Sugar Research Australia

FINAL REPORT 2019/015

Situation analysis and opportunities for pest, disease and weed RD&A (including biosecurity) in Australian sugarcane

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ABSTRACT

Given Sugar Research Australia's (SRA) investment in and management of a portfolio of RD&A projects that drive productivity, profitability and sustainability for the Australian sugarcane industry, SRA is committed to setting the right targets, managing research investments to maximise the likelihood of success, and ensuring the delivery and adoption of project outcomes and impacts across the Australian sugarcane industry.

SRA's Key Focus Area 3 Pest, disease and weed management, aims to drive "Reduced or avoided yield losses and/or added input costs".

SRA commissioned a review of RD&A over the last 15 years that identifies emerging technology and best practice strategies for addressing pest, disease, weed and biosecurity threats in sugarcane.

The review focused on four themes:

- Biosecurity: enhance capacity to manage biosecurity risks
- Pest control: enhance capability to deal with pests
- Disease management: improve disease management strategies and technologies
- Weed management: improve weed management strategies and technologies

The review provides a set of recommendations for further RD&A that can be used by SRA to consult with industry and leading technical experts on the current management practices and threats, opportunities and priorities for pest, weed, disease and biosecurity management.

EXECUTIVE SUMMARY

Sugar Research Australia's (SRA) investment in and management of a portfolio of RD&A projects that drive productivity, profitability and sustainability for the Australian sugarcane industry means that SRA is committed to setting the right targets, managing research investments to maximise the likelihood of success, and ensuring the delivery and adoption of project outcomes and impacts across the Australian sugarcane industry.

SRA's Key Focus Area 3 Pest, disease and weed management, aims to drive "Reduced or avoided yield losses and/or added input costs".

SRA commissioned a review of RD&A over the last 15 years that identifies emerging technology and best practice strategies for addressing pest, disease, weed and biosecurity threats in sugarcane. Published and unpublished project reports were reviewed.

The review focused on four themes:

- Biosecurity: enhance capacity to manage biosecurity risks
- Pest control: enhance capability to deal with pests
- Disease management: improve disease management strategies and technologies
- Weed management: improve weed management strategies and technologies

The review provides:

- A critical literature review and summary on each of these themes, focusing on priority issues that are likely to justify RD&A investment and covering work in reports and publications over the last 15 years from the Australian sugarcane industry, other sugarcane industries, and other agri-industries.
- A review of techniques and technologies that address specific opportunities and threats in the current (and future) industry, including what R&D would be required to develop, adapt and commercialise/extend these techniques and technologies.
- A summary of the opportunities for RD&A identified as informed by the above two bodies of work.

The set of recommendations for further RD&A can be used by SRA to consult with industry and leading technical experts on the current management practices and threats, opportunities and priorities for pest, weed, disease and biosecurity management.

TABLE OF CONTENTS

1. BACKGROUND

Sugar Research Australia (SRA) invests in and manages a portfolio of RD&A projects that drive productivity, profitability and sustainability for the Australian sugarcane industry. As an industry-owned company, SRA is committed to setting the right targets, managing research investments to maximise the likelihood of success, and ensuring the delivery and adoption of project outcomes and impacts across the Australian sugarcane industry.

The SRA Strategic Plan details eight Key Focus Areas (KFAs) for research investment in order to respond to industry's needs through to 2021-22. KFA3, Pest, disease and weed management, aims to drive "Reduced or avoided yield losses and/or added input costs".

SRA is developing an investment strategy for KFA3 that will identify, prioritise and address the needs of the Australian sugarcane industry. For this it has commissioned a review of RD&A over the last 15 years that identifies emerging technology and best practice strategies for addressing pest, disease, weed and biosecurity threats in sugarcane.

The findings of the review will be used by SRA to consult with industry and leading technical experts on the current management practices and threats, opportunities and priorities for pest, weed, disease and biosecurity management.

2. PROJECT OBJECTIVES

The review focused on four themes:

A. Biosecurity: enhance capacity to manage biosecurity risks

B. Pest control: enhance capability to deal with pests

C. Disease management: improve disease management strategies and technologies

D. Weed management: improve weed management strategies and technologies

The review provides:

1. A critical literature review and summary on each of these themes, focusing on priority issues that are likely to justify RD&A investment and covering work in reports and publications over the last 15 years from the Australian sugarcane industry, other sugarcane industries, and other agri-industries.

2. A review of techniques and technologies that address specific opportunities and threats in the current (and future) industry, including what R&D would be required to develop, adapt and commercialise/extend these techniques and technologies.

3. A summary of the opportunities for RD&A identified as informed by the above two bodies of work.

3. BIOSECURITY THREATS

3.1. General

3.1.1 Responsibilities

The arrival and establishment of a new exotic pest, disease or weed could have significant impact on the economic, sustainability and social aspects of the Australian sugarcane industry. The incursion of

sugarcane smut in 2006 provides a salutatory example (Croft *et al*. 2008; Willcox *et al*. 2008). The risk and subsequent impact can be moderated by appropriate pre-border, border and post-border actions (Plant Health Australia 2016, 2017).

The national border is clearly the responsibility of the Australian Government and is controlled by the federal Biosecurity Act 2016. Management of a pest incursion is by cooperative arrangements between the federal and state governments with input from Plant Health Australia. State governments manage incursions under state government legislation. The initial stages of an incursion of a pest or disease are managed under the Emergency Plant Pest Response Deed (EPPRD), held under the auspices of Plant Health Australia. The sugar industry is a signatory to the EPPRD and is an industry member of Plant Health Australia. SRA is an associate member of Plant Health Australia. Once deemed not eradicable, responses move to management by industry and state governments. Weeds are not covered by the EPPRD, with any response the subject of individual agreements.

Post-border responses are the responsibilities of state governments and industry. Since the 2006 incursion of sugarcane smut, there have been significant changes in Queensland and New South Wales biosecurity legislation. The Queensland (*Biosecurity Act 2015; Biosecurity Regulation 2016*) and the New South Wales (*Biosecurity Act 2015* and *Biosecurity Regulation 2017*) made many changes to regulations regarding threats to the sugar industry. Biosecurity zone boundaries, which control the movement of cane between regions within Queensland, were redrawn to reflect the current distribution of important diseases (Thompson & Wilson 2017). These biosecurity zones protect the industry from movement of pests and diseases between the zones. Previously, Queensland regulations limited growers to planting and growing approved varieties that met minimum disease resistance standards. The varieties of cane that can be grown in different regions is now a voluntary industry code of conduct. SRA staff are not currently inspectors under the Queensland *Biosecurity Act 2015* with powers to impose quarantine and to enter and search properties for suspected pests and diseases. SRA still provides technical advice and cooperates with Biosecurity Queensland, Plant Health Australia and the Federal Department of Agriculture. Two recent publications, a Biosecurity Plan for the sugarcane industry and a grower Biosecurity Manual, provide a guide to farm biosecurity measures to reduce the risks of weeds, pests and diseases impacting production (Plant Health Australia 2016, 2017; project 2014/088). The Biosecurity Plan contains lists of exotic threats and a risk assessment of each threat. Dossiers detailing the symptoms, including photographs, distribution and references of major sugarcane exotic threats can be found at Pest and Disease Illustrated Library (PaDIL, www.padil.gov.au/pests-and-diseases). The SRA website summarises biosecurity arrangements in the sugar industry and has links to information sheets on a number of high-risk threats (https://sugarresearch.com.au/growers-and-millers/biosecurity/).

Pre-border preparedness is where the sugar industry plays the major role and where it should focus its R&D effort. Activities include identifying, understanding and prioritising threats, determining where they occur, working with overseas partners to understand the pests/diseases/weeds in sugarcane systems and how to manage them and to understand the impact they might have on the Australian industry, particularly in terms of the susceptibility of Australian germplasm. This requires ‘insurance’ investment, without short-term benefits and with benefits that are difficult to quantify until the ‘insurance’ is needed – the impact of sugarcane smut was significantly reduced through the pre-emptive screening of all Australian contemporary cultivars and breeding lines in Indonesia.

Saunders (2017) recommended a set of biosecurity actions of importance to SRA:

- Finalise the Industry Code of Practice for varietal selection and receive endorsement from industry and government through the appropriate channels.

- Promotion of General Biosecurity Obligation/Duty to industry to raise awareness of the new legislation in Queensland and New South Wales.
- Investigate the current state of industry and state government surveillance of sugarcane high priority pests in order to develop a surveillance strategy. It is suggested that consultation in the form of a Workshop(s) may be required to identify current activities and gaps.
- Conduct preliminary trials of industry surveillance data capture with Productivity Services and SRA research plantings.
- Investigate the development of an online industry surveillance data capture system, such as an app, as well as incorporation of surveillance recording with Smartcane.
- Establish a biosecurity reference panel that builds on the Industry Biosecurity Group and the Biosecurity Act Working Group to help coordinate, prioritise and monitor implementation of the Biosecurity Plan annually.
- Develop a distribution plan and communications strategy to ensure the Biosecurity Manual is distributed widely. Although industry events may serve as the basis for material distribution, any awareness programs and distribution of information outside of these events will need to be defined.
- Undertake on-farm biosecurity training exercises in major growing regions, including farm-visits and promote what could be implemented at the farm level, including awareness about the GBO, Code of Practice, Smartcane and possible levy proposals.
- Review and develop detailed information sheets on key high priority pests and publish them on the SRA website.
- CANEGROWERS and SRA develop categorisation requests for key high priority pests to initiate the process of categorization through the Emergency Plant Pest Response Deed.
- Review, develop and finalise dossiers / contingency plans for key high priority pests.
- Prioritise efforts in relation to the containment and management of established pests and weeds.
- Investigate the possibility of conducting Fiji leaf gall surveys to determine its distribution and feasibility of eradication
- Determine whether there is interest in conducting a cost-benefit analysis of the annual eradication of *Eumetopina flavipes* in northern Australia
- Review, develop and submit final National Diagnostic Protocols for high priority plant pests to the Sub-Committee for Plant Health Diagnostics.
- Conduct Emergency Plant Pest Response Deed training for key stakeholders/decision makers in the sugarcane industry.
- Identify Industry Liaison Officers and Coordinators in major growing areas, such as cane grower district managers and conduct a training workshop(s) on their roles and responsibilities.
- Run an industry wide simulation exercise to demonstrate the preparedness of an industry and government(s) to an emergency response.

- To be most effective, development of biosecurity R&D priorities that are listed and agreed to in the Biosecurity Implementation Table of the Biosecurity Plan should have a mechanism to feed into the SRA investment planning process, allowing prioritisation within the overall R&D portfolio.
- Prepare submissions to the APVMA for emergency chemical use permits for high priority pests

Recommendation: There is no simple summary of whether these actions have occurred. They need to be reconsidered and those not yet undertaken need to be progressed.

3.1.2 Germplasm exchange – post-entry quarantine

SRA operates an Australian Department of Agriculture, Water and the Environment approved post-entry quarantine facility that imports foreign sugarcane clones. SRA has exchange agreements with most of the major sugarcane breeding organisations overseas. The imported clones are used in the SRA breeding program and imported clones have made important contributions to improved varieties.

Thompson & Wilson (2020) described the comprehensive quarantine procedure and testing to ensure the imported clones do not introduce new diseases. SRA encourages overseas countries to quarantine the varieties and hot-water treat the stalk pieces before dispatch to Australia. This reduces the risk of the canes being infected or contaminated with diseases. The quarantine procedure involves a 12-month quarantine with regular inspections within the SRA high-security quarantine glasshouse, followed by a cold soak-long hot-water treatment. The treated cane is replanted into a separate glasshouse chamber and the ratoon kept for observation for approximately 6 months. The clones are tested for RSD, *sugarcane mosaic virus*, *sorghum mosaic virus*, *sugarcane yellow leaf virus*, *Fiji disease virus*, phytoplasmas, *sugarcane streak virus*, leaf scald and other diseases if relevant. At the end of the quarantine period, SRA applies to the Department of Agriculture for release of the clones, if they are free of diseases. From 2001 to 2019, BSES/SRA has imported 834 clones from a wide range of countries. Diseases that have been intercepted during this period were leaf scald, RSD, sugarcane streak mosaic, sugarcane mosaic, sorghum mosaic and sugarcane yellow leaf (Thompson & Wilson 2020). Sugarcane yellow leaf is not considered quarantinable by the Department of Agriculture because it is present and widespread in Australia. Sugarcane yellow leaf is the disease most often detected in quarantine. In 2001, there was a *Diatraea* mothborer intercepted in a consignment from South America.

CSIRO, in cooperation with BSES, imported true seed from China as part of cooperative research on introgression to improve sugarcane resistance to drought and diseases (Foreman *et al.* 2007; ACIAR funded project).

Diagnostic tests play an important role in post-entry quarantine and the tests that have been developed and used in SRA quarantine are described below for each of the exotic diseases. Mollov and Malapi-Wight (2016) of the USDA quarantine service have shown next generation DNA sequencing (NGS) is a reliable diagnostic tool for rapid virus identification that can be applied in sugarcane quarantine, certification, and breeding programs. This technique can identify known and unknown viruses. The Australian Government Department of Agriculture, Water and the Environment as part of its Rural R&D for Profit program has funded a project (ST16010) aimed at producing a next generation DNA sequencing (NGS) toolkit for exotic disease threats. The project is investigating NGS as a generic test for known and unknown viruses in post-entry quarantine in Australia.

3.2 Exotic pests

The diversity and distribution of potential exotic pests is strikingly different from that of diseases. FitzGibbon *et al.* (1999) provided an assessment of these, focusing heavily on lepidopterous stemborers, a few sucking bugs (some of which are disease vectors) and some whitegrubs that could be confused with endemic species. These remain the most obvious threats, but over 1300 species are known to attack sugarcane and the maxim "Expect the expected, but also expect the unexpected" remains true as shown by the recent arrival of fall armyworm. Dossiers on each were provided in FitzGibbon *et al.* (1998).

The dossiers for the exotics was later updated by Saunders (2017) to comprise: the mothborers *Chilo* spp., *Sesamia grisescens*, *Eldana saccharina* and *Scirpophaga excerptalis*; sugarcane planthoppers (*Perkinsiella vastatrix* and *P. vitiensis*); sugarcane pyrilla (*Pyrilla perpusilla*); root borer (*Polyocha depressella*); sugarcane whitefly (*Aleurolobus barodensis*) and sugarcane woolly aphid (*Ceratovacuna lanigera*). The remainder are now woefully out of date.

Apart from these 'true exotics', two species established in Australia but not in commercial canegrowing areas need to be considered as biosecurity threats. Island planthopper (*Eumetopina flavipes*), the vector of Ramu stunt virus, is established in the Torres Strait, and *Perkinsiella thompsoni*, a potential additional vector of Fiji disease virus, occurred on sugarcane in the Ord region but, with the demise of that industry, its current status is unknown.

3.2.1 Mothborers

Unlike all overseas industries except Fiji, Australian commercial sugarcane has no significant lepidopterous mothborers; only one species of little economic importance, *Bathytricha truncata* (Noctuidae), attacks sugarcane in Australia (Sallam 2006). Of the 36 exotic species recorded on sugarcane, 22 have the potential to invade Australia and cause economic damage to sugarcane (Sallam & Allsopp 2005; Sallam 2006).

Economic damage can be very high; for example, *Scirpophaga excerptalis* can cause up to 40-50% loss in the yield of sugarcane crops (Goebel *et al.* 2014; Kuniata *et al.* 2019) and infested cane contains products that disrupt milling and lower sugar quality. This has led to a series of off-shore projects that have researched the occurrence and management of mothborers (2009/033, Magarey *et al*. 2015; project 2015/046, Magarey *et al.* 2018a, 2019; ACIAR HORT 2006 147; project 2018/010; ACIAR HORT/2012/083.).

Five groups of borers occur as key pests in other industries:

- *Sesamia* spp. (Noctuidae) in Africa and southern Asia through to Papua New Guinea (PNG), with *S. grisescens* an important species in PNG;
- *Chilo* spp. (Crambidae, Chiloini) mainly in southern Asia through to PNG, with *C. sacchariphagus* an important species in Indonesia, *C. terrenellus* an important species in PNG to the northern Torres Strait islands, and *C. crypsimetalla* and an unidentified species on several islands in the Torres Strait (Allsopp *et al.* 2005; Grimshaw & Donaldson 2007);
- *Diatraea* spp. (Crambidae, Chiloini) in the Americas, especially *D. saccharicida*;
- *Eldana saccharina* (Pyralidae) in sub-Saharan Africa, particularly southern Africa;
- *Scirpophaga excerptalis,* topshoot borer, (Crambidae, Schoenobiinae) through south-eastern Asia to Papua New Guinea.

Each of these groups has a dossier on the SRA e-library site that outlines the taxonomy, distribution, host plants, damage type and symptoms, morphology, detection methods, control techniques, means of movement and phytosanitary risk to Australia. For use in a rapid response to an incursion, it is important that these dossiers are updated each 5 years – when this was last done is not given on the dossiers.

Management of borers is usually based on a combination of scouting, geographic risk assessment, natural enemies, inundative releases of reared parasites, plant resistance, insecticides, and scheduling of harvesting and planting to avoid periods of high damage. The best published example is from Ramu Sugar in Papua New Guinea, where Kuniata (1999) developed a system combining all of these tactics. These schemes work best in a plantation-style system where centralised decision-making can ensure appropriate and timely implementation and costs are borne centrally. How such an area-wide system might work in an Australian setting with a large number of individual decision makers and no simple system for allocating costs is difficult to envisage. In very small-scale farming, e.g. India, manual removal of egg masses and young larvae gives good control.

Taxonomy and identification

Accurate identification of moth borer species is very challenging as the larvae that attack cane cannot be reliably identified unless reared through to adults, or DNA-based methods are used. Adults generally lose key external diagnostic characters through incorrect handling, although genitalia dissections yield robust identifications even though those features are subtle. However, the lack of a modern systematic review of stemborers, hampers identification. The presence of these species in neighbouring countries requires Australia to be able to detect incursions and identify species before establishment and spread, in order to implement targeted eradication or management.

Rapid diagnosis of some exotic species is achievable using the DNA-based methods devised by Lange *et al.* (2004), but molecular diagnostics are more effective when they use a universal marker system, such as the COI gene, which is the standard for DNA barcoding. However, the uncritical use of DNA barcode sequences has been problematic, due to incorrectly identified specimens used to generate publicly available DNA barcodes. These issues were addressed for sugarcane mothborers by integrating morphological studies of adult specimens with the generation of new DNA barcodes and nuclear genes using high-throughput ('next-generation') sequencing from all seven high-risk species (*Scirpophaga excerptalis*, *Chilo infuscatellus*, *C. auricilius*, *C. sacchariphagus*, *C. terrenellus*, *Sesamia inferens* and *S. grisescens*), 12 of the 14 medium-risk species, and other low-risk and related species (project 2016/041 and ST16010 (CON-001354) – iMapPESTS – Modernising sugarcane diagnostics; Mitchell *et al.* 2019; Lee *et al.* 2019).

Within the Noctuidae, relationships among *Bathytricha* spp. showed little correspondence to current taxonomy, with *B. truncata* recovered in two different clusters, while *B. monticola* and *B. leonina* shared barcode sequences and, several unique sequence clusters contained samples that could not be assigned to any described species.

Of the two high-risk *Sesamia*, *S. inferens* separated into two distantly related clusters. Both clusters contain samples from China and Pakistan, and one cluster also had samples from India. Each cluster appears more closely related to other species; it is likely the two clusters represent different species.

For *Chilo* the dataset included 18 identified species, each separated from other species in the tree, and seven additional clusters or singletons separated from other species but without identification due to damaged or female voucher specimens or being undescribed species. All four high-risk *Chilo* species are monophyletic groups. *Chilo sacchariphagus* subdivided into three clusters that matched

the subspecies descriptions and distributions, although dissection of additional well-preserved adult specimens is needed to confirm this tentative result.

Scirpophaga excerptalis subdivided into at least five clusters showing a strong geographic structure, with clusters from PNG, China and India/Pakistan well separated from Indonesian samples.

The barcode analyses also clearly indicated that 24 DNA sequences downloaded from online databases were misidentified. *Chilo* species were often assigned to the wrong species and *Sesamia inferens* sequences were mis-assigned to *Chilo*. While these sequences are still misidentified on GenBank, their identities have now been updated on BOLD.

In further (unpublished) work, Lee *et al.* applied multilocus molecular species delimitation techniques to the resulting sequences to estimate species diversity among the samples and determine whether potential cryptic species identified using COI are corroborated by nuclear markers, and tested the congruence of the gene trees. They also estimated divergence dates among lineages, and samples for four *Wolbachia* markers. Of those species previously found to be highly diverse in the COI marker, (particularly *Sesamia inferens*, *Scirpophaga excerptalis* and *Chilo sacchariphagus*) some exhibit similar high diversity in nuclear markers (*S. inferens* and *C. sacchariphagus*) while some do not (*S. excerptalis*). Gene trees were found to be generally congruent, with a few outliers. Four samples were found to be infected with *Wolbachia* from supergroups A and B. Low levels of *Wolbachia* infection indicate that the presence of this endosymbiont may not be the main cause of nuclear/mitochondrial discordance in this group. Further work is now needed to determine whether any distinguishing morphological characters can be found in the delimited species.

Overall, the study clarified the identity and distributions of the major pest species and demonstrated that DNA barcoding is effective as an identification tool when used correctly. It did, however, highlight the need for taxonomic revisions, with least one case of a previously undetected species. Further specimens are being analysed.

Recommendation. Continue the research on DNA methods for mothborer identification and promote comprehensive taxonomic revisions of Sesamia and Chilo.

Insecticides

As shown in the pragmatic Ramu borer management system, insecticides can be an important and useful component of minimising borer impact; this is despite the ideological antipathy to insecticides shown by some previous research partners.

Kuniata (2017) summarised the use of insecticides against *Sesamia* at Ramu. Data from initial whole field size trials (n=20) indicated that highly significant increases in cane yields were achieved due to reduced infestations and damage in sprayed compared with unsprayed cane. In other field trials, an increase of up to 200% in cane yield and up to 150% in sugar yields has been observed in sprayed cane over the unsprayed control plots.

Products used are:

- Tebufenozide (Mimic ®), an insect growth regulator (IGR) specific for Lepidoptera, is used during July to October. It is an expensive product in PNG but is used on a smaller area of young cane (<1000 ha) that will not be harvested that year.
- The pyrethroids permethrin and lambda-cyhalothrin are knockdown products that have limited residue life in the field and are cheaper than the IGR. These are used between December and April, using a spray plane when large areas need to be covered in a limited

space of time. Withholding periods of up to 7 days mean that they are only used on young cane once harvesting starts in April.

- Mospilan® (acetamiprid), was introduced towards the end of 2001 and has performed quite well. It is an expensive product but with a smaller area treated, it has been utilised in the November–December period.

Successful use of these insecticides relies on sampling to provide an early warning that pest populations/damage could reach economically important thresholds. Initially, destructive sampling was used to monitor pests and the damage caused. The method involves taking 200 stalks, sampled at random in a block, the stalks are split open and life stages of *S. grisescens* and damage recorded. These are then used to direct releases of parasitoids and insecticide spraying. The cost of this sampling technique was estimated at about US $2 per tonne of sugar, about 3% of potential crop loss (US $2.75 million). Currently, a pheromone is used to monitor numbers of *Sesamia* moths. An economic threshold of 2 moths per trap per week is used as the basis for scheduling insecticide spraying so as to have insecticide on the plants when eggs hatch. The estimated cost of this monitoring technique is about US $25 000 per annum, or <1% of the value of potential crop losses. The increased effectiveness (reliability) and efficiency compared with destructive sampling (splitting cane) have resulted in lower pest numbers and damage.

Use of a spray plane is expensive and difficult on small fields as found in many Australian farms. A treatment applied to the soil at fill-in and that has a long enough active period to kill borers after they have hatched would be more appropriate. In addition, there are newer active ingredients available that could also be effective against canegrubs – this would reduce any registration costs.

In project 2018/010 (Moth borers – How are we going to manage them when they arrive? – commercial in-confidence, not completed yet), trials were established in Indonesia and PNG to assess the impact of a range of soil applied systemic insecticides applied in plant or ratoon cane against mothborers in both countries. Preliminary data observations show that stemborer damage in PNG trials was caused *by Scirpophaga excerptalis, Chilo terrenellus* and *Sesamia grisescens,* whilst in the Indonesian trials only *Chilo sacchariphagus* and *S. excerptalis* were present. There was a higher incidence of mothborer damage in the PNG trials than in the Indonesian trials, although mothborers were at reasonable infestation levels at all sites to determine insecticide efficacy at the rates and combinations used. Trials, when completed, will provide further information which could ultimately lead to optimised insecticide treatment rates and timing for mothborers in the event of an incursion. Milestone reports do not detail if or how this information has been conveyed to the respective insecticide companies.

Recommendation: Pre-incursion registration of an insecticide is not possible in Australia. SRA has produced dossiers on potential insecticides that could be supplied quickly to APVMA to secure emergency-use permits (Saunders 2019). These need to be updated regularly (say every 3 years) and thought given as to how they would be used commercially in Australia (aerial or ground application, what type of pre-use monitoring, withholding periods, etc).

Inundative release of parasitoids

This tactic relies on the mass production of parasitoids in a purpose-built facility and released, ideally at critical times in the target lifecycle. Egg parasites (usually *Trichogramma* and *Telenomus*), larval parasites *(Cotesia*) and pupal parasites (*Xanthopimpla stemmator*) are most commonly used (Magarey *et al.* 2012a). These are usually reared on surrogate hosts that are more amenable to mass rearing than are borers.

Although widely used in plantation-style enterprises, there is little data available on useful release levels, effectiveness or cost-benefit of these treatments. Many times, releases appear to be made with whatever is available and not strategically on the basis of borer risk, appropriate timing or effective release numbers – a culture of 'any release must be beneficial'. Rearing is also very labour intensive, dependent on low-cost labour that would not apply to the Australian situation.

Sallam (2006) reviewed the natural enemies of 18 key pest species and concluded that the braconid '*Cotesia flavipes*' (a species complex – see below) stood out as a promising candidate for introduction into Australia following a borer incursion; it is capable of parasitising 15 of 18 stemborer pest species distributed in Asia and Indian Ocean islands. However, a range of natural enemies attacking different host stages may be needed to successfully control a target pest, although the interactions may not be simple. For example, introducing egg parasitoids into South Africa had no impact on *Eldana saccharina* populations, since a large proportion of eggs and neonate larvae is already eaten by predators. Alternatively, the pupal parasitoid *Xanthopimpla stemmator* (Ichneumonidae) was introduced to Mozambique, where post-release surveys showed a sharp reduction in the introduced *Chilo sacchariphagus* population in all release fields. No direct competition between *C. flavipes* and *X. stemmator* is expected as they attack different host stages and use different attack strategies.

However, the presence of different strains (notably the Pakistan and Kenyan strains) of *C. flavipes* and several morphologically similar species and synonyms have complicated the understanding of its effectiveness against different borer species and its geographic distribution. In Australia, the name *nonagriae* had been cited as a synonym of *flavipes* and this required resolution before any potential import application could be made.

Muirhead *et al.* (2006) examined the genetic variation among worldwide populations of the *C. flavipes* complex using mitochondrial gene regions, 16S rRNA and COI. This initial analysis supported the monophyly of the complex and the existence of genetically divergent populations of *C. flavipes* and *C. sesamiae*. The geographically isolated Australian haplotypes formed a distinct lineage within the complex and were ~3.0% divergent from the other species. Muirhead *et al.* (2008) reinstated *C. nonagriae* based on molecular, morphological and biological differences

Further work (Muirhead *et al.* 2012) generated nucleotide sequence data for two mtDNA genes (COI, 16S) and three anonymous nuclear loci (CfBN, CfCN, CfEN) for taxa in the *C. flavipes* complex. All phylogenetic analyses provided strong support for monophyly of the complex and the presence of at least four species, *C. chilonis* (from China and Japan), *C. sesamiae* (from Africa), *C. flavipes* (originating from the Indo-Asia region but introduced into Africa and the New World), and *C. nonagriae* (from Australia and Papua New Guinea). Haplotype diversity of geographic populations relates to historical biogeographic barriers and biological control introductions and reflects previous reports of ecological variation in these species. Strong discordance was found between the mitochondrial and nuclear markers in the Papua New Guinea haplotypes, which may be an outcome of hybridization and introgression of *C. flavipes* and *C. nonagriae*, possibly due to intermixing of collections in mass rearing at Ramu Sugar. The position of *C. flavipes* from Japan was not well supported in any analysis and was the sister taxon to *C. nonagriae* and *C. flavipes* (nDNA) and may represent a cryptic species. The concatenated five gene phylogenetic analyses did not support the overall separation and monophyly of clades associated with different host species, although some clades did show specific host associations, possibly due to localized host availability, rather than host specificity. Given the limitations of morphological based identification for members of this complex, molecular identification was recommended prior to any biological control introductions.

Muirhead *et al*. (2008, 2010) compared the biology of *C. nonagriae* on the native noctuid stemborer host *Bathytricha truncata* with *C. flavipes* life history traits by undertaking a detailed study of the life history traits of *C. nonagriae*, including adult longevity and the potential and realised fecundity of

females. In addition, the influence of learning on microhabitat location and foraging behaviour were investigated. Duration of the larval stages and adult longevity of *C. nonagriae* were longer than previously recorded for other members of the species complex. The potential fecundity of females was similar to *C. flavipes* (~200 eggs); however, *C. nonagriae* oviposited an average of over 100 eggs into each host, almost three times more than for other species in the *C. flavipes* complex (30–40). The propensity of *C. nonagriae* to allocate a large number of eggs to each host may be an evolutionary strategy due to the high mortality rate (50–57%) of ovipositing adult wasps. During microhabitat location, both naïve and experienced females demonstrated a strong response towards the plant host complex, with experienced wasps benefiting by having a more rapid response time to host-induced volatiles and cues.

These studies form a strong basis for the importation of a suitable overseas species in the event of an introduction into Australia, particularly as there is difference in thermal resilience and phenologies between sympatric populations of *C. sesamiae* and *C. flavipes* (Mutamiswa *et al.* 2018) and at least three main lineages of *C. sesamiae* in sub-Saharan Africa (Kaiser *et al.* 2017).

Plant resistance

To a grower, a variety resistant to mothborers would have great inherent attractiveness – ease of use, no application costs, little planning needed, no impact of adverse weather, etc. However, borer resistance is not a single, simple trait that can be easily selected for in a breeding program. Non-preference, tolerance and antibiosis components all play roles in 'natural' resistance – from tight leaf sheaths that restrict oviposition, to tough outer rind that restricts initial boring by neonate larvae, to tough nodal tissues that restrict larvae to a single internode, to tough internode tissue that restricts feeding, to toxic chemicals. The release of transgenic cultivar CTC20BT in Brazil expressing the Cry1Ab protein (Cheavegatti-Gianotto *et al.* 2019) has changed this, especially if a second Bt gene can be incorporated to minimise the chance of resistance (as in Bt cotton) and if the Bt genes can be incorporated into normal breeding lines.

However, selection for borer resistance would undoubtably restrict improvement in other desirable traits and impose additional costs on breeding programs. Just how such selection for multiple traits can be done remains a conundrum – focusing on one trait risks losing other resistance traits and there are often two or more borer species with different phenologies and damage potentials and characteristics. The simplest method is to plant trials in infested areas and simply select the clones that do best in terms of cane and sugar yield and ratoonability– logically these will have better resistance, but some monitoring is needed to confirm infestation to be sure that the results reflect resistance levels, and, especially, that what is seen is not simple nonpreference that will not hold up if that clone is planted widely.

Pre-emptive screening of Australian clones or selection programs needs to be conducted off-shore, much in the way that pre-emptive screening for smut resistance was achieved (Croft *et al.* 2008). This requires close relationships between Australian breeders and overseas organisations; Indonesia (Indonesian Sugar Research Institute) and Papua New Guinea (Ramu Sugar) are the obvious locales. Both would require substantial funding, placement of Australian staff and careful consideration of cultural differences. The Indonesian Government's attitude to foreign scientists and the de-emphasis of sugar relative to oil palm at Ramu and the likely retirement of Dr Kuniata in the next few years are likely to make relationships at both places more problematic.

Resistance screening methods were investigated in a series of off-shore projects (2009/033, Magarey *et al*. 2015; 2015/046, Magarey *et al.* 2018a; ACIAR HORT 2006 147) with field trials at Ramu Sugar and in Indonesia.

General techniques

Information on procedures for screening sugarcane varieties for resistance to moth borers and associated research was summarised from the literature and by a 2-week visit to SASRI (South African Sugarcane Research Institute) by Samson (project 2009/033). Screening procedures are mainly of two types:

- in-field plots where plants are infested by naturally occurring populations of moths, sometimes encouraged by planting of susceptible host plants near the experimental plots and/or augmented by the release of additional moth borers from laboratory culture
- in pots where plants are infested artificially, often using moth-borer eggs.

Each of these has advantages and disadvantages. Natural infestation of field plots allows the full range of resistance mechanisms – antixenosis, antibiosis and tolerance – to operate under commercial conditions, but results are subject to considerable experimental variation due to variable environmental conditions and inconsistent numbers of borers. Artificial infestation of potted plants allows the experimenter to control environmental variables and apply a constant infestation pressure, but some components of resistance (especially ovipositional antixenosis) may be missed. While artificial infestation has been used in numerous studies to elucidate resistance mechanisms, it seems to be currently used as a routine method of screening varieties for borer resistance only at SASRI against *Eldana*.

Molecular markers for resistance have been identified that can help with choice of parents in breeding programs. There is some evidence of ovipositional antixenosis, larval antixenosis and antibiosis and plant tolerance as mechanisms of genotypic sugarcane resistance against different species of moth borers. Of these, antixenosis or antibiosis acting against early-stage larvae seems the most common, preventing larval penetration of the stalk or delaying penetration so that small larvae are exposed to abiotic and biotic mortality factors. Near-infrared spectroscopy (NIR) has potential as a method for predicting resistance that is associated with stalk surface chemistry. Phenotypic resistance may be altered by plant nutrition; water stress or increased levels of nitrogen may increase susceptibility while increased silicon may promote resistance.

Data collected in resistance trials always includes a measure of borer damage, typically a count of bored internodes, and sometimes a measure of larval performance such as number or weight of borers or number of emergence holes indicating successful production of adults. A few studies have included plants from which borers are excluded, usually with insecticide, which allows crop tolerance to be measured, or have estimated tolerance by rating plant response according to indirect measures such as side-shooting or stalk breakage.

Systematic screening of varieties ideally includes a set of standard varieties covering the range of expected responses from susceptible to resistant. Most screening programs rate varieties as susceptible, resistant or intermediate, but the program at SASRI rates varieties on the 1-9 scale familiar to plant pathologists, with the results weighted according to experimental precision.

The SASRI system provides a model that would be useful in an Australian setting post-incursion. It has adopted an annual program of screening varieties from stages 4 and 5 of their 5-stage breeding program in pots in a shadehouse for resistance to *Eldana.* The method ensures uniform infestation of plants, allows better control of environmental variables and requires less labour than similar field trials. However, yield trials in stages 4 and 5 are also sampled for *Eldana* damage but with fewer variables measured than in the shadehouse. Plants in the shadehouse are assessed for damage as bored internodes and larval performance as number of live larvae. Length of bored internodes and weight of larvae and pupae were initially measured but were strongly correlated with the other two variables and so were dropped from the procedure. Six standard varieties are included in each screening trial and both standard and test varieties are rated on a 1-9 (resistant-susceptible) scale by

a statistical method that weights the measured variables according to their variance and then calculates ratings according to the precision of the experiment; the latter calculation is fundamentally different from the method used by SRA plant pathologists.

Screening at Ramu

As an initial step, Korowi *et al.* (2011) analysed the results of two variety trials planted in 2003 and 2007 with the aim of evaluating varietal responses to these borers particularly to S*esamia grisescens*, the major species at Ramu:

- For *Sesamia* and *Scirpophaga*, damage and/or numbers differed significantly among clones in at least one trial.
- Varietal differences were not detected for *Chilo*.
- There was a negative correlation between damage from *Chilo* and *Sesamia* in one trial and it is possible that damage from the latter species may interfere with the activities of *Chilo* or destroy evidence of its presence.
- Cane yield was measured in one trial and was negatively correlated with damage from *Sesamia* but not from *Chilo* or *Scirpophaga*.

The study confirmed that screening of Australian commercial varieties for resistance to *Sesamia* in PNG is worthwhile, but apparent varietal differences in crop response to borers in PNG may be influenced by interactions among species.

Two further trials showed highly significant differences among clones for internode damage from *Sesamia* and stalk damage from *Scirpophaga* (Korowi & Samson 2013; Magarey *et al.* 2012b; Samson *et al.* 2017). Over all trials, there was a greater and consistent difference among clones for damage from *Sesamia* and *Scirpophaga* than from *Chilo*. This consistency indicates potential to develop a rating system for *Sesamia* and *Scirpophaga* that will allow test varieties to be grouped into categories of resistance or susceptibility, but this is less likely for *Chilo*.

However, the response of the standard clones was not consistent among trials. This indicates interference between infestations of *Chilo* and especially *Sesamia* related to their different biologies. Selection for one species therefore is confounded by the presence of other species and resistance mechanisms probably act differently.

In an effort to develop faster, more efficient screening, a short-term shadehouse technique was trialled with *Scirpophaga* (Magarey *et al*. 2018b) with the aims of assessing the tolerance/resistance of six varieties, and to confirm that larval dispersal to adjacent plants, after hand placement on selected stalks, provides for a reliable resistance assessment.

Overall, the technique proved useful with a noticeable effect of variety on dead-heart symptoms, tunnel length and larval weights. Of the Australian clones, Q135 was the least affected and Q219$^{\phi}$ the most affected; Q208$^{\phi}$ demonstrated low damage levels, while Q235$^{\phi}$ appeared to be more susceptible. No Australian variety showed significantly lower severity measurements than RQ117, a clone selected under borer pressure at Ramu.

Screening in Indonesia

Sallam *et al.* (2014) examined damage levels caused by *Chilo auricilius*, *C. sacchariphagus* and *Scirpophaga excerptalis* in 931 sugarcane farms across Java, Indonesia. Of 39 varieties used in Java,

only the main 7 commercial varieties were studied. Varieties were ranked according to levels of borer damage expressed as either dead hearts, leaf damage or bored internodes. No patterns were clear for either dead heart, leaf damage or internode damage symptoms caused by *C. auricilius*. For *C. sacchariphagus*, Kidang Kencana had the highest mean dead heart and leaf damage and PS951 sustained highest bored internode damage, while PSJT941 showed lowest internode damage. For *S. excerptalis*, PS951 showed the highest dead heart symptoms and PSJT941 suffered the least damage, while PS851 had significantly the lowest leaf damage symptoms. This study will serve as a basis to identify candidate varieties for borer resistance in future breeding programs.

Future directions on resistance

- A system similar to that used at SASRI appears the most useful for screening clones in the breeding program and for screening current Australian cultivars, particularly post-incursion.
- Pre-emptive screening needs to be done off-shore, with Indonesia and Papua New Guinea the most logical locations, but this requires adequate funding to overseas partners and close attention by Australian entomologists and breeders.
- Off-shore screening of new Australian cultivars has a significant time lag due to biosecurity constraints in these countries and the need to bulk-up material off-shore before useful trials can be done.
- A range of resistance types exist in sugarcane germplasm, so trials and associated sampling need to be designed to account for these.
- Different borer species have different damage types and infestation by one may mask the impact of others – this complicates the interpretation of results from trials infested by multiple species.
- No one parameter is adequate to assess resistance.
- SRA should attempt to obtain overseas clones with significant levels of resistance and incorporate these as parents in the current breeding program or as a sub-program along the lines of what was done for sugarcane smut.
- The use of GM technology in Brazil for mothborer resistance needs to be followed carefully and re-assessed for use in the Australian industry.

Cultural controls

The crop production system used at Ramu incorporates a range of control tactics. Initial work (Kuniata 1999) used an interaction matrix to identify production factors that have a significant effect on *Sesamia* populations. The most significant include varietal resistance, time of planting/ratooning, and the use of natural enemies. Sites with a high environmental risk from spraying have been identified on the plantation and more resistant varieties are used in these areas. These are usually along riverbanks where it is important that spray drift is reduced. More than 60% of the 1 500 ha of cane to be planted each year is planted during March-June, thus presenting a semi-mature crop (less attractive to *Sesamia*) when populations start to increase in February-March the following year. Cane planted/ratooned from September to November will be highly susceptible to borer damage, but this area is smaller and can be easily sprayed for borer control, thus reducing insecticide usage further.

Management of the system at Ramu is relatively simple, with only a few people involved in decisions for the entire enterprise. A similar system in Australia would be much more difficult with individual on-farm decision makers and much more geographically disperse production areas.

Recommendation: Consideration needs to be given as to how area-wide management could work within the Australian system.

Training

The hands-on experience with borers in Indonesia, Papua New Guinea, South Africa and USA from the early 1990s gave the SRA entomologists good understanding of the biology, ecology and management of these pests. All of these people (Chandler, Sallam Samson, Allsopp) have since retired or moved to other roles – it is vital that current staff, as well as breeders and staff from Cane Productivity Services, gain a similar understanding.

3.2.2 Whitegrubs

Whitegrubs (canegrubs) are minor pests in other sugarcane industries. A complex of species exist in Indonesia, Papua New Guinea and through the Solomon Islands and Vanuatu to Fiji. Their taxonomy is not well understood, and all are similar to endemic Australian species. Any introduced species is likely to be overlooked in Australia and not identified until well established. However, the risk of introduction is low as most would require the transport of soil in which the larvae could survive.

Control would be the same as used for endemic Australian species; for example, Magarey *et al.* (2012a) and Achadian *et al.* (2013) tested imidacloprid against the Indonesian species *Lepidiota stigma* and found it effective.

No further pre-incursion R&D is warranted.

3.2.3 Sucking pests

Island planthopper

The island planthopper, *Eumetopina flavipes*, is the only known vector for Ramu stunt disease of sugarcane. Ramu stunt disease appears confined to Papua New Guinea (PNG), but disease-free populations of the vector are known to occur throughout the Torres Strait island archipelago (TS) and on the northern part of Cape York Peninsula (Anderson 2011; Grimshaw & Donaldson 2007; Anon. undated). In Papua New Guinea, *E. flavipes* occurs on *Saccharum officinarum*, *S. robustum*, *S. edule* and *Saccharum* hybrids. The species was the subject of a PhD thesis by Kylie Anderson (2011) (STU052).

The genus *Eumetopina* is thought to have evolved in New Guinea. Seven described species (and some synonyms) and up to 25 undescribed species have been collected, but many more may be present. Michael Wilson, at the National Museum Cardiff, was revising the genus during the early 2000s but this has not been published and the status of specimens from surveys through TS, PNG and Indonesia that were sent to him is unknown. A contemporary taxonomy is required, although the species in TS is probably *flavipes* given its colouring (flavipes = yellow legs).

Adults and nymphs reside in the ‘spindle’ roll, or growing tip of the sugarcane plant. Large numbers of individuals (i.e. >100) are not uncommon in PNG. The ability of *E. flavipes* to hitch-hike on cut sugarcane stalks moved by people throughout the region was assessed by Anderson *et al.* (2007).

They found that almost half of the initial population of nymphs and almost one third of the adults survived six days *in situ* on cut stalks; indicating that *E. flavipes* is capable of surviving extended periods of time on deteriorating cane. These results imply that human-mediated movement may play an important role in the dispersal of *E. flavipes* and may be a route to the introduction to commercial cane areas in Australia.

As a first step in establishing the invasion potential through TS, Anderson *et al.* (2009) showed that *E. flavipes* utilises a wide range of *Saccharum* host species in PNG and that the occupation rates and abundances differed significantly among host types. For hosts in common, the proportion of plants occupied in PNG was significantly greater than in TS. This is likely the result of greater overall host density and connectivity in PNG. *E. flavipes* abundance per plant did not differ significantly between the two regions suggesting a possible plant-specific abundance and/or dispersal threshold independent of location. Whilst *E. flavipes* presence and persistence was highly variable at some TS locations, large and stable infestations occurred down the western edge of the TS archipelago. These populations appear to link PNG to the Northern Peninsula Area and offer a potential incursion route for Ramu stunt disease. The stability of these populations appears to be associated with the availability and persistence of host material, which in turn is significantly affected by variation in cultivation practices.

Wind-assisted immigration from Papua New Guinea was predicted to occur widely throughout the Torres Strait islands and the tip of mainland Australia, especially in the presence of tropical depressions and cyclones (Anderson *et al.* 2010). Simulation showed potential for a definite, seasonal immigration which reflected variation in the onset, length and cessation of the summer monsoon, but in general, simulation predictions did not explain *E. flavipes* observed infestations. The discrepancy suggests that post-colonization processes such as the temporal and spatial availability of host may be equally or more important than possible wind-assisted immigration in determining population establishment, persistence and viability. An analysis of eight microsatellite loci from 648 individuals suggested that frequent, wind-assisted immigration from multiple sources in PNG contributes significantly to repeated colonization of far northern TS islands (Anderson & Congdon 2013). However, intermittent wind-assisted immigration better explains patterns of genetic diversity and structure in the southern TS islands and on the tip of mainland Australia. Significant population structuring associated with the presence of clusters of highly related individuals results from breeding *in situ* following colonization, with little post-establishment movement. Results also suggest that less important secondary movements occur between islands; these appear to be human mediated and restricted by quarantine zones.

Overall, control of the planthopper may be very difficult on islands close to PNG given the apparent propensity for multiple invasion but may be achievable further south where local populations appear highly independent and isolated. In TS, implementation of pre-emptive management of *E. flavipes* via cultivation techniques, such as simultaneous tip-pruning, may be an effective means of control for the pest, and would be simpler and preferable to the direct management of Ramu stunt disease should it be detected in TS.

Recommendation: Work with Torres Strait and Bamaga communities to emphasise the potential importance of Eumetopina to the Australian industry and what measures they should undertake to prevent its spread.

Sugarcane woolly aphid

This species, *Ceratovacuna lanigera*, is a serious pest particularly in the Indian subcontinent, but its distribution extends from India through south-east Asia, southern China, PNG and the Solomon Islands to Fiji. It lives in large colonies on the leaves, sucking phloem and excreting large amounts of

honeydew that provides an ideal environment for the development of sooty mould. The direct and indirect feeding reduces cane yield (up to 30% loss) and lowers the quality of the juice that causes problems in extraction and sugar quality. Joshi & Viraktamath (2004) provide a good summary of its impact, life cycle and management, and some experience has been gained by Australian entomologists in Indonesia and PNG (Anon. 2016).

Infestations can be controlled with insecticides, but there is a wide range of natural enemies that usually keep them under some control. Similar aphid-feeding natural enemies are found in Australia and would be expected to exert some control. Soil-applied imidacloprid (for canegrub control) could help reduce populations in Australia. Varieties also vary in their attractiveness (properties of the leaf surface, e.g. Aravind & Kajjidoni (2007).

Given the extensive research in places such as India, no Australian-funded off-shore research is recommended. The dossier on this pest was updated in project 2014/088 (Saunders 2019).

Sugarcane pyrilla

This planthopper, *Pyrilla perpusilla*, is a serious pest in the Oriental region through to Vietnam and Indonesia. It sucks phloem sap from leaves and excretes honeydew onto foliage, leading to development of sooty mould. This direct and indirect damage affects sugar yield and quality. Kumarasinghe & Wratten (1996) provide a good summary of its impact, life cycle and management.

Infestations can be controlled with insecticides, but there is a wide range of natural enemies that usually keep them under some control (e.g. Kumarasinghe & Wratten 1996; Gangwar *et al.*2008). Soil-applied imidacloprid (for canegrub control) could help reduce populations in Australia. Varieties also vary in their attractiveness.

Given the extensive research in places such as India and Sri Lanka, no Australian-funded off-shore research is recommended. The dossier on this pest was updated in project 2014/088 (Saunders 2019).

Sugarcane whitefly

Sugarcane whitefly, *Aleurolobus barodensis*, is a serious pest on sugarcane throughout the Indian subcontinent and through to Thailand. The nymphs of whiteflies suck the sap from the undersurface of the leaves and the severe whitefly infestation may result in reduction in cane yield as well as sugar recovery; cane yield up to 24 % and loss in sugar up to 2.9 units (Bhavani & Narasimha Rao 2013). They are particularly pests under low nitrogen conditions. Insecticides, cultural controls and natural enemies all play roles in controlling this pest.

Given the extensive research in places such as India and Thailand, no Australian-funded off-shore research is recommended. The dossier on this pest was updated in project 2014/088 (Saunders 2019).

3.3 Exotic diseases

3.3.1 Ramu stunt

Ramu stunt is caused by a virus and causes severe stunting and death of plants (Braithwaite *et al.* 2007, 2019; Magarey 2015;2018; SRA projects 2009/033 and 2015/046). In the early 1980s, there was an epidemic of the disease at the Ramu sugar plantation in Papua New Guinea that caused devastating losses. The susceptible variety, Ragnar, was planted over 90% of Ramu plantation and

the whole plantation had to be replanted within 12-18 months to prevent further losses. Ramu stunt has only been reported from Papua New Guinea where it is found in both commercial sugarcane and in chewing canes (*Saccharum officinarum* L.) grown in village gardens. Mollov *et al.* (2016) sequenced the six RNA segments of the virus and showed the virus had similarity to *Tenuivirus* and *Phlebovirus.* Ramu stunt is spread by infected planting material and the planthopper, *Eumetopina flavipes* Muir (Braithwaite *et al.* 2019). The insect vector has been reported in Cape York and Torres Strait (see Pest section of this review). Surveys of commercial crops at Ramu and in village gardens found the disease was less common than predicted from early surveys based on symptoms alone. A distinctly different genotype of the virus was found near Alotau 700 km south east of Ramu on the southern side of the Owen Stanley Range.

Braithwaite *et al.* (2007, 2012) described a diagnostic RT-PCR (reverse transcriptase – polymerase chain reaction) assay for Ramu stunt. The assay has been used in surveys (Braithwaite *et al.* 2014), validation of disease resistance trials (Braithwaite *et al.* 2012), vector transmission studies (Braithwaite *et al.* 2019) and in post-entry quarantine (Thompson & Wilson 2020). Prior to the availability of the assay, surveys were conducted in Papua New Guinea that found that Ramu stunt symptoms were widespread throughout Papua New Guinea and possibly present in Indonesia (Magarey *et al.* 2007; ACIAR project HORT/1996/147). However, the presence of the virus was not confirmed, and later surveys using the diagnostic assay suggest that the virus was not common. The diagnostic assay is an essential component of preparation for any incursion of Ramu stunt.

Resistant varieties have successfully controlled Ramu stunt and, once the susceptible variety, Ragnar, was replaced by resistant varieties, the disease became rare on Ramu plantation. SRA has screened some Australian varieties for resistance to Ramu stunt in preparation for an incursion of this disease (Braithwaite *et al.* 2012; Magarey *et al.* 2012b; Magarey 2018). Ramu stunt resistance trials are conducted by planting the test varieties between rows of Ramu stunt infected cane and relying on natural spread by the insect vector. Kuniata *et al.* (2010a) reviewed past Ramu stunt resistance trials and reported that none of the 47 older Australian varieties tested were rated susceptible and only two, Q125 and Q134, had intermediate resistance. The remaining 45 varieties were rated resistant. Magarey (2015) reported the results for three series of Ramu stunt resistance trials containing 39 Australian varieties. Two important commercial varieties, KQ228$^{\phi}$ and Q231$^{\phi}$, showed moderate infection levels. In these Ramu stunt resistance trials, the level of disease was low, and Magarey (2015) did not consider the ratings as reliable. Of the current recommended varieties, 23 out of 85 have been screened for Ramu stunt resistance. Only one variety, CP74-2005, is rated susceptible.

It is difficult to assess the risk of Ramu stunt. The low incidence of the disease in commercial fields at Ramu and the low incidence of the disease in surveys of garden canes makes it unlikely that the disease would spread to Australia. The low number of susceptible Australian varieties would decrease the risk of serious loss, if an incursion occurred. The presence of the insect vector in Australian territory on Cape York and in the Torres Strait increases the risk. If there was an incursion of Ramu stunt and the vector, the rate at which it spread would depend on suitability of the environments in the sugarcane growing areas for the vector. Little is known about the environmental factors controlling populations of the vector. Efforts should continue to obtain reliable ratings for the major commercial varieties.

Recommendation: Continue research to develop a reliable method of rating varieties for resistance to Ramu stunt and rate the major commercial varieties.

3.3.2 Sugarcane downy mildew

Downy mildew in sugarcane is caused by the oomycetes, *Peronosclerospora sacchari* (Papua New Guinea and Fiji) and *P. philippinensis* (Philippines and some other Asian countries) (Suma & Magarey 2000). Both species also infect corn and some other grasses. The disease is characterized by chlorotic streaks on the leaves and down on the underside of the leaves that is produced on warm humid nights. Infected plants can produce tall thin stalks that grow rapidly and are much taller than surrounding stalks. The organism is present in all plant tissues and infected plants are unthrifty and yield losses of 60-75% have been reported (Rauka *et al.* 2005). The down on the underside of the leaves contains the conidia or asexual spores of the organism. The spores only spread short distances and are very delicate, losing viability by 10 or 11 am on the day in which they were produced. The organism also produces oospores within the leaves that can cause the leaves to split and shred. These spores can survive in the soil and can infect plants but their role in transmission of the disease in nature is not well understood.

The taxonomy and identification of species of *Peronosclerospora* is difficult. Recent studies of the molecular diversity of downy mildew in Papua New Guinea have been conducted by Thompson *et al.* 2013 and Magarey (2015, 2018) (SRA projects 2009/033 and 2015/046). Symptoms of downy mildew have been found in Papua New Guinea in species closely related to sugarcane, including pit pit (*Saccharum edule*), noble canes (*S. officinarum*), wild cane (*S. robustum*) as well as more distantly related species such as *Miscanthus* and corn (*Zea mays*). The DNA studies have shown strong evidence that there are two species of downy mildew infecting commercial crops at Ramu, but the dominant species is *P. sacchari*. DNA studies suggest there are a further three species infecting wild sugarcane and other grasses. One DNA type infects *Miscanthus* (probably a new species of *Peronosclerospora*, genetically different from the *P. miscanthi* already described), one infects *S. robustum* in the lower Madang and Gusap Province, and another that can infect a wide range of hosts. No *P. philippinensis* was detected in Papua New Guinea but some of the samples had DNA profiles similar to *P. philippinensis.*

P. sacchari was present in Australia until 1972. It was common and widespread and in 1940 was considered the most important disease of sugarcane in Australia (Croft *et al.* 2000). By the mid-1950s the disease had been eradicated from commercial crops and in 1972 the last downy mildew disease resistance trials on the BSES Pathology Farm at Eight Mile Plains in Brisbane were destroyed and the disease has not been seen in Australia since then. *P. sacchari* is common in Papua New Guinea and moderate infection is seen in some commercial varieties grown on the Ramu plantation. *P. sacchari* was common in Fiji but is now rare. *P. philippinensis* causes serious disease of sugarcane and corn in the Philippines.

Downy mildew can be eliminated from planting material by hot-water treatment at 50°C for 2 hr or by treatment with metalaxyl biocide. Metalaxyl applied to the setts and soil at planting will protect plants from infection for up to 3 months (Suma & Magarey 2000).

Resistant varieties are the primary method of managing downy mildew and rating Australian varieties for resistance was one of the main objectives of Magarey (2015, 2018) (SRA projects 2009/033 and 2015/046). The method used to screen varieties for resistance is to plant one row of downy mildew infected cane to every two rows of the test varieties and to rely on natural spread of the disease. Plants are inspected for symptoms in the first ratoon crop. Kuniata *et al.* (2010b) reviewed the downy mildew resistance trials conducted at Ramu from 1986-2008. They reported that 42% (7/33) of older "Q" varieties were too susceptible to be grown at Ramu and only 18% were resistant. The remaining 40% of varieties were rated intermediate. Magarey (2015) reported the downy mildew incidence in 39 Australian varieties screened in 2010, 26 in 2011 and 37 in 2013. This data is available in the SRA plant breeding database, SPIDNet. Only 34% (29/85) of the currently recommended varieties have ratings for downy mildew resistance. Forty one percent of the varieties with ratings were rated susceptible. Five of the top seven varieties grown in Australia, accounting for

60% of the crop, are rated susceptible to downy mildew (Q183$^{\Phi}$, Q200$^{\Phi}$, Q208$^{\Phi}$, KQ228$^{\Phi}$ and Q242$^{\Phi}$). The other two varieties in the top seven, Q232$^{\Phi}$ and Q240$^{\Phi}$, do not have ratings. The Australian crop is very vulnerable to an incursion of downy mildew.

Magarey *et al.* (2014, SRA project 2015/046) described development of a rapid method of screening clones for resistance to downy mildew by inoculating the soil with oospores collected from infected leaves. The test varieties were planted into pots containing the oospores. Initial results were very promising with high levels of disease in two experiments. Magarey (2018) reported that further experiments with oospore inoculation were inconsistent. The technique is promising but needs further testing and development.

