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1 Acknowledgments

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2 Executive summary

Sugarcane streak mosaic (SCSM) caused by sugarcane streak mosaic virus (SCSMV) is a relatively recently recognised disease of sugarcane that is now widespread through S.E. Asia and widely infecting commercial crops, both in terms of incidence and in the percentage of plants infected within individual fields. Crop losses, though not spectacular in individual plants, is very significant since whole crops are very often affected meaning that accumulated smaller losses in individual plants leads to very significant losses for cane farmers. A number of aspects related to the disease were unknown at initiation of this research, including: distribution across Indonesia (Sumatera, Sulawesi, and the eastern archipelago); transmission mechanisms (mechanical or insects or planting material only); the epidemiology and speed of spread; crop resistance; sensitive, specific and cheap detection technologies; and an effective integrated disease management (IDM) strategy. All these points were addressed in associated research activities.

Research has clearly shown that the disease is at greater incidence on the island of Java, with findings in other regions generally associated with the movement of planting material from this location. A reliable, cheap antiserum-based detection technique was developed as well as a very sensitive qPCR assay for use in quarantine and other applications. Variation in the pathogen was detected in molecular assay of samples from different Indonesian locations. Significant mechanical transmission was only associated with rubbing extracted, infested stalk and leaf sap onto the leaf surface of test plants after surface abrasion; this will be a reliable technique for use in resistance screening. Knife transmission was minor. No insect or arthropod transmission has as yet been detected and confirmed, though the virus was found associated with some arthropods. Spread of the disease, apart from planting material, was found to be relatively slow in sites in eastern Java but has been reported to be very rapid in Myanmar; the outcomes of a small project investigating arthropod transmission there is the focus of a separate report. Some resistance to the disease has been detected, though a number of Indonesian varieties are susceptible. The essential elements of an effective IDM include: disease-free planting material, varietal resistance and termination of heavily-diseased crops.

Project impacts so far have included better guidance to farmers on how the disease is likely to affect their crops, recommended management options to reduce crop yield losses, an ability to test planting material for the presence of the disease (to enable better selection of disease-free planting material) and improved farmer understanding of the disease.

Scientific impacts have included a much better understanding of pathogen variation, the development of optimised molecular and serological assays for virus detection in planting materials and a better understanding of the speed of spread and distribution of the disease.

3 Background

Sugarcane is an important agricultural crop in Indonesia and contributes Rupiah 11,500 billion ($AUD 1.57 billion) to the Indonesian rural economy. Sugarcane is grown by over 140,000 farmers, and the industry supports over 1.3m workers in associated industries. Almost all sugarcane farmers are small-holders and there is a strong reliance on sugarcane as a cash crop. The profitability of sugarcane farming operations is therefore important for alleviating poverty amongst the sugarcane farming community.

There are 58 sugarcane factories in Indonesia processing 30m tonnes of sugarcane from 380-400,000 ha land. Over three-quarters of the sugarcane production occurs on the island of Java. The Indonesian Government has set a target to become self-sufficient in domestic sugar production and this will lead to an even greater reliance of the Indonesian economy on the sugarcane crop.

Over the last 40 years, productivity has been declining. Pests and diseases are major contributors to this production slide, and stem borers are dramatically affecting crop yields in Java. Major stem borers include *Scirpophaga excerptalis*, *Chilo auricilius* and *C. sacchariphagus*; losses have been quantified in an ACIAR-funded project titled 'Integrated pest management of stem borers and insect vectors of viral diseases of sugarcane in Indonesia'. Project research led to the formulation of a modified IPM strategy that is being implemented in the Indonesian sugarcane industry.

Previous research (Putra *et al.*, 2014) and previous project research identified *sugarcane streak mosaic virus* (SCSMV) as a widespread disease of high significance to the current Javan-based sugarcane industry; an estimated $AUD 50-100m is lost annually to SCSMV. The disease is also significant to sugarcane production in expansion areas, for instance West Papua.

The disease has only relatively recently been identified and was found in >80% of the 931 crops surveyed in Java. A significant proportion of commercial varieties are susceptible to the disease. Only two popular commercial varieties are rated as resistant, which leaves commercial cane farmers with few options for disease control. Disease-free nursery material is a key strategy needing strong implementation; many nurseries are affected by the disease. There are many facets to the disease that needed researching prior to this project. Project HORT /2006/147 provided evidence to suggest that insect vectors and mechanical equipment may transmit the disease; the latter is unusual for this type of disease and may help explain why the disease is widespread through the industry. The rate of disease spread, and the pattern of spread were unknown, as was the resistance of Indonesian and Australian varieties.

Since SCSM is so widespread in Indonesia, there remains a strong need to implement effective control programs, especially the implementation of an effective Integrated Disease Management (IDM) strategy. This applies not only to Java, but also Sumatera, Sulawesi, Sumba and potentially the West Papua region. Such a strategy is likely to include disease-free planting material, resistant varieties and the termination of heavily-diseased, susceptible crops.

Questions that were addressed in project research were: -

Question 1: Where does the recently-identified disease occur in Indonesia?

Question 2: What are the practices needed to ensure the production of disease-free nursery material?

Question 3: How is the disease transmitted?

Question 4: How quickly does it spread?

Question 5: What is the best (most rapid / cheapest method) for disease detection?

Question 7: What is the resistance of Indonesian and Australian commercial varieties?

Question 8: What economic losses are associated with SCSM in Indonesia?

Results from previous projects (HORT 2006/147 and CP 1996/ 140*)* were drawn on in the research reported here. HORT 2006/147 provided important disease information for Java, as well as proven networks for researching/extending information in Indonesia. The Indonesian Sugar Research Institute (ISRI) has been an excellent research partner in previous ACIAR-funded research.

Limited yield loss studies had been conducted prior to this project and methods for resistance screening had not been adequately researched – due to lack of knowledge on transmission. Limited epidemiological studies had been conducted; such research can provide clues to the most common form of disease transmission in the field and the likely rate of disease spread under commercial cropping conditions. Molecular detection techniques had been developed previously (RT-PCR) but serological and more modern molecular detection methods had not been investigated. There was a lack of knowledge on the incidence of SCSM in cane production areas outside of Java.

SCSMV poses an important threat to the Australian sugarcane industry and is one of the more probable exotic invasive pathogens. The gaining of resistance ratings for Australian commercial varieties is a valuable asset and was needed to reveal whether the industry is vulnerable to the disease. Yield loss studies are important for understanding the potential losses that could result if SCSMV was ever to become endemic in Australia. Sensitive, accurate and rapid molecular assays are needed to detect the disease during an incursion. The development of an effective IDM is needed for our industry to manage any disease outbreak.

It was estimated prior to this project SCSMV costs the Indonesian industry an estimated $AUD50-100m annually in direct yield losses. The disease makes it very difficult to locate disease-free planting material, not only for sugarcane crops in Java, but also for the establishment of new plantations in other Provinces. This is a challenge to the commercial viability of these plantations. For these reasons, the influence of SCSMV on the sugarcane industry was a high priority for Indonesia.

Sugarcane mosaic-type diseases are of serious concern in S.E. Asia and are generally poorly controlled. In many countries in the region (including Thailand, China, Vietnam and the Philippines), the disease is readily observed in commercial fields, with a number of fields 100% diseased. SCSMV was thought to be spread by insect vectors; not enough was known about these vectors and their control before project commencement.

In Australia, mosaic-type symptoms are caused by a different virus, sugarcane mosaic virus (SCMV). SCMV is not a severe as SCSMV. A SCSMV incursion would be a serious development for the Australian sugarcane industry. A previous ACIAR-funded project, CP/1996/140, also identified an unknown form of mosaic in East New Britain; no assay was able to detect the pathogen. Research conducted in this project may have implications for PNG also. For these reasons, SCSMV research was an important priority for the Australian sugarcane industry.

A smut incursion in the Australian sugarcane industry in 2006 was addressed by SRA scientists who used GPS technologies and extensive crop surveys to determine the speed of disease escalation, spread across regions and the influence of varietal resistance. This work was supported by SRDC (project BSS302) and provided excellent experience for SRA staff. Farmers were advised when the disease would reach their farm, how soon susceptible crops should be terminated, and an overall appreciation of the nature of the disease. Similar strategies were adopted in SCSMV project research.

This project aligned with ACIAR's stated operational plan. ACIAR's program in Indonesia directly supports the Australia-Indonesia Partnership 2008–13 (AIP). The AIP is a comprehensive plan of Australia's support to Indonesia that focuses on poverty alleviation. ACIAR's support, through Pillar 1 of the plan—Sustainable growth and economic management—focuses on improved economic opportunities for rural people through increases in productivity, access to markets, and better infrastructure and growth of small- to medium-sized enterprises in target provinces. In this project, the productivity and profitability of small-scale sugarcane farming operations was targeted through the development of a successful IDM strategy to minimise the effects of SCSMV.

The AIP emphasises that 'support for applied research will be increasingly important in Indonesia. The project directly targeted applied research for commercial disease control.

4 Objectives

1. Assess the importance of SCSM in Indonesia by defining the distribution and incidence of the disease in Java, Sumatera, Sumba and Sulawesi.
2. Determine the potential for disease escalation and the conditions required to maintain disease-free nursery cane by investigating SCSMV epidemiology
3. Identify the resistance of varieties in order for farmers to minimise the level of SCSMV in their crops by developing a rapid varietal resistance screen
4. Assess the economic importance of SCSM in Indonesia by defining the yield losses caused by the disease
5. Enable the Indonesian farming community to reduce disease incidence in newly-planted crops by identifying SCSMV in nursery cane through a cheap, rapid detection technique
6. Reduce SCSM crop incidence through the adoption of an effective IDM strategy

5 Methodology

5.1 Disease distribution

Introduction

In this report, the disease (called sugarcane streak mosaic, SCSM) will be distinguished from the causal agent (sugarcane streak mosaic virus, SCSMV).

Information on the distribution of SCSMV around Indonesia is important for both the Indonesian and Australian sugarcane industries; for the former it provides information on where disease management is needed; while for the latter, it provides a guide on incursion risks. Specific regions were targeted for surveys, namely Sumatera, West Java, Sulawesi, Lombok, Sumba, Sumbawa and the Moluccas. Unfortunately, surveys of West Papua were not possible, though this Province holds strategic importance in the context of the Indonesian, Australian and PNG sugarcane industries.

Surveys were conducted in Sumatera and Western Java (August 2016), Sulawesi (October 2017) and the Eastern Indonesia Archipelago / Moluccas (November 2018). The focus was on SCSM but the incidence of other significant sugarcane pests and diseases was also noted.

5.1.1 Sumatera / West Java

The first survey was conducted over a 7-day period in August 2016. Staff were from SRA (Nicole Thompson, Lisa Derby), ISRI (Etik Achadian, Lilik Putra) and IFCSRI (Titiek Yulianti). Five mill areas were targeted in Sumatera and one in West Java (Figure 1), with 31 fields inspected (Table 1). The main focus was on commercial nursery plots but inspections also included commercial crops and a few other stools of several sugarcane species. SCSM was the main target disease, but other significant sugarcane pests and diseases were also looked for - including leaf scorch, several moth borers / parasitoids, *Xylaria* stool rot, phytoplasma diseases (white leaf / grassy shoot / green grassy shoot), downy mildew, Ramu stunt, weevil borer, rust diseases, and others exotics.

Table 1: Sugar factories and fields surveyed in the six designated mill areas in Sumatera and West Java.

Sugar Factories	Province	No. crops
Kuala Madu	North Sumatera	4
Komering	South Sumatera	4
Cinta Manis	South Sumatera	8
Bunga Mayang	Lampung	4
Gunung Madu	Lampung	9
Subang	West Java	2
	Total	**31**

Figure 1: Mill areas visited in Sumatera and western Java during the August 2016 survey

5.1.2 Sulawesi

The survey was conducted over a 7-day period in October 2017. Staff were from SRA (Nicole Thompson, Liz Wilson), ISRI (Etik Achadian, Helwan Adi), IFCSRI (Heri Prabowo) and for part of it, CIRAD (Regis Goebel). Sulawesi is a mountainous island with recently established sugarcane factories. There were few individual farmers as the cropping area is managed by sugar factory staff. Seven different factory areas were surveyed (Figure 2).

Figure 2: Sugar factory areas in Sulawesi surveyed during the 2017 SCSMV survey.

As in the previous survey, plots within commercial crops were marked and the occurrence and incidence of SCSM and other pests and diseases recorded. Adjacent cane of interest (other *Saccharum* species) were examined and relevant collections made (both plant material and pests; Table 2).

Table 2: Sugar factory areas in Sulawesi and the number of fields (crops) inspected.

Sugar Factories	Province	No. crops
Bone SF	South Sulawesi	8
Camming SF	South Sulawesi	7
Takalar SF	South Sulawesi	6
Marketindo Selaras, SF	South East Sulawesi	2
Disbun Kendari	South East Sulawesi	2
Kilau Indah Cemerlang	South East Sulawesi	1
Gorontalo SF	Gorontalo	5
	Total	**31**

Potential SCSMV vectors

Sweep net-capturing of crop associated arthropods was undertaken during the survey; the specimens were preserved in absolute ethanol for SCSMV molecular assay. Some of these species were identified to enable characterisation of the crop-associated arthropods.

Training

A training workshop on pest and disease recognition and agronomic issues was held with factory staff. Attendees completed questionnaires related to their knowledge of sugarcane pests and diseases both before and after the workshop – this enabled an assessment of training effectiveness.

5.1.3 Eastern Indonesia

The survey was conducted over a 7-day period in November 2018. Staff were from SRA (Nicole Thompson) and ISRI (Etik Achadian, Ari Kristini and Herwan Adi). There are two big private companies cropping sugarcane in the Eastern Archipelago but permission was gained to visit one of them only - namely PT Success Mantap Sejahtera. Similarly, crops of a private company in Mollucas were visited, with one field belonging to the Estate Crop Agency. Garden sugarcane was also inspected. Survey locations are listed in Figure 3 and Table 3.

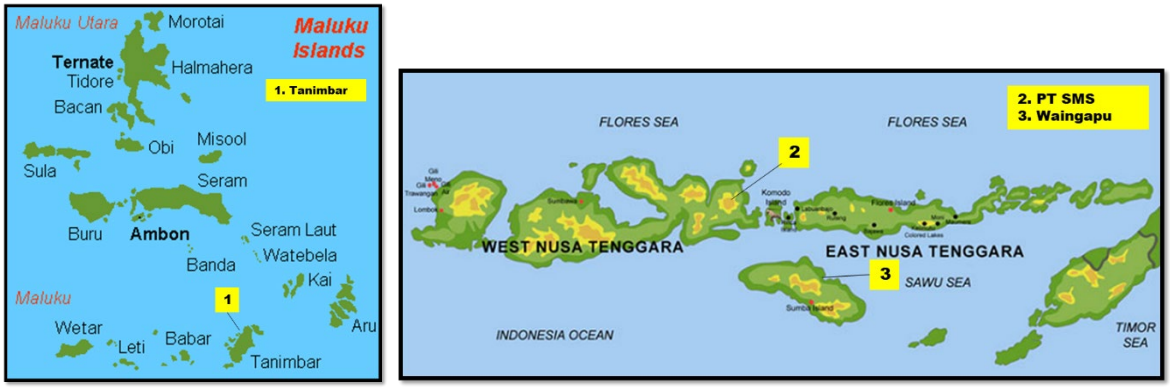

Figure 3: Sugarcane areas in Moluccas and Nusa Tenggara surveyed during the 2018 SCSMV survey.

Potential SCSMV vectors

Sweep net-capturing of crop-associated arthropods was undertaken during the survey; specimens were preserved in absolute ethanol for SCSMV molecular assay. Some of these species were also identified to characterise the arthropods associated with sugarcane.

Table 3: Sugar factory areas and crops / sugarcane sampled during the Eastern Archipelago survey.

Sugar Factories	Province	No. Fields
People's yard	South Moluccas	10
Moluccas State Crop A.	South Moluccas	1
PT Tanimbar Nanim Sejahtera	South Moluccas	1
Cane growers/yard	Sumbawa	5
PT Sukses Mantap Sejahtera	Sumbawa	5
Cane growers/yard	Sumba	6
PT Muria Sumba Manis	Sumba	1

5.2 Epidemiology (spread)

5.2.1 Virus transmission

SCSMV is known to spread via diseased planting material – this form of transmission is of prime concern in an effective IDM strategy. Two potential additional modes of SCSMV transmission are mechanical (via contaminated cutting surfaces or infection of wounded leaf surfaces via infested juice) or arthropod transmission. Disease spread via both potential mechanisms (arthropods and mechanical means) would make the disease extraordinarily hard to manage. Field spread suggests that in some circumstances the disease does spread both within, and between crops, quickly. Detailed below are experiments conducted during the project seeking to quantify the speed of disease spread and to identify spread mechanisms.

5.2.1.1 Mechanical transmission

5.2.1.1.1 Juice inoculation of setts (52111)

Experiment 1

Stalks of the susceptible variety PS 864 were selected and cut into single-eye setts. The setts were immediately dipped in virus-infested or disease-free sugarcane juice, freshly extracted from diseased or healthy stalks. The setts were then planted in soil contained in black plastic bags and grown within mesh bags (to exclude insects). The mesh was strung over the plants and supported by wires (Figure 4). Appropriate control plants were also grown (positive and negative SCSMV controls; Figure 5).

The experiments were established in June-November 2015.

Treatments

1. SCSMV-infested juice sett dip
2. Disease-free juice sett dip
3. SCSMV-diseased cane: no inoculation
4. Healthy cane: no inoculation

Figure 4: Inspecting the transmission trial

Figure 5: symptoms of SCSMV in test plants in the field in Pasuruan.

The experiment was established in June 2015 and inspected monthly after inoculation. Diseased plants used as a source of virus-infested juice were tested for the presence of SCSMV and the closely-related SCMV, to ensure the correct virus was present and also to confirm disease-freedom in the healthy control treatments. The experiment was terminated in early November 2015.

5.2.1.1.2 Knife transmission

Introduction

Significant knife transmission was reported in previous research; knife transmission would make the disease very difficult to manage since many sugarcane cropping operations could lead to mechanical transmission.

Experiment 1

Several treatments were established in June 2015 to investigate knife transmission.

Treatments

1. Diseased cane stalk cut: followed by the cutting of 10 single-eye setts of healthy cane
2. Healthy cane stalk cut: followed by 10 single-eye setts of healthy cane

3. Disease control: 10 setts of diseased
4. Healthy control: 10 setts of healthy

Disease inspections were undertaken fortnightly until the plants were three months in age.

