Turning a Blind Eye to Ratoon Stunting Disease of Sugarcane in Australia

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The Invisible Disease

Captain Horatio Nelson was pushing home an advantage against the Danish Fleet off the coast of Copenhagen in 1801. His signals officer brought to his attention an unexpected message from the Admiral of the Fleet: “Discontinue the action.” Incredulous, Nelson demanded the telescope before raising it to his one blind eye, rightfully claiming he couldn’t see the signal. He continued the rout, winning the battle, and bringing great glory to himself and the British Fleet. At the same time, he brought the term “turning a blind eye” into common usage. When it comes to ratoon stunting disease (RSD), there is evidence that the Australian sugar industry has turned a blind eye, but unlike Nelson, it is likely that significant losses are being sustained.

It is relatively simple to turn a blind eye when the target is invisible. Although RSD can cause sugarcane yield losses of over 50%, it lacks obvious symptoms (Steindl 1950) (Fig. 1). Caused by a xylem-inhabiting bacterium *Leifsonia xyli* subsp. *xyli* (Davis et al. 1984; Evtushenko et al. 2000), water and nutrient use efficiency are impacted. Reduced germination and growth rates promote indirect losses due to greater weed competition (Gillaspie and Teakle 1989). Established through planting infected vegetative propagation material, RSD is further transmitted throughout the crop during each harvest, so that eventually it affects so many of the stools that it is not economically viable to ratoon the crop further (Steindl 1950) (Fig. 2). The disease has been affecting crops for so long that growers tend to attribute the poor ratooning performance to lack of rainfall or weed competition, or unexplained failure of a particular once-loved variety, before plowing

the crop out and starting again (Young and Brumbley 2004). Although RSD is essentially invisible, there has been no coordinated industry survey to delimit its incidence in Australia. Despite this, the Australian sugar industry assumes the management systems in place are working, and there is a very low incidence, according to the Sugar Research Australia (SRA) website (Sugar Research Australia 2017). The potential that RSD plays a significant, industry-wide role in reduced yields and crop deterioration in Australia has been widely overlooked.

When the sugarcane plant senses *L. xyli* subsp. *xyli*, it generates a localized response aimed at preventing further spread through the vascular tissues. This can be observed as internal red spots when lower nodes are cut transversely with a sharp knife and as electron dense material in xylem vessels when observed with electron microscopy (Davis et al. 1980; Hughes and Steindl 1956; Kao and Damann 1978; Steindl and Hughes 1953; Teakle et al. 1978; Zhang et al. 2016a). There is also some deformation and degradation of cell walls (Zhang et al. 2016a). Thus, the infected xylem vessels become occluded, which impedes water mobility, and explains why RSD causes significant yield losses, particularly under drier conditions (Steindl and Hughes 1953; Teakle et al. 1978). As may be expected, photosynthetic rates are reduced and defense enzyme activities up-regulated (Zhang et al. 2016a, b). Yields may only be marginally affected under irrigation or adequate rainfall (Rossler 1974). In any given infected crop, not all stools are infected, giving crops an uneven appearance. Relative yield losses of 5 to 67% have been recorded, and under extreme conditions, infected cane will die more rapidly than healthy cane (Steindl 1950).

The exact magnitude of losses associated with the disease in any area are difficult to gauge. With the cost of Australian control measures being cited as exceeding AU \$2 million annually, RSD was estimated as costing between AU \$10 million and AU \$20 million in lost productivity for the Australian industry (Croft and Smith 1995). The basis for these calculations was not provided, but the authors did consider the possibility that the losses were underestimated due to diagnostic difficulties. Thus, for over 20 years, this must be considered as the minimal

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annual cost of living with RSD in Australia. Projecting observed incidence levels with the total proportion and known infection impacts of different varieties, it was calculated that in 1988–89, RSD cost Florida US \$37 million in raw sugar alone (Dean and Davis 1990). It is likely that the proportional losses to RSD are higher in industries that lack the management infrastructure available in Florida or Australia. For

example, although only 40 fields were surveyed, the minimum inferred RSD rate in East Java was 50% (Young and Nock 2017). The insidious nature of the disease led respected Bureau of Sugar Experiment Stations (BSES) pathologist Graham Hughes to conclude that RSD is the most economically significant disease of sugarcane (Hughes 1974).