Magarey (2018) reported experiments using conidia (asexual spores) and oospores from corn, sugarcane and *S. robustum* to infect commercial sugarcane, *S. robustum, S. edule* and *S. officinarum* (garden canes). They found that conidia from corn were highly infectious to sugarcane and that corn, *S. edule* and *S. officinarum* are all susceptible to downy mildew. The oospores taken from *S. robustum,* which are probably a different species to that infecting commercial cane, were able to infect sugarcane.

The data clearly shows that the potential for losses from downy mildew are high with the disease capable of causing severe losses. Experience in Australia from earlier outbreaks has shown that the disease can establish in all regions. The high level of susceptibility of Australian varieties makes the industry particularly vulnerable. The risk of spread of downy mildew however is low. The disease has no recent history of spread between countries. Spread into Australia by the asexual conidia (spores) is extremely unlikely because the spores are very fragile and are unable to spread long distances. The only likely source of an incursion is by movement of plant material from an infected plant. The disease is not common in garden canes but is common on the Ramu plantation, the two most likely sources of infected planting material. There is considerable movement of people between Papua New Guinea and Australia, including traditional movement through the Torres Strait. Strict Australian border quarantine reduces the risk of plant material entering Australia illegally. Overall, the risk from downy mildew is rated as moderate to high.

Recommendation: SRA should continue to screen Australian varieties for resistance to downy mildew. More efficient and reliable methods for screening varieties should be investigated.

3.3.3 Mosaic viruses

Mosaic diseases of sugarcane are characterized by a pattern of contrasting shades of green on the leaf blade. In the past, all mosaic diseases were attributed to S*ugarcane mosaic virus* (see section 5.7) with many different strains of the virus. DNA sequencing of the different strains has allowed a reorganization of the viruses causing mosaic in sugarcane and now the group has been split into different species. The three most important species in sugarcane are *Sugarcane mosaic virus, Sugarcane streak mosaic virus* and *Sorghum mosaic virus.* Only *Sugarcane mosaic virus* is present in Australia.

Sugarcane streak mosaic

A mosaic infected sugarcane plant intercepted in post-entry quarantine in the USA was studied by Hall *et al.* (1998). They found that the virus causing the symptoms was in a different genus of the *Potyviridae* to *Sugarcane mosaic virus* and they named the virus, *Sugarcane streak mosaic virus. Sugarcane streak mosaic virus* belongs to the genus *Rymovirus.* Subsequently, *Sugarcane streak mosaic virus* has been reported to be extremely widespread and common in Indonesia, India,

Pakistan, Bangladesh, Myanmar and Thailand (Chatenet *et al.* 2005, Hema *et al.* 2008; Damayanti & Putra 2011). It is believed that this virus was previously named *Sugarcane mosaic virus* strain F.

Streak mosaic in Indonesia is considered a high risk to Australia and has been investigated in two Australian Centre for International Agriculture Research (ACIAR) funded projects (Magarey 2006, 2019, ACIAR HORT 1996 147 and HORT/2012/083).

Streak mosaic can easily go undetected by growers and industry advisors. The symptoms of streak mosaic are indistinguishable from the symptoms of the other viruses causing mosaic and assays are required to help detect the disease and determine the species causing the disease. Diagnostic RT-PCR tests for streak mosaic have been developed (Damayanti & Putra 2011; Magarey *et al.* 2018c; Thompson *et al.* 2019). The RT-PCR tests have been used to survey plantations in Indonesia. Damayanti & Putra (2011) reported that more than 50% of plantations were infected and there was a high incidence in variety PS864. Putra *et al.* (2014) conducted an extensive survey during 2008-2009 in 30 mill areas across Java and found that 30% of fields in 28 of the mill areas were affected by the streak mosaic. Most commercial varieties were infected by the virus, but the highest incidence was in the variety PS864. They also reported streak mosaic in sorghum, corn and the weed, *Dactylactonium aegypticum*. Magarey (2019) found that streak mosaic was present in four of the six mill areas surveyed in Sumatera and West Java with infection levels as high as 35% infected plants. Magarey (2019) reported streak mosaic for the first time in Sulawesi.

Streak mosaic is transmitted by infected planting material. It can also be transmitted experimentally by rubbing leaves with an abrasive pad dipped in infected juice (Magarey 2019). Putra *et al.* (2014) reported that the disease could be spread by knives contaminated with juice from infected stalks but Magarey (2019) found no transmission by knives.

Generally, *Potyviridae* are transmitted by insects, often aphids. Putra *et al.* (2014) failed to transmit the disease with *Rhopalosiphum maidis* (corn aphid) and *Ceratovacuna lanigera* (sugarcane woolly aphid). Magarey (2019) reported the incidence of the virus in different insect species collected from streak mosaic infected plants. This information demonstrates the potential for these insects to transmit the disease but does not prove that they are vectors and further research is needed to confirm that the insects can transmit the virus to healthy plants. Virus was detected in the planthoppers, *Perkinsiella saccharicida* and *Eumetopina* sp.*,* the aphid, *Melanaphis sacchari,* a lace bug (Tingidae sp.*)* and a palm planthopper. Magarey (2019) also conducted epidemiological studies to determine the rate of spread within blocks at two sites on Java. At one site the spread was moderate, increasing from 0 to 12% infected plants from plant to second ratoon and at the other site the spread was slower, only reaching 4% by second ratoon. This rate of spread combined with spread in infected planting material could lead to unacceptable increases in disease over time. It is important to identify the natural vectors of the disease so that the presence of the vectors in Australia can be determined. This will significantly affect the risk of establishment and spread in Australia.

Putra *et al.* (2014) reported yield losses of 20% when there were 50% infected plants. Magarey *et al.* (2019) reported yield losses of between 17-26% in plant, first and second ratoon crops. Streak mosaic is a significant limit to productivity in susceptible varieties. It was estimated that streak mosaic is causing losses of approximately Indonesian Rupiah 447M ($46,000) in Java.

Putra *et al.* (2014) screened 16 commercial varieties grown commercially in Indonesia by artificial inoculation with infected juice and abrasive pad and found that 31% were resistant, 38% intermediate, 19% susceptible and 13% highly susceptible. Magarey (2019) screened 10 Australian varieties in a glasshouse and a field trial with a similar artificial inoculation method. In the glasshouse, there were only a small number of plants, but 8/10 Australian varieties showed some infection, with 100% infected plants in Q231$^{\Phi}$ and 67% in KQ228$^{\Phi}$. Only preliminary results were available from the field trial and there were generally low levels of disease. Four Australian varieties

were showing some infection. KQ228$^{\phi}$ had the highest infection at 22% followed by Q200$^{\phi}$ with 7%, Q240$^{\phi}$ with 6% and Q138 with 5%. Knowledge of the resistance of Australian varieties is a key component of a response plan.

Magarey (2006) reported that hot-water treatment of setts at a range of temperature-time combinations provided no control of streak mosaic. Tissue culture from streak mosaic infected plants produced 70% plantlets apparently free of the disease. Tissue culture appears to be a valuable method of producing virus-free plants to establish seed-cane plots. Disease-free seed plots would need to be positioned in a location at low risk of reinfection. Disease-free seed plots are an option to reduce losses from streak mosaic.

Streak mosaic remains a significant threat to the Australian sugar industry. It causes moderate yield loss and there is a high likelihood that insect vectors will be present in Australia. This disease has a history of spread between countries. Australia's strict border quarantine regulation and enforcement reduces the risk of an incursion. Streak mosaic has already been intercepted in germplasm imported into Australia from Asia (Thompson & Wilson 2020). Spread to Australia through the Torres Strait is a high risk as the disease is present in West Papua only a short distance from Australian territories. Streak mosaic is rated as a moderate to high risk.

Recommendation: Continue cooperative research with Indonesia to identify vectors of streak mosaic and to screen Australian varieties for resistance to the disease.

Recommendation: Conduct targeted surveillance in the south-eastern provinces of Papua New Guinea and Torres Strait to monitor for spread of streak mosaic.

Recommendation: Screen any germplasm imported into Australia that has originating from Asia intensively for streak mosaic.

Sorghum mosaic

Sorghum mosaic virus (previously sugarcane mosaic strains H and I) causes mosaic in sugarcane in North and South America and has been recorded in China (Xu *et al.* 2008; Perera *et al.* 2009; Rice *et al.* 2019). In Louisiana, mosaic caused by *Sorghum mosaic virus* has been historically an important disease. Successful breeding for resistance in the 1980s and 1990s reduced the disease to low incidence in commercial cultivars. However, recent reports of mosaic at multiple locations lead to uncertainty concerning the current distribution and incidence. Field surveys were conducted in Louisiana from 2016 to 2018 in breeding program yield trials and experimental clone seed-cane increase fields. Mosaic symptomatic plants were observed in a newly released variety, HoCP09-804, in three of five production areas, with incidences ranging from 0 to 10%. Mosaic also was observed in nine experimental clones. Evidence pointed to distribution in seed cane and that spread within fields was low.

Thompson & Wilson (2020) reported intercepting clones from Asia infected with *Sorghum mosaic virus* in 2010. SRA quarantine uses both serological and RT-PCR assays to detect *Sorghum mosaic virus.*

Sorghum mosaic virus is a moderate threat to the Australian sugar industry. It causes moderate yield loss and has the potential for spread by insects and in planting material. This disease is widespread in North and South America and in China, but Australia's strict border quarantine regulations and enforcement reduces the risk of an incursion. SRA should monitor spread of *Sorghum mosaic virus* and ensure all germplasm imported from the Americas and China is screened for *Sorghum mosaic virus.*

3.3.4 White leaf/grassy shoot phytoplasmas

Phytoplasmas are bacteria with no cell walls that infect plants and the insects that spread them between plants. The vectors of phytoplasmas belong to the insect groups known as planthoppers, leafhoppers and psyllids. Phytoplasma taxonomy is complicated and is based not on traditional methods used for prokaryotes but on DNA sequence of the ITS genes or genome. The phytoplasma diseases that have been reported in sugarcane are white leaf (Taiwan, Thailand Vietnam, Laos) (Chen & Kusalwong 2000), green grassy shoot (Thailand, Vietnam) (Pliansinchai & Prammanee 2000) and grassy shoot (India, Pakistan, and South-east Asia) (Viswanathan 2000). The molecular relationship between the phytoplasmas infecting sugarcane has been investigated by Nasare *et al.* (2007), Marcone & Rao (2008), Hoat *et al.* (2013) and Rao *et al.* (2014). These studies found the phytoplasmas were closely related but with slight differences.

The symptoms of the three phytoplasma diseases are similar, only differing in the extent of the white leaf symptom. The diseases cause severe stunting, profuse tillering giving the plants a grassy appearance and varying degrees of white discoloration of the leaves (lack of chlorophyll). The diseases can cause total crop loss.

The diagnostic method for phytoplasmas uses ribosomal ITS primers in a nested-PCR assay followed by sequencing of any PCR product detected (Thompson *et al.* 2012, Braithwaite *et al.* 2017). These assays are applied to any clones imported by SRA from Asia while the plants are growing in quarantine.

Hot-water treatment is reported to be ineffective against the sugarcane phytoplasmas. Wongkaew & Fletcher (2004) found that tissue cultured sugarcane plantlets treated with 200-500 mg/mL of oxytetracycline went into remission or were free of phytoplasma. They also showed that meristem tip cultures from infected plants produced 60% of plantlets that were free of phytoplasma through serial subcultures.

All sugarcane infecting phytoplasmas are transmitted through infected planting material and by insect vectors. The knowledge of the vectors of sugarcane phytoplasmas is complex. White leaf is transmitted by the leafhoppers *Matsumurattetix hiroglyphicus* (Chen & Kusalwong 2000) and *Yamatotettix flavovittatus* in Thailand (Hanboonsong *et al.* 2006). Grassy shoot is reported to be transmitted by *Deltocephalus vulgaris*, *Maiestas portica* and *Cofana unimaculata* (Srivastava *et al.* 2006; Tiwari *et al.* 2017). The vectors of grassy shoot in India are poorly understood.

Magarey (2009, SRDC study tour SR09002) investigated the incidence and severity of green grassy shoot in Vietnam. He found a severe green grassy shoot epidemic developing in sugarcane in the NAT&L Sugar Factory area at Quy Hop, Vietnam.

Phytoplasmas have the potential to cause severe losses. The successful establishment of a phytoplasma disease would require both the phytoplasma and a vector to be present. The disease could establish if there was an incursion of a phytoplasma-infected vector, a vector and an infected plant, or an infected plant was introduced and the phytoplasma found an endemic insect that could act as a vector. The phytoplasma diseases are widespread and relatively common in Asia but do not occur in Australia's near neighbours. Overall, we would rate the current risk from phytoplasmas as low to moderate. SRA should keep a watch for spread of the disease and insects into Indonesia and be vigilant when importing germplasm from Asia.

Recommendation: Screen any germplasm imported into Australia that has originating from Asia intensively for phytoplasmas.

3.3.5 Leaf diseases including leaf scorch and exotic rusts

Leaf scorch is a fungal disease caused by *Stagonospora sacchari* (Lee & Liang 2000). The disease causes elongated red to straw coloured lesions extending to the leaf tip. In susceptible varieties, the lesions can destroy all the leaf tissue except for the mid-rib. The disease is favoured by hot, humid and rainy weather. Yield losses of 10-30% have been recorded. The disease occurs in Japan, Taiwan, Philippines, south-east Asia and Indonesia. In Indonesia, the disease was initially recorded on Sumatera but then spread to Java. It caused yield losses in some varieties. There have been no reports of the disease in Indonesian West Papua. There has been no recent research on leaf scorch. If leaf scorch spreads to West Papua, where Indonesia has been planting sugarcane in preparation for developing a sugar industry, the risk to Australia would increase. Australia should keep a watch on the spread of this disease.

A new species of rust was observed on sugarcane in Swaziland and South Africa in 2008 (Martin *et al.* 2017a). Phylogenetic DNA studies showed that the new rust was closely related to *Macruropyxis fraxini.* The name proposed for this newly discovered rust species was *Macruropyxis fulva* and the disease was called tawny rust. The economic importance of this new disease is unknown. A PCR assay to distinguish this new species of rust from the other two rust diseases of sugarcane was reported by Martin *et al.* (2017b). SRA should access this PCR assay in case this rust spreads to Australia.

3.3.6 Other potential exotic disease threats

Fiji leaf gall is discussed in detail in the section on established diseases. Fiji leaf gall is now rare in Australia and may be eradicated in coming years. An incursion of Fiji leaf gall from overseas is already a threat to most areas of the Australian sugar industry that are free of the disease. Fiji leaf gall is widespread in Papua New Guinea and Fiji and is probably present in other south Pacific islands. It has been reported in Malaysia and the Philippines. The close relative of sugarcane, *Saccharum edule,* is highly susceptible. This species is widely grown as a vegetable in Papua New Guinea, West Papua and Fiji and is known as pit pit, daruka, tebu ikan and Fijian asparagus in different areas by south Pacific islanders. The aborted flowers are eaten as a vegetable. Movement of this plant represents a risk as it is highly prized by islanders living in Australia. One of the vectors of Fiji leaf gall, *Perkinsiella saccharcida,* is present in all areas of the Australian industry (recent research on this species is covered in the section on pests). Currently, the restrictions on growing susceptible varieties are being relaxed in some areas and the potential for establishment of the disease in areas now free of the disease will increase. SRA already treats Fiji leaf gall as a threat in germplasm exchange (Thompson & Wilson 2020). Fiji leaf gall is a higher risk than downy mildew and Ramu stunt. SRA should continue to conduct targeted surveillance in the Torres Strait to monitor for spread of Fiji leaf gall and ensure federal and state biosecurity staff are aware of the risk of Fiji leaf gall to the sugar industry.

There are other viruses that have been reported in sugarcane that represent a biosecurity threat to Australia. They include *Peanut clump virus* which causes red leaf mottle in Africa (Rott & Chatenet 2000) and *Sugarcane streak virus* (including Mauritius and Egypt strains) which causes streak disease (Rott & Peterschmitt 2000, note this is different to streak mosaic).

Sugarcane yellow leaf virus is present in Australia, but various strains of the virus have been reported overseas. Thompson & Wilson (2020) reported that since 2012, 10 to 50% of clones imported from overseas have been infected with *Sugarcane yellow leaf virus.* These clones were destroyed to prevent the introduction of new strains of the virus. There has been extensive research on epidemiology, yield losses and management of *Sugarcane yellow leaf virus* in many overseas sugar industries (ElSayed *et al.* 2015; Rott *et al.* 2016) but little is known about the incidence and

importance of *Sugarcane yellow leaf virus* in Australia. Several project proposals have been submitted but have not been funded.

Recommendation: Continue research into NGS/metagenomics as a generic assay for detection of pathogens in sugarcane in post-entry quarantine.

Recommendation: Investigate the incidence and importance of Sugarcane yellow leaf virus to the Australian sugar industry.

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4. PEST MANAGEMENT

4.1. Canegrubs

Canegrubs, larvae of a complex of endemic melolonthine scarabs, are the key pests in Australian sugarcane. They feed on the roots and underground stalk material (the stool that produces new shoots) of sugarcane plants, reducing plant vigour, crop yield, and sugar content. Where root loss is severe, the plant's ability to regenerate and produce subsequent ratoon crops is severely impaired, and stools may be removed inadvertently during mechanical harvesting, further reducing subsequent yield potential and adding undesirable levels of soil to milling. It is the third-instar larvae that cause the most damage.

The genera *Lepidiota* (10 species), *Antitrogus* (4 species), *Alepida* (3 species), *Dermolepida* (1 species) and *Rhopaea* (1 species) are involved. *Dermolepida albohirtum*, (greyback canegrub), which has a one-year life cycle, is the most damaging species and occurs in all areas except southern Queensland and New South Wales. Either *Lepidiota negatoria* (negatoria canegrub) or *L. frenchi* (French's canegrub) occurs in all areas—both have two-year lifecycles. *Antitrogus consanguineus* (southern one-year canegrub, one-year lifecycle) and *An. parvulus* (Childers canegrub, two-year life cycle) are significant pests in sandy and heavy clay soils, respectively, of southern Queensland. The other species are more localized. Many species show a strong preference for specific soil types controlled by oviposition responses and/or differential larval survival.

Canegrub management is only one component of the farming system and tactics must integrate with other farming practices - benefits must be weighed carefully against costs and impacts. There must be a net contribution to the economics and sustainability of sugarcane production. This is often difficult to value, as there are productivity, profitability, environmental, and social benefits. Newer cropping systems have delivered profitability gains to Australian sugarcane growers and canegrub management has contributed to this. The challenge is to continue to provide more effective,

cheaper, and more integrated options with lower environmental impacts that ensure grower profitability and supply security to sugarcane millers.

Allsopp (2010) reviewed the development of a comprehensive integrated pest management (IPM) strategy for the management of Australian canegrubs. This research covered a wide range of topics during the 1990s and 2000s such as basic taxonomy, species identification, ecology and biology of different species within the sugarcane system, possible identification of pheromones, development of new insecticides and new formulations of insecticides, potential development of genetically modified pest-resistant canes, methods for predicting risk and level of new infestations, and a wide-ranging extension program that then saw broadscale adoption of the new strategies. However, since then, commercial management of canegrubs has lost that broadscale focus and become totally reliant on the use of one insecticide, imidacloprid. This mirrors the situation during the 1950-1980s when the industry was complacent and the focus on canegrub RD&E diminished greatly.

Allsopp (2010) outlined a series of challenges for the sugar industry in improving canegrub management. These, albeit with some updating, continue to be relevant after 10 years if the industry is not to go through another high-impact period as in the mid-late 1990s:

- Broadening the types of insecticides available. Chlorpyrifos is no longer being used, and the dominant use of imidacloprid is under scrutiny by regulatory and environmental agencies and risks the development of resistance. New actives, new formulations and possibly biological insecticides will provide increased competition in the insecticide market but require commitment from both insecticide companies and industry researchers to obtain registration.

- Tools to warn of impending insecticide resistance. To be realistic, such tests need to mimic the mode of acquisition and action of the insecticide from both controlled-release (point source) and liquid (diffuse source) applications.

- Effectiveness and application methods for chemical and biological insecticides in a farming system dominated by controlled traffic, wider row spacings, legume rotations and minimum tillage. This system potentially provides a very different environment for canegrubs than did the burnt-cane, high tillage, continuous cane of the 1990s.

- Understanding adult (and larval) behaviour to provide a better basis for forecasting and risk prediction. This is particularly important for greyback canegrub, whose adults move to and from fields.

- Better methods for risk prediction coupled with better acquisition of field, farm, and regional data. Growers detest sampling for canegrubs, and the relatively low value of the crop makes scouting difficult to justify. Geographic information systems and remote sensing for detection of damage and population monitoring provide significant opportunities.

- Continued development and adoption of farming practices that minimize populations. The retention of crop residues in the Burdekin region is restricted because it interferes with furrow irrigation.

- Continuing the extension message with an emphasis on pre-emptive rather than reactive management. It is difficult to maintain interest and momentum when other issues, in 2010 the then-recent incursion of sugarcane smut, and during the 2010s the appearance of Yellow Canopy Syndrome and the regulation of fertiliser use, are more important in growers' minds.

4.1.1 Taxonomy

Understanding what one is dealing with is the key to information on the biology, ecology and management options of a pest.

Larvae and adults of canegrubs were comprehensively described by Miller & Allsopp (2000). Since then, the only change to the taxonomy (Allsopp 2018) has been the removal of Bundaberg canegrub (*crinita*), Froggatt's canegrub (*froggatti*) and picticollis canegrub (*picticollis*) from *Lepidiota* to a new genus *Alepida* Allsopp, 2018. The combinations now are: *Alepida crinita* (Brenske), *Alepida froggatti* (Macleay) and *Alepida picticollis* (Lea).

The genera appear to be well founded, with the similarity of the cuticular hydrocarbon profiles showing good correlation with the understanding of the phylogeny (Fletcher *et al.* 2008). Major components are polymethylated hydrocarbons, 3-methyl substituted n-alkanes, 9,10-allenes and the corresponding C9 alkenes. The four genera examined are well separated with the two *Lepidiota* spp., *negatoria* and *noxia* more closely related to each other than either is to *Alepida picticollis*. The dissimilarity of *Antitrogus consanguineus* and *Antitrogus parvulus*, in both chain length and the extent of methylation in the two parvulus hydrocarbons, is surprising given their reasonably close taxonomic relationship. One C25 allene was shown to have a potential role in mate recognition in *A. consanguineus*.

4.1.2 Broadening the types of insecticides available

The Australian sugar industry has a history of reliance on one insecticide - BHC, then chlorpyrifos, now imidacloprid. This brings benefits, but has considerable risk – withdrawal by regulatory agencies, insecticide resistance, reduced market competition.

Recent concerns with non-target and off-site impacts, coupled with more intensive sampling of waterways, has seen increased scrutiny of imidacloprid use (Chandler 2017). Given that it is long out of patent protection, no manufacturer is likely to be overly interested in supporting R&D to justify its continued use. This exposes the industry to considerable risk. The failure of chlorpyrifos-based insecticides in high pH soils of the Burdekin during the 1990s and the losses incurred should provide a lesson for the development and commercialisation of new active ingredients and new formulations suitable for the target species and the sugarcane system.

Experience with the development of the suSCon® family of controlled-release insecticides, organophosphate- and imidacloprid-based liquid 'knockdown' insecticides and *Metarhizium*-based insecticides shows that there must be:

- A willingness in an insecticide producer to invest in R&D to test, formulate and secure registration and to market a new product – presumably, seeing a profit from so doing – - and having a commercial 'champion' to drive the process.
- A willingness in the industry to invest in R&D organisations (primarily Sugar Research Australia) to partner with insecticide producers to test active ingredients and formulations under a variety of conditions and against major target species, with the understanding that the insecticide producer will make a profit from a successful product.

With the poor performance of chlorpyrifos-based suSCon® Blue in the mid-late 1990s, Crop Care Australasia (CCA) developed the imidacloprid-based suSCon® maxi (50 g imidacloprid/kg). This saw some new formulation chemistry from CCA (a more biodegradable matrix), careful definition of the characteristics of a useful product (CCA/BSES), access to imidacloprid that was still under patent (CCA), release-rate studies (CCA/BSES), large series of field trials (mainly BSES), development of a registration package (mainly CCA), registration and then commercial marketing (CCA).

In field trials, initially, greyback canegrub populations in plant crops were markedly reduced by treatment with 10–15 kg/ha of product applied into the planting furrow (Chandler & Tucker 2010). In first-ratoon crops, effects on populations were much less pronounced. Yield benefits from treatment with suSCon® maxi relative to untreated controls were more pronounced in first ratoons than in plant crops. suSCon® maxi was equally or more effective than other registered CR products.

Later data (Chandler & Tucker 2011) allowed registration of suSCon ® maxi against Childers canegrub, negatoria canegrub and southern one-year canegrub up to second ratoon. Populations of all three species were markedly reduced in first- and second-ratoon crops by treatment with 10 kg/ha of product applied into the planting furrow either at-planting or at drill fill-in. Cane yield increased in first- and second-ratoon crops, following these population reductions. Additional data was sufficient to support registration to control Childers canegrub for up to four crop-years (third ratoon). Other observations showed control of large Childers canegrubs present at planting with suSCon® maxi (Chandler & Allen 2014).

Further work on formulations and release rates resulted in a change to the product to provide extended control in later ratoons - suSCon® maxi Intel.

To measure the effectiveness of suSCon® maxi Intel relative to four other candidate formulations and the standard, 30 field trials were established between 2010 and 2013 in far-north Queensland, the Burdekin, Central and Southern production districts. These trials were monitored for infestation and the control efficacy recorded. Data from these trials supported the initial registration of suSCon® maxi Intel in 2015 (Tucker *et al.* 2015).

In SRA project 2014/006 (Ward 2016), observations of these trials continued to gain additional data to support further label extensions providing even longer protection from grubs. The trials demonstrated that suSCon® maxi Intel provides protection from greyback, negatoria, consobrina and Bundaberg canegrubs for three years and Childers and southern one-year grubs for four years, representing an increase by one year for all species over that provided by suSCon® Maxi. The data was used to register suSCon® maxi Intel, providing Australian cane farmers with between 33 and 50% longer protection from canegrubs depending on species for the same upfront cost. However, the data did not support any further extension of label claims from 3 to 4 years in the case of greyback, negatoria, consobrina and Bundaberg canegrubs and from four to five years in the case of Childers and southern one-year grubs.

The success of this work and the translation to new registrations demonstrated the benefits of close commercial-industry partnership – neither party would have been successful without the other. The project delivered significant social, environmental and economic benefits to the Australian sugar industry. In an economic sense, growers gained access to a control method that is “essentially set and forget”, ensuring that for growers that have a high risk of grub damage the risk is largely alleviated. Equally importantly, suSCon® maxi Intel was marketed for the same cost as suSCon® Maxi, meaning that the cost of control per crop-year has been reduced by between 33 and 50%. If the cost of additional control with a liquid product in the final year is included, the total cost of management falls further. In an environmental sense, the total amount of imidacloprid applied has been reduced compared with annual applications through extending the protection provided from a single application of controlled release product. This also has social implications, firstly through reducing the potential impact from sugarcane farming on the environment, and secondly through reducing the time required for growers to manage canegrubs on their properties.

Field trials during the late 1990s and early 2000s in cooperation with Bayer provided data for the registration of liquid formulations of imidacloprid (initially Confidor®) into ratoon crops (Vitelli *et al.* 2001; McGill *et al.* 2003; Chandler 2003). This provided a replacement for granular organophosphates and extended ratoon treatments to species with 1-year lifecycles (especially

greyback canegrubs). Again, interest and input from 'champions' in Bayer, combined with extensive BSES field work, resulted in registration and market adoption.

However, alternative active ingredients would provide market competition and lessen the likelihood of resistance developing in target species. Alternatives might also be less susceptible to being implicated in off-site movement in water. Project 2016/003 (Powell 2020) tested chemical insecticides, both commercially available and under development, to determine their efficacy towards canegrubs compared with imidacloprid. In initial laboratory bioassay trials, larval mortality of greyback canegrub was high for all treatments tested compared with the untreated control, suggesting that all tested products have the potential to either kill larvae or adversely affect larval feeding and development. Microplots were established in Mackay and Bundaberg to quantify the relative persistence of selected products in the soil and all tested products showed relatively good persistence compared with imidacloprid, ranging from 3-9 months. Multiple field trials were then conducted, in the Burdekin, Central and Southern regions targeting greyback canegrub and Childers canegrub. In addition, in some regions the impact on Bundaberg canegrub, negatoria canegrub and French's canegrub were also assessed. Obtaining consistent trends across field trials proved problematic because in most field trials canegrub populations were low which made it difficult to statistically determine relative treatment efficacy. However, some treatments did show consistent trends as potential alternatives to imidacloprid. Further screening of these insecticides is needed together with a more robust process of site selection and pre-screening prior to field trial evaluation and a more intensive sampling regime at field sites.

The 1990s also saw attempts at the development of bioinsecticides based on the fungus *Metarhizium anisopliae*. Initial development was very much in-house, with little thought to the registration of a product and its commercial production and distribution. A commercial product (BioCane) only succeeded after a three-way collaboration was established between BSES, CSIRO and Bio-Care (summarised in Samson *et al.* (2000, 2002) and Allsopp (2010)) (subsequent to this Samson *et al.* (2010) provided data on efficacy against southern one-year canegrub).

However, BioCane provided only partial control and, being a biological product, it required refrigerated storage and could not be left exposed on hot soil in furrows during application. Production was labour intensive and required pre-ordering some month before expected application. It could not compete with imidacloprid insecticides once these were registered and production was discontinued.

The main molecular factors involved in the complex interactions occurring between greyback canegrubs and *M. anisopliae* were investigated by comparing the proteomes of healthy canegrubs, canegrubs infected with *Metarhizium* and fungus only (Manalil *et al.* 2009). Differentially expressed proteins from the infected canegrubs were subjected to mass spectrometry to search for pathogenicity related proteins. Immune-related proteins of canegrubs identified in this study include cytoskeletal proteins (actin), cell communication proteins, proteases and peptidases. Fungal proteins identified include metalloproteins, acyl-CoA, cyclin proteins and chorismate mutase. Comparative proteome analysis provided a view into the cellular reactions triggered in the canegrub in response to the fungal infection at the onset of biological control.

Larvae of greyback canegrub can also be infected by the coccidian pathogen *Adelina* sp. The pathogen invades the fat bodies of the host reducing glycogen accumulation and ultimately destroying the cells. *Adelina* sp. is frequently recovered from larvae the Wet Tropics and acts as a density-dependent mortality factor in that region, while grub populations remain high in the Burdekin where *Adelina* is not common. Adelina oocysts are produced in large numbers following the death of the host (usually around May) and remain dormant in soil until they are ingested by a new host in the following season (January-February). However, *Adelina* requires living host cells to complete its life cycle.

Project 2013/356 (Sallam & Marshal 2016; SRA and AgResearch New Zealand) investigated the possibility of mass-producing *Adelina* either *in vitro* via the use of a cell line, or *in vivo* via infecting canegrub larvae or a surrogate host. Oocysts, sporocysts and sporozoites of *Adelina* sp. isolated from infected greyback canegrubs were introduced into an African black beetle cell line grown and oocysts successfully produced sporocysts which in turn released sporozoites into the cell line. However, no further development was evident. This was also the case when sporozoites were isolated and inoculated directly into the cell line. Cell lines from other insect species failed to acquire the pathogen.

Tissues sourced from adult canegrub beetle ovaries and fat bodies as well as embryonic cells obtained from fertilized eggs or fresh hatchlings were inoculated into three media types in an attempt to start a cell culture. Apparent cellular activity was detected in the SDM medium inoculated with ovarian tissues, but no further progress was evident. Tissues sourced from developed embryos inoculated into the PS100 medium noticeably multiplied within weeks of inoculation and required repeated sub-culturing into fresh media.

Conventional *Adelina* breeding via the infection of young canegrub larvae was feasible in the laboratory. The highest infection rate (70%) was obtained by infecting young second-instar larvae using *Adelina*-contaminated pieces of carrot as food. However, breeding greyback grubs in the laboratory or in a greenhouse is labour intensive and would not provide a cost-effective method of *Adelina* production.

The key to any successful development of *Adelina* as a bioinsecticide is the development of a mass-production system, presumably based on a cell culture. However, development, production and registration costs are likely to be high and it would have to compete with low-cost chemical insecticides. Without a commercial partner, a commercial product is not feasible.

The third technique to the development and delivery of biocides revolves around genetic modification of the crop (conventional GM) or of an organism intimately associated with canegrub development (paratransgenesis).

Potential antimetabolites and their incorporation into conventional GM sugarcane were researched through the 1990s (reviewed in Allsopp 2010). Although the technique proved feasible, further development was not pursued when the industry decided to solely focus on the development herbicide-resistant GM sugarcane.

Symbiotic bacteria residing in the hindgut chambers of canegrubs could be useful in paratransgenic approaches to reduce larval root-feeding activities. Pittman *et al.* (2008a) compared the bacterial community profiles associated with the hindgut walls of individual *Dermolepida albohirtum* third-instar larvae over 2 years and those associated with their plant root food source across different geographic regions. Denaturing gradient gel electrophoresis analysis was used with universal and Actinobacteria-specific 16S rRNA primers to reveal several taxa that were found consistently in all *D. albohirtum* larvae but not in samples from their food source, sugarcane roots. These taxa included representatives from the “Endomicrobia,” Firmicutes, Proteobacteria, and Actinobacteria and were related to previously described bacteria from the intestines of other scarab larvae and termites.

Eight species of gut bacteria from the Proteobacteria, Firmicutes, and Actinobacteria phyla were assessed for their potential to be used in paratransgenic strategies to target greyback canegrubs (Pittman *et al.* 2008b). RNA-based DGGE analysis of 16S rRNA was used to detect the persistence of these isolates in the hindgut environment. One of these isolates (Da-11) remained metabolically active in the hindgut for 19 days postconsumption. Da-11 most likely forms a new genus within the Burkholderiales order, along with taxa independently identified from larvae of the European scarab pest, *Melolontha melolontha*. Using the EZ::Tn5 transposon system, a kanamycin resistance gene was inserted into the chromosome of Da-11, thus establishing a stable transformation technique for

this species. A second feeding trial that included inoculating approximately 400 transgenic Da-11 cells onto a food source resulted in a density of 1 × 106 transgenic Da-11 cells/mL in the hindguts of larvae at 9 days post-consumption. These populations were maintained in the hindgut for at least another 12 days.

The successful isolation, genetic transformation, and establishment of transgenic Da-11 cells in the hindguts of *D. albohirtum* showed the potential for paratransgenesis but further development was not followed. Obviously, there would be a difficult process in obtaining regulatory approvals for a commercial product and there would need to be considerable research to develop a rearing and production system to supply such a product.

4.1.3 Tools to warn of impending insecticide resistance

Widescale use of any one insecticide risks the development of resistance in the target species. A method for detecting incipient resistance would provide early warning and allow appropriate alternative products and use strategies to be implemented before products fail to give field control.

Chandler & Erbacher (1997) showed that different species of canegrubs had different susceptibilities to the then-used chlorpyrifos. There is no reason to suspect that the situation would be different for imidacloprid.

What is required is a robust testing system that mimics the acquisition and symptomology of the insecticide. This was simple with a contact-based insecticide such as the organophosphates, where insecticide could be mixed with soil at known doses and mortality was obvious after a set time of exposure. Imidacloprid is probably acquired through dermal, ingestion of soil and ingestion of cane roots; mimicking such a system is difficult. Imidacloprid also acts in a more subtle way, causing cessation of feeding, a long period of little activity and finally death – this makes assessment of toxicity difficult.

During 2000s and 2010s Chandler did some preliminary work to establish a suitable testing system for imidacloprid (described in various unpublished reports on collaborative work between BSES/SRA and Crop Care). This work was never completed prior to the cessation of the collaboration and prior to Chandler's retirement.

4.1.4 Application methods under current farming systems

Samson *et al.* (2010, 2011a) conducted efficacy trials against greyback canegrub in the Central and Herbert districts, to evaluate the impact of new planting configurations, including the use of minimum-tillage planters and dual-row beds, which may impact on the efficacy of insecticides. The controlled-release insecticide granules suSCon® Blue and suSCon® maxi gave satisfactory control of greyback canegrub when applied between the discs of a double-disc opener planter. Confidor® Guard gave at least one year of control of greyback canegrub in dual rows when applied at planting or using coulters. Twin coulters were slightly more effective than a single coulter. Effective application rates of suSCon® granules and Confidor® Guard in dual rows were within the range currently listed on the registered labels for single-row application when expressed per 100 m of single- or dual-row bed. Results were reported to the relevant insecticide companies and changes to the suSCon® Maxi label to include dual rows and double-disc opener planters were made subsequently.

With increased monitoring of agricultural chemicals in rivers discharging into the Great Barrier Reef Lagoon, the potential for off-site movement of imidacloprid (reviewed in Chandler 2017) has drawn

some attention focusing on formulation type and application methods (particularly depth of placement of liquid formulations).

Chandler (2017) suggested three groups of variables possibly influencing risk of imidacloprid moving offsite. Firstly, insufficient filling and/or closure of coulter-slots into which liquid imidacloprid is applied facilitates escape of the insecticide into runoff; this situation being exacerbated by site variables e.g. soil type and slope. Deviations from the registered pattern of use (e.g. side-dress applications) may disproportionately exacerbate loss. Secondly, data interpolation suggests that the liquid formulation has higher potential for off-site movement than the granular controlled-release (CR) formulation; with independent observation to support this. Thirdly, the simplicity to routinely treat for canegrub control rather than to use Integrated Pest Management Process to decide if, where, and with what formulation to treat, results in more-than-necessary treatment; especially if the liquid option is applied more than once in a 4–5 year crop-cycle. Despite the liquid formulation's inherently greater environmental hazard, its contribution to risk can probably be mitigated by managing the other two sets of variables. Amenity for efficient canegrub control is justification for having both formulation types, each with respective advantages.

Fillols & Davis (2020) looked to define the best application methods for ratoon treatment with imidacloprid to reduce off-site movement. They established two rainfall-simulation trials in the Burdekin and in the Wet Tropics to assess the impact of depth and slot coverage on imidacloprid runoff when the liquid formulation is applied with a stool-splitter tine implement. An additional runoff trial under overhead irrigation was set up in the Wet Tropics to test the efficacy of the StoolZippa™ (Hughes & Gonzalez 2019) to close the slot and reduce imidacloprid runoff losses when the product is applied at the correct depth of 100 mm. Higher imidacloprid concentrations occurred in runoff from a shallow application at 50 mm compared to the recommended minimum 100 mm application depth. A press wheel reduced the imidacloprid concentration to nil when the product was applied at the correct depth of 100 mm; however, it slightly increased the concentration in the case of the shallow application. The StoolZippa™ increased the imidacloprid concentrations in runoff versus the slot left open, but these concentrations were still extremely low and not of environmental concern. Overall, the trials indicate that ensuring the product is consistently applied at 100 mm depth is the best way to reduce imidacloprid loss via runoff when the product is applied with a stool-splitter tine implement.

Bayer and Nufarm subsequently created an instructional video, training package and an application slot-depth measurement gauge for use in ratoon cane applications. These tools allow growers to easily measure slot depth across several locations in their blocks to ensure they are achieving the minimum depths for efficient treatment.

Testing of off-site movement from both liquid and controlled-release formulations is currently underway (Fillols, pers. comm.)

4.1.5 Understanding adult and larval behaviour

Sallam (2010) summarised the biology and ecology of greyback canegrub. He indicated several research foci that would improve knowledge and potentially open avenues to new management techniques (some of these were expanded on by Goebel *et al.* 2010):

- Emergence rate of adults under different climatic conditions and rainfall and its impact on the proportion of females that mate and then oviposit.
- Dispersal distance and direction of beetle flight (and what proportion oviposits in the same field from which it had emerged).
- Reasons why adult beetles are attracted to certain fields and not others.

- Role of crop height in attractiveness in non-Burdekin areas.
- Proportion of females that mate and then successfully oviposit.
- Decline in grub densities over time in absence of pathogens and at different grub densities.
- Role of larval combat as opposed to dispersal.
- Disease carry-over rates; a transmission coefficient is needed to describe the relationship between pathogen levels and subsequent disease probability and further inoculum production.
- Identification of unknown mortality factors.
- Impact of tillage on pathogen levels in soil.

Most of these are applicable to the other canegrub species, and all remain relevant today. However, the success of imidacloprid in reducing canegrub numbers means that much of this work is currently difficult.

Horsfield *et al.* (2008) used historical data on Burdekin populations of greyback canegrub to study the impact of climatic variables and insecticide application on subsequent damage in sugarcane. Irrigation and extensive sugarcane cropping in the Burdekin are highly conducive to maintaining high greyback population densities. However, spring is a critical period for these populations due to their annual life cycle and relatively short window of opportunity to feed, mate, and oviposit. An environmental stress during spring such as prolonged hot and dry conditions may influence the total number of beetles emerging, the timing or synchrony of emergence and the ability of beetles to feed, mate, and oviposit. Dry spring conditions limit the total number of beetles emerging from all pupation sites ranging from cane crops to fallowed blocks and surrounding areas such as riverbanks. Mild and wet spring conditions, however, maximize and synchronize beetle emergence from all pupation sites and provide more favourable conditions for feeding, mating, and oviposition. Therefore, over a larger spatial and temporal scale, spring weather does play a role in determining levels of subsequent damage.

Goebel *et al.* (2010) sought to define for greyback adults: (1) the dispersal distances and individual likelihood estimates of beetles leaving a cane field upon emergence; (2) the attraction of different species of 'feeding trees' outside of the cane field; and (3) the subsequent dispersal from feeding trees back to cane fields for oviposition. They used small radio transmitters attached to the prothorax of beetles, but the size of them apparently hampered normal beetle behaviour. Little new data came out of this work.

4.1.6 Better methods for risk prediction

Most pest-crop situations allow for decisions on management treatments to be made following sampling of the pests and comparison with economic thresholds (sample-decide-treat). In most cases with canegrubs this is not possible – damaging populations are present after the crop cannot be accessed for treatment and the wet season means access to fields is impossible anyway (2-year grubs in the south may be an exception where treatment is in Spring). This requires a set-and-forget strategy, with decisions to treat made well in advance of any possible sampling (at planting or after harvest of the previous crop) and, if made rationally, based on an assessment of risk. Risk can be based on previous infestation history, potential for development of damaging populations, age of crop, etc, and perception of allowable risk obviously varies among individuals. Predictive systems could improve growers' decision-making.

Risk-assessment models for greyback canegrub take account of the intrinsic uncertainty in the system by providing estimates of probability for the occurrence density classes. An initial model used yes/no answers to eight questions on previous history of damage and management practices to assess risk of high or low subsequent infestations. More recent models aim to classify risk according to low (≤0.5 grubs per stool),moderate(>0.5–2 grubs per stool), and high (>2 grubs per stool) density).The models use combinations of measures of infestation levels in the monitoring fields, especially visible damage before harvest and presence of gaps after harvest; crop measurements, including soil texture, pH, distance to the nearest treelines, method of ground preparation, cultivar, insecticide use, crop class, harvest date, and height during beetle flights; and pest measurements focusing on the level of infection by pathogens in each field and indications of infestation pressure from nearby fields. The most reliable models include information on current numbers of canegrubs in both individual fields and the district, as well as measurements of canegrub health as affected by known pathogens. However, predictions are possible without some or all these data, provided there are good records of current levels of canegrub damage in individual fields and other factors that might predispose fields to canegrub attack. Theoretically, once refined, these models would provide important input for decision-making in the GrubPlan approach (Sallam & Lowe 2012; Samson & Eaton 2012).

However, these monitoring and forecast methodologies relied on extensive field work (digging up cane plants, laboratory breeding of recovered canegrubs, assessment of canegrub pathogen rates, records of cultivar, landscape assessment etc.) which are very demanding tasks and are needed when the crop has become impenetrable. This project 2011/342 (Sallam 2015; Johansen *et al.* 2014, 2018) addressed the potential of using remote sensing/satellite imagery technology to detect emerging grub damage and produce risk maps to advise growers. They used object-based image analysis (OBIA) and high spatial resolution satellite imagery to map canegrub damage. The OBIA mapping approach used was based on four key steps for three selected study sites in Queensland, each covering 50-100 km^2 around Mackay, Home Hill and Gordonvale: (1) initial segmentation of sugarcane block boundaries based on existing GIS layers provided by the respective mills and further segmentation of each block into smaller homogenous objects; (2) classification and subsequent omission of fallow/harvested fields, tracks and other non-sugarcane features within the block boundaries; (3) identification of 'potentially' grub-damaged areas within each block with the lowest amounts of green leaves (low Normalised Difference Vegetation Index (NDVI) values) and highest level of image texture; and (4) the further refining of 'potentially' grub damaged areas to 'likely' affected areas based on the absolute difference in the amount of green leaves (NDVI values) and texture between the 'potentially' grub damaged areas and the remaining parts of each block. The initial validation based on field observations of greyback canegrub damage at the time of the satellite image capture in June 2013 yielded overall accuracies between 53–80%. However, this included a number of false positives resulting from sprawling, drainage issues, weed and pig damage. They recommended further research to focus on reducing these false positives as well as investigating the inclusion of additional data layers to increase the predictive accuracies. Such data layers may include distance from damage to tree corridors, distance to neighbouring grub damage and, potentially, soil type, cane variety and treatment information. Analysis of archived imagery may also provide some insight into the historic location and distribution of grub damage, thus assisting with improved understanding of potential risk for the subsequent year.

Further work developed an eCognition rule set for classifying grub damage using satellite images. This yielded overall damage detection accuracies of up to 90% or higher in several cases but still included a number of false positives resulting from sprawling, water logging, weed and pig damage.

Powell (2019) (project 2015/038) continued this theme but using SPOT-6 imagery with Geoeye-1 imagery to determine its comparative efficiency in mapping canegrub damage. Depending on the spatial resolution (as determined by pixel size) of the acquired imagery, the images can potentially show differences in canopy reflectance, texture, stool tipping and gaps within the crop. Both

Geoeye-1 and SPOT-6 have the capacity to obtain panchromatic and multi-spectral (red, green, blue and& near infra-red) imagery. However, the resolution or ground sampling distance (GSD) differs, with SPOT-6 at 1.5 m and Geoeye-1 at 0.46 m. SPOT-6 imagery is also cheaper to obtain than Geoeye-1 imagery.

Imagery was acquired from two satellite platforms, Geoeye-1 and SPOT-6, covering three major cane-growing regions, Mackay, Mulgrave and the Herbert. At a smaller scale, SPOT-6 imagery was also compared to cheaper but lower resolution (3 m) Planet imagery. However, Planet image data was deemed unfeasible for assessment of multi-temporal characteristics of canegrub damage due to relatively poor spatial resolution. Validation of canegrub algorithms, developed using the geographic object-based image analysis GEOBIA and a rule set devised for use in eCognition software, was conducted. The rule set classified potential canegrub damage detected within fields as having a low, medium or high likelihood of representing actual grub damage. The rule set was validated and modified according to ground-truthing for grub presence based on site selection from imagery obtained from both helicopter and satellite sources. Overall, although the imagery could identify sites with canegrub damage, there were different relative amounts of false positives and false negatives and this varied between regions and seasons. This finding, combined with the requirement for annual calibration, the high cost of imagery and the phasing out of both Geoeye-1 in 2018 and the SPOT-6 satellite in 2022, may ultimately prove a major limiting factor in potential utilization of this type of satellite imagery. However, this study did form the basis for future potential studies on damage mapping using spectral sensors to detected canopy changes caused by soil-dwelling insect pests of sugarcane. It also opened up the opportunity to investigate the potential use of other imagery types or different platforms for future spectral data collection.

Obviously, further work is required to develop an economically viable and user-friendly system. Any monitoring and prediction system needs to be paid for – who is going to do this if growers are unwilling to pay?

Any sampling of canegrubs requires an appropriate sampling strategy. For greyback canegrub, strategies were first evaluated by Sallam *et al.* (2008) and refined further by Samson *et al.* (2011). Canegrubs were counted annually from March-May under 20 cane stools in infested field using two sampling schemes: stools dug in each of four transects the length of each field, five per transect, or from the four corners and the centre of each field, four per position. Relationships between the mean and variance of grub counts did not differ significantly between the two schemes when calculated over a similar range of means. Mean grub counts did not differ significantly between the inner and outer transects or the central and corner positions in either scheme. Taylor's power law was used to describe the mean-variance relationships for individual fields and for whole districts, and these relationships were used to derive optimal sampling plans. To estimate grub densities in individual fields with a standard error equal to 0.25 of the mean, a sample of 20 stools per field will only be adequate at high grub densities of more than 2 per stool. However, to estimate district-wide grub densities with the same precision, it will usually be better to sample fewer than 20 stools per field and sample more fields. For a given grub density, the optimal number of fields and samples per field depend on the time it takes to sample stools within fields and the cost of travel between fields. The latter will vary greatly depending on the layout of the district and the way a survey is structured - whether a grub survey is a stand-alone activity or done in conjunction with other activities, and the size of the sampling team. More sampling is needed to achieve higher precision of density estimates.

An alternative to manual inspection of soil samples is acoustic detection – this was tested by Mankin *et al.* (2009). Field surveys were conducted to detect greyback and Childers canegrubs. Computer analyses were developed to identify distinctive scrapes and other sounds produced by both species and to distinguish them from sounds of nondamaging white grubs (Rutelinae, Dynastinae), as well as from extraneous, wind-induced tapping signals. Procedures were considered for incorporating acoustic methods into surveys and sequential sampling plans. Digging up and inspecting sugarcane

root systems requires 10–12 min per sample, but acoustic assessments can be obtained in 3–5 min, so labour and time could be reduced by beginning the surveys with acoustic sampling. In a typical survey conducted in a field with low population densities, sampling might terminate quickly after five negative acoustic samples, establishing a desired precision level of 0.25 but avoiding the effort of excavating and inspecting empty samples. With a high population density, sampling might terminate also if signals were detected in five samples, in which case it would be beneficial to excavate the samples and count the white grubs. In intermediate populations, it might be necessary to collect up to 20 samples to achieve desired precision, and acoustic methods could help determine which samples would be best to excavate.

4.1.7 Development and adoption of farming practices that minimize populations

Little recent work has been undertaken on this aspect.

Chandler *et al.* (2016) attempted to quantify or rank relative tolerance of different cane varieties using insecticide field-trial data; this could improve the 'guesstimates' of varietal tolerance to canegrub damage within the QCANESelect™ decision-support system for variety selection. Differences in cane yield between the highest-yielding insecticide treatment and the untreated control were used to estimate % cane loss suffered by each variety relative to canegrub population pressure. Results indicated:

- Grub-pressure induced 14-43% cane loss, across all eight varieties considered. None were resistant.
- Regressions of cane loss to canegrub numbers were not significant; probably due to confounded factors of crop-class, previous damage, and site variation.
- Analysis of variance suggests varieties do differ in proportionate cane loss associated with grub damage.

They recommended a series of field-trials comparing cultivars suited to specific sites, each with and without luxury-rate insecticide protection from damage, should be a relatively timely and efficient means to rank for susceptibility. Overall, these results are similar to those obtained in the 1990s with a wide selection of germplasm (reviewed in Allsopp 2010).

Frew studied the effect of soluble forms of silicon and associated vesicular arbuscular mycorrhizae (AM) on the development of greyback canegrubs (Frew 2016, Frew *et al.* 2016, 2017a,b,c,d). Results showed that:

- Canegrub performance positively correlated with root phenolics, while correlating negatively with root Si. A negative correlation between phenolics and Si suggested positive responses by root feeding insects to high phenolic concentrations may be a response to low Si concentrations.
- Elevated CO_2 decreased sugarcane root nutritional value while increasing canegrub growth rate and root consumption by 116% and 57%, respectively. Silicon decreased performance of the canegrub under both ambient and eCO_2, highlighting the potential role of Si in future pest management strategies.
- The impacts of two AM fungal communities on sugarcane and canegrub performance within different soil types known to have different concentrations of Si showed that both AM communities had the same effect on sugarcane and canegrub responses. AMs promoted sugarcane growth and photosynthesis by 81% and 39%, respectively, while also increasing root Si concentrations, but only in soil with low Si concentrations. Similarly, AM fungi

decreased canegrub performance, but only within the low Si soil. This suggests that AM fungi promote Si accumulation within Si depleted soil environments, negatively impacting canegrub performance.

- The effects of Si and AM fungi on plant growth alongside their impacts on canegrub performance, root consumption and immune function were evaluated with two different sugarcane varieties. Si decreased canegrub performance and consumption, while AM fungi decreased canegrub performance when Si was not applied and only on one plant variety. AM fungi increased canegrub immune function by 62%, a response that was not explainable by any measured plant trait. Canegrub immune function negatively correlated with canegrub mass, suggesting a trade-off between growth and immunity.

Frew's research showed how the impacts of common agricultural management practices can potentially exacerbate canegrub problems. It demonstrated that Si and AM fungi can promote plant growth and reduce canegrub performance, although the effects of AM fungi can be context dependent, specifically on soil Si availability and plant variety. Future pest management strategies should look to exploit plant Si defences through targeted application of Si fertiliser in Si depleted soils. Practices that encourage native AM communities also hold potential in reducing soil pest persistence, through mechanisms including increased Si uptake, or perhaps even through direct interactions with soil insects.

Although the work showed potential, no follow-up studies have been undertaken. The usefulness of soluble silicon is constrained by the lack of a commercial product at a reasonable price, and the differences among cultivars and soil types means that providing useful and comprehensive advice for its use would be difficult.

4.1.8 Continuing the extension message

The GrubPlan extension package (Samson *et al.* 2007, Samson 2008, Hunt *et al.* 2012 and references in Allsopp 2010) was developed to allow growers or their advisers to assess the risk of greyback canegrub damage in each block of sugarcane, map these to their farms, and select appropriate control strategies. It was supported by a formal one-day training program based on adult-learning principles and a reference booklet. The workshops were initially conducted in four parts: situation assessment, education, application of knowledge, and evaluation. Each part began with each participant mapping the distribution and intensity of damage and cultural practices on a map of their farm. Each participant, in concert with the workshop staff, then developed their own whole-farm risk reduction strategy. The approach was later extended to the complex of canegrubs in southern Queensland, where a different suite of strategies is needed for species with different biologies and ecologies.

In 2001, its first year, GrubPlan engaged more than 900 growers and advisers in 70 workshops in northern and central Queensland. A survey in 2002 showed that 25% had fully complied with the plans they developed the previous year. Another 32% had applied most of these management strategies. Many growers used a combination of strategies, including controlled-release and annually applied insecticides, the *Metarhizium*-based insecticide BioCane, and changes to their farming practices through ploughing, trap-cropping, later planting, and reduced tillage. The impact of this process on damage was difficult to quantify, but crop loss to greyback canegrubs in 2002 was 80% lower compared with levels in 2001. Workshops were advertised again in 2002, but attendance was only about one-fourth of that in 2001. Some of this may be attributed to the success of the 2001 training, as growers commented, "I now know what to do, and I don't need to attend follow-up workshops" and "grubs are no longer a problem, they have gone away." The workshop structure was changed in 2003 to include farm walks and less formal activities to present a new look to encourage

participants. However, with the registration of imidacloprid insecticides growers lost interest and most workshops ceased.

Samson & Eaton (2012) implemented a monitoring and risk-assessment system for greyback canegrub on 10 farms near Mackay during 2008-2010. They counted numbers of greyback canegrubs per field and assessed disease in these grubs, surveyed fields for damage, and obtained other information necessary to make decisions on future risk of canegrub attack using the previously developed predictive models. Maps showing currently effective insecticide treatments and numbers of canegrubs in the sampled fields and a report stating predicted risk levels and treatment recommendations were developed for each farm. There was considerable unexplained variation in canegrub numbers outside of what was accounted for by the models, but they judged the predictions as adequate, particularly when expressed as density classes (low, moderate, high).

Similarly, Sallam & Lowe (2012) worked with Mulgrave growers to assess risk of greyback canegrub based on monitoring grub numbers and damage levels in 2008 to predict grub densities in 2009. Growers were advised whether or not to treat these fields according to the predicted level of risk, and the majority of growers accepted the recommendations. The same fields were sampled again in 2009 to validate predictions. There was a significant reduction in grub numbers where growers applied a chemical treatment following their recommendations. Grub numbers did increase in fields that were not treated; however, where growers were advised to refrain from treatment, grub numbers were still well below economic levels.

Sallam *et al.* (2013) used population monitoring data collected in the Herbert in 2011 and prediction models to forecast greyback canegrub damage levels for 2012. Heavy rainfall and flooding that followed from cyclone Yasi disrupted the work program and caused inaccurate predictions to be generated for 2012. Damage and population assessment methods restricted the commercial use of this system and, although the Herbert Cane Productivity Service were heavily involved in the research, they did not continue the service into subsequent years.