Effect of stalk age

Two experiments were conducted to test whether stalk or bud age affects transmission.

Sett age x disease transmission.

Experiment 2

Healthy stalks were selected as well as cane showing definitive SCSM symptoms (source of the virus). Juice was extracted from the diseased stalks before a knife was dipped for 3 seconds in the freshly-extracted juice; this was then used to cut successive single-bud setts from the top of the stalk (sett 1) and successively down the same stalk (sett 7). The original bud position on the stalk was recorded via labelling on each pot. A healthy control treatment was established by cutting healthy cane in a similar fashion with a knife dipped in disease-free juice. A positive disease control was established via the leaf-rub inoculation procedure, using virus-infested juice. There were 10 replicates of the nine treatments. Test plants were the susceptible variety PS864 (Figure 6).

The experiments were established in August 2015-December 2015.

The observations were done fortnightly from 2 weeks old plant until 4 months old plants. The percentage of infected plants for each treatment was calculated by dividing the number of infected plants with the total plants in the experiments (10) and times with 100%.

Figure 6: Cutting the cane stalk with the SCSMV-contaminated knife blade

Experiment 2

This was a repeat of experiment 1 and again evaluated the effect of bud position on knife transmission. The same treatments were applied, starting from the top of the stalk (position 1) to lowest part of the stalk (position 7). Ten replicates were again included in the experiment. The experiment was planted in February 2016 and the final results recorded in July 2016. Fortnightly observations were again made for SCSM development.

5.2.1.1.3 Transmission via abrasive pad

Sugarcane mosaic virus (SCMV), compared to SCSMV, is readily transmitted by applying infested juice to leaves using an abrasive scourer pad. The minor wounding of the leaf surface allows virus entry and leaf infection. The following treatments were established in June 2015 to test the effectiveness of this technique with SCSMV. A scourer was dipped in cane juice before rubbing the leaf blade of six-week old test plants.

Treatments

1. Diseased stalk juice: applied with a scourer pad to the leaf surface
2. Healthy stalk juice: applied as above
3. Diseased leaf extract: applied as above
4. Disease control: diseased test plants
5. Healthy control: healthy test plants

Fortnightly observations were again made for SCSM development with the final disease inspection at three months of age (September 2015).

5.2.1.1.4 Transmission via pin-prick (sein) method

SCMV (vs SCSMV) may be transmitted by the sein technique – pushing a pin through a diseased leaf into the spindle (unrolled) leaf of a healthy test plant. This technique was used to establish an experiment in June 2015 with the treatments listed below; treatment details describe how the needle was contaminated (where applicable) as it was pressed into the spindle leaves.

Treatments

1. Infested cane juice: the needle was dipped into the juice
2. Disease-free cane juice: as above
3. Needle pushed through diseased leaf as it was wrapped around the spindle leaf
4. Diseased test plants (control)
5. Healthy test plants (control)

Test plants were grown for 6 weeks before all plants were assessed for SCSM.

5.2.1.2 Vector transmission

Introduction

There are currently no known arthropod vectors for SCSMV. Personal enquiries made with a prominent Indian pathologist / virologist (GP Rao, pers. comm.) suggested that a vector is unknown in India. However, the disease has rapidly spread in some commercial crops, so the possibility of an insect / arthropod vector was real and required more research. A viral disease of wheat, with a similar causal agent (*wheat streak mosaic virus* (WSMV)), has an eriophyid mite vector (wheat curl mite – *Aceria tosichella*). A similar mite could be involved in SCSMV transmission in sugarcane. Rapid spread of SCSMV in countries such as Myanmar also suggests the possibility of an arthropod vector. A mid-term project review (conducted by ACIAR Program Manager, Dr Richard Markham) raised the possibility of travelling to Myanmar to further investigate arthropod transmission in this country. This has since been effective and is reported elsewhere.

Arthropods from several groups were identified and these provided the focus for transmission investigations.

Method

Cage transmission: using vectors of other crop viruses

Six potential arthropod vectors were identified: -

- *Melanaphis sacchari* (sugarcane aphid)
- *Perkinsiella saccharicida* (Delphacid planthopper)
- *Aleurolobus barodensis* (white fly)

- *Ceratovacuna lanigera* (woolly aphid)
- *Saccarococcus sacchari* (mealy bug)
- *Oligonychus exsiccator* (mite)

Each of these species had been observed in sugarcane crops, and representatives of these general groups are known viral vectors. Populations of each species were established by collecting individuals from disease-free plants and transferring these to SCSMV-infected sugarcane. Additional diseased plants and individuals of each of the vectors were added to each specific cage on a regular basis to help ensure that any potential vectors became infested with the virus. It was hoped to breed successive generations of each species on diseased sugarcane.

After an initial two weeks feed time, individual arthropods were collected, placed into absolute ethanol and assayed using a molecular assay to determine if they acquired SCSMV. Collections were made every two weeks thereafter. Arthropods collected over the first six-week period were assayed initially with the view that if acquisition hadn't occurred by then, it may not be justified to continue assaying additional specimens. Where possible, the larger insects (e.g. *Perkinsiella*) were separated into males, females and nymphs and each were analysed separately.

Survey arthropod collections

A second approach to vector identification came via SCSMV assay of specimens collected from diseased cane crops during each of the crop surveys. While detection of the virus in collected insects does not prove vector status, a consistent and significant association could suggest likely vector candidates.

Arthropods were collected from Sumatera, West Java, Sulawesi, Moluccas, Sumba and Sumbawa in each of 2016, 2017 and 2018. These were assayed for SCSMV, using qPCR to determine whether the virus was consistently associated with both diseased plants and the insects feeding on them.

5.2.2 Epidemiology

Introduction

Spatial pattern information can be critical for identifying mechanisms for disease transmission; epidemiology information is foundational for formulating an effective IDM strategy. Epidemiology studies were therefore initiated to provide an understanding of how quickly the disease may spread both between and within crops, how far away nurseries need to be located to avoid disease contamination, and how quickly farmers need to terminate crops of susceptible varieties to avoid excessive yield losses.

Method

The basic experimental approach consisted of planting known diseased and disease-free sections of crops (first experiment) or identifying individual crops where there was a significant disease incidence. These crops were then monitored regularly for disease on a stool-by-stool basis, with stool health tracked using GPS measurements. GPS data then provided sequential spatial disease incidence information, thus potentially assisting with characterisation of the spread pattern.

Kediri experiment

The first trial was established at Kediri in July 2015 and monitored for three years. Plots of healthy and diseased cane were planted adjacent to healthy (test) cane; SCSM observations were limited to the original disease-free plot (100 rows x 70 m) (Figure 7). Disease monitoring occurred on a monthly basis.

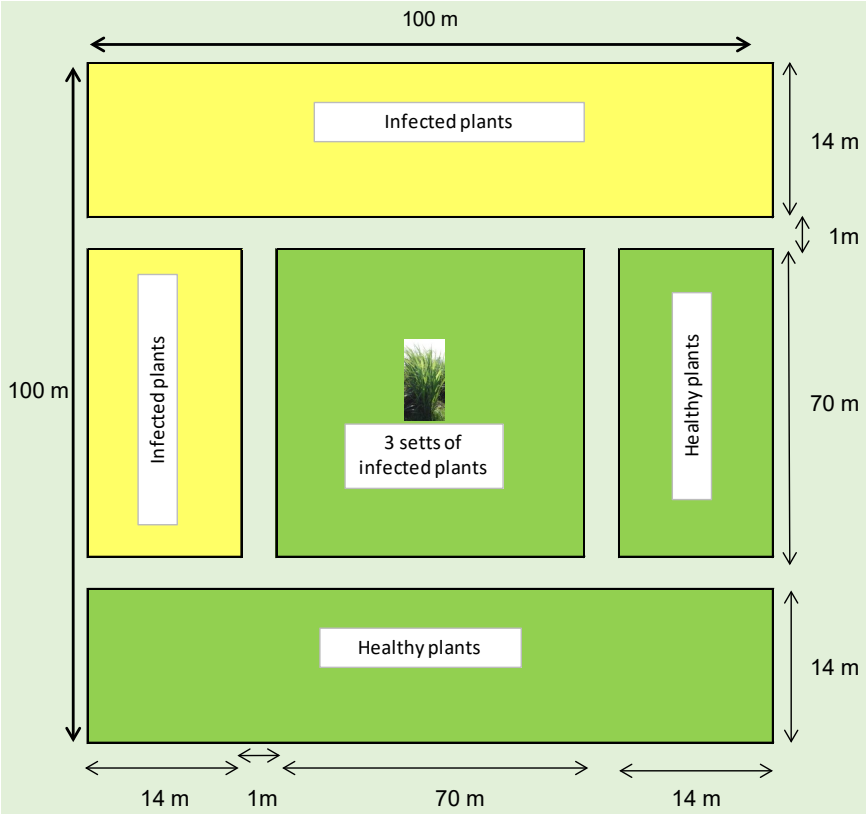

Figure 7: Design of the epidemiology trial at Kediri, planted in July 2015.

Kejobo, Pasuruan

A nursery site at Pasuruan (eastern Java) was also selected as it had a higher initial disease incidence. Though monitoring began well after planting (the site had already been established before selection as a study site) disease incidence data provided another opportunity to monitor the speed and pattern of disease escalation.

The crop consisted of the susceptible variety PS881; both plant and ratoon crops were monitored (100 rows x 32 m row lengths). The plant crop was harvested on 22 December 2015 and the first ratoon, on 26 November 2016.

5.3 Resistance screening

5.3.1 Glasshouse trial

Introduction

Transmission research clearly showed that virus-infested juice applied to test plant leaves, using an abrasive pad, reliably leads to high levels of infection in susceptible varieties. Disease expression has been seen within 12 weeks suggesting that short-term pot experiments are suitable for a rapid varietal resistance screen.

A preliminary glasshouse experiment was therefore conducted in Pasuruan; test plants were initiated on 24 April 2019, with inoculation on 28 May 2019 using the normal abrasive pad method and infested cane stalk juice. There were four replications of each test cane with each replicate consisting of one pot - each supporting two plants. Inspections were undertaken every two weeks. There were 47 varieties with 10 of Australian origin (Table 4).

Table 4: Origin of the test varieties screened for SCSMV resistance.

Experiment \ Source	Australia	PTPN 10	PT Kebon Agung	PTPN 11	Resistant standards	Susceptible standards
1	10	10			2	2
2			10	10	2	2

Standards in both experiments were: -

- Resistant: PS 091, GMP 7
- Susceptible: PS864, PS881

5.3.2 Field trial

Introduction

The field screening experiment was undertaken with similar varieties as used in the glasshouse experiment. Some of the variety results cannot be published without permission from the associated company – these have been excluded from this report.

Method

The experiment was planted at the Indonesian Sugar Research Institute (ISRI), Pasuruan in December 2018. The screening trial consisted of 44 varieties. Disease was initiated using a scourer pad soaked in infested cane stalk juice, as per the glasshouse experiment. There were three replicates, with plot size being 1 row x 3 metres. Twelve inspections were undertaken over the experimental period, at two-weekly intervals.

5.4 Yield loss

Introduction

Quantification of SCSMV-associated yield losses is an essential project output. The yield and economic effects of SCSM in Indonesia are relatively poorly understood but are essential for assessing IDM strategy priorities. It is likely the disease is not receiving the industry focus it deserves because it does not kill or severely restrict the yield of individual plants or portions of a crop. This makes disease incidence less dramatic; however, because it affects whole crops, actual yield losses remain profound.

5.4.1 Yield loss in a susceptible crop

Method

In 2015, a disease yield loss trial was established at Kediri by planting different mixtures of healthy and diseased setts of a susceptible variety (PS864). This created disease incidences ranging from zero to 100%. Yield loss assessments were based on the measurement of several yield parameters (stalk population, stalk weight, stalk diameter, sugar content).

A randomised complete block design with five replicates was employed. Plots consisted of four rows, 12 m in length of the susceptible PS 864. All plots were surrounded by 4 width plots of the more resistant ‘BL’ variety, to limit disease spread between plots.

Treatments

There were 10 treatments with the following percentage of diseased buds planted: 0, 5, 10, 15, 20, 25, 30, 50, 75 and 100%.

Disease monitoring

Plots were monitored monthly until the cane became too big to inspect (January in any one year generally). The cane was cut by hand, transferred via mechanical equipment and then weighed (Figure 8).

Figure 8: Harvesting the yield loss experiment at Kediri at the end of the plant crop.

5.4.2 Yield loss as influenced by resistance

A decision was made to further explore yield losses, particularly the influence of varietal resistance. Such information provides data on the level of resistance needed to minimize crop losses – essential information required for the development of an effective IDM strategy.

Method

Six varieties were selected in mid-2017 (resistant, intermediate and susceptible) and diseased and healthy plant sources of these canes created via SCSMV leaf-abrasion inoculation, when the plants were around knee-high. Plants were potted in small polythene bags before planting into field plots at the Indonesian Sugar Research Institute, Pasuruan. The cane was grown from May 2018 until harvest in May 2019.

Plot yields were assessed by hand-harvesting and weighing stalk material from each plot. Measurements included stalk populations, stalk diameter, stalk height and total yield.

5.5 Diagnostic tests

Introduction

A molecular (PCR) test, available pre-project, provided an accurate and useful method for detecting and confirming the presence of SCSMV in specimens, particularly early on in project research. This has been important for confirming the disease status of test canes and sources of inoculum for project experiments. However, a reliable and cheap method for disease detection is needed to assay plant sources under commercial conditions. Several possibilities were investigated including an antiserum-based DIBA assay, a rapid molecular assay (Loop-mediated Amplification (LAMP)) and molecular assay of samples using a nitrocellulose membrane. Each of these assay types was investigated by project staff, either at SRA Indooroopilly or at the University of Bogor, Bogor.

SRA and University of Bogor researchers collaborated, with Bogor undertaking diagnostics for ISRI specimens and SRA developing new tools for survey sample collection and their associated assay. SRA took the lead on molecular and serological assays, while Bogor staff initially focused on serological assays.

5.5.1 Molecular assays (SRA research)

Introduction

Diagnostic tests for SCSMV had previously been developed (Chatenet *et al.*, 2005). An RT-PCR assay for SCSMV was available, a result of previous Indonesian research; primers for the assay were developed and published by Tri Asmira Damayanti (Bogor University) and Lilik Putra (ISRI) (Damayanti and Putra, 2011). The assay is relatively expensive, especially for use on larger numbers of samples; CPf and AP3 primers were available and were tested on project samples.

Quantitative PCR (qPCR), a more sensitive assay, was also investigated. This method offers specific advantages, including suitability for small samples (single insects), reduced cross-contamination (single-tube assay), high sensitivity, high throughput – but it is expensive and requires specialist equipment.

LAMP, a rapid and potentially cheaper molecular assay, was also researched (Mori and Notomi, 2009; Notomi *et al.*, 2000). This technique may find field application under the specific circumstances.

In any molecular diagnostic research, pathogen variation is a potentially serious consideration. Some variation was suspected and was investigated within this project. Australian research focused on the development of a cheap molecular test. Close cooperation between Australian and Indonesian researchers also addressed a serological assay; such tests are specific and cheaper, but less sensitive than molecular tests.

5.5.1.1 RT-PCR test / FTA cards

The RT-PCR assay was used initially as the means to accurately identify SCSMV infection of experimental test and survey plants. The assay successfully diagnosed SCSMV infection in dried and preserved (irradiated) plant samples collected on surveys, and in other experiments.

A significant issue investigated was whether the assay detected the virus only in preserved plant samples or in an alternative preservation system (FTA cards) as well. The cards allow for the extraction, deposit and drying of sap samples on a simple-to-use card system. This allows for easier movement and security of sample extracts, especially when shifting samples between countries. FTA cards are commercially available.

Method

13 plant samples were collected from a Kediri project experiment; sap was applied directly to the cards after leaf maceration. The samples were dried, imported through quarantine into Australia and then assayed using RT-PCR. The following variations in assay were tested: i. one-step RT-PCR using CPf-AP3 primers, ii. two-step RT-PCR using Poty1 RT and CPf-AP3 primers, and iii. two-step RT-PCR using Poty1 and qPCR primers.

5.5.1.2 Quantitative PCR

qPCR is generally suitable only for research purposes, a consequence of the cost of the equipment and the reagents. A big advantage is its high sensitivity and reduced cross contamination.

To further develop the test for SCSMV, known SCSMV sequences were aligned and a phylogenetic tree constructed in Geneious (v8) to show any potential groupings of SCSMV isolates. The alignment was used to target primer design. Greater similarity was found at the 3’ end of genome; 10 primer sets were developed for this end of the viral genome. The most conserved sequences were selected to form a generic primer set.

5.5.1.3 Rapid ‘LAMP’ assay

To further develop the LAMP assay, an extract was obtained from fresh leaf material using the following method: the leaf material was ground (shaken) for two minutes in a 5 ml tube,

using a 12 mm hardened steel ball bearing, in 1 ml of TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA). The extract was then allowed to settle on ice. Undiluted extract, or 1:10, 1:100 or 1:1000 dilutions in TE buffer were used to determine the most appropriate tissue:TE ratio for assay. Four x 0.5 cm^2 pieces of leaf material from green leaves (usually second to fifth unfurled leaf) were selected for routine LAMP assays and these were ground in 1 ml of TE buffer. This was used undiluted in the LAMP assay using 1 µl of supernatant. Deleting sample dilution minimised cross contamination.

Primers were designed using LAMP Designer 1.15 software (PREMIER Biosoft) and based on SCSMV full genome sequences in the GENBANK database. Primer sets were compared initially using the hydroxyl naphthol blue detection method (Goto *et al*., 2009). This worked well on purified RNA (Figure 1) from other countries held at SRA but didn't for the Indonesian samples. Primers were re-designed and tested using both the colorimetric and Genie II systems (Optigene, Ltd). In Indonesia the colorimetric system didn't work well so all final assays were completed using the Genie II. Better results were obtained. LAMP assays used Isothermal Master Mix (Optigene) in a 25 µl reaction with primer concentrations as recommended (F3/B3 at 5 pmol each, LoopF/LoopB at 10 pmol each and FIP/BIP at 20 pmol each). Cycling conditions consisted of 20 minutes at 65°C amplification followed by annealing from 98°C to 80°C ramping at 0.05°C per second. The melt curve was used to confirm a positive or negative result.