Fig. 1. Diagnosis of an invisible disease. *Leifsonia xyli* subsp. *xyli*-infected cane field (A) confirmed using LSB-PCR. There are no obvious external symptoms. When an infected stalk is sliced longitudinally through the lower nodes with a sharp knife, a red discoloration of the vascular bundles can often be observed (B). Accurate field diagnosis of RSD is virtually impossible.

Fig. 2. Transmission pathways for ratoon stunting disease (RSD). RSD can be spread while cutting plants (A), planting (B), and harvesting (C). Even after thorough attempts at cleaning, there remain inaccessible parts of the harvester where infectious debris can occur (D) (arrows indicate obvious debris). The use of sterilizing agents is recommended, but of limited efficacy.

A major issue with RSD is the absence of specific external symptoms, making accurate field identification virtually impossible. Prior to its discovery in 1945, other workers had apparently recognized unusual and unexplained instances of crop failure that may now be confidently attributed to the emergence of RSD (Anon 1934; Anon 1935; Bell 1935a, b; Denley 1938; Edgerton 1939; Hill 1935; Stevenson 1947; Tapiolas 1934; Young 2016a). However, RSD was discovered by chance when BSES pathologist David Steindl went to Mackay, Queensland, to investigate an issue with the promising new hybrid variety Q28. A single field had been planted from two different plant sources, and one side was growing vigorously, while the other was heavily stunted (McDougall et al. 1948). After confirming that it was not a variety mix-up, Steindl demonstrated that the condition could be transmitted to healthy Q28, and later, to other varieties. Shortly after, the characteristic red comma-shaped spots at the lower nodes were discovered (Steindl and Hughes 1953). As cane growing throughout Australia and in quarantine exhibited the same symptoms, it was clear that the disease was widespread, and that international germplasm exchange was the likely mode by which it had become widespread.

The key RSD control measures have been practiced for more than 60 years. These are hot water treatment (HWT) of planting stocks, maintenance and provision of clean seed schemes, diagnosis of prospective planting material, and farm hygiene (Steindl and Hughes 1953). While effective in theory, each of these steps can be compromised. Given the volumes of sugarcane treated, in practice HWT does not kill all of the infection (Damann and Benda 1983; Koike et al. 1982; Roach 1987; Victoria et al. 1986). For example, Damann and Benda (1983) found 17% disease carryover for susceptible variety L 62-96 following a 2-h HWT at 50°C, while with the same treatment, 19 out of 20 varieties tested had disease carryover (Koike et al. 1982). Therefore, there is a high likelihood of transmission to new crops after HWT, particularly when whole stalks are treated (Victoria et al. 1986). Harvesters cannot be completely sterilized (Taylor et al. 1988), and there are severe deficiencies with the most commonly used diagnostic method in Australia (Hoy et al. 1999; Young et al. 2016). Without strict application of control measures, RSD infiltrates crops and has been associated with the historic elimination of susceptible, but otherwise elite, varieties (Abbott 1959; Hughes and Steindl 1956; King and Steindl 1953; Steib and Chilton 1968). Being essentially invisible, the effects of RSD would have been ascribed to various factors that are themselves exacerbated by RSD, such as insufficient rainfall, harvester damage, poor weed control, soil health factors, or inadequate nutrition (Steindl 1950). Extraordinarily, the extent to which RSD is effectively managed in Australia has never been satisfactorily established. Determining the incidence of RSD is the logical first step, but this has never been done.

What is the RSD Incidence in Australia?