Stanley & Chandler (2013) tested a similar approach in the Isis with mainly 2-year canegrubs using monitoring of canegrub risk as the basis for more cost-effective and efficient canegrub management and then taking the most appropriate option. Over the crop years 2009-11, 50 growers were involved. Key at-risk fields (218) were identified in discussion with growers and sampled for canegrubs and/or signs of activity. Most growers participated in the monitoring. Monitoring programs successfully detected or confirmed broad categories (general/ light/ or nil) of infestation, and current and future risk, and growers generally were willing to discuss various options. Grub damage remained low in the vicinity of monitoring fields for the duration of the project, although several non-participating growers experienced substantial damage. These follow-ups confirmed no major false negatives (under-estimates of grub-damage potential), in the fields surveyed during 2009-12, although follow-up monitoring detected several infestations regaining intensity from 2012-14.

Isis Productivity Limited (IPL) Board members, many of whom had participated in the activity, considered that the results of grub-risk-monitoring in this format were beneficial to their enterprises. However, the IPL Board considered monitoring in the format tested may not be a profitable or efficient core-business role for them with only two full-time staff, and that there would not be much uptake by the canegrowing community to pay for an annual commercial service. They did consider that, with added efficiency of focusing monitoring activity on growth constraint areas through remote (satellite) sensing, monitoring to confirm canegrub risk and/or other crop conditions could be profitable and useful. Similarly, Mackay growers would have liked more certainty in the predictions and the system as delivered would be costly if supplied by a consultant – approx. $2000 (2012 values) per farm. Most of that cost was in sampling for canegrubs, pointing to the need for more efficient sampling schemes or by using other detection systems such as remote sensing.

General learnings from these studies are:

- Growers see potential benefits in the concept of being able to predict risk and implement appropriate strategies;
- Growers are unwilling to sample canegrubs to provide input data and would like this to be done through remote sensing or some other less-labour-intensive method;
- Growers would like more definitive predictions, rather than categorical predictions (high, medium, low);
- Growers do not see enough value for them to pay for a commercial service;
- Productivity services do not see the provision of a commercial service as part of their core role;
- The effectiveness and cheapness of imidacloprid mask the benefits of any system.

4.1.9 Future opportunities

- Over the last 15 years growers have become conditioned to using imidacloprid to provide safe, reliable, cheap and set-and-forget control of canegrubs – whilst this continues, it will be impossible to break them of this mindset.
- If imidacloprid was withdrawn or banned or resistance developed in canegrubs, then the industry would face significant losses – the experience of the 1990s provides a salutary lesson.
- New insecticide chemistry or an effective biological is needed to provide an alternative/replacement for imidacloprid.
- Insecticide companies need to see a return on any investment in new products before proceeding with development. The success of imidacloprid means that a return in the short term would be unlikely and there appears to be no 'champion' in any of the companies to drive internal processes.
- Nufarm (current owners of suSCon technology) now have a business model of marketing out-of-patent products – they do not have the focus on new products and the R&D skills that Crop Care had.
- Prediction and insecticide-minimisation programs resonate with some growers, but how the requisite data for modelling can be obtained, the skills needed to interpret the data and how it could be turned into a commercial service are significant barriers to enthusiasm and adoption.
- Stemborers are the major pests in virtually all other sugarcane industries – the only significant improvement in control has been the recent release of Bt-based GM sugarcane in Brazil (Cheavegatti-Gianotto *et al.* 2019) (not toxic to canegrubs, but other antimetabolites known). This provides a reliable, cheap and set-and-forget method for controlling those pests but requires significant and expensive development and a willingness to embrace such technology and sell sugar from those crops on the open market. Recent experience with GM sugarcane shows that the Australian industry is not willing to do this.

Recommendation: Continue the research to provide an alternative registered product to imidacloprid for the control of canegrubs.

Recommendation: Develop tools to warn of impending insecticide resistance to registered products.

Recommendation: Improve understanding of adult (and larval) behaviour to provide a better basis for forecasting, risk prediction and resistance management. This is particularly important for greyback canegrub, whose adults move to and from fields.

Recommendation: Continue development of better methods for risk prediction coupled with better acquisition of field, farm, and regional data.

Recommendation: Update and continue the extension message with an emphasis on pre-emptive rather than reactive management.

4.2 Soldier flies

Soldier flies are a complex of at least two endemic species, *Inopus rubriceps* and *I. flavus*, that occur in sugarcane south from the Herbert region, more extensively from Proserpine south. The larvae feed on the roots of sugarcane and other plants. Larvae burrow their heads and foreparts into the roots, making visible cavities where they feed. Infestations may reduce germination when these are high, but more commonly cause poor ratooning with underground buds failing to germinate after harvest and affected stools producing few or no shoots. Some of the effect on plants appears to be associated with the injection of a 'toxin', but its presence and characteristics have not been resolved.

Allsopp & Robertson (1988) reviewed the taxonomy and identification, distribution, host plants, population biology and dynamics, and chemical and cultural controls for soldier flies to that date. They nominated several areas that required further research (list is modified slightly):

- Taxonomy and distribution;
- Effects of host plants on development on different hosts and sugarcane cultivars;
- Processes involved in the inhibition of sugarcane growth;
- Damage and intervention thresholds and sampling schemes in sugarcane;
- Contribution of predators, parasites and diseases to mortality;
- Control with insecticides;
- Control strategies other than insecticides.

Considerable research, particularly by Samson and associates, was carried out during the 1990s covering aspects of biology, insecticides, biopesticides, plant resistance and alternative host plants (combinations of these were tested by Samson *et al.* (2004a). None of these alone provided a 'miracle cure'. The combined portfolio was reviewed by Matthiessen (2014) when there was an apparent further 'outbreak' of soldier fly. He outlined:

- The absence of 'silver bullet' control methods does not imply a lack of sufficient knowledge about soldier fly biology and ecology.
- No further basic biology studies of soldier fly are warranted. They would be time consuming, costly and unlikely to lead to new knowledge useful for practical control.
- An upsurge in the abundance of a pest insect frequently reflects no more than natural cycles that will eventually settle back to a lower level. That is small comfort to severely affected growers, but the reality is that against a backdrop of already extensive research information,

the chances of researching and implementing a radically new, effective and economic control option in a short time are nil.

- Often, pest outbreaks lead to control measures that merely coincide with the natural decline of the outbreak with the result that they are often thought to be far more effective than they really are.
- Insecticidal control is generally not limited by toxicity of existing products but rather by the intractable problem of there being no effective delivery systems in established crops.
- Biological controls, and similar, are ineffective and inappropriate – and always will be.
- Resistant or sufficiently tolerant varieties of sugarcane will not be cost-effective to develop.
- Cultural management remains the only effective option

In line with this review's terms of reference, the focus here is on R&D in the last 15 years.

4.2.1 Taxonomy and distribution

The assumption has been that there are two species of *Inopus* as pests of sugarcane, but their taxonomy and distribution have not been well defined.

Allsopp & Robertson (1988) reviewed the previous work that indicated that there might be several species of *Inopus* and/or other genera present in sugarcane and surrounding grasslands. This was born out when Daniels (2016) described *Metridius robertsoni*, a new genus and species. with winged males and apterous females from adults and larvae found infesting sugarcane stools from near Mackay, central Queensland.

Braithwaite *et al.* (2019) analysed the COI gene in samples of soldier fly larvae collected from the Atherton Tablelands, Ayr, the Central region (Habana, Finch Hatton, Hay Point and Carmila) and the Southern region (Bingera, Gooburrum, South Kolan, Maryborough and Cordalba) between September 2017 and June 2018. Phylogenetic results showed that there were five major genetic groups of soldier fly in Queensland cane fields, not two as expected. The clustering pattern was strongly linked to the geographic location for all larvae except for those collected from the Atherton Tablelands.

Generic concepts within the Australian Chiromyzinae were refined by Lessard *et al.* (2020), with two species formerly in *Inopus* being transferred to a different genus but with *rubriceps* and *flavus* and 3 other described species still retained in *Inopus*. They did not address the species-level taxonomy other than listing the described species and noting that there are at least 12 undescribed species. They only presented distribution maps at the genus level. Curiously, *Inopus* is shown only from about Mackay south, despite the type locality of *I. hitchcocki* being Gordonvale and *Inopus* sp. being recorded from the Atherton Tableland, whilst *Chyromiza* is shown as far north as the Wet Tropics. In addition, their key to genera only covers adults – they note "Considering the significance of some species as known pests of sugarcane, development of a robust key to species would be useful for future workers".

The situation is obviously more complicated that thought previously and, without a workable taxonomy, it is difficult to make any sense of previous biological, ecological or control data. A robust taxonomy needs to be developed and this needs to be mapped to previous research to allow that to be understood fully.

4.2.2 Effects of host plants on development on different hosts and sugarcane cultivars

Past emphasis on varietal choice for soldier fly management has been on tolerance, i.e. choosing sugarcane varieties that are able to ratoon despite the presence of larvae. There has been less emphasis on choosing varieties that inhibit soldier fly development, mainly because there was little or no evidence that this type of resistance exists. However, Samson *et al.* (2004b) monitored two field trials near Mackay that gave the opposite results. Crop yields and soldier fly populations were measured annually with 15 or 18 varieties in each trial. The number and average weight of soldier fly larvae differed significantly among varieties and the two were significantly correlated in some years; varieties hosting fewer larvae also tended to host smaller larvae. The effect of this weight difference on subsequent larval development was assessed in one trial for two varieties, Q138 and Q135, chosen because larvae were smaller under Q138. A lower proportion of larvae had pupated in winter under Q138 than under Q135, suggesting that the smaller larvae under Q138 may have required two years to complete their development whereas most larvae under Q135 may have developed in one year. Q138 ranked second of 18 varieties in sugar yield over a plant and three ratoon crops in that trial. This shows that it may be possible to choose varieties that are associated with both poorer establishment of larvae in any year as well as a longer generation time and so a slower rate of increase of soldier fly populations, while also having good sugar yields.

Lenancker *et al.* (2020) attempted to assess varietal tolerance in four randomized-block trials for 3 years. Each trial contained 7 to 10 varieties. Numbers of soldier flies were too low in two of the trials for statistical analysis. In the remaining trials, some varieties (not stated) tended to host less larvae, although post-hoc tests were not significant. They recommended additional research into varietal tolerance to soldier fly to determine whether varietal choice can help limit damage.

4.2.3 Processes involved in the inhibition of sugarcane growth

The presence of an inhibitor/toxin to plant growth has long been suspected in the soldier fly/sugarcane interaction, although studies have proven inconclusive (reviewed in Allsopp & Robertson 1988; Birch 2002).

Etebari *et al.* (2020) and Furlong & Etebari (2020) used a transcriptomic approach to characterize the composition of salivary glands in soldier fly larvae. This approach enabled them to produce the first gene expression profile in soldier fly salivary glands. They identified noticeable differential gene expression in the salivary glands of starved and fed soldier fly larvae, with significant modulation of 850 transcripts in salivary glands upon exposure to plant roots or starvation stress. However, further comprehensive investigations to characterise the proteins that these genes code for are required, followed by functional studies in sugarcane plants. There are many other sequences in the solder fly transcriptome which have completely unknown functions. These need to be identified and their role in the interaction between soldier fly and its sugarcane host plant investigated.

4.2.4 Damage and intervention thresholds and sampling schemes in sugarcane

No recent work has been conducted on this theme, although, without an effective insecticide, intervention thresholds would be of academic interest only. As indicated above, such thresholds are likely to be affected by tolerance in the sugarcane cultivar grown, and also possibly by growing conditions.

4.2.5 Contribution of predators, parasitoids and diseases to mortality

Morris & Samson (2006) studied populations of soldier fly, potential predators, other soil fauna and various abiotic factors in canefields and grasslands during 1998-2001, corresponding to the plant crop to young third ratoon of the canefields. Numbers of soldier fly larvae were higher in plant crops of sugarcane that had been fallowed and planted by June compared with fields planted later after a long break. Trash blanketing appeared to favour increased numbers of some predatory groups such as staphylinid beetles in soil cores or pitfall traps. However, results from pitfall trapping must be interpreted with caution, as catches are influenced not only by absolute numbers of the faunal groups but also by surface activity, which may change depending on trash cover. No relationship was seen between these other fauna and soldier fly population dynamics. Soldier fly remained at sub-economic levels in most of the fields studied. Disappearance of eggs and early-stage larvae, that is, the difference between the number of larvae expected to be present in spring as calculated from empty pupal cases (an indicator of adult soldier fly emergence) and the number of larvae actually measured, was the key mortality that determined the change in soldier fly numbers between generations. Key factors that influenced population dynamics presumably acted during this period. However, the actual factors responsible for regulating numbers were not identified.

4.2.6 Control with insecticides

Following the withdrawal of dieldrin in the early 1990s, no insecticide has been registered for the control of soldier fly in Australian sugarcane, despite extensive testing and this being seen by affected growers as a high priority.

Previous field trials with now-superseded insecticides did not provide positive results. Samson (2015) evaluated thiamethoxam, imidacloprid and clothianidin for their efficacy against soldier fly in field trials. Thiamethoxam was not effective when applied using coulters in a ratoon crop in the one trial conducted. Imidacloprid was not effective as either a liquid form applied using coulters in ratoons (three trials) or a controlled-release formulation applied in plant cane (one trial), despite application rates several times greater than used successfully against canegrubs. Clothianidin significantly reduced numbers of soldier fly larvae when applied using coulters in ratoons in one of two trials, but only at an application rate more than double the rate registered against canegrubs. None of these insecticides from the neonicotinoid group is likely to have any practical use for soldier fly management.

More recent work reported briefly by Lenancker *et al.* (2020) saw the establishment of four randomized-block insecticides trials where soldier fly larvae annually were quantified for 3 years. Each trial tested 5 to 8 insecticides (not stated) at different application rates applied at fill-in on plant cane. The number of soldier fly larvae collected in one of the trials was too low for statistical analysis. In all remaining trials, there was no difference in numbers of soldier flies among untreated plots and any of the insecticide-treated plots. Additionally, the number of soldier flies collected for two 3-year trials increased with ratoon number (1.7–3.2 times more larvae/year) regardless of treatment. They recommended that future research needs to include laboratory bioassays to determine whether the active ingredients are ineffective against soldier fly larvae, or whether the application method is suboptimal.

Development of strains of the fungus *Metarhizium anisopliae* as a bioinsecticide showed some promise in field trials but required strains other than those then registered for canegrub control (Samson *et al.* 1994, 2000; Samson & Milner 1997) However, overall the likelihood of a commercial product with a specifically soldier-fly-active strain would be negligible and any potential product was never registered.

4.2.7 Control strategies other than insecticides

Samson (2007) combined six possible controls, particularly the effect of long breaks, in a series of field trials. Numbers of soldier fly larvae in newly planted sugarcane crops were reduced when crops were planted after a long break (up to 1 year) compared with a short break of about 3 months, and this difference was maintained into the first ratoon in the second year after planting. Long breaks managed with herbicide or that included a soybean rotation were equally as effective as a fallow maintained bare by cultivation. The addition of organic matter as mill mud (mill waste) or cane trash did not affect numbers of soldier flies. Numbers of predatory beetles (Carabidae, Staphylinidae and Elateridae) were not affected by the different practices, numbers of ants were greater in short-break plots in one of the study years, and numbers of earthworms were greater in plots with mill mud. The significance of factors that might increase mortality of soldier flies was examined by correlation between annual intergenerational mortality and densities of other faunal groups. There was evidence for an association between an increase in mortality of the pest and densities of ants and of predatory Coleoptera, particularly the staphylinid *Thyreocephalus chalcopterus*, in some datasets. Soil concentrations of spores of the entomopathogenic fungus *Metarhizium anisopliae* in the root zone of new plant crops were higher after minimum tillage planting back into the old cane rows than after conventional soil preparation and planting. Sugar yields were lower when cane was planted after a long break than when cane was planted sooner, perhaps because of the shorter growing time of the crop but perhaps also because of the use of experimental planting equipment that might not have been optimal. Despite this, a long break is likely to reduce the risk of damaging soldier fly infestations in subsequent cane crops, with either a rotation with soybeans or spraying out the old crop with herbicide being the preferred options.

4.2.8 Current management options

The above results, as well as those from work during the 1990s, were synthesised into a set of management options that will reduce numbers of soldier flies and their effect on the crop (Anon. 2013):

1. Take out affected blocks early in the harvest season. This will lengthen the break from cane and destroy the larval food while the new generation is still small and vulnerable.
2. Have a grass-free break from cane, e.g. a long herbicide fallow under trash after spray-out of the old ratoon, or a short fallow followed by a non-grass crop such as soybean. Larvae will eventually starve as grasses are their natural food.
3. Plant the next cane crop after the flight period (i.e. after June). Flies are less likely to lay eggs when there is no cane or grass during the flight period.
4. Plant sugarcane with minimum tillage following the herbicide fallow. Keep cultivation for the break-crop at minimal but adequate levels. Extra cultivation does not effectively kill soldier fly and will harm natural enemies.
5. Grow varieties with strong root systems that ratoon quickly.
6. Harvest plant and early ratoon crops when conditions are good for ratooning. Soldier flies will have less impact if ratoons come away quickly.

In addition, do not:

1. Plough-out and immediately re-plant infested blocks.
2. Plant sugarcane early (in autumn) following an infested ratoon. Plant after the flight period (after June).

4.2.9 Future opportunities

Some affected growers harbour the hope that an effective insecticide rather than the cultural controls above will be the 'magical' answer to soldier flies. Field trials have yet to find an appropriate insecticide. In addition, given the potential market size, no agrochemical company is going to invest in testing and registering an insecticide solely for soldier fly in Australian sugarcane. An insecticide already registered for say canegrub control might be more attractive to a marketer but probably only if they were supplied with efficacy and residue data supplied by an industry source (Sugar Research Australia). This is especially so with insecticides that are out of patent.

Any on-farm or area-wide management of soldier fly would rely on effective sampling of target fields. Given the sampling methods (soil cores that are washed out and larva collected and counted), growers would not be capable of or enthusiastic about doing this. A commercial service or one based on a productivity service would not be economically viable.

The recommendations made by Matthiessen (2014) remain appropriate today. He recommended:

- What is NOT worth considering
    - Further studies of soldier fly biology. The existing information is sufficient.
    - Further work on biological insecticides. They are highly unlikely to ever be effective.
    - Trapping, baiting and similar labour-intensive ideas that may seemingly reduce the pest or be a warning system. They are unlikely to be rigorously implemented, especially during non-outbreak periods when it is at the end of which they would be most useful.
    - Breeding for soldier fly resistance or resistance to the induced 'disorder' that causes stools to fail to bud. It would take too long, be very costly and unlikely to be fully effective in elite sugar cultivars.
    - Fanciful notions like incorporating endophyte into sugarcane, pheromones (soldier fly is unlikely to have them), sterile male release technique, attractants.
    - Fanciful notions of applying molecular biology techniques ('genetic engineering') for soldier fly control or resistance in sugarcane.
- What IS worth considering
    - Development of methods for much earlier prediction of outbreaks through the use of remote sensing and weather information.
    - Using the early detection for an industry-sanctioned group-action response plan to take out infested patches or blocks as soon as possible after being identified in order to short-circuit population upsurge and limit spread. In other words, work to prevent or limit the outbreak in its early stages. Intervention at a late stage is actually likely to be more counterproductive than effective.

To this can be added:

- Develop a robust taxonomy and map that to previous research to allow full understanding of that work.

4.3 Endemic armyworms

Until the recent incursion by fall armyworm, armyworm infestation of Australian sugarcane has been attributed to day-feeding armyworms (*Spodoptera exempta*) and night-feeding armyworms (*Mythimna* spp.). The former occur sporadically during late summer and autumn, whilst the night feeders are a conspicuous feature of the green-cane trash-blanket system – adults are attracted to the fermentation products and larvae feed on new shoots during spring and early summer. There is only one generation in each of these fields as by the time the first generation emerges as adults that field is no longer attractive.

Conventional advice to growers is that, by the time damage is apparent, there is no economic benefit from applying insecticides – most of the damage has already occurred and insecticides will kill armyworm parasites that reduce successive populations (Chandler & Benson 1991). However, modelling suggests that early intervention may be economically beneficial, particularly in the south where temperatures are lower (Liu & Allsopp 1996). There is also a range of resistance in sugarcane germplasm (Allsopp *et al.* 2000), but poorly performing clones are likely to be eliminated during the breeding-selection program due to lower cane yields.

No further work on endemic species is likely to yield industry-wide benefits.

The incursion of fall armyworm (*Spodoptera frugiperda*) may change this situation, but there have been no significant reports from through southern Asia of significant damage to sugarcane from this species. Endemic parasites and predators may also impact on this species.

4.4 Earthpearls

During the 1990s pink earthpearls *(Eumargarodes laingi*) were chronic problems on red kraznosem soils around Bundaberg and a few other districts. Much of the infested area near Bundaberg is now growing vegetable or tree crops and current impact on the overall sugarcane industry is minimal.

Samson & Harris (1998) showed that cysts of *E. laingi* were widely distributed in soil, occurring in equal numbers in both the planting rows of sugarcane and most or all of the inter-row space. Cysts were smaller at increasing distance from the sugarcane plants. Most cysts were found in the top 20 cm of soil, but some occurred to a depth of at least 50 cm. Cysts of *Promargarodes* spp. were also found in one field. These had a similar lateral distribution to *E. laingi,* but a greater proportion occurred more than 20 cm deep. Adults of *E. laingi* were found from October to February in the four fields examined in 1993 and 1994. Adults were found in the greatest numbers in November, but they comprised less than 10% of the total *E. laingi* population at any time. A large number of cysts of all sizes was present throughout the year, and the results indicate that most individuals had a life cycle of at least 2 years. The large reservoir of cysts during the adult emergence period explains why *E. laingi* has proved difficult to control with insecticides and with cultural methods during this supposedly vulnerable time.

Allsopp (2001) provided the most recent summary of control measures (metham-sodium is no longer available, and I have removed it from the summary below):

- Conventional insecticides were not effective (although imidacloprid now used for canegrub control might be providing unrealised control of earthpearls):
- Cultural controls of removing infested crops and long fallowing reduce numbers significantly (although bare fallows are now not recommended as the best way to grow cane);
- Appropriate varieties can minimise impact (although the profile of current varieties is unknown);

- Farm hygiene is important in restricting spread of the pest.

Pink earthpearls are also pests in turfgrass, especially bowling greens; no insecticides are registered for control in these situations (D Loch, pers. comm. 2019)

4.5 Wireworms

Wireworms (Coleoptera: Elateridae) damage the eyes and young shoots of germinating cane setts in many cane-growing regions. Insecticides can be applied at planting to avoid damage. However, there are no guidelines for deciding on whether treatment is warranted and a useful sampling method to detect wireworms before planting has not been developed.

Samson & Calder (2003) tested baits of rolled oats as a method for detecting wireworms in preplant fallows. In one year at Bundaberg, the reduction in crop establishment when treatment was withheld was positively correlated with the catch of wireworms at baits before planting. However, no similar relationship was seen in a second year or at Mackay in two years; severe damage was recorded on one farm at Mackay where no wireworms had been found. A sampling plan was developed to estimate wireworm numbers at baits with a fixed level of precision. Wireworms collected at the baits and in samples from established canefields in Queensland and New South Wales included five named and 21 unnamed species from five genera, the most abundant being *Agrypnus, Conoderus* and *Heteroderes*.

As Samson & Calder (2003) conclude, development of a reliable monitoring system would require considerably more research, the cost of which relative to industry benefits could not be justified.

4.6 Sugarcane weevil borer

Sugarcane weevil borer (*Rhabdocscelus obscurus*) is native to New Guinea and is common in all areas north from the Herbert. Larvae burrow into the internode tissue, packing the tunnels with chewed fibre and excreta. They require mature or semi-mature cane with expanded internodes to complete their development but can develop in sound billets and tops that resist drying. During the 1980-1990s they caused significant damage in the Wet Tropics at a time when green-cane trash-blanketing was being adopted and provided shelter for adults and food for larvae that allowed large carry-over populations.

Development of higher-fibre cane varieties and harvesting that smashes the cane billets that are left in the field into smaller pieces mean that its importance has reduced dramatically, obviating the need for alternative controls.

The most recent Australian R&D involved assessing the usefulness of lures to capture adults (Sallam *et al.* 2001), similar to that used against a related species in oil palms in central and South America. However, this did not yield a practical control component.

Research at Ramu Sugar in Papua New Guinea has indicated variability amongst clones in their resistance to the pest (Samson *et al.* 2017), but this is unlikely to form part of a breeding and selection program in Australia.

This species is also thought to be the same as that which infests palms in northern Queensland, although there is some thinking that more than one species might be involved there and in sugarcane in Hawaii. Resolution of this would have implications on the movement of potted palms from north Queensland into southern areas where the weevil is not known to be a pest of sugarcane.

4.7 Planthoppers

Established planthoppers, as themselves, are only minor pests of Australian sugarcane and do not warrant control measures. Two species have been studied recently – linear bug (*Phaenacantha australica*) as a possible vector or causal agent of Yellow Canopy Syndrome (YCS), and the sugarcane planthopper (*Perkinsiella saccharicida*) as the vector of Fiji disease virus (FDV). The island planthopper (*Eumetopina flavipes*), found in the Torres Strait and nearby areas, is considered in the biosecurity section of this report.

In initial investigations on YCS, linear bug was identified as a possible pest causal agent. However, field populations did not always match the appearance of symptoms, and cage experiments with high population densities of *P. australis* did not elicit YCS-like symptoms (Olsen *et al.* 2015).

Only during the 1970s did *P. saccharicida* attain populations large enough to cause primary damage (although it was probably disguised by FDV symptoms), and then only because of the widespread planting of the favourable host cultivar NCo310. Four recent studies as summarised below have looked at the taxonomy, biology and parasitoids of *P. saccharicida* in relation to the overall management of FDV.

One conundrum has been the presence of *P. saccharicida* in all canegrowing areas of Queensland and New South Wales, but the disease occurs only in and south of the Central district. Ridley *et al.* (2006) tested whether northern Queensland populations of *Perkinsiella* are poorer vectors of the disease. They found that the ITS2 sequences of the Western Australian *P. thompsoni* and the Fijian *P. vitiensis* were distinguishable from those of *P. saccharicida* and there was no significant variation among the 26 *P. saccharicida* populations tested. Reciprocal crosses of a northern Queensland and a southern Queensland population of *P. saccharicida* were fertile, reinforcing that there is only one species present in eastern Australia. Single vector transmission experiments showed that a population of *P. saccharicida* from northern Queensland had a higher vector competency than either of two southern Queensland populations. The frequency of virus acquisition in the vector populations was demonstrated to be important in the vector competency of the planthopper. The proportion of infected vectors that transmitted the virus to plants was not significantly different among the populations tested. This study shows that the absence of FDV from northern Queensland is not due to a lack of vector competency of the northern population of *P. saccharicida*.

As a first step to understanding the biocontrol agents of *P. saccharicida*, Hughes *et al.* (2008) devised a laboratory-based technique was devised to rear the vector using sugarcane leaves as a food source. Planthoppers were reared on sugarcane leaf segments embedded in agarose enclosed within plastic containers. This allowed a non-destructive assay for determination of the inoculation potential of planthoppers using RT-PCR.

Later, Hughes *et al.* (2011a), as a step in a future paratransgenic approaches targeting FDV transmission, surveyed *Wolbachia* infections in populations of *P. saccharicida* and showed variable frequencies, low-titre infections, and high phylogenetic diversities of strains. The frequencies of infection of *Wolbachia* in planthoppers varied among populations, from 4% to 100%. *Wolbachia pipientis* is a maternally inherited endosymbiotic bacterium that infects a wide range of arthropods and nematodes. *Wolbachia* is renowned for inducing dramatic reproductive phenotypes, such as cytoplasmic incompatibility and parthenogenesis, that manipulate host reproduction to enhance *Wolbachia* transmission.

They (Hughes *et al.* 2011b) also identified used PCR to identify two distinct yeasts within *P. saccharicida*. One was related to yeast-like symbionts from other planthoppers. The second yeast was a member of the *Candida* genus, a group that has been identified in beetles and recently described in planthoppers. Microscopy revealed the presence of yeast in the fat body of *P. saccharicida*. The *Candida* yeast was cultured, and transformation was accomplished by

electroporation of *Candida albicans* codon optimized plasmids, designed to integrate into the genome via homologous recombination. Transgenic lines conferred resistance to the antibiotic nourseothricin, and expression of green fluorescent protein was observed in a proportion of the yeast cells. Stably transformed yeast lines could not be isolated as the integrative plasmids presumably replicated within the yeast without integration into the genome. This yeast may be useful as an agent for a paratransgenic control of FLG, but no further development has occurred.

4.8 Mealybugs

Sugarcane mealybugs (*Saccharicoccus sacchari*) occur throughout the industry but cause very minor primary damage. However, they form colonies behind the leafsheaths, and their excreta collects there and forms an ideal medium for the development of bacteria that cause the formation of floc. As such, they excited considerable interest during the 1980-90s when an in-field solution to floc was thought feasible. The most recent publication is Allsopp *et al.* (1993).

With the advent of in-mill reduction in floc levels and mixing of shipments of raw sugar to dilute levels, there has been no interest in them as pests and they do not warrant research attention.

4.9 Feral pigs

The damage caused by feral pigs (*Sus scrofa*) in Wet Tropics cane growing regions can be significant, with losses in cane proceeds exceeding $1 million in some years within the Herbert region alone. The management of the pest is difficult, due to the landscape in which the feral pigs are found, the varying success of hunting, baiting and trapping activities and the overall intelligence of the pest being managed.

In project GGP066 (Kemp 2014), the Hinchinbrook Community Feral Pig Management Program (HCFPMP), a group of community partners, utilised a multiple approach to manage feral pigs within the landscape through the use of GIS, genetic mapping and targeted on-ground activities (shooting, dogging, baiting and trapping).

The project :

- highlighted feral pig management strategies for Wet Tropics sugarcane production areas to cane farmers working within this region and produced written resources that are being used by landholders in the cane industry.
- highlighted the opportunity for landholders to work together instead of alone, to manage a pest that knows no boundaries.
- generated the realization by industry what financial impacts feral pigs cause to the cane growing industry.
- provided grower training in the effective management of feral pigs using baiting, fencing and trapping techniques.
- highlighted the importance of 1080 as an effective tool for the management and control of feral pigs.
- identified appropriate bait carrier options for the management of feral pigs in a Wet Tropics sugarcane production area.
- Genetic data and population mapping has assisted the HCFPMP better target feral pigs within the landscape. Data identified two populations of feral pigs within the Herbert area.

Whilst the project undoubtably had short-term benefits, it is difficult to gauge 6 years later whether the interaction between neighbouring cane growers to manage feral pigs has continued. This is a common problem where dedicated resources are not available to continue area-wide programs and

local industry, especially through cane productivity services, do not rate the problem as a high priority.

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5. DISEASE MANAGEMENT

5.1. Ratoon stunting disease

5.1.1 Background

Ratoon stunting disease (RSD) is caused by the bacterium *Leifsonia xyli* subsp. x*yli* (*Lxx,* Davis *et al.* 1980, 1984). The bacterium lives in the xylem cells and causes stunting but no other external symptoms. When mature stalks are sliced longitudinally with a sharp knife, small red dots or commas can sometimes be seen in the vascular bundles in the nodes of infected stalks. The disease is spread by planting infected setts/billets and by contaminated cutting implements including knives, planting equipment and harvesters. RSD causes yield losses from 5 to 55% depending on the susceptibility of the variety and the environmental conditions (Croft 1995). RSD restricts water flow in plants and is more severe when plants are suffering moisture stress. The bacterium is not known to occur naturally in any hosts other than sugarcane and its close relatives (Davis & Bailey 2000).

RSD was discovered in 1948 when growth differences were observed in plots of the variety Q28 planted from different plant sources (Steindl 1961). This review will look at diagnostic methods, the status of RSD incidence and management in Australia and the debate about the need for breeding RSD resistant varieties. It will also consider research on genomics of *Lxx* and interaction of the pathogen with the plant and how this research may lead to more innovative management of the disease.

5.1.2 Diagnosis of RSD

Most diseases can be diagnosed by visual symptoms on leaves, stalks or roots. RSD has no external diagnostic symptoms. The internal symptoms of RSD are small red/brown dots in the nodes when stalks are sliced open. This symptom is quite diagnostic in some varieties but in other varieties the symptoms are faint or can be confused with other diseases such as leaf scald and chlorotic streak. Some varieties have discoloration of the nodes unrelated to RSD. Prior to 1972, RSD was thought to be caused by a virus but the distinctive bacterium that causes the disease was observed under an electron microscope and then it was found that they could be seen in xylem extracts with phase-contrast microscopy (Steindl 1976). BSES initially offered phase-contrast microscopy as a service for Productivity Boards but in the early 1980s, BSES encouraged Productivity Boards to purchase microscopes and trained staff in the use of the microscopes (Egan 2015). This was successful in some districts (Amiet 1985), but in other districts staff had difficulty learning to use the microscopes. In 1993, BSES established a laboratory to diagnose RSD with a serological method known as EB-EIA (Croft 1994, SRDC project BSS030)). The EB-EIA had similar sensitivity to phase-contrast microscopy by an experienced microscopist but allowed more samples to be processed in a day by one person. This method continued to be used by the industry until 2016-2017 (Croft *et al.* 2004). In 2016, SRA

commenced a project (project 2015/078) to implement a new PCR method developed by Young *et al*. (2014, SRA project 2014/086).

Taylor *et al.* (2003), Pan *et al.* (1998) and Fegan *et al.* (1998) reported PCR assays for *Lxx* in xylem extracts. Maclean *et al.* (2001, SRDC project UQ024) developed a quantitative real-time PCR (qPCR) assay for *Lxx* that was more sensitive than the normal PCR assays. The report analysed the economics of implementing the qPCR for routine RSD diagnosis and found that it was not cost competitive and there were questions about the IP of qPCR for commercial applications.

Grisham *et al.* (2007) described an RSD assay using DNA extracted from leaves. Croft *et al.* (2012) identified that leaf sheaths contain high populations of RSD bacteria. This was the first report of leaf sheaths being considered as a source for RSD diagnosis. Croft *et al.* (2012) recommended that leaf sheaths be considered for diagnosis using PCR methods. Young *et al.* (2014) and Young *et al.* (2016, SRA project 2014/086) described a PCR method for diagnosis of RSD using leaf sheath biopsies (LSB-PCR or LSB-qPCR). They showed that this method was more sensitive than the EB-EIA and allowed more simple and non-destructive sampling. The ease of sampling would allow field staff to collect more samples from blocks and increase the probability of detecting the disease.

An SRA-funded project (Berna *et al.* 2015, SRA project 2013/001) looked at volatiles produced in sugarcane infected with RSD to determine if specific volatiles could be identified that could be used for rapid in-field diagnosis. No specific volatiles were identified but changes in the levels of some volatiles were detected in response to infection. The changes in volatiles were not consistent across seasons and between varieties and this method does not appear to be an option for diagnosis.

SRA commenced a transition from the EB-EIA to the LSB-qPCR in 2016 in its RSD laboratory (Thompson 2017, SRA projects 2015/078 and BIOBRSD). The first year of the project encountered several problems and the concluded further development was required before full implementation. Samples from 673 fields were analysed with both LSB-qPCR and EB-EIA. Of the 673 samples, 91.4% were negative by both methods. Fifty-eight samples were positive (either positive by LSB-qPCR or EB-EIA or both). LSB-qPCR detected 74% of the positive samples and EB-EIA detected 62% of the positive samples. All samples positive by EB-EIA and negative with LSB-qPCR were confirmed by qPCR of the xylem extract. LSB-qPCR detected 22 positive samples missed by EB-EIA and EB-EIA detected 15 samples that were missed by LSB-qPCR. The problems with operation of the RSD laboratory in 2016 led to an external review of the laboratory that made recommendations for improvement in operation of the laboratory and future research. The current SRA project BIOBRSD resulted from the review and is comparing LSB-qPCR and qPCR of xylem extracts. The project is also examining factors such as age of crop, sampling intensity, storage and handling of samples and inhibition associated with discoloured leaf sheaths. The final report of this project was not available at time of writing.

5.1.3 Incidence and economic importance

The incidence of a disease that has no obvious external symptoms relies on field workers taking samples and either visually looking for internal symptoms or sending samples to a diagnostic laboratory for analysis. Young (2018) claimed the Australian sugar industry has “turned a blind eye” to RSD, that RSD has been mismanaged to the detriment of the industry and incidence of the disease is much higher than claimed by SRA (SRA Fact Sheet IS13007). However Hoy (2019) stated that “A careful, objective evaluation of the contents of this article [by Young (2018)]” does not support the allegations.

Productivity Services companies in Queensland reported their best estimate of the area infected with different diseases from 1980 to 2002 (Magarey 2006a). Productivity Services used data from RSD surveys and plant source inspections to give their best estimate of RSD infection. The

Productivity Services estimated 3% of Queensland cane fields were infected with RSD during this period with 5% in the northern region, 6% in the Herbert, <1% in the Burdekin, 2% in the central region and 5% in the southern region.

Although plant source inspections are not representative of all commercial crops, they allow trends in RSD infection to be followed over time. RSD infection in plant sources in Harwood were 40% in 1994 (Croft *et al.* 2004a; McGuire *et al.* 2009). In Harwood, the management strategies of disease-free seed, plant source inspections and hygiene resulted in a fall in incidence to 11% infected plant sources by 2002 (Croft *et al.* 2004a), 9% in 2008 (McGuire *et al.* 2009), 9% in 2004 to 2011 (Young *et al.* 2012) and 11% in 2019 (Young & Knight 2020). All the plant source samples were assessed by the EB-EIA assay except in 2019 when the samples were screened with the LSB-qPCR assay (Young and Knight 2020). The estimates of percent infection in plant sources in Harwood since 2009 were similar irrespective of assay method used. It should be noted that in Harwood 50-60% of the plant sources reported in Young *et al.* (2012) were from replant blocks. SRA recommends that planting material should not be sourced from replant blocks because of the risk of volunteers from the previous crop. In Condong, the incidence of RSD in plant sources fell from 40% in 2000 to 16% in 2008 after the introduction of approved seed plots and plant source inspections.

The Innisfail-Babinda Cane Productivity Services sampled 288 proposed plant source blocks in 2019 and found 29% of blocks infected (Young & Knight 2020). Innisfail-Babinda Cane Productivity Services did not have a history of sending samples to the RSD laboratory before 2019.

McGuire *et al.* (2009) reported surveys in Broadwater mill area of commercial crops on 25% of the total number of farms in the mill area each year from 2006 to 2008 and they found 4.7% RSD incidence.

The data from the SRA laboratory (Croft *et al.* 2004a) indicates that many districts have sent samples for RSD diagnosis but the results of these samples is not available in a format that allows interpretation except by the Productivity Services and the interpreted results have not been published to make the results available to the wider sugar industry community. For example, Mackay Productivity Service surveyed 3,019 commercial fields and found 1.3% of fields infected (Croft *et al.* 2004a). Croft *et al.* 2004a also reported the results for 30,592 plant sources collected in Mackay from 1995 to 2002 that found 0.7% of plant sources infected. SRA has requested that Productivity Services provide more detailed documentation when sending samples to enable SRA to identify the purpose and source of samples which will allow SRA to better collate and interpret the results (Magarey pers. comm.).

SRA project 2019/003 “RSD detection at the sugar factory – disease detection blueprint” is examining whether RSD can be detected in juice samples collected at the mill. Mills sample juice from every consignment (rake) of cane for sugar content (CCS). If this project is successful, it would provide a cost-effective method to conduct large surveys for RSD. The labour-intensive components of an RSD survey are the travel to farms, taking samples and dispatching samples to the laboratory. This could be avoided by taking sub-samples from CCS samples that are already collected by the mills. Mills have details of the source of each sample because the CCS samples are used for payment.

In conclusion, the success of the current recommended management programs can be monitored by the incidence of RSD in the industry. Productivity Service companies have more recent data that has not been published. Most mill areas conduct plant source inspections, and these are an indicator of the RSD status of an area, although they may under-estimate the incidence of the disease in the commercial crops. Recent data collected by the Productivity Services from commercial crops and plant sources should be collated to determine if there is existing information on RSD incidence or whether more surveys should be conducted. This will allow a more informed assessment of the current situation.

Young & Knight (2020) estimated the losses from RSD as $20M if the incidence is 5% infested fields and $126M if the incidence is 30%. Croft (1995) estimated losses of $168M/year in 1995 if no RSD management is practiced. It was estimated that an efficient approved seed scheme could recover more than 80% of this potential loss after costs.

Before conclusions can be made about the success or failure of the current management program, information should be gathered on the adoption of the management program in each area. Information on why the management program is adopted to differing levels in different areas should be investigated and barriers to adoption identified. If the barriers to adoption are for legitimate reasons or there is a fundamental failure in the management program to adequately control the disease, alternative management strategies may be necessary.

5.1.4 Disease-free seed-cane and hygiene

RSD-free seed-cane was recognised as an important method of managing RSD (Steindl 1961). Hot-water treatment (HWT) for 2-3 hr at 50°C was found to eliminate the bacterium from 95-99% of infected stalks (Steindl 1961). HWT tanks were established in nearly all mill areas in Australia during the 1950s and continue to be used as a component of RSD management (Egan 2015). Initially, local Pest and Disease Control Boards (now called Productivity Services) hot-water treated stalks or setts supplied by growers who planted the treated cane on their farms. The problem with this system was that some disease escapes can occur if heavily infected cane is treated. Germination failures were not uncommon as the heat treatment can affect germination and the buds on stalks become soft and easily damaged during transport. Hot-water treatment for individual growers is still done to a limited extent in some mill areas but during the 1980s to 1990s most Productivity Services established Approved Seed Plots (ASP) which supply a nucleus of disease-free seed to growers (Egan 2015). Quality control guidelines for the operation of ASP have been published by SRA (Croft & Cox 2013). The guidelines recommend a strict program of repeated hot-water treatment each year, hygiene and quality control checks to ensure seed-cane is true-to-type (DNA fingerprinting) and free of RSD and a range of other diseases including chlorotic streak, Fiji leaf gall, mosaic, leaf scald and smut. The Productivity Services act as agents for SRA to supply growers with new varieties from their ASP. Some Productivity Services have purchased their own farms to grow the ASP while others lease land or have other arrangements with growers. ASP play an important role in the Australian sugar industry. Quality control is essential as any failure could lead to rapid dissemination of diseases or incorrectly labelled varieties. Extensive quality control checks for RSD have been an integral part of the ASP and SRA seed plots that supply the new varieties to ASP.

Tissue culture derived plantlets (SmartSetts™) have been used to replace HWT by some Productivity Services in recent years (Geisjkes *et al.* 2003; Lakshmanan *et al.* 2004, SRDC project BSS242). SRA uses repeated HWT and inspections to ensure the source material for the tissue culture laboratory is free of RSD and other important diseases (Croft & Cox 2013). SmartSetts™ have increased in popularity with Productivity Services.

The ASP cane is distributed to growers as whole stalks or billets and growers plant the cane on their farms. The recommendation is that ASP cane should never be planted in replant blocks because of the high probability that volunteer plants will be present from the previous crop. Mixing ASP cane with volunteer plants defeats the purpose of providing a disease-free seed source. Growers ideally plant their commercial crops directly from the blocks planted with the ASP cane, but in many cases, growers need to multiply the ASP through one or more multiplication steps on their farm to provide sufficient quantities of cane to plant their commercial fields. Ensuring planting equipment is disinfected before planting the ASP, and when planting subsequent blocks derived from the ASP cane, is essential to prevent reinfection from equipment that might be contaminated. Commercial disinfectants such as Steri-Max® are registered for disinfecting sugarcane equipment (SRA Fact Sheet

IS13007). It is also important to ensure that billet planters do not carry-over billets from the blocks they were planting prior to planting ASP cane, that may be of at a higher risk of infection with RSD.

Disinfection of harvesters between harvesting commercial fields is best practice but is hard to achieve. Harvester contractors are reluctant to take the time to thoroughly clean and apply disinfectant which can take up to an hour. SRA has placed the emphasis on ensuring disease-free seed and hygiene are practiced at planting. Spread by harvesters into disease-free cane is relatively slow, especially if the harvesters cut at or below ground level where the friction provides some self-cleaning (Taylor *et al.* 1988; Hoy *et al.* 1999).

5.1.5 Resistant varieties

Davis *et al.* (1988) and Harrison & Davis (1988) showed that the population of bacteria in RSD resistant varieties is lower than in susceptible varieties. Comstock *et al.* (1995) developed a tissue blot immunoassay for rating varieties for resistance to RSD and implemented the screening in the USDA Canal Point plant breeding program (Comstock *et al.* 2001). Comstock *et al.* (1996) found that the incidence of RSD in commercial fields of resistant varieties is lower than in susceptible varieties. Hoy *et al.* (1999) found that spread of RSD during planting was greater than by harvesters and that the rate of spread varied significantly between varieties.

Croft *et al.* (1994, 2012), Croft (2002), and Croft & Johnston (2013) report the resistance of Australian sugarcane varieties to RSD. Resistance was assessed by the EB-EIA and tissue blot assays. The RSD resistance ratings are available to growers in variety information sheets and SRA on-line variety information site QCANESelect. A simple method of inoculation of the resistance trials was described by alternating stalks of RSD-infected cane and stalks of the test variety through a whole-stalk planter or cutting setts with lopping shears connected to an automatic spray that sprays RSD-infected juice onto the blades. Croft (2002) compared the relative population of bacteria estimated by EB-EIA absorbance and the percent infected plants of 25 commercial varieties. Only one variety, H56-752, had less than 40% infected plants, all other varieties had >80% infected plants. Croft *et al.* (2012) and Croft and Johnstone (2013) reported data for most of the major varieties currently grown commercially and found a higher proportion of resistant varieties than in earlier studies. The most widely grown variety in Australia for the last 11 years, Q208, was rated resistant and two other widely grown varieties, Q183$^{\phi}$ and Q200$^{\phi}$ were moderately resistant. These three varieties contributed 48% of the crop in 2018.

RSD resistance trials are labour intensive compared with trials for other diseases that are rated on visual symptoms (Croft *et al.* 2012). The collection of samples and processing at a laboratory is much more labour intensive.

Croft (1995) related the incidence of RSD in the Herbert to resistance ratings of varieties and found some relationship. The RSD resistance ratings of varieties was related to incidence of RSD in plant sources in Innisfail (Young & Knight 2020) and Harwood (Young *et al.* 2012; Young & Knight 2020). These papers found a relationship between SRA resistance ratings and incidence of disease. In replant blocks, there is a significant probability that the RSD could be detected in a volunteer plant of a different variety to that listed as the variety present in the block. This would lead to some confusion when comparing incidence of RSD from surveys and resistance ratings.

Croft *et al.* (1995, SRDC project BSS101S) conducted an economic analysis comparing management of RSD with disease-free seed plus hygiene and varietal resistance. They calculated that breeding for resistance would give a small return on investment ($0.41/$ invested) compared with management of RSD that allowed 10% escapes, with the proviso that the plant breeding program was expanded to ensure selecting for RSD resistance did not affect genetic gains. If there was no expansion in the breeding program, and genetic gains for yield were restricted by 50%, growers would lose $22/ha/yr

or $220/ha across the whole industry in 10 years and $440/ha in 20 years from losses in gains from potentially higher yielding varieties. The economic analysis found that a sub-program breeding specifically for RSD resistance for districts that have difficulty controlling RSD would only give small returns on investment. A review of the incidence of RSD in the wider industry and the adoption of RSD management practices would allow an informed decision about the need for breeding for RSD resistance. Young & Knight (2020) have shown moderate to high levels of incidence in two mill areas but these mill areas only represent a small percentage of the industry. A more thorough investigation of current knowledge on RSD incidence and adoption of management practices is needed to make an informed decision.

Grisham *et al.* (2007) showed that qPCR could be used to quantify the bacterial population in leaves of different varieties. The SRA RSD laboratory is now using LSB-qPCR or qPCR of xylem extracts and these methods could be used to assess RSD resistance trials instead of the EB-EIA or tissue blot methods.

Young & Knight (2020) reported that 20 new SRA varieties do not have RSD resistance ratings in QCANESelect. SRA should ensure that all new varieties are rated for resistance to RSD to assist growers that have a high incidence of RSD on their farms to manage RSD.

5.1.6 Genomics and host-pathogen interactions

In 1993, a research project to study the interaction of the *Lxx* bacterium and sugarcane using molecular tools was commenced with funding from SRDC (BSS099) and the CRC for Tropical Plant Pathology (Brumbley *et al.* 2006). The aim of this research was to better understand the genes responsible for pathogenicity of *Lxx* with the potential outcome to produce GM varieties with resistance to RSD. Tools were developed to produce transposon mutants of *Lxx* that were unable to infect sugarcane. The genes that were mutated and caused loss of pathogenicity were identified. There has been no attempt to produce genetically modified sugarcane with resistance to RSD.

A large international project successfully sequenced the genome of *Lxx* (Monteiro-Vitorello *et al.* 2004). They described the gene structure of the genome and identified some genes that may be involved in pathogenicity. Young *et al.* (2006) found that there was no diversity in the ribosomal genes of 105 isolates of *Lxx* sourced from 9 countries around the world. An isolate of *Lxx* from China was recently sequenced with modern whole genome sequencing techniques. The Chinese isolate was found to have 93.6% similarity to the Brazilian isolate that was used for the initial genome sequencing project (Wang *et al.* 2016). This shows that there is variation in the genome of *Lxx* between countries, but the implications of this variation are unknown.

Cia *et al.* (2018) conducted an extensive study of the expressed genes and proteins associated with RSD infection in sugarcane. They identified many genes and proteins that were up-regulated in association with infection. This study increased our understanding of the interaction but did not suggest any immediate outcomes that will lead to better control of the disease.

Decisions about developing GM resistant varieties will depend on the future policy of the sugar industry to GM sugarcane. Although research on the interaction of RSD and sugarcane is of scientific interest, there is no obvious direct application to control of the disease unless there is a change in the policies for RSD management and/or commercialisation of GM sugarcane.

Recommendation: Coordinate a review in collaboration with Productivity Services to interpret past RSD laboratory results and document RSD incidence in commercial blocks and plant sources in recent years. The review should also record the level of adoption of the key components of the RSD management program such as percentage of growers obtaining ASP plot cane and tonnes of ASP

cane/grower, percentage of blocks planted from progeny of ASP cane, number of plant source inspections, seed-cane sources from fallow and replant blocks and adoption of hygiene practices by planting and harvesting contractors.

Recommendation: Investigate industry support for conducting a coordinated industry wide RSD survey. If the diagnosis of RSD in juice samples at the mill is successful, this method would provide a cost-effective way to conduct a survey.

Recommendation: SRA should evaluate qPCR of LSB and/or xylem extracts for assessing bacterial populations in varieties in RSD resistance trials. SRA should ensure that new varieties are rated for resistance.

Recommendation: The need for breeding for RSD resistance should be reassessed after an industry-wide review of the RSD incidence and the RSD management program. A new economic analysis of the benefits of breeding for RSD resistance could be conducted given the new information on the RSD situation in the industry and the current knowledge of the level of resistance in Australian germplasm.

5.2 Smut

5.2.1 Background

Sugarcane smut is caused by the fungus, *Sporisorium scitamineum* (formerly *Ustilago scitaminea*) (Comstock 2000). It infects plants through dormant or young germinating buds and causes plants to produce a whip-like structure from the meristematic tissues. The whip is made up a central core of plant tissue which is surrounded by masses of spores of the fungus. Infected plants can be severely stunted with profuse tillering. Yield losses of 60% or more are common in susceptible varieties. Smut is spread by infected planting material and by wind-blown spores. Spores can travel large distances and the disease has spread around the world. SRDC funded a pest risk analysis that identified smut as the highest risk to the Australian sugar industry and an incursion management plan was prepared (Croft 1996, SRDC project BS172S). The first record of smut in Australia was in 1998 in the Ord River district of Western Australia (Croft & Braithwaite 2006). The disease spread rapidly in the susceptible varieties NCo310 and Q117 in the Ord, but extensive surveys in the main sugarcane growing areas on the east coast failed to find any smut (Croft 1999, SRDC project BSS230).

Smut was found for the first time in Queensland in June 2006 on a farm in the Isis mill area near Childers (Croft *et al.* 2008b). The Queensland government, BSES and Productivity Services conducted extensive surveys throughout the east coast sugar industry and the disease was found in the central and Herbert regions a few months after the initial finding. Indications were that the disease had been in these areas for at least two years before detection. Spore trapping combined with microscopic or PCR examination of the spore trap tapes were used as an early warning of spread to new districts (Magarey *et al.* 2009a). Smut spread to all farms in the Herbert, central and Bundaberg/Isis districts within 3 years of the initial finding (Magarey *et al.* 2009b). Spread within crops of highly susceptible varieties was also rapid with greater than 85% of plants in the highly susceptible variety Q157 becoming infected within 18 months in the Herbert (Magarey *et al.* 2009b).

Magarey *et al.* (2010) estimated direct losses of $32M in the Herbert alone in 2010 and losses would have increased if susceptible varieties had not been replaced. Smut was found in all regions within 3 years (Cox *et al.* 2010). At the time of the incursion, 60-70% of the Australian crop was planted to susceptible varieties. Antony *et al.* (2010) compared the predicted losses from smut at the time of the incursion (Watson 2007) with the actual losses 3 years on. They found that smut spread faster than predicted but the emergency response helped to alleviate the worst of the potential losses.

Growers commenced a major replanting program to replace susceptible varieties and the Queensland government assisted the Bundaberg/Isis region to import large quantities of planting material of resistant varieties from the Burdekin and resistant varieties were rapidly multiplied and distributed as tissue culture plantlets (Cox *et al.* 2010).

5.2.2 Resistant varieties

SRDC funded a project to screen Australian varieties for resistance to smut in Indonesia in 1997 and the project was due to commence in 1998 (SRDC projects BSS214). After the incursion of smut in the Ord, the project was expanded. Screening varieties also commenced in Western Australia (SRDC projects CTA043 and WAA002). The Ord had strict quarantine regulations which slowed the movement of varieties from Queensland. Testing in Indonesia was conducted on an island isolated from the Indonesian sugar industry and allowed larger numbers of clones to be shipped, pass through a shortened quarantined/propagation period, and be screened for smut resistance. By 2007, 1705 Australian clones had been screened for smut resistance in Indonesia (SRDC projects BSS214, BSS256, BSS265) and 481 clones had been screened in the Ord (Croft *et al.* 2008a). In 2007, the screening program in Indonesia ended and smut screening commenced in Bundaberg. A new project, BSS325 "SmutBuster", was jointly funded by the Queensland government and SRDC.

The incursion of smut continues to have a large impact on the SRA plant breeding program. At the time of the incursion, 69% of clones in the BSES breeding program were susceptible to smut (Croft *et al.* 2008a). In 1998, the BSES breeding program set a policy to make 50% of crosses each year with a smut mid-parent resistance rating of 6.5 or less (rating scale 1-9 where 1 is highly resistant and 9 is highly susceptible). In 2004, the policy was reviewed and although there was progress in meeting the target, it was found that very few crosses were made with a resistant mid-parent rating (mid-parent rating <3.5). The review recommended a new target of 25% of crosses with a mid-parent rating < 3.5 and 25% with a mid-parent rating <6.5. The target was exceeded in the 2006 crossing season before smut was found in Queensland (Croft *et al.* 2008a). After the smut incursion, a new policy was formulated and in 2007 more than 90% of crosses had a mid-parent rating of resistant or intermediate and only a small number of susceptible crosses were made. In 2010, only a small number of special purpose susceptible crosses were made and 99% of crosses had a resistant or intermediate mid-parent rating. Plant breeders estimated this new policy would restrict genetic gain as many of the best parents used to increase yield were susceptible to smut.

Overseas research has shown that, even in crosses between two highly smut susceptible parents, between 6–20% of progeny will be smut-resistant (Chao *et al.* 1990; Wu *et al.* 1983). In an attempt to recover the genes for high yield and sugar content from the susceptible parents, projects BSS325 SmutBuster and SRA 2012/325 SmutBuster II planned to screen large populations from crosses between the high-breeding-value susceptible parents, identify resistant clones and rapidly recycle these clones as parents in the breeding program. They were also tested as potential new varieties.

A new method of screening true seedlings of sugarcane for smut resistance was developed for the SmutBuster projects (Cox *et al.* 2010). The true seedlings were dipped in a suspension of smut spores at the time they were transplanted from the germination trays into pots for hardening-off. The SmutBuster project inoculated approximately 40,000 seedlings with smut each year for 5 years. From the seedling populations, approximately 10,000 disease-free clones were selected and inoculated in a second round of smut screening using the standard sett-dip inoculation method. Any clone that showed smut infection in either of the two-stage screening program was discarded. Approximately 2,500 clones from these populations were selected each year and planted in clonal assessment trials in each of the four regional selection programs. A selection of clones was recycled directly back into the parent collection for crossing (Cox *et al.* 2010). The best clones from the clonal assessment trials were merged with the clones coming from the core breeding program in the final

assessment trials. This project effectively doubled the first two stages of the plant breeding program for five years and the last clones to progress through the final assessment trials were planted in these trials in 2016. Three recently released varieties, SRA15(ф), SRA16 and SRA20, came from the SmutBuster program and 19 clones that have been maximum propagated for potential release in the next few years are from the program. The clones from the SmutBuster program selected for use as parents are actively being used in crossing and will continue to contribute to the breeding program.