RT-PCR was used to check the accuracy of the LAMP and DIBA assays by testing the same plants for the presence of SCSMV. This was undertaken using the primers described by Damayanti and Putra, 2011, with some modifications. RNA was extracted using QIAGEN RNEasy Mini Kit and the quality and quantity checked by a nanodrop spectrophotometer. A One-Step RT-PCR (QIAGEN) kit was used for detection of SCSMV in a 20 µL reaction using 1 x OneStep RT-PCR Buffer, 1 x Q-Solution, 0.2 mM dNTP primer mix, 0.4 µM of AP3 and CPF primers, 0.2 U RNAsin, 0.8 µl OneStep RT-PCR Enzyme Mix and 2 µL of template RNA. Thermal cycling included reverse transcription at 50°C for 30 minutes, followed by denaturation at 95°C for 15 minutes. This was immediately followed by 35 cycles of 94°C for 30 seconds, 62°C for 30 seconds and 72°C for 1 minute and a final extension at 72°C for 10 minutes. Amplicons were analysed by gel electrophoresis.

Using sequence information already available, suitable LAMP primers were developed. The program 'LAMP Designer' was used to design 6 primer sets. The method involves a simple DNA extraction, dilution, addition of an enzyme and reaction components before a single incubation.

Initial research found that the original primers did not detect all viral isolates – detection of SCSMV in Sri Lankan and Myanmar samples worked well, but SCSMV was not detected in the Indonesian samples. The LAMP primers were therefore re-designed to detect all sources of the virus.

The blue-purple colour reaction was not sufficiently different to differentiate a positive result; a different colour reaction was then researched (yellow / black).

5.5.2 Antiserum assay (SRA / University of Bogor)

5.5.2.1 Antiserum development

SRA

Australian diagnostic research, though focusing on molecular techniques, also had a small component that suited SCSMV antiserum development. Sequence variation in the coat protein region was searched for, and variation detected, in samples from countries other than Indonesia. An Australian commercial company capable of generating antisera, based on sequence data, was employed to undertake antiserum development. These antisera were tested in experiments undertaken at Bogor in August 2018. Specificity was initially investigated using the Western blot technique and dot-blot assay.

Serological detection methods such as DIBA (dot immuno-binding assay) and ELISA (enzyme-linked immuno-sorbent assay) potentially could be employed using the antiserum.

DIBA

Antisera for DIBA were produced to four different SCSMV targets: this included a complete expressed coat protein based on GENBANK accession sequence GQ388116; and three epitopes identified by GenScript and designated as Peptide 1; Peptide 2; Peptide 3 (GenScript, USA). Antisera was received dried and resuspended to a concentration of 500 µg/ml in sterile water. The aliquots were stored at -80 °C. The peptides were also received and resuspended and stored at 10 mg/ml.

Identical dot blots were made for analysis: 0.5 g of fresh leaf material was ground in 1 ml of PBS buffer (Phosphate Buffered Saline pH 7.5, Sigma) using either a mortar and pestle, or in a 5 ml tube with a 12 mm steel ball by shaking vigorously for 2 minutes. Two asymptomatic (healthy) plants, one SCSMV symptomatic, one SCMV symptomatic, one suspected mixed infection was prepared. The extract supernatant was diluted in a series from 1:10 to 1:1000 in PBS, and 1 µL of each was spotted onto a nitrocellulose membrane and allowed to air dry. The peptides were likewise diluted to 100 µg/mL prior to making a dilution series and dotting on the membrane as positive controls. PBS was dotted as a negative control. The membranes were air dried prior to detection.

The DIBA assay used was a modification of established methods (Graddon and Randles, 1986; Hibi and Saito, 1985). The following steps were completed at room temperature with constant shaking. The protein-binding sites of the membrane were saturated by blocking with Blocking Buffer A (PBS solution containing 2.6 % skim milk powder) or Blocking Buffer B (Blocking buffer A plus 0.1 x volume of healthy plant extract ground as above) for 1 hour. The blocking buffer was replaced with antisera diluted in Blocking Buffer A (1:1000 and 1:10,000) and incubated at 1 hour. The membrane was washed three times for 3 minutes each using Blocking Buffer A, draining the membrane each time. The secondary antibody (goat-anti-rabbit-AP; Sigma) was diluted 1:1000 in PBS + 1% BSA, and the membrane incubated at 30 minutes. The membrane was washed twice for 3 minutes each in buffer AP 7.5 (0.1 M Tris-HCl pH 7.5, 0.1 M NaCl, 2 mM $MgCl_2$ and 0.05 % Triton X-100) and twice for three minutes each in buffer AP 9.5 (0.1 M Tris-HCl pH 9.5, 0.1 M NaCl, 5 mM $MgCl_2$). SigmaFast NBT-BCIP Tablets (Sigma) were prepared as per manufacturer's recommendations and added to the membranes. The membranes were incubated in the dark with constant shaking until dots appeared, approximately 30-60 minutes. The reaction was stopped by removal of the SigmaFast solution and addition of stop buffer (10 mM Tris-HCl pH 7.5, 5 mM EDTA).

University of Bogor

The University of Bogor research focused on the development of SCSMV antisera based on the production of antibodies in rabbits. An essential requirement is the location of a pure source of SCSMV. In Indonesia, dual sugarcane infections with SCSMV and SCMV (sugarcane mosaic virus) are common. There were six main thrusts to the University of Bogor approach: i. identification of a pure SCSMV source, ii. virus propagation for

purification, iii. production of a polyclonal antibody to SCSMV, iv. choice of the serological assay method, v. cloning of the coat protein (CP) for SCSMV

5.6 Integrated Disease management

Introduction

The ultimate aim of the research outlined in this report was to develop a SCSM management strategy that is practical, effective, cheap and able to minimise disease incidence and crop losses. Research outcomes were considered and an IDM proposed to take all the above requirements into account. Industry-specific considerations were taken into account as well as feedback from extension activities undertaken during the course of the project.

5.6.1 Extension activities

Project extension activities related to the project were undertaken both during surveys of the different cane-farming regions of Indonesia as well as at several Indonesian research institutions: i. In January 2017 a focus group discussion (FGD) was held at the Indonesian Sugar Research Institute (ISRI), Pasuruan. Project research outcomes were extended in the presence of Government officers, scientists, sugar factory staff, sugarcane farmer and University lecturers; ii. During the Sulawesi survey in 2017, the survey team extended project results to factory staff and local sugarcane farmers; iii. a little later in the same year, a two-day training was offered to around 50 staff from Camming Sugar Factory; iv. In November 2018, a national workshop was conducted at the Indonesian Fibre Crop and Sweetener Research Institute, (IFCSRI) in Malang, with about 200 participants; v. In December 2018, training similar to that conducted in Sulawesi was conducted in Sumatera; vi. On 25 June 2019, the project team presented results from the whole project a range of staff from PTPN X, a major factory conglomerate. This was well received and enabled many questions about project work, and indeed other diseases, to be answered.

6 Achievements against activities and outputs/milestones

Objective 1: Assess the importance of SCSMV in Indonesia by defining the distribution and incidence of the disease in Java, Sumatera, Sumba and Sulawesi.

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No.	Activity	Outputs/ milestones	Completion date	Comments
1.1	Assess the occurrence and severity of SCSM in Sumatera, Sulawesi, Sumba and Sumbawa.	Spatial distribution map for each Province and the whole of Indonesia. Assessment of the health of commercial crops and nurseries.	November 2018	Surveys were undertaken over a three-year period. SCSMV was found for the first time in several Provinces of Indonesia for the first time and seemed to be associated with the transfer of planting material from Java.

PC = partner country, A = Australia

Objective 2: Determine the potential for disease escalation and the conditions required to maintain disease-free nursery cane by investigating SCSMV epidemiology

No.	Activity	Outputs/ Milestones	Completion date	Comments
2.1	Collect, identify and breed the 2 known insect vectors of SCSMV and re-test their ability to transmit SCSMV.	Confirmed role of two aphid species in SCSM transmission.	April 2019	The two suggested vectors of SCSMV were not found to be vectors of the disease. No other arthropod vectors have yet been associated with transmission, but the virus was detected in some insects collected during SCSM surveys.
2.2	Test freshly expressed infected cane juice as a mechanism for SCSM transmission.	Confirmed mechanisms for disease spread; results will be used to design resistance screening tests.	November 2018	Dipping sett material in fresh juice or applying the same juice on cutting equipment did not lead to significant disease transmission. Mechanical transmission therefore does not appear to be significant in the epidemiology of SCSMV.
2.3	Monitor disease escalation in selected commercial crops	Data on the speed of escalation of the disease and the pattern of spread.	January 2019	Disease spread data was gathered for two sites – Kediri and Pasuruan. Disease spread was not as rapid as expected and there did not appear to be strong spread patterns.
2.4	Test mechanisms for disease spread in simulated field situations.	Data confirming mechanisms for disease spread under field conditions.	June 2019	All attempts to spread the disease, apart from infected planting material and application of infected juice to wounded leaf surfaces, were not very effective.

PC = partner country, A = Australia

Objective 3: Assess the economic importance of SCSM in Indonesia by defining the yield losses caused by SCSM

No.	Activity	Outputs/ Milestones	Completion date	Comments
3.1	Evaluate the relationship between % infected stools and final yield	Yield and monetary losses from SCSM identified in a highly susceptible variety.	June 2018	There was a strong correlation between infection levels in plots and yield – in plant, first ratoon and second ratoon crops. Losses were between 17-26% cane yield (tonnes cane / ha).
3.2	Evaluate the relationship between disease resistance and SCSM-associated yield loss.	Resistance threshold for minimising yield losses identified; data on spread in varieties of differing resistance.	May 2019	There were minimal losses from SCSMV in resistant varieties and significant losses in susceptible varieties. This highlights to magnitude of potential losses but also that resistant varieties are an effective management strategy.

Objective 4: Identify the resistance of varieties in order for farmers to minimise the level of SCSMV in their crops by developing a rapid varietal resistance screen

No.	Activity	Outputs/ Milestones	Completion date	Comments
4.1	Develop a resistance screen for assessing the resistance of important clones and varieties.	Resistance ratings for important clones and varieties	June 2018	Application of infected stalk juice to wounded leaf surfaces appears to be a very suitable method for assessing varietal resistance
4.2	Develop a resistance screen that provides for testing very large numbers of clones in the plant breeding program	A resistance technique that can be used in early stage plant breeding selection programs.	June 2018	As above – the same technique can be used in early stage screening (depending on the size of the screening program)
4.3	Conduct a plant breeding workshop to consider implications from the results from resistance screening research	An efficient breeding and resistance screening strategy for implementation in Indonesia. Identification of ramifications for the Australian industry	Not completed	Funding for the work did not allow this to be completed.

Objective 5: Enable the Indonesian farming community to reduce disease incidence in newly-planted crops by identifying SCSMV in nursery cane through cheap, rapid detection techniques

No.	Activity	Outputs/ milestones	Completion date	Comments
5.1	Development of antibody-based diagnostic tools	Produce a specific antiserum and develop diagnostic tests	June 2019	Effective antisera were developed to SCSMV with initial testing of a DIBA assay.
5.2	Development of nucleic acid-based diagnostic tools	Use existing sequence data to develop LAMP	June 2019	Both RT-PCR, qPCR and LAMP assays were developed and shown to be effective and suitable for use in quarantine and in industry applications (depending on the assay)
5.3	Compare 5.1 and 5.2	Compare anti-body-based and nucleic acid-based diagnostic techniques under different conditions	June 2019	Limited comparisons were made but sufficient for the Indonesian sugarcane industry to decide how to deploy the tests.

Objective 6: Reduce SCSM crop incidence through the adoption of an effective IDM strategy

No.	Activity	Outputs/ milestones	Completion date	Comments
6.1	To provide appropriate training in IDM	600 smallholder farmers and factory field staff trained in IDM strategies.	July 2019 (partial completion)	The nature of the disease was much better understood during the course of the project and training was provided to industry staff in various Provinces around Indonesia (e.g. Sulawesi and in Java)
6.2	To identify industry leaders and champion farmers (Government and private sector).	Selection of target farmers to use in extension activities	July 2019 (alternative actions)	No champion farmers were selected but rather personal communication with sugar factory staff and farmers was instigated. As a result, staff from the milling groups PTPN X, PTPN XI and private companies in Java and other provinces were educated on IDM strategies. Staff from these milling groups are also sending varieties for SCSMV resistance screening at ISRI
6.3	To conduct hands-on training, workshops (200 participants), seminars and field days to enable adoption of improved practices.	Raised awareness amongst the general farming community of IDM strategies for stem borers and mosaic disease	July 2019	Factory staff, farmers and industry researchers were given hands-on training at various locations around Indonesia. Staff from a key conglomeration of factories, that controls crop processing at a number of locations, was addressed just after the final project meeting. These staff are influential at several sites; project researchers not only addressed key issues associated with SCSMV but also a range of other diseases and pests

6.4	To establish demonstration plots and production systems at 7 sites.	Clear examples of successful IDM strategies enabling public/farmer visualisation of IDM.	July 2019 (alternative actions)	The 7 sites were not selected as the focus remained on completing research into such things as the potential vectors of SCSMV and screening for varietal resistance to the disease. The delay in establishing issues related to the epidemiology of the disease meant that selection of demonstration sites was not the highest priority for SCSMV research and extension. Such extension will remain an on-going activity for the Indonesian industry.

PC = partner country, A = Australia

7 Key results and discussion

7.1 Disease distribution

7.1.1 Sumatera and West Java

Diseases

SCSM was seen in four of the six mill areas surveyed with infection levels as high as 35% infected stools. Communication with local staff suggested that most of the SCSMV-infested crops arose from planting material recently sourced from Java; nurseries established from other material were affected to a much-lesser degree. SCSM was the most commonly observed sugarcane disease.

A number of other diseases were observed during the survey; these included smut, leaf scald, *Xylaria* root / stem rot, target blotch, ring spot, yellow spot and orange rust. Inspection timing was not ideal for leaf diseases so their incidence was low and not necessarily indicative of their prevalence in the area. There was some evidence that white speck disease was present; this may be a first record for Indonesia (this needs confirming by specific identification of the pathogen).

Potential SCSMV vectors

A range of insect species were collected from sugarcane exhibiting SCSM symptoms; these ranged from planthoppers (including *Perkinsiella* sp), aphids, a psyllid, brown weevil, whitefly and *Proutista* sp. The results of SCSMV assay are presented in Table 5.

Table 5: The number of arthropod specimens collected during the Sumatera survey and the percentage testing positive for SCSMV.

Species	Number	Negative	Positive	%
Perkinsiella sp.	28	25	3	10.7
Proutista sp.	3	3	0	0.0
Whitefly nymph	4	4	0	0.0
Dicladispa sp.	5	5	0	0.0
Brown weevil	3	3	0	0.0
Psyllid	2	2	0	0.0
Aphid	2	2	0	0.0

A small percentage of *Perkinsiella* individuals tested positive to the virus, but no other potential vectors.

Pests

The two major moth borer species (*Scirpophaga excerptalis* and *Chilo sacchariphagus*) were the most common pests detected. Others included *Chilo auricilius*, giant borer (*P. castanea*), longicorn beetle, woolly aphids, white flies, army worms, the planthopper *Perkinsiella saccharicida, s*cale insects and meally bugs.

7.1.2 Sulawesi

Diseases

SCSM was observed in crops in four out of the seven factory areas. Further questioning revealed that as for Sumatra, SCSM was present in crops established from planting material sourced in Java. Molecular assay of leaf samples with suspect mosaic symptoms confirmed the presence of SCSMV. This is a first record for Sulawesi. In one factory area, assays suggested two mosaic pathogens are present in the region – SCSMV and SCMV.

Smut was also observed in crops in a similar number of mill areas and this too is a first record for Sulawesi. Its characteristic symptoms make it an unmistakeable disease. Other observed diseases included leaf scald, yellow spot, red leaf spot, orange rust and pokkah boeng. The results of the survey are outlined in Figures 9-12.

SCSMV associated with arthropods

As for the Sumatra arthropods collected, a range of species were collected from the Sulawesi survey. *Perkinsiella* sp. were common while a range of other potential vector species were also collected (Table 6). Other pests included, woolly aphids, white flies, *s*cale insects and mealybugs.

Table 6: The number of arthropod specimens collected during the Sulawesi survey and the percentage testing positive for SCSMV.

Species	Number	Negative	Positive	%
Planthopper	12	10	2	16.7
Pink mealybugs	7	7	0	0.0
Wasps	4	4	0	0.0
Palm planthopper	1	0	1	100.0
Perkinsiella sp.	3	3	0	0.0
Lace wing (Tingidae sp.*)*	9	3	6	66.7
Aphids	4	0	4	100.0
Woolly aphids	5	5	0	0.0
Proutista sp.	5	5	0	0.0

Pests

The two major moth borer species (*Chilo auricilius* and *Chilo sacchariphagus*) were also observed in some crops. These borers have been seen on Sulawesi previously, but published reports are lacking. Minor occurrences of *Sesamia inferens* were noted, especially close to rice fields; rice is a preferred host for *S. inferens*.

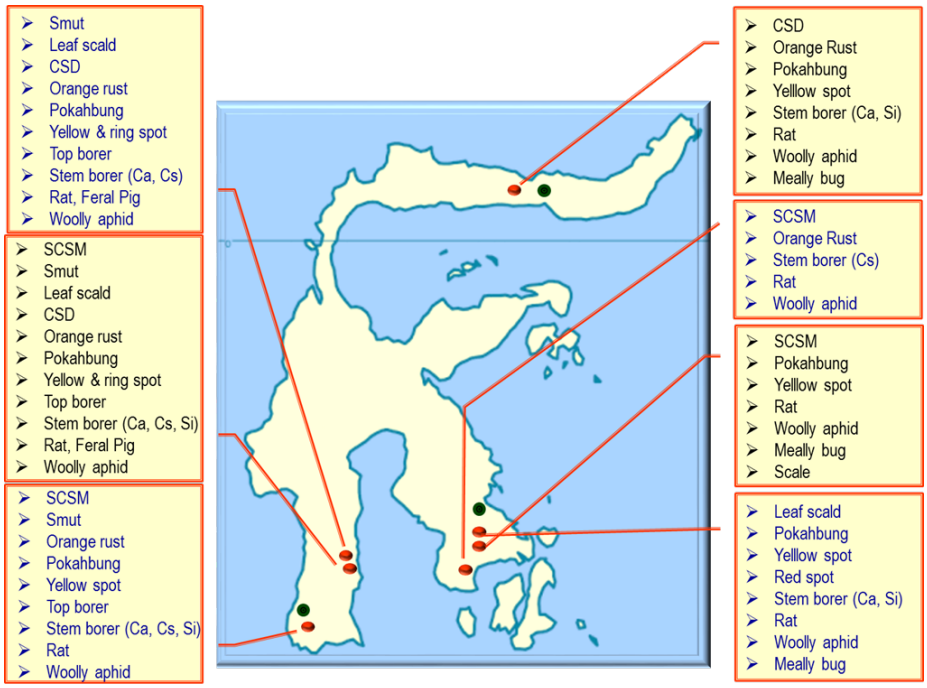


Figure 9: Broad overview of the pests and diseases detected in the crops inspected in Sulawesi.