Determining the incidence of what is essentially an invisible disease has always been problematic, particularly when you are not looking for it. With the exception of the initial surveys that demonstrated the presence of RSD throughout Australia's key industries, there has never been an industry wide coordinated survey to determine its incidence. Surprisingly, Australian sugar industry fact sheets on RSD, both by SRA and its predecessor BSES, claim that: "Generally, RSD is present in fewer than 5% of fields in Australia, although in some districts incidence is much higher" (Fig. 3). More recently, the SRA website claims that: "Due to improved diagnostics and management practices, this disease affects fewer than 5% of crops." How this 5% figure is determined cannot be established, nor can any published data be found to support it. In fact, the disparate reports available on the incidence of RSD in Australia all tend to suggest that most areas fall into the category where incidences are higher than 5% (Amiet 1985; Croft et al. 1995; Dominiak et al. 1992; Leaman et al. 1992; McGuire et al. 2009; Roach et al. 1992; Young 2016b; Young et al. 2012, 2014, 2016). It can only be conjectured that the RSD incidence rates suggested by the fact sheets are based on the SRA RSD laboratory results (Croft et al. 2004). If so, there is cause to question their accuracy and the extent to which they are representative of the Australian industry.

The evaporative-binding enzyme immunoassay (EB-EIA), which has been used to screen xylem sap samples in Australia since 1994

(Croft et al. 1994, 2004), is a demonstrably poor performing diagnostic (Hoy et al. 1999; Young et al. 2014, 2016). Typically, 16 stalks are selected from each field to be tested, with the xylem samples of four stalks collected into a single tube, yielding four tubes per field (Croft and Cox 2013). It is recommended to target the largest stalks in the smallest stools (Croft and Cox 2013) as it is difficult to extract xylem sap from smaller stalks (which are more likely to be stunted). The estimated EB-EIA sensitivity is only approximately 10^6 cells ml^{-1} (Croft et al. 1994), which is higher than the bacterial titers supported by some sugarcane varieties (Davis et al. 1988). This problem is further compounded if a pooled xylem sample comes from a mixture of infected and uninfected stalks, which may dilute the target bacterium below the detection threshold. EB-EIA has been found to produce an average of 21% false negative diagnoses compared with other serological techniques and microscopy (Hoy et al. 1999). Furthermore, the imposition of a phase contrast microscopy (PCM) confirmatory step can lead to more false negatives because it is highly dependent on the skill of the microscopist (Young et al. 2016). Despite the limited sensitivity of the EB-EIA/PCM platform, the first wave of PCR diagnostics (Fegan et al. 1998; Pan et al. 1998; Taylor et al. 2003) were never adopted. Thus, there is a strong potential for significant under-diagnosis of RSD.

An early demonstration of likely underdiagnosis using EB-EIA is revealed by analysis of data from the Herbert production zone in Queensland (Table 1) (Croft et al. 1995). Over a period of 4 years, surveys were conducted on four fields per farm, eventually covering 90% of the farms in the region. As only four stalks were tested per field, the infection rates represent a very conservative estimate of the actual RSD incidence. During the first 3 years, all samples were examined using PCM, resulting in an average field infection rate of 12.8%, and an average farm infection rate of 31.9% (that is, farms with at least one field positive for the disease). However, in the final year, the new EB-EIA diagnostic was used, with PCM used only as a confirmatory diagnostic. Field and farm infection rates dropped 40%, to 7.5% and 19.2% respectively, which is statistically significant at a 1% confidence level. The authors struggled to interpret the drop in incidence using EB-EIA, because all positive samples were checked by PCM. However, as they only checked 20% of EB-EIA-negative samples by PCM, it was more likely than not that they failed to detect any of the EB-EIA false negatives (see Table 1). It may be no coincidence that the Herbert Cane Productivity Services routinely use PCM instead of EB-EIA for their plant source inspections (Lawrence Di Bella, personal communication).