Parfitt *et al*. (2016, SRA project 2011/343) investigated extending the seedling inoculation with smut that was developed in the SmutBuster project to the core breeding program. They found that the method successfully increased the percentage of resistant varieties selected from the seedling stage in trials conducted in the northern trials (Meringa) but made little difference in southern trials. They suggested that spreader rows of smut-infected cane planted between the rows of seedlings be considered as an alternative to inoculating seedlings and that any decision to implement smut inoculation or spreader rows be made at a regional level. The adoption of markers, as discussed below, may mean that inoculating seedling with smut is unnecessary as markers may be a more efficient way to eliminate susceptible clones in early stages of the breeding program.

The method of screening clones for resistance to smut adopted by SRA for routine trials is to dip setts in a suspension of smut spores and inspect trials in a plant and first-ratoon crop. This method is widely used overseas. To speed up the trials, SRA ratoons the plant crop at 3-4 months and the first ratoon crop is inspected at 4 months after ratooning (Bhuiyan *et al.* 2013a). The incidence and severity of symptoms in the test clones is compared with disease levels in a set of standard varieties. Environmental factors that influence the success of the trials were investigated by Bhuiyan *et al.* (2009). They found that temperature was a critical factor both in the dip suspension and post dipping when the setts were germinating. The optimum temperature for smut spore germination and infection was found to be 30-31°C. Water quality in the dip suspension was also important with significantly fewer spores germinating in bore or town water compared with distilled water. Dipping and germination of setts in SRA smut resistance trials is conducted in a temperature-controlled germination cabinet at 30°C.

A criticism of the sett dip technique is that it is too severe and does not reflect the field resistance of varieties. Bhuiyan *et al.* (2018a) conducted three field trials where the test varieties were planted between rows of smut-infected cane of a susceptible variety. The trials were inspected in a plant and two ratoon crops. The incidence of smut in the varieties in the field trials was highly significantly correlated with the smut ratings obtained in the dip inoculation trials. This confirmed that dip inoculation is a good method for estimating field resistance. Ratings for resistance in smut trials is based on a formula that combines incidence and severity. Bhuiyan *et al.* (2020) described a new method of statistically analysing and assigning smut ratings. The new method provides information on the confidence of the rating and gives a visual representation of the data that will help pathologists, plant breeders and advisors to assess the reliability of the ratings.

An indirect method of rating varieties for resistance to smut using NIR spectroscopy to identify spectral properties of sugarcane buds related resistance was investigated by Purcell *et al.* (2010, SRA projects BSS307 and BSS325). This method has the advantage, like molecular markers, that no specific trials of the test varieties need to be planted, as the measurements can be made on propagation plots or plant breeding yield trials. Initial experiments developed two models which were further evaluated on a blind set of 300 clones. When the two models were combined, the NIR correctly rated only 40% of the 300 clones. It was also found that the NIR equipment used in the final year of testing was unreliable and no further work has been conducted.

Barden *et al.* (2010) and McNeil *et al.* (2018, SRA projects 2012/026 and 2012/325) showed that microscopic examination of the extent of fungal hyphal colonisation in buds of varieties a few weeks after inoculation is highly correlated to resistance. This method could be used to rate varieties

without the need to plant inoculated plants to the field and results would be obtained within a few weeks instead of 12 months. The disadvantage of the method is that it is very labour intensive and may not be suitable for the large numbers of clones required by the breeding program. The method has been used as an aid in understanding the interaction of smut with sugarcane.

5.2.3 Genomics and host-pathogen interaction

Understanding genetic variation within pathogens is important when screening for disease resistance. Varieties need to be screened for resistance to the genetic types present in an area. The propensity of pathogenic species to mutate to overcome resistance can lead to new epidemics in previously resistant varieties. While various countries have reported the presence of races of *S. scitamineum* (Comstock & Heinz 1977), there is limited information on the number of races and their prevalence (Ferreira & Comstock, 1989).

Braithwaite *et al.* (2004, funded by CRC Tropical Plant Pathology) found that 38 collections of smut spores from 13 countries had a low level of variation at the genomic DNA level, but a divergent group of isolates from south-east Asia was identified. Croft & Braithwaite (2006) found that smut collected from the Ord River, Western Australia and Indonesia were identical, suggesting Indonesia was the likely source of the smut incursion in the Ord. This was an important finding because BSES was screening Australian varieties for resistance in Indonesia. During the early stages of the smut incursion in Queensland, an anomaly was observed in the Mackay district. On one of the first farms on which smut was found in Mackay, low levels of smut were found in the varieties Q205$^{\Phi}$ and Q170. These varieties were rated highly susceptible in the Indonesian screening trials and were showing heavy infection in the Bundaberg/Isis region. Other susceptible varieties on the farm were heavily infected. Bhuiyan *et al.* (2015b) found that smut spores collected from the Mackay farm produced significantly less infection in the varieties Q170, Q205$^{\Phi}$, Q174 and Q138 than spores collected from Bundaberg under controlled conditions. When smut spread more widely in Mackay, Q205$^{\Phi}$ and Q170 showed high levels of smut on many farms. Bhuiyan *et al.* (2015b) suggested that the anomaly in the reaction of Q205$^{\Phi}$ and Q170 on this farm may be due to a founder effect where the initial infection on this farm established from a small number of smut spores that varied genetically from the spores that established the infections in other areas. Over time, the smut genetic types would have spread between regions in Australia and the mixing would swamp the genetic variants that initially established on this Mackay farm.

Past research has shown that there are two mechanisms of resistance to smut, external resistance from chemical and/or physical barriers in the bud scales and internal resistance within the plant cells. Varieties with external resistance are resistant when setts are dipped in a spore suspension but behave like susceptible varieties if the external bud scales are damaged or the spores are injected into the bud. Varieties with internal resistance remain resistant even if spores are injected into the bud. Bhuiyan *et al.* (2013b) found that 59% of Australian resistant varieties exhibited external resistance and 41% internal resistance. Widely grown commercial varieties, KQ228$^{\Phi}$ and Q200$^{\Phi}$, have external resistance and Q183$^{\Phi}$ has internal resistance. Internal resistance is more stable and will not breakdown if buds are damaged during planting or in the growing crop but both forms of resistance have been found to be generally stable under field conditions. When investigating host-pathogen interactions or the genes controlling resistance, it is important to understand which type of resistance is present in the varieties or families being studied.

The mechanisms of both types of resistance have been the subject of many studies. External resistance is the combination of structural characteristics (i.e. physical barrier) (Waller 1970), bud phenylpropanoids and glycosyle-flavonoids (Lloyd &Naidoo 1983; Fontaniella *et al.* 2002; Millanes *et al.* 2005). Internal resistance is expressed when the external resistance barrier is breached and is governed by several defence responses including increase in lignin concentration (Santiago *et al.*

2012), production of glycoprotein, phytoalexins, salicylic acid and polyamines (Legaz *et al.* 1998; Borrás-Hidalgo *et al.* 2005; Blanch *et al.* 2007).

The genome of *S. scitamineum* has been sequenced by Taniguti *et al.* (2015). This has enabled comparisons with other smut fungi that have been more extensively studied and will assist with studies of the interaction of the fungus and the plant at a molecular level. Taniguti *et al.* (2015) investigated the interaction of smut and sugarcane and found that genes coding for plant cell wall degrading enzymes, proteases, lipases, chitin modification and lignin degradation enzymes, sugar transporters and transcriptional factors were differentially expressed during the interaction. The fungus also modulates transcription of genes that help protect the fungus from reactive oxygen species and other toxic metabolites produced by the plant.

The genes associated with the response to smut infection and the microscopic interaction between the fungus and the plant cells were studied by McNeil *et al.* (2018) (Aitken 2015, SRA project CPI026). They used global expression profiling with RNA-seq to investigate the genes involved in smut resistance. They concluded that the interaction was complex with many genes differentially expressed during the interaction. The expression of 13 differentially expressed genes with putative roles in smut resistance were confirmed by quantitative real-time reverse transcription PCR (qRT-PCR) analysis, and the results were consistent with the RNA-seq data. They identified one large effect QTL originating from a *S. spontaneum* hybrid that was different to QTLs for smut resistance found in existing commercial hybrids.

Overseas research on changes in gene expression in sugarcane in response to smut infection has been conducted by Bedre *et al.* (2019). They reported changes in expression of genes with putative functions in cell wall modification, transcriptional regulation, ROS homeostasis, and defence hormone signalling. Barnabas *et al.* (2017) studied the proteins expressed by the smut fungus *in vitro* in response to sugarcane tissue. They then examined the expression of these proteins in plants in response to smut infection and found five of the *in vitro* secreted proteins were expressed in distinct patterns by *S. scitamineum* during different stages of infection, with relatively higher expression at 1 day after inoculation. This suggests that these proteins could be aiding *S. scitamineum* early in the penetration and colonisation of sugarcane cells. Xuipeng *et al.* (2016) identified expressed sequence tags associated with smut infection up to 4 days after inoculation. They found that the response to infection was complex and involved many genes, some of which have been reported to be associated with resistance to pathogens in other crops.

The Australian studies, along with the growing overseas literature on the interaction of smut and sugarcane, could lead to the identification of resistance genes that can be converted into perfect markers for use in the breeding program. Sugarcane smut and the smuts infecting maize and sorghum are related and the research on corn and sorghum may provide leads to help identify genes for resistance in sugarcane (Wisser *et al.* 2006).

5.2.4 Marker-assisted breeding

Marker-assisted breeding is being implemented in breeding programs for a range of crops species with the aim of introducing the target genes into elite genomic backgrounds without associated genetic drag from unwanted traits (Cobb *et al.* 2019). Perfect markers are the actual genes controlling a trait. Perfect markers for disease resistance have been identified including a perfect marker for sugarcane brown rust resistance (Raboin *et al.* 2006, SRDC Project CTA046). BSES/SRA has invested in development of molecular markers since the 1990s. There have been many projects in this field with involvement of BSES/SRA, CSIRO, Southern Cross University and the International Consortium of Sugarcane Biotechnologists. Aitken *et al*. (2013) screened progeny from two crosses involving one parent that displayed internal resistance to smut and the progeny revealed 1:1

segregation, indicating a possible major gene for resistance. This suggests there may be major resistance genes that could be converted into markers.

A preliminary quality trait loci (QTL) study carried out by Raboin *et al.* (2001) on a small bi-parental population with a limited number of markers found four markers associated with smut resistance. Researchers from Japan filed a US patent for DNA sequences of markers for smut resistance in 2012 (Enoki *et al.* 2012). Aitken (2016, SRA project 2012/025) found many SNP markers that were highly correlated with smut resistance. Aitken & McNeil (2019) found that the markers for smut resistance had a greater effect than markers for resistance to Pachymetra root rot and root-knot nematodes and this probably reflects the fact that smut resistance in sugarcane is controlled by fewer genes than the other diseases. They found a large effect QTL in a cross between sugarcane and a *S. spontaneum* clone The QTL was inherited from the *S. spontaneum* parent and explained approximately 50% of the variation in this population This is the largest marker effect identified for smut resistance in Australia.

A SNP marker panel which included markers for smut was assessed for implementation and the results of a validation experiment of these SNPs for increased smut resistance demonstrated that the markers were ready for use in the breeding program (Aitken & McNeil 2019). The markers were identified through both RNA-seq gene expression analysis, association analysis in a set of breeding lines and through QTL analysis of an introgression population. The ability of this project to cross validate the SNP markers using the three different discovery methods increased confidence in the selected SNP markers. These SNP markers have been delivered to the SRA breeding group and are being used for early stage selection as part of project 2018/005 (Sun *et al.* 2019; Aitken *et al.* 2020).

The international effort to map the genome of sugarcane (Garsmeur *et al.* 2018;, SRDC/SRA projects CTA035, ICB008, 2010/019, 2013/030, 2015/027) is an important tool for further research to understand resistance to smut and other diseases and to identify and deploy markers for disease resistance. The markers that have already been discovered can be mapped to the sugarcane genome and improved markers with closer linkage to the actual resistance genes can be identified. In the future, the aim is to identify the resistance genes and determine their role in resistance. This is the goal of the marker and genomics work and will further increase the efficiency of markers in the breeding program. Investigation of resistance genes in introgression crosses will allow new resistance genes to be identified in wild germplasm that can be introduced into the breeding population to give even greater resistance or protect the industry from changes in the pathogen that might overcome existing resistance genes. Recent progress suggests that markers will a provide major step forward in breeding for disease resistance and yield.

5.2.5 Other management practices

Fungicides play a minor but important role in the management of smut. Maintaining smut at a low level in ASP plots is important for supply of disease-free seed. Hot-water treatment is used to eradicate or reduce smut in planting material, but overseas research suggested moderately resistant varieties become more susceptible to infection by spores in the soil when they are planted after hot-water treatment (Comstock 2000). Bhuiyan *et al.* (2015a,c) screened several fungicides for treatment of setts at planting to prevent reinfection from smut. The fungicide Sinker® (flutriafol) protected setts from reinfection and killed smut already established within the buds. This fungicide is taken up by the roots and, when applied in the soil around setts at planting, it protects the buds from infection and protects the young plants for a few months after planting. The fungicide has been registered for smut control and is recommended for use in ASP plots and for commercial plantings of moderately resistant varieties.

DNA assays conducted by SRA were used to confirm that the initial sample of smut was *S. scitamineum* (James *et al.* 2007). The SRA laboratory screened all subsequent samples at the request of Biosecurity Queensland to prove they were sugarcane smut during the emergency response phase of the incursion. The laboratory also screened disinfects and heat treatments to assist in the emergency response.

Recommendation: Monitor the success of implementation of markers for smut resistance by determining the increase in the proportion of smut resistance of clones after implementation compared with prior to implementation.

Recommendation: Determine the proportion of clones with each smut resistance marker to monitor whether the clones selected predominantly have one or different markers for resistance. This is important to see if markers are leading to clones with one or more resistance types.

Recommendation: Markers (resistance genes) discovered in introgression clones (derived from S. spontaneum and /or Erianthus), that are different to the resistance genes in core breeding population, should be introduced into the core breeding population to provide different sources of resistance that may be stacked into resistant varieties as an insurance and protection against changes in the pathogen that might overcome the existing resistance genes in the core program.

Recommendation: Continue research to identify "perfect" markers (key resistance genes) for smut resistance to improve our understanding of the resistance genes present in the breeding program and how these can be deployed to provide high level and sustainable resistance. Perfect markers would also increase the efficiency of implementation of markers in the breeding program and limit the risk that linkage between the marker and the trait is lost due to recombination within chromosomes.

Recommendation: Review the current program of screening for smut resistance in the light of the success of markers. The stage in the selection program where screening for disease resistance is conducted may be reassessed, if markers are successful at eliminating a large proportion of the susceptible clones.

5.3 Soil health

5.3.1 Pachymetra root rot

Background

Pachymetra root rot was discovered in the 1980s (Croft & Magarey 1984) and is caused by the oomycete *Pachymetra chaunorhiza* (Dick *et al*. 1989). Pachymetra root rot was first discovered in association with a condition known as northern poor root syndrome but was then recognised to occur in all regions of the Australian sugar industry (Croft & Magarey 2000). The disease has only been found in Australia. The oospores of *P. chaunorhiza* are relatively large, 40-60 µm in diameter, and have large characteristic blunt projections. The oospores are produced in large numbers in rotted roots and can easily be seen under low magnification with a microscope.

Pachymetra root rot causes a soft rot of the primary and secondary roots and yield losses have been estimated at up to 40% in susceptible varieties (Magarey 1994). The loss of the main anchor roots can lead to stool tipping and plants are dislodged during harvest. The soil picked up by the harvesters when plants are dislodged, or stools that have tipped from the ground, causes serious maintenance problems in the sugar mill. No biocides other than fumigants are effective at controlling pachymetra root rot and the *Pachymetra* oospores can survive in the soil for many years. Short-term fallows, as practiced in the sugar industry, have little effect on reducing the carryover of oospores from one crop to the next. Varieties resistant to pachymetra root rot were identified by

Croft (1989) and resistant varieties have reduced losses (Croft & Magarey 2000). Magarey (1989) developed a microscopic method to quantify populations of *Pachymetra* oospores in soil and the method has been used to advise growers on the level of resistance needed when selecting varieties for planting.

Shannon *et al.* (2019) estimated losses in the Tully district in 2018 at $3.5M. Similar losses could be expected in at least 10 other mill areas making losses in the order of $30-40M, even though varieties rated as intermediate in resistance dominate the industry.

Incidence and soil assay

The microscopic method developed by Magarey (1989) to quantify the characteristic oospores in soil is relatively labour intensive. Magarey (2018, SRA project 2016/047) developed a molecular DNA assay to quantify populations of *Pachymetra* and two species of nematodes in soil; this project is a collaborative project between SRA and SARDI Molecular Diagnostics Centre. SARDI has an established commercial laboratory that provides DNA assays for soil-borne pathogens (PREDICTA®B) and beneficial organisms in grain crops. They have a proprietary DNA extraction method from soils. Their method can extract DNA from 500 g of soil. Other available DNA soil extraction methods use small quantities of soil which introduce significant variability due sampling errors. The DNA assay for *Pachymetra* was highly significantly correlated with the microscopic assay but was less sensitive at detecting *Pachymetra* at low populations. Because the main purpose of the assay is to quantify populations into high, medium, or low categories, the DNA assay should provide an effective alternative to the microscopic assay. The DNA assay has the advantage that with the one DNA extraction, *Pachymetra* and two nematode species can be quantified, and the assay could be extended to other beneficial and harmful soil organisms. The cost of the new assay is likely to be higher than the microscopic assay, but the cost could be offset by testing for a range of soil organisms with the one DNA extraction.

SRA project 2018/009 aims to commercialise DNA assays in the sugar industry. The project is cooperating with other projects, 2017/005, 2019/903 and 2019/905, to extend the assay to quantify beneficial soil organisms.

Surveys have been conducted in many districts to monitor the populations of *Pachymetra* oospores and to assess the success of management programs. This information will help breeders and pathologists to determine the level of resistance required in the breeding program for each region (Magarey *et al.* 2003, 2004, 2006a, 2013, Holzberger *et al.* 2016, Shannon *et al.* 2019). Magarey *et al.* (2013) reported that yield losses of 18 to 64% could be expected in the fields surveyed from 1998 to 2010 in Mulgrave to Condong. Significant yield losses would be expected in over 50% of sites surveyed in Mulgrave, Proserpine, Babinda, Plane Creek and Mackay. The only exceptions were the Burdekin where none of the 33 fields surveyed in 2003 contained *Pachymetra* spores and the Tablelands where only one infected farm was reported in 1993.

Resistant varieties

The method used to screen varieties for resistance to pachymetra root rot has not basically changed since it was developed by Croft (1989). *Pachymetra* oospores are grown in the laboratory on artificial media and then mixed with potting mix. Plants of the test varieties are grown in the potting mix with the pots enclosed in a temperature-controlled bench in a glasshouse for 12 weeks. The root system is then rated for the extent of rot (Croft *et al.* 1998). The resistance ratings obtained with this method are highly correlated with field populations of oospores under varieties with different resistance and with yield loss (Magarey & Bull 2011, Jensen *et al.* 2019). Magarey *et al.* (2005)

showed that the breeding program for pachymetra root rot resistance has seen a reduction in the percentage of susceptible varieties coming through the program from 45%, before breeding for resistance commenced, to less than 20% after 10 years. This was achieved by restricting crosses to parents with a mid-parent rating of <6.5 (1-9 scale where 1 is highly resistant and 9 is highly susceptible). The limit is only applied to regions where pachymetra root rot is present.

Surveys have shown that *Pachymetra* oospore populations are high under varieties with intermediate resistance in some locations and that more highly resistant varieties are required to reduce losses. The smut incursion in 2006 resulted in the loss of several pachymetra root rot resistant varieties and a large increase in the percentage of varieties with intermediate resistance planted in the industry (Figure 1).

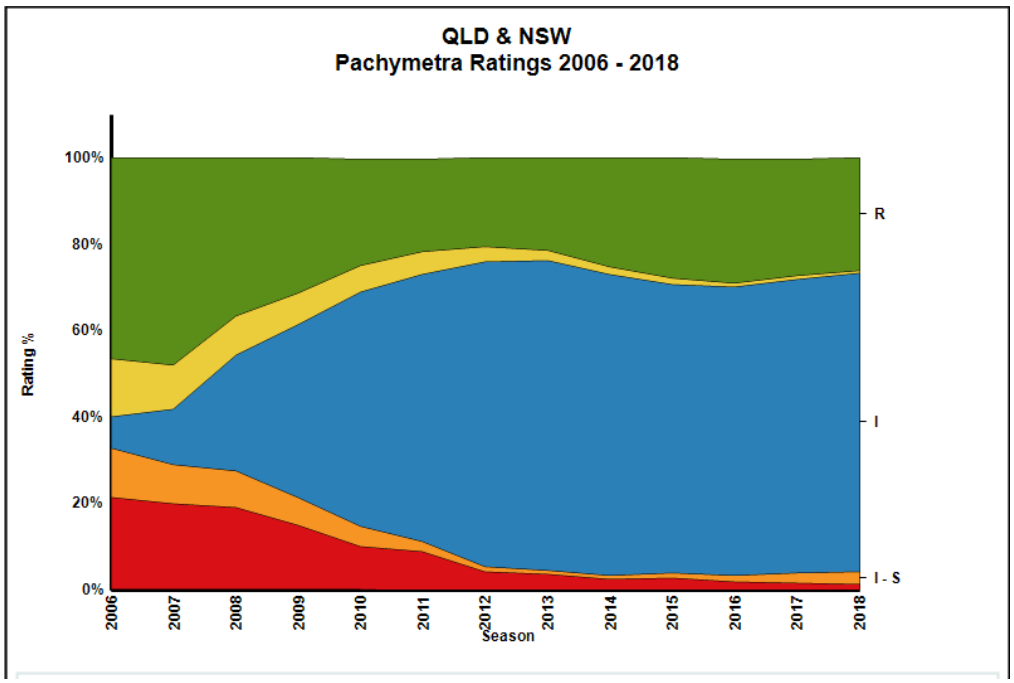

Figure 1. Percent of tonnes sourced from varieties of different pachymetra root rot resistance ratings in Australia, 2006-2018 (QCANESelect).

Bhuiyan *et al.* (2016a) compared *Pachymetra* oospore populations under 15 current commercial varieties in a field trial at Bundaberg. *Pachymetra* oospore populations were as high or higher under the intermediate resistant varieties Q208$^{\phi}$ and Q232$^{\phi}$ compared with susceptible varieties but the intermediate varieties gave significantly higher yield than susceptible varieties. Resistant varieties had oospore populations below levels predicted to cause yield loss and the resistant varieties generally gave the highest yields. Oospore populations under the intermediate varieties were 4 times higher than levels predicted to cause serious yield loss. This research showed the potential of resistant varieties to reduce losses from pachymetra root rot at sites conducive to the disease. Intermediate varieties can still suffer significant losses at these sites.

Stringer *et al.* (2016, SRA project 2014/054) used mill data to show that planting a pachymetra root rot susceptible variety following susceptible variety was associated with a 15% yield loss. Jensen *et al.* (2019, SRA funded PhD project PANPAC) estimated 21% yield loss in the intermediate resistant variety Q208$^{\phi}$ in plots with high *Pachymetra* oospore populations compared with plots with lower populations. The variety Q208$^{\phi}$ was planted over the replicated variety trial at the same site in Bundaberg as in the study by Bhuiyan *et al.* (2016a). There was no increased loss from planting

Q208$^{\phi}$ in two consecutive crop cycles compared with planting Q208$^{\phi}$ after other intermediate resistant varieties. Jensen *et al.* (2019) found no significant yield loss occurred in Q208$^{\phi}$ in similar experiments at sites in the Herbert and central region where *Pachymetra* oospore populations were below the oospore population threshold listed for losses in SRA Pachymetra root rot information Sheet IS13005. This research shows that significant yield losses from pachymetra root rot can occur in varieties with intermediate resistance and that high-yielding resistant varieties could improve yields on soils conducive to the disease. Resistant varieties would give growers more flexibility to rotate intermediate and resistant varieties to gain the yield benefits of some intermediate varieties while still reducing losses from the disease.

Introgression breeding

Magarey & Croft (1996) reported that 10 clones of *Erianthus arundinaceus,* a close relative of *Saccharum,* were highly resistant to pachymetra root rot and resistance was also present in some clones of *S. spontaneum.* Croft *et al.* (2015, SRA project 2011/344) summarised the resistance of clones derived from introgression breeding in Australia and China to all the major diseases, including pachymetra root rot. *Erianthus* back-cross 3 generation clones (back-crossed 3 times with true sugarcane) were significantly more resistant than clones in the core breeding program. For many years, SRA has been attempting to cross *Erianthus* with sugarcane to introduce valuable genes for resistance to pachymetra root rot from this species, as well as other valuable traits such as drought resistance (projects BSS008, BSS115 and projects funded by Australian Centre for International Agricultural Research and CRC for Sugar Industry Innovation through Biotechnology). The first true hybrids with *Erianthus* were produced by researchers in China. CSIRO and SRA researchers were able to prove that the crosses made in China produced true fertile hybrids and clones derived from these crosses were imported into Australia (Foreman *et al.* 2007). New introgression material with Chinese *S. spontaneum* clones were also imported. These potential new sources of valuable disease resistance genes have been further characterised and back-crossed with Australian high breeding-value parents in SRA projects 2013/358, 2014/053 and 2016/044.

PCR markers were developed for *Erianthus* and *S. spontaneum* chromosomes and QTL markers for pachymetra root rot resistance in introgression families by Piperidis (2017b, SRA project 2013/358). These molecular markers were exploited by Piperidis (2017a, SRA project 2014/053) to identify true hybrids carrying genes/chromosomes from the *Erianthus* parents. A major QTL marker for pachymetra root rot resistance was identified in a cross between the commercial variety ROC25 and a Chinese *S. spontaneum* clone that was inherited from the variety ROC25 and another QTL marker was identified from the *S. spontaneum* parent (Piperidis 2017b). These markers have been included in marker panels for use in the breeding program. The chromosome containing the large effect QTL from this cross has been identified and could be sequenced with the aim of identifying the pachymetra root rot resistance gene.

The high level of pachymetra root rot resistance of *Erianthus* clones originally collected in Indonesia was reported by Magarey & Croft (1996). Piperidis *et al.* (2019) reported an exciting breakthrough in utilising *Erianthus* clones from Indonesia. Previously, these clones had resisted all attempts to produce fertile off-spring when crossed with sugarcane. Fertile hybrids with the Indonesian *Erianthus* clones were produced by crossing the clones with parent clones that contain genes/chromosomes from the Chinese *Erianthus* hybrids. The new *Erianthus* hybrid families had a high percentage of pachymetra root rot resistant clones with half of the resistant clones showing no root rot. This work, along with the back-cross families from the Chinese *Erianthus* hybrids, offer an exciting new source of resistance to pachymetra root rot.

Marker-assisted breeding

Pachymetra root rot resistance was identified as one of the main targets for development of molecular markers by SRA because it is a high priority for the industry and the SRA breeding program. Aitken & McNeil (2019, SRA project 2015/025) aimed to identify and develop a low-cost SNP marker panel using markers identified in earlier projects (Aitken 2016, projects 2012/025, BSS358 and CPI025). The markers were converted to a high-throughput cost-effective marker system that could be incorporated directly into the early stages of the breeding program. The markers were tested to determine their conversion efficiency and then a series of experiments were carried out to validate the markers in new sugarcane populations to determine how useful they would be in the breeding program. Markers for resistance to smut, pachymetra root rot disease and root-knot nematode were converted to a SNP platform that can be implemented into the SRA breeding program. The markers will be used to increase the frequency of clones resistant to smut, pachymetra root rot and root rot nematodes in the early stages of the breeding program (McNeil *et al.* 2017). The integration of molecular markers, including markers for pachymetra root rot resistance, into the SRA plant breeding program is the aim of the current SRA project 2018/004.

Genomics, host-pathogen interaction and pathogen variation

Genetic variation in *Pachymetra* was first reported by McGhie (1998) with one genetic type found in districts north of Townsville and a second type in central and southern districts. McGhie found that the northern isolates were more pathogenic than the central and southern isolates. Heelan (2002) confirmed that there were two distinct groups and found a single nucleotide difference in the ITS region of the ribosomal DNA. Jensen (2019, SRA funded PhD project code PANPAC) screened a wider sample of isolates from NSW to northern Queensland and screened ITS, COX-2 and COX-2-1 genes and next generation sequencing (NGS) of selected isolates. Again, two distinct groups of isolates were found that conformed to the same geographic regions as the groups identified previously. Jensen (2019) found differences in pathogenicity between isolates but the differences were not related to the two genetic groups. The NGS identified more genetic differences between isolates and differences in putative pathogenicity genes. Further research is required to understand the importance of these differences to varietal resistance. Characterisation of the isolates used to screen varieties for resistance may help in reducing variability between screening trials.

Jensen (2019) identified several putative pathogenicity-related genes in *Pachymetra*, including crinkler effector, glycosyl hydrolases and pectate lyase genes. These are the first pathogenicity related genes reported in *Pachymetra.* These genes have been studied in other host-pathogen systems and may assist in identifying resistance genes in sugarcane.

Aitken & McNeil (2019) conducted experiments that investigated the genes involved in the interaction of *Pachymetra* and sugarcane roots. They found genes such as ILITYHIA, a myb transcription factor and a circadian clock-associated-1 gene that have been implicated in plant immunity. A selection of these putative resistance genes was converted into markers and screened for association with resistance in sugarcane varieties.

The genes activated by the interaction between the pathogen and the plant will assist future development of markers linked to resistance with the ultimate aim to identify “perfect” markers (the key genes that control resistance). This research has increased our understanding of the complex gene networks that are involved in the interaction between plant and pathogen and gene sequences are stored in the sugarcane DNA hub.

Aitken & McNeil (2019) have assembled a draft of the genome of *Pachymetra. Pachymetra* is an oomycete with few other close relatives. The genome sequence was used by Aitken and McNeil (2019) to distinguish between sugarcane and *Pachymetra* genes expressed during studies of the

interaction of the plant and pathogen. Jensen (2019) also used the draft genome sequence to identify potential pathogenicity genes. The full sequence of *Pachymetra* is close to completion and will be a valuable resource for future host-pathogen research.

Recommendation: Introgression breeding has the potential to give a major advance in breeding for Pachymetra resistance and support should be given to exploit the introgression crosses fully. Research should be supported to identify the Erianthus homology group/chromosomes that carry resistance to Pachymetra root rot and to develop rapid DNA tests to identify the genes in progeny from the hybrid crosses to expedite the introduction of the genes into elite sugarcane varieties.

Recommendation: Markers for pachymetra root rot resistance should be a high priority. Existing markers should be validated, and new markers identified. Markers should be implemented as a priority in the breeding program.

5.3.2 Nematodes

Background

Nematodes are microscopic, eel-shaped worms which live in all soils (SRA information sheet I31040). Plant-parasitic nematodes attack the roots of plants including sugarcane. There are many species of plant-parasitic nematodes, with the two most important pests of sugarcane being root-knot nematode *(Meloidogyne* spp.*)* and lesion nematode *(Pratylenchus zeae)*. Stunt nematode *(Tylenchorhynchus annulatus)*, dagger nematode *(Xiphinema spp.),* stubby root nematode *(Paratrichodorus minor),* reniform nematode *(Rotylenchulus spp.)* and spiral nematode *(Helicotylenchus dihestera)* also attack sugarcane. Most plant-parasitic nematodes have broad host ranges, for example, root-knot nematodes attack both broadleaf and grass species. In addition to plant-parasitic nematodes, there are many species of free-living nematodes that feed on bacteria, fungi and other soil organisms. High populations of free-living nematodes are a sign of a “healthy” soil, as they indicate high organic matter levels and high diversity of soil organisms.

Plant-parasitic nematodes were recognized as contributing to yield decline and were extensively studied within the SRDC and Queensland government funded projects within the Sugar Yield Decline Joint Venture (Garside 2000, 2006, SRDC projects YDV001, YDV002). Blair & Stirling (2007) obtained yield responses of 15% to repeated application of nematicides in plant crops and 12% in ratoons, indicating that nematodes are subtle but significant pests of sugarcane. Lesion nematode was the species most strongly correlated with yield losses. Blair & Stirling (2007) estimated that plant-parasitic nematodes conservatively cost the Australian sugar industry about AU$82M/year.

Incidence and soil assay

SRA and Biological Crop Protection have provided a nematode extraction and microscopic counting service to quantify the populations of nematodes in soil samples. A CD-ROM containing descriptions of plant parasitic nematodes common in sugarcane soils to aid research staff to identify nematode species was produced by Nobbs (2003, SRDC funded project SAI001).

Blair *et al.* (1999a,b) found that lesion nematode had high populations in all soil types and root-knot nematode had high populations in sandy soil types.

Magarey (2018, SRA project 2016/047) developed DNA soil assays for *Pachymetra chaunorhiza, Pratylenchus zeae* and *Meloidogyne* species for sugarcane soils in collaboration with SARDI. The nematode DNA assays were highly correlated with the microscopic methods and the current SRA project 2018/009 aims to commercialise the DNA assays. The project is cooperating with other

projects, including 2017/005, 2019/903 and 2019/905, to extend the assay to quantify beneficial soil organisms including beneficial free-living nematodes.

Management of nematodes

Several chemicals are registered for nematode control in sugarcane including Rugby® 100G, Nemacur® 100G (granules) and Nemacur® 400 (liquid) (SRA Information Sheet IS14027). These nematicides also kill natural nematode enemies, only reduce nematode populations for a short period of time and are not widely used. Halpin *et al.* (2015) and Jakins (2015, SRA project 2013/071) found that applications of nematicide reduced nematode numbers but did not increase yield.

The Sugar Yield Decline Joint Venture identified nematodes as a significant component of soil health and proposed a farming system that would lead to a reduction in the impact of nematodes and other harmful soil organisms (Garside 2000, 2006, SRDC projects YDV001 and YDV002). Three of the farm management practices that can contribute to suppressing nematode damage are minimal soil disturbance, conservation of plant residues (trash or green manure cover crops) and crop rotation. These practices were the basis of new projects aimed at refining and promoting the adoption of the new farming system.

Salter *et al.* (2010, SRDC project BSS286) found that addition of sugarcane trash and reduced tillage were associated with a reduction in lesion and root-knot nematode populations. Soybean residue added to the soil caused a rapid increase in the beneficial free-living nematodes.

Stirling *et al.* (2005) found that addition of soybean residue increased readily oxidisable carbon, microbial biomass, microbial activity and numbers of free-living nematodes. The soybean residue was associated with a reduction in lesion nematodes of 95% 24 weeks after sugarcane was planted. A bioassay showed that no suppression of nematodes occurred after 47 weeks. The amount of organic matter in soil (total C, total N, and labile C) from crop residues and the size of the free-living nematode community was found to be associated with the suppressiveness of soil to nematodes (Stirling *et al.* 2011). Nematode-trapping fungi were identified as one of the biological agents associated with suppression of nematodes. Soil amendment with mill mud/ash and compost produced from mill mud/ash, bagasse and wood waste reduced lesion nematode populations by 60% up to 2 years after application (Stirling *et al.* 2018b).

Pasteuria spp. are bacteria that cause a disease of nematodes. In a survey of all sugar production areas in Australia, *Pasteuria* spp. were detected in 56% of the fields sampled (Stirling *et al.* 2017). *Pasteuria* endospores were seen on root-knot nematode (*Meloidogyne* spp.), lesion nematode (*Pratylenchus zeae*), stunt nematode (*Tylenchorhynchus annulatus*) and spiral nematode (*Helicotylenchus dihystera*). At most sites, the infection was low, but two sites were identified that had high infection levels and *Pasteuria* spp. appeared to be contributing to suppression of root-knot and lesion nematodes. Bhuiyan *et al.* (2018b) showed that high endospore concentrations of *P. penetrans* reduce populations of root-knot nematode by 70-99% in the glasshouse. They found that *P. penetrans* was present at high populations in 3 of 126 sugarcane fields. Populations were higher in fields with controlled traffic and minimum tillage practices. Bhuiyan *et al.* (2017) found that a field soil from Bundaberg, naturally infested with *P. penetrans,* reduced root-knot nematode populations by >95% in glasshouse experiments and that there was a highly significant correlation between the population of *P. penetrans* and suppression of root-knot nematode. *Pasteuria* species are difficult to culture in the laboratory, but Syngenta has released a product CLARIVA™ containing *P. nishizawae* for control of cereal cyst nematodes. This breakthrough may open the way for other *Pasteuria* species to be cultured and released as biocontrol agents.

Stirling (2018, SRA project 2014/004) investigated the benefits of the new farming system on suppression of nematodes over several crop cycles. He found that, although nematode populations

were decreased and root health improved just below the trash blanket, nematode populations were high and root health was poor in the bulk of the soil. Attempts to increase suppressiveness of the bulk of the soil by addition of organic amendments was sometimes successful but the benefits obtained depended on the composition of the amendment, application rate and the method of application. This finding is typical of biological control in general where many complex interactions are involved in the success of biological control and our ability to manipulate or control these interactions is limited. This project shows that growers are being encouraged to adopt a farming system that only partially addresses the problem of plant-parasitic nematodes.

Resistant varieties

Little was known of the resistance of Australian varieties to nematodes before research by Stirling (2006). He found that no variety was completely resistant to root-knot nematode (*M. javanica*) but Q152 and Q158 supported much lower population densities than the other varieties tested. He concluded it would be technically feasible to breed for more resistant varieties. Ogden-Brown *et al.* (2010) reported that crosses between *Erianthus* and sugarcane showed some resistance to root-knot nematode, *M. javanica,* and lesion nematode, *P. zeae.*

New methods were developed to screen large numbers of sugarcane varieties for resistance to lesion and root-knot nematodes in the glasshouse (Croft 2016; Bhuiyan *et al.* 2014a, 2016b, SRA project 2011/344). These methods were used to screen more than 260 introgression clones and more than 30 commercial varieties. Seven commercial varieties were shown to have moderate resistance to root-knot or root lesion nematode and the variety CP84-1198 had moderate resistance to both nematode species. The wild sugarcane relatives, *Erianthus arundinaceus* and *S. spontaneum,* were highly resistant to root-knot and lesion nematodes. Some advanced backcross clones from the Chinese *E. arundinaceus* and *S. spontaneum* retain high levels of resistance to root-knot and lesion nematodes. The resistance present in the wild species tended to decrease with each successive backcross generation. The SRA plant breeding team is using the clones derived from the wild relatives to boost the resistance of varieties to nematodes. The project also found that a few commercial varieties have moderate resistance to root-knot and lesion nematodes.

A selection of the introgression clones that were shown to be resistant to nematodes in SRA project 2011/344 were planted into field trials in the Herbert, Mackay and Bundaberg/Isis (Piperidis 2017a, SRA project 2014/053) to confirm their resistance under field conditions and to determine if the level of resistance is sufficient to obtain a yield advantage. The nematode populations were manipulated at the trial sites by growing different fallow crops and by application of nematicides. Lesion nematode was the main nematode present in trials in the Herbert and Mackay. In these trials, a few introgression clones had fewer lesion nematodes than the commercial varieties, but the differences were not great. In the trial at Bundaberg, results showed that 7 of the 8 introgression clones consistently maintained lower numbers of root-knot nematode than the six commercial varieties (Bhuiyan *et al.* 2019). These results confirmed the results of the glasshouse resistance trials which rated these clones more resistant than commercial varieties. In Bundaberg, the commercial varieties Q208$^{\phi}$ and Q240$^{\phi}$ maintained high yield despite high numbers of root-knot nematode, suggesting that these varieties might be tolerant to this nematode. The introgression clones could not match the CCS in the commercial varieties and sugar yield per hectare was well below the commercial varieties. Further backcrossing is required to increase sugar content in the introgression lines.

Jakins (2015, SRA project 2013/071) compared nematode populations and yield of four commercial varieties. Q183$^{\phi}$ had five times more root-knot nematodes than Q245$^{\phi}$ and 20% lower yield. This project recommended SRA screen varieties for resistance to the two major nematode species attacking sugarcane.

The research conducted to date has shown some hope that varietal resistance could contribute to management of nematodes but much more work is required to breed nematode resistance into varieties with commercial potential.

Marker-assisted breeding

Molecular markers for root-knot nematode resistance in *Erianthus* and *S. spontaneum* breeding populations were identified by Piperidis (2017b, SRA project 2013/358). Molecular markers have been identified in both *Erianthus* and *S. spontaneum* backcross populations linked to root-knot nematode resistance. The markers require validation on a larger number of clones. The markers have been converted to high throughput markers in project 2015/025 to screen introgression clones within the SRA introgression program.

The genes expressed in response to attack by root-knot nematode have been investigated in two backcross *S. spontaneum* clones (McNeil *et al.* 2020). They identified numerous defence-related SNP markers differentially expressed in plants in response to root-knot nematode attack. These markers were validated in other clones of known resistance to root-knot nematode. The results indicate that there is potential for the SNP markers to select clones with increased resistance to root-knot nematode in the breeding program.

Recommendation: Existing projects aiming to increase the understanding and adoption of practices that improve soil health may lead to improved suppression of damage from nematodes and should be continued (see section on general soil health).

Recommendation: The commercialisation of DNA assays for plant parasitic nematodes should be continued and extended to include free-living beneficial nematodes and possibly bacterial pathogens of nematodes such as Pasteuria.

Recommendation: Introgression appears to offer the best chance of increasing the resistance of varieties to nematodes. Clones derived from the introgression program should be screened for nematode resistance and the resistant clones should be fed back into the program to be used as parents and assessed for yield potential.

Recommendation: Markers for nematode resistance should be used to increase the frequency of root-knot nematode resistance genes in the introgression breeding program.

5.3.3 General soil health

A healthy soil is one that promotes the optimum growth of the crop you are interested in growing. Stirling *et al.* (2018a, SRA project 2016/025) lists common characteristics of healthy soils: 1) a physical structure that will infiltrate water, drain readily and provide homes for the soil fauna that are partly responsible for disease suppression; 2) levels of soil organic matter that are high enough to stimulate biological nutrient cycling processes and improve the supply of nutrients to plants; 3) a large and diverse population of beneficial soil organisms to cycle nutrients, decompose organic matter, improve soil structure and suppress pathogens and pests. Stirling *et al.* (2018a) conducted master classes on soil health that were attended by 252 sugar growers, productivity services staff and others in 2017 and 2018. The classes provided information and practical demonstrations of the health of root systems, microbes that can affect root health and practices that can promote healthy soils.

Brackin *et al.* (2017) reviewed the current knowledge of soil biological health in other crops and in sugarcane and the latest methods for studying soil biology. They describe the complexity of soils and

the gaps in our knowledge of soil organisms. Soil biology is a rapidly growing area of research in crops and ecosystems and new molecular methods are increasing our understanding of the complexity of soils.

Soil microbiome is a name to describe the vast array of species that inhabit soils and interact with plants and each of other in a complex web. *Pachymetra* and plant-parasitic nematodes are part of the soil microbiome and have been discussed in detail above because they are two components of sugarcane soils that have been extensively studied.

There have been several projects that have investigated the soil ecosystem or microbiome to describe or identify organisms associated with healthy and unhealthy soils. Schmidt *et al.* (2010, SRDC project UQ043) conducted microbial functional analysis by treating soil as a "super organism" Most studies identify microbial taxa using DNA profiles, but this approach is biased towards known microbial taxa. They used molecular DNA analysis to look at expressed genes contributed by many organisms with emphasis on nitrogen metabolism. The expressed genes in soils from farms practicing the new farming systems were compared with soils from farms using traditional farming practices. Harvey (2018, SRA project 2016/025) used DNA sequencing and isolation to quantify differences in fungal communities between continuous sugarcane and cane-legume rotations. Differences in fungal species and/or genera were observed between treatments. Young *et al.* (2020) used DNA barcoding to study the species of free-living nematodes in sugarcane soils. They propose to use the molecular techniques to quantify free-living nematodes to aid the understanding of treatments applied to improve soil health.

The following projects focusing on soil health are currently in progress:

- 2017/005 Measuring soil health, setting benchmarks and driving practice change in the sugar industry
- 2018/008 Establishing sugarcane farming systems to improve soil health.
- 2018/009 Development of commercial molecular biological assays for improved sugarcane soil health and productivity.
- 2018/003 Implementation of root systems diagnostics to deliver a field-based measure for root health.
- 2019/903 Australian sugar industry soil health benchmarking in the Central region of Qld - increasing profit and transforming soil health practices through competitive industry research, extension and adoption.
- 2019/904 Australian sugarcane industry soil health benchmarking in the Wet Tropics region of QLD – increasing profit and transforming soil health practices through cooperative industry research, extension and adoption.

The extensive research portfolio on soil health highlights its importance and the industry interest in this subject. Research on known pathogens such as *Pachymetra* and nematodes is likely to provide advances that can readily be adopted by industry. General farming system research may lead to improved soil health and improved yield, but the proposed farming systems have not been widely adopted by industry. There are limited options for crop rotation in sugarcane and rotations longer than the current practice of 6-10 month fallow between sugarcane crop cycles can impact on supply of cane to sugar mills.

Our understanding of the complex soil microbiome is still in its infancy. It will be many years before this research will provide outcomes that can be adopted by growers. There is limited evidence that the farming system currently being promoted improves root health and increases growers' financial returns. The new farming systems can improve moisture and nutrient retention, suppress weeds and improve soil structure but there is no clear evidence they improve root health. Salter *et al.* (2010, SRDC project BSS286) reported variable responses in yield to components of the new farming system. Stirling (2018) found that farms where the new farming system has been practiced for

several crop cycles still have poor root systems and high nematode populations. Bell *et al.* (2006) reported that the new farming system had little effect on *Pachymetra* populations. Croft & Saunders (1996) showed that planting directly in the row of the previous crop with minimum tillage resulted in the new crop being planted in the highest concentration of *Pachymetra* oospores and caused significant yield loss compared with planting in the interspace of the previous crop where oospore populations were much lower. Jensen (2019) showed that cultivating soil diluted *Pachymetra* oospores by 60-80% compared to the population in the row of the previous crop. These papers show that the new farming system is not a panacea that will overcome all soil health issues. Until the new farming systems can clearly demonstrate tangible benefits to growers, adoption is likely to continue to be slow. Varieties with resistance to pachymetra root rot have clearly demonstrated that they can improve root health, yield, and reduce populations of this soil-borne pathogen. Varietal resistance to nematodes is also showing promise. The genetics of the sugarcane crop has a direct impact on root health ("soil health") and is readily adopted by growers. New farming systems will contribute to soil health but varieties resistant to the known soil-borne pathogens are likely to give the greatest improvement to root health and financial returns to the industry.

5.4 Leaf scald

5.4.1 Background

Leaf scald is caused by the bacterium *Xanthomonas albilineans* and is present in most overseas countries and in all areas of the Australian sugar industry (Rott & Davis 2000; Croft *et al.* 2000). The bacterium lives in the xylem cells and is spread by wind-blown rain, infected planting material and by contaminated harvesters and planting machines. Leaf scald can infect a range of grass species that are common in sugarcane areas and often reappears after many years when a susceptible variety is planted, presumably re-entering the sugarcane crop from infected grasses. Leaf scald causes a white line on the leaves, chlorosis of patches on the leaves, burning or scalding of the leaf tips, side-shooting of buds on the stalk and, in some cases, sudden death of whole plants.

Leaf scald is currently rare in commercial crops because nearly all varieties have adequate levels of resistance (Magarey 2006a). The disease is regularly found in susceptible clones in the early stages of the breeding program on SRA experiment stations. The disease currently causes no direct economic losses but there are indirect losses due to loss of susceptible varieties and impacts on genetic gains in the breeding program.

5.4.2 Diagnosis

Diagnosis of leaf scald is important in post-entry quarantine of introduced varieties because variation in pathogenicity has been reported in *X. albilineans* in overseas countries. Australia needs to prevent introduction of any new strains of the pathogen. James (2000, SRDC project BSS187) and James *et al.* (2004) compared several diagnostic methods for detecting *X. albilineans* in post-entry quarantine and found the most sensitive method was the bio-PCR developed by Wang *et al.* (1999).

Duarte Dias *et al.* (2018) compared loop-mediated isothermal amplification (LAMP) assay and nested PCR but these assays still failed to detect some infections. They recommended a cultural step as used in bio-PCR to improve detection from plants. Australian Government Department of Agriculture, Water and the Environment as part of its Rural R&D for Profit program has funded a project (ST16010) which aims to develop a next generation DNA sequencing (NGS) toolkit for disease threats including leaf scald. The research is still in progress.

5.4.3 Resistant varieties

The SRA breeding program does not make crosses between leaf scald susceptible parents and only about 10% of clones coming through the selection program are rated as susceptible to leaf scald. All clones in the advanced stages of the SRA breeding program are screened for resistance and susceptible clones are discarded (Magarey *et al.* 2005). The methods used to screen clones for resistance have remained the same for many years. Stalks of 4-month-old plants are decapitated and painted or sprayed with leaf scald-infected juice. The plants are then allowed to grow for another 9-10 months before they are inspected. Croft & Greet (2017) investigated inoculating setts at planting to shorten the time required to obtain leaf scald resistance ratings. They found this method was reasonably successful with highly susceptible varieties showing symptoms after a few months, but in some years moderately susceptible varieties did not show symptoms until 12 months.

Gutierrez *et al.* (2016) tested qPCR to quantify bacterial populations in varieties 8 weeks after inoculation. They found that qPCR can provided an improved method to evaluate resistance to leaf scald in sugarcane, however, multiple experiments were needed to accurately determine a variety's resistance.

5.4.4 Genomics, host-pathogen interaction, pathogen variation and GM sugarcane

Birch & Patil (1983, 1985, 1987) described a toxin produced by *X. albilineans* that they called albicidin, and its role in causing the chlorosis symptom in leaf scald-infected plants. Extensive research was conducted to understand the role of albicidin as a pathogenicity factor in sugarcane and its biosynthesis. Birch (2001, SRDC project UQ010) produced genetically transformed plants of susceptible varieties with resistance to albicidin and leaf scald. The plants were transformed with a gene from a different bacterium that blocked the activity of albicidin and the transformed plants of the susceptible variety were resistant to the disease. The GM resistant plants have not been commercialized. This resistance gene is available for production of GM resistant varieties if the sugar industry changes its policy on GM sugarcane. Because there are high levels of resistance to leaf scald in the SRA breeding program, the cost-benefit of GM would have to be assessed relative to conventional breeding for resistance.

Mensi *et al.* (2016, 2017) studied the attachment of *X. albilineans* to the sugarcane leaf surface. They found that surface polysaccharides are involved in attachment and survival of the bacterium on the leaf surface and they developed a bioassay to compare varieties for suitability to attachment of the bacterium. Attachment to the leaf is the first stage of the infection process and may be a key determinant of resistance to spread by wind-blown rain.

There have been several studies of the molecular interaction of *X. albilineans* and sugarcane. Santiago *et al.* (2009) examined the response of leaf discs exposed to elicitor proteins from cultures of *X. albilineans.* They found increases in phenolic acids, phenylalanine ammonia-lyase and peroxidase. Ntambo *et al.* (2019) used comparative transcriptome analysis with the RNA-seq platform to compare reaction to *X. albilineans* in resistant (LCP 85-384) and susceptible (ROC20) sugarcane cultivars. They identified over 100,000 differentially expressed genes. The relative expression levels of 10 differentially expressed genes involved in plant hormone production were studied in more detail and it was found that plant hormone (auxin and ethylene) signal transduction pathways played an essential role in infection.

Rott *et al.* (2011) identified potential pathogenicity factors produced by *X. albilineans* by transposon mutagenesis.

Zhang *et al.* (2020) reported the sequencing of the complete genome of *X. albilineans* and the sequence allowed detailed comparison of accessions of the bacterium from different countries,

highlighting variations between isolates. This genome will greatly assist future investigations of the interaction between *X. albilineans* and sugarcane.

5.4.5 Marker-assisted breeding

Markers for leaf scald resistance were investigated using the association mapping population and 39 SNP markers that were highly significantly associated with leaf scald resistance were identified (Aitken 2016, SRA project 2012/025) but these markers have not been verified in other populations. Markers for leaf scald resistance have been given a lower priority than markers for smut and pachymetra root rot but should be reassessed once markers have for smut and pachymetra root rot have been implemented in the breeding program.

Gutierrez *et al.* (2018) identified eight QTL markers associated with leaf scald resistance in a biparental cross between a resistant and a susceptible parent. Comparative genomic analysis with *Sorghum bicolor* allowed them to pinpoint the location of three major effect SNP markers on the sugarcane genetic map.

5.4.6 Other management practices

Approved seed and plant source inspections plays an important role in preventing spread of leaf scald within districts and are particularly important for varieties with moderate resistance. The risk of leaf scald in planting material can be reduced by soaking the cane for 40 hr in water at ambient temperature followed by heat treatment at 50°C for 3 hr (Croft & Cox 2013). Approved seed plots supply growers with a source of disease-free seed cane.

Disinfectants used to prevent spread of RSD are also effective for leaf scald.

Recommendation: Leaf scald should be the next target for molecular markers after markers for smut and pachymetra root rot are integrated into the breeding program. Existing markers identified in earlier projects should be validated and markers identified in overseas research should be evaluated in Australian germplasm. Marker discovery projects should be considered to identify new markers for resistance to leaf scald.

5.5 Fiji leaf gall

5.5.1 Background and current status

Fiji leaf gall, caused by the virus *Fiji disease virus,* was responsible for a major epidemic during the 1970s in southern Queensland and NSW. In the early 1980s, the disease spread for the first time to central Queensland (Egan *et al.* 1989). Fiji leaf gall has never been recorded in commercial crops north of Proserpine.

The disease is spread in Australia by the planthopper *Perkinsiella saccharicida* and by planting infected planting material. The virus infects and multiplies in *P. saccharicida* and the infected planthoppers can transmit the virus throughout their lives. Fiji leaf gall is characterized by the formation of leaf galls on the underside of the leaves. It causes distortion and shortening of the young leaves, giving the top a bitten-off appearance. Plants become severely stunted and have profuse tillering.

The epidemic in Bundaberg/Isis coincided with an explosion in the populations of the insect vector that resulted in rapid spread of the disease (Egan *et al.* 1989). The moderately susceptible variety NCo310 accounted for 90% of the crop in Bundaberg and 80% of the crop in central Queensland.

NCo310 was susceptible to Fiji leaf gall and highly favourable to the insect vector. The epidemic was so severe that even fields that were planted with disease-free planting material of NCo310 were ploughed out after first ratoon to prevent further losses and spread of the disease. During the 1980s, planting of NCo310 was banned and growers were forced to plant highly resistant but poorer yielding varieties such as Q110. The control program was successful and by the end of the 1980s there was little, if any, Fiji leaf gall in the Bundaberg/Isis region (Magarey *et al.* 2019). In the central region, Fiji leaf gall spread to 179 farms, but growers replaced the susceptible variety NCo310 before the disease caused any serious losses. The last known field to have infected plants in the central region was ploughed out in 1996. Plots of susceptible varieties were planted throughout the Mackay district in 2003 in a sentinel crop program and this program continued until recently. The plots were inspected for Fiji leaf gall and no Fiji leaf gall was found. Magarey *et al.* (2019) calculated that the probability that Fiji leaf gall was still present in the central region was extremely low and proposed that the area be declared free of the disease. Area freedom will mean growers will have access to a wider range of varieties and plant breeding can make greater genetic gains without the restrictions imposed by breeding for resistance to the disease. The last report of Fiji leaf gall in Queensland was in 2012 in the Rocky Point mill area and the last report in New South Wales was around the same time (Magarey pers. comm.). SRA and local industry staff have conducted surveys in the last known infested farms in southern Queensland and New South Wales in recent years, but no Fiji leaf gall has been detected. Potentially, if the southern and New South Wales regions are found to be free of the disease, they can also gain the benefits of access to a wider range of varieties and greater genetic gains in the breeding program. Fiji leaf gall resistant trials are conducted at SRA Woodford. If Australia is to completely eradicate Fiji leaf gall, SRA will have to stop conducting Fiji leaf gall screening trials at Woodford.

5.5.2 Diagnosis

Visual diagnosis of Fiji leaf gall relies on inspection for the characteristic gall on the underside of leaves. In some cases, these galls are difficult to see and can take from 6 to 12 months after infection before they develop. Strict quarantine on movement of cane between the Fiji leaf gall infested districts to districts north of Proserpine has been practiced. This has meant that SRA has quarantined clones before sending from central and southern districts to SRA Meringa for use as parents and for assessment for release in the northern districts. Cane varieties imported from countries such as Papua New Guinea and Fiji where Fiji leaf gall is present are also screened for this disease.