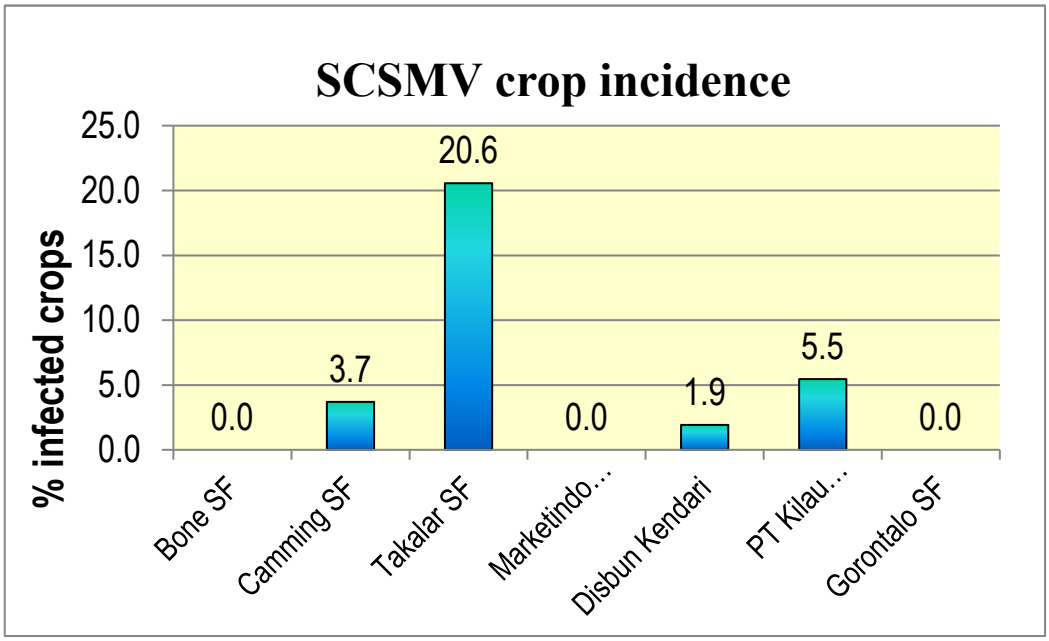


Figure 10: The in-crop incidence of SCSM in crops inspected in each factory area; more disease was observed in those where planting material had been sourced from Java.

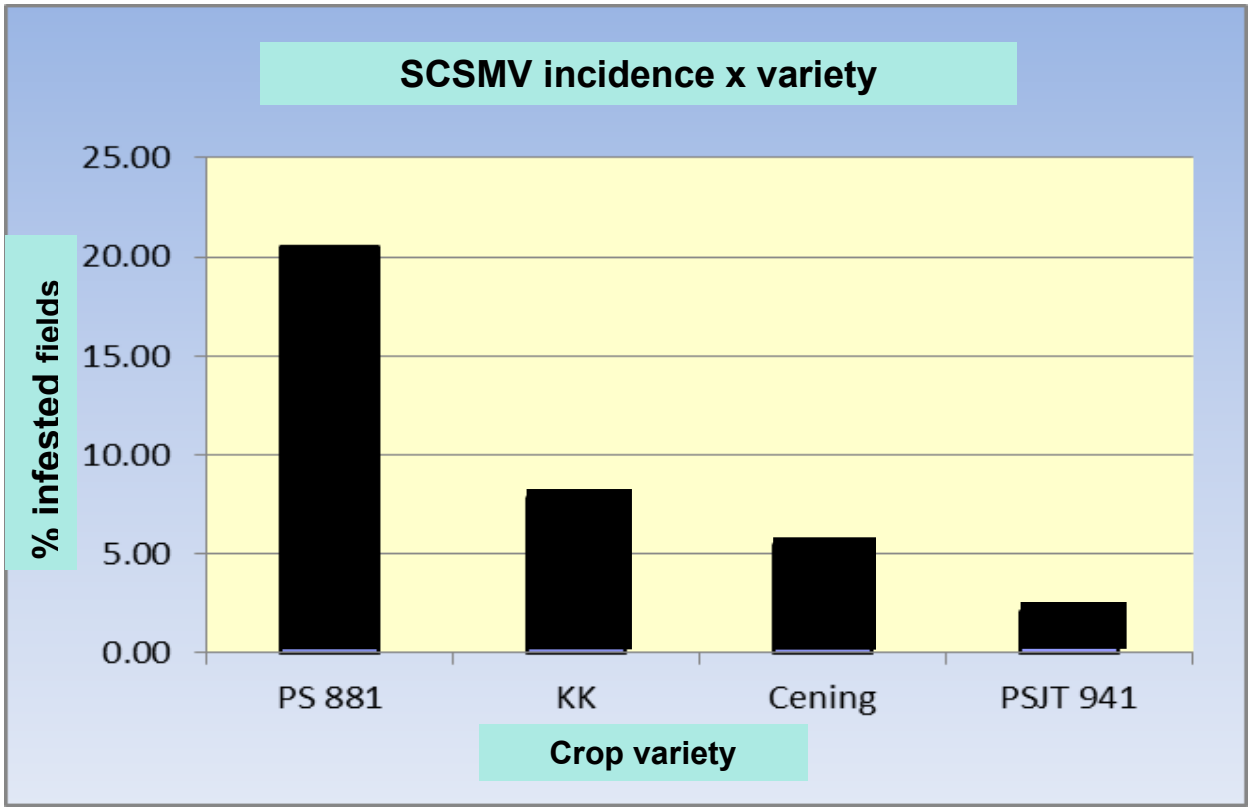


Figure 11: The relationship between variety and SCSMV incidence in the Sulawesi survey.

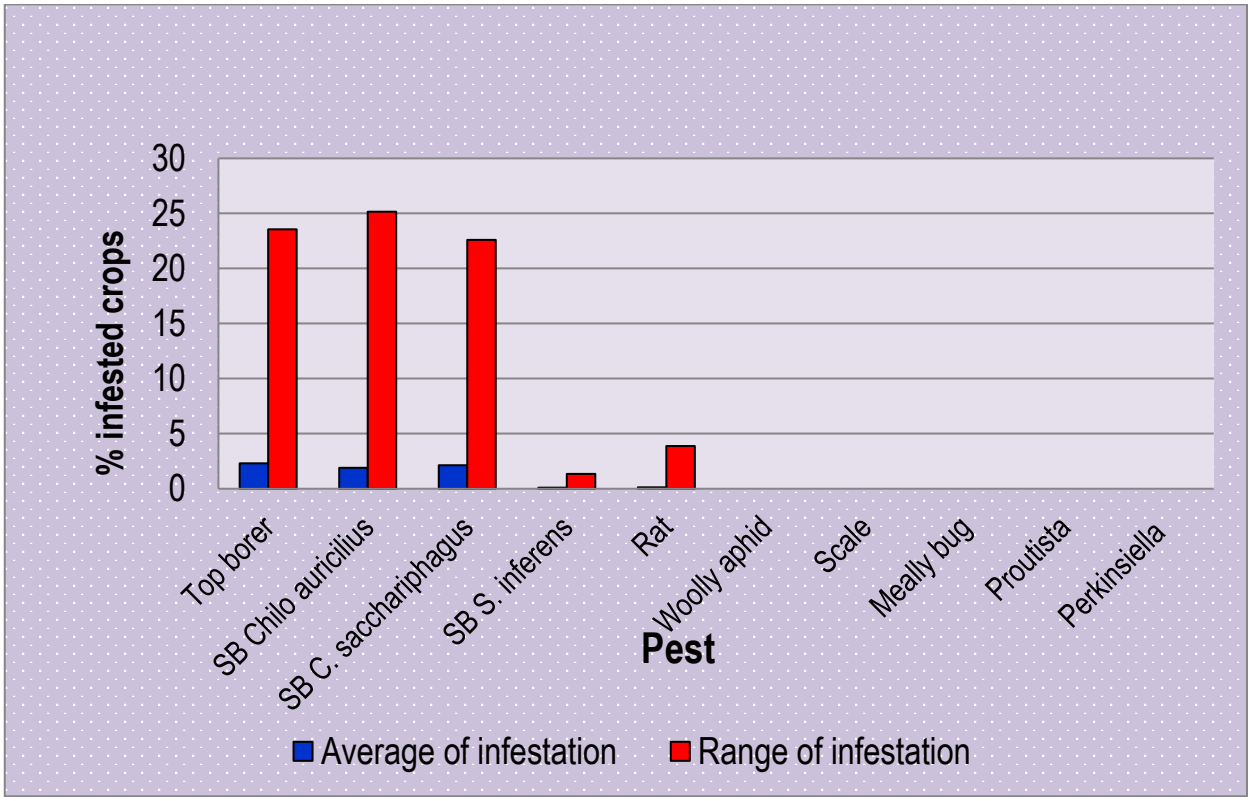


Figure 12: The incidence of various pests in crops in the 2017 Sulawesi survey

SCSMV associated with arthropods

7.1.3 Eastern Archipelago

SCSMV associated with arthropods

A smaller range of species were collected from the Eastern Archipelago survey. *Eumetopina* sp were not uncommon (not *Perkinsiella* sp.) and little else likely to host the virus. SCSMV was found associated with a significant percentage of *Eumetopina* (planthopper) specimens (Table 7).

Table 7: The number of arthropod specimens collected during the Eastern Archipelago survey and the percentage testing positive for SCSMV.

Species	Number	Negative	Positive	%
Thrips	1	1	0	0.0
Beetles	11	11	0	0.0
Eumetopina	12	10	2	16.7

Discussion

The surveys suggested that SCSMV has spread to neighbouring islands and production areas via diseased planting material. This is most unfortunate since it means that the virus is now affecting previously disease-free regions. Action is needed that reflects the gravity of the situation; Governments and businesses need to understand the immense economic implications of disease spread. Keeping pests and diseases out of a production area has huge economic implications for the longer-term viability of an agricultural operation. All too often, short-term monetary return governs decisions rather than long-term crop profitability. Once a disease invades a region, it is usually impossible to eliminate it; this will now be the case for SCSMV in regional Indonesia.

Accurate and reliable identification of both pests and diseases in survey material is important for future reference. Specimens, especially first records, need to be confirmed using both morphological and molecular assays (where possible). An aim of project staff is to publish any reliable first records in a recognised journal to ensure accurate data on sugarcane pest and disease incidence is available to others, both now and in the future. Further identifications are required to complete this process.

An interesting finding was the association of SCSMV with several species of insects collected on diseased sugarcane; these include species of *Perkinsiella*, *Eumetopina*, a *Tingidae* (lace wing) species and aphids. A species of aphid is known to transmit SCMV; there is a possibility a similar species may transmit SCSMV. It should be noted that association does not mean causation and simply finding the virus in association with ese species doesn't imply their involvement in disease transmission.

7.2 Epidemiology

Any consideration of the study of disease spread inevitably draws in two different aspects – transmission and spread. These are addressed in the results presented below.

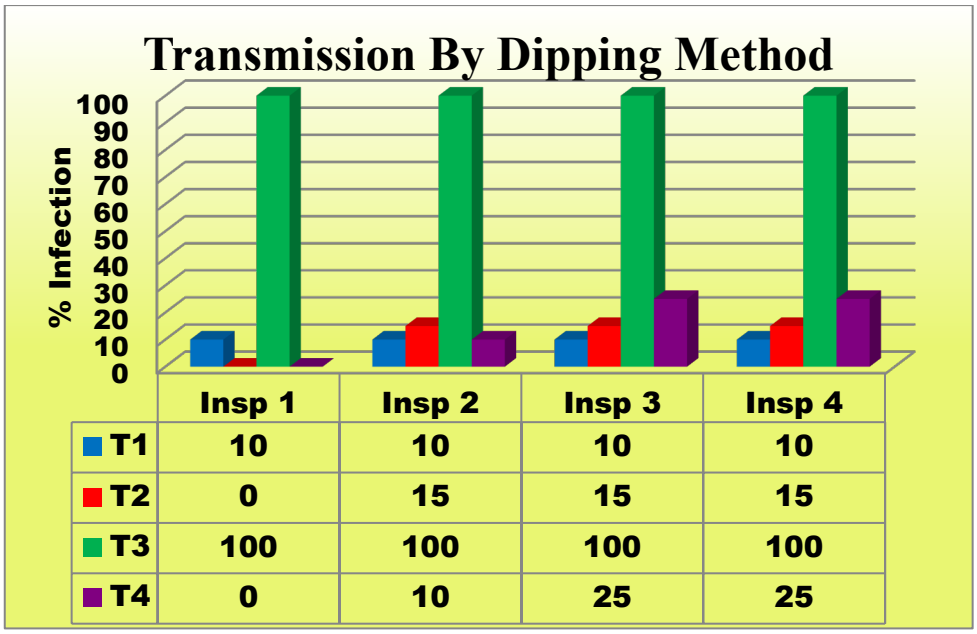
7.2.1 Virus transmission

7.2.1.1 Mechanical transmission

There are several ways pathogens may be transmitted mechanically; these include via cutting equipment (knives, machines such as planters and harvesters), in infested juice, and even by windblown rain (the latter especially with bacterial diseases).

7.2.1.1.1 Juice inoculation of setts

There was very little disease in plants arising from setts dipped in infested juice, while cane grown from diseased stalks (positive control) had a high disease incidence (nearly 100%) (Figure 13). The presence of SCSM in both healthy controls (disease-free test plants and healthy-juice inoculations) suggests some cross contamination occurred. The data suggested that infection of the cut end of setts via infected juice is not a major form of disease transmission.

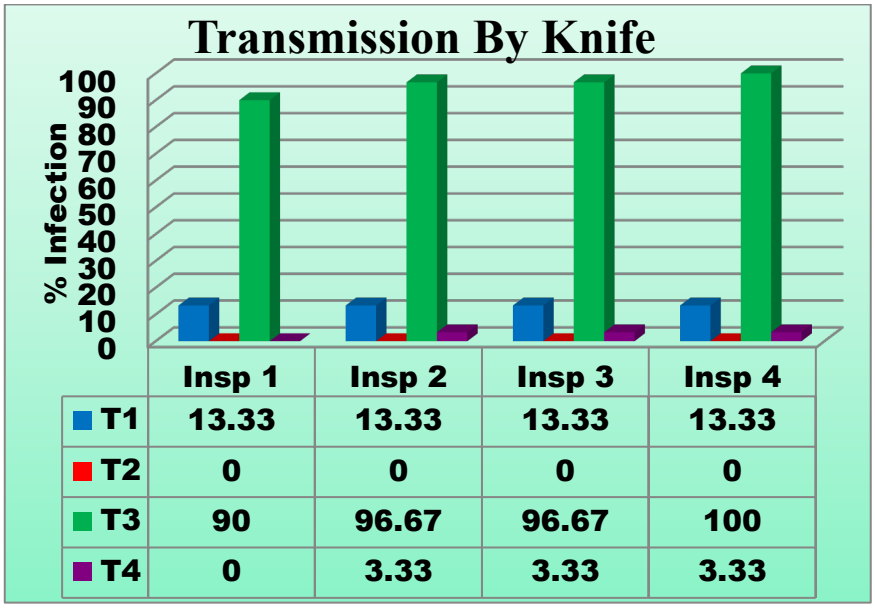


	Insp 1	Insp 2	Insp 3	Insp 4
T1	10	10	10	10
T2	0	15	15	15
T3	100	100	100	100
T4	0	10	25	25

Figure 13: Sett dip inoculation with infested with SCSMV. Treatments: 1=infested juice; 2=disease-free juice; 3=disease control; 4=healthy control. Limited disease transmission occurred via dipping in infested juice.

7.2.1.1.2 Knife transmission

There was an apparent low transmission when secateurs were used to cut diseased cane before healthy stalks. High disease incidence characterised the positive control (diseased planting material) and there was a low incidence in the negative control (Figure 14).



	Insp 1	Insp 2	Insp 3	Insp 4
T1	13.33	13.33	13.33	13.33
T2	0	0	0	0
T3	90	96.67	96.67	100
T4	0	3.33	3.33	3.33

Figure 14: Results from experiment 2 investigating SCSMV knife transmission. Treatments: 1=diseased cane cut – then healthy stalk; 2=healthy cane cut then healthy stalk; 3=disease control; 4=healthy control.

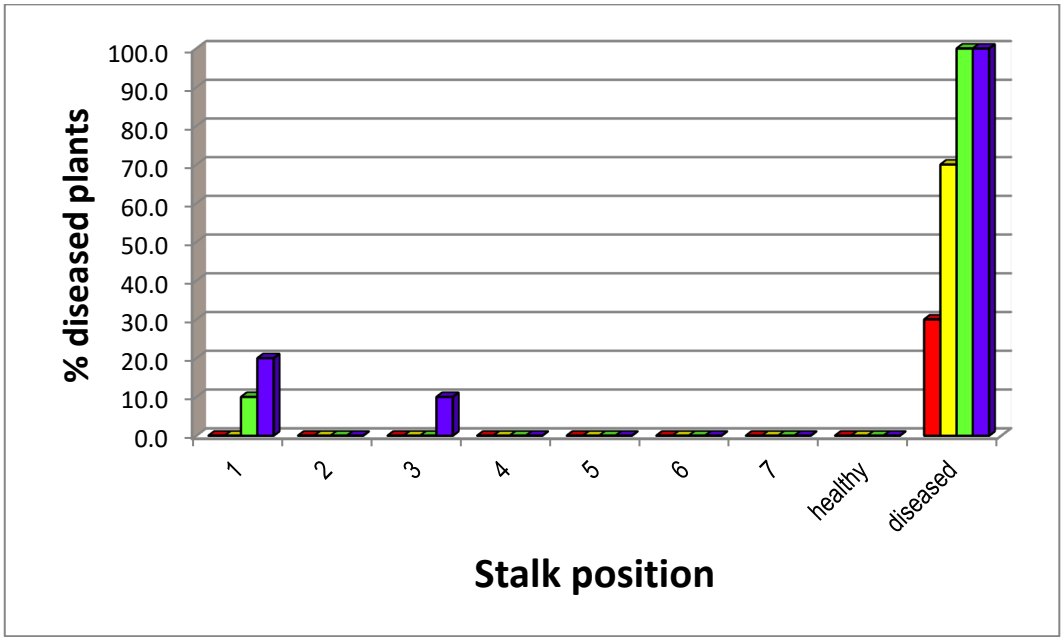


Figure 15: SCSM incidence in single-bud setts cut with a SCSMV–contaminated knife in order from the top of the stalk downwards; the variety used was the susceptible PS864.

The negative and positive control treatments exhibited zero and 100% disease, as expected, showing that no contamination of the test plants had occurred and that juice used to contaminate the knife surface was infective. Low levels of SCSM were seen in just a couple of treatments; this suggests a small amount of knife transmission may occur but that this is unreliable and at low frequency (Figure 15).

In a repeat experiment where a contaminated knife was used to cut single-eye setts consecutively down a stalk (from top to bottom), low transmission was again observed. The results are outlined in Figure 16.

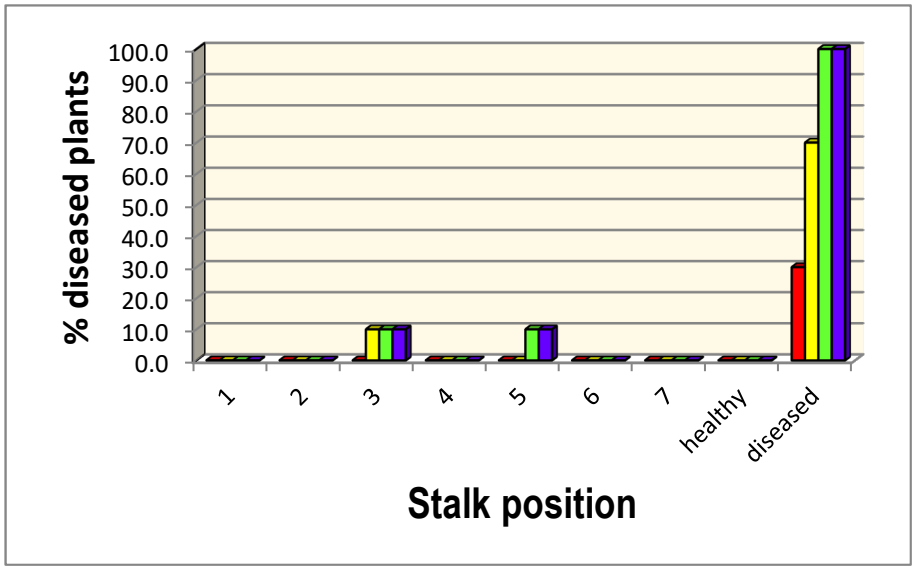
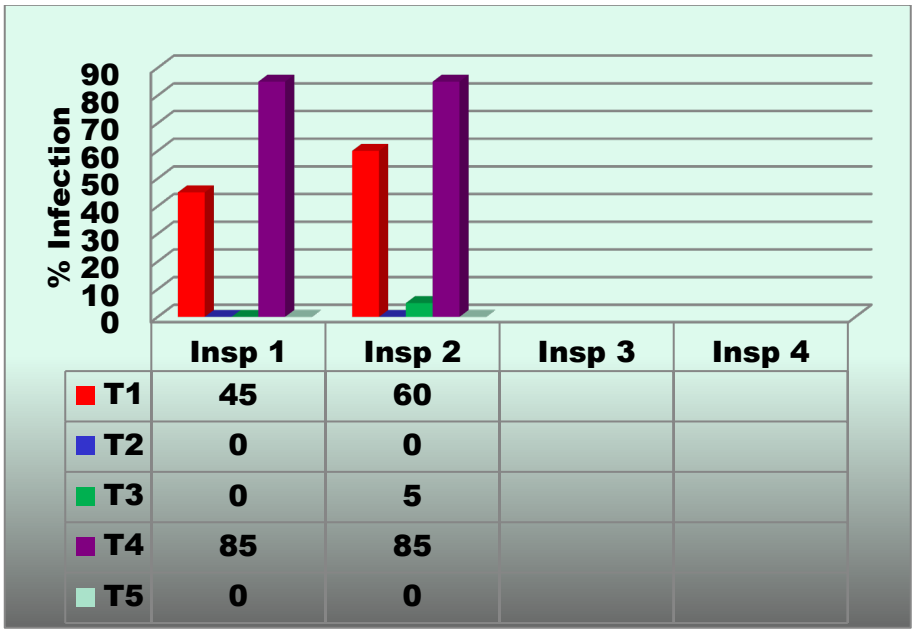


Figure 16: SCSM development in plants grown from setts cut with an SCSMV-infested knife. The cutting number refers to setts cut consecutively after the infested knife was contaminated (sett 1=first sett cut after the knife was dipped in diseased juice; sett 2= second sett cut etc).

The negative and positive control treatments again exhibited zero and 100% disease. The efficiency of knife transmission is very low and appears not to be a major form of disease transmission.

7.2.1.1.3 Transmission via abrasive pad

The best transmission of SCSMV in all transmission experiments was via the application of infested stalk juice to leaves using an abrasive (scourer) pad (Figure 17). Up to 60% of test plants became diseased using this method. In contrast, when cane leaf juice (vs stalk juice) was used as the inoculum source, infection rates were very low.



	Insp 1	Insp 2	Insp 3	Insp 4
T1	45	60		
T2	0	0		
T3	0	5		
T4	85	85		
T5	0	0		

Figure 17: Results from experiment 3 investigating leaf inoculation with SCSMV-infested stalk / leaf juice and an abrasive scourer. Treatments: 1=diseased stalk juice; 2=healthy stalk juice; 3=diseased leaf extract; 4=diseased planting material (disease control); 5=healthy planting material (healthy control).

7.2.1.1.4 Transmission via the pin-prick (sein) method

Transmission occurred when a needle was inserted through a diseased leaf into the young test plants; however, the level of transmission was not high (20%). No infection occurred when the needle was dipped into infested juice and then inserted directly into the spindle leaf of young test plants (Figure 18).

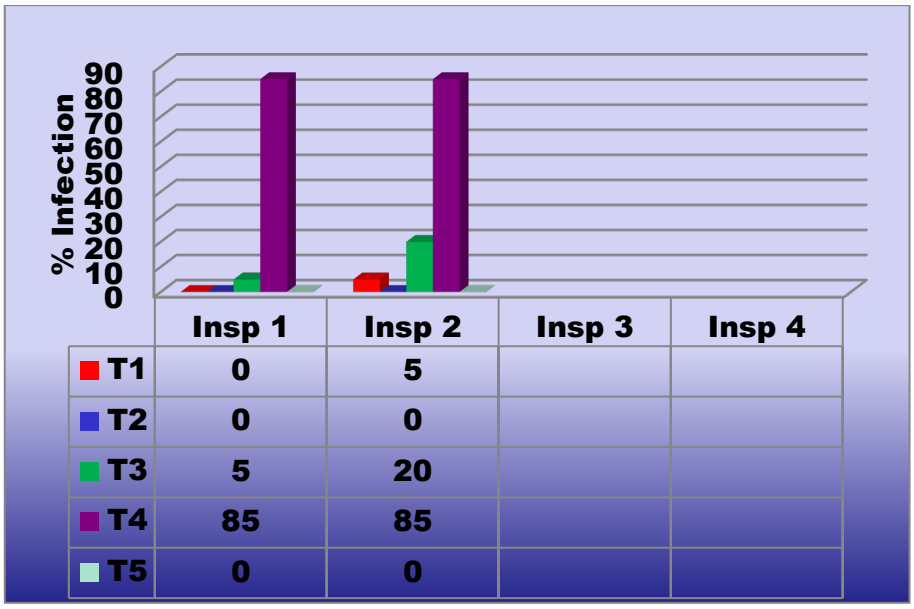


Figure 18: Results from experiment 4 investigating a SCSMV transmission using the pin prick method. Treatments: 1=needle dipped in infested cane juice; 2=needle dipped in healthy cane juice; 3=needle pushed through diseased leaf into the spindle leaf; 4=diseased test plants; 5=healthy test plants.

Discussion

The most effective SCSMV transmission was with the application of infested cane juice (from diseased stalks) to leaves using a scourer pad. There is little doubt that under normal circumstances knife transmission provides an insignificant mode of SCSMV transmission, contrary to pre-project reports. This is a positive development and makes management of SCSM simpler. However, it should be noted that low levels of transmission did arise from a contaminated knife – so knife transmission is possible. It is yet to be decided where and when this may be of significance. There is no logical explanation for why SCSM symptoms were observed in plants growing from buds at different heights on the original stalk material; this is likely to be a random effect.

A sett-dip in infested juice also does not show promise for disease transmission. This contrasts with some reports from the previous project where this form of transmission may have been successful.

Sugarcane mosaic virus (SCMV, another 'poty' virus) is known to be transmitted mechanically by rubbing infested juice on the leaf surface of disease-free test plants. This method is simple and provides a suitable inoculation method for screening varieties for disease resistance - and is used world-wide. This looks to be the best method for varietal resistance screening.

7.2.1.2 Vector transmission

It was difficult to breed populations of some of the vectors on diseased sugarcane, however populations of the sugarcane aphid (*Melanaphis sacchari*) and planthopper (*Perkinsiella saccharicida*) multiplied particularly well. There were moderate populations of the mite (*Olygonyrcus exsiccator*) and the mealy bug (*Saccharococcus sacchari*), while the sugarcane white fly (*Aleurolobus barodensis*) failed to colonise the host plants.

There was no trend in the SCSMV data with male vs female Perkinsiella individuals so combined results only are summarised in Table 8.

Table 8: Insect SCSMV acquisition experimental results summary. The numbers indicate the number of individuals or groups of individuals that tested positive for SCSMV from each replicate at each timepoint. Only those species are presented in the table where some association with SCSMV was found – there were no positive SCSMV assays for the other species.

Week collected	**Rep 1**	**Rep 2**	**Rep 3**
Perkinsiella saccharicida (analysed as individuals)			
2 weeks	1 / 5	1 / 5	0 / 6
4 weeks	1 / 12	1 / 12	0 / 15
6 weeks	1 / 11	0 / 7	0 / 9
Melanaphis sacchari (analysed in batches of 5-6 aphids each)			
2 weeks	0 / 5	0 / 5	0 / 5
4 weeks	1 / 5	0 / 5	1 / 3
6 weeks	0 / 5	0 / 5	0 / 5

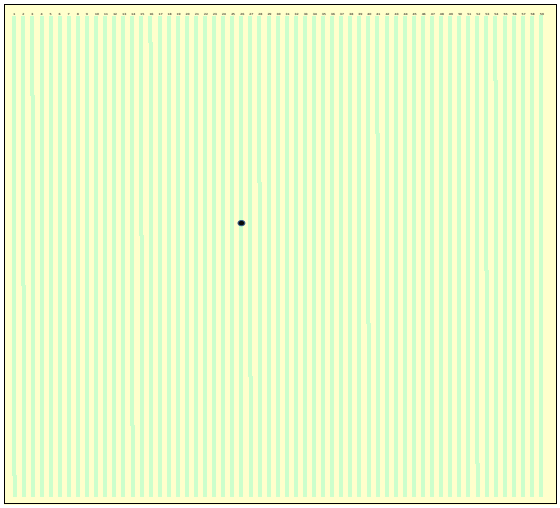
The woolly aphid (*Ceratovacuna lanigera)* was analysed in batches of 4-6 aphids each, and no positive results were found. The planthopper (*Eumetopina* sp) samples were only collected at one timepoint and separated into 1 nymph, 6 males and 10 females; 1 female only returned a positive result.

Mites were analysed in groups as they are extremely small. There were no conclusive assay results; one sample had a possible faint band however this was not sufficient to draw any conclusions and the positive result could not be replicated.

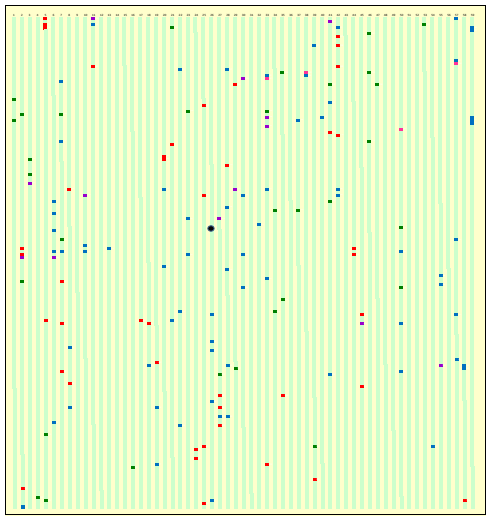
Unfortunately, there were no consistent results: in no case was a high proportion of any insect found to be carrying the virus in the first 6 weeks, therefore the remainder of the samples were not analysed. These results indicate that none of the species tested are efficient vectors of the virus.

7.2.2 Epidemiology

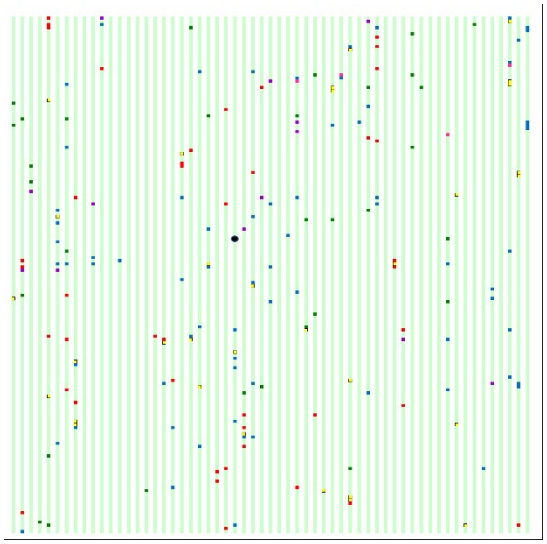
Kediri site: GPS data highlighted only a low level of disease spread. If an arthropod vector exists at Kediri, it did not seem to be very active during the experiment. There was no strongly-pronounced spatial pattern at the Kediri site (Figures 19 and 20).



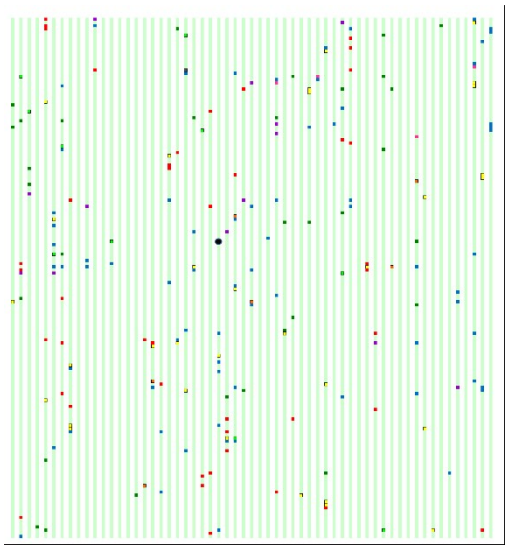
July 2015 (Planting)



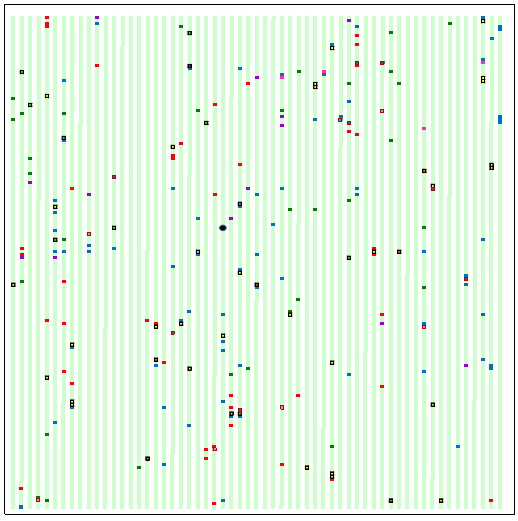
February 2016 (plant crop)



September 2016 (first ratoon)



February 2017 (first ratoon)



January 2018 (2R)

Figure 19: The build-up of SCSMV in the epidemiology experiment at Kediri, July 2015-January 2018. Dots refer to SCSMV-diseased stools within the crop.

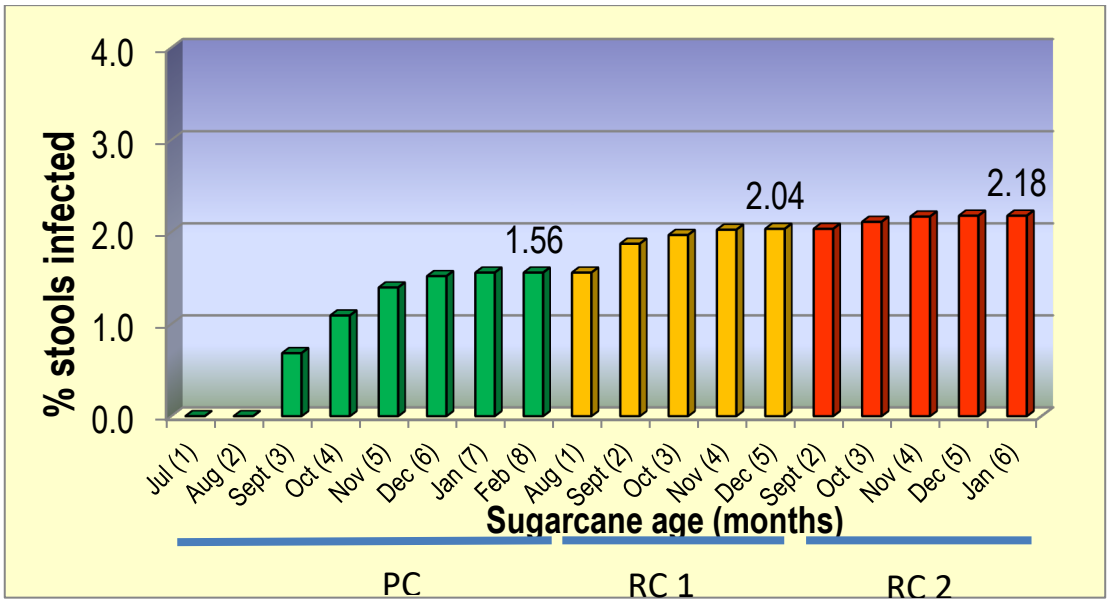
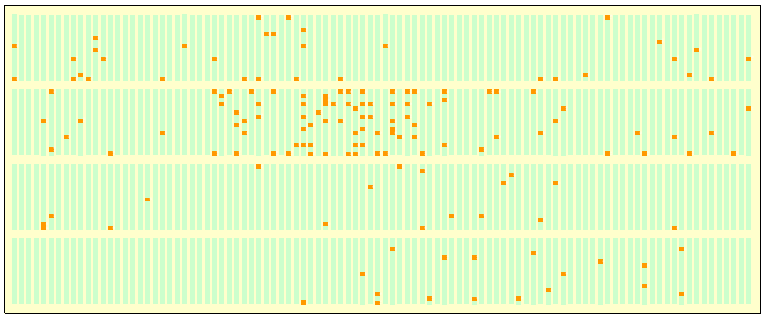


Figure 20: Graphical presentation of the increase in SCSM at the Kediri site.