The failings of the EB-EIA/PCM platform are more evident when compared with molecular diagnostics. One hundred prospective plant sources were screened by conventional PCR and EB-EIA/PCM conducted on duplicate xylem sap samples collected as per the standard protocol. Of the 400 xylem samples, 26 were PCR positive, representing 12 fields with at least one positive sample. A total of 12 of these PCR-positive samples were negative using EB-EIA. Of the 14 samples that did register an initial EB-EIA positive, only six, representing samples from three fields, were confirmed positive using PCM (Young et al. 2016). Thus, of the 12 fields that yielded PCR-positive xylem sap, only three were deemed positive via the EB-EIA/PCM platform. In this case, reliance on the EB-EIA/PCM diagnostic platform would have resulted in nine infected fields being used as plant sources, further feeding into "unexplained" RSD infections (Dominiak et al. 1992). The majority of the Australian industry has relied on EB-EIA/PCM to screen plant sources for many years (Croft et al. 2004). Therefore, there is a high likelihood that RSD has been inadvertently spread through infected plant sources that were diagnosed healthy.

Even the xylem sap sampling strategy employed is largely ineffective at detecting *L. xyli* subsp. *xyli*. In addition to the xylem sap samples screened in the aforementioned study, each field was tested by PCR on 50 pooled leaf sheath biopsy samples (LSB) (Young et al. 2016). Conventional PCR on these samples (LSB-PCR) identified 18 infections, while quantitative PCR (LSB-qPCR) confirmed these and revealed another nine infections. From the 30 fields diagnosed with RSD using PCR and/or qPCR on either LSB or xylem sap samples, 78% of xylem samples were negative for *L. xyli* subsp. *xyli* using PCR. That is, from confirmed infected fields, *L. xyli* subsp. *xyli* was only detected in 22%

of expressed xylem sap samples. Although increased sample number and removal of bias against smaller stalks are advantages for the LSB platforms, there are additional biases that operate against extraction of *L. xyli* subsp. *xyli* from infected stalks. As it is more difficult to extract sap from infected stalks (Teakle et al. 1978), these may contribute less sap than healthy stalks in a pooled sample. This same principle presumably applies to fibrovascular bundles, with greater ease of extraction from uninfected (and thus non-occluded) vessels leading to a higher proportion of xylem sap coming from uninfected vessels. Thus, in a mixed sample of healthy and infected stalks, a greater proportion of pooled xylem sap may be expected to come from healthy fibrovascular bundles, potentially diluting the target bacterium below the detection threshold. This is particularly problematic when using a weak diagnostic platform such as EB-EIA/PCM.

Evidence that may be construed as supporting the “fewer than 5% of Australian fields” assertion is presented by Croft et al. (2004), which celebrated 10 years of service of the EB-EIA test. Calculating from the reported percent positives from northern, central, and southern Queensland and NSW, the estimated 10,669 positives from 266,124 samples gives an overall incidence of 4% (Table 2). At first glance, this seems to confirm an RSD incidence of less than 5%. However, the percentage of positive samples is not necessarily a measure of the positive fields, as typically four or more samples are submitted for each field. Thus, the minimum number of fields tested can be calculated by dividing the total number of samples by four. As a field is considered infected if

one or any number of submitted samples are positive, the average field incidence based on these results could range from a minimum of 4% up to a maximum of 16%, depending on whether all four tubes were infected, or only one in four. To illustrate, over 8 years at Harwood, NSW, a total of 538 fields were diagnosed positive for RSD (Young et al. 2012). These diagnoses were based on 1,262 EB-EIA/PCM-positive xylem sap samples, giving an average of 2.3 positive tubes per infected field. There is no way of determining the proportion of infected samples that registered false negative results, or infected fields that yielded target-free xylem sap samples. However, using this conservative estimate as an indication, it may be projected that the average percentage of positive fields from the EB-EIA/PCM data of Croft et al. (2004) is actually 6.8%. While higher than the claimed 5% incidence, the very conservative nature of this estimate is further evinced by consideration of the material sampled.

The majority of samples sent for EB-EIA/PCM screening are sourced from prospective planting material during annual seedbed inspections. This cane can be considered the very healthiest material a grower has available on their farm. Additionally, a significant proportion of all samples sent for analysis are quality control samples from approved seed plots (ASP) and motherplots (MP) maintained by productivity services companies. These typically involve intensive sampling of each variety in the propagation plots that are grown from hot water treated material (MP), or cane grown from MP cane (ASP). Thus, the 6.8% projected field incidence of RSD includes a

Fig. 3. Industry fact sheets on ratoon stunting disease (RSD) have continuously claimed that the disease is “Generally present in fewer than 5% of fields in Australia, although in some districts RSD incidence is much higher.” Although the fact sheets are no longer available, the Sugar Research Australia website now claims: “Due to improved diagnostics and management practices, this disease affects fewer than 5% of crops.” (Sugar Research Australia 2017). There is no evidence that supports the “fewer than 5% of fields” claim and it is impossible to determine where this number comes from.