Smith & van de Velde (1994) developed a RT-PCR assay for Fiji leaf gall which has been used in domestic and international quarantine for many years. Clones being sent from the central, southern and NSW regions to SRA Meringa and imported from countries where Fiji leaf gall is present are screened (James 2000, James *et al.* 2004, SRDC project BSS187). Thompson & Wilson (2017) described the use of this assay, combined with tissue-culture production of plantlets of clones, to reduce the quarantine period from 12-18 months to 3-4 months.

5.5.3 Resistant varieties

At the peak of the Fiji leaf gall epidemic, new varieties were screened for resistance in plant breeding trials in Bundaberg by relying on natural spread from surrounding infested farms. Varieties were also screened in specific field trials at the BSES Pathology Farm where test varieties were planted between spreader rows of infected cane. The SRA Pathology Farm at Woodford is situated in an area isolated from commercial crops to reduce the risk of Fiji leaf gall spreading to commercial farms. During the 1990s, the level of disease in the Pathology Farm trials decreased and the trials became unreliable (Croft *et al.* 2004b). Low numbers of the planthopper vector were responsible for the declining spread of the disease. A new method of screening clones for resistance was developed

where insect vectors that are bred on infected plants are allowed to feed on the test clones for two weeks in the glasshouse and then are transplanted into the field (Croft *et al.* 2004b, Croft 2005, SRDC project BSS255). The plants are inspected for symptoms of the disease 5-6 months after transplanting to the field. The incidence and severity of symptoms are recorded, and the clones are rated for resistance based on a combination of these measures relative to a set of standard varieties. This method continues to be used in the SRA breeding program.

Magarey *et al.* (2005) reported that about 20% of clones screened for Fiji leaf gall resistance are rated as susceptible and would not be released in central, southern or NSW regions. In 2015, the central region relaxed its restriction on susceptible varieties and now releases some susceptible varieties. Sixteen of the 85 varieties currently recommended in Australia (19%) are rated susceptible and are not recommended in southern and NSW regions.

An indirect method of rating varieties for resistance to Fiji leaf gall using NIR spectroscopy to identify spectral properties of sugarcane leaves related to resistance was investigated by Purcell *et al.* (2005). They developed a model based on the 10 varieties used as standards in the conventional Fiji leaf gall resistance trials that could explain 90% of the variation in resistance. This work has not been validated in a broader sample of clones.

5.5.4 Genomics, host-pathogen interactions and GM sugarcane

The *Fiji disease virus* has 10 segments of double stranded RNA (dsRNA). The 10 segments have been sequenced (Smith *et al.* 1998 – SRDC project BS86S; McQualter 2003 – SRDC project STU025; Harding *et al*. 2006, McQualter *et al.* 2003, 2004b). McQualter (2004a) described the production of plants transformed by introduction of a gene sequence derived from the *Fiji disease virus* genome. The plants were more resistant to the disease than the original variety. The GM plants have not been commercialized.

Jiang *et al.* (2008) compared the variation in 2 of the 10 dsRNA segments of the *Fiji disease virus* genome in 25 collections of *Fiji disease virus* from Malaysia, Papua New Guinea and Australia. There was less than 2% sequence variation within the 12 accessions from Australia. The Malaysian accessions had up to 15% sequence variation to the Australian and PNG accessions. Five of the 10 accessions from PNG were similar to the Australian accessions but 5 were up to 7% different. The variation in genetic sequence among *Fiji disease virus* in different countries highlights the need for strict quarantine to prevent new strains of the virus entering Australia.

Ridley *et al.* (2006a, SRDC project BSS255 PhD student) examined the variation in *P. saccharicida* collected from different regions within Australia and two other species, *P. thompsoni* from Western Australia and *P. vitiensis* from Fiji. The internal transcribed spacer (ITS2) sequences of *P. thompsoni* and *P. vitiensis* were easily distinguishable from those of *P. saccharicida.* There was no significant sequence variation among the 26 *P. saccharicida* populations from within Australia. Reciprocal crosses of a northern Queensland and a southern Queensland population of *P. saccharicida* were fertile, so they can be considered conspecific. Single vector transmission experiments showed that a population of *P. saccharicida* from northern Queensland had a higher vector competency than two southern Queensland populations. These results showed variation in the planthoppers was not responsible for the absence of Fiji leaf gall in northern Queensland and that if there was a disease incursion into northern Queensland the vector is more than capable of spreading the disease.

Candy *et al.* (2001) examined the genes expressed by sugarcane in response to infection with *Fiji disease virus.* They found a range of stress related genes were upregulated.

Hughes *et al.* (2008) described a laboratory test to determine the inoculation potential of planthoppers. Planthoppers were fed on leaf segments embedded in agarose within plastic containers and the presence of virus within the leaf segment was determined by RT-PCR.

Dhileepan *et al.* (2006) (Ridley 2005; Croft 2005; SRDC project BSS255 with funding from CRC-Tropical Plant Pathology) found that the incidence of virus infection in *P. saccharicida* was much higher when the planthoppers were bred on infected variety NCo310 (50-90% infection) compared to those that were bred on plants of the tolerant variety WD1 (10%). WD1 had been used in spreader rows in field trials during the 1990s and the low level of infectious planthoppers obtained on this variety may have partially accounted for the poor success of trials during this period. Ridley (2006b) found that galls contained 200 times more virions than non-gall tissue and that the acquisition of the virus by planthoppers was related to the percentage of leaf area affected by galls but not to the concentration of virus in leaves of different varieties.

Dhileepan & Croft (2003) found that there was no clear correlation between planthopper preference for varieties susceptible to Fiji leaf gall compared to resistant varieties in the glasshouse. Populations of the planthoppers in the field on varieties differing in susceptibility were correlated to resistance (Dhileepan *et al.* 2003). Populations were low in the field on all varieties in this study.

5.5.5 Other management practices

Quarantine to prevent the spread of Fiji leaf gall between districts has been an important and successful management practice (see section on diagnosis). Approved seed and plant source inspections have also played a role in preventing spread of the disease within districts.

Management of the vector is discussed in the pest section of the report.

Recommendation: Surveys to establish whether Fiji leaf gall is still present in commercial crops in Australia should be a high priority. If Australia can be declared free of Fiji leaf gall in commercial sugarcane crops, there would be a number of significant benefits including:

- ***Wider access to existing varieties in central, southern Queensland and NSW***
- ***Improved genetic gains in the breeding program as restrictions on use of parents and loss of susceptible clones due to Fiji leaf gall in the selection program would no longer occur***
- ***Reduced costs to SRA as screening for Fiji leaf gall at Woodford is scaled back or discontinued. SRA would need to reassess the need for Fiji leaf gall resistance screening at Woodford and decide whether to eradicate Fiji leaf gall from Woodford to eliminate the risk of spread from Woodford to commercial crops***
- ***Reduced costs to SRA if quarantine for clones moving from central and southern regions to the north are relaxed***

5.6 Chlorotic streak

5.6.1 Background

Chlorotic streak is a disease that occurs in all regions of the Australian sugar industry and many overseas countries (Magarey & Egan 2000). The disease is associated with poorly drained soils or areas subject to flooding. The disease is spread by planting infected planting material and in soil water, flood water and recycled irrigation water. Chlorotic streak is reported to infect a wide range of common grasses found in sugarcane fields. The symptoms of the disease are irregular white streaks on the leaves and red streaks in the vascular tissues within stalks. Chlorotic streak causes losses between 10 and 60%. Higher losses occur when fields are planted with infected planting material (Magarey 2002, SRDC project BSS243). Infected setts can be cured by hot-water treatment

at 50°C for 30 min. Chlorotic streak was the most widely distributed disease in Queensland from 1980 to 2002 (Magarey 2006a). Incidence of the disease increases in years with above average rainfall (Young & Ensbey 2015).

Varieties vary in resistance to chlorotic streak but currently there is no standard method of rating varieties for resistance. Magarey (2002, SRDC project BSS343) and Magarey *et al.* (2006b) describe field and glasshouse hydroponic trials to rate varieties for resistance to chlorotic streak. The field trials provided comparisons of the resistance of varieties, but the trials were slow, taking at least two years to obtain ratings and were dependent on weather. The hydroponic glasshouse method was not successful. Young *et al.* (2013) rated varieties for resistance in Harwood based on natural spread into seedbed plots.

5.6.2 Causal agent and diagnosis

The cause of chlorotic streak was a mystery despite extensive research efforts during the 1960s. The disease was presumed to be caused by a virus because no causal agent could be found. Two PhD students failed to identify viruses associated with the disease using electron microscopy and nucleic acid technologies (Rogers *et al.* 2001; Magarey 2002).

Braithwaite & Croft (2013) investigated virological and bacterial molecular technologies to identify the cause of chlorotic streak but like previous studies no viruses or bacteria were associated with the disease. They then tested a wide range of 'universal' fungal and oomycete PCR primers and a primer set was identified that generated a DNA fragment highly correlated with chlorotic streak. The primers were initially designed to detect oomycetes that cause downy mildew. The primers detected the actin gene which is highly conserved across all organisms (viruses do not have actin genes). This provided the first firm evidence that the causal agent was not a virus. Unfortunately, because the actin gene is highly conserved, searches of the DNA databases using the chlorotic streak sequence matched a wide range of organisms.

The primers were highly specific to chlorotic streak infected sugarcane extracts and provided the first diagnostic assay for the disease. The assay has been used for further research and for diagnosis of chlorotic streak in industry samples and quarantine.

The new diagnostic assay identified xylem extracts as the best plant sample for further investigation of the cause of chlorotic streak. Ngo *et al.* (2018) (Braithwaite 2017, SRA project 2013/357) used metagenomic high-throughput sequencing to identify ribosomal and nuclear gene sequences strongly associated with chlorotic streak infected plants. The sequences had homology to protozoans in the phylum Cercomonodida but did not match any known species. They were then able to isolate an organism that was morphologically similar to Cercozoa and the DNA of the pure cultures matched the specific sequences identified in infected plants. This was the first report of a Cercomonodida causing a plant disease and the first Cercozoan that could be grown in a simple culture medium. The organism was most closely related to the genus *Cercomonas* and was given the name *Phytocercomonas venanatans.* Braithwaite *et al.* (2018) proved that the new species, *P. venanatans,* is the cause of chlorotic streak by inoculating healthy plants with pure cultures of the organism, reproducing the symptoms of the disease and re-isolating the organism from the inoculated plants. The pure cultures reproduced the symptoms of the disease when they were injected into the roots, leaves and stalks of plants. The organism moved from the roots to the above ground stalks and from the leaves to the stalks. The organism has two flagella and can swim through water, explaining the association of the disease with flooded fields.

Other plant pathogenic protozoans include *Plasmodiophora brassicae*, the causal agent of club root in brassicas, *Spongospora subterranea*, which causes powdery scab of potato, and *Polymyxa*

graminis, which infects grasses and is a vector of several viruses. These species are not closely related to *P. venanatans* and are restricted to the underground parts of the plant.

The ribosomal genes were used to develop a more sensitive and specific PCR assay (Ngo *et al.* 2018). This assay could be used to screen plant sources for the disease using the same samples used for RSD diagnosis.

The basic biology of *P. venanatans* and its interaction with sugarcane is still largely unknown. A better understanding of the biology of the organism could help in the development of methods to screen varieties for resistance and is of significant scientific interest. Research on basic biology and interaction of *P. venanatans* and sugarcane would be an excellent student project as it covers all aspects of plant pathology and all findings would be new to science.

5.6.3 Resistant varieties

Before the discovery of the organism that causes chlorotic streak, attempts were made to develop a hydroponic method of screening varieties for resistance to chlorotic streak using infected plants planted in the hydroponic trays with the test varieties (Braithwaite 2017). The method did not give reliable results.

Ngo *et al.* (2019) showed that inoculating varieties with a pure culture of *P. venanatans* through roots or setts could be used to compare varieties for resistance to the disease. A new project, 2017/010, titled "Delivering solutions for chlorotic streak disease" aimed to develop methods to screen varieties for resistance but was terminated due to budget issues. Growers in flood prone and wet environments value knowledge on chlorotic streak resistance of varieties so they can avoid planting highly susceptible varieties in these environments.

Recommendation: Methods for screening varieties for resistance to chlorotic streak should be developed using the new knowledge of the causal organism.

Recommendation: SRA should consider funding student projects investigating the biology of P. venanatans and sugarcane. This would be an opportunity to train future plant pathologists.

5.7 Sugarcane mosaic

Mosaic caused by *sugarcane mosaic virus* (SCMV, *Potyviridae*) is found in the Bundaberg/Isis region and Nambour district (Magarey 2006a). In recent years, the incidence has been low in these districts. The virus is spread by a range of aphid species and in infected planting material. Mosaic, as the name suggests, causes a pattern of contrasting shades of green on the leaf blade (Grisham 2000). Mosaic causes losses of 10-20% and, although the losses are not as severe as some other diseases, the rapid spread under the right conditions can mean that losses are experienced over large areas. Mosaic can infect a range of grasses including sorghum and corn. The disease is spread by a range of species of aphids and in infected planting material. Diagnosis of mosaic is by a commercial serological assay that can detect many strains, and a specific RT-PCR assays (Smith & Van de Velde 1994, Thompson *et al.* 2012, Thompson & Wilson 2017).

Modern hybrid sugarcane varieties are generally much more resistant to mosaic than the original *S. officinarum* varieties. Varieties are rated for resistance to mosaic at the last stage of the SRA selection program. The Bundaberg/Isis industry is consulted on release of any variety that is rated susceptible. In other regions, where mosaic is absent, susceptible varieties are released. Nine of the 85 varieties recommended for planting in Australia are rated susceptible to mosaic.

Clones moving from southern to northern districts are screened for mosaic by RT-PCR (Smith & van der Velde 1994, Thompson & Wilson 2017) while in quarantine.

Mosaic was used as a model system for development of transgenic sugarcane using a gene derived from the coat protein of the virus (Smith 1997, SRDC project BS94S). Plants of a susceptible variety were transformed with the virus gene and were resistant in glasshouse and field trials. This proof-of-concept project proved that transformation of sugarcane for disease resistance was possible.

5.8 Brown rust

There are two species of the genus *Puccinia* that cause rust of sugarcane in Australia.

Brown rust, caused by *P. melanocephala,* was first recorded in Australia in 1978 (Croft *et al.* 2000). The symptoms of the disease are small brown elongated pustules on the leaf surface. The pustules disrupt the normal water management of the plant, allowing moisture to escape and increasing moisture stress. The spores can spread long distances in the wind. The disease is favoured by cool nights with a light dew and is most severe in spring (Taylor *et al.* 1986). Heavy rain and warmer temperatures during the wet season lead to a decrease in brown rust.

In 1978, several varieties were susceptible, including Q90, which accounted for 90% of the crop from Tully to Mossman. The disease spread throughout Queensland and NSW within the first twelve months after it was detected. Taylor *et al.* (1986) measured yield losses of up to 18% in tonnes sugar/ha in Q90 and 31% in the highly susceptible variety, Q105, in Mourilyan. Brown rust continues to affect moderately resistant varieties (Magarey 2006a) but losses are not great.

Management of brown rust is primarily through resistant varieties. Natural selection for rust resistance occurs in plant breeding trials and this, combined with observations made by plant breeding technicians during the 12-14 years a clone is assessed in the breeding program, has prevented the release of highly susceptible varieties.

Hoy *et al.* (2016) reported that brown rust can overcome the resistance of varieties and that 9 of the last 12 varieties released in Louisiana have become susceptible to brown rust while under cultivation. Comstock *et al.* (2010) reported breakdown in resistance in four commercial varieties in Florida. There have been no confirmed reports of changes in races of brown rust in Australia. The severity of brown rust in moderately susceptible varieties varies from year to year but there is no evidence of changes in rust reaction (Taylor 1992).

Two genes that control resistance to brown rust, *Bru*1 and *Bru*2, have been identified and the location of the *Bru*1 gene on the sugarcane genome has been mapped (Daugrois *et al.* 1996; Raboin *et al.* 2006; SRDC Projects ICB004, ICB006 & ICB009, CTA046). A molecular marker for this gene has been developed and has been used extensively in overseas countries to determine the proportion of clones carrying this gene in breeding programs (Costet *et al.* 2012; Glynn *et al.* 2013). Breeding programs in some countries have unknowingly selected for *Bru*1 and a high proportion of clones have this gene. Brown rust resistance from *Bru*1 has been durable and effective in many countries. Louisiana and Argentina, which have experienced recent breakdown in brown rust resistance in commercial varieties, have a low frequency of *Bru*1 in their commercial varieties (Parco *et al.* 2014, Racedo *et al.* 2013). Costet *et al.* (2012) included results for 23 older "Q" varieties and the 9 resistant varieties all had the *Bru*1 gene. Most CSR/Wilmar resistant clones included in the study also contained the *Bru*1 gene, but 4 resistant clones did not have *Bru*1 and must have another resistance gene. The high level of reliance on this one gene for resistance around the world places many countries at risk if there is a change in the pathogen that allows it to overcome this gene.

5.9 Orange rust

Orange rust caused by the fungus, *Puccinia kuehnii,* was considered a disease of minor importance in Australia until 2000 when it caused the most devastating disease epidemic ever experienced in the Australian sugar industry (Magarey 2000, 2010). The outbreak of orange rust occurred in the variety Q124 which was planted over 89% of the central region, 59% of the Herbert, 44% of the Bundaberg/Isis and 47% of the whole Australian industry. Orange rust was restricted to Asia and the western Pacific regions until 2008 when it was detected in North and Central America (Comstock *et al.* 2008; Ovalle *et al.* 2008) and it is now present in South America (Funes *et al.* 2016).

Orange rust causes orange-brown pustules on leaves. The disease is distinguished from brown rust by the colour of the pustules and by microscopic examination of the spores. The disease is favoured by extended periods of high humidity and therefore the peak of disease is in the wet season (Staier *et al.* 2004).

Orange rust can cause severe scorching of leaves as pustules coalesce and stalks become thin and rubbery. Yield losses of 40% were recorded in Mackay (Staier *et al.* 2003). Magarey *et al.* (2011) estimated the direct economic losses from orange rust to the Australian sugar industry at $200M in 2000. Severe losses occurred in the central, Herbert and southern regions. Indirect costs associated with the large replanting program affected the industry for the next 3-4 years until Q124 was replaced.

Fungicides (propiconazole, cyproconazole and mancozeb) were used to reduce losses during the years following the outbreak, until Q124 could be replaced with resistant varieties (Staier *et al.* 2003).

There was an obvious change in the fungus and molecular analysis was used to compare collections of spores from the new outbreak with historical and overseas collections. The analysis found no differences in the ITS sequences between historical Australian samples (1898) and samples collected from Q124 in 2000 (Braithwaite *et al.* 2009). Samples collected from Indonesia and Papua New Guinea did show some variation to the collections from Australia. Spores collected from the new outbreak in Central America were similar to the Australian collections.

Resistant varieties are the main method for management of orange rust and the Australian sugar industry is fortunate that less than 5% of clones in the breeding program are susceptible (Magarey *et al.* 2005). The resistance of parent clones and clones in the advanced stages of the breeding program are assessed annually at Meringa (Magarey & Bull 2009c). Natural selection for orange rust resistance occurs in plant breeding selection trials and plant breeding technicians record observations on incidence of orange rust in clones in trials. Any clones that show high levels of orange rust in trials are discarded.

Apan *et al.* (2004) demonstrated that Hyperion satellite imagery can be used to detect orange rust disease in sugarcane crops. Magarey (2006b, SRDC project BSS295) investigated the potential of a range of remote sensing techniques for measuring disease levels of orange rust, brown rust and yellow spot in commercial crops and plant breeding trials. Remote sensing could be a simple way of rating clones for resistance to these diseases. No research has been conducted to apply remote sensing for leaf diseases in the sugar industry.

Considerable research is being conducted on orange rust in North and South America where it is causing significant losses. Currently, this research does not appear to have any direct application in Australia as orange rust remains at very low levels in commercial crops and the vast majority of commercial varieties and breeding clones are resistant.

5.10 Yellow spot

Yellow spot caused by the fungus, *Mycovellosiella koepki,* is characterized by yellow to red spots on the leaves (Autrey & Saumtally 2000). The disease occurs in many countries and all regions of the Australian sugar industry. The disease develops during extended periods of overcast weather with high humidity and severe disease is usually restricted to the wet tropics from Tully to Babinda and parts of the Herbert (Magarey 2006a; Magarey *et al.* 2007). Yield losses of 10-15% have been associated with yellow spot in the Tully to Babinda mill areas in northern Queensland (Magarey *et al.* 2008).

Magarey & Bull (2009a) reported on the resistance of 114 commercial varieties from Australia and overseas and 245 Australian clones used as parents in the SRA breeding program. The resistance of clones was assessed by estimating the percent leaf area affected compared with a set of standard varieties; 20% of commercial varieties and 33% of the Australian parent clones were rated susceptible. Magarey and Bull (2009b) analysed the environmental conditions that favoured yellow spot infection at SRA Meringa and found that in only 50% of years were the conditions conducive for yellow spot. Rating clones for resistance at Meringa was only reliable in years conducive to yellow spot infection.

5.11 Pineapple sett rot

Pineapple sett rot is caused by the soil-borne fungus *Thielaviopis paradoxa* (anamorph = *Ceratocystis paradoxa*) (Girard & Rott 2000). The fungus rots the setts of cane after planting before they have a chance to germinate. It can cause complete failure of germination and fields have to be planted again. Pineapple sett rot gets its name from the fruity smell, like over-ripe pineapples, produced by the fungus in rotting setts. The disease is present world-wide. Pineapple sett rot is favoured by any condition that slows the germination of the setts, including cool soil temperatures, setts that are damaged (splits, cracks, punctures of the rind), short setts with only one node, excess soil moisture, drought or poor soil-sett contact. Billet planting has seen an increase in pineapple disease. Harvesters used to cut billets for planting need to be set up specifically for producing billets of adequate length and with minimal damage. Large quantities of organic matter added to the soil shortly before planting, such as occurs in plough-out replant of sugarcane or when a large green manure crop of a species like sorghum is ploughed in, can lead to a burst of sporulation of the fungus that can place the newly planted setts under severe disease pressure. Planting is the most expensive operation conducted on a sugarcane farm, costing in the order of $1800/ha. The success of establishment of the new crop affects the yield for the next 4-6 years of the crop cycle. Germination failure can severely affect grower profits.

Management of pineapple sett rot involves ensuring planting material and conditions for germination are optimized and application of a fungicide to protect the setts from the fungus. Almost all sugarcane planted in Australia is treated with a fungicide and sugarcane planting machines have either a spray or dip mechanism to apply the fungicide.

The most widely used fungicide is Shirtan® (methoxy ethyl mercuric chloride, MEMC). The APVMA cancelled the registration for agricultural products containing mercury in August 1995. The only exception was Shirtan® for use on sugarcane. Australia is a signatory to the Minamata treaty for the reduction of mercury in the environment and the continued registration of Shirtan® is under constant review.

Bhuiyan *et al.* (2014b) compared two fungicides registered for pineapple sett rot control, Tilt® (propiconazole) and Shirtan®, with the fungicide Sinker® (flutriafol) which is registered for control of smut in planted setts. They found that all three fungicides effectively prevented pineapple sett rot and Sinker® is now registered for control of smut and pineapple sett rot.

Wickramasinghe *et al.* (2015) assessed the efficacy of the fungicides Vibrance® (6.62% difenoconazole, 1.65% metalaxyl-m and 1.38% sedaxane), Dynasty® (6.64% azoxystrobin, 1.11% fludioxonil and 3.32% mefenoxam) and Mirador® (25% azoxystrobin) against pineapple sett rot of sugarcane compared to the registered fungicides, Sinker® and Shirtan®. The results demonstrated that Dynasty® and Vibrance® can effectively control pineapple sett rot with similar effectiveness to Sinker® and Shirtan®. Mirador® was not effective.

Holzberger *et al.* (2018) compared Sinker® and Shirtan® and investigated whether adding mill ash to the soil affected the efficacy of the fungicides. Sinker® gave the best control, but Shirtan® was also effective. There was no interaction between the fungicides and mill ash content in the soil.

Zhang (2017, SRA project 2014/402) showed that several *Trichoderma* and *Paenibacillus* strains were antagonistic to *Thielaviopis paradoxa in vitro.* The potential biocontrol agents can be grown on media based on sugarcane bagasse and molasses. Further testing is required to demonstrate that the organisms can prevent pineapple disease when applied to sugarcane setts under field conditions.

Fungicides will play an important role in pineapple disease control into the future and there are several viable alternatives to Shirtan®.

5.12 Red rot

Red rot is caused by the fungus *Glomerella tucumanensis* (anamorph = *Colletotrichum falcatum*) (Singh & Lal 2000). The disease occurs wherever sugarcane is grown but is particularly severe in India and south-east Asia. The disease causes a rot of the mature stalks and can severely affect sugar quality. Complete crop loss can occur in some situations. The fungus is present in the soil and on external plant parts and enters the stalk through growth cracks, root primordia or insect damage. Mature cane that suffers stress from drought or waterlogging is particularly susceptible. The incidence of red rot in Australia is generally low (Magarey 2006a) but severe damage occasionally occurs in individual fields. Southern regions, where drought is more common, have a higher incidence of the disease.

Red rot is managed with resistant varieties and all varieties are screened for red rot resistance at the last stage of the SRA breeding program. It is rare for varieties to be rated as highly susceptible to red rot in Australia (Magarey *et al.* 2005). The variety Q209$^{\phi}$ has shown moderate levels of red rot in recent years. The incidence of red rot in this variety has restricted adoption of the variety in the central region.

Kalaimani *et al.* (2012) and Singh and Lal (2000) describe research into management of red rot in India. Extensive research has been conducted into varietal resistance, variation in the fungus and interaction of the pathogen with sugarcane. Aitken *et al.* (2020) described research that will attempt to identify markers for red rot and smut resistance in a biparental population from a cross of Q208 x Q209. This project aims to develop cooperative research with India but will also provide useful information on markers for red rot resistance that can be incorporated into the SRA breeding program.

5.13 Yellow canopy syndrome

Yellow Canopy Syndrome (YCS) was first observed in 2012 in the central and northern cane-growing regions in Australia and has since been observed in southern districts. This review will only describe the research on possible pathogens associated with YCS and will briefly mention current research on insecticides. Extensive research has been conducted on physiological response to YCS and the

relation of the syndrome to stress (Botha 2015, SRA project 2014/090; Scalia 2020, SRA project 2014/049). Olsen *et al.* (2015) (Olsen 2015, SRA project 2013/807) found no known disease, pest or nutritional factor that was consistently associated with YCS symptom development. They monitored the development of symptoms and the distribution of YCS in fields. The epidemiology of YCS was not consistent with known sugarcane diseases.

Braithwaite *et al.* (2017) screened samples showing YCS with specific tests for known sugarcane pathogens. They also used a range of generic tests for viruses, bacteria and fungi, with an emphasis on pathogens that cause yellowing symptoms, such as luteoviruses and phytoplasmas. Traditional aseptic pathogen isolation and culturing, light microscopy and electron microscopy were also performed. The pathology investigations found that both YCS and asymptomatic cane contained viruses, bacteria and fungi but no organism was consistently associated with YCS. Several new and uncharacterised viruses were found that were not associated with YCS. The significance of these viruses to sugarcane production in Australia is unknown.

Singh (2018, SRA project 2014/082) used next-generation DNA sequencing techniques, including amplicon, metagenomics and metatranscriptomic sequencing along with several soil health measures, to identify the cause of YCS. They stated that fungal and viral infection, and soil nutritional health are unlikely to cause YCS. Their data suggested links between bacteria (e.g. *Curtobacterium* spp.) and phytoplasmas and YCS, but there was no firm evidence for involvement of these organisms. They found a badnavirus with a genome similar, but not identical, to the known sugarcane bacilliform in YCS and asymptomatic leaves.

Research investigating the role of fungi, oomycetes, viruses and phytoplasmas in YCS was conducted by Geering *et al.* (2020, SRA project 2016/064). YCS-affected plants were treated with a range of anti-microbial agents active against fungi, oomycetes and bacteria. The antibiotic streptomycin was the only anti-microbial agent that reduced YCS symptoms, but the antibiotic also caused some phytotoxicity that complicated the interpretation of the results. Streptomycin is active against bacteria and phytoplasmas. The phytoplasma, *Candidatus* Phytoplasma aurantifolia, was detected in YCS-affected and healthy plants but populations of the organism were higher in plants with YCS symptoms. The detection of the phytoplasma could not be reproduced in the SRA laboratory. Further research is required to clarify whether phytoplasmas are associated with YCS.

Olsen & Ward (2019) showed that soil application of insecticides including neonicotinoids (thiamethoxam and imidacloprid), and foliar applications of the pyrethroid, bifenthrin, resulted in a delay in the onset of YCS symptoms and a significant reduction in the severity of symptoms. The insecticide treatments also resulted in a significant yield improvement relative to cane in the untreated control. An acaricide treatment was ineffective in reducing YCS symptoms. Further research is currently underway to confirm the response to insecticides, determine which insects are being controlled and explain the mechanism by which the insecticides are reducing YCS symptoms. No recommendations can be made until the results of this research are available. The response to insecticides is a strong lead that may help identify a cause of YCS and should be thoroughly investigated.

The conclusion from these studies is that no pathogens are consistently associated with YCS. Some previously undescribed viruses were detected that were not associated with YCS, but their significance to sugarcane is unknown.

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6. WEED MANAGEMENT

6.1. Introduction

Weed control is the botanical component of pest control, which attempts to stop weeds from competing with desired flora and fauna including domesticated plants and livestock, and in natural settings preventing non-native species competing with native species. Weeds compete with crops for space, nutrients, water and light. The presence of weeds does not necessarily mean that they are damaging a crop, as during the very early growth stages both weeds and crops may grow without interference. However, as growth proceeds, they each begin to require greater amounts of water and nutrients. General estimates suggest that weed and crop can only co-exist harmoniously for about 3 weeks before competition becomes significant. Weed control is critical in agriculture and weed control methods include chemical control with herbicides, powered cultivation with cultivators, smothering with mulch, hand cultivation with hoes, thermal lethal wilting, and burning.

In sugarcane, the major biotic limiting factor to productivity is the direct interference caused by weeds, especially in the first 3 months of growth, when canopy closure has not been completed because crop growth is slow. Weeds also serve as host plants of pests and diseases and interfere with crop-management practices such as fertilising, field inspection, as well as with mechanical harvesting. Weed control in sugarcane is usually performed either by powered cultivation or the application of chemical herbicides, but the emergence of environmental considerations is prompting the search for new control alternatives.

The term Integrated Pest Management was first used in agriculture beginning in the 1970s in response to growing knowledge about the negative side-effects of pesticide overuse. Efforts were needed to reduce crop losses due to pests through the implementation of Integrated Pest Management (IPM) (resistant crop varieties, rational use of pesticides, biocontrol and better cultural practices) without harmful side-effects.

Among the pests, weeds are considered an important biotic constraint to food production. Their competition with crops reduces agricultural output (quantity and quality) and increases external costs by spreading them across farm boundaries. By extension, the term Integrated Weed Management was developed.

6.2 Integrated Weed Management

6.2.1 Definition

Integrated weed management (IWM) is the control of weeds through a long-term management approach, using a combination of weed management techniques such as:

- Physical control (e.g. strategic tillage, harvest weed seed control),
- Chemical control (e.g. herbicides sprayed broadcast, herbicides precision sprayed using weed detection technology),

- Biological control (e.g. *Neurostrota gunniella* Busck (Lepidoptera: Gracillariidae) against *Mimosa pigra*),
- Cultural control (e.g. increasing crop competitiveness through adjusting seeding rates and row spacing, growing competitive crop varieties, using crop rotation (Chauhan 2016), retaining cane trash residues).

Any integrated weed management plan or strategy should focus on the most economical and effective control of the weeds whilst including ecological considerations. The long-term approach to integrated weed management should reduce the extent of weeds and reduce the weed seed stock in the soil. It should consider how to achieve this goal without degrading the desirable qualities of the land, such as its native ecology or agricultural crops. By using several techniques to control weeds, weed species are less likely to adapt to the control techniques, which is likely if only one technique is used. For example, if a herbicide is used over a long period of time, a weed species can build up a resistance to the chemical.

There are various descriptions of IWM in the literature. In sugarcane, IWM follow these principles (identified in the IWM guide on SRA website http://sugarresearch.com.au/wp-content/uploads/2017/02/IS13038-Integrated-weed-management.pdf):

- Know what weed species are present,
- Identify the cause and pressure of the weed issue,
- The critical crop stage to minimise weed competition is during the first 3-4 months after cane germination (to approximately 12 cm height to top visible dewlap),
- Prevent new weeds entering the farm and the spread of weeds across a farm,
- Deplete the soil weed seed bank by controlling weeds before they set seed,
- Rotate herbicide groups to reduce the risk of resistance,
- Use fallow crops to suppress weeds and to allow for different herbicide selection,
- Make the most of cultural practices (e.g. trash blankets) to reduce the need for herbicides,
- Select herbicides that have lower environmental footprints, given efficacy and cost considerations,
- Record herbicide usage and review effectiveness for different situations and conditions,
- Always read the label and the Safety Data Sheet.

6.2.2 IWM models

Traditionally, agronomists, crop consultants and growers build their IWM skills via training workshops and hands-on experience. IWM recommendations are complex and many parameters need to be considered to develop an IWM plan. Nowadays, computer simulation modelling is a new tool for helping understand and predict crop-weed competition and it can play a role in integrated agricultural systems to increase crop yields and manage weeds. By integrating crop simulation models, such as APSIM, with other models that handle the weed population dynamics, models could be used to investigate impacts of many parameters on weed populations: crop planting density, crop row spacing, row orientation, weed density (influenced by management actions), spatial patterns (i.e. interrow spraying or cultivation), spread of seeds by harvesting equipment, soil environmental conditions that may cause non-homogeneous germination, emergence time of the crop and of the weeds, etc (Renton & Chauhan 2017).

In Cuba, Diaz *et al.* (2004b) developed a computer-based decision support system to assist integrated weed management in sugarcane which covers: -a Knowledge Base component containing extensive information on the 32 most widespread weed species (with colour images of plants at different stages), herbicides and herbicide treatments currently in use, costs and productivity of all chemical, mechanised and manual weed control operations and spray nozzle specifications; - an

Immediate Herbicide Treatment Recommendation Subsystem recommending best immediate herbicide treatments for any area, according to the control of prevalent weed species, treatment costs and existing weed-crop-environment conditions and, optionally, the amount and mixing order of each product to be placed in the sprayer tank; - and an Annual Weed Control Planning Subsystem to develop chemical, mechanical, and manual weed control plans, of both areas and required inputs, by fortnights and annual total, for the whole estate, farms or sections and individual fields. Implementation in a few estates in Cuba during several years has shown greatest acceptance among growers of the two former sections, and in the latter, a close relationship of total planned farm herbicide budget with expected crop yields, due to variable yield thresholds included to allocate herbicide treatments.

In Louisiana, a collaborative effort between weed scientists and agricultural economists has resulted in the development of the *Sugarcane Fallow Weed Control Program - Producer Decision Aid*, a simple Excel-based spreadsheet model that can be used as a farm planning tool for producers to determine costs of current fallow programs and to compare costs for alternative fallow programs (e.g. controlling *Cyperus rotundus* using chemical versus mechanical strategies) (Griffin *et al.* 2011).

Similar tools exist in grain cropping systems in Australia. The *Ryegrass Integrated Management (RIM) model* is a decision-support and educational tool specifically designed for evaluating ryegrass management options based on average conditions over a series of average past seasons. A version for wild radish also exists.

The *Weed Seed Wizard* is a more advanced model using specific soil, climate and weed data to predict the effect of management options on a number of the most problematic weeds across Australia's cropping zones. The *Weed Seed Wizard*, currently used in the Australian grain industry: -applies to all Australian grain growing areas; -helps growers understand and manage weed seedbanks on their farms; -uses farm management records to simulate how different crop rotations, weed control techniques, irrigation, grazing and harvest management tactics can affect weed numbers, the weed seedbank and yields; -uses farm-specific management and site-specific weather; -is multi-species (up to nine weed species included for the Northern region). The *Weed Seed Wizard* is a collaboration between several organisations (Department of Primary Industries and Regional Development, Grains Research and Development Corporation, University of Western Australia, University of Adelaide, NSW Department of Primary Industries, Queensland Department of Agriculture and Fisheries) https://www.agric.wa.gov.au/weed-seed-wizard-0.

Further research needs: ***Extending the Weed Seed Wizard to the sugar industry would prove particularly relevant in the recent context of increased herbicide scrutiny and limitations or withdrawal of some active ingredients. The Weed Seed Wizard would help identify the best weed management strategy using the remaining registered products and alternative practices. Knowing that three years sufficed to produce the wizard model for grains, including a useful representation of a number of important weed species, we would expect a weed management decision-support package for sugarcane could be developed within 5 years (to take into account the cane crop cycle) and would include a good representation for 10-20 species (including vines, broadleaves and grasses). The extension of the model to cane would involve the collection of empirical data for key weed species, cultivars and ecotypes at different densities, different emergence times, and in different environmental conditions. Such data collection is expensive, but its cost can be justified by the benefits of accurate weed management predictions.***

6.3 Weed ecology

Some basic aspects of weed ecology include weed characteristics and weed classification. Some characteristics of plants that support weediness include rapid seed germination, rapid growth, the

ability to take up and utilise large amounts of nutrients, prolific seed production, seed characteristics that promote dispersal, seed dormancy mechanisms, continual flushes of germination, the ability to adapt to various environmental conditions, and high tolerance to stresses. Furthermore, the classification of weeds can be comprised of population dynamic factors (i.e. habitat, growth form, life cycle, reproduction), seed production (amount of seed produced and in what form), and seed dispersal through abiotic and biotic factors. Weed ecology is directly correlated with the plant community composition, the evolution of weeds (potentially through factors such as herbicide resistance), allelopathy, and competition. The importance of weed ecology to both natural and agricultural systems cannot be stressed enough. Once the basic ecology of a weed is known, proper management strategies can be implemented to prevent the weed from producing seeds. In fact, the ecology of a weed species could be the most important tool to determine the correct course of weed management.

6.3.1 Weed identification

To allow identification of weeds in sugarcane, a special edition of the *BSES Bulletin* was released in 1989 (Anon. 1989). This edition, widely used by growers throughout the years, is now out of print. Its content has been made available on the SRA website and is also phone- and tablet-enabled. Both printed edition and web content provide users with the necessary tools to identify the main weeds they may find on Australian cane farms. Two identification keys are provided:

- Key to leaf shape for broadleaves (narrow lance shaped, broad lance shaped, club shaped, oval or ellipticals, round shaped, heart shaped, shallow lobes, many or deep lobes, divided leaves, finely divided leaves, thistle like, three leaflets, even number of leaflets, and odd number of leaflets),
- Key to seed head shapes for grasses, sedges, and water plants (spike or plume, millet like, finger like, few spikes, many spikes, small flower heads on short stalks, cone shaped, etc).

A consortium of countries has developed a web platform called the *Weed Identification and Knowledge in the Tropical and Mediterranean areas portal* (WIKTROP portal) that stores photographs, feature characterisation, distribution and ecological information of weeds in tropical crops. WIKTROP is a geographical extension of the Weed Identification and Knowledge in the Western Indian Ocean portal (WIKWIO portal) to tropical and Mediterranean areas around the world. WIKTROP aims to build and leverage a network, which will consolidate existing scientific and technical knowledge and facilitate sharing of new information on weeds and weed management. The action aims at enhancing the capacities of researchers, reinforce the institutional capabilities of the National Agricultural Research System and Universities, empower extension services and improve their quality of service, through a participatory, technology facilitated platform. As the portal is used in areas such as Reunion and Mauritius where sugarcane is the dominant crop, a large proportion of the Australian weeds are already present on the portal. https://portal.wiktrop.org/

Further research needs: ***SRA is currently updating the Weed in Australian Cane Fields manual as new weeds have emerged in some districts and are not encompassed in the old edition. Additional photographs of weeds at different growth stages are also being captured and uploaded to facilitate identification. Progress is slow and dependent on staff availability and, currently, confirmation of specimen identification by the herbarium is not possible.***

Adding all Australian sugarcane weed species to the existing WIKTROP portal would make it relevant for use by Australian sugarcane growers, agronomists and consultants. As the platform is already created and running, minimum funding would be necessary to upload the missing species and create an Australia-specific section in the portal. The reporting capacity of the WIKTROP portal

would also be an extremely convenient way to capture and understand the weed distribution dynamic in the sugarcane industry.

6.3.2 Weed distribution

Very limited work has been done to scope the weed distribution in the sugar industry. Osten (2010) undertook a weed scoping study at the request of the Grains Research and Development Corporation that encompassed seven cropping regions within Central and Northern Queensland. Weed species, weed management issues, and associated RD&E needs were identified using an informal industry grower consultation process. 134 weeds representing 19 genera were noted. 30 were grasses, 4 were sedges, with the remaining 100 being broadleaved weeds (18 were leguminous, 25 had vine growth habit). Each region had unique weeds with the North Queensland Coast having the most, butsome problem weeds such as Feathertop Rhodes grass, Fleabanes and Sida were common across the cane, grains, legumes, horticulture and cotton industries.

Environmental weeds have received more attention, with governmental agencies undertaking extensive work to determine their distribution. Some of these environmental weeds can be found in sugarcane fields and their management or movement may be controlled under state legislation. When found in sugarcane fields, the landowner is required to take specific actions to satisfy governmental obligations:

- Balloon vine (*Cardiospermum grandiflorum*) – Widely distributed in the coastal regions of Eastern Australia and particularly common in Southern Queensland and NSW. It is a category 3 restricted invasive plant under the *Biosecurity Act 2014*. It must not be given away, sold, or released into the environment. The Act requires everyone to take all reasonable and practical steps to minimise the risks associated with invasive plants under their control.
- Giant sensitive plant (*Mimosa invisa = M. diplotricha)* - Found in Far North Queensland around Mackay and from Ingham to Cooktown. It is a category 3 restricted invasive plant under the *Biosecurity Act 2014*.
- Itch grass (*Rottboellia cochinchinensis)* - Occurs in coastal areas from the NSW-Queensland border to North Queensland and also present in the Northern Territory. Itch grass has been locally declared and considered an invasive biosecurity matter in the Burdekin shire.
- Olive hymenachne (*Hymenachne amplexicaulis)* - Found in Cape York in Queensland to Casino in NSW. It is a category 3 restricted invasive plant under the *Biosecurity Act 2014*.
- Red witchweed (*Striga asiatica)* – It is a prohibited invasive plant under the *Biosecurity Act 2014*. Prohibited invasive plants must be reported to Biosecurity Queensland within 24 hours of detection. Red witchweed must not be introduced, kept, moved, supplied, or released into the environment without a permit issued by Biosecurity Queensland. An eradication process is ongoing in the Mackay region.
- Siam weed (*Chromolaena odorata)* - Found in North Queensland. Siam weed is a restricted invasive plant under the *Biosecurity Act 2014*.
- Sicklepod (*Senna obtusifolia)* - Found in Darwin and surrounding areas of the NT and in Queensland around Mackay, Ingham, and parts of the Atherton Tablelands. All sicklepods are restricted invasive plants under the *Biosecurity Act 2014*.
- Singapore daisy (*Sphagneticola trilobata)* - Found along the east coast of Queensland. Singapore daisy is a category 3 restricted invasive plant under the *Biosecurity Act 2014*. https://www.farmbiosecurity.com.au/crops/sugarcane/sugarcane-pests-and-weeds/

  https://www.daf.qld.gov.au/business-priorities/biosecurity/invasive-plants-animals/fact-sheets#weeds

In addition to studying the distribution of invasive weeds, governmental agencies also investigate their ecology to help determine the timing and duration of control treatments. Biosecurity Queensland currently conducts weed ecology projects such as a study of weed seeds dynamics; the control and ecology of *Stevia ovata*; sicklepod ecology and control; the ecology and control of aquatic weeds of northern Australia; the management and ecology of fireweed (*Senecio madagascariensis*); the ecology and management of *Chromolaena odorata* and *Clidemia hirta*; and the eradication progress and biology of tropical weed eradication targets (Anon. 2020).

In sugarcane, weed distribution studies are uncommon. Fillols *et al.* (2015) inspected 2603 and 1936 cane blocks in the Mackay district in 2012 and 2013, respectively, for vine species presence. Vines were found in 26% and 17% of the surveyed blocks with pink and red convolvulus (*Ipomoea triloba, I. hederifolia*) being the main vine species. Siratro (*Macroptilium atropurpureum*) was the other most observed vine species, found in 3% and 2% of the paddocks in 2012 and 2013, respectively. In both years, Ipomoea vines occurred mostly in blocks planted with cane varieties with erect foliage such as Q209$^{\Phi}$ and Q200$^{\Phi}$. Blocks planted with newer varieties (often better cared for) and varieties not self-trashing (i.e. KQ228$^{\Phi}$ and Q232$^{\Phi}$) were less likely to host vines. Most vine species were found to grow in many soil types with the exception of pink convolvulus which seems more adapted to challenging soil conditions such as soloths. These results explained some vine species preferences in terms of cane varieties or soil type and highlighted variety choice as a strategic way to reduce favourable vine-growing conditions. Knowing the weeds' distribution assists our understanding of some aspects of weeds ecology like their interaction with the crop, soil, and environment.

Information on weed distribution at the farm and paddock scale can also provide relevant insights on weed ecology to design more comprehensive weed management decisions. Ferraro *et al.* (2008) surveyed the weed communities in 91 crop fields in sugarcane farms of northern Argentina during the spring of 2004 and 2005. Using classification and regression trees (CART) for partitioning the clustered groups of weed community change into subsets, and non-metric multidimensional scaling (NMS) and CART models, the authors were able to explain almost 50 % of weed composition changes due to several explanatory factors (farm, latitude, number of ratoons, field crop area, yield and cane variety). Moreover, the CART models suggested different weed management methods for both changes in presence and abundance in sugarcane weed communities.

Further research needs: Weed distribution studies on specific problem weeds such as Guinea grass and nutgrass in all districts, Balsam pear and calopo in the Wet Tropics; and sicklepod in the Wet Tropics and Central region, would reveal crucial ecological information on these troublesome weed species that could be used to improve their management.

Common weed issues across several cropping industries could result in an opportunity for co-funding of biology / ecology research on specific weeds.

6.3.3 Weed spread

In addition to the inherent opportunities for weed seed spread (transport by wind, flood water and animals), mechanical operations such as harvesting and baling are the other two main vectors for seed dissemination.

In sugarcane, unclean machinery is a major path for weed seed dispersal, both from block to block on farms and between farms. Growers should clean down machinery, especially when moving from known weedy blocks onto other parts of the farm. They should also have clean-down agreements with contractors. Harvesters are a major contributor to weed seed spread within and between farms.

In a survey of harvesters in Mackay during 2012, thousands of convolvulus vine seeds were collected from the spirals, shoe, and floating rear shoe after harvesting a block infested with vines. In the case of pink convolvulus, the majority of seeds were viable throughout the season. Simple hygiene measures such as blowing down with an air compressor removed most of these seeds (Ross & Fillols 2017).

Baling the trash blanket is another potential source of weed dissemination, which can lead to transport of weed seeds long distances and to other cropping industries. Trash baling was a major source of concern during the witchweed outbreak in the Mackay region.

Further research needs: No other studies have been found regarding dispersal of weed seeds with mechanical operations or the potential dissemination of weeds via baling. The extent of dissemination of weed seeds ejected by the harvester extractor fans is unknown but could have a large impact on weed spread, which could potentially be alleviated by a weed-seed destructor system as used in the grain industry.

6.3.4 Weed classification

There are many ways to classify weeds into groups for convenience of planning, interpreting, and recording control measures against them. In sugarcane, they are essentially classified according to their cotyledonous character.

Monocots

Grasses

Grasses can be either annual (germination, vegetative development, flowering, seeding, and dying each year) or perennial (survival beyond one season). Grasses are the most prolific weeds as they germinate in the early stage of cane development, grow quickly (compete vigorously with the crop) and will significantly reduce the yield if left uncontrolled. Perennial grasses are very difficult to control, especially in their more mature stages, and may necessitate a premature plough-out of the sugarcane crop. Green-cane trash-blanket is an effective suppressant for grass seeds, but large-seeded grass still tends to germinate through the mulch. Perennial grasses that have been cut during harvest also regrow readily through the mulch.

The most common grasses occurring across all districts are barnyard and awnless barnyard grass (*Echinochloa crus-galli, E. colona*), crowsfoot (*Eleusine indica*), summer grass (*Digitaria ciliaris*), feathertop Rhodes grass (*Chloris virgata*), green summer grass (*Brachiaria subquadripara*), Guinea grass (*Megathyrsus maximum* var. maximum), couch grass (*Cynodon dactylon*), paragrass (*Brachiara mutica*) and wild sorghum (*Sorghum* spp.). Other regional problem grasses are itch grass (*Rottbellia cochinchinensis*), green panic (*Megathyrsus maximum* var. publiglumis), sour grass (*Paspalum conjugatum*) and hymenachne (*Hymenachne amplexicaulis*).

Osten (2010) found three grasses common to all cane growing regions: barnyard grass, summer grass, and feathertop Rhodes grass.

Research work on the ecology of some grass species has revealed important findings that can support their management.

Walker *et al.* (2020) studied the seedling emergence of two grass species, *Echinochloa colona* and *Urochloa panicoides,* and showed that *E. colona* seedlings emerged as a series of flushes predominantly in the first year from 0-2 cm depth whereas *U. panicoides* emerged mostly as a single large flush in the first two years mainly from 5 cm depth. Grass seed longevity was short with <5%

remaining after burial at 0-2 cm for 24 months. Seed persistence increased significantly with burial depth.

Other ecology studies on species of the genus *Echinochloa* showed that, in the absence of human intervention, weeds such as *Echinochloa crus-galli* dominate their ecological niches. Fan (2016) revealed that the comparative genome analysis of *E. crus-galli* identified more than 10,000 *Echinochloa*-specific genes and extremely high copy numbers of cytochrome P450 monooxygenase (P450) and glutathione S-transferase (GST) genes, which are two key enzymes for the detoxification of allelopathic compounds or herbicides. This provided new insights into the adaptation strategy of weeds by evolving more adaptation-related genes than crops to gain a competitive advantage.

Sedges

Sedges are a large group of plants which are usually perennial with triangular stems. Nutgrass (*Cyperus rotundus*) is the most prevalent sedge in all districts and all soil types (Osten 2010). Nutgrass has underground runners and tubers that can survive for many years. Nutgrass can grow through the cane trash blanket and be troublesome in ratoons, but the biggest competition occurs in plant cane. Tillage has proven to be quite unsuccessful to control nutgrass as it breaks underground runners connecting tubers and individual tubers can grow new plants.

Navua sedge (*Cyperus aromaticus*) is another problem weed localised in North Queensland. Navua sedge is extremely aggressive and competes strongly for nutrients, light, and moisture. Navua sedge can be a problem in sugarcane where the crop is light with poor canopy cover. In pasture, Navua sedge is capable of forming dense stands that can smother many tropical pasture species. Spread occurs through the normal extension of the rhizome system, by seed and by dispersal of viable rhizome fragments during cultivation. Navua sedge is classified as an invasive plant in Queensland.

Dicots

Broadleaves

Broadleaf weeds can be annual or perennial. In general, they are easier to control than grasses due to the use of selective herbicides. There is a wide range of broadleaves and they are often district or soil specific.

Some common broadleaves present across most Australian sugarcane regions are blue top (or billygoat weed) (*Ageratum conyzoides*), blackberry nightshade (*Solanum americanum*), rattlepods (*Crotalaria* spp.), pigweeds (*Portulaca oleacera*), sensitive weed (*Mimosa pudica*), fleabanes (*Conyza* sp.), willow primrose (*Ludwigia octovalvis*) and square weed (*Spermacoce latifolia*). Some particularly troublesome broadleaves are sicklepod (*Cassia obtusifolia*), giant sensitive weed (*Mimosa invisa*) and milkweed (*Euphorbia heterophylla*).

In Louisiana, a large perennial broadleaf weed identified as *Solanum nigrescens* (divine nightshade) is an introduced species to the United States, which has recently become a problematic weed in agricultural systems. Its rapid spread to non-infested areas, ability to compete with sugarcane, and tolerance to traditional pre-emergence and post-emergence herbicide programs in Louisiana sugarcane production shows the potential problem of this nightshade species (Orgeron *et al.* 2018).

Vines

Vines are climbing plants that entangle the sugarcane plants. Compared to other weeds, they can germinate over a long period as late germinators under a close crop canopy can still manage to climb through to access light.

Vines are easy to kill using the suite of herbicides available in cane, however their extended germination period makes it difficult to achieve control in one pass. Paddock access to control late vine germination after canopy closure requires specific spraying equipment (high-rise sprayers or aerial spraying). Unlike most annual weeds, with seed weights between 0.1 and 5.0 mg and which usually emerge from depths of less than 5 cm, seedlings of larger-seeded species like ipomoeas may do so from 10 cm or more as their larger seed size is associated with a greater food reserve enabling the seedlings to reach the surface. Vines seeds also have enough resources to germinate through thick trash blankets. Since the adoption of green cane trash blanketing which can suppress many grass and broadleaf weeds, vine weeds have become a greater issue in sugarcane fields. If left uncontrolled, vines greatly compete with the crop for light, they entangle and flatten the crop and impede harvest. The lodged crops attract rats, which increase the damage further.

The most common vines across the sugarcane regions are pink and red convolvulus (*Ipomoea triloba, I. hederifolia*), common and blue morning glory (*Ipomoea purpurea, I. indica*), bell vine (*I. plebeia*), star of Bethlehem (*I. quamoclit*), stinking passion flower (*Passiflora foetida*),horned cucumber (*Cucumis metuliferus*), siratro (*Macroptilium atropurpureum*), centro (*Centrosema mole*), calopo (*Calopogonium mucunoides*) and balsam pear (*Momordicha charantia*).

An ecology research study by Gaungoo *et al.* (2010a) in Mauritius showed that *Ipomoea triloba* and *Mikania micrantha* grew more quickly and more vigorously than *Passiflora suberos*a. *M. micrantha* also proved to be more resistance to drought than *I. triloba* whereas *P. suberosa* appeared to thrive in very wet environmental conditions.

Gaungoo *et al.* (2010b) also revealed that *Ipomoea nil, I. obscura* and *I. triloba* growing in sugarcane fields have the ability to emerge from depths of up to 12 cm, although emergence was found to decrease relative to 8 cm depth. The three vine species displayed the same capacity for producing longer hypocotyls at depth beyond 4 cm.

Parasitic weeds

Red witchweed (*Striga asiatica*) is a parasitic plant that grows attached to the roots of certain commercially important grasses and summer cereals, including sorghum, corn, rice, and sugarcane. Three *Striga* species are major pests of grain crops in Africa, where they cause an estimated $7 billion damage each year. Witchweed species rank as some of the world's worst agricultural weeds. Red witchweed is exotic to Australia and is a prohibited invasive plant under the *Biosecurity Act 2014*. It was found on a property near Mackay in Queensland in 2013. The weed remains contained within the Mackay region, with approximately 87 ha known to be infested.

In July 2015, industry and government reached an agreement on a 10-year (2015-16 to 2024-25) response plan, to eradicate red witchweed from Mackay. The response plan was agreed to the value of $5.86 million and will be cost-shared on a 50:50 basis between governments (the Australian, state and territory governments) and industry groups (CANEGROWERS, Grain Producers Australia; Cattle Council of Australia and the live export industry and CANEGROWERS on behalf of the Australian Cane Industry).

Eradication will take at least decade until authorities can be sure that the eradication activities have worked. Success is also dependent on the cooperation and ongoing efforts by growers and graziers in the region as well as the affected community.

Further research needs: Research on weed ecology for environmental weeds is well funded, but limited weed ecology research has been carried out for weeds in agricultural systems. Sugarcane integrated weed management would greatly benefit from further knowledge of the basic ecology of the most troublesome weeds. However, some specific ecological questions related to the interaction between some weed species and the sugarcane crop trash blanketing system have been researched and their outcomes are presented in other sections of this review.

6.4 Weeds and climate change

The dynamics of competition between weeds and crop plants are affected by environmental conditions and have been shown to change with CO_2 enrichment and temperature, with consequences for plant community composition, weed-crop interactions, and chemical management.

Ziska (2016) showed that increased minimum temperatures are associated with a greater increase in perennial relative to annual weeds in cultivated soybean, along a North-South latitudinal gradient in the midwestern U.S., with consequences for herbicide application rates. Differential selection for weed fitness could potentially occur in response to CO_2. Carbon dioxide represents the source of carbon for photosynthesis and is less than optimal in about 94% of all plant species (i.e. those that rely solely on the C3 photosynthetic pathway). Long-term (seasonal) exposure to elevated CO_2 during the soybean growing season facilitated the number of C3 weeds relative to C4 grasses from the weed seed bank with significant changes in weed demographics and populations (Kiska & Goins 2006). However, Patterson &Flint (1980) reported that C4 plants also showed better growth with increase in temperature.

The Canegro model was used to simulate growth and development of sugarcane crops under typical management conditions at three sites (irrigated crops at Ayr, Australia, and rainfed crops at Piracicaba, Brazil, and La Mercy, South Africa) for current and three future climate scenarios (Singels *et al.* 2013). Future cane yields were expected to increase at all three sites, ranging from +4% for Ayr to +9% and +20% for Piracicaba and La Mercy. The uncertainty of these predictions correlates with the magnitude of the predicted yield increase. Canopy development was accelerated at all three sites by increased temperature, which led to increased interception of radiation, increased transpiration, and slight increases in drought stress at rainfed sites. The authors suggested that quicker canopy development could lead to a lower vulnerability to weeds, requiring a different approach to weed control. However, the model does not simulate weeds growth and the potential competitive advantage of C3 weeds.

In parallel to the impact of climate change on weed and crop competition dynamic, there are an increasing number of studies that demonstrate a decline in chemical efficacy with rising CO_2. The basis for this reduction is unclear. Ziska *et al.* (2004) studying Canada thistle grown in monoculture under field conditions suggested a greater root to shoot ratio and subsequent dilution effect of glyphosate when grown at elevated CO_2. If CO_2 does reduce efficacy, then additional work is needed to determine herbicide specificity, concentration, and application rates as possible means of adaptation.

Warmer temperatures or an increase in weather extremes (e.g. precipitation) could also reduce field access necessary for herbicide application. Climate change induced inconsistencies in rainfall, wind, relative humidity or soil or air temperature could reduce spray coverage or retention times of active ingredients following application. Increases in precipitation (as single extreme events or higher averages) could dilute the herbicide following application as well as exacerbate runoff and leaching; windier conditions could increase the risk of drift. Higher temperatures could increase both absorption and translocation of foliar applied herbicides adding to efficacy, but also increase

volatility and microbial breakdown. High humidity can reduce droplet drying after application and increase absorption of the herbicide. Overall, increased environmental variation exacerbated by climate change could influence delivery times, spray coverage, volatilisation, movement, and accidental injury associated with herbicide application (Ziska 2016).

Future research needs: Explore the impact of climate change on the dynamic of competition between weeds and sugarcane crop and investigate potential management issues. This could be done by combining climate models with models specifically developed for weed/crop competition dynamics (i.e. Weed Seed Wizard).

6.5 Economic value of weed management

6.5.1 Yield loss from weed competition

Competitiveness is the relative ability of a plant to obtain a specific resource when in competition with another plant. According to Aldrich & Kremer (1997), competition between the weed and crop occurs when some factor, such as water, nutrients, or sunlight, is insufficient to meet the needs of both.

Yield loss from weed competition, combined with the cost of weed control in sugarcane in Australia, was estimated to exceed $70 million annually in 2000 (McMahon *et al.* 2000).

In plant cane

Weed competition in sugar cane is critical in the early stages of crop establishment. Experimental work demonstrated that even a small weed population present early in the growth stage of the cane plant could have a significant competition effect, resulting in yield loss. Weed growth 4 weeks after spiking caused a yield reduction of 11%, while delaying control until 8 and 12 weeks resulted in large yield losses of 23% and 34%. On average, the yield loss due to delayed weed control is 2.8% yield loss per week (McMahon *et al.* 2000). O'Grady & Murphy (2001) also found that weed growth early in the crop establishment phase causes significant loss in cane yield of the order of 13% to 50% depending on the period of competition, the weed population, and the climatic conditions. The cost to sugarcane production where these weeds are not controlled was estimated at up to $100 per hectare per week (Kerr 2011). Updating Kerr's reference to a price of $35/tonne of cane, and a yield potential of 85 t/ha, the current economic loss of delaying weed control would be about $85 per hectare per week. Another unverified reference to Gilmour (2008) estimated the annual average value of lost yield due to sub-optimal weed management to be $338/ha.

In cane planted as one-eye-setts

When planting sugarcane as one-eye-sett seedlings, weed competition and the periods in which weed communities need to be controlled in this system has not been widely researched. Beluci *et al.* (2018) showed the critical period of interference prevention in a one-eye-sett sugarcane system was 103 days, with weed community interference (predominated by *Ipomoea hederifolia* and *Merremia aegyptia)* starting at 35 days and lasting until 138 days after planting. Weed coexistence during the entire sugarcane cycle reduced productivity by 60% and affected qualitative characteristics such as the total reducing sugars. Both species affected the vegetative development of the crop, mainly the tillering. *I. hederifolia* plants developed ahead of the three vine plants, but in late evaluations the interference caused by *M. aegyptia* (77%) was greater ($P<0.01$) than that caused by *I. hederifolia* (72%).

Future research needs: Determine the critical period of weed intervention when planting NEF CEEDS™ technology in the Australian sugarcane cropping system.

In ratoon cane

In ratoons, weed infestation significantly reduced cane yield by 21 to 31%% where weeds were allowed to compete with the crop beyond the initial 30 days after ratoon initiation. Similarly, keeping the field free of weeds for a minimum of 60 days after ratoon initiation minimised yield losses to less than 10%. If weeds were only controlled for the first 30 days after ratoon initiation, 19% yield loss could be expected. The critical period for intervention in ratoon was defined between 30–60 days after ratoon initiation (Srivastava *et al.* 2002).

Future research needs: The dynamic between cane and yield growth is intrinsically linked to access to nutrients and water. In the current environmental context, which promotes lower nitrogen application, limited input of nutrients is likely to impact on the dynamic of weed/crop interaction and potentially affect the intensity of competition from weeds. Some research efforts should be targeted on assessing the impact of lower nitrogen rates on weed competition in cane.

6.5.2 Yield loss from specific weed species competition

Some particularly troublesome weed species such as nutgrass, itchgrass, Guinea grass, couch grass, sour grass and vines can infest cane fields and be largely dominant over other weed species. Species specific competition research studies have been published that determine their economic impact on the crop.

In Central Queensland, significant yield losses up to 27% and 18% were caused by nutgrass left uncontrolled for 12 weeks in two field trials in plant cane on dry land and irrigated land, respectively. Cane and sugar yield losses were proportional to the duration of nutgrass competition. 12% yield loss occurred where nutgrass was left uncontrolled for 4 weeks. An efficient herbicide treatment costs about $70/ha in the fallow and $200/ha in the crop. In all cases, it was worth applying one or more nutgrass herbicide treatments to limit nutgrass competition and maintain yield (Fillols 2011). In New South Wales, Aitken *et al.* (2011) showed cane yield loss of around 30% in both plant cane and ratoon where nutgrass was left totally uncontrolled. Allowing nutgrass to grow for 4 to 8 weeks resulted in reduction of cane growth, mainly attributed to the competition for soil moisture and nutrients (25 to 45 kg of N/ha and 45 to 50 kg of K/ha taken up by nutgrass). In 4 to 8 days, nutgrass removed the equivalent of 11-12 mm of rain from the plough layer. Aitken *et al.* (2011) also calculated that total control of nutgrass resulted in large economic benefit ($350-$450 /ha), while one single application of an effective herbicide resulted in a short-term benefit of $200-400/ha.

Etheredge *et al.* (2010) planted four nutgrass tubers in pots along with a single node cutting of four sugarcane varieties. Averaged across varieties, 4 purple nutsedge tubers/pot reduced shoot dry weight an average of 62% and root dry weight an average of 71% at 64 days after planting and dry weight for one variety averaged 2 and 1.7 times that, respectively, of the other three varieties, highlighting a varietal response to nutgrass competition. For 30% shade, nutgrass shoot population was reduced 53% at 56 days, compared with the full sunlight (no shade) control. For the 70 and 90% shade treatments, nutgrass shoot population was reduced an average of 92% compared with full sunlight, showing that nutgrass should not be as competitive in established sugarcane.

In Louisiana, itchgrass (*Rottbellia cochinchinensis*) growth during the sugarcane grand growth stage reduced sucrose yield by 7 and 17% after 30 and 60 days of competition, respectively; whereas up to 43% reduction in sucrose yield were recorded when itchgrass competed season-long with sugarcane. Season-long divine nightshade (*Solanum nigrescens*) competition reduced sucrose yield 33% when

compared with sucrose yield when divine nightshade was controlled in mid-March (Spaunhorst & Orgeron 2019).

In Florida, Odero *et al.* (2014) studied the interference caused by fall panicum (*Panicum dichotomiflorum*) to sugarcane yield parameters. Fall panicum must be controlled 2 to 7 weeks after emergence to prevent 10% cane yield loss. Season-long interference of fall panicum resulted in 25 to 63% cane yield loss. The sugarcane variety CP 00-1101 was the most tolerant to fall panicum interference while CP 89-2143 was the least tolerant.

Competition between couch grass (*Cynodon dactylon*) and sugarcane was studied by Fontenot *et al.* (2016) in a pot trial. After 56 days of growth in pots, sugarcane shoot number and weight were each reduced 51% when grown with a single couch grass plant. Sugarcane root weight was reduced 36% when grown with two couch grass plants.

Although the critical period of weed competition in sugarcane is up to 90 days after planting, the twining weeds pose a further problem by their nature of growth and by occupying the top plane of the cane, thus extending the critical competition period. Jones & Griffin (2009) showed that red morning glory (*Ipomoea coccinea)* competition reduced sugarcane yield and sugar yield around 27% when the weedy treatment competed with sugarcane from May until harvest. They observed that their trial was cut by hand, whereas in commercial mechanical sugarcane production, when wrapping of sugarcane stalks with red morning glory is extensive, harvest may be abandoned resulting in total yield loss. Red morning glory has been reported as the most common and troublesome broad-leaved weed in Louisiana sugarcane fields (Jones *et al.* 2006); its competition with the crop can reduce yield as much as 30% over a season (Millhollon 1988).

Thakar & Singh (1954) reported that *Ipomoea hederacea* infestations left uncontrolled caused losses of 20–25% in sugarcane in the Pusa area of Bihar.

Future research needs: Define the economic impact from the competition of troublesome weeds such as Guinea grass, sour grass and Ipomoea vines in the Australian sugarcane industry.

***Define the tolerance of Australian sugarcane varieties to the most troublesome weed issues*.**

6.5.3 Yield loss from sugarcane pathogens hosted in weeds

By harbouring sugarcane pathogens, weeds can have an indirect economic impact on cane production. Although the specific role of weeds as hosts to crop pathogens is well documented in other crops, very little research has been carried out in sugarcane.

Sugarcane mosaic virus has a wide host range, including families of Gramineae and Potyviridae, and occurs in many regions of the world.

In recent surveys in Florida for alternative hosts of sugarcane yellow leaf virus (SCYLV), 123 of 141 (87%) plants of *Sorghum almum* tested positive for this virus. *Sorghum almum* is a common weed growing in the Everglades Agricultural Area, and further studies will determine its importance as a reservoir for sugarcane infecting viruses in Florida (Rott *et al.* 2017).

Chenopodium album, *Amaranthus spinosus, Portulaca oleracea* and *Senna occidentalis* and *S. obtusifolia* are common weeds in sugarcane fields in Florida that are suitable food sources and oviposition sites for *Diaprepes abbreviatus* (sugarcane root weevil). Adult residence was highest on *S. occidentalis* followed by *A. spinosus* and *P. oleracea* and caused feeding damage on all weed species. Few adults were observed on sugarcane varieties and *C. album*. Little adult feeding damage was observed on sugarcane varieties. Oviposition of *D. abbreviatus* was observed on all sugarcane

varieties and weed species with exception of *C. album* and *S. obtusifolia*. Significantly more egg masses were found on sugarcane varieties compared to weed species (Odero *et al.* 2010).

Rainbolt & Cherry (2007) evaluated the effect of chemical and mechanical weed management systems on wireworm populations in fallow sugarcane fields. They found higher wireworm counts coincided with greater weed densities and indicated that controlling weeds during fallow periods either mechanically or chemically can result in reduce wireworm populations.

Future research needs: Investigate the role of weeds as hosts of the key pathogens in sugarcane in Australia.

6.5.4 Rapid assessment methods

Remote sensing can be used to assess the economic impact of weed infestation at a district scale, as shown by Simoes *et al.* (2005) who used Landsat 7 images to compare two highly infested areas in the Piracicaba region of Brazil to areas with no infestation. The areas were mapped using GPS and overlaid on Normalised Difference Vegetation Index (NDVI) images to quantify the difference in the spectral behaviour of the various field situations. The results showed significant differences between the infested and weed-free areas in the satellite and NDVI images. The infested areas had significantly lower NDVI values compared with areas with only sugarcane.

Future research needs: Optimise the use of satellite imagery and imagery processing to assess weed infestation at a district scale and target weed management efforts to areas with the highest economic loss due to weeds.

6.5.5 Yield loss from herbicide phytotoxicity

Herbicide toxicity to the cane plant is also an important contributor to yield loss, which has not been measured at the industry scale. The assessment of sugarcane cultivar reaction to herbicides is presently undertaken annually by SRA to assess potential yield losses on newly released varieties (refer to the Sugarcane variety herbicide tolerance section of this review).

6.6 Weed management using herbicides

Chemical methods, known as herbicides, offer a great potential for weed control in crops. Certain herbicides even function on the basis of selectivity by killing only the weed plants and not affecting the crop or valuable plants. Herbicides were first invented in 1933 with Dinoseb; MCPA and 2,4-D were created in 1945. Worldwide, the consumption of herbicides is 43% of all agrochemicals, followed by insecticides (34%) and fungicides (21%). The herbicide market has increased many fold in past 20 years or so.

The popularity of herbicides to control weeds is linked to numerous advantages:

- most effective as compared to other methods of weed control,
- very suitable for closely spaced crops,
- provide early season weed control,
- suitable for adverse soil conditions,
- control many perennial weeds very effectively.

The main drawbacks of chemical methods of weed control are:

- they require some technical knowledge to use,
- they must be applied with proper care using adequate PPE,
- they can produce harmful residues which can affect the succeeding crop,
- they may have unintended off-site impact on neighbouring crops and the environment.

Herbicides kill weeds through various mechanisms within the plant or germinating seed. The way a particular herbicide affects a plant at cellular level is called its mode of action. Herbicides that have similar modes of action are categorised into Groups (chemical family). Using the mode of action group is the easiest way to work out how to rotate herbicides to minimise the risk of resistance developing:

- Soil applied residual herbicides (also called pre-emergent herbicides) are taken up by various parts of germinating seedlings. Most residual herbicides translocate to other parts of the germinating seedling. Soil moisture is important to allow maximum uptake by germinating roots and/or shoots.
- Post-emergence (foliar applied) systemic herbicides translocate to other parts of the weed and although coverage is important, it is not as critical as with contact herbicides. Active weed growth is needed for maximum translocation within the weed. Suitable adjuvants may also increase the absorption of the herbicide, especially by weeds with hairy or waxy leaf surfaces.
- Post-emergence (foliar applied) contact herbicides do not translocate. Coverage of foliage is important to ensure efficacy. Poor coverage may only cause localised burn-off of foliage without killing the weeds. Contact herbicides work best on smaller weeds and tend to not effectively control established perennial weeds.

The *Weed Management in Sugarcane Manual* available to download from the SRA website contains valuable information related to herbicides' modes of actions, their suitability and application, and includes a herbicide selection guide depending on the crop stage or the problem weeds. http://sugarresearch.com.au/wp-content/uploads/2017/03/Weed_Management_in_Sugarcane_Manual.pdf

The South African Sugar Research Institute (SASRI) has recently developed their herbicide selection tool, available as an MS Excel application. The electronic version tool considers the weed spectrum, weed growth stage and soil % clay and suggests a range of active ingredients that will work for the chosen criteria (Campbell & Govender 2019).

6.6.1 Herbicide efficacy to control weeds

Herbicide labels contain valuable information related to their efficacy on target weeds that have been tested during the herbicide registration process. However, many weed species are not covered by most herbicide labels and labels do not compare the performance of herbicides with each other and provide only scarce information related to mixing with other active ingredients. Therefore, the efficacies of currently and formerly registered herbicides in sugarcane have been extensively reviewed in scientific reports and published papers. In contrast to the scarcity of papers studying the ecology of weeds in the sugarcane farming system, an abundance of published papers focus on herbicide efficacy to control weeds in sugarcane. In this review, only a selection of papers of relevance to the present sugarcane farming system context in Australia is presented.

Pre-emergent herbicides efficacy

Many published research papers compared the performance of registered pre-emergent herbicides on specific weed targets and the importance of identifying and validating combinations of active ingredients to improve weed control.

Fillols *et al.* (2020) compared the efficacy and economic costs of a range of registered pre-emergent herbicides in a study in green-cane trash-blanket (GCTB) ratoons in the Wet Tropics region of North Queensland. Several herbicides like amicarbazone, imazapic and isoxaflutole were effective on certain weed species but lacked broad spectrum control. For example, amicarbazone was particularly effective against broadleaves and vines but its efficacy against grasses was limited. Better efficacy results were obtained from products with multiple active ingredients like Bobcat®i-MAXX (imazapic, hexazinone) or Barrage® (diuron, hexazinone) at full rate and demonstrated the benefits of using mixtures of active ingredients to widen the spectrum of weed control efficacy. Barrage at 0.9 kg/ha (maximum rate for the Wet tropics and during some specific windows of application during the wet season in other regions) was not controlling enough weed species and would require a mixing partner.

Seeruttun *et al.* (2007) tested trifloxysulfuron + ametryn (Krismat® WDG75) and amicarbazone alone or in tank-mixes in both plant and ratoon cane. When applied pre-emergence to weeds, Krismat® (1.5 and 1.8 kg ai/ha) and amicarbazone (1.05 and 1.4 kg ai/ha) tank-mixed at low rates overcame their individual weaknesses compared to being applied alone, while maintaining a residual activity over 14 to 16 weeks. The efficacy of Krismat® on many grass species including *Rottboellia cochinchinensis* and some broad-leaved weeds such *Euphorbia heterophylla* has also been reported in Cuba (Diaz *et al.* 2004a).

Fillols (2013) showed that Flame® (imazapic 240 g/L) applied pre-emergence totally prevented the production of new active tubers of nutgrass (*Cyperus rotundus*). Fillols (2014) also found Flame® efficacy on nutgrass varied with the soil type. Near Mackay, it was effective when applied in acidic soil (Kuttabul) or in soil with low CEC and organic carbon (Marian): the production of new tubers was reduced by 99% and their viability by more than 60%. In less acidic, moderate CEC and organic C soil type (Calen), Flame® did not perform well: it only decreased tuber viability by 29%. Flame® was not efficient when applied on clay soil with neutral pH and high CEC (Victoria Plains). The efficacy of Flame® on nutgrass also varied with the quality of its incorporation. Incorporation by irrigation that moves the herbicide down to the tuber zone was the most efficient option.

The efficacy persistence of pre-emergent herbicides throughout the wet season has been studied by Azania *et al.* (2010). During the 120 days following spraying, 698 mm of rainfall was observed and the herbicides control of the weed species in sugarcane were: amicarbazone (91.2% control), imazapic (90.8%), imazapic + sulfentrazone (89.6%), amicarbazone + isoxaflutole (89.2%), imazapic + isoxaflutole (85.6%), diuron + hexazinone + imazapic (84.4%), tebuthiuron (76%), sulfentrazone (70.8%), flumioxazin (19.2%).

Other studies have been focused on optimising the volume of water for specific pre-emergent herbicides. As a general rule, pre-emergent herbicide labels recommend high water rates (250-400 L/ha) to maintain ground coverage when using larger droplets by increasing the number of droplets produced. However, using high water rates reduces the farmer profitability as more tank refills are necessary. Ramos *et al.* (2010) evaluated the efficacy of different volumes of water (70, 100, 150, 200 L/ha) when compared to the standard (250 or 300 L/ha) for the application of imazapic in sugarcane and its effect on soil spray coverage. They found that imazapic applied with spray volumes from 70 L/ha, with a soil coverage of 8.34%, efficiently controlled the weeds in sugarcane when applied either at the beginning, in the middle, or at the end of the crop season, providing gains up to 29.8% in the application operational performance.

Specific research work has been carried out by Gaungoo *et al.* (2010b) to determine the efficacy of some pre-emergence herbicide treatments against *Ipomoea triloba* sown at soil depths of 2, 6 and 10 cm. At 6 weeks after spraying, all herbicide treatments provided satisfactory control, irrespective of seed depth. The residual activity of the various herbicide treatments tested over a period of 18 weeks revealed that only sulfentrazone was found to be significantly superior to the untreated control for seed sown at a depth of 2 cm.

The timing of herbicide application is also an important aspect in the sugarcane weed-management program. Timing for vine control is critical and often not synchronised with the timing of pre-emergence application to control other weed species. Late germination and growth of vines often necessitate specific timing of the pre-emergent herbicides and potential post-emergent herbicides follow up. Viator *et al.* (2002) reported high rates of diuron at 3.36 kg/ha controlled red morning glory 83 to 99% when applied in the first year, and 73% to 75% when applied in the second year. Metribuzin applied at 1.12 kg/ha controlled 96 and 60% of the vine in the first and second years, respectively; the difference in control between the two years was attributed to timing of an activating rainfall, soil pH, and organic matter. Therefore, control of *Ipomoea* spp. with soil-applied herbicide can be inconsistent and post-emergence herbicides are often required. Jones & Griffith (2009) reported red morning glory control 77 days after the application on May 26 was 48% for pendimethalin plus atrazine and 93% for pendimethalin plus sulfentrazone. When application was delayed until June 23, red morning glory control 49 days after treatment was 71% for the atrazine mix and 95% for the sulfentrazone mix. Late season directed application of paraquat with atrazine or sulfentrazone in July controlled red morning glory no more than 39% and sugarcane and sugar yield were reduced an average of 39 and 50%, respectively, compared with the application in June.

Post-emergent herbicides efficacy

A range of papers compared the performance of registered post-emergent herbicides depending on specific weed targets and the importance of identifying and validating combinations of active ingredients to improve weed control. Research papers on post-emergence herbicide efficacy have mainly been targeted to hard-to-control weed species.

On grass species

As Guinea grass (*Megathyrsus maximum*) is from the same Poaceae family as sugarcane, no selective herbicide options are available. Guinea grass growth being faster or similar than sugarcane, it is also difficult to find options for physical selectivity. Fillols (2018) studied the efficacy of a selection of post-emergent herbicides strategies and directed spray application methods to control established perennial Guinea grass as a rescue operation. None of the tested herbicide options and spray application strategies resulted in acceptable control of established Guinea grass in the cane row. Mixing isoxaflutole + MSMA generated strong phytotoxicity symptoms and growth reduction on the grasses but did not kill them. On the other hand, it resulted in strong phytotoxicity on cane and reduced cane yield. Asulam also delayed grass growth (no kill), but it was a safer option on cane and therefore resulted in the highest cane yield. Although all tested application methods (shield, QDAF dual spray bar, 'octopus' leg) failed to control Guinea grass in the row, they were effective to control Guinea grass in the interrow.

Fillols (2018) also compared several herbicides for spot spraying Guinea grass and found that a mixture of isoxaflutole (Balance® at 75 g/100 L) and MSMA (Daconate® at 1.5 L/100 L) was the most effective herbicidal option to use as a spot spray, with the least off-target impact on the adjacent cane stools, however this specific use is not endorsed by the products' labels.

Fall panicum (*Panicum dichotomiflorum*) is the most troublesome annual grass weed associated with sugarcane production in Florida. Like Guinea grass, fall panicum must be controlled very early when it is less than 4 cm in height by combinations of herbicides like atrazine or metribuzin with ametryn. Rescue treatments of fall panicum with asulam applied alone or in combination with trifloxysulfuron were assessed by Odero &Negrisoli (2019). At 28 days after treatment, fall panicum at the booting stage was controlled 93 to 98% with asulam alone (3,740 g ai/ha) or in combination with trifloxysulfuron. Fall panicum control at the heading stage was controlled 90 to 96% with asulam in combination with trifloxysulfuron compared to 28 to 41% control with asulam alone. Similarly, asulam in combination with trifloxysulfuron provided better fall panicum control (88 to 91%) at anthesis compared to asulam alone (31 to 49%). No fall panicum control was observed with asulam alone or in combination with trifloxysulfuron at the seed-filling stage. The authors also showed that fall panicum seed production was only inhibited when rescue treatments (asulam at 3,740 g ai/ha + trifloxysulfuron at 15.8 g ai/ha) were applied between booting and heading stages.

In Louisiana, variability in bermudagrass (*Cyperus rotundus*) morphology and growth characteristics and in control with glyphosate has been observed. Of the 19 bermudagrass biotypes evaluated, five specific biotypes were found least sensitive to glyphosate and seven other specific biotypes were found most sensitive. The least sensitive biotypes were tall-growing with long internodes and wide leaves and were able to retain green foliage later into the winter (Fontenot *et al.* 2015).

In the marginal sugarcane production region of South Florida, growing Giant reed (*Arundo donax* L.) has been proposed as a potential feedstock for bioenergy production. Strategies with asulam at 3.7 kg/ha decreased the probability of giant reed regrowth and therefore are a potential control option for giant reed plant in cane which can curtail its highly invasive potential (Odero & Gilbert 2011).

Further research needs: Specific Guinea grass studies to determine the impact of timing of herbicide application on seed viability need to be conducted to provide growers with more accurate long-term Guinea grass control. Similar studies need to be conducted on other troublesome grass such as wild sorghum, itch grass and sour grass.

*On nutgrass (*Cyperus rotundus*)*

In response to the large economic impact from nutgrass, many research studies have been targeted to assess herbicide efficacy to control nutgrass.

Fillols (2013) showed that both single and double applications of Roundup® CT (450 g/L glyphosate) were very efficient in regard to desiccation of aerial parts and reduction of the production of active tubers by 78–98%. They also reduce the viability of the tubers produced (0–42% viable). As glyphosate is a non-selective herbicide, this strategy is mainly limited to nutgrass control in fallows. Sempra® (750 g/kg halosulfuron-methyl) at 90 g/ha reduced by 90–92% the production of active tubers and only 0–25% of these were viable. Sempra being non phytotoxic to cane, makes it the number one strategy to control nutgrass in crop. Hero® (ethoxysulfuron 600 g/kg) impacted on the production of active tubers by up to 98% and 0–20% of these were viable, butthis active is no longer available in Australia. With Krismat®WG (trifloxysulfuron sodium 8 g/kg, ametryn 731 g/kg), 87% fewer active tubers were produced but 17%–65% were still viable. Flame® applied post emergence reduced the production of active tubers by 77%–88% and their viability ranged from 0 to 56%. Other tested products (2,4-D, MSMA, S-metolachlor) did not sufficiently reduce the tuber production and/or their viability to be considered suitable long-term nutgrass management options. Aitken *et al.* (2011) found similar conclusions related to product efficacies on nutgrass control in their trials in New South Wales.

Further research needs*: *Sempra® (750 g/kg halosulfuron-methyl) is the only selective herbicide registered for Navua sedge control on pasture. Neither the label nor the minor use permit PER80065 allow for application in cane. Furthermore, due to the competitive and persistent nature of Navua sedge, regular applications of herbicide are required. A specific permit for using Sempra® in cane should be applied for.

On vines

As vines are particularly troublesome due to their extended period of germination, their control using post-emergence herbicides is often required. Seeruttun *et al.* (2005) showed that fluroxypyr at rates varying between 0.3 and 0.4 kg ai/ha provided effective control of *Ipomoea nil, I. obscura* and *Cajanus scarabaeoides*, whereas *Paederia foefida* and *Passiflora suberosa* required higher rates (0.4 to 0.6 kg ai/ha). The addition of Actril-DS (2,4-D, ioxynil) to fluroxypyr did not improve the level of control over that of fluroxypyr applied alone. A synergistic effect was observed when fluroxypyr was tank-mixed with atrazine, allowing for lower use rates of fluroxypyr. Earlier studies indicated that an application of 2,4-D was highly effective for control of *Ipomoea* spp.; however, the response was variable with application rates and size (Griffin et *al.* 2000). Jones & Griffin (2009) reported that, when environmental conditions are conducive to prolific growth of *Ipomoea* spp. and they climb sugarcane stalks, 2,4-D remains the most effective treatment. Therefore, pre-emergence herbicides followed by post-emergence herbicides is the best combination for adequate control of *Ipomoea* spp. in sugarcane.

Pre-emergent herbicides with post-emergent properties

Some pre-emergent herbicides also have post-emergent properties and can be applied on early germinating weeds. Pre-emergent herbicides labels include this type of application; however, as labels do not cover the efficacy of many potential mixing active ingredients, weed scientists have studied some of these specific interactions.

Seeruttun *et al.* (2007) showed that both Krismat® (trifloxysulfuron sodium, ametryn) and Dinamic® (amicarbazone) were effective on most broadleaved weeds and some grasses when applied early post-emergence of the weeds. The efficacy of Krismat® on *Paspalum* spp*., C. rotundus* and other sedges, and that of Dinamic® on *Digitaria horizontalis,* compensated for their individual inefficacies when they were tank-mixed at a low individual rate. The tank-mixes were also well tolerated by both young plant and ratoon cane.

The efficacy of Krismat® as early post-emergence on many grass species including *Rottboellia cochinchinensis* and some broad-leaved weeds such *Euphorbia heterophylla* has also been reported in Cuba (Diaz *et al.* 2004a).

Hernandez *et al.* (2004) also reported that Krismat® effectively controlled annual grasses including *Rottboellia cochinchinensis*, *Echinochloa colona*, *Eleusine indica* when applied postemergence under moderate to high soil moisture, while *Sorghum halepense* was not controlled. 2-2.5 kg/ha commercial product, plus 0.1 % v/v non-ionic surfactant, is recommended: the lower rate in a younger weed growth (5-15 cm) stage, and the higher rate in a more advanced (20-40 cm) stage. Under low soil moisture, herbicide efficacy was overall poor.

6.6.2 Herbicide performance management

Timing of application

In rainfed systems, it is recommended to apply pre-emergent herbicide before a (non-inducing runoff) rain front, that would perform the required level of incorporation.

A worst-case scenario for pre-emergent herbicide efficacy would be to apply herbicides after a rainfall event that has induced weed germination, without any following rain events (no herbicide incorporation). The herbicide would be concentrated on the soil surface (or on the trash blanket) and weeds could germinate and grow from below.

Post-emergence applications must be carried out at the weed growth stage as indicated in the product label. In general, small weeds are easier to control with lower herbicide rates. Crop stage, weather and environmental conditions must also be considered for spraying to maximise efficacy and minimise off-target impacts.

Weed emergence models based on hydrothermal degree can be used to predict the ideal timing of weed control measures. These models rely on equations such as Gompertz, Weibull or logistic, in which daily soil temperature and moisture are required inputs. These models are good predictors of weed emergence patterns at local and regional scales where fields have similar climatic and soil conditions but lose accuracy when extrapolated to different scenarios. Izquierdo *et al.* (2016) showed that, when using the differential form of the sigmoid equation used in these models, validation at local scale and starting date were not required because calculations were based on sigmoid relationships between data recordings. The model showed that the best time to spray is when weed emergence reaches 95%.

Weed emergence models are included in most complex models such as the Weed Seed Wizard and will recommend the best timing of application based on the percentage weed emergence for specific weed populations.

Drift management

Herbicides must be applied under the appropriate weather conditions to minimise the movement of chemical by drift, increase efficacy and reduce costs. To optimise herbicide performance, it is important to:

- Use nozzle types and operating pressures to produce a coarse spray quality or larger, booms should only be 50 cm above the top of weeds for a 110˚ nozzle at 50 cm boom spacing.
- Avoid surface inversion conditions. These conditions exist some evenings one to two hours before sunset and persist until one to two hours after sunrise. During inversion conditions, damaging concentrations of agricultural chemicals can drift for many kilometres and affect sensitive crops. The following signs can be indicators of surface inversion conditions: mist, fog, dew, frost, smoke or dust hangs in the air and moves sideways just above the ground surface, wind speed is constantly less than 11 km/h in the evening and overnight, distant sounds become clearer and easier to hear.
- Spray when there are consistent light winds, 3 to 20 km/h (3-15 km/hr for Group I herbicides) blowing away from sensitive areas (crops, plants, houses), mild temperatures and high relative humidity.

Growers and spray contractors are required to research the weather before spraying, monitor the weather while spraying, and record the weather details.

As of 3 October 2018, the Australian Pesticides and Veterinary Medicines Authority (APVMA) has issued new spray drift label instruction requirements for 2,4-D herbicides. These instructions now include new requirements relating to droplet size, travel speed, boom height, buffer distance, record keeping, and inversion weather conditions targeted at reducing spray drift.

Specific management considerations for soil-applied herbicides

Amongst herbicides, pre-emergence herbicides require higher technical knowledge as many factors that can impact on their efficacy must be considered. These considerations are extensively described in a reference manual published by Congreve & Cameron (2019).

Photodegradation

Photodegradation is the process in which the herbicide is broken down in the presence of sunlight and lost to the weed control system. It occurs when an herbicide is sprayed onto a dry soil surface or trash, with no following rainfall or mechanical incorporation. To limit photodegradation, standard incorporation practices such as cultivation or sufficient rainfall after application are adequate. Herbicides registered in cane that are particularly susceptible to photodegradation include the Group C herbicides atrazine, terbuthylazine and diuron, the Group D dinitroaniline herbicides pendimethalin and trifluralin, and the Group K herbicide S-metolachlor.

Volatilisation

Volatile herbicides transition to a gaseous phase after application if left on the soil surface without incorporation. Volatility is measured as vapour pressure: herbicides with a low vapour pressure (i.e. less than 1 mPa) are generally referred to as 'non-volatile', whereas products with a vapour pressure above 1 mPa may convert into a gaseous phase. Trifluralin, S-metolachlor and pendimethalin have a vapour pressure >1mPa and therefore need quick incorporation. In addition to the volatility of the compound, the speed of herbicide loss to volatility increases with environmental factors (i.e. high temperature, high moisture, low binding soil types, presence of trash, high wind speed).

Incorporation

To reduce loss from volatilisation and photodegradation of some pre-emergent herbicides, they need to be incorporated within hours or days of application. For other pre-emergent active ingredients with a very low vapour pressure and UV stability, they can remain on the soil surface for weeks. When incorporation is advisable, the objective is to move the herbicide into the top few centimetres of soil where it will be protected from UV degradation and volatilisation, yet still keeping it in the zone required for weed control.

Mechanical incorporation - This form of incorporation using light to moderate mechanical cultivation works well for highly volatile products such as trifluralin provided it is done within hours of the spray application. However, in a GCTB situation or in situations where minimum tillage practices are implemented, mechanical incorporation is unsuitable.

Incorporation by rainfall - Spray applications should be timed prior to a forecast rainfall event. However, the unreliability of rainfall forecasts often leads to inconsistent results. Herbicides with low water solubility often require larger volumes of rainfall to achieve incorporation and need good moisture conditions after application and also for the period of desired weed control (i.e. trifluralin,

pendimethalin, flumioxazin, isoxaflutole, terbuthylazine, atrazine, diuron). Conversely, herbicides with moderate solubility (S-metolachlor) are relatively easy to incorporate with limited rainfall and prefer to remain in the soil moisture phase where they are more freely available to the plant or weed. However, if the herbicide is highly soluble (i.e. metribuzin, hexazinone, imazapic, amicarbazone), it will tend to move with the soil moisture and be more likely to leach under the weed root zone or cause off-target effects.

Incorporation by irrigation - Overhead irrigation can be used to incorporate some herbicides. The volume of water required will depend upon the soil type, ground cover, solubility of the herbicide and the existing soil moisture. Typically, a 5 to 10 mm irrigation event is usually satisfactory for herbicides with higher solubility, while 20 to 50 mm may be required for herbicides with 'low' solubility. It is important not to over water and risk moving the herbicide down the soil profile before binding has occurred. Furrow irrigation is not recommended for herbicide incorporation for the following reasons: -unevenness of soil wetting between the start and finish of the furrow; -too much irrigation water usually applied; -runoff into tail ditches; -the herbicide located in the row is only incorporated by capillary action from below which may result to an inadequate level of herbicide incorporation in these zones.

Incorporation by sowing - This type of incorporation is not practiced in cane. It is a tactic used extensively in reduced and zero till farming systems in the grains industry. After the herbicide has been applied broadcast, a knife point seeder is set up to 'throw' a small amount of treated soil out of the sowing furrow and onto the interrow to cover the herbicide which has been previously applied to the soil surface. Typically, this will only work with seeders set up to plant on approximately 25 to 30 cm row spacing and a higher herbicide application rate is often recommended to compensate for the losses due to the herbicide bound to the stubble, the herbicide in the crop row being largely displaced into the interrow and/or incomplete herbicide coverage.

Further research needs: Incorporating pre-emergent herbicides while sowing an intercrop in sugarcane interrows could be investigated.

Incorporating herbicides by furrow irrigation is a practice used in the Burdekin, but this practice is causing pre-emergence efficacy / phytotoxicity issues. Further research would be needed in the area to better optimise the use of pre-emergence herbicides.

Impact of soil type

The type of soil often impacts on the performance of the pre-emergent herbicide. Soil texture (the ratio of sand, silt, and clay) and soil organic matter will have an effect on the binding ability of the herbicide (adsorption). Heavier clay soils and soils with higher organic matter have more binding sites (higher CEC) and often require higher herbicide application rates, as less herbicide is available in the soil water for uptake by germinating weeds. Increased binding also generally results in less leaching. Conversely, in sandy or low organic matter (lower CEC) soils, more herbicide is likely to be available in the soil water. This may lead to increased risk of injury to crops soon after application, especially for highly soluble herbicides. As a result, some labels recommend a lower application rate in lighter soils (e.g. isoxaflutole, S-metolachlor). Duplex soils with a sandy shallow topsoil over a heavier B horizon can be particularly challenging. Low binding and high availability may apply in the A horizon, but strong binding and therefore persistence of the herbicide may occur in the B horizon. This can lead to high levels of exposure to the crop just after herbicide application, with long lived persistence for some products which could affect subsequent crops.

The degree of herbicide binding can be better predicted by considering the Herbicide Soil/Water Adsorption Coefficient (Kd). The herbicide Kd value is the ratio of herbicide adsorbed onto the soil in comparison to the amount remaining in the soil water. As binding is highly influenced by the level of

organic matter, the binding coefficient is often normalised to consider organic carbon levels in different soils and is presented as a Koc value. The Koc value is calculated by the equation: Koc = Kd / soil organic carbon. Herbicides with high Koc value are more tightly bound to the soil (e.g. pendimethalin, trifluralin). Herbicides with a low Koc are less tightly bound to the soil and more freely available in the soil water (e.g. metribuzin, amicarbazone, hexazinone). For some herbicides, Koc is very sensitive to soil pH, in particular the imidazolinone herbicides (e.g. imazapic) which bind tighter at low pH.

Fillols (2013) confirmed the relationship between soil pH, soil CEC, and OM and imazapic in an efficacy pot trial on nutgrass. Imazapic was effective when applied in acidic soil (Kuttabul) or in soil with low CEC and organic C (Marian). In less acidic, moderate CEC and organic C soil type (Calen), Flame® did not perform well: it only decreased tuber viability by 29%. Flame® was not efficient when applied on clay soil with neutral pH and high CEC (Victoria Plains).

Warne & Peta (in review) have demonstrated that KOC values were the most adequate predictor of pesticide mobility as it correlated with solubility, predicted KOC and experimental pesticide loss. They developed the Pesticide Decision Support Tool based on pesticide relative mobility and calculated by dividing the KOC of each active ingredient by the largest KOC pesticide (diquat dibromide) used in the sugar industry.

Within field, the heterogeneity of the soil affects the efficacy of pre-emergence herbicides. Metcalfe *et al.* (2018) observed that soil with high organic matter had more surviving weeds with higher biomass than the low organic matter soil. In high organic matter soil, modelling results show these surviving plants recovered to produce the same amount of seed as if no herbicide had been applied (less weed competition than in untreated plots) showing that weeds surviving pre-emergence herbicides could compensate for sublethal effects. The ED50 (median effective dose of pre-emergence herbicide) was higher for controlling weed seed production than seedling mortality or biomass with greater difference on high organic matter soil.

These results show that the application rate of herbicides should be adjusted to account for within-field variation in soil organic matter. Modelling results emphasised the importance of crop competition in limiting the capacity of weeds surviving pre-emergence herbicides to compensate and replenish the seedbank.

In plant cane, Fillols (2018) confirmed the accuracy of soil type recommendations on product labels when medium and high rate of isoxaflutole and imazapic resulted in phytotoxicity on cane when used in some light soil types with low CEC.

Impact of soil moisture

With low soil moisture (available soil water), lower levels of herbicide will be dissolved in the available soil water resulting in less root uptake. This explains why pre-emergent herbicides with low solubility may fail to provide good weed control under dry or ‘lower soil water‘ conditions. To maximise their performance, good soil moisture is required from incorporation through to the desired period of weed control. After an herbicide is incorporated, it typically takes several days to establish an equilibrium between the amount available in the soil water and that binding onto soil colloids and organic matter. Labels generally have a statement ‘Do not irrigate’ or ‘Do not apply if runoff rainfall is expected’ within 2 or 3 days after application, to allow time for soil binding to take place and the equilibrium to be established. Once an equilibrium is established, it is an active process with herbicide constantly sorbing or desorbing from binding sites in ratios defined by its binding coefficient values.

6.6.3 New herbicides - registration work

The latest registered active ingredients or combination of already registered actives for use in sugarcane in Australia include:

- Bobcat® imaxx (imazapic + hexazinone) registered in 2015 and Bobcat® imaxx SG in 2018,
- Amitron® (amicarbazone) registered in 2018,
- Valor® (flumioxazin) registered in 2018,
- Palmero® TX (terbuthylazine + isoxaflutole), registration pending.

Chemical companies can carry out efficacy trials required for registration work if they have the capacity, however that role is often being outsourced to private companies or individual contractors. Some of these contractors in North Queensland are Darren Westerhuis (ex-Bayer), Charissa Rixon (ex-Syngenta), Bill Farnsworth (ex-Eurofins). There are others further south in Bowen, Mackay, and Southern Queensland. Sugar Research Australia or productivity services are rarely involved until the product is available on the market.

Further field studies on newly registered actives are always required to fine tune the manufacturer information in terms of: weed control on weed species not tested in the registration process; the effect of farming systems and soil types not tested on the compound efficacy; the toxicity of the compound to newly released cane varieties; the efficacy of the compound mixed with other herbicide partners; and the runoff loss potential of the compound in different soil types compared to other products. This additional field work is essential to assist growers in optimising product use. Unfortunately, due to the risk of a new product's application for registration failing, these further trials tend to start after the product has been released on the market. In the meantime, growers often tend to make their own assessments and opinions which are often anecdotal and potentially incorrect.

Of potential interest to control weeds in the Australian sugarcane cropping system, the pre-emergent herbicide Lumax®, consisting of three active ingredients mesotrione (0.0375 kg ai/L), terbuthylazine (0.125 kg ai/L) and S-metolachlor (0.375 kg ai/L), has been evaluated as a substitute for atrazine in Mauritius in field trials in both plant and ratoon sugarcane. Lumax® at rates varying between 3.5 and 5.0 L/ha proved effective on a wide spectrum of broad-leaved weeds and some grasses, including *Digitaria horizontalis*. In general, Lumax® was superior to the standard S-metolachlor + atrazine. In all situations, it provided a residual activity varying between 10 and 12 weeks and showed no phytotoxicity on the various sugarcane varieties tested (Seeruttun *et al,* 2010).

Orgeron & Spaunhorst (2018) also show that Lumax® EZ (S-metolachlor + atrazine + mesotrione) tank mixed with pendimethalin (202 plants/ha), clomazone (120 plants/ha) or metribuzin (209 plants/ha), provided greater itchgrass control as compared to non-treated check (2,803 plants/ha).

The post-emergent herbicide topramezone, registered in 2017 in the U.S., provides an additional option to sugarcane growers. Topramezone exhibited activity on a wide range of difficult to manage grass and broadleaf weed species, including bermudagrass (*Cynodon dactylon*) and fall panicum (*Panicum dichotomiflorum*). Sugarcane showed good tolerance to the active at labelled rates, with a potential for minor transient foliar chlorosis (Caffrey *et al.* 2017).

6.6.4 Interactions between herbicides and the sugarcane farming system

Pre-emergence and post-emergence herbicides in sugarcane directly interact with the specific environment they have been sprayed in. In the case of sugarcane, a thick trash blanket presents particularly challenging conditions for some pre-emergence herbicides. On the other hand, ashes resulting from burning the trash residues can also interact with some pre-emergent herbicides. For

the post emergence herbicides, application in crop can present some challenging spray applications issues and fallow spraying may represent the best application opportunities.

Controlling weeds on trash blanket

Herbicide intercepted by standing organic material is subject to a certain level of binding depending on the herbicides' characteristics (see previous section on herbicide performance management). Some herbicides can bind tightly to crop residues and become lost to the system in terms of weed control, despite subsequent rainfall (e.g. trifluralin). Others are loosely bound and relatively soluble and can be returned to the soil by rainfall that 'washes' herbicide off the organic material (e.g. isoxaflutole, imazapic). To understand the potential level of binding of a herbicide to the trash residues, its binding coefficient (Kd or Koc) and solubility must be considered. However, even if a herbicide's properties make it suitable for spraying on crop residues, it still may be prone to loss due to volatility and photodegradation before it is incorporated into the soil by rainfall.

Specific research work has been done to better understand the interaction of pre-emergent herbicides with a trash blanket. Selim *et al.* (2001), Perez & Chao (2004) and Fillols & Callow (2010) showed a selection of pre-emergent herbicides applied on ratoons were efficient even when applied on trash blanket. Early applications (just after harvest) of pre-emergent herbicides such as diuron, hexazinone, isoxaflutole or imazapic applied on fresh trash blanket (in dry soil and incorporated 20 days later) were effective weed management strategies. Their incorporation and activation with the first rainfall coincided with weed emergence and need for weed control. These herbicides were effective at controlling weed emergence for up to three months (Fillols 2012). Similar results were reported in previous trials when the incorporation was delayed for one month after spraying (Fillols & Callow 2011). Fillols & Callow (2010, 2011) also noted that pre-emergent herbicides applied on trash were efficient regardless of the thickness of the trash layer. However, moist soil under trash at application followed by no rapid incorporation negatively affected pre-emergence herbicide efficacy. Weeds emerged under the trash while the herbicide was still sitting on the trash surface.

Selim *et al.* (2010) investigated the effectiveness of trash residue on retention of applied atrazine on sugarcane. Their results indicated that the rate of atrazine retention by the residue was similar for the entire growing season with an average value of Kd = 17.9 L/kg. They found that retention capacity of the residue for atrazine did not change significantly with the age of the decaying residue over the growing season.

Late application (before canopy closure) of pre-emergence strategies on weathered trash blanket was also tested by Fillols (2012). Late applications of pre-emergent herbicides achieved good results from their time of application onwards but were not significantly better to control the weeds than a knockdown-only application. The best options in a wet year were the early pre-emergence strategies or the late knockdown.

Fillols & Callow (2010, 2011) and Fillols (2012) also noted that application of pre-emergent herbicides on GCTB rarely generated significant increase in cane yield, because the weed infestation was partially suppressed by the GCTB itself. Nonetheless, weed emergence reduction has the long-term positive impact of reducing the weed seed bank and future weed infestations.

To reduce the level of spray droplet interception by the surface residues in grains, Congreve &Cameron (2019) recommend some spray techniques that can increase the proportion of herbicide reaching the soil. These techniques include: -using rear facing nozzles where the angle offsets the travel speed to have droplets moving predominantly downwards through the stubble; -using larger droplets travelling at higher speed; -keeping water rates high to maintain coverage when using larger droplets; -narrowing nozzle spacing (25 cm vs 50 cm); -reducing travel speeds (i.e. < 16 km/h)

to reduce horizontal movement (forward trajectory of droplets), and; -minimising boom height while ensuring at least double overlap.

Further research needs: Some techniques to improve pre-emergent herbicides application on crop residues used in the grain industry have not been tested in cane. Optimising the application of pre-emergent herbicides in cane could result in better weed control and potential opportunities to use a wider range of pre-emergent herbicides or reduce the label rates of currently effective herbicides on trash blanket, with consequent economic and environmental benefits.

Controlling weeds on burnt trash

Although most of the Australian sugar industry harvests green and lets the subsequent ratoon crop grow through the green-cane trash-blanket, in some cases the remaining trash blanket is burnt for agronomic or practical reasons. The Burdekin region also mostly burns the cane before harvesting. A hot burn can assist with weed control as it ensures maximum mortality of the weed seeds, but also maximise the amount of residue converted to ash and minimise the amount left as charcoal. Herbicides do not generally bind tightly when sprayed onto ash. However, a thick layer of ash may prevent even soil coverage, unless a rainfall event has occurred between the burning and the herbicide application to disperse the ash. Conversely, herbicides do usually bind to charcoal to an even greater extent than they do to green organic matter or stubble. Where charcoal is left after a burning event, then it is likely that less herbicide will be available for weed control and herbicide performance may be compromised (Congreve & Cameron 2019).

Use of soil conditioners

Soil conditioners like organic amendments or mill by-products can bind pre-emergent herbicides tightly and reduce their efficacy to control weeds. In the current project SRA 2017/008, mill mud-ash mix applied broadcast and incorporated in plant cane before pre-emergent herbicides application reduced the efficacy of amicarbazone + isoxaflutole by 24% and imazapic + hexazinone by 44%. When spread as a band over the row in ratoon, mill mud-ash reduced the efficacy of amicarbazone + isoxaflutole up to 17% and imazapic + hexazinone up to 54%, however results vary greatly between trials (unpublished data).

Evenza *et al.* (2005) also found a significantly greater weed incidence on amended than non-amended soil, with weed (mainly *Cyperus* spp.) ground cover reaching 62.5% on amended plot versus 6.5% on non-amended plots. Therefore, agronomic management programs that utilise organic amendments to improve organic matter of sandy soils in south Florida will need to address potential problems associated with achieving proper weed control.

Further research needs: The interaction between soil conditioners and pre-emergent herbicides has been poorly studied and may result in frequent weed control failure. Better understanding of these interactions and development of alternative control strategies would greatly improve weed management for farms that use soil conditioners.

Controlling weeds in fallows

In addition to improving soil productivity and reducing other pest populations, a fallow period helps overcome weed infestations. Fallows offer the best opportunities to reduce the weed seed bank as a new range of weed management strategies and herbicides become available. In bare fallow, the use

of non-selective herbicides like glyphosate is a very cost-effective option to reduce flushes of weeds before they set seeds and gradually exhaust the weed seed bank.

A bare fallow provides a unique opportunity to control difficult weeds such as nutgrass, Guinea grass, sour grass, and couch grass with glyphosate. The use of selective herbicides like haloxyfop, fluazifop-P or acifluorfen makes it possible to control these weeds within legume fallow, but fallow blocks heavily infested with problem weeds should not be planted with legumes so as to facilitate access and use of repeated applications of inexpensive, non-selective herbicide if needed to reduce the weed seed bank.

Etheredge *et al.* (2005) found that bermudagrass (*Cynodon dactylon*) control when tillage and Roundup® UltraMAX were used was excellent (less than 5% ground cover). When bermudagrass was not controlled in fallow, sugarcane and sugar yields were reduced approximately 40%.

When going back to cane, the false seed bed technique offers a further opportunity to reduce the weed seed bank. This technique consists of inducing the germination of weed seeds with soil preparation and/or rainfall before planting the crop so that a few flushes of germinated weeds can be sprayed and killed.

6.6.5 Application methods

Chemical application accreditation

To apply restricted agricultural pesticides, the spray operator must have followed a AQF3 Chemical Accreditation course and hold a "Chemical Handling Certificate" or "Chemical ticket". The course focuses on upskilling chemical users on the industry's best practice methods and national standards. Participants are provided with practical advice covering industry's updates and local resistance and spraying issues to minimise chemical costs, limit spray drift and improve spraying outcomes. The ChemCert AQF3 Accreditation includes the two units AHCCHM304 Transport and Store Chemicals and AHCCHM307 Prepare and Apply Chemicals to Control Pest, Weeds and Diseases. It is nationally recognised for five years and enables the holder to legally use restricted chemicals unsupervised.

In Queensland, the Agricultural Chemicals Distribution Control (ACDC) / Commercial Operators licence is required for those who ground spray herbicides with a powered machine as part of a job on land they do not own or occupy.

Spray technology

Spray pumps

Spray pumps are a critical part of every spray system, ensuring adequate flow and pressure required for specific application. Diaphragm pumps are the most popular type seen in the sugar industry. They easily produce the higher pressures needed for applying herbicides. Available models provide maximum outputs ranging from 16 L/min to 200+ L/min and maximum pressures ranging from 144 kPa to 1378 kPa. Diaphragm pumps are extremely durable because all moving parts are sealed in an oil bath and spray solutions. Smaller electric diaphragm pumps are available for use for ATV or interrow tractors to apply herbicides (i.e. a spray system mounted on an ATV for spraying interrow for vine control).

Rate controllers

Automatic rate controllers ensure consistent application volumes. They control the spray liquid pressure by opening or closing a bypass valve. The rate controller allows the applicator to enter a

desired application volume and the controller sets the spray pressure that gives the necessary flow for the application volume and sprayer travel speed being used. In practice, this means that higher travel speeds result in higher spray pressure, and vice versa.

Unfortunately, rate controllers do not take into consideration how pressure affects nozzle performance. Pressure affects the flow rate of the nozzle, the spray pattern (fan angle) and the spray quality (droplet size range), which impact on coverage, overlap, and spray drift.

A new technology Pulse-Width Modulation (PWM) system has been developed by a range of manufacturers (Case, John Deere, Teejet, Miller) to alleviate the variable pressure generated by rate controllers. PMW uses solenoids that rapidly open and close to control spray rate at individual nozzles. PWM holds the pressure constant while it varies the duty cycle on the nozzles to match the application rate required. PWM maintains droplet size and improves coverage at varying speed and rate ranges.

Direct injection

The process of direct injection means the primary tank contains only water, and since mixing happens in-line, there is no chemical entering the main tank. The result is less chemical waste, faster, more efficient mixing in the field and quicker clean-out.

Raven manufactures a two-tank, two-pump direct injection system called Sidekick Pro. AgChem, John Deere and Hagie use the Raven's system. Chemicals are stored in separate tanks, each with its own injection pump. The main drawbacks of the technology are the cost (15 to 25% of the price of the sprayer) and the need to pour concentrated chemical into holding tanks.

Dosatron Chemical injectors are water-powered, non-electric chemical injectors which provide an easy and reliable way to accurately inject chemicals into water lines. Dosatron injectors work using volumetric proportioning, ensuring that the chemical mixture remains the same regardless of variations in pressure and flow. Dosatron chemical injectors have become popular to apply Confidor® Guard for canegrub control. They could be also used for herbicide application to reduce left over chemical brews in the tank. Dosatron users pump the chemical straight from the original chemical container to reduce the handling risk. https://www.dosatronusa.com/

Guidance systems

Guidance systems and advanced controls have made sprayers easier to operate. Guidance monitors can help monitor and map fields more accurately with options such as straight or curved A|B lines, last pass, fixed contour and pivot guidance options. Improved accuracy and precision allow users to cut down on input costs and save time in the field. Other benefits of using guidance monitors include cutting down on driver fatigue and the ability to work in lower visibility settings. Serving as a foundation to many other precision technologies, guidance monitors can work with other systems such as section control (turn a section of the boom on or off, to reduce overlap and overspray at the row ends) and assisted-steering systems (ensure parallel pass-to-pass accuracy).

Teejet is proposing some new options like the droplet size monitor and the tip flow monitor to identify plugged spray tips. http://teejet.it/english/home/literature/image-library.aspx

Nozzles

The main hydraulic nozzles used for spraying herbicides in the cane industry are flat fans and flood jets. Tapered fans are used in boom sprays while even fans are used for band spray over the cane row. Flood jets are often use with droppers to cover the interrow: one flood jet will cover 1.5 m, but

it does not deliver an even spray coverage. WILGA Spraying technology is now offering COMBO-JET® spray tips which use a patented 'all-in-one' design, so the cap, tip, O-ring, and strainer all snap into one piece, making them safer and easier to handle. The Combo-Jet® strainers also are 40% longer than other brands, so they plug less. https://www.wilger.net/sprayers/

For spot spraying, a hand wand with a flat fan nozzle is normally used; however, to control some problem weeds such as Guinea grass with herbicides that require an optimum spray coverage, a hand gun nozzle (i.e. Spray Turbo 400 handgun with a 2.5 mm ceramic jet delivering up to 5 L/min at 2.5 bar) is more effective. https://sugarresearch.com.au/wp-content/uploads/2017/02/IS17011-Spot-spraying.pdf

Further research needs: Other types of nozzles and spray tips are now available on the market and their relevance for the sugarcane cropping system still needs to be established.

- ***3D NOZZLE (Pentair, John Deere). This new nozzle model reduces drift while maintaining full efficacy and coverage (drift potential reduction of up to 50–75% compared to conventional flat fan nozzles). It is particularly efficient on vertical targets and soil clods. This nozzle could be tested to spray grasses and nutgrass and for pre-emergent herbicides applied on bare soil and on trash blanket.***
- ***ULTRA LOW-DRIFT MAX. Compared to flat fan nozzles, it features a 95% reduction in drift, with a 130° wide spray angle for effective pattern overlap at low boom heights. This nozzle could potentially optimise interrow spraying (i.e. with Irvin leg) https://www.deere.com.au/assets/pdfs/common/parts-and-service/parts/JD1377-sprayer-nozzle-brochure.pdf***

Boom sprays

A boom spray is used to control weeds in fallow and to broadcast pre-emergence and selective post-emergence in the early growth stage of the cane crop. A range of new technologies have been developed to improve boom spraying. None of these technologies have been adopted in the sugarcane industry because of their upfront cost and the uncertainty in terms of added profitability.

- To reduce crop damage, tractor control technologies have been developed to manage the draw bar or the steering axle of a towed crop sprayer and steer the wheels of the sprayer to precisely follow the tram line, minimising crushing of the crop.
- To increase accuracy, boom control can avoid the problem with rigid booms that can sway up and down at their ends as the tractor moves over bumps and hollows in the paddocks, impacting on spray accuracy and efficacy. Boom levelling control technologies have been recently developed (i.e. Visio BLC system). They adjust the boom with precision, thanks to ultrasonic sensors that measure the distance from the ground. Another technology that improves spray accuracy is the ExactApply nozzle control (John Deere). This retrofit kit allows nozzles to be controlled individually from the sprayer cab. Herbicide can be placed more precisely and overlap minimised. Built-in turn compensation varies application rates along the boom through the turn to reduce crop damage. The system also allows to switch between two pre-positioned nozzles on the fly in order to adapt to changed conditions.
- To increase herbicide penetration, the spray air boom technology (Miller) controls droplet size, spray pattern, and the speed of the air blast spraying into the crop. Air nozzles, spaced every 10 inches along the air boom, blast the spray droplets deep into the crop canopy, ensuring complete top-to-bottom leaf surface coverage. Because of the smaller controlled droplets and directed air blast, the technology allows for a very efficient use of water, often in the range of 20 to 50 L /ha for herbicides.

Direct spray

Wide row spacing enables two different methods of weed control to be implemented, with non-selective chemical or physical control methods utilised in the wide interrow zone, with or without selective chemicals used on the row only (Peltzer *et al.* 2009). Lundkvist *et al.* (2016) showed that a combination of interrow hoeing and intra-row spraying in spring oilseed rape gave similar weed control and crop yields as broadcast spraying and diminished the overall use of herbicides up to 65-70% in comparison with broadcast spraying. Cultivation of the interrow zone in plant cane is still common in some regions like the Far North Queensland, however the practice is not recommended from soil health and erosion perspectives.

Zonal herbicide applications are the recommended practices in sugarcane and directed herbicide applications targeted either at the row or at the interrow are common.

- Directed applications over the row are generally done using a single nozzle centred directly over the row. Applications of pre-emergence herbicides to control the weed emergence over the row can be done this way. In ratoon cane on trash blanket, weeds tend to be more abundant over the row than in the interrow. The interrow is subject to more compaction, higher amount of trash blanket and more wheel traffic that all impede on weed emergence. The best distribution for band application over the row is obtained using even-spray nozzles, whereas standard flat-fan nozzles have an uneven distribution with a peak just below the nozzle centre and less spray towards the edges of the spray swath.
- Directed application in the interrow can be done using droppers or Irvin legs that spray the interrow and the row edges. Irvin legs and droppers are the most common spray delivery systems in the Australian sugar industry. They allow post-emergent herbicide applications to be applied under the cane canopy, minimising the contact with the crop. However, Irvin legs are often incorrectly set up with inadequate nozzles and/or nozzles angled incorrectly (Fillols, pers. obs.). The Dual Herbicide Sprayer (DHS) has recently been developed by QDAF to allow the use of systemic knock-down herbicides in sugarcane without the requirement for a shielding hood. The DHS is a relatively low-cost modification to the common Irvin type boom consisting of replacement spraying heads for the Irvin leg, a small herbicide tank, separate plumbing, pressure gauge, regulator, and electric pump. The DHS uses an air induction nozzle to apply glyphosate to the interrow while 'conventional' herbicides that a grower would normally use such as paraquat, PSII's and 2,4-D are applied to the row edges through low drift flat fans. Despite some concerns about glyphosate toxicity on cane, trials showed no significant impact on cane yield or growth measurements. Presumably paraquat, a desiccant herbicide, prevented uptake of any non-target glyphosate applied to the row edges. The new spray bar had potential to become a useful weed-management tool in sugarcane production in the Wet Tropics region and it could also assist growers to both comply with the Reef Regulations and usage change (Blair *et al.* 2019).

Further research needs: Investigate the impact on herbicide efficacy of nozzle types and nozzle angles fitted to Irvin legs and droppers to demonstrate to growers the importance of correct nozzle configurations.

Shields or Hoods

Spray Shields or hoods are a safer way to apply non-selective herbicide in the interrow. When spraying glyphosate in the interrow, shields are important to protect the cane from the spray and the drift. Shields are not widely used by sugarcane growers for a range of reasons:

- They increase the sprayer weight, which can limit access to wet paddocks.

- Some cane heights or canopy structures may be unsuitable to spray with shields (some actively growing cane leaves positioned close to the ground could be sprayed).
- Herbicide can drip from the edge of the shield if nozzles have not been set up correctly. Glyphosate dripping close to the cane row has occasionally resulted in crop phytotoxicity. The *Weed Management in Sugarcane Manual* offers instructions to address this concern and set up nozzles correctly.

Shielded sprayers can be efficient to control some particularly troublesome weeds in the interrow. Fillols (2018) studied the efficacy of directed spray application methods to control established perennial Guinea grass as a rescue operation. While the interrow shielded sprayer failed to control the grasses in the row, it was 100% effective in controlling them in the interrow. For some grass species with a creeping habit or with stolons, shields are more beneficial as the herbicide can translocate and affect weeds located in the row. Griffin *et al.* (2012) observed that even though spray hoods confined glyphosate spray to the row middles and row sides, some control of bermudagrass (*Cynodon dactylon*) was obtained when stolons originating from the sugarcane drill were contacted with herbicide. At 210 days after treatment, bermudagrass control on the row tops was 85 and 33% for glyphosate at 3.36 kg/ha at each trial location.

High-rise sprayers

High-rise sprayers are used to access the cane field after canopy closure. Late vine germinations that have escaped previous herbicide applications can be controlled after out-of-hand stage by fitting a boom or droppers to these machines.

Aerial spraying

Aerial spraying is an alternative to high rise sprayers to control vines after the out-of-hand stage. Aerial contractors using planes or helicopters are delivering such services. Spraying with UAV technology is recently being explored with the main limitations being the spray tank size and the flight duration (see next section).

Latest spray technology advances

Precision application

Automatic spot-spraying weeds instead of spraying the bare ground or the crop can have many economic and environmental benefits. Major technological progress in weed detection and precision application has been made in the recent years, and the technology is becoming available for many crops.

Green from brown sensors

The optical spot spray technology relies on infrared sensors to detect chlorophyll in the leaves of weeds in fallow paddocks (i.e WEEDit technology https://www.croplands.com.au/Products/WEEDit-Optical-Spotspraying#.XrS5q9bivyQ, Weedseeker technology http://www.mcintoshdistribution.com.au/machinery/show/weedseeker-australia). The sensors detect “green from brown” and only trigger the nozzles to spray the green weed patches. This technology is successfully used in broadacre fallow spraying and more rarely under shield in row crops. Bramley *et al.* (2015) compared using a Weedseeker® to spot-spray four sugarcane fallow

fields with different soil types to a blanket herbicide application. The herbicide savings ranged from 14.5% to 80%. However, only the fallow trial on black soil tested with the Weedseeker® had a lower total cost than the broadcast application, due to the higher price of the Weedseeker® technology.

Fillols *et al.* (2013) assessed the technology in cane by equipping an interrow shield to allow for spot spraying glyphosate in the interrow. Herbicide savings for the spot-sprayed treatments using the Weedseeker® were not as large as expected when weed infestation was light and uniform in coverage, not in patches. When weeds were small but scattered, the sensors triggered regularly and generated a large spray footprint. The best savings were obtained when patches of sensitive weeds were predominant (small number of big plants). Similar outcomes were reported by Griffin *et al*. (2012), where the use of the sensor-equipped hooded sprayer reduced herbicide application of glyphosate by 17 to 45%. The difference in savings was related to bermudagrass infestation level at application, 25% and 40% ground cover, respectively. Benefiting from co-funding from the government, two Australian sugarcane growers have invested in Weedseeker® sensors and mounted them under shields. The inconvenience of using shields and the cost of the technology are the main limitations for adoption.

Osten (2010) noted there was no registration for applying herbicides with WeedSeeker® units in cane.

Green from green sensors

To improve adoption of precision spraying in row crops, researchers have been busy developing autonomous machine vision technology able to identify in-row weeds from growing crop. For a machine-vision system, the challenge is to identify reliable discriminating features to delineate between crop and weeds. Different types of sensors can be considered to discriminate weeds from crops:

- Sophisticated sensors like hyperspectral imagery, stereovision, and LiDAR (a sensing approach of light detection and ranging) provide additional dimensions of imaging data,
- Machine-vision systems based on commonly available colour cameras provide a robust technology solution in commercial conditions due to their low-cost and high-end specifications, e.g. high number of pixels and numerous configuration, lensing and image-analysis options.

To discriminate visual differences between weeds and crop, two types of image analysis methods can be explored:

- conventional methods in which image analysis features are hand-crafted by the system designer for crop and weed discrimination. McCarthy *et al.* (2012) developed an approach involving the application of line detection techniques to high quality in-field colour camera images to enhance sugarcane/weed discrimination. The result from one trial was an 85% hit rate in discriminating Guinea grass, herbicide saving of 70% with 1% overspray on crop (project NCA011). SRA Project 2015/055 with the University of Southern Queensland (USQ) further aimed to refine the machine vision-based weed discrimination algorithms and to integrate them with commercial spray control systems from a spray equipment manufacturer. The prototype machine vision-based spot sprayer achieved average hit rates ranging from 67 to 96% with less than 1% false triggers on big, medium, and small Guinea Grass, respectively, in agronomic field trials for large cane. The spot sprayer was inconsistent in agronomic trials for small cane; however, weed detection accuracy of 89% correct detection of Guinea grass with 8.6% false triggers was achieved during post-processing analysis. Simultaneous to research in the sugar industry, development of the machine vision-based spot sprayer also occurred for application in the cotton industry to control volunteer

cotton and other weeds. In cotton, potential herbicide cost savings were calculated as up to $8 per hectare for a glyphosate-based strategy, and $30 to $40 per hectare for alternate modes of action. When the commercial partner hardware becomes available, additional spray efficacy field trials and finetuning of the algorithms are required to finalise the project outputs of delivering a commercial sensor detecting weeds from cane (McCarthy, 2019; Brett *et al.,* 2019).

- deep learning methods in which the computer determines discriminating features between crop and weed without human input, based on a vast training dataset. It is now the most widely used technology for computer vision when it comes to complex images (recognising weeds within crops, or on bare soil). Deep learning is part of the family of machine learning and is inspired by the way the human brain works (deep learning often uses deep neural networks architecture). The classical process to develop a deep learning algorithm with supervised method is:
  - Define algorithm usage and objectives (i.e. recognise Guinea grass in cane with > 90% accuracy),
  - Gather data (i.e. capture pictures of Guinea grass in cane fields in a diversity of situations),
  - Sort and label data (i.e. indicate on each picture what is Guinea grass, what is cane). Also, all images are separated into 2 sets, training set and testing set,
  - Train algorithm: the training set needs to be processed (thousands of times) by the algorithm so that it can learn patterns,
  - Test algorithm: the test set is processed (one time) by the algorithm to compare the results of the algorithm with the reality and test in the paddock,
  - Repeat the process until the field test is successful.

James Cook University (JCU), in partnership with AutoWeed Pty Ltd, have developed a new and innovative system that can detect weeds within a target crop and allow for precise robotic weed control to be applied per plant. The Autoweed system, developed and spun out from postgraduate research at JCU, utilises deep learning on images in the visible spectrum to detect grass and broadleaf weed species within any target crop or pasture environment. This cutting-edge technique allows for a tremendous reduction in herbicide usage. Early trials on-farms across Queensland and New South Wales indicates that up to a 95% reduction in use of herbicides is achievable compared to traditional blanket spraying of the target crop, subject to existing weed density (Olsen, 2020). In pasture, an average of 89% herbicide reduction was achieved by using selective spot spraying application to target Chinee Apple, Navua Sedge and Harrisia Cactus in Townsville, Malanda, and Boggabilla grazing lands (Olsen, 2020). Two broadacre cropping trials were also conducted that revealed a 95% reduction in herbicide targeting turnip weed in oats at Spring Ridge NSW, and a 96% reduction in sow thistle weed in wheat at Arcturus QLD (Olsen, 2020) http://autoweed.com.au/. A new 2-year project funded by GBRF Reef Fund Partnership - Innovation and Systems Change, Water Quality Program will start late 2020 to assess the potential of this technology to control weeds in sugarcane (JCU Townsville – project leader).

Some other examples of companies, start-ups, large corporations, and universities who are developing systems with green on green capability are listed below. The technology used is similar: artificial intelligence with cameras (sometimes RGB/colour cameras, sometimes hyperspectral cameras).

- Bilberry, a French AI based start-up that specialises in cameras for recognising weeds using deep learning approach. In the Australian grain industry, several sprayers are equipped with Bilberry cameras. https://www.bilberry.io/
- Blue River Technology, acquired by John Deere in September 2017 for more than $300M, developed a See and Spray technology operating on a limited basis for cotton weeding. See & Spray features multi-camera arrays (front and rear facing sensors) and dedicated graphic

processing units for each individual row unit, which provide the computer vision and deep learning, while custom-designed robotic nozzles only initiate when directly over a weed with a very small footprint. http://smartmachines.bluerivertechnology.com/

- Ecorobotix, a Swiss based start-up developing an autonomous solar robot that kills weeds, using deep learning. https://www.ecorobotix.com/en/
- AgroIntelli, a Danish company developing an autonomous robot to replace tractors, that will also include spraying capacity. They are developing the RoboWeedMaPS technology which combines deep learning and big data approaches. http://www.agrointelli.com/roboweedmaps.html#roboweedm
- Bosch, the German company, that is more and more involved in agriculture has launched a project call Bonirob, an adaptable robotic platform that includes smart cameras to kill weeds in a more efficient way.

Further research needs: Automated spot spray technology is the future of chemical spraying while reducing the environmental impact. Investments in this area should focus on reviewing which technologies successfully developed in other cropping systems are the best candidate to adapt to the sugarcane farming system.

Weed mapping

The emergence of remote sensing technology and Unmanned Aerial Vehicles (UAVs) has allowed for faster mapping and understanding of weed distribution, dissemination and their dynamics. UAVs can fly at very low altitudes to generate fine spatial and temporal resolution imagery, their acquisition costs are low, and different sensors with diverse spectral ranges can be embedded. These characteristics facilitate the procurement of high spatial, spectral and temporal resolutions, which are required for the agronomic goal of detecting weeds.

Pena *et al.* (2013) developed a robust and entirely automatic object-based image analysis (OBIA) procedure using a six-band multispectral camera (visible and near-infrared range) with the ultimate objective of generating a weed map in an experimental maize field in Spain. The OBIA procedure combines several contextual, hierarchical and object-based features and consists of three consecutive phases: 1) classification of crop rows by application of a dynamic and auto-adaptive classification approach, 2) discrimination of crops and weeds on the basis of their relative positions with reference to the crop rows, and 3) generation of a weed infestation map in a grid structure. In addition, the OBIA procedure computes multiple data and statistics derived from the classification outputs, which permits calculation of herbicide requirements and estimation of the overall cost of weed management operations in advance. The estimation of weed coverage from the image analysis yielded satisfactory results. The relationship of estimated versus observed weed densities had a coefficient of determination of R^2=0.89 and a root mean square error of 0.02. A map of three categories of weed coverage was produced with 86% of overall accuracy. In the experimental field, the area free of weeds was 23%, and the area with low weed coverage (<5% weeds) was 47%, which indicated a high potential for reducing herbicide application or other weed operations. Santi *et al*. (2014) who studied the phytosociological variability of weeds in a soybean field under no-tillage in southern Brazil also found that weeds occur in patches with different densities, and site-specific weed management in soybean based on infestation zones could lead to herbicide savings ranging from 30% to 70%. Further work from Pena *et al.* (2016) has allowed weed maps to be obtained early in the growing season of maize. Weed emergences could be discriminated with accuracy higher than 90% if image resolutions were correctly selected for each type of crop and scenario.

However, UAVs come with limitations and technical problems, e.g. stabilisation may not be constant at high flight altitudes (e.g. 100 m) due to stronger wind, the battery determines the duration of the flight and flight altitude may be restricted by the country aviation regulations. This affects the pixel

size and dimensions of the surface covered by each flight because the lower the flight altitude, the higher the spatial resolution but lower the surface coverage. Using machine vision and consumer-grade UAV technology, the University of Southern Queensland also showed that colour loss at higher altitudes has a greater impact on weed-recognition performances than spatial resolution alone when using green-from-brown differentiation (Smith *et al.* 2019).

Consequently, a set of overlapping images from low flight altitude is required to cover the whole study area. These images must be stitched together to create an ortho-mosaicked image that requires aero-triangulation and ortho-rectification, which is facilitated by a number of invariant features and geo-locating targets. In the case of a row crop such as sugarcane, these steps become more complicated because of the repetitive pattern and the difficulty in identifying invariant or specific features. Recent research has investigated and overcome some of the difficulties related to weed mapping in herbaceous row crops. Borra-Serrano *et al.* (2015) have found that resampling -a mathematical technique used to create a new version of a remotely sensed image with a different width and/or height in pixels – is a useful way to deal with this issue. Resampling allows a flight at low altitude to be used as a baseline (high spatial resolution) to obtain a new image at a higher altitude (lower spatial resolution) without carrying out the real flight at a high altitude. Research showed resampling accurately extracted the spectral values from high spatial resolution UAV-images at an altitude of 30 m and the resampled-image data at altitudes of 60 and 100 m was able to provide accurate weed cover and herbicide application maps compared with UAV-images from real flights.

Timing between generating the weed map and the spray operation is critical as the weed map would only be relevant for a short period of time. A succession of weed maps over time would be the best indicator of the weed distribution at a paddock scale and could help inform decisions related to the seed bank management with pre-emergence herbicides. Gonzalez-Andujar & Saavedra (2003) found substantial evidence to show that, in many circumstances, the distribution of some weed species, including the major grass weeds, in cereal crops is patchy (not uniform) and that these patches are relatively stable within a season and from season to season.

In Queensland, USQ is currently developing a software to automatically generate prescription spray maps using machine vision and consumer-grade UAV technology (Smith *et al.* 2019).

QDAF is also investigating the mapping of vine zones using UAV imagery in sugarcane but weed recognition and data processing are not automated. https://www.daf.qld.gov.au/news-media/media-centre/fisheries/news/airborne-weed-killer-helps-farmers-and-the-reef

Further research needs: Investigate the best approach to develop weed maps in sugarcane and determine how they can be used to predict future weed patches to reduce the application of pre-emergent herbicides to weed patches only.

Nanotechnology

Novel smart delivery systems, nano-sensors, and nanomaterials (e.g., nanoparticles) are receiving increasing attention for possible applications in agriculture.

Nanotechnology has emerged as a promising tool for the development of new herbicide formulations with active ingredients containing particles in the range of 1–100 nm in size. Nanoformulations allow controlled release of the compounds, both in terms of time or place, or trigger it only under certain environmental conditions. They could also be used for natural herbicides to overcome their short natural half-life. Several nanocarriers based on different basic frames such as nanoparticles, nanocapsules, nanoclays or liposomes could be used to attach or load inside the metabolite to protect it against degradation. Controlled release formulations could minimise, if not

prevent, the leaching of chemicals as well as reduce volatilisation and degradation losses (Korres *et al.* 2019). Nano-based biosensors could also enable better and more efficient use of herbicides while maintaining environmental safety (Duhan *et al.* 2017).

6.6.6 Sugarcane variety tolerance to herbicides

Inherent tolerance

Some crops are inherently more tolerant to a particular herbicide. Usually this tolerance comes from the crop being able to rapidly detoxify that herbicide. There may also be differences among individual varieties in their ability to detoxify a particular herbicide. Visual symptoms on crop foliage after spraying a particular herbicide can be misleading of the real herbicide impact on the plant crop growth and final yield: a poor relationship exists between the visual and measured reaction to phytotoxicity caused by herbicides (i.e. up to 45% cane yield loss measured but not assessed visually in the early growth stages). To measure the real impact of a particular herbicide on a particular crop cultivar beyond the initial visual symptoms, specific herbicide tolerance screening trials must be carried out.

From 1985 to 1992, most herbicide tolerance trials on cane varieties in Australia were done annually by BSES in the field at different locations. Plots were 2 rows by 4 m long, with no repetitions or two repetitions, and only visual assessments were carried out. Hernandez *et al.* (2004) also used visual field assessments to report on good selectivity of ametryn + trifloxysulfuron in four assessed sugarcane cultivars, applied broadcast on the crop foliage, showing very slight and short lasting to no phytotoxicity symptoms.

In 2001, O'Grady &Murphy (2001) concluded that spraying, at the 3-4 leaf stage, cane varieties grown in pots and measuring elongation and biomass 10 weeks after spraying was a robust technique; however, no correlation with yield from field trials was established. Field trials in sugarcane are the only way to assess a yield impact of the treatments; however they take a long time (12-13 months), are costly (to assess many combinations of herbicide by varieties) and are high risk (external factors likely to affect yield more than the tested herbicide treatment). To assess visual damage and measure early impact on growth, pot trials represent less risk than field trials: they are more homogeneous and less susceptible to external factors.

In 2007 and 2008, BSES carried out pot trials with 6 repetitions and assessed mainly shoot elongation and carried out visual assessments. Pot trials to assess varietal herbicide tolerance have also been the method selected by Galon *et al.* (2009) who evaluated the tolerance of three sugarcane genotypes to three herbicides at 0.0, 0.5, 1.0 and 3.0 times their recommended commercial dose. They assessed phytotoxicity symptoms (measured in %) at several dates and measured leaf area and shoot dry matter at 80 days after crop emergence.

In 2014, after reviewing further the variety screening process for herbicide tolerance, SRA implemented a two-stage evaluation program (as in grain crops). The first stage screens in pots all relevant newly released varieties against a range of commercially available herbicides. It is implemented in year 1 in pots and visual damage and biomass after 10 weeks growth are measured. The second stage in field only tests the combinations of variety by herbicide that displayed significant symptoms in the first stage by comparison with susceptible varieties. The field trial, implemented in year 2, measures the impact of the herbicides on cane yield (Fillols 2018). Following this methodology since 2014, SRA has been carrying out yearly trials at SRA Meringa. Results from this routine herbicide tolerance screening of newly released sugarcane varieties are communicated to growers via the QCANESelect platform and the regional variety guides.

As these trials are only adequate to test post-emergence herbicides that are sprayed on the cane foliage, an SRA innovation project (INNOVA5) is currently developing a methodology to test the varietal tolerance to pre-emergence herbicides.

Further research needs: Improvement to the current variety screening process for herbicide tolerance would include a second rate of herbicide at twice the maximum label rate to take into account potential overlapping in the field and inclusion of the pre-emergence herbicide testing methodology (once developed).

Determine the impact of herbicides on varieties planted using NEF CEEDS™ technology.

Tolerance by spatial separation

A non-selective herbicide that is toxic to the crop may still be able to be used where it can be spatially separated from the crop. For post-emergent herbicide, this is achieved using directed spray on the weeds while limiting contact with the sugarcane leaves. For pre-emergent herbicides, they may be able to be applied in a situation where the crop is planted at a depth below the herbicide band. While this may be an effective strategy for some herbicides such as imazapic and isoxaflutole, crop injury may still occur in situations where herbicide is moved down into or below the crop root zone, particularly if heavy rainfall occurs as the first incorporating rainfall. Crop damage is often a function of one or more of the following: shallow planting depth, heavy rain after application, soil with low binding characteristics and/or products with high solubility and/or low binding.

As reducing the depth of soil cover at planting supposedly enhanced the rate of sugarcane emergence, Richard & Dalley (2006) studied the effect of reducing soil cover on the potential for pre-emergent herbicide injury in Louisiana. Terbacil, azafenidin, and terbacil + diuron reduced plant cane yield, comparing 5- with 10-cm of soil cover at planting. In the first ratoon crop, cane yield was reduced by terbacil, comparing 5- with 10-cm of soil. The authors concluded that reducing soil cover left sugarcane more vulnerable to injury from certain herbicides.

In the case of planting seedlings instead of cane setts, spatial separation cannot occur, and non-selective pre-emergent products cannot be used.

Herbicide-resistant (HR) crops

Application of biotechnology in weed management, in the form of HR crops, is the most widely adopted modern tool for weed management in conjunction with herbicides. HR crops are generated with transgene technology (e.g., for glyphosate, glufosinate, 2,4-D, dicamba resistance) or mutation breeding (e.g., for imidazolinone resistance). The rapid and widespread adoption of glyphosate-resistant (GR) crops was unprecedented, reaching 73%, 80%, and 93% of corn, cotton, and soybean planted in the United States, respectively, in 2012. Despite herbicide-resistant crop technology being an ideal theoretical tool for integrating into weed management practices, simplicity of the technology and economics caused farmers to use it with little integration with other tools and have accelerated the selection, and contributed to the increasing number, of GR weed species. To mitigate the increasing herbicide resistance problem, trait stacking has become the norm of the latest cultivars of HR. In addition to the existing resistance traits to glyphosate, glufosinate, acetolactate synthase (ALS) inhibitors, and acetyl coenzyme-A carboxylase (ACCase) inhibitors, resistances to dicamba, 2,4-D, and 4-hyroxyphenylperoxidase (HPPD) inhibitors are being added to the mixture of HR crop traits.

Nasir *et al.* (2013) used four sugarcane varieties (CPF-234, CPF-213, HSF-240, and CPF-246) to develop glyphosate herbicide tolerance. A glyphosate-tolerant gene of 1368 bp cloned directionally under the 35S promoter with the β-glucuronidase (GUS) reporter gene was used as the transgene.

Through the biolistic transformation of sugarcane, calli of all cultivars were transformed with glyphosate-tolerant gene constructs. Based on initial screenings through GUS assay, the transformation efficiency was 22%, 32%, 17%, and 13% for cultivars 246, 234, 213, and 240, respectively. In transformed sugarcane plants, the transgene of 1368 bp was amplified. It was revealed that all acclimatised transgenic sugarcane plants survived the glyphosate spray application of 900 mL/0.404 ha, except for the control non-transformed plants. However, at spray application of 1100 mL/0.404 ha, transgenic plants having the transgene protein OD of 0.2 to 1.0 did not survive, while those that had a transgene protein OD range of between 1.0 and 2.0 did. In addition, weeds growing alongside transgenic sugarcane plants turned brown and subsequently died at glyphosate spray applications of both 900 and 1100 mL/0.404 ha.

In 2017, the Brazilian Comissão Técnica Nacional de Biossegurança (CTNBio – National Biosafety Technical Commission) has approved the commercial use of the first genetically-modified sugarcane (Bt Sugarcane) developed by the Brazilian sugarcane breeding and technology company Centro de Tecnologia Canavieira (CTC). The variety CTC 20 BT is resistant to crop damage caused by the main sugarcane pest in Brazil, the sugarcane borer (*Diatraea saccharalis*). In the coming years, CTC plans to develop other varieties that are resistant to other insect pests and tolerant to herbicides. https://ctc.com.br/en/genetically-modified-sugarcane-developed-by-ctc-in-brazil-is-approved-at-ctnbio-2/

In Australia, SRA Joint Venture with DuPont also sought to develop HT cane using genetic modification (GM) technology. Given the significant resources required to develop and deliver a GM crop, herbicide tolerance was chosen to focus on a single trait: glyphosate resistance. The technology was successfully introduced into Q208$^{\phi}$ and Q240$^{\phi}$. SRA developed more than 2000 independent (sugarcane transformation) lines and conducted field trials and other related activities over five locations. However, market sentiment shifted as the anticipated returns on investment did not arise beyond maize, soybeans, cotton, and canola, while consumers and regulators did not embrace the technology as anticipated. Monsanto (now merged with Bayer), Syngenta and DuPont (now merged with Dow Agrosciences and called Corteva) all made significant investments but subsequently stopped or notably reduced their focus on sugarcane. Sugarcane also faced additional hurdles compared to other crops when it came to commercialisation of the technology, due to the crop cycle and planting method and how the economic benefit of GM technology would be shared among all stakeholders, including growers. In that context, DuPont approached SRA indicating that they were reducing their investment in sugarcane and terminated the alliance with SRA.

The South African Sugar Research Institute (SASRI) is currently developing traits that include enhanced nitrogen-use efficiency and herbicide and drought tolerance. Research in this area includes the development of technologies and resources required for genetic engineering, mutagenic breeding, and the preservation of valuable germplasm, as well as the demonstration of proof-of-concept regarding the performance of the novel lines produced. They are developing an integrated field programme for deploying imazapyr-tolerant sugarcane. Rutherford *et al.* (2017) studied seven imazapyr-tolerant mutant (Mut1-Mut7) sugarcane plants, previously generated by in vitro mutagenesis (N12 callus exposed to 16 mM ethyl methanesulfonate and selected on imazapyr-containing medium). The imazapyr concentrations required to inhibit their acetolactate synthase (ALS basal activity) were 0.77 – 5.36 times greater than that of the N12 'control parent'. Five of the seven mutants tested showed greater imazapyr-resistance in the field than the control N12. The basal ALS activities of Mut1 and Mut6 were 1.4-fold higher than that of N12. No differences in sucrose, fibre and estimated yield were observed amongst lines in untreated plots. Mutant plants germinated and grew in soil treated with the herbicide (at the lethal dose of 1248 g a.i./ha). Multiple resistance mechanisms seem to be present across the tested mutants, e.g. point mutations in the ALS gene, increased basal expression, and may include exclusion by transporters and increased detoxification.

Ramdoyal *et al.* (2010) studied the inheritability of the herbicide tolerance trait as varieties with one or both parents in common have been observed to show a similar tolerance level to the tank-mix of diuron and Actril-DS®. Sixty parents established in pots and later transplanted in replicated field trials were evaluated for their tolerance to the test herbicide tank-mix. Crosses were made with some selected parents with known tolerance and 15 families, comprising 40 seedlings each, were subsequently evaluated for their response to the same tank-mix. A clear-cut segregation towards either tolerant or susceptible groups was not evident that could indicate the action of a major gene. Partitioning of variance indicated a high component of additive genetic variance, high narrow-sense heritability, and the possibility of mutation breeding for the character through a judicious choice of parent varieties.

Further research needs: SRA board has recently approved some plant breeding investments into non-GM herbicide tolerance varieties, which will require a study of the inheritability of the herbicide tolerance trait amongst SRA breeding parent varieties, as well as investments in specific herbicide efficacy trials and the development of a stewardship program.

Plant back

Herbicide residues in soils can limit crop performance if not managed correctly. Typically, in sugarcane, herbicides applied in the last ratoon cane (i.e. diuron, imazapic) can impede the growth of fallow crops or plant cane in case of plough-out/replant. Also, herbicides applied in the fallow crop (e.g. haloxyfop to control grasses, imazethapyr to control broadleaves) can pose a problem in the following plant cane.

For many herbicides, microbial degradation is the primary path of degradation. Conditions that encourage soil microbes (warm soil, good soil moisture, adequate oxygen, organic matter, nutrients and neutral pH) will typically see faster degradation and shorter persistence of the herbicide, whereas extended dry periods which do not support the sustained activity of microbial populations can substantially increase the persistence of these herbicides. A mobile herbicide such as imazapic (high solubility/low binding) may persist for long periods deeper in the soil profile where there is little microbial activity. These herbicides can carry over to following seasons and affect rotational crops when the crops' roots get down to access the herbicide at depth. Imazapic is an exception amongst the pre-emergent herbicides: at acidic pH, binding of imidazolinones increases, thus reducing bioavailability to the microbes required for breakdown, resulting in increased persistence. Osten (2010) observed real and perceived re-cropping issues with several residual herbicides (particularly imazapic) for many crops across many soil types.

For herbicides that breakdown via hydrolysis, the speed of breakdown is influenced by temperature, moisture and is often highly influenced by pH. The Group C sub-group of triazines and the Group B subgroup of sulfonylureas typically undergo chemical hydrolysis in neutral or acid soils. They persist much longer in alkaline soils. In duplex soil with an alkaline and impermeable sub-soil, herbicide that is moved deeper in the profile can persist for many years.

The rate of herbicide persistence is usually reported as a DT50 value or the number of days that it takes for 50% of the herbicide in the soil to breakdown. As this rate varies between different soils and environmental conditions, the DT50 is often reported as a range of values or an average. Non persistent pre-emergent herbicides (DT50 < 30 days) like flumioxazin, metribuzin, S-metolachlor, fluazifop-P or terbuthylazine often have minimal plant back constraints as they tend to breakdown relatively quickly. However, these herbicides can still be useful pre-emergent herbicides if applied at a high enough rate to allow them to provide the desired length of residual control. Persistent pre-emergent herbicides (DT50 > 100 days) such as trifluralin, imazethapyr or imazapic often result in plant back issues and long re-cropping intervals. Many labels have a plant back period specifying the

number of months and a rainfall requirement from application until susceptible crops can be sown. Product labels are legal documents which seek to set 'one size fits all' recommendations to manage plant-back risks, but they are unable to account for the combinations of soil types and weather conditions that can affect herbicide persistence in soil. Some strategies may provide additional data on which to assess or reduce plant back risk:

- Soil testing from a laboratory specialising in herbicide residue testing. However, the cost may be prohibitive, and interpretation of results is difficult.
- Conducting a simple bioassay by seeding some crop seeds into the field a few weeks prior to the desired sowing date and observing their establishment. However, this test will not cover injury from mobile herbicides that have moved further down the soil profile.
- Cultivating aggressively prior to sowing of a sensitive crop to dilute herbicides tightly bound to the soil surface (this is a process mainly carried out in dry country in the grain industry) (Congreve & Cameron 2019).

There are currently very few tools to assist growers to determine the level of herbicide residues present, and if they negatively affect soil and crop performance. Dr Michael Rose's Project 4.2.001 https://soilcrc.com.au/current-projects/ is developing new knowledge and tools to better understand the factors regulating herbicide persistence and bioavailability. The outcome will be that farmers are better equipped to react to variable environmental and soil conditions, and major losses after planting will be avoided.

In South Africa, imazapyr is registered for *Cynodon dactylon* control in sugarcane fields due for replanting, with a waiting period of four months and at least 600 mm of rainfall between application and replanting. As summer fallows benefit from a break crop, Campbell *et al.* (2017) investigated the plant back impact of imazapyr on sun hemp (*Crotalaria juncea*) and velvet bean (*Mucuna pruriens*). In the clay soil, sun hemp emergence was reduced, leaves were chlorotic and deformed ("broccoli" growth) and plants were stunt, whereas in the humic soil, sun hemp emergence was nil. Velvet beans were also severely impacted in both soils, however some plants recovered. The plant back impact was less severe with 106- and 152-mm rainfall occurring between herbicide application and sowing in the clay and humic soil, respectively. This study concluded that velvet beans might be a valuable cover crop option when planted in clay soil, one month after imazapyr application (and after 106 mm rainfall).

Further research needs: There is little understanding and acknowledgement of establishment issues and yield loss issues related to plant back. The technique of using simple bioassays in the field or in pots using soil from the tested field should be investigated as a potential standard method to be promoted to growers and advisors.

6.6.7 Weed resistance to herbicides

Globally, the first case of herbicide resistance in weeds was identified in 1964. Currently, there are more than 250 resistant grass and broadleaf weed species in more than 70 countries worldwide (Heap 2019). Herbicide resistance has developed a strong foothold in Australian agriculture since it was first reported in annual ryegrass in 1982. It has spread and diversified to become a key constraint to crop production in all states generally with a history of intensive herbicide use. Today, resistance has been confirmed in a range of grass and broadleaf weed species and has now developed to 11 distinctly different herbicide mode of action groups which can significantly reduce herbicide options for the grower. Cases of multiple resistance have also been commonly reported (i.e. annual ryegrass resistant to two or more chemical groups) (Anon. 2019).

In 2015, cases of resistance to paraquat have been reported in the Australian sugarcane cropping system for *Gamochaeta pensylvanica, Solanum nigrum* and *Eleusine indica*. To date, these cases

have been restricted to Southern Queensland where sugarcane is grown in close proximity with tomato, peanut and avocado crops https://www.agronomo.com.au/latest-news/2016/4/5/paraquat-resistance-is-gaining-momentum-in-australia.html, http://www.weedscience.com/details/Case.aspx?ResistID=11025.

In other Australian cropping systems such as grain or cotton, many weed species have developed resistance to several herbicide groups. Some of these weed species are also common to the sugarcane industry and should be closely monitored.

- Awnless barnyard grass (*Echinochloa colona*) resistant to group M (>200 sites),
- Crabgrass (*Digitaria sanguinalis*) resistant to Group A and B,
- Crowsfoot grass (*Eleusine indica*) resistant to Group A and L,
- Feathertop Rhodes grass (*Chloris virgata*) resistant to Group M,
- Blackberry nightshade (*Solanum nigrum*) resistant to Group L,
- Flax-leaf fleabane (*Conyza bonariensis*) resistant to Group M, B, L,
- Tall Fleabane (*Conyza sumatrensis*) resistant to Group M,
- Small square weed (*Mitracarpus hirtus*) resistant to Group L,
- Tridax daisy (*Tridax procumbens*) resistant to Group M.

Other weeds that have developed resistance to some herbicide groups in sugarcane in other countries should also be closely monitored:

- *Echinochloa colona* resistant to Group C in Iran,
- *Commelina diffusa* resistant to Group I in US,
- *Chloris barbata* resistant to Group C in US.
  http://www.weedscience.com/Summary/Crop.aspx

Another potential threat is the risk of itch grass (*Rottbellia cochinchinensis*) developing resistance. Failure to control itchgrass in soybean (*Glycine max*) with fluazifop-P-butyl has been documented in Louisiana, Bolivia, and Costa Rica; and itchgrass resistant to foramsulfuron, iodosulfuron-methyl-sodium, and nicosulfuron was reported in a Venezuelan corn (*Zea mays*) field in 2004 (Avila *et al.* 2007; Castillo-Matamoros *et al.* 2016; Hea 2019).

Currently in cane, there is a relatively high reliance in glyphosate, which would have been aggravated if glyphosate-resistant cane varieties were to be released. In the absence of GM cane varieties, resistance to glyphosate has not yet been identified (Osten 2010).

In 2019, SRA collected samples of crowsfoot grass populations suspected resistant to glyphosate in the Herbert district and they all tested negative (Herbicide Resistance Screening Centre, Charles Sturt University). In 2020, SRA will also check other suspect populations of crowfoot grass and feathertop Rhodes grass from the Burdekin area, for their resistance to glyphosate.

Computer simulation modelling is an essential aid in building an integrated understanding of how different factors interact to affect the evolution and population dynamics of herbicide resistance, and thus in helping to predict and manage how agricultural systems will be affected (Renton *et al.* 2014). Because herbicide resistance evolves in very large populations over periods of many years, modelling is an important tool for investigating the dynamics of the problem. A diversity model was developed to track the simultaneous evolution of resistance to multiple herbicides, using multiple genetic pathways, in several weed species at once. The model was used to test weed management strategies for new cropping cotton varieties with multiple herbicide tolerances ('triple-stacked' varieties), in an Australian context. Assuming some glyphosate resistance was already present, simulations showed that glyphosate-resistant summer grass populations reach 20,000 seeds/m^2 within 12 years using the triple-stack herbicides (glyphosate, glufosinate and dicamba) and a minimum of other tactics. Adding three pre-emergent modes of action plus cultivation to the system

effectively controlled glyphosate-resistant grasses for over 30 years. In conditions where resistance genes were as frequent as 1 in 100, however, highly fecund weeds such as *Conyza bonariensis* were hard to control beyond 15 years even with very highly diverse management (Thornby *et al.* 2018).

Harvest weed seed destruction (HWSD) is a means to control weed seeds by way of mechanical impact trauma to the seed and can be included in an IWM program to help control weed populations and combat herbicide resistance. The Weed Seed Wizard computer model has calculated the impact of employing HWSD every second year. The model gave reasonable predictions of *Lolium rigidum* seed production each year (when compared to the actual seed production in the field trial), and accurately predicted when *L. rigidum* seed numbers would reach very low levels due to annual HWSD. HWSD used in every second year could not reduce *L. rigidum* seeds to the same extent as annual HWSD, but the *L. rigidum* population was reduced to and maintained at less than one plant/m^2 at harvest within four years. The results highlighted both the benefits of HWSD as a weed management tactic and the value of the Weed Seed Wizard as a tool to investigate different IWM programs (Borger *et al.* 2018).

Further research needs: Very limited seed samples of weed populations suspected to have developed resistance to some herbicide group have been collected by SRA. Some productivity services and growers become increasingly worried about the rise in efficacy failures of certain herbicides on certain weeds, justifying the need for a wider weed resistance survey. As the screening protocol is relatively simple, it could be carried out in-house to reduce the cost of screening for numerous samples.

6.6.8 Herbicide off-site impacts

Drift

Spray drift issues (especially with 2,4-D and glyphosate) on sensitive crops are commonly reported (Osten 2010). In Louisiana, dicamba-containing products are applied in sugarcane crop while soybean is growing in proximity in fallow blocks. Bauerle *et al.* (2011) found that when dicamba was applied directly to soybean at 1/64 of the sugarcane use rate (rate that can be expected with herbicide drift), soybean yield was reduced by 8% when applied at 2 to 3 fully expanded trifoliate leaves and by 43% when applied at first flower.

Low-drift and non-drift nozzles are available on the market to limit off-site impacts and specific drift regulations are in place to use coarse spray droplet size for products like 2,4-D. Osten (2010) observed that the required coarse droplet spectrum to limit drift with 2,4-D is compromising efficacy in certain weed types.

Herbicide drift can also enter close sensitive environmental areas and water bodies.

Drift reduction measures are essential to avoid the entry of plant protection products into non-target areas. Gil *et al.* (2015) highlighted the need for objective methods for drift evaluation as well as robust procedures for the classification of sprayers according to their risk of contamination. As a complementary tool to actual drift measurement methodologies in the field or in laboratory conditions, the authors have developed a new quick and objective method for the quantification of the potential drift and the influence of wind velocity and direction on the drift potential value (DPV) using an ad hoc test bench The results indicated that wind velocities below 1.0 m/sec have a negligible influence on the DPV. Front wind led to higher DPVs than lateral wind.

Runoff

Herbicidal impact on the health of the Great Barrier Reef (GBR) lagoon came to the forefront in 2009 with the Queensland Government's *Great Barrier Reef Protection Amendment Act 2009*.

According to Brodie & Landos (2019), the management for environmental protection at the Australian level by the regulator, the Australian Pesticide and Veterinary Medicine Authority, has serious deficiencies in process and practice. However, Federal and Queensland government programs have maintained the spotlight on both freshwater and marine water quality and action is being taken at the Queensland Government level to reduce pesticide pollution of waterways, including research, monitoring, risk assessments and application of better pesticide application methods.

Label restrictions on diuron use in sugarcane started in 2013 but only limited reductions in above-guideline detections have been recorded by 2018 (e.g. O'Brien *et al*. 2016; Wallace *et al.* 2017). In addition, the alternatives to diuron (Smith *et al.* 2015), which are now beginning to be used in greater quantities (e.g. pendimethalin, metolachlor and metribuzin), still pose substantial off-site risks to aquatic organisms (Davis *et al*. 2014). In a comprehensive study of Sandy Creek south of Mackay (draining a sugarcane cultivation dominated catchment), the concentration of diuron was very high in all monitored sub-catchments with 84% of samples exceeding the ecosystem protection guideline trigger value for freshwater ecosystems (0.2 µg/L) (King *et al.* 2017a) and the concentration was 17–85 times greater than the ecosystem protection guideline trigger value (excluding Cut Creek and Oaky Creek). Metolachlor, MCPA and atrazine were also detected above guideline values (King *et al.* 2017a, b). Imidacloprid (insecticide), imazapic, hexazinone, diuron, atrazine and 2,4-D were detected in greater than 75% of all samples. Fluroxypyr, MCPA, isoxaflutole metabolite, metribuzin and metolachlor were detected in more than 50% of all samples (Wallace *et al*. 2017).

Subsequent to these repeated pesticide exceedances in watercourses, ambitious pesticide load reduction targets have been set by the Reef 2050 Plan, as one of the means to improve water quality and the resilience of the GBR ecosystem.

Research has shown specific weed management practices that can result in environmental benefits.

Timing the application of pre-emergence herbicides

Herbicide loss in runoff are greater when occurring within the 20–25 days following spraying. Masters *et al.* (2013) found that the event mean concentrations of all herbicides declined significantly as herbicides had time to dissipate from day 1 to day 21. Ametryn and atrazine concentrations were about 8-fold lower at day 21 compared with day 1, whilst diuron and hexazinone were 1.6–1.9 fold lower. Consequently, herbicide loads and percent loss (of applied) declined from day 1 to day 21. At day 1, the loss was in the order of hexazinone (9.8%) > atrazine (7.9%) > diuron (6.1%) > ametryn (5%). Losses at day 21 were hexazinone (5.9%) > diuron (2.8%) > atrazine (0.7%) > ametryn (0.4%). These findings have resulted in implementing windows of applications on labels for herbicides such as diuron and amicarbazone that prohibit their application during the wet season.

Incorporating pre-emergent herbicides

Incorporating the pre-emergent herbicides by non-runoff inducing irrigation or rainfall reduces their risk to be lost in the following runoff event. Herbicide losses in runoff also approximately halved with

every 50 mm of non-runoff causing rainfall or irrigation, before the first runoff event (Rohde *et al.* 2013).

Zonal application of pre-emergent herbicides

Herbicide runoff losses are proportional to the amount applied. Melland *et al.* (2015) showed the percentage of the plot area covered by applied herbicide was positively related to both the dissolved and particulate event mean concentration in runoff of the herbicides studied. The multi-rate response measured in their study demonstrated a proportional reduction in the loss of herbicide by rainfall runoff effect for a wide range of herbicide spray coverages, sites, soils, cane crop phases, and groundcover levels. Of relevance to both the knockdown and pre-emergent herbicides, at least a 50% reduction in herbicide loss in runoff could be achieved if the area normally sprayed by blanket spraying was halved using zonal spraying.

When the trash blanket is thick enough in the interrow to provide sufficient weed suppression, it is often possible not to apply pre-emergent herbicides in the interrow. If necessary, post-emergent herbicides can assist in keeping the interrow clean.

In furrow irrigated systems such as the Burdekin, Davis & Pradolin (2016) showed that banding herbicides on the cane row provided herbicide load reductions extending substantially beyond simple proportionate decreases in amount of active herbicide ingredient applied to paddocks. Losses of atrazine and metribuzin applied broadcast typically range between ~4 and 6% of active ingredient applied. Banding produced significant load loss reductions of >80% for both atrazine and metribuzin applications ($p < 0.05$), despite only ~60% less paddock area being treated compared to conventional broadcast applications for both herbicides. Oliver *et al.* (2014) found similar conclusions: applying diuron and atrazine to only the raised beds decreased the average total load of both herbicides moving off-site by 90% compared with the conventional treatment. This was despite the area being covered with the herbicides by the banded application being only 60% less than with the conventional treatment. However, the efficacy of pre-emergence herbicides applied on the row is compromised by an inadequate incorporation in the furrow irrigation system (refer to section "Specific Management considerations" in this document).

Using alternative pre-emergent herbicides

A range of alternative residual herbicides to Photosystem II (PSII) herbicides can reduce the environmental risk. In a study in the Wet Tropics region of North Queensland, PSII and alternative pre-emergent herbicides behaved quite similarly in terms of their propensity for off-site movement in water (surface runoff losses generally >10% of active applied), with their losses largely driven by their application rate with the exception of pendimethalin and flumioxazin (because of their higher soil sorption). Therefore, herbicides with lower application rates such as alternative herbicides imazapic and isoxaflutole consistently contributed less to the total herbicide loads measured in surface runoff (with 4 to 29 times less risk to aquatic species). (Fillols *et al.* 2018, 2020; Ross *et al.* 2017).

Warne and Peta (in review) have developed the Pesticide Decision Support Tool for each chemical active ingredient used in the sugar industry. When released, this tool will enable growers to make more informed decisions when selecting pesticide in regard to their environmental impact on water quality.

Further research needs: Additional research and effective grower extension activities are required to address information gaps on issues such as specific weed control efficacy of newly registered alternative herbicides and herbicide mixes, while also minimising environmental risks.

From an environmental perspective, on-going research is required to assess the eco-toxicity of pesticide combinations and newly registered pesticides on freshwater and marine organisms relevant to Queensland watercourses and the GBR.

Adding soil-binding adjuvants

Fillols & Davis (2020) studied the ability of three oil-based adjuvants (Grounded®, Atpolan®soil Maxx, Ad-Here™), a terpene-based adjuvant (Flextend®) and a polyol-based adjuvant (Watermaxx®2), to reduce runoff losses as well as improve the weed control efficacy of pre-emergent herbicides. When sprayed on trash blanket, all oil-based adjuvants significantly increased herbicide runoff, Flextend® did not affect herbicide runoff and Watermaxx®2 slightly reduced herbicide concentration in runoff by up to 25%. When sprayed in bare ratoon, Grounded® did not reduce herbicide concentration in runoff (unpublished data). In freshly tilled bare soil, Grounded® reduced herbicide concentration in runoff by about 35%. Most of the tested products slightly increased herbicide efficacy on weeds in the efficacy trials. Adding Grounded® to the spray tank cost $72 per hectare (2019 retail price). To date, none of the tested soil-binding adjuvants has been shown to greatly reduce herbicide runoff in ratoons, which occupy most of the sugarcane cropped area.

Further research needs: Other adjuvants need testing for their impact on herbicide runoff and leaching (in collaboration with QDAF). Flextend® which generated moderate herbicide reduction in runoff in trash blanketed ratoon should be revisited.

Freshly tilled plant cane

Pre-emergent herbicides applied in freshly tilled plant cane have a reduced chance to runoff compared to herbicides applied on compacted soil. When comparing the impact of several sugarcane farming systems on herbicide loss, Nachimuthu *et al.* (2016) found a reduction in atrazine loads (by 50%) and metribuzin loads (by >10-fold) when the cane rows were sprayed with pre-emergent herbicides after the rows were zonal tilled versus minimum tilled (one pass of a single tine ripper before planting). Fillols (2018) observed similar results on fully tilled plant cane where herbicide loads in runoff were only 2% of the amount applied (versus > 10% in compacted soil). Walton *et al.* (2000) also reported that a tilled soil with small aggregates provide more binding sites to herbicides which become less prone to runoff.

Impact of trash blanket on herbicide runoff losses

The impact of trash blanket on herbicide runoff is still unclear. Cowie *et al.* (2013) carried out rainfall simulations on trash versus bare soil in ratoons in Ingham and concluded that cane trash blanket reduced the runoff loss of PSII herbicides from 15% of applied to 9 %. Aslam *et al.* (2013) concluded that the degree of mulch decomposition enhanced the adsorption of non-ionic pesticides (with the exception of imazapic, most pre-emergence herbicides in sugarcane are non-ionic). Herbicide residues on cane trash were substantially lower over time after application and were more resistant to runoff or had a smaller transferrable component, thereby resulting in lower runoff losses. A number of studies have demonstrated that the highest risk of rainfall runoff from the cane trash was shortly after application (Dang *et al.* 2016). Fillols *et al.* (2018) found that herbicide loss coming from trash blanketed plots was similar to bare soil plots when spraying was done on fresh trash blanket shortly after harvest, suggesting no impact of fresh trash blanket on herbicide runoff in ratoons.

Impact of mill biproducts on herbicide runoff

Duhan *et al.* (2020) studied the sorption potential for a range of herbicides using mill muds containing organic matter (47.6 to 65.1%) produced by sugar mills and applied as soil conditioners by farmers. All mill muds had significant sorption capacity, especially for diuron, atrazine and metribuzin, which was 6 to 26 times higher than the soil with 3.5% organic carbon (OC). The inclusion of ash in three mill muds did not significantly affect the herbicide sorption of the mill muds. However other studies in the literature have previously noted enhancement of sorption of diuron herbicide on ash/chars when ash was added to the soil (up to 2500 times more effective). Generally, sorption of the five herbicides assessed in all mill muds followed the order diuron > atrazine=metribuzin > hexazinone=imazapic. Application of mill muds at 40 t/ha increased sorption of studied herbicides by 2 to 10 fold. Soil amendment with mill muds also reduced the rate and extent of desorption of herbicides, especially mobile herbicides like metribuzin. Nearly 79% release of metribuzin was observed after three desorption steps in amended soil, whereas in unamended soil, 100% of metribuzin was released during the first desorption step.

Fillols (2020) has also been investigating the impact of mill biproducts (mud, ash, mud/ash mix) on herbicide runoff. Results from small plot rainfall simulated field trials showed that mud and mud/ash mix increased herbicide concentrations in runoff whereas herbicide concentrations were reduced by up to 50% with the ash. The results with mud are in contrast with Duhan *et al.* (2020) potentially because each biproduct also interferes with the hydrology of the plot according to their own water absorption properties. It is possible these interferences supersede the herbicide sorption properties of each biproduct, especially at a small plot scale.

Further research needs: Further research using paddock-scale trials monitoring runoff throughout the wet season would assist in better understanding the full impact of mill biproducts to runoff quality. These trials should also assess the impact of the mill biproducts on the pre-emergent herbicides' efficacy to control weeds.

Further reading

The GBR is a data rich region due to its iconic status and associated world heritage values, prompting the government to make significant investment in research and monitoring. The amount of literature review produced in the space of herbicide runoff is mind boggling. A selection of research papers not cited in this review but still relevant to the eco toxicity or the management of pesticides in sugarcane farming is available from Emilie Fillols.

Leaching

A preliminary survey of groundwater quality within two catchments of the Wet Tropics region, the Tully-Murray and Johnstone, carried out in 2012-2013 found the most commonly detected herbicides were hexazinone (6 out of 7 bores), atrazine (4), and diuron (4). To a lesser extent the herbicides bromacil (2), simazine (2), metsulfuron methyl (1) and glyphosate (1) were also detected. The insecticide imidacloprid was also detected in two bores and at the highest concentrations of all the pesticides within the study (1.5 µg/L). In previous pesticide monitoring of the Johnstone catchment in 1995 and 1996, atrazine was present in four bores. Furthermore, attention has been drawn to the potentially high losses of pesticides via the extensive network of constructed drains throughout sugarcane paddocks in the Wet Tropics region. It is likely that a major loss pathway to groundwater in sugarcane is through constructed drains, which is an area that is currently poorly understood (Masters *et al.* 2014).

Experiments in soil columns have been carried out to better understand the leaching potential of some herbicides used in sugarcane. Deuber *et al.* (2008) used a bioassay to study the leaching potential of flumioxazin. Flumioxazin at 50 g/ha was applied over the soil surface in plastic columns, rains of 50 and 100 mm were dripped over the surface, then columns were cut longitudinally in halves and *Cucumis sativus* and *Avena sativa* seeds were sown all along the columns as test-plants. Flumioxazin showed very little leaching in both sandy-loam and clay tested soils, with phytotoxicity symptoms detected no more than 27 mm deep in the clayish soil and 20 mm deep in the sandy-loam soil. Phytotoxicity symptoms were also observed in the bottom of the columns, showing that some flumioxazin was leached through the entire height of soil.

Dos Reis *et al.* (2017) investigated in soil columns the impact of soil texture (sandy or clayey) in the total leaching of the commercial mixture diuron + hexazinone + sulfometuron-methyl, and of each isolated compound. In the sandy soil, hexazinone leaching was higher compared to diuron and sulfometuron. Most of the applied diuron remained at the top layer of the soil, indicating that this herbicide has low soil mobility.

To reduce leaching, soil binding adjuvants have been commercialised. Blair & Robertson (2020) found that two of fifteen adjuvants tested in a pot trial at application rate 15 times higher than recommended showed promising results in reducing herbicide leaching.

Further research needs: Pesticide monitoring in groundwater and drains is not carried out on a regular basis and could reveal exceedances that could contribute to the poor water quality in waterways. Further studies in soil binding adjuvants and mill biproducts need to be carried out to find strategies to reduce leaching of herbicides into the environment as well as assessing their impact on herbicide efficacy.

6.7 Weed management using non-chemical methods

Even if herbicides remain a simple and cost-effective way to control weeds, they create adverse reactions to plant crops, can harm the user, and can have significant environmental impacts. To control weeds through a long-term management approach, IWM principles also promote the use of alternative weed management techniques such as physical control, cultural control, or biological control.

6.7.1 Mechanical

The mechanical methods of weed control are being used since man began to grow crops. They include various manual methods like hand hoeing, tillage, mowing, etc. However, each of these methods is labour and time consuming and often does not achieve complete weed control. Weeds can also be controlled by various mechanised tillage operations such as ploughing, harrowing, planking, or levelling. Many perennial weeds can also be controlled by deep ploughing continuously for a period of 3 or 5 years. Implements that cultivate or disturb the soil surface also dig out weeds. With their root systems exposed in the sun, weeds die, but these operations must be timed precisely: -if the conditions are too wet, weeds would not dry but only be transplanted to a new position; -if the conditions are too dry, the loss of soil moisture due to the operation will penalise the crop growth.

In some Australian sugarcane growing regions, mechanical cultivation is still widely used to control weeds in plant cane up to the out-of-hand stage. The first tillage operations will also remove excess soil on top of the planted cane to improve germination, further cultivations will ensure the gradual filling of the planting furrow and later operations will hill-up the row profile to present the crop for harvest. All these operations are used to keep the weed pressure low.

Many opportunities for automated mechanical weed control exist but their economic viability varies widely among cropping systems and locations. High-value conventional and organic horticultural crops, for example, are cropping systems best suited for automated mechanical weed control. Cultivators use a variable-speed rotating, semicircle-shaped disc blade while a camera and computer-controlled guidance system adjust the rotational speed of the disc in real time so that the opening of the disc blade passes around the crop. A wide range of inter- and intra-row weeders are already available including interrow hoes, basket weeders, brush hoes, powered vertical axis tines, finger weeders, spring tine harrows, torsion weeders, mini-ridgers, rotating wire weeders, and pneumatic weeders (Korres *et al.* 2019). Weather alters soil physical properties and can significantly affect the efficacy of many weeders except the brush weeders and mini-ridgers, which perform well regardless of soil moisture or weather conditions. These non-discriminatory weeders require the crop to be tolerant or resistant to the weeding method. Robotic arms and grippers or precision high-voltage electrical probes are some examples of discriminatory weeders. It is unlikely that the current cost associated with automated mechanical weeding could be supported by Australian canegrowers, however as the technology becomes more broadly adopted and the production cost reduces, it will open new ways to control weeds without herbicides.

In fallow, mechanical control is not recommended as it greatly increases the risk of erosion during the wet season. Research has also shown there was no benefit of mechanical control in fallow from a weed control perspective. In Louisiana, Jones and Griffin (2010) evaluated the impact of tillage versus herbicide to control red morning glory (*Ipomoea coccinea*) in fallow. Total season emergence of red morning glory was 34% greater where soil was tilled compared with no-tillage. In October, seed population in soil for the tilled and no tillage treatments was equivalent and had decreased an average of 35% from June. Griffin *et al.* (2010) also evaluated the effect of tillage versus glyphosate application (no-tillage) treatments in fallowed fields on itchgrass (*Rottboellia cochinchinensis*). Itchgrass seedling emergence following tillage was greater or equal to the no-tillage glyphosate treatment depending on the sampling dates. Itchgrass seed population in soil core samples collected in November each year was equal for the tillage and no-tillage glyphosate treatments and averaged 94% less in 1991 and 99% less in 1994 compared to the undisturbed control.

6.7.2 Thermal

Thermal weed control methods can be divided into direct heating by hot water, steam, flame, infrared, hot air and indirect heating by electrocution, microwaves, laser radiation, or UV-light. Cryogenic techniques comprise a third thermal method for weed control

Flame

In Louisiana, burning is a standard practice to remove post-harvest sugarcane trash residue before spring regrowth and the residue condition can range from dry to damp. In live-fire simulations, seeds of divine nightshade (*Solanum nigrescens*) and itchgrass (*Rottboellia cochinchinensis*) were exposed to dry and moistened post-harvest sugarcane residue at four densities (6.1 to 24.2 t/ha) and a non-burned control. Burning the highest densities of post-harvest residue with 44% moisture when wind speeds were lower allowed the fire to smolder, which reduced weed emergence by 23% compared to burning post-harvest residue with 30% moisture during breezy conditions. The moistened 6.1 t/ha post-harvest residue treatment resulted in 53% more divine nightshade and itchgrass emergence after burning when compared to dry 6.1 t/ha post-harvest residue. The fluid-filled and fleshy content that comprises divine nightshade fruit protected seed from short durations of high temperatures. This research demonstrates that burning post-harvest residue from fields with poor stands or older ratoon, especially when post-harvest residue is wet, will not produce temperatures lethal to divine

nightshade and itchgrass seed (Spaunhorst & Orgeron 2019). Ball-Coelho *et al.* (1993) showed burned post-harvest sugarcane residue could result in a maximum flame temperature of 121 to 288°C.

Many studies have shown successful control of problematic weed seeds in numerous cropping systems across the world using heat (Walsh *et al.* 2007). White & Boyd (2016) showed that exposing seeds to direct flame rapidly reduced germination, with less than 1 sec of exposure required to reduce seed germination of witchgrass (*Panicum capillare*), spreading dogbane *(Apocynum androsaemifolium*), and meadow salsify (*Tragopogon pratensis*) by > 90%. However straw burning did not consistently reduce germination of hair fescue (*Festuca filiformis*) or winter bentgrass (*Agrostis hyemalis*), indicating that a surface burn occurring above weed seeds may not be consistently effective at reducing seed viability. The authors concluded that thermal technologies that expose weed seeds to direct flame will be the most consistent in reducing seed viability.

The process of propane-fuelled flame weeding was first engineered in 1852 by John A. Craig who patented the first flame weeding machine for use in sugarcane fields in the US. It applies intense heat which causes the fluid inside the plant's cells to expand and rupture the cell walls. Within a few days, the plant wilts and dies. In general, an exposure time of up to 130 milliseconds is sufficient to kill leaf tissue. Flame weeding has several advantages compared to other methods of weed control: the risk of injury to crop roots is low, especially in the case of heat-tolerant agronomic crops like maize, cotton or sugarcane, or when the flame is directed to the base of the crop to control weeds intra-row. Flaming is compatible with no-tillage systems, ideal for fields with erosion problems. Compared to other methods of thermal weed control, flaming is cheaper; however, there is a high risk for crop injury, injury to the user, or ineffectiveness on some species and on large weeds. In general, plants with an upright habit and thin leaves are more susceptible to flaming than prostrate plants with protected growing points, such as grasses. Timing for flaming is crucial, as it is most effective when plants are 1–5 cm tall or in the 3–5 leaf stage (Korres *et al.* 2019). Safety is a serious concern with flame weeding, especially with tractor-mounted units and with high volume of crop residues in the field, which is the case for cane ratoons with trash blanket.

Steam

The use of steam, instead of hot water, has been reported as a quicker, more effective, and sustainable method of weed control. Engineering research is needed to improve the efficiency and availability of equipment for various crop production systems.

Soil steaming can also kill weed seeds. Compared to the false seed bed technique (i.e. weed seeds are allowed to germinate and then killed prior to crop planting with minimal soil disturbance), soil steaming reduces the weed seedbank more by killing weed seeds buried up to 20 cm deep (Barberi *et al*. 2009). Soil steaming is usually more expensive than other non-chemical preventive methods, but cheaper than chemical soil fumigation. Steaming can also be performed as a "banded treatment" where only the intra-row area is treated, reducing the energy used (Korres *et al,* 2019).

Nishimura *et al.* (2015) determined how seed mortality varied with steam conditions and treatment season in open fields. Regardless of treatment season, saturated steam produced heat at a more stable temperature than superheated steam. Seed mortality of 90% was achieved at an operating speed of 0.3 - 1 km/h for *Ipomoea lacunosa* and 0.3 - 1.6 km/h for *Lolium multiflorum*, suggesting that the required operating speed differed across treatment seasons. Suitable steam conditions also differed across weed species and treatment seasons. Melander & Kristiansen (2011) also showed that seedling emergence was reduced by 90% when the maximum soil temperature reached 60°C. A further rise in temperature to 70°C reduced seedling emergence by 99%. Soil type, soil moisture content, and soil structure influenced the lethality of soil steaming when the maximum soil

temperatures were below 70°C. Steaming was more effective in a sandy soil than in a loamy soil. The efficacy of soil steaming also increased with soil moisture content as soil water conducts heat, resulting in better kill of weed seeds.

Raffaelli *et al.* (2016) designed a new prototype band-steaming machine and tested it in field cultivated carrot. The prototype has a 3265 MJ/h steam generator, which applies steam in 12 soil bands, 180 mm wide. The steam was mixed with the soil by mean of an opposite rotary cultivator. The results showed that a maximum temperature of 63^{o}C at a 25 mm depth was observed with a steam dose of 2.78 kg/m^2, an operative time of 14 h/ha and total fuel consumption of 768 kg/ha.

Weedtechnics, an Australian company, has developed saturated steam generators that kill weeds in a single pass (https://www.weedtechnics.com/products/). Satusteam © is a patented method of producing a mixture of saturated steam and boiling water. Their current range of Weedtechnics' machines is designed for nurseries, intensive small acre farms, vineyards and orchards. The SW3800KD can be trailer mounted to a customise size trailer. It can support multiple heads that can cover a swept path width of 120 cm such as in orchards or be mounted on a hydraulic arm to control weeds on the row edge.

Ercane in Reunion is currently testing a hot water and steam weeding machine for weed control in sugarcane (Houat 500, Oeliatec http://oeliatec.co.uk/houat-500/). Their preliminary tests showed promising results to control all weeds at the 20-30 cm growth stage (including *Cynodon dactylon* and *Brachiaria decumbens*). However, they noted a very high water consumption (900 L/h) and a very slow speed of treatment (500 h/ha). They believe the technology could be useful as replacement for chemical spot spraying (for hard to control species such as couch grass or Guinea grass) since glyphosate is going to be banned in Reunion in 2022. However, the high cost of the equipment at 30,000 € is another constraint (pers. comm., Alize Mansuy).

Electricity

The practice of weed control via electric shock is called electrocution. Weeds can be killed by spark discharge (high voltage, short duration pulses) or electrical contact (an electrode connected to a high-voltage source touches the plant with current flow for the duration of the contact time) with 20kV. The strength of electric shock, contact or exposure duration, weed species, morphological features and growth stage significantly affect the success of electrocution. The severity of damage is aggravated in drought conditions. However, because of higher energy requirement, high financial costs involved, and hazards to operators, this technology is not widely adopted in agriculture. (Korres *et al.* 2019).

Zasso Electroherb is a technology developed by the Zasso group which has already been commercialised in sugarcane in Brazil (https://zasso.com/). Zasso Electroherb technology uses electrodes to direct high voltage discharges through the whole plant. In Brazil, PTO powered units have been used to successfully control weeds in sugar beet.
https://www.asaja.com/horizontales/categoria_14/agxtend_xpower%E2%84%A2_-_exitoso_control_de_las_malas_hierbas_en_el_azucar_remolachas_3712

The risk of lighting up the trash blanket make this strategy difficult to implement in trash blanketed ratoon cane.

Microwaves

Brodie (2016) has demonstrated that microwave heating, using a suitable device to project the microwave energy onto plants and the soil, can kill weed plants and their seeds in the field. The

following species were successfully controlled with microwave heating: ryegrasses – annual and perennial; barnyard grass; barley grass; bellyache bush; brome grass; clover; feather top Rhodes grass; fleabane; hemlock; *Mimosa pigra*; parthenium; rubber vine; wild oats; and wild radish. The microwave energy density required to kill plants varied according to the species. Microwave treatment is not affected by weather conditions such as wind or rain. Brodie hopes to have some preliminary designs for commercial prototypes available mid 2020 with the idea to have the machine set up on a trailer and running from the power take-off (PTO) of a tractor. https://www.futurefarming.com/Machinery/Articles/2019/11/Growave-kills-weeds-using-microwave-technology-495304E/

Further research needs: When commercially available, the microwave technology should be investigated for its potential use in sugarcane.

The Zasso Electroherb should also be further investigated for its potential application in cane.

6.7.3 Cover crops

Fallow management that promotes soil cover may reduce weed germination due to attenuation of soil temperature and reduced light transmission (due to high biomass production or to the production of allelochemicals). Residues from fallow species may favour losses from the weed seed bank due to germination, loss of seed vigour, or seed decay. In addition, fallow vegetation can influence weed seed predation. Enhancement of soil productivity should increase the vigour of the following crop to better compete with weeds. The burning of fallow residues may result in weed seed death due to extreme temperatures or may induce seed germination. Short-term improved fallows can be an important component of integrated weed management (Gallager *et al.* 1999).

Well-managed legume cover crops in the sugarcane cropping system have improved cane yield compared to plough-out replant. Apart from producing nitrogen that will be available for the new cane crop, fallow crops improve many soil properties. The inclusion of cover crops in the crop rotation, at a time when the land might otherwise lie uncropped, is also an effective method for suppressing permanent weeds. However, Osten (2010) observed that growers generally do not effectively control their weeds during the cane cycle, resulting in maintenance or increase of the weed seed bank. Similarly, very little to no weed control in green manure legume crops also increases the weed seed bank, with legume and vine weeds in legume crops being common issues to all regions. The main weeds in cane are the main weeds in rotational crops. Growers awareness of the role of good weed management practices in fallow must be improved.

Traditionally a bare fallow provides an opportunity to control difficult weeds such as nutgrass, Guinea grass and couch grass with glyphosate. The use of more specific herbicides such as Verdict, Blazer or Fusilade makes it possible to control these weeds within legume fallow, however fallow blocks heavily infested with problem weeds should not be planted with legume to facilitate access and use of repeated applications of herbicide if needed to reduce the weed seed bank.

Fillols (2018) tested some strategies to control weeds in fallow cover crops in the Wet Tropics without using herbicides. Field trials showed that cowpea (*Vigna unguiculata*) alone or cowpea mixed with lablab (*Lablab purpureus*) and Japanese millet (*Echinochloa esculenta*) were the best weed suppressants, as long as the cover crops were sown at twice the standard sowing rate and before any weeds germinated. These cover crops performed well in no-till, zonal till, and full tillage systems. In wet conditions, Ebony cowpea performed the best. Japanese millet performed well in all farming systems: the early germinating millet outcompeted the weeds in the early stages after sowing, while the legumes emerged and competed with the weeds a few weeks later.

Combining cowpea and lablab (high sowing rate) with or without millet were the cheapest of the non- herbicide strategies, but they were still $60 to $90 /ha more expensive than traditional legume

scenarios that relied on herbicides. This extra costing did not consider additional benefits: no plant back effect in the following plant cane from herbicide residues, mixes of cover crop species in fallow are beneficial to soil structure, high green manure biomass provides more organic matter.

Further research needs: Soybean is currently the most planted legume fallow in Queensland (25% of growers acknowledge planting soybean in their fallow according to the results of the 2017 SRA grower survey), however soybean does not provide an early ground coverage to compete with weeds. Increasing soybean rates, variable sowing configurations and mixing with other seeds are strategies that need investigating from a weed management perspective.

Intercropping

Intercropping is a farming method that involves planting or growing more than one crop at the same time and on the same piece of land. In sugarcane, some plant species sown in the interrow can be used to compete with the weeds.

Mansuy *et al.* (2016) tested the effectiveness of legume crops to control weeds in sugarcane fields in Reunion. *Canavalia ensiformis* and *Vigna unguiculata* sown in the interrows of ratoon cane 1.6 to 3.3 months after harvest maintained the weed coverage in the interrow below 30%. However, cane yield losses ranging between 10 to 22% were observed, probably due to the competition between the cover crop and the cane. Hogarth & Allsopp (2000) also noted that the low-growing non-twining pinto peanut (*Arachis pintoi*) could also be used as a permanent understory in the cane farming system, however in some experiments it had some adverse effect on plant cane yield.

Yang *et al.* (2013) conducted a field experiment with four crop arrangement patterns (soybean monoculture, sugarcane monoculture, 1:1 row sugarcane–soybean intercropping, 1:2 row sugarcane–soybean intercropping) in Guangzhou, China. Land equivalent ratio (LER) was used to evaluate the potential advantages of the intercrops, aggressivity (AG), and competitive ratio (CR) which are based on crop yield and nitrogen acquisition were used to evaluate interspecific competition between sugarcane and soybean. The results indicated that the sugarcane–soybean intercropping system had intercropping advantages based on total LER in the three-year study. The partial land equivalent ratio for sugarcane varied between 0.84 to 1.16 during the three years of study, indicating that sugarcane yield could either benefit or be impacted by the presence of the intercrop depending on the weather. Sugarcane had lower AG and CR values than soybean.

Conlong & Campbell (2010) studied an innovative solution of planting a non-invasive tufted grass, *Melinis minutiflora*, to compete with the troublesome *Cynodon dactylon*, which commonly encroaches into fields from surrounding areas, e.g. cane breaks and roadsides, requiring frequent and costly control. In trials at Port Shepstone and Mount Edgecombe (South Africa), results indicated that biomass of *C. dactylon* was severely or completely suppressed under *M. minutiflora*, which had formed an effective barrier against this weed and prevented encroachment into the field. *M. minutiflora*, in return, did not encroach into the adjacent sugarcane.

Many other overseas studies indicate the economic benefit of intercropping, but in all these cases the intercrop is harvested as a cash crop and chemical or manual weed control is still required.

In North-Western India, four cropping systems (sole sugarcane, sugarcane-cabbage, sugarcane-peas, and sugarcane-garlic) and six weed control treatments were investigated. Intercropping of sugarcane with peas, cabbage and garlic produced cane yield (75.3–88.3 t/ha) similar to sole sugarcane (76.1–86.0 t/ha) and intercrop yields of 6.28–6.57 t/ha for garlic, 7.45–7.88 t/ha for peas and 15.3–15.8 t/ha for cabbage. The intercropping of these vegetables in sugarcane increased the net monetary returns by 1.74–2.66 fold as compared to sole sugarcane. Pre-emergence application of oxyfluorfen and pendimethalin provided similar level of weed control to hand-weeding, and increased the yield

of cabbage, peas, and garlic compared to the weedy check. The use of herbicides increased the net returns by USD 229–725/ha as compared to the weedy check (Kaur *et al.* 2015).

Sugarcane-oilseed rape and sugarcane-Indian mustard intercropping systems produced cane yield (73.6–88.6 t/ha) similar to sole sugarcane (78.4–85.3 t/ha). Oilseed rape produced seed yield of 1.47–1.59 t/ha while Indian mustard produced 2.51–2.95 t/ha. Sugarcane–oilseed rape and sugarcane-Indian mustard systems increased the net returns by 1.3 and 1.7-fold as compared to sole sugarcane. Indian mustard exhibited higher weed suppression than oilseed rape and sole sugarcane, which may be associated with greater production of secondary branches and planting arrangement of Indian mustard (2-rows) as compared to oilseed rape (1-row). Pre-emergence application of pendimethalin and alachlor provided adequate control of weeds in these intercropping systems and increased the seed yield of oilseed rape and Indian mustard relative to the weedy check by an average of 41% and 15%, respectively (Kaur *et al.* 2016).

Further research needs: Review literature and establish trials to determine the best cropping method to grow an intercrop cover crop species to control weeds without impeding on cane growth in several farming systems in Australia.

Shade

Light availability significantly influences plant life cycles and is important in regulating the competitive relationship between crop and weed species. One of the ways to control weeds in cane is to take advantage of the intense shading under the sugarcane canopy which limits the density and biodiversity of plant populations underneath the crop. At canopy closure, the light environment underneath can exclude a considerable number of plant species which show high sensitivity towards canopy shade and eventually die in this condition. However, some species can escape shade by increasing their internode length and decreasing their leaf size to compete with the crop for light. Some species also seen under canopy shade have subterranean organs which explain their persistence. Sun flecks may also play an important role in the maintenance of the weed population under the sugarcane canopy (Ascencio & Lazo 2012).

Research has proven that alternation of cultural practices, and selection of competitive crop cultivars, could be a possible strategy to minimise the light available to weeds and reduce their competitiveness.

- Manipulation of crop row spacing and orientation is a reliable way to increase light interception by the crop and reduce light interception by weeds. Row orientation perpendicular to the path of the sun enhances crop water, nutrient and light use efficiency and consequently results in increased crop growth and reduced weed biomass and density (Ali *et al.* 2017). Row configuration from single- to twin- or paired-row pattern is also effective for weed control. Twin-row planting of soybean or peanut allows earlier canopy closure, with consequent increases in crop competitive ability and reduction in weed germination and growth, decreasing the need for herbicide application (which reduces crop stress), thereby benefiting crop yield (Korres *et al.* 2019). Planting pattern in sugar beet (single row planting with 50 cm row width, single row planting with 60 cm row width and twin row planting with 60 cm row width) had an impact on weed biomass, with the best results obtained in twin row planting at 60 cm (Zargar *et al.* 2017).
- Mixing cultivars with different growth rates uses positive effects of competition and light compensation between cultivars to increase growth and yield and potentially reduce weed competition. Takaragawa *et al.* (2016) compared two cultivars with different canopy structures, one with high stalk weight and the second with many tillers. Stalk length of one cultivar in the mixture by-row (each cultivar alternating every second row) was shorter,

which overall resulted in better light-intercepting characteristics of this canopy during later growth stage. Mixture-by-plant (cultivars mixed within the row) could promote competition for light during the earlier growth stage.

Adding light as a key factor after temperature and moisture could improve the accuracy of thermal time (TT) or hydrothermal time (HTT) based emergence models that have been developed for predicting weed species emergence. Field trial results showed that the effect of light is variable depending on the species, but in general light-adjusted models usually improve the accuracy of those based only on HTT (Royo-Esnal *et al.* 2016).

Further research needs: In Australia, no research has been carried out on the competitiveness of the sugarcane varieties or the benefit of row configurations on weed management. This area should be explored further.

6.7.4 Harvest seed destruction

Harvesters are a major contributor to weed spread, resulting in soil seedbank increases. Harvest weed seed control (HWSC) methods used in the grains industry include both cultural and mechanical practices to prevent seed dispersal.

- The chaff cart consisting of a chaff collection and transfer device, attached to the harvester, delivers the crop residues along with weed seeds into a bulk collection bin that will be removed from the field.
- Narrow-windrow burning consists of using a chute mounted on the rear of the combine that delivers the bulk of the chaff into a narrow windrow. Burning these windrows as soon as possible secures higher weed seed destruction; however, wind speed, type of crop, air temperature, and soil moisture can affect the efficacy of narrow-windrow burning. The amount of chaff could be another factor that can alter the efficacy of this system. In soybean, narrow-windrow burning reduced the *Amaranthus palmeri* population by 73% and the soil seedbank by 62% over 3 years (Norsworthy *et al*. 2016).
- The Harrington seed destructor (HSD) is integrated directly into the chaff stream of a combine harvester (Walsh *et al*. 2013). The latest mechanical-drive system can be mounted on the rear of the combine, behind the sieves for later model John Deere, Case IH, New Holland and Claas harvesters, with no permanent modifications required. http://www.mcintoshdistribution.com.au/machinery/show/harrington-seed-destructor-harvesting-australia. The HSD technology is highly effective in Australia wheat cropping and results in 93–99% seed destruction including seeds of rigid ryegrass, wild radish, bromegrass, and wild oat (Storrie 2014). In soybean, >97% of the most common weed seeds were destroyed by the HSD (Schwartz-Lazaro *et al.* 2017).
- The bale-direct systems (Walsh & Powles 2007) method consists of a large baler directly attached to the combine that bales the chaff as it is exiting the harvester. This system captures the weed seed and bales can be exported as feed for livestock.

Nevertheless, it might be expected that continuous use of HWSC in the grain industry will select for early maturing weed phenotypes. Overreliance on this tool may lead to the same result as overreliance on herbicides.

Further research needs: None of the HWSC strategies used in the grain industry has been considered in sugarcane, where the amount and type of weed seeds ejected along with the trash by the extraction fan is yet to be evaluated.

6.7.5 Trash blanket

Physical barriers

The benefit of sugarcane trash as a physical barrier that impedes weed germination and growth has been extensively researched. GCTB impedes the development of most weed species, especially grasses (Villegas *et a*l. 2007; Manechini *et al.* 2005; Sampietro & Vattuone 2006; Fillols & Callow 2010) and the control of broadleaf weeds and grasses improves with an increasing level of trash. In rice (*Oryza sativa*), Bolfrey-Arku *et al*. (2011) reported that rice residue exceeding 4 t/ha on the soil surface reduced itchgrass emergence by 50%.

In contrast, nutgrass (*Cyperus rotundus)* was observed to develop under thick trash blanket conditions (Novo *et al*. 2008; Fillols & Callow 2010). Variable outcomes have also been observed regarding the impact of trash residues on vines. Fillols & Callow (2010, 2011) reported that 9 t/ha of trash in one trial and 13 t/ha in another significantly impeded the development of *Ipomoea* species. A similar study conducted by Richard (1999) showed 79% less *Ipomoea* species emergence from 6.4 t/ha of post-harvest sugarcane residue retained on the soil surface when compared with emergence from soil with no cover. Azania *et al*. (2002) reported a reduction in vines emergence at 20 t/ha. Gaungoo *et al.* (2010b) found that seed germination for *I. triloba, I. obscura* and *I. nil* was not affected by a trash layer of 5 cm whereas a significant reduction was observed as trash thickness was increased to 10 cm. Manechini *et al.*(2005) argued that *Ipomoea* species were not affected by the trash. The outcomes seem to vary depending on the vine species, the trial site, the seed depth in soil and environmental factors such as temperature and soil/trash moisture (Gaungoo *et al.,* 2010b). For example, when seeds were sown at 2 cm depth under a trash layer of 10 cm, results showed germination of *I. triloba > I. nil> I. obscura* (Gaungoo *et al.* 2010a). Fillols & Staier (2015) found that siratro (*Macroptilium atropurpureum*) with 78% emergence was less impacted by trash residues compared to red convolvulus (*Ipomoea hederifolia*) and centro (*Centrosema molle*) (60 and 66% emergence respectively). Vine hypocotyls were proportionally longer with increase in trash level and the hypocotyl elongation/ trash level ratio was higher for Ipomoeas than legumes, suggesting a higher plasticity of *Ipomoeas* that would allow them to grow through even thicker trash. When studying the impact of trash residue quantity on vine germination, Fillols & Staier (2015) also found that 18 t/ha of trash residue reduced the final number of emerged vine seedlings by 66% on average compared to 6 t/ha trash residues. 12 t/ha trash residues reduced seedling emergence by 32% on average.

Allelopathy

Allelopathy is the direct or indirect effects of chemicals produced by plants or microorganisms on the growth, development, and distribution of other plants and microorganisms in natural and agricultural ecosystems. Use of allelopathic interactions to favour the crop and reduce weed infestation has been used for centuries without understanding the chemical basis of the phenomenon. Allelochemicals are chemically diverse, e.g., terpenes, alkaloids and non-proteinaceous amino acids, phenols, and sugars/glycosides. Plants with allelopathic potential are considered sustainable alternatives for weed control and are means to minimise reliance on herbicides (Korres *et al.* 2019). Several studies in crops where plant residue was left on the soil showed that low residue densities stimulated plant grows while high residue densities were inhibitory. Sugarcane straw leachate concentrations at 6 g/L significantly promoted root growth of pigweed, radish, sorghum, wheat and wild mustard. However, a significant decline in root length was observed at leachate concentrations higher than 6 g/L. The identified leachates responsible for allelopathic effects were the trans-ferulic, cis-ferulic, vanillic and syringic acids (Sampietro & Vattuone 2006). Villegas *et al.* (2007) also measured the allopathic effect of the sugarcane residue that lowered weed populations in pots irrigated with a fresh residue extract. The author also noted

that the allopathic effect of green cane residues affected the germination of some cane varieties. Only 23% of the Colombian sugarcane variety MZc 74-275 germinated when setts were irrigated with residue extract, leading to removal of residues to the interrow and hilling of the cane row in commercial fields to minimise allelopathic effects on sett germination. Viator *et al.* (2006) came to similar conclusions: large amounts of post-harvest cane residue (up to 24 t/ha), deposited on the field surface when harvesting green generated high concentrations of residual extracts, which exhibited auto toxicity by delaying early leaf development. The authors also noted low extract concentration increased sugarcane germination by 45% compared with the control, indicating hormetic effects and suggesting a small amount of cane residues on the row could prove beneficial.

Occasionally, crop cultivars are developed in consideration of their allelopathic potential. In organic rice production, an allelopathic cultivar "Rondo" was grown to reduce weed pressure (Gealy & Yan 2012). Gealy *et al.* (2013) used conventional breeding to develop new rice cultivars with improved weed-suppressive ability and better yield. Allelopathy being quantitatively inherited, they bred a rice cultivar with almost equal weed-suppressive ability as the allelopathic parent.

Further research needs: Evaluate the allelopathic potential of the residues produced by Australian sugarcane varieties and use the trait in conventional breeding.

6.7.6 Biological control

Biological control methods involve the utilisation of natural living organisms, i.e. bioagents such as insects, pathogens and competitive plants, to limit the weed infestation, the objective of biological control not being the complete eradication of the weed populations but bringing the populations below the economic injury level. Weed biocontrol agents can only be released following a process of risk analysis that considers the potential for nontarget species to be attacked, the status of potential nontarget species (e.g., rare), the likelihood of spatial and temporal overlap between target and nontarget species, the predicted effect of attack on the target weed, and the effect of other control methods (including no control). Agents may be released even if nontarget effects are predicted by host-specificity testing, but only if the benefits of control greatly outweigh the potential cost to the nontarget. The advantages of biocontrol agents are their relative cheapness, their comparatively long-lasting effects and least impact on environment and non-target organisms. Biological weed control methods are commonly explored for controlling environmental weeds, with some outstanding results:

- Control of *Eichhornia crassipes* (water hyacinth) using *Necochetina eichhorniae* (hyacinth weevil),
- *Salvinia molesta* (water fern) is controlled by *Crystobagus* spp.,
- *Lantana camara* in India has been effectively controlled by the moth *Crocidosema lantana*,
- *Zygograma bicolorata* beetle feeds on *Parthenium* plants during the rainy season.

Currently Biosecurity Queensland is investing in eleven biocontrol research projects to manage environmental invasive weeds such as bellyache bush (*Jatropha gossypiifolia*); prickly acacia (*Vachellia nilotica* ssp. *indica*); cat's claw creeper (*Dolichandra unguis-cati*); Navua sedge (*Cyperus aromaticus*); parthenium (*Parthenium hysterophorus*)*;* parkinsonia (*Parkinsonia aculeata*); *Cylindropuntia spp*.; Siam weed *Chromolaena odorata*; mother-of-millions (*Bryophyllum* spp.); and giant rat's tail grass (*Sporobolus* spp.) (Anon, 2020).

As Navua sedge is also found in sugarcane in Far North Queensland, this research from Biosecurity Queensland is of particular interest for the sugarcane industry. Biosecurity Queensland is searching for specialist natural enemies as biological control agents for Navua sedge in its native range that includes countries in equatorial Africa and islands in the Indian Ocean, off the coast of southeast Africa. A smut pathogen that attacks Navua sedge's flower head and seeds has been identified as a

prospective biocontrol agent. The smut pathogen on Navua sedge was shown to represent a new species of *Cintractia* that differed in a molecular phylogenetical analysis from smut pathogens found on other sedges in East Africa. Field host range studies conducted in a quarantine facility in CABI-UK and molecular studies suggest that the smut pathogen is likely to be host specific. If proven host specific, approval will be sought for its release in Australia, which would benefit the sugarcane industry in Northern regions. https://www.daf.qld.gov.au/business-priorities/biosecurity/invasive-plants-animals/research/current/landscape-protection-and-restoration#6

According to data from several reviewed studies evaluating biological weed control outcomes, approximately 25% of biocontrol projects have achieved complete control (e.g. *Azolla filiculoides* Lam. in South Africa), 50–70% of biocontrol projects have achieved at least substantial control, and the remaining 5-25% have achieved no control (e.g. *Lantana camara* in India). Efforts to improve the success of weed biocontrol include choosing target weeds with a higher probability of successful control, determining the most sensitive life stage(s) of a weed to target, and determining which biocontrol agent(s) will most likely be able to reduce the population growth of the target weed (Hinz 2020).

Classical weed biological control programs which often consist of deliberate introductions of exotic organisms are increasingly criticised for their lack of rigorous evaluation of the ultimate result. In a typical program, most resources are invested in surveying the native range of the invader for natural enemies and experimentally demonstrating that the candidate agents do not pose an unacceptable risk to non-target plant species, but limited time and energy are spent assessing the effectiveness of candidate biological control agents in suppressing the target weed. Morin *et al.* (2009) highlighted the need to develop sound plans to evaluate the effectiveness of weed biological control agents at various phases throughout a program. For example, detailed data on weed populations collected at representative sites in the introduced range several years before the release of agents can be compared with similar data collected later to assess agent effectiveness. Hinz *et al.* (2020) also emphasised the need for further investigation to better understand the underlying reasons for spatial or temporal variability of biocontrol success, which could be addressed through post-release monitoring over multiple years across environmental gradients and in different habitats. Taylor *et al.* (2007) reinforced this need for comprehensive post-release studies when an agent is released that demonstrated a potential to attack nontarget species during host testing. In Australia, *Euclasta whalleyi* Popescu-Gorj and Constantinescu (Lepidoptera: Pyralidae) has been released to control rubber vine despite host testing results indicating closely related native species could be attacked. Post-release studies showed the impact on nontarget species was negligible. *Neurostrota gunniella* Busck (Lepidoptera: Gracillariidae) is another agent approved and released in Australia, despite a known potential for attack on nontarget plants. It is one of 14 agents that have been released in Australia against *Mimosa pigra* as it reduces its seed production by 60%. Although an average of 61% of *Neptunia major* plants (non-target plant) growing adjacent to *M. pigra* thickets in the field had evidence of *N. gunniella* attack, the intensity of attack was relatively low, which supports the predictions made during pre-release studies of *N. gunniella.*

Morin *et al.* (2009) noted that, despite information on yield reduction due to a weed and financial costs of its control being generally readily available for many intensive agriculture industries, classical weed biological control is rarely contemplated for such systems.

Smith *et al.* (1997) studied the head smut *Sporisorium ophiuri*, which forms systemic infections sterilising the weed, as a potential classical biological control agent for itch grass (*Rottboellia cochinchinensis*). A difference equation model of the population dynamics of itchgrass in maize cropping system was developed and used to estimate the constant annual infection rate by *S. ophiuri* that would be necessary to provide long-term control of the weed. The model suggested that with the smut as the sole control agent, an annual infection rate of about 88 % would be required to reduce itchgrass density to 10% of the level achieved with no control. Where seed set or seedling

survival were low, the required infection rate could be substantially reduced. When combined with one or two weeding operations per year, the level of infection necessary for satisfactory control could also be reduced. Since the maximum infection rate achieved in experiments was about 80%, the smut may not always achieve satisfactory control when used alone but could be a useful adjunct in integrated control programmes.

Further research needs: Funding should be allocated to explore biological control agents to control some troublesome sugarcane weeds. For example, the hawk moth* Agrius convolvuli *feeds on convolvulus vines in sugarcane crops in Australia and serious damage on vine leaves from feeding larvae can often be observed by sugarcane growers.* Agrius convolvuli *is a pest in New Guinea and Indonesia on Sweet Potato (*Ipomoea batatas*). Agrius convolvuli could be evaluated for its potential use as biological control for Ipomoea vines in sugarcane.

6.7.7 Bio-based herbicides

Some of the most successful drugs and agrochemicals on the market have been developed from secondary metabolites (SMs) of natural compounds. When searching for natural compounds having herbicidal effects, the process is to search for fungal pathogens able to cause symptoms such as necrosis and chlorosis and try to identify the fungal SMs whose macroscopic effects resemble those due to the pathogen's attack. Natural bioactive compounds have the advantage of a low environmental impact due to their rapid degradation in the environment; however, this rate of degradation may be their downfall in terms of efficacy persistence. Undeniably, there are many other limitations in using metabolites of biotic origin: difficulties in scaling-up the production/fermentation process, complex chemical structures difficult to synthesise, low stability or persistence of bioactive compounds and unacceptable off-target effects.

While there are abundant literature reviews on the isolation and characterisation of phytotoxins from many sources, with many having been patented for potential use as herbicides, there are very few effective natural herbicides in the market (Korres *et al.* 2019).

Examples of bio-herbicides are:

- Corn gluten meal is used as pre-emergent. It needs to be applied at high rate which makes it very expensive.
- Acetic acid is a contact herbicide only and non-selective.
- Fatty acids (herbicidal "soap") such as pelargonic acid are contact herbicides only, non-selective and expensive.
- Essential oils such as pine oil, clove oil, lemongrass oil, manuka oil are contact herbicides and non-selective. They work better when applied with surfactants.
- Microbial products such as bialaphos tripeptide obtained from the fermentation of the actinomycete Streptomyces hygroscopicus and marketed as a herbicide in East Asia. Bialaphos is a pro-herbicide, metabolised by plants into its herbicidal form, phosphinothricin (L enantiomer of glufosinate). Like gluphosinate, bialaphos is a broad-spectrum, post-emergence herbicide. Glufosinate is a model for the development of a synthetic herbicide from a natural compound.
- Brassicaceae seed meal common field application rates are between 1 t and 2 t/ha of seed meal and provide 53–59 kg N/ha which could substitute for the application of chemical N fertiliser, in addition to weed control. Oilseed meals such as those from Indian mustard (*Brassica juncea*), rapeseed (*Brassica napus*), and yellow mustard (*Sinapis alba*) contain glucosinolates which become herbicidal upon enzymatic hydrolysis. Weed species have differential response to these compounds. Experiments conducted to determine the herbicidal activity of mustard seed meal (*Sinapis alba* 'Ida Gold' and *Brassica juncea* 'Pacific

Gold') in two weed species, large crabgrass (*Digitaria sanguinalis*) and Palmer amaranth (*Amaranthus palmeri*) show that 'IdaGold' might have better herbicidal efficacy on Palmer amaranth (broadleaf weeds), whereas 'Pacific Gold' was more effective on large crabgrass (grass weeds) (Wang *et al*. 2015).

6.8 Grower engagement

Osten (2010) observed that weed management in Northern and Central Queensland cropping systems was undertaken for yield impost purpose only, with little consideration of the weed seed bank and highlighted the short-term view of weed control by growers. The author also noted that weed management considerations were not a driver of the cropping systems, but the systems appeared to be driving the weeds.

Since 2014, Smartcane BMP has been working with cane farmers across Queensland to record and verify their practice improvements to help secure growers' reputation as stewards of the land. Smartcane BMP has focused on the three core modules so growers can become accredited and be independently recognised for their management of soil health and nutrients (module 1), irrigation and drainage (module 2), and weeds, pests and diseases (module 3) https://smartcane.com.au/wp-content/uploads/2018/10/SmartcaneBMP_fact-sheet_Module-3.pdf.

The weed, pest and disease module focuses on responsible pesticide use and records growers' current practices, while identifying options for further improvement. Industry standards are described as a weed management plan based on the SRA 2020 Integrated Weed Management Plan template that has been endorsed and recognised by SmartCane BMP https://sugarresearch.com.au/growers-and-millers/weeds/. The module includes correct chemical application in line with label requirements and legislation, keeping records of chemical management for each field, selecting appropriate nozzles, calibrating equipment at the start of each season and with each change in product, including a chemical management plan within the weed management plan, and timing chemical application to minimise run-off. The module also includes the development and implementation of a weed management plan that focuses on controlling weeds during the fallow period, minimal use of residual herbicides, using GPS technology to identify and manage weed problem areas, using automatic flow rate controllers and precision application equipment, and continuous monitoring and calibration that are considered above industry standard practices.

A district facilitator or productivity officer assists in follow-up on additional information, training, or expert advice. Participation is entirely voluntary.

As of 3 July 2020, 565 sugarcane farm enterprises, equivalent to 126,047 ha, were accredited for modules 1,2 and 3, which represents 14% of farms and 35% of hectares harvested. https://smartcane.com.au/

The Reef 2050 Water Quality Improvement Plan identifies priority catchments and targets for reducing pollution from catchments flowing to the Great Barrier Reef. Funding under the Reef Trust Partnership aims to deliver measurable progress towards those targets. As sugarcane growers have been identified as large contributors to pesticides that contaminate watercourses and ultimately the GBR, a range of extension projects currently funded by the Reef Trust Partnership have been undertaken to assist growers change their weed management behaviour. Currently funded projects include:

- Cane Changer project. A behaviour change program that elicits improved practices through accreditation in the SmartCane Best Management Program and other forms of 'commitment' towards improved practices in the Wet Tropics region. In April 2019, Cane

Changer was expanded into the Burdekin, Mackay-Whitsunday and Southern sugarcane growing regions of Queensland (Pickering *et al.* 2019; Moore *et al.* 2020).

- Cane to Creek 2.0 project. This project undertakes farm activities with small canegrower groups to address nitrogen and pesticides. The program breaks down the barriers between scientists and growers, maximises peer-to-peer learning opportunities and improves understanding of the drivers of water quality impacts. It operates in the Mossman, Mulgrave-Russell, Johnstone, Murray, Herbert and Haughton catchments.
- Project Bluewater. This project reduces the runoff of pesticides into the Great Barrier Reef lagoon through the adoption of improved sugarcane farming practices. The project directly engages over 70 growers, managing over 12,000 ha of land, in the catchments of Haughton, Pioneer and O'Connell Rivers and Plane Creek, which were identified as high priority in the Reef 2050 Water Quality Improvement Plan.
- The Sandy Creek Sub-catchment Water Quality Monitoring Project. This project was initiated by growers and leading industry bodies in response to continued exceedance of water quality guidelines, with the aim of identifying where in the catchment problems exist, thereby enabling growers to make informed decisions to improve management practices and improve the health of Sandy Creek. Local growers together with Mackay Area Productivity Services and Farmacist have monitored water quality at 13 sub-catchment sites in Sandy Creek.
- An Evidence Based Approach to Improving Water Quality in Barratta Creek Catchment (Stage 2). This farmer led project raises awareness and drives practice change through improved fertiliser application, modifying pesticide type and quantity and improving irrigation efficiency in the Burdekin River Irrigation Area.
- Reef Alliance Project, Phase 2. This project supports cane farmers and graziers by using one-to-one agricultural experts (extension officers) to move 462 land holders, covering 209,750 ha, towards best practice to reduce sediment, nitrogen and pesticides in the Wet Tropics, Burdekin, Mackay/Whitsundays, Fitzroy and Burnett Mary regions.

New project proposals that will directly aim to reduce nitrogen, sediment and pesticide loads from priority catchments through a series of regional water quality improvement programs are being assessed for funding from July 2021. Target pollution load reductions have been set for each catchment, and regional programs and associated projects will need to demonstrate how they will contribute towards these targets. https://www.barrierreef.org/what-we-do/reef-trust-partnership/water-quality-improvement

Further research needs: In addition to meet with adoption or extension officers, growers like to be involved in research projects and have direct contact with researchers. SRA project 2014/050 included a research and an extension component, which regularly invited growers to visit herbicide efficacy research trials. The unprecedented attendance to the field days demonstrated the appetite growers have to be involved in on-going research and the interest in increasing their knowledge on weed management. More grower participation in herbicide efficacy field trials when compared to non-chemical alternatives field trials showed lower interest in non-chemical strategies. This limited interest is understandable as non-chemical strategies are considered novel and unproven methods by growers. Non-chemical strategies must receive adequate funding to be validated by adequate research work and gradually gain growers' confidence.

Future funded weed management research projects should include an on-field-based extension component to maximise the impact on the industry.

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7. RECOMMENDATIONS FOR FURTHER RD&A

Biosecurity threats

- Reconsider Saunders' set of biosecurity actions of importance to SRA, determine which have been undertaken, and progress those not yet undertaken.
- Ensure industry staff are familiar with and have experience with potential exotic threats.
- Ensure dossiers on potential pests are updated at least every 3 years.
- Continue the research on DNA methods for mothborer identification and promote comprehensive taxonomic revisions of *Sesamia* and *Chilo*.
- Update regularly (say every 3 years) thee dossiers of insecticides potentially useful in Australia and give thought to how they would be used commercially in Australia (aerial or ground application, what type of pre-use monitoring, withholding periods, etc).
- Continue off-shore research to develop a reliable method of rating varieties for resistance to mothborers and rate the major Australian varieties; the system used at SASRI provides a good model.
- SRA should attempt to obtain overseas clones with significant levels of resistance to mothborers and incorporate these as parents in the current breeding program or as a sub-program.
- The use of GM technology in Brazil for mothborer resistance needs to be followed carefully and re-assessed for use in the Australian industry.

- Consider how area-wide management of mothborers could work within the Australian system.
- Work with Torres Strait communities to emphasise the potential importance of *Eumetopina* to the Australian industry and what measures they should undertake to prevent its spread.
- Continue research to develop a reliable method of rating varieties for resistance to Ramu stunt and rate the major Australian varieties.
- SRA should continue to screen Australian varieties for resistance to downy mildew. More efficient and reliable methods for screening varieties should be investigated.
- Continue cooperative research with Indonesia to identify vectors of streak mosaic and to screen Australian varieties for resistance to the disease.
- Conduct targeted surveillance in the south-eastern provinces of Papua New Guinea and Torres Strait to monitor for spread of streak mosaic.
- Intensively screen any germplasm imported into Australia from Asia for streak mosaic.
- Intensively screen any germplasm imported into Australia from Asia for phytoplasmas.
- Continue research into NGS/metagenomics as a generic assay for detection of pathogens in sugarcane in post-entry quarantine.
- Investigate the incidence and importance of Sugarcane yellow leaf virus to the Australian sugar industry.

Pest management

Canegrubs

- Continue the research to provide an alternative registered product to imidacloprid for the control of canegrubs.
- Develop tools to warn of impending insecticide resistance to registered products.
- Improve understanding of adult (and larval) behaviour to provide a better basis for forecasting, risk prediction and resistance management. This is particularly important for greyback canegrub, whose adults move to and from fields.
- Continue development of better methods for risk prediction coupled with better acquisition of field, farm, and regional data.
- Update and continue the extension message with an emphasis on pre-emptive rather than reactive management.

Soldier flies

- Test any insecticide newly registered for use on sugarcane for efficacy against soldier flies.
- Develop methods for much earlier prediction of outbreaks through the use of remote sensing and weather information.
- Use the early detection to implement an industry-sanctioned group-action response plan to take out infested patches or blocks as soon as possible after being identified in order to short-circuit population upsurge and limit spread.
- Develop a robust taxonomy and map that to previous research to allow full understanding of that work.
- Update and continue the extension message with an emphasis on pre-emptive rather than reactive management.

Disease management

Ratoon stunting disease (RSD)

- Coordinate a review in collaboration with Productivity Services to interpreting past RSD laboratory results and document RSD incidence in commercial blocks and plant sources in recent years. The review should also record the level of adoption of the key components of the RSD management program such as percentage of growers obtaining ASP plot cane and tonnes of ASP cane/grower, percentage of blocks planted from progeny of ASP cane, number of plant source inspections, seed-cane sources from fallow and replant blocks and adoption of hygiene practices by planting and harvesting contractors.
- Investigate industry support for conducting a coordinated industry wide RSD survey. If the diagnosis of RSD in juice samples at the mill is successful, this method would provide a cost-effective way to conduct a survey.
- SRA should evaluate qPCR of LSB and/or xylem extracts for assessing bacterial populations in varieties in RSD resistance trials. SRA should ensure that new varieties are rated for resistance.
- The need for breeding for RSD resistance should be reassessed after an industry-wide review of the RSD incidence and the RSD management program. A new economic analysis of the benefits of breeding for RSD resistance could be conducted given the new information on the RSD situation in the industry and the current knowledge of the level of resistance in Australian germplasm.

Smut

- Monitor the success of implementation of markers for smut resistance by determining the increase in the proportion of smut resistance of clones after implementation compared with prior to implementation.
- Determine the proportion of clones with each smut resistance marker to monitor whether the clones selected predominantly have one or different markers for resistance. This is important to see if markers are leading to clones with one or more resistance types.
- Markers (resistance genes) discovered in introgression clones (derived from *S. spontaneum* and /or *Erianthus*) that are different to the resistance genes in the core breeding population should be introduced into the core breeding population. This would provide different sources of resistance that may be stacked into resistant varieties as an insurance and protection against changes in the pathogen that might overcome the existing resistance genes in the core program.
- Continue research to identify “perfect” markers (key resistance genes) for smut resistance to improve our understanding of the resistance genes present in the breeding program and how these can be deployed to provide high level and sustainable resistance. Perfect markers would also increase the efficiency of implementation of markers in the breeding program and limit the risk that linkage between the marker and the trait is lost due to recombination within chromosomes.
- Review the current program of screening for smut resistance in the light of the success of markers. The stage in the selection program where screening for disease resistance is conducted may be reassessed, if markers are successful at eliminating a large proportion of the susceptible clones.

Pachymetra root rot

- Introgression breeding has the potential to give a major advance in breeding for pachymetra resistance and support should be given to exploit the introgression crosses fully. Research should be supported to identify the *Erianthus* homology group/chromosomes that carry resistance to pachymetra root rot and to develop rapid DNA tests to identify the genes in progeny from the hybrid crosses to expedite the introduction of the genes into elite sugarcane varieties.

- Markers for pachymetra root rot resistance should be a high priority. Existing markers should be validated and new markers identified. Markers should be implemented as a priority in the breeding program.

Nematodes

- Existing projects aiming to increase the understanding and adoption of practices that improve soil health will also lead to improved suppression of damage from nematodes and should be continued (see section on general soil health).
- The commercialisation of DNA assays for plant parasitic nematodes should be continued and extended to include free-living beneficial nematodes and possibly bacterial pathogens of nematodes such as *Pasteuria*.
- Introgression appears to offer the best chance of increasing the resistance of varieties to nematodes. Clones derived from the introgression program should be screened for nematode resistance and the resistant clones should be fed back into the program to be used as parents and assessed for yield potential.
- Markers for nematode resistance should be used to increase the frequency of root-knot nematode resistance genes in the introgression breeding program.

Leaf scald

- Leaf scald should be the next target for molecular markers after markers for smut and pachymetra root rot are integrated into the breeding program. Existing markers identified in earlier projects should be validated and markers identified in overseas research should be evaluated in Australian germplasm. Marker-discovery projects should be considered to identify new markers for resistance to leaf scald.

Fiji leaf gall

- Surveys to establish whether Fiji leaf gall is still present in commercial crops in Australia should be a high priority. If Australia can be declared free of Fiji leaf gall in commercial sugarcane crops, there would be several significant benefits including:
  - Wider access to existing varieties in central and southern Queensland, and NSW
  - Improved genetic gains in the breeding program as restrictions on use of parents and loss of susceptible clones due to Fiji leaf gall in the selection program would no longer occur
  - Reduced costs to SRA as screening for Fiji leaf gall at Woodford is scaled back or discontinued. SRA would need to reassess the need for Fiji leaf gall resistance screening at Woodford and decide whether to eradicate Fiji leaf gall from Woodford to eliminate the risk of spread from Woodford to commercial crops
  - Reduced costs to SRA if quarantine for clones moving from central and southern regions to the north are relaxed

Chlorotic streak

- Methods for screening varieties for resistance to chlorotic streak should be developed using the new knowledge of the causal organism.
- SRA should consider funding student projects investigating the biology of *P. venanatans* and sugarcane. This would be an opportunity to train future plant pathologists.

Weed management

- Develop and extend the Weed Seed Wizard to the sugar industry

- Add all Australian sugarcane weed species to the existing WIKTROP portal to make it relevant for use by Australian sugarcane growers, agronomists and consultants.
- Conduct weed distribution studies on specific problem weeds such as Guinea grass and nutgrass in all districts, Balsam pear and calopo in the Wet Tropics; and sicklepod in the Wet Tropics and Central region, to reveal crucial ecological information on these troublesome weed species that could be used to improve their management.
- Conduct studies on the dispersal of weed seeds with mechanical operations or the potential dissemination of weeds via baling and assess the potential of a weed-seed destructor system as used in the grain industry.
- Conduct research on the basic ecology of the most troublesome weeds in sugarcane to improve sugarcane integrated weed management.
- Explore the impact of climate change on the dynamic of competition between weeds and sugarcane crop and investigate potential management issues.
- Determine the critical period of weed intervention when planting NEF CEEDS™ technology in the Australian sugarcane cropping system.
- Aassess the impact of lower nitrogen rates on weed competition in cane.
- Define the economic impact from the competition of troublesome weeds such as Guinea grass, sour grass and Ipomoea vines in the Australian sugarcane industry.
- Define the tolerance of Australian sugarcane varieties to the most troublesome weed issues.
- Investigate the role of weeds as hosts of the key pathogens in sugarcane in Australia.
- Optimise the use of satellite imagery and imagery processing to assess weed infestation at a district scale and target weed management efforts to areas with the highest economic loss due to weeds.
- Undertake specific Guinea grass studies to determine the impact of timing of herbicide application on seed viability to provide growers with more accurate long-term Guinea grass control. Similar studies need to be conducted on other troublesome grass such as wild sorghum, itch grass and sour grass.
- Apply for a specific permit for using Sempra® in cane for control of sedges.
- Investigate incorporating pre-emergent herbicides while sowing an intercrop in sugarcane interrows .
- Undertake further research to better optimise the use of pre-emergence herbicides that are incorporated by furrow irrigation.
- Optimise the application of pre-emergent herbicides in cane to use a wider range of pre-emergent herbicides or reduce the label rates of currently effective herbicides on trash blanket.
- Investigate the interaction between soil conditioners and pre-emergent herbicides to improve weed management for farms that use soil conditioners.
- Determine relevance for the sugarcane cropping system of new spray nozzles
- Investigate the impact on herbicide efficacy of nozzle types and nozzle angles fitted to Irvin legs and droppers to demonstrate to growers the importance of correct nozzle configurations.
- Review automated spot spray technology successfully developed in other cropping systems are the best candidate to adapt to the sugarcane farming system.
- Investigate the best approach to develop weed maps in sugarcane and determine how they can be used to predict future weed patches to reduce the application of pre-emergent herbicides to weed patches only.
- Improve the current variety screening process for herbicide tolerance to include a second rate of herbicide at twice the maximum label rate to take into account potential overlapping in the field and inclusion of the pre-emergence herbicide testing methodology (once developed).

- Determine the impact of herbicides on varieties planted using NEF CEEDS™ technology.
- Continue plant breeding investments into non-GM herbicide tolerance varieties, through a study of the inheritability of the herbicide tolerance trait amongst SRA breeding parent varieties, as well as investments in specific herbicide efficacy trials and the development of a
- Investigate the use of simple bioassays in the field or in pots using soil from the tested field should be investigated as a potential standard method to test for plant-back issues to be promoted to growers and advisors.
- Establish a screening protocol to test for resistance to herbicides in weed species.
- Additional research and effective grower extension activities are required to address information gaps on issues such as specific weed control efficacy of newly registered alternative herbicides and herbicide mixes, while also minimising environmental risks.
- On-going research is required to assess the eco-toxicity of pesticide combinations and newly registered pesticides on freshwater and marine organisms relevant to Queensland watercourses and the GBR.
- New adjuvants need testing for their impact on herbicide runoff and leaching. Flextend® which generated moderate herbicide reduction in runoff in trash blanketed ratoon should be revisited.
- Research using paddock-scale trials monitoring runoff throughout the wet season would assist in better understanding the full impact of mill biproducts to runoff quality. These trials should also assess the impact of the mill biproducts on the pre-emergent herbicides' efficacy to control weeds.
- Pesticide monitoring in groundwater and drains is not carried out on a regular basis and could reveal exceedances that could contribute to the poor water quality in waterways.
- Further studies in soil binding adjuvants and mill biproducts need to be carried out to find strategies to reduce leaching of herbicides into the environment as well as assessing their impact on herbicide efficacy.
- When commercially available, the microwave technology should be investigated for its potential use in sugarcane.
- The Zasso Electroherb should also be further investigated for its potential application in cane.
- Increasing soybean rates, variable sowing configurations and mixing with other seeds are strategies that need investigating from a weed management perspective.
- Review literature and establish trials to determine the best cropping method to grow an intercrop cover crop species to control weeds without impeding on cane growth in several farming systems in Australia.
- Determine the competitiveness of the sugarcane varieties or the benefit of row configurations on weed management.
- Evaluate the HWSC strategies used in the grain industry in sugarcane, where the amount and type of weed seeds ejected along with the trash by the extraction fan is evaluated.
- Evaluate the allelopathic potential of the residues produced by Australian sugarcane varieties and use the trait in conventional breeding.
- Explore biological control agents to control some troublesome sugarcane weeds.
- Involve adoption or extension officers and growers in research projects.
- Future funded weed management research projects should include an on-field-based extension component to maximise the impact on the industry.