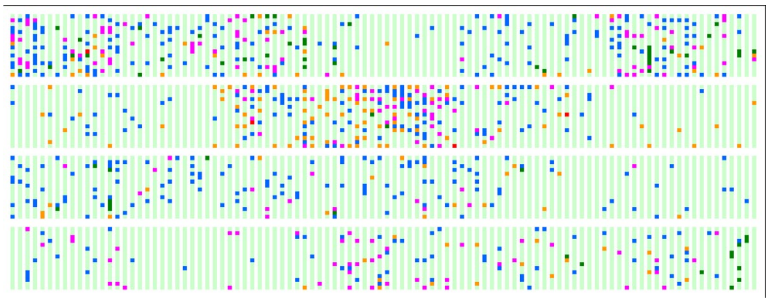
Though build-up is occurring, disease incidence at the end of the second ratoon crop remains at only around 4% diseased stools.

Pasuruan

Disease escalation at Pasuruan has been slightly faster than at Kediri, with around 12% diseased stools recorded at the end of the monitoring period (Figures 21 and 22).



Plant cane (7 months old crop)



First ratoon cane (4 months old)

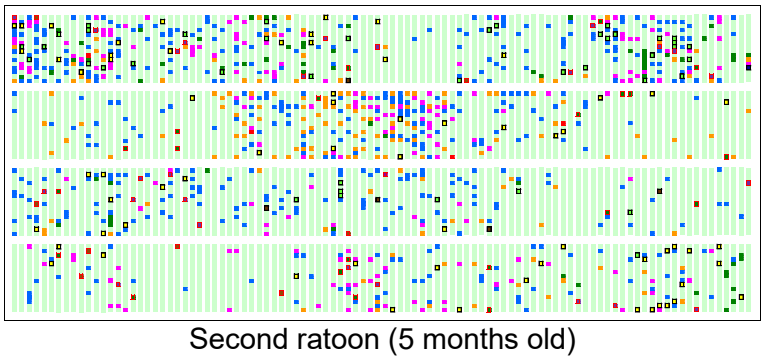


Figure 21: Increase in SCSMV in nursery cane at Pasuruan, from late plant crop (top) to early second ratoon (bottom).

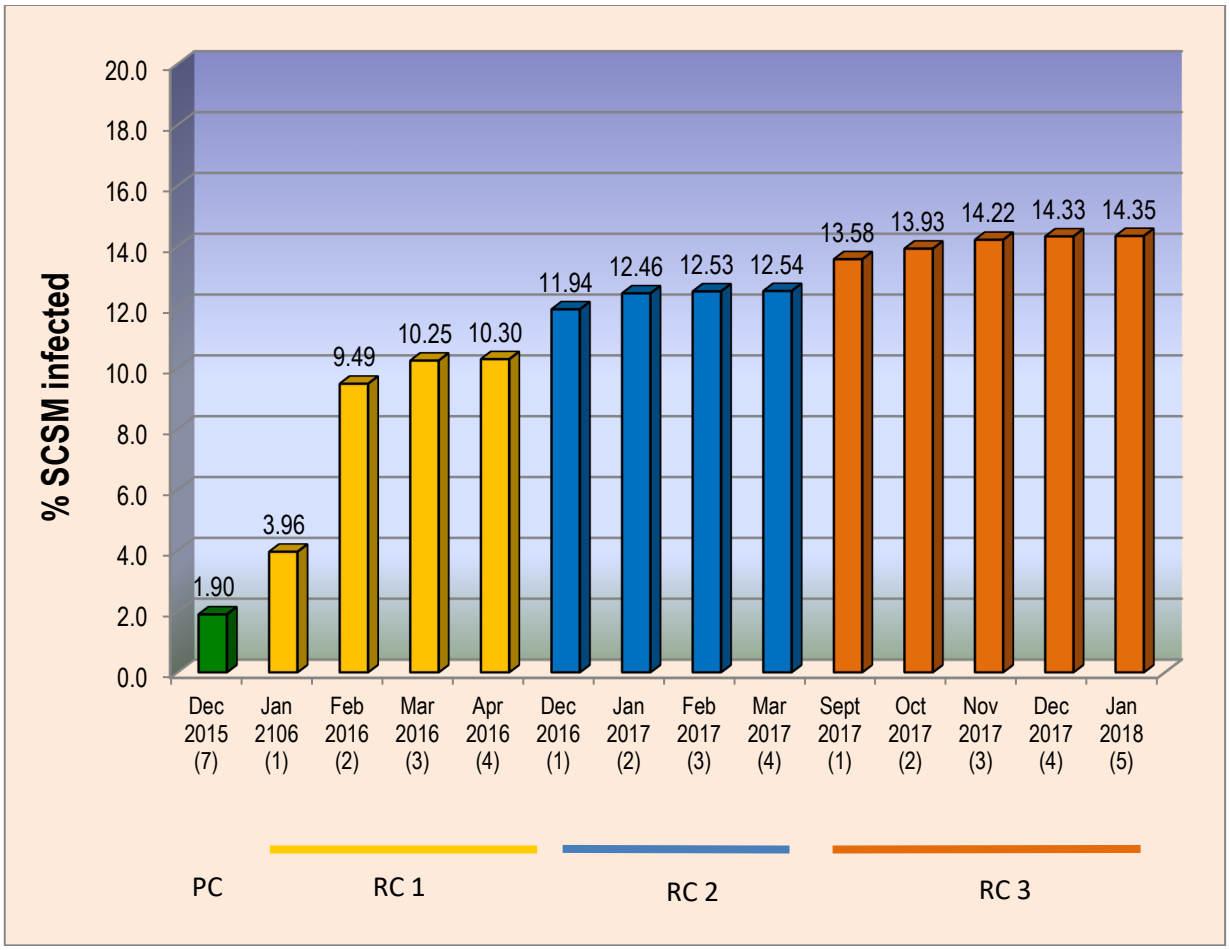


Figure 22: SCSM increase in the monitored crop at Pasuruan.

Discussion

Disease spread, and the opportunity to analyse meaningful spatial pattern data, was limited in the data obtained from Java (Kediri / Pasuruan). The speed of spread was generally low. At the Pasuruan site, higher disease levels prevailed with the possibility of some clumping in disease incidence; this tends to suggest an arthropod may be involved in disease spread.

In contrast, previous *ad hoc* observations of SCSMV spread in Myanmar (tissue culture plants imported directly from Australia), suggest much faster transmission; all plants in a batch of around 10 varieties became diseased within 5 months of planting in the field. It is quite possible that an arthropod vector is present in Myanmar. This was investigated in a small project undertaken in conjunction with this project (reported separately to this report).

Low observed spread rates in at least some crops in Java is positive from a crop loss and management perspective; a slower-spreading disease provides greater opportunity for management to minimise economic losses. There must however be significant spread in parts of Java as many nursery plots and commercial crops are heavily diseased; the disease does not appear to entirely rely on spread via planting material. The Indonesian sugarcane industry needs to be aware of the variation in the speed of disease spread.

7.3 Resistance screening

7.3.1 Glasshouse trial

Results of the final inspection of plants in the glasshouse resistance screen suggest the following % plants diseased (Table 9): -

Table 9: SCSM disease incidence in Australian varieties inoculated with SCSMV in a glasshouse experiment.

Variety	% diseased plants	DIBA assay
Q 138	0	Not tested
Q 183	0	- (negative)
Q 200	50	+
Q 208	50	+
KQ 228	67	+
Q 231	100	+
Q 232	50	+
Q 238	50	+
Q 240	25	+
Q 242	25	+
GMP 7	0	-
PS 881	50	+
PS 864	50	+

The low number of test plants in each treatment means that care should be taken in the interpretation of the varietal resistance. Further testing of these varieties is needed to confirm the initial results. There does appear to be some variation in disease resistance amongst the varieties.

7.3.2 Field trial

The results below focus on Australian varieties; data for other corporately-owned varieties requires specific permission from those corporations. The results (Table 10) are only

preliminary and there were some varieties and plots which were unexpectedly negative (for instance, PS864).

Table 10: SCSM incidence in varieties included in the field resistance screening experiment.

Variety	% diseased plants
Q183[A]	0
Q208[A]	0
Q231[A]	0
Q232[A]	0
Q238[A]	0
Q242[A]	0
Q138	4.8
Q240[A]	5.6
Q200[A]	7.4
KQ228[A]	22.2

Discussion

The experiments reported here (both glasshouse and field) suggest that there may be some variation in varietal resistance to SCSMV in the Australian germplasm. Further testing is needed to confirm this variation. Differing resistance to SCSMV is known to occur in Indonesian varieties, and concerted breeding for resistant, high-yielding commercial varieties will be needed to reduce and then maintain low disease levels in commercial crops.

7.4 Yield loss

7.4.1 Yield loss in a susceptible variety

Results

Disease incidence remained fairly constant within plots over the plant, first and second ratoon crops, suggesting little spread both within and between plots (Table 11).

Table 11: Diseased plots within the yield loss trial and recorded disease incidence to the second ratoon crop.

Treatments	% diseased stools at trial establishment	% infected plants: 6-8 crop growth		
		P	1R	2R
T1	0	0.52	0.91	1.72
T2	5	6.27	8.50	8.71
T3	10	9.95	12.86	13.37
T4	15	16.06	18.77	18.92
T5	20	19.86	23.99	24.12
T6	25	24.58	25.90	26.17
T7	30	29.91	30.36	31.93
T8	50	49.78	49.25	52.12
T9	75	74.92	74.74	76.71
T10	100	100	100	100

Stalk populations

There appeared to be little effect on stalk populations across the treatments in plant cane, but at the two highest disease levels, lower stalk populations were apparent in the two highest disease levels.

Stalk height

As with stalk populations, there was little difference in stalk heights at all but the highest disease levels, where some reductions appeared to be present in the first ratoon crop.

Total yield

Plant crop and second ratoon yield losses were around 22% (total yield, Figure 23) while first ratoon losses were approximately 17%.

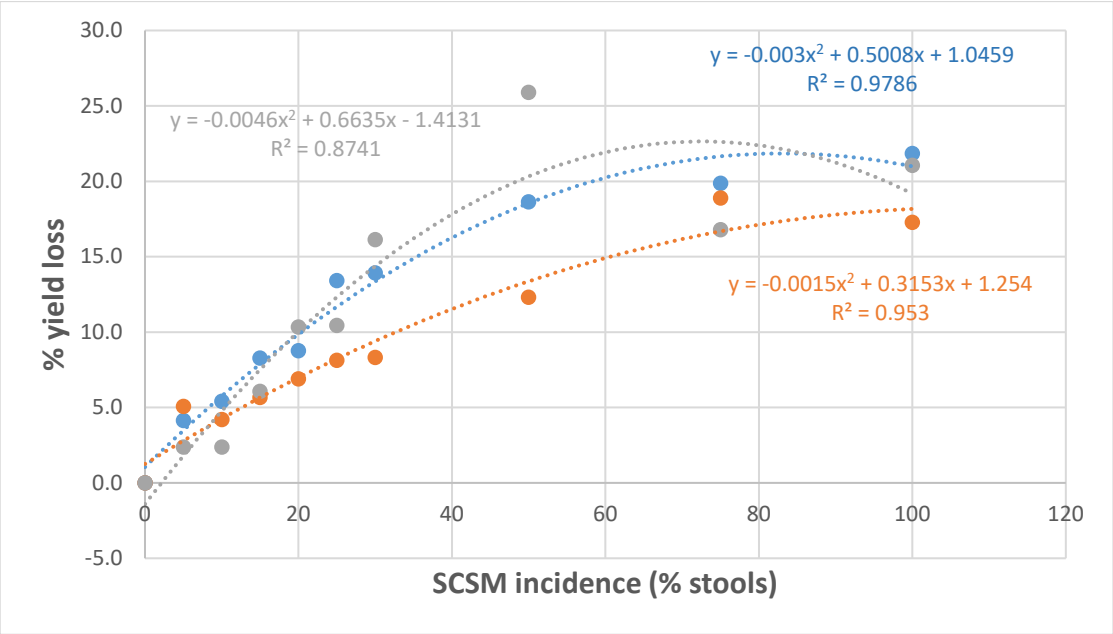


Figure 23: The effect of SCSM on crop yields in the Kediri yield loss experiment (blue=plant crop; orange=first ratoon; grey=second ratoon).

7.4.2 Yield loss as influenced by resistance

Monitoring of SCSM in plots of each variety during the experiment showed that up to 4% infection was present in control (disease-free) plots – suggesting some disease transmission had occurred during the course of the experiment. In this trial, there were no 'buffer plots' of a resistant variety planted to limit disease spread. Such buffer zones could well have limited disease spread in the other yield loss work undertaken at Kediri.

There were definite effects of SCSM on yield parameters in the susceptible varieties, most notably on total cane yield. As expected, yield effects were much more limited in intermediate and resistant varieties.

Yield loss in the two susceptible varieties was between 15-18%. This compares favourably to the yield losses seen in the susceptible PS864 (P, 1R and 2R crops) in the yield loss trial established at Kediri. There appeared to be very limited effect on stalk numbers (Table 12); this was consistent in all six varieties.

Table 12: The effect of SCSM on stalk populations in susceptible, intermediate and resistant varieties in a field yield loss experiment (S=susceptible; I=intermediate; R=resistant)

Variety	Resistance	% stalk population reduction
PS864	S	-0.7
PS881	S	-0.7
BL	I	8.0
PS862	I	1.6
PS091	R	4.8
GMP 7	R	-2.1

There were also very limited effects on stalk height (Table 13)

Table 13: The effect of SCSM on stalk height in susceptible, intermediate and resistant varieties in a field yield loss experiment (S=susceptible; I=intermediate; R=resistant)

Variety	Resistance	% Stalk height reduction
PS 864	S	1.0
PS 881	S	-9.1
BL	I	-0.7
PS 862	I	-0.8
PS 091	R	6.7
GMP 7	R	-2.8

Yield losses in the intermediate varieties were close to zero and seemingly of little consequence. In the resistant varieties, there appeared to be some losses in PS091 while the yield in GMP7 appeared to be higher in the inoculated cane (Figure 24). There is a difference between resistance and tolerance: resistance refers to the ability of a variety to limit pathogen infection, while tolerance is the ability of the variety to maintain yield in the presence of the pathogen. It is possible that PS091 exhibits a lack of tolerance to SCSMV while GMP7 may be both resistant and tolerant.

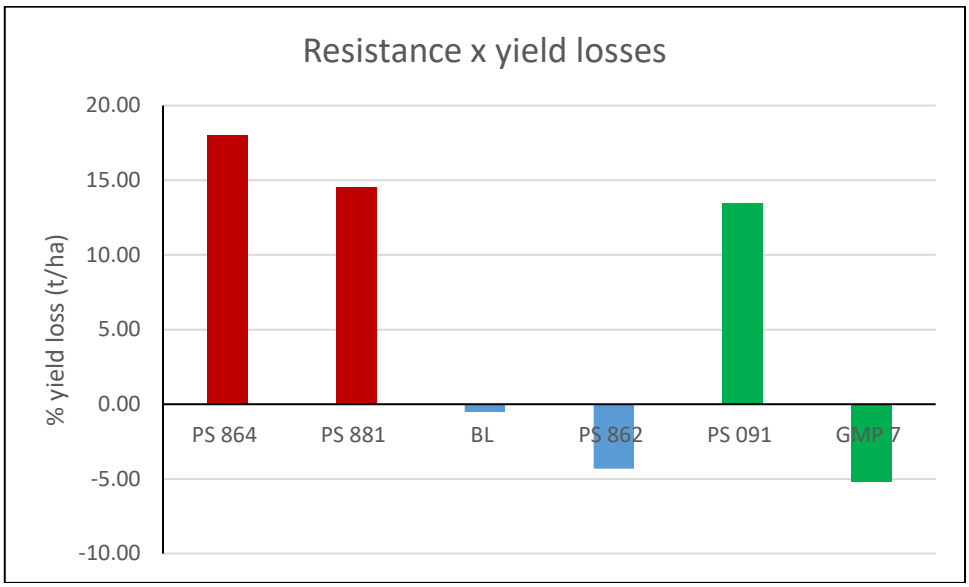


Figure 24: Yield losses in varieties of differing resistance to SCSMV.

These data suggest that varietal resistance is an effective disease management strategy; if sufficient resistance is bred into commercial varieties, the disease can be managed well.

7.4.3 Economic considerations

The data obtained in yield loss research allows for an estimate of the effects of the disease on sugarcane production in Indonesia. While crop data for 2019 is lacking, and the actual percentage of diseased crops for the same period, data from the previous project (HORT 1996 147) provided data on SCSMV crop incidence in Java in the 2009-2010 period. The following assumptions were made when calculating probable production and economic losses for Java: -

- Percentage of susceptible crops in Java: 30% (based on ISRI advice)
- Current crop mean yields: 67.1 tonnes cane / ha
- Current sucrose % crop yield (CCS): 7.5

- Mean % crops infected: 34% (based on previous project survey data; 217 diseased out of 931 crops surveyed)
- Mean % stools diseased in these crops: 20% (based on assessments made in the previous survey)
- Estimated yield loss (mean 20% diseased stools – 2009-2010 data): 10% (based on current project yield loss estimates)
- Sugar price (white refined, 2019 values): Rp12,200
- Percent white sugar / tonnes cane: 7.5%
- Total yield loss in Java crops: 489,126 tonnes (based on 10% loss; 34% of crops; 67.1 t/ha)
    - Sugar lost: 36,684 tonnes
- Estimated lost value: Rp447,550,000

Discussion

Very useful data were obtained in the yield loss experiments that confirm the disease significantly reduces many yield parameters associated with cane growth; total losses varied between 17-26%, depending on crop class. The lack of either dead stools or stools suffering severe yield effects has meant that the significance of the disease has gone unrecognized in many countries. A high percentage of crops in some Asian countries are 100% diseased with little recognition of the yield effects. Those pests and diseases that severely affect stools or patches of crops often attract far more attention. Diseases of lesser severity, but with a whole crop influence, are often not given adequate priority; SCSM falls into this latter category.

The influence of varietal resistance on SCSM-associated losses was clearly shown in the 2019-assessed yield loss trial. Significant losses (up to 26%) occurred in the most susceptible variety, while insignificant losses were observed in the resistant cane. This clearly shows that: i. there is sufficient resistance in some varieties to manage disease incidence, and ii. that varietal resistance is a key control measure to be included in an IDM strategy.

Using survey data from the previous project (CP 2006 147), yield losses and lost revenue from SCSM was calculated for Java. This suggests significant losses are occurring from the disease (nearly 500,000 tonnes cane). This is a conservative estimate since it takes no account of the losses occurring in the Indonesian industry outside of Java. It also takes no account of the spread of the disease over the last 10 years. It should also be noted that two other diseases, smut and ratoon stunting disease (RSD) are also significantly influencing cane yields across the sugarcane industry.

7.5 Diagnostics

7.5.1 Molecular assays - SRA research

Molecular genome research showed significant variation in SCSMV related to source of the virus. There are potentially several different SCSMV groups, with isolates from India and Pakistan showing variation compared to isolates from Indonesia and some other SE Asian countries. Project research has identified significant variation amongst isolates but not as much as with some other viral pathogens. Quantification of this variation is an important component of any research related to diagnostics. Sufficient variation was found to alter the status of some assay results.

7.5.1.1 RT-PCR test / FTA cards

Assay of the FTA card samples containing extracts from Kediri gave the following results (Table 14)

Table 14: The results from testing the ability of FTA cards to preserve specimen assays during surveys and other similar applications.

FTA card Samples from Kediri 25-11-2015		CPf AP3 one-step	CPf-AP3 two-step	qRT-PCR two-step
1	T0 Rep 3			
2	T8 no symptoms			
3	T4 Rep 2 unclear symptoms	Positive	Positive	Positive
4	Disease trial, disease escalation centre		Positive	
5	T6 Rep 3			
6	T0 75% Possible mixed infection	Positive	Positive	Positive
7	T10 Rep 2	Positive	Positive	Positive
8	T10 Rep 2 Bad symptoms	Positive	Positive	Positive

The data show that RT-PCR assay of FTA extracts can successfully detect the virus. DNA sequencing was undertaken to confirm correct amplification. One-step RT-PCR was not as decisive as the two-step reaction on the gel analysis.

7.5.1.2 Quantitative PCR

In order to design robust primers, the sequence variability of the virus was analysed. Known RNA sequences of SCSMV isolates were aligned and are represented in a phylogenetic tree (Figure 25), along with the closely related SCMV and wheat streak mosaic virus (WSMV). This shows that sequence variation needs to be considered when developing tests to all isolates of the virus.

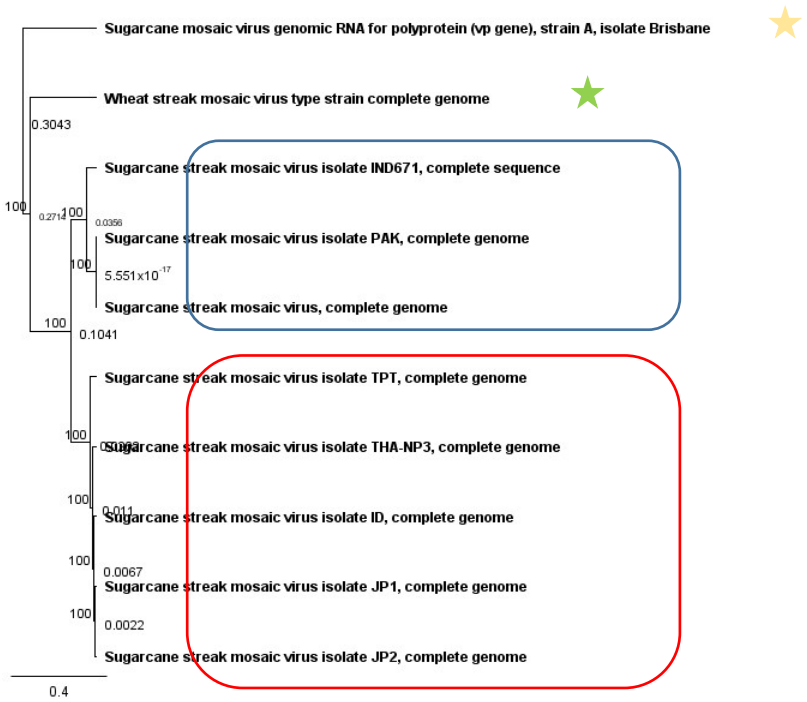


Figure 25: The relationship between SCSMV isolates obtained from various Indonesian, Indian, Pakistani, Sri Lankan sources and other closely-related viruses (SCMV and wheat streak mosaic virus (WSMV)). Two main groups of SCSMV can be seen in the red and blue boxes. SCSMV is more closely related to WSMV than SCMV.

Sequence alignment was used to target primer design. Ten sets of primers at the 3' end of the virus were constructed, with a focus on the most conserved areas. Real time PCR was

used to test samples from Indonesia, Sri Lanka and healthy controls and showed good amplification of the specimens with no contamination of the healthy control (Figure 26). It is suspected that the sensitivity of the real time PCR was higher than the standard RT-PCR; some existing samples that were negative with older PCR tests have now proven to be positive. The results were found to be repeatable and consistent.

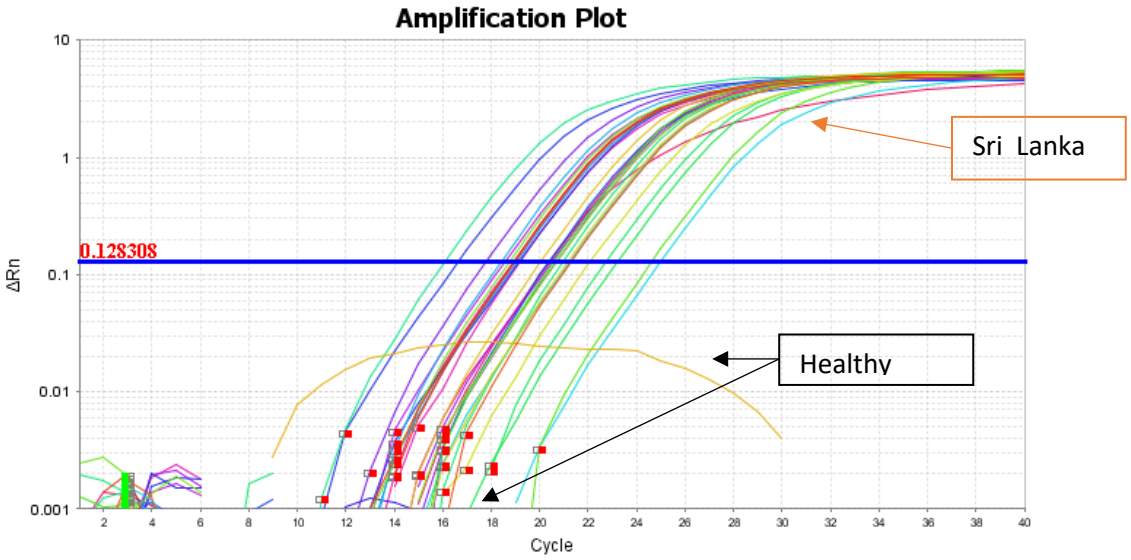


Figure 26: Quantitative PCR analysis of SCSMV-infected samples (Sri Lanka = sample from Sri Lanka; healthy control; all others are specimens from Indonesia collected in previous project)

7.5.1.3 Rapid 'LAMP' assay

LAMP successfully amplified RNA from SCSMV-infected leaf material, using colorimetric detection (AB563503 primers) when undertaken in Australia (Figure 27). In Indonesia, the colour distinction using fresh material was not as good, so the samples were re-analysed in Australia. Figure 28 shows the typical amplification curve and diagnostic melt curve for LAMP when using purified RNA from known infected leaf material. The method was highly prone to contamination, especially in a non-sterile environment, giving false positive results. The melt curve of the anneal derivative should therefore be used in LAMP assays.

Indonesian assay of samples, using the described method (and Genie II) produced excellent results. A series of unknown specimens were used to further test the system. Figure 29 shows the results of ISRI samples (gmp7 and PS864 as negative and positive controls) as well as a selection of surveillance samples (41-53) which were collected a few days prior on the eastern Archipelago survey. The melt curve showed a clear distinction between specific and any non-specific amplification: those with a sharp peak >15,000 fluorescence units at a temperature between 87.5 °C and 88.5 °C were positive. Non-specific amplification were clearly different.

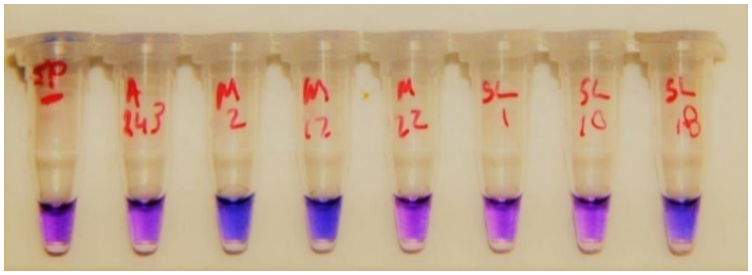


Figure 27: Example of colorimetric LAMP assay. Samples are from surveys and controls in the SRA collection. M2, M12 and SL18 show the reaction typical for SCSMV. The others show no reaction, including the negative control in tube 1.

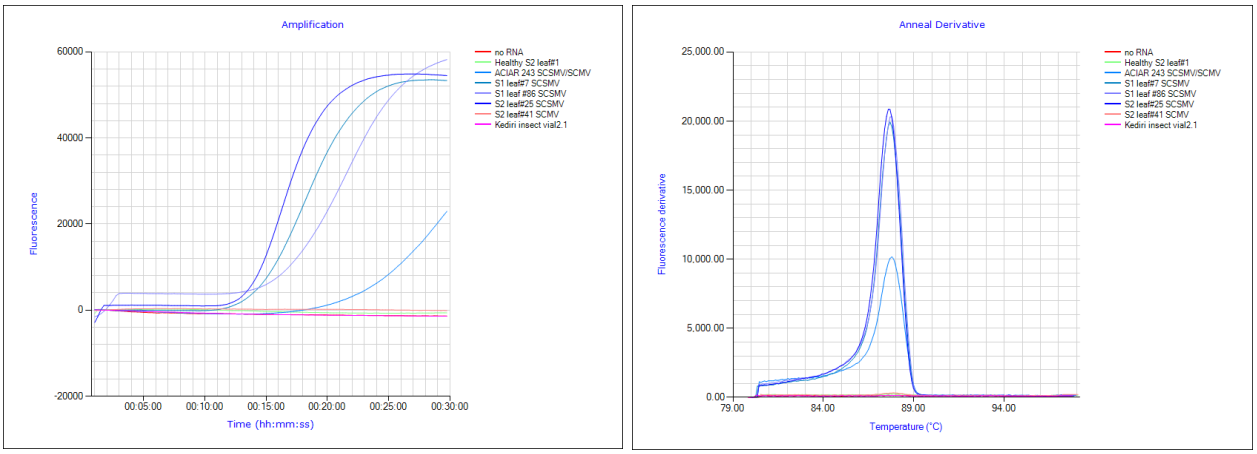


Figure 28: Results from GENIE II LAMP run for SCSMV using purified RNA. LHS shows exponential amplification. RHS shows melt curve of derivative amplicon. Lines in Blue are RNA extracted from SCSMV specimens and SRA laboratory controls.

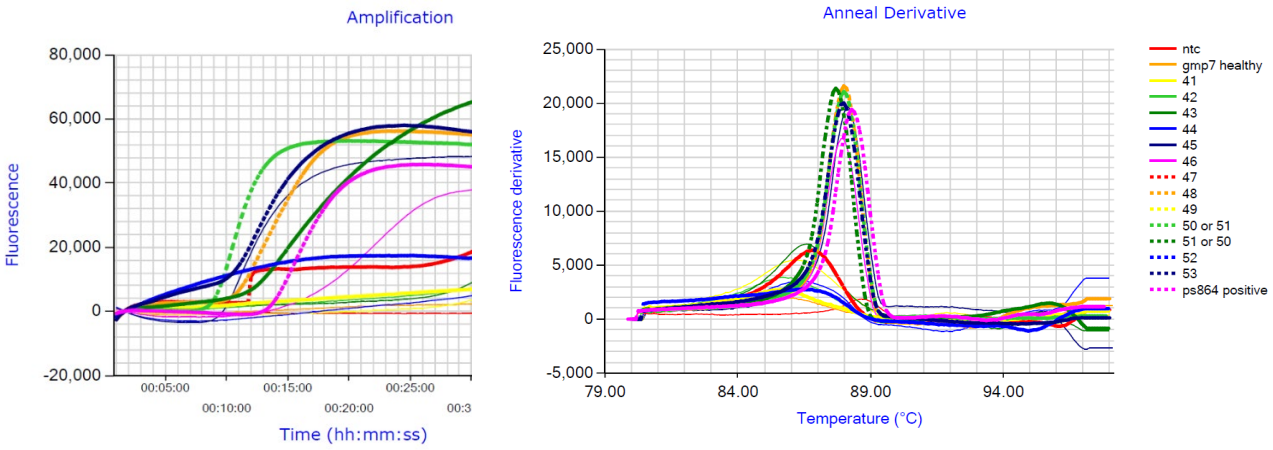


Figure 29: Results from GENIE II LAMP for unknown samples taken on survey of Indonesia. The LHS is amplification curves for 30 minutes, RHS the anneal derivative (melt curve) data for these specimens. ntc is no template control; gmp 7 is healthy control and ps864 is positive control. All others are samples taken from Indonesia field sites.

7.5.2 Antiserum assay

University of Bogor research

Selection of a pure SCSMV source

Difficulty was experienced in the propagation of a pure source of SCSMV; mixed infections (SCSMV / SCMV) in the original disease sources made the identification of a source much harder.

Selection of test plant material

Difficulty was also experienced in growing disease-free host material. Sugarcane dispatched from Pasuruan, and testing disease-free on dispatch, tested positive to SCSMV after several months propagation at Bogor.

Coat protein purification

Experimentation to purify the coat protein for antiserum production also struck trouble, with no purified protein produced in the attempts made.

For these reasons, Bogor research failed to develop a suitable antiserum assay.

Australian research (SRA)

Of the four antisera developed by the commercial company, two showed no reaction to the SCSMV infected leaf extracts and so were not used further. Of the two other peptides, one reacted slightly with the healthy control but the fourth (CP-specific antisera) had no reaction to the healthy extract. The CP antisera was able to detect a 1:100 dilution of the extract, though the clearest reaction was at a 1:10 dilution. The suggested outcome is to use of the antisera at 1:1000 dilution and the extract at 1:10 - for routine assay.

DIBA application

The results from the application of DIBA to known diseased and healthy specimens, with dilutions testing assay sensitivity are outlined in Figure 30.

DIBA Plan:

H1 – Healthy
H2 – Healthy
SCMV – Sugarcane mosaic virus
SCSMV – Sugarcane streak mosaic virus
SC Mix – suspected mixed infection
Pep Mix – Mixture of peptides used to produce Peptide antisera
PBS – negative control

Figure 30: DIBA showing specificity of Coat Protein antisera (L) and Peptide 3 antisera (R). The columns are Undiluted, Diluted 1:10, 1:100 and 1:1000. The rows are shown on the plan (Right) Healthy (1 and 2), SCMV, SCSMV, SCMV, Peptide mix, PBS

Discussion

ISRI and SRA have different requirements for an accurate assay; i. ISRI: require rapid detection with in-field sampling to provide an assay for nursery cane prior to distribution – with little specialist equipment; ii. SRA needs the most accurate diagnosis to use in international quarantine and to identify a possible future incursion. Table 15 highlights the main considerations related to diagnostic assays and how this relates to each assay developed in this project. A comparison is also made with RT-qPCR.

Table 15: Comparison of SCSMV diagnostic tests developed during this project

	Nucleic acid-based		**Antibody based**
	RT-qPCR	**LAMP**	**DIBA**
Equipment needs, cost	Specialist, expensive	Specialist, moderate	General, low
-Have at ISRI	No	No/some	Yes
-Have at SRA	Yes	Yes	Yes
Consumables, cost	Specialist, moderate	Specialist, moderate	General, low
Test sensitivity	High	High	Moderate/Low
Test specificity	High	High	High/Moderate
False positives (eg. contamination)	Moderate	High	Low
False negatives (eg. missed positive)	Low	Low/moderate	High
Time for result	2 hours	< 1 hour	4 hours
Suggested use	Quarantine	In-field/demonstration	Routine
Detection of SCSMV in plant	Yes	Yes	Yes
Downstream assay	May be multiplexed with other disease detections	May be able to detect variation; multiplexing may be possible	ELISA development Tissue blot development

Each assay developed has an immediate application, with the DIBA assay likely to be the main high-throughput method in Indonesia. There are three reasons: i. ISRI have the required materials; ii. DIBA is a robust method with sufficient sensitivity for general use; iii. the method is easily adaptable for future use / research.

The antisera could be used in a tissue blot assay and/or ELISA, depending on requirements. There is good scope for use of LAMP for in-field detection, with less expensive equipment and training needed compared to RT-PCR (or RT-qPCR) – so this could be adopted in Indonesia also. Many examples of genetic diversity and change within the SCSMV genome (Chandran and Gajjeraman, 2013; Liang *et al*., 2016; Moradi, Nazifi, and Mehrvar, 2017; Wang *et al*., 2017) may mean that LAMP and RT-qPCR assays need to be periodically optimised to cater for evolving strains. It seems that RT-qPCR and LAMP are the most likely candidates for quarantine use, alongside the established RT-PCR.

Project research has thus demonstrated two new SCSMV diagnostic assays for potential use in Indonesia as part of an IDM strategy.

Loop-mediated amplification (LAMP) assay development was completed and focused on the development of suitable primers. The program ‘LAMP Designer’ was used to design 6 sets of primers. The method involves a simple DNA extraction, dilution, addition of an enzyme and reaction components before a single incubation. A blue-purple detection method was initially employed.

Initial research found that the original primers did not detect all viral isolates – detection of Sri Lankan and Myanmar virus samples worked well, but the Indonesian samples were not detected. The LAMP primers were re-designed to detect all sources of the virus.

7.6 Integrated disease management (IDM)

Introduction

In considering a suitable IDM strategy for managing SCSM in Indonesia, it is first worth summarising the most significant project outputs, particularly mechanisms for transmission, varietal resistance and propensity to spread. Comments on these findings are summarised below and an IDM strategy discussed with reference to these findings.

Transmission

Research conducted in this project identified two clear means of disease transmission: these are: i. diseased planting material, ii. mechanical transmission involving the application of infested juice to a disrupted leaf surface.

The main transmission mechanism appears to be diseased planting material. In the project we identified spread to outlying regions was likely via planting material. It is imperative for both local farmers, and larger plantation managers, that disease-free sources of planting material are propagated and sourced to establish new crops – both locally and when transferring planting material to other regions.

Transmission via an arthropod vector has not been established and neither has significant mechanical transmission, other than purposeful inoculation of leaf surfaces with infested juice. For these reasons, mechanical transmission is unlikely to be a significant avenue for disease spread. Sanitation of cutting equipment therefore appears unimportant for containment of SCSMV.

Resistance

Research has clearly identified a means to screen commercial varieties for resistance to SCSMV. Resistant commercial varieties are present within the Indonesian sugarcane germplasm. This offers both hope and scope for disease management based on the cropping of varieties that will resist infection. The Indonesian industry should therefore develop a strategy to breed for disease resistance. This will most effectively be undertaken via the following: -

- **Using resistant parents in crossing programs**: selecting resistant parents will improve the probability of producing resistant seedlings – which can then be selected based on genetic yield potential. Researching inheritance of SCSMV resistance would provide a clear understanding of how effective this strategy will be.
- **Selection for resistance – promising clones**: it will be important that Indonesian plant breeders collaborate with local pathologists to screen promising clones for SCSMV resistance in the plant breeding selection program. This will enable highly susceptible clones to be discarded from the selection program at an early stage – ensuring that elite clones reaching the final stages of selection possess sufficient commercial resistance to the disease.

Disease-free planting material

For both sugarcane farmers and industry research staff, the production of disease-free sugarcane in nurseries will be imperative for managing the disease (along with resistance). Central nurseries in each factory area should be established from disease-free sources. Where current nurseries exist, it is likely they will need to expand in size, be regularly tested for the presence of the disease and of sufficient size to supply cane to both local farmers and larger Estates. Several issues need to be considered to produce this cane: -

- **Diagnostic tests:** project research has identified and developed several sensitive assays – some for quarantine application and others that potentially could be developed for commercial application in the sugarcane industry. It will be important to establish a diagnostic assay lab service for the Indonesian sugarcane industry, to ensure that nurseries remain free from the disease. A serological assay (for instance, DIBA) may be the best assay to incorporate into a disease testing service. An industry body (ISRI?) should consider providing this assay service.
- **Visual inspections:** trained staff will need to inspect nursery cane for disease symptoms and be shown how and when to take suitable leaf samples for virus testing.

Termination of heavily diseased crops: this is a significant, but not the most important, strategy. Termination of heavily diseased crops will minimise the often unrecognised losses resulting from the disease. Though in most situations SCSMV does not appear to spread rapidly (if it did, termination of heavily diseased crops would be a high priority), a reduction in the area affected will lead to higher productivity and reduce the probability of crops being established from diseased material. There are a number of economic issues for managers to consider regarding termination of a crop: i. current crop yield – does it remain high or have yields declined? ii. availability of suitable planting material – does the farmer or estate owner have suitable disease-free plant sources of a high yielding variety to plant the succeeding crop? iii. are there other potential rotation crops that could provide another income source (rice or maize for instance)? These points need to be taken into consideration when crop termination is contemplated. Where a disease spreads rapidly, termination of heavily diseased crops is much more urgent and important.

In summary, an ideal IDM for SCSMV will include: -

- **Disease free nursery plots**: of sufficient size in each sugar factory area
    - plots will need to be established from disease-free sources
    - cane growing in plots will need to be tested regularly for the presence of the virus using a suitable assay.
- **Resistant varieties**: commercial high-yielding, resistant varieties should be developed
    - This will require collaboration between plant breeders and pathologists
    - Early screening of clones in the selection program will be beneficial; use of resistant parents will lead to a higher proportion of resistant seedlings to select for desirable agronomic traits
- **Termination of heavily diseased crops**: this will reduce the possibility of disease spread, either via the use of this material for planting new crops or from the lower probability of disease spread via mechanical means or arthropod vectors (as yet unidentified).

8 Impacts

The impact of the research reported here has begun to flow to the industry through a greater awareness of the disease in both expansion areas (in the regions) and in the cane-growing areas of Java. This began with the first project (CP/2006/147) that addressed the disease. Surveys quantifying the widespread disease incidence, in association with extension meetings, highlighted the need for farmers and estate managers to control and limit crop losses from SCSM. The current project has built on these results and assisted with additional impacts within the Indonesian industry.

8.1 Scientific impacts – now and in 5 years

Current scientific impacts include development of much improved assay technology. Molecular assays of a very sensitive nature have been developed that are suitable for use in quarantine and to address specific and varied research questions. The assessment and specifics of pathogen variation has provided valuable information in the context of the use of sensitive and specific assays. The research has provided a scientific basis to accurately assay planting material using the latest technology (such as LAMP). Pathogen variation research significantly added to our knowledge of the pathogen. This is important information for SCSMV researchers across Asia (SE Asia, south Asia and in Australia).

An assay was also developed that was able to investigate the presence of SCSMV in individual arthropods; this was used to determine any association between captured arthropods feeding on diseased plants and the presence of the virus. Such technology could be used on an ongoing basis to assist with confirming whether there is an arthropod vector for SCSMV.

The virus was found for the first time in several regional Provinces in Indonesia; this included Sulawesi. This is a first record and should be published in a scientific journal. Confirming spread of the pathogen is a significant scientific finding and is one of concern to the Indonesian sugarcane industry.

The failure to confirm knife and arthropod transmission of the virus challenges some research publications (Dayamanti and Putra, 2014) who claimed that such transmission was significant. We could not substantiate this; if these are insignificant forms of disease transmission, it has very positive implications for SCSMV management – it is always harder to control a disease that is readily spread by several mechanisms. Further research in Myanmar, where spread seems to be very rapid, will be important from a scientific perspective.

8.2 Capacity impacts – now and in 5 years

Project staff in both Indonesia and Australia benefitted significantly from this project. Assay skills were learnt and adopted; Australian scientists gained valuable experience with first-hand contact with the disease, experience in developing and using the molecular assays and a much better knowledge of the Indonesian sugarcane industry.

Staff at the Indonesian Sugar Research Institute (ISRI) used the new serological test (DIBA) successfully and now have the capacity to operate the assay within the industry. They also gained valuable experience in visiting the regional production areas, something which doesn't normally fit within their work roles.

In five years time, use of the new serological assay should be on a much larger scale with increased assay capacity leading to a much better understanding of the presence of the virus across the cane-growing regions, the quality of industry propagation plots and the need for further extension of IDM strategies. This will be important for gaining maximum adoption of the SCSMV IDM strategy in Indonesia.

The capacity to screen promising clones for resistance to SCSMV should also be much higher in five years. This will require strategising and the provision of more resources but will be critical for bringing SCSM under control. It will also require close collaboration between the plant breeders and the pathologists.

8.3 Community impacts – now and in 5 years

Better disease management should have a flow-on effect in the farming communities associated with the sugarcane industry. The extent of this impact will depend on the success of follow-up socialisation programs, that highlight to farmers and estate managers the benefit of disease management. Effective IDM is needed to reduce both the number of crops affected and the severity of the disease.

8.3.1 Economic impacts

It was estimated that SCSMV is causing approximately losses of Rupiah 447m to the Indonesian economy. If disease levels were reduced by one-half as a result of project research, this would lead to much higher industry and farmer returns. This would not only benefit sugar factories, but also individual farmers and landholders. Most of this benefit will be in 5+ years, when diseased crops are terminated and new crops established using disease-free sources. A complicating factor is the amount and severity of sugarcane smut affecting a SCSMV-resistant variety. If smut increases in severity, and even more important, it may slow the potential economic benefit from SCSMV management. This shows the need not only for an IDM for individual diseases, but an overall IDM for diseases.

8.3.2 Social impacts

Social impacts may include higher annual incomes arising from higher crop yields.

8.3.3 Environmental impacts

There will be few environmental benefits from this work; the only one of significance will be a small decrease in the proportion of crops terminated (once heavily diseased crops have been eliminated) which will result from healthier cane and longer-ratooning cycles. A decrease in exposure of bare soil (at replant) will lead to reduced soil erosion.

8.4 Communication and dissemination activities

There were significant extension activities related to the project; these were undertaken with the purpose of socialising project activities, including important results from research. Other activities were conducted in association with specific surveys; survey data were incorporated into these training activities. This enabled a much better understanding of pest and disease issues by staff at these locations in the context of the disease surveys.

In January 2017 a Focused Group Discussion was conducted at ISRI, Pasuruan (Figure 31). The meeting was attended by government officers, scientists, sugar factory staff, sugarcane farmers and university lecturers. The purpose of the meeting was to extend project findings and the significance of SCSM. Approximately 100 people attended the meeting.

Figure 31: FGD on the development of SCSM disease in Indonesia in January 2017.

Following the FGD meeting, the Indonesian project team extended project results to sugar factory staff and sugarcane farmers in Sulawesi. It was clear that factory field staff needed pest and disease training. Later that year (December 2017), factory staff and cane farmers in Camming Sugar Factory area received training with a focus on SCSM; around 50 people attended this meeting (Figure 32). The meeting was conducted over a two-day period, one discussing the diseases and pests inside with a second day associated with field identification. This training was the first in the area for 12 years and was welcomed.

Figure 32: Training on sugarcane pests and diseases at Camming SF.

In November 2018, a national workshop was conducted at the Indonesian Fibre Crop and Sugar Research Institute (IFCSRI; Figure 33). The workshop was attended by about 200 participants. The participants consisted of government officers, scientists, sugar factory staff, sugarcane farmers, lecturer at the universities, etc. ACIAR-project outcomes were communicated to workshop participants and some scientists from IFCSRI outlined their sugarcane research activities.

Figure 33: Some participants at the IFCSRI workshop in November 2018.

In December 2018, training, similar to that undertaken in Sulawesi, was delivered in Sumatra (Figure 34). The project team focused on pest and disease in Bungamayang Sugar Factory, specifically targeting sugarcane farmers., with about 58 attending. The training was conducted over 2 days, one day for seminars and second day focusing on a field visit. The effectiveness of the training was gauged pre- and post-training tests.

Figure 34: Training on sugarcane pests and diseases at Bungamayang SF.

There was consensus that further post-project extension should target higher management in sugar milling conglomerates with presentations on the importance of disease-free nurseries. Disease-free planting material is of prime concern in an SCSMV IDM strategy and the key influencers in establishment of these nurseries need to be convinced of their importance.

9 Conclusions and recommendations

The project made definite conclusions on some aspects of the disease, while several others require additional and on-going research.

9.1 Conclusions

The main conclusions from the project are: -

- The disease does not appear to be transmitted mechanically, other than by rubbing infested cane juice on disrupted leaf surfaces.
- Arthropod transmission was not evident in the transmission research conducted but SCSMV assays found the virus associated on an *ad hoc* basis with several species, including *Perkinsiella* sp., *Eumetopina* sp., an aphid species and the lace wing *Tingidae sp.* There was insufficient evidence to suggest that any of these species are vectors for the virus.
- Planting material appears to form the main disease transmission mechanism. Growing disease-free nursery cane will be an important aspect of disease management.
- Yield losses were measured at between 17-26% in plant, first and second ratoon crops, making the disease a very significant limit to productivity in susceptible varieties.
- A method for screening varieties and clones for resistance to SCSMV was developed, opening the way for screening clones for resistance in the breeding selection program.
- Resistance was identified in both Indonesian and Australian commercial varieties.
- Molecular and serological assays were developed to test both plants and arthropods for the presence of the virus. Tests include RT-PCR, qPCR, LAMP and the serological assay DIBA.
- SCSM has been recorded for the first time in Sulawesi – suggesting spread is occurring from the first finding in Java in the early 2000s.
- Recommended IDM strategies include: -
    - Disease-free planting material
    - Resistant varieties
    - Testing of all plant sources for SCSMV
    - Termination of heavily-diseased crops

9.2 Recommendations

The following are key recommendations arising from project research: -

- **Recommendation 1**: implement the suggested IDM strategy immediately. This will require significant socialization resources and industry staff training – for instance in how to multiply sufficient disease-free nursery material for distribution to farmers and factory estate managers.
- **Recommendation 2**: train local factory staff in taking relevant samples for SCSMV assay. This will be needed in order to ensure nursery cane is free from SCMSV infection.
- **Recommendation 3**: establish a central SCSMV assay service based on the serological DIBA test (cheap / rapid). Continue commercial development of the

assay examining quality control mechanisms and optimized test conditions – including how samples should be stored and dispatched.

- **Recommendation 4:** continue to socialize the Indonesian sugarcane community (farmers, estate managers) on outcomes from project work. Activities could include yield loss demonstration plots that highlight the effect of the disease on productivity along with key messages that reinforce the recommended IDM strategy. It will be important that the Indonesian sugarcane industry takes ownership of disease management recommendations.
- **Recommendation 5**: alert Indonesian quarantine authorities to the spread of SCSMV to regional Indonesian production areas. Encourage such personnel to extend the importance of quarantine to sugarcane companies and the general community, to minimize further disease spread across regional borders.
- **Recommendation 6**: continue to research the possibility of an arthropod vector for SCSMV. Rapid spread in Myanmar suggests a vector could be present there. Continue experimentation testing the ability of arthropods to transmit the virus, particularly species of *Perkinsiella*, *Eumetopina*, relevant aphid species and the lace wing *Tingidae sp.* – those identified as the most likely vectors of the disease, should one exist.
- **Recommendation 7:** finalise formal identification of all diseases and insects collected during disease surveys and publish 'first records' in an appropriate journal. This includes first reports of SCSMV in regional Indonesia, as well as a first report for diseases such as leaf freckle etc.
- **Recommendation 8:** update Australian contingency plans for SCSMV incorporating project research outcomes.
- **Recommendation 9:** plant breeders and pathologists working in the Indonesian sugarcane industry meet soon to develop a strategy for breeding commercial varieties with sufficient SCSMV resistance to minimize yield losses. Issues to be considered are: i. development of a resistance screen capable of testing the resistance of clones in the latter stages of the plant breeding selection program, ii. testing of parent varieties for resistance to SCSMV, iii. how and when clones will be discarded (for instance, deciding on a resistance threshold for clonal discard) and the stage in the selection program at which this will be effected.
- **Recommendation 10:** maintain on-going monitoring of SCSMV research undertaken in other countries - in order to utilise all available research outcomes in a revised IDM strategy for SCSMV in Indonesia.

10 References

10.1 References cited in report

Chandran, V., and Gajjeraman, P. (2013). A simple precipitation approach for isolation and enrichment of sugarcane streak mosaic virus. *Sugar Tech* **15**(4)**,** 417-419.

Chatenet, M., Mazarin, C., Girard, J. C., Fernandez, E., Gargani, D., Rao, G. P., Royer, M., Lockhart, B., and Rott, P. (2005). Detection of sugarcane streak mosaic virus in sugarcane from several Asian countries. *Sugar Cane International* **23**(4)**,** 12-15, 28.

Damayanti, T. A., and Putra, L. K. (2011). First occurence of sugarcane streak mosaic virus infecting sugarcane in Indonesia. *Journal of General Plant Pathology* **77,** 72-74.

Goto, M., Honda, E., Ogura, A., Nomoto, A., and Hanaki, K. I. (2009). Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxy naphthol blue. *Biotechniques* **46**(3)**,** 167-+.

Graddon, D. J., and Randles, J. W. (1986). Single antibody dot immunoassay - a simple technique for rapid detection of a plant virus. *Journal of Virological Methods* **13,** 63-69.

Hibi, T., and Saito, Y. (1985). A dot immunobinding assay for the detection of tobacco mosaic virus in infected tissues. *Journal of General Virology* **66,** 1191-1194.

Liang, S. S., Alabi, O. J., Damaj, M. B., Fu, W. L., Sun, S. R., Fu, H. Y., Chen, R. K., Mirkov, T. E., and Gao, S. J. (2016). Genomic variability and molecular evolution of Asian isolates of sugarcane streak mosaic virus. *Archives of Virology* **161**(6)**,** 1493-1503.

Moradi, Z., Nazifi, E., and Mehrvar, M. (2017). Molecular characterization of two sugarcane streak mosaic virus isolates from Iran with emphasis on its population structure. *Acta Virologica* **61**(4)**,** 428-437.

Mori, Y., and Notomi, T. (2009). Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. *Journal of Infection and Chemotherapy* **15**(2)**,** 62-69.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., and Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* **28**(12)**,** E63.

Putra, L. K., Kristini, A., Achadian, E. M., and Damayanti, T. A. (2014). Sugarcane streak mosaic virus in Indonesia: Distribution, characterisation, yield losses and management approaches. *Sugar Tech* **16**(4)**,** 392-399.

Wang, X. Y., Li, W. F., Huang, Y. K., Zhang, R. Y., Shan, H. L., Yin, J., and Luo, Z. M. (2017). Molecular detection and phylogenetic analysis of viruses causing mosaic symptoms in new sugarcane varieties in China. *European Journal of Plant Pathology* **148**(4)**,** 931-940.

10.2 List of publications produced by project

1. RC Magarey, A Kristini, E Achadian, N Thompson, E Wilson, M Reynolds, N Sallam, R Goebel, L Putra (2018). Sugarcane streak mosaic-researching a relatively new disease in Indonesia. *Proceedings of the Australian Society of Sugar Cane Technologists*: **40**, 257-266.

2. EJ Wilson, N Thompson, R Magarey, EM Achadian, A Kristini, SH Hidayat (2016). Australian collaboration for development of detection methods for sugarcane streak mosaic virus (poster paper). *Proceedings of the Australian Society of Sugar Cane Technologists.* **38**, 12

3. NP Thompson, EJ Wilson (2019). Comparison of diagnostic tests developed for sugarcane streak mosaic virus. *Proceedings of the International Society of Sugar Cane Technologists* **30,** (In Press).

11 Appendixes

11.1 Appendix 1:

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