimental program in the mid-1980s, but it is thought that the smut was introduced in subsequent movement of cane by the plantation staff.

Leaf scald was also found on the survey and was thought to be spread by the same mechanism. Other serious diseases seen include chlorotic streak, mosaic, yellow spot, eye spot and orange rust.

Sesamia grisescens was not found on the survey but another less important pest species was (*S. inferens)*. The vector of Ramu stunt, *Eumetopina flavipes,* was found in high numbers on several islands; there is a need to confirm whether Ramu stunt is present in these locations. *Perkinsiella* species were also observed and these insects may also transmit important viral diseases (such as Fiji leaf gall).

Northern Australia

No smut or ratoon stunting disease was found, except for the known smut outbreak at Kununurra on the Ord River. Very few pests or diseases were seen, probably a result of the low sugarcane population density across the north. Widespread distribution of hybrid sugarcane was observed and this was the most common sugarcane found. Individual stools within gardens were generally seen in the backyards of residences. *Saccharum officinarum*, *S. edule* and *S. spontaneum* were located, but only rarely. *S. spontaneum* occurs in quite large areas on the lower Daly River. Herbarium reports suggest it has been there at least 55 years. The area poses a potential target for entry of pests or diseases into the Northern Territory.

Cape York Peninsula and Torres Strait

Sugarcane, while common in the Torres Strait, was at low incidence on Cape York Peninsula (Magarey *et al*., 2004) reflecting the traditions of the indigenous peoples. The lack of cane on the Peninsula is providing a natural barrier to the spread of sugarcane pests and diseases from the Torres Strait into commercial production areas.

A number of common pest species were found in the Torres Strait, most being minor pests. The vector of Ramu stunt, *Eumetopina flavipes* was common on some islands and at Bamaga; this infestation was recorded previously (Gough and Petersen, 1984; Chandler and Croft, 1986; Allsopp, 1991; Magarey, 1997). The infestation at Bamaga could be eradicated, as it is the only mainland infestation. There was some evidence of *Chilo* borer damage on Mabuiag Island, and further observations should be made for this pest on Mabuiag and nearby islands. *Chilo terrenellus* has been found previously in the northern Torres Strait islands of Saibai and Dauan (Gough and Petersen, 1984; Chandler and Croft, 1986; Magarey, 1997). Both of these records may refer to a native species of *Chilo* identified recently through DNA testing of material from Thursday and Hammond Islands (Sallam Mohamed, pers. commun.)

Minor surveys

Eastern New Britain

The focus of this survey was on an unidentified sugarcane mosaic disease at Kerevat. Assays using conventional probes for mosaic failed to identify the normal causal agents in specimens collected during the first PNG survey. Specimens were again collected from the National Agriculture Research Institute NARI Research Station at Kerevat. Samples were assayed at BSES Indooroopilly; no recognised pathogens that cause sugarcane mosaic-type symptoms were detected.

Hybrid sugarcane was again found to be widespread in the area suggesting movement away from Ramu Sugar.

Tip of Cape York communities

Further specific inspections of sugarcane around Bamaga revealed large populations of *Eumetopina flavipes* in some gardens. One individual was collected from New Mapoon and later identified as *E. flavipes*. This is the first record of the pest from New Mapoon, or from any of the other Cape York Peninsula communities (known from Bamaga previously).

Darwin and outlying areas

A brief survey was undertaken to see what pests and diseases were present in residential gardens in Darwin. Although sugarcane was found, there were few pests or diseases of note on these plants. Sugarcane smut was not found, nor any of the other significant leaf diseases.

Java

Mosaic-type diseases of sugarcane are not uncommon in commercial crops in Indonesia; mosaic is one of the most common diseases of importance in the region. Assays conducted at BSES Indooroopilly on specimens collected from Java, suggested sugarcane mosaic virus and sugarcane streak mosaic virus were present in eastern Indonesia. This was the first report of sugarcane streak mosaic in Indonesia. This disease has subsequently been found to be widespread on Java. The visual symptoms of sugarcane mosaic virus and sugarcane streak mosaic are indistinguishable. The project has established facilities and trained staff in Indonesia to screen for these diseases with molecular techniques.

Other recorded diseases include orange rust, red leaf spot, ring spot, target blotch, pokkah boeng, yellow spot, smut and chlorotic streak. Smut and sugarcane mosaic were relatively common; RSD is also known to be common.

Research

Resistance to sesamia stemborer

A number of 'standard' (of known resistance) varieties were identified in these trials and will be used in future work. There is still no clear indication of a character that can be used to give over 90% confidence in determining *S. grisescens* resistance. A potential character may be the number of pupae produced per variety. Greater pupal numbers would mean the variety supports more larvae (borers).

This parameter will be used in the short term until other studies define a more appropriate resistance/tolerance parameter. Data obtained previously suggested that either '% bored stalks' or number of 'dead hearts' (that is, dead young shoots) are likely to be the best plant parameters to measure in assessing pest resistance.

Causal agent of Ramu stunt

Preliminary reports suggest a phytoplasma related to the sugarcane white leaf phytoplasma is associated with the disease (Cronje *et al.*, 1999). However, phytoplasma assays failed to associate these pathogens with the disease in BSES research. Attempts to locate a viral agent proved more successful. Some unique proteins were associated with diseased, but not healthy, leaf material.

Viral minipreps, and running the extracts on PAGE, consistently showed a 36 kDa protein in diseased, but not healthy, leaves. This band is very clear in good quality tissue but its presence is variable and degrades quickly if the leaves are in poor condition (Braithwaite *et al.*, 2007).

Sequencing of the protein suggests the virus does not specifically match any known virus, but sequencing of nucleic acids associated with Ramu stunt suggests that the virus has some homology with tenui-viruses. Isometric viral particles have been seen under the electron microscope in one variety (Ragnar) (Braithwaite *et al.*, 2007).

Sugarcane smut

More than 1500 Australian clones have been screened for smut resistance in Indonesia. Collection of smut spores from the field for the screening trials is very arduous with up to 1 kg of spores required for each trial. Research conducted in Indonesia compared the viability of spores stored in a desiccator at room temperature (previous method used by ISRI) with spores stored at –20°C. It was found that 60 % of spores were still viable after 12 months when stored at –20°C, and 30% were viable when stored in the desiccator. Storage at –20°C will become the standard storage procedure, and will allow smut spores to be collected when smut whip development peaks in the field.

Sugarcane mosaic

Assays for the various agents responsible for sugarcane mosaic-type symptoms in sugarcane can now be undertaken by BSES Indooroopilly staff. Research has shown the variation in the pathogens present in Australia and Indonesia. There remains a need to determine the causal agent of the disease in PNG.

Discussion

It is important the Australian sugar industry does not sit back and wait for incursions of exotic pests and diseases. A wait-and-see approach will not lead to adequate preparation or established strategies to reduce the risk of incursions, and severe financial hardship may ensue for cane farmers. In project work reported here, a serious attempt was made to determine where important pests and diseases are located, to become familiar with their management, and to learn from overseas scientists about their aetiology and control. To this aim surveys, research and training were undertaken. *Saccharum* germplasm present in the centre of diversity (West Papua and Papua New Guinea) needs to be preserved if genes for high yield are in the future to be incorporated into commercial germplasm. The preservation of *Saccharum* germplasm should remain a long-term aim of the Australian industry, even when these project activities cease.

The work reported achieved some very significant outcomes, including mapping of the distribution of the most important pests and diseases in the region, identification of an assay for Ramu stunt (with information on identity of the causal agent), improved methods for screening varieties for sesamia stemborer resistance, significant training of quarantine staff in northern Australia and PNG, and knowledge of the distribution of *Saccharum* throughout northern Australia, PNG and Indonesia. Networking with overseas scientists has also been profitable. The presence of an international scientific team in communities all over the region significantly raised the profile of sugarcane and the importance of quarantine.

Information contained in pest and disease databases will be used to update sugarcane pest and disease records for each country and this will be a significant resource for quarantine personnel and scientists alike. These records need to be stored in a freely available form; web-based systems would be the most accessible. It is unlikely a similar set of surveys will be undertaken for some time and the data obtained in this project provide a valuable resource.

Much work remains to be undertaken. This includes restricting the spread of commercial sugarcane hybrid material in each country. In PNG in particular this is a cause for concern. Ramu Sugar is now infested with leaf scald and ratoon stunting disease (RSD). In the 2001 PNG survey, no RSD was detected in sugarcane in village gardens. In 2004, the disease was detected at Ramu Sugar (Kuniata *et al.*, 2005) for the first time and has now been detected in garden canes (*S. officinarum*). As the disease is widely distributed in Ramu crops, and very easily transmitted on bush knives, spread to the rest of PNG seems inevitable. The movement of hybrid canes away from the Ramu Sugar Estate will also undoubtedly spread the disease. RSD and leaf scald together are likely to cause significant loss of *Saccharum* germplasm in the region. There is a need to map the spread of these two diseases and to determine the magnitude of the losses they are causing to help assess the priority for control. The spread of hybrid material will also directly affect conservation of some *Saccharum officinarum* varieties. In the 2001 PNG survey, we saw local villagers had a preference for the more vigorous hybrid material. Unless further work is done, significant loss of germplasm may go unnoticed, and particularly important genes may be lost.

It would be beneficial to test archived samples from the PNG and eastern Indonesia surveys for the presence of the Ramu stunt-associated virus, now that an assay has been developed. Some symptoms seen during the Indonesian survey showed a resemblance to Ramu stunt. Unless samples are assayed, there will be no confirmation of the distribution of Ramu stunt in these areas.

Many species of *Eumetopina* occur in PNG. There remains a need for these to be classified/identified and for their status as vectors of Ramu stunt to be clarified. It is unlikely this work will be completed anytime soon, since there are few insect taxonomists around the world with the time or competence to complete the identifications, nor scientists with freely available time in the field to perform the necessary transmission studies. The unknown form of sugarcane mosaic found on East New Britain is a cause for concern for Ramu Sugar and for the Australian and Indonesian sugarcane industries. The failure to identify a causal agent with the normal mosaic-type assays suggests this could be a new pathogen of unknown influence on current commercial varieties.

There also remains a need for further identification/clarification of the species of *Peronoslcerospora* causing downy mildew in PNG. The disease may affect several *Saccharum* species, and several *Peronosclerospora* species are known within the region. Currently there are no readily available molecular assays for the species causing this disease, though some research is current in the United States.

Changes in strain of *P. sacchari* have been noted at Ramu Sugar and this had a significant effect on the cropping of two previously important commercial varieties. Molecular tools for identifying *Peronosclerospora* species would be very helpful in this situation.

Other needs include the strengthening of quarantine practices in both PNG and Indonesia. This will be an ongoing issue: to keep the awareness of sugarcane quarantine issues at the fore-front in these countries. Further development of quarantine materials with a sugarcane focus and manuals/field guides on sugarcane pests and diseases would be helpful.

Another option for *Saccharum* germplasm conservation would be the collection of unique PNG germplasm and the establishment of a working breeding program. The development of novel phenotypes would allow the long-term incorporation of diverse genes into commercial varieties and this would broaden the genetic base of the crop.

This would require long term funding and a determination to see the program to completion. Without such a strategy, the consistent encroach of new pests and diseases into the centre of *Saccharum* diversity, and the subtle replacement of traditional canes with hybrid material, are likely to erode the genetic base of one of our valuable resources. Whether the international community will heed the warning and initiate further work is another question. It appears unlikely at this stage.

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