Table 1. Ratoon stunting disease (RSD) incidence in the Herbert as determined by phase contrast microscopy (PCM) and evaporative binding enzyme immunoassay (EB-EIA) (data reproduced from Croft et al. 1995). If the 1990–1992 average infection rate (12.8%) is applied to the 729 samples from 1993, a total of 93 infections would be expected. Thus, a total of 38 EB-EIA false negatives are expected to exist within the 674 remaining samples (approximately 5.6%). If 20% of EB-EIA negative samples were checked by PCM, the likelihood of detecting a single false negative is 20% of 5.6%, or 1.1%. Therefore, in the 135 samples screened, it may be expected that 1 to 2 (1.5) were infected. None were reported.

Year	Diagnostic	Fields				Farms			
		Samples	Inf.	Uninf.	% inf.	Farms	Inf.	Uninf.	% inf.
1990	PCM	468	60	408	12.8	128	40	88	31.3
1991	PCM	707	96	611	13.6	183	63	120	34.4
1992	PCM	709	85	624	12.0	165	49	116	29.7
1993	EB-EIA	729	55	674	7.5	182	35	147	19.2

significant number of fields of the healthiest material available, which is least likely to be infected by *L. xyli* subsp. *xyli*.

In contrast, there is a significantly higher amount of RSD in commercial fields. During 2015, LSB-PCR detected *L. xyli* subsp. *xyli* in 28.2% of commercial fields, which was fourfold higher than the seedbed incidence of 6.5% (Young 2016b). Again, this represents a minimum incidence. LSB-PCR is 50% less sensitive than LSB-qPCR (Young et al. 2016), and there are bound to be cases where all samples were collected from uninfected stalks in otherwise infected fields. Thus, there are significant biases that operate throughout the standard diagnostic framework that act toward lowering the perceived incidence of RSD (Fig. 4).

There are further factors that limit the utility of diagnostics based on expressed xylem sap. Sampling is generally restricted to the mornings, before transpiration prevents adequate extraction, limiting the extent and duration of surveys. It is difficult to extract xylem sap during drier periods, while during wetter periods, or when cane is under irrigation, there is the potential that the higher relative water content could dilute the pathogen below the detection threshold. This might explain historically low RSD detections in regions under irrigation, such as central Queensland (Croft et al. 2004). The presence of splashed dirt at the base of stalks in high rainfall areas has led to some workers sampling nodes from a meter above ground level (Graham Cripps, personal communication), despite the lower bacterial titers present further up the stalk relative to basal nodes (Bailey 1977; Harrison and Davis 1988). Likewise, as it is more difficult to extract xylem sap from the lowest nodes, many workers progressively pump sap from higher nodes, a technique colloquially referred to as “chasing lollies” (Steve Lokes, personal communication). The laborious nature of collecting stalks, scrubbing them free of extraneous matter, cutting, and pumping means that it is logistically infeasible to collect a greater number of samples for analysis, so the sample size is necessarily limited. As such, commercial fields are rarely, if ever, screened. Instead, only prospective propagation material, representing the healthiest, most promising plant sources available to growers, is typically tested. This leads to the most glaring hole in our understanding of the incidence of RSD in Australia: the vast majority of fields are never tested.

Multiple factors have combined to downplay the perceived incidence of RSD in the Australian industry. There are significant biases in the material screened, ranging from the fields that are tested, the stalks selected for extraction, and the differential flow rates between infected and uninfected vascular bundles. The EB-EIA diagnostic platform is demonstrably weak, and the PCM confirmation step provides another pathway to deliver false negative diagnoses. There has been no coordinated survey for RSD, and no published figures that support the “fewer than 5% of Australian fields” claim. In fact, all available data indicate a much higher incidence than what the Australian industry formally acknowledges. The actual incidence of RSD in Australia is unknown, but it cannot be determined using projections from a second rate diagnostic applied to a biased sample of first rate material. If the actual incidence of RSD is higher than previously

thought, it may be expected that its effects may be evident throughout the industry, even if they are not recognized as such.

Hidden Impacts of RSD

It is impossible to determine the impact of RSD within a given industry without a clear knowledge of the proportion of infected fields, its incidence within fields, and the relative yield losses of different varieties under different conditions. None of this is currently known in Australia. Instead, potential losses may be guessed based on susceptibilities inferred through the relative bacterial titer of xylem sap expressed from inoculated plants (Comstock et al. 1995; Croft et al. 1994; Davis et al. 1988). In contrast to other major diseases of sugarcane, RSD susceptibility is not considered as a selection trait in the Australian plant improvement program because the disease is considered to already be “economically managed” (Croft and Johnson 2013). Consequently, most varieties are not rated for RSD susceptibility until they are released, and many varieties even then are not rated (Young et al. 2012). In fact, resistance has been so far dismissed as a management tool that RSD ratings did not appear on QCANESelect, the SRA web-based variety selection tool, until 2012 when calls were made to include them (Young et al. 2012). Even now, RSD is the only pest or disease with an opt-in requirement for QCANESelect, requiring each productivity services company to specifically request RSD susceptibility information to be displayed on their regional settings. While there may be theoretical ratings for most varieties in most areas, there are no actual yield trials for these varieties that can provide an indication of relative yield loss in the field. At best, then, the impact of RSD in Australia can only be guessed as there are no reliable incidence data and incomplete direct yield loss data upon which to make projections.

An indirect way to gauge the possible impacts of RSD is to examine the effects of the imposition of differential levels of disease control. This may be among different grower groups, regions, or when there has been a significant change in RSD management practice within a given industry. Thus, if, as claimed, the incidence of RSD is indeed very low, then the imposition of additional control measures is unlikely to result in significant yield increases. However, there is evidence that yields are directly linked to the level of RSD control deployed, indicating a high background level of RSD.

There has been historically high incidence of RSD at Harwood, NSW (Croft et al. 2004; McGuire et al. 2009; Roach et al. 1992; Young et al. 2012). It is not known whether this reflects poor management, greater susceptibility of varieties grown, lack of irrigation, more favorable environmental conditions for *L. xyli* subsp. *xyli*, or even if it is potentially related to the fact that Harwood has historically had the most comprehensive screening for RSD in the Australian industry (Croft et al. 2004; McGuire et al. 2009). In 2013, Harwood embarked on an enhanced RSD management program (Young 2016b, 2017). This included changing to the LSB-PCR diagnostic platforms (Young et al. 2014, 2016) and expanding the size of the MPs and ASPs. As Harwood growers have near universally adopted billet planting, and

Table 2. RSD infection rates inferred from data reproduced from Croft et al. (2004). These are pumped xylem sap samples and contain a significant number of check samples from cane from approved seed plots and motherplots that have been treated for RSD.

Area	No. samples	Positive samples ^a	% positive samples	Estimated fields tested ^b	Positive fields			% inf. fields		
					Max. ^c	Min. ^d	Est. ^e	Max.	Min.	Est.
North QLD	13,770	299	2.2	3,443	299	75	127	8.7	2.2	3.7
Central QLD	162,885	1,191	0.7	40,721	1,191	298	508	2.9	0.7	1.2
South QLD	28,587	1,056	3.7	7,147	1,056	264	450	14.8	3.7	6.3
NSW	60,882	8,123	13.3	15,221	8,123	2,031	3,463	53.4	13.3	22.8
Total	266,124	10,669	4.0	66,531	10,669	2,667	4,548	16.0	4.0	6.8

^a The number of positive samples is inferred from the % infection provided in Croft et al. (2004).

^b The estimated number of fields tested is calculated at four samples per field.

^c The maximum number of infected fields is based on each field being diagnosed positive based on just one out of four positive xylem sap samples.

^d The minimum number of infected fields is based on infected fields yielding four out of four infected xylem samples.

^e The estimated number of positive fields is based on applying 2.3 positive tubes for each infected field, as was the case between 2004 through 2011 at Harwood, NSW.

as the need for wholestalk planting was seen as an impediment to clean seed uptake, distribution of billets was given priority. To overcome distance-related barriers to clean seed uptake, a billet delivery system was constructed and deployed. To lower the overall susceptibility in the variety mixture grown, preferential variety recommendations were made based on RSD resistance. These changes resulted in a tripling of the already high clean seed output (Young 2016b), and prevented inadvertent spread of RSD from many fields that were deemed negative via the EB-EIA/PCM platform, but were LSB-PCR positive. The overall 2015 yield of 151 t/ha was the second highest on record, and was only marginally lower than that recorded in 1962 when cane was harvested by hand and significant areas of virgin land were brought into propagation (Young 2017). Significantly, the 1-year-old proportion yielded 120 t/ha, which is the highest 1-year-old yields ever recorded for Harwood. The following year, despite significantly drier conditions, the combined 1- and 2-year-old average yield was 146 t/ha, making the 2015 and 2016 seasons the highest consecutive yields on record. In contrast, these yields were respectively 11 and 14% higher than neighboring Broadwater, which has a similar cropping system and climatic conditions, but had not yet implemented the RSD control program pioneered at Harwood (Young 2017). That other factors may be involved is indisputable. However, record yields immediately following redoubling of control measures is evidence for a significant underlying RSD problem.

A similar observation has been recorded in the Herbert (Stringer et al. 2016). Average yields were compared among grower groups whose ASP attendance was classified under regular, frequent, infrequent, and never. Growers who regularly purchased clean seed had yields that were 8% higher than frequent attendees, and 13% higher than those who never purchased clean seed. Interestingly, there was

less than 1% yield difference between the infrequent and never groups. This suggests that it is only by applying the very best standard of RSD control that any significant advantages are achieved. This observation is irreconcilable with claimed very low RSD incidences.

A previously unsuspected impact of undiagnosed RSD is its probable influence on significant numbers of agricultural research trials. For example, RSD was identified in five out of 15 trial sites that examined the role of harvester speed and cutter height on yields and subsequent ratooning (Young 2016b). These harvesting trials not only investigated yield differences between different cutting heights and forward speed, but also germination rates of the resulting ratoons. Both of these factors are known to be deleteriously impacted by RSD. Therefore, any observed differences in the data cannot be confidently disentangled from the impacts of RSD. It cannot be determined how much sugarcane trial work has been confounded by the presence of RSD because it is generally assumed that the disease is not present. Like commercial fields in general, trial blocks are rarely if ever tested.

Given the cryptic nature of RSD, it is impossible to prove that the disease has had an undiagnosed yield-eroding impact throughout the Australian sugar industry. However, a historical precedent exists in the form of “varietal yield decline,” which confused Australian and international sugarcane technologists from the 1930s through to the 1950s (King 1951). This phenomenon was characterized by the rapid failure of promising new sugarcane hybrids, which soon after release had to be replaced by new varieties that likewise failed and so on (King and Steindl 1953). Like RSD, the symptoms of this condition were most noticeable in ratoons or under drier conditions. Without control measures, RSD infiltrated all available planting stocks, leading to the “decline” of varieties over a 5- to 12-year period (Abbott 1959;

Fig. 4. For over 20 years, xylem sap samples have been screened using an ELISA and microscopy-based method that has been demonstrated to be a weak diagnostic. However, several other factors have tended to downplay the incidence of ratoon stunting disease (RSD). **A**, Only the best material available is typically tested. This is cane vegetatively propagated from hot water treated material (motherplots), cane grown from motherplots (approved seed plots), and prospective seedbeds from which a grower may wish to propagate a commercial crop. RSD is spread through infected planting material, contaminated planting equipment and harvesters, but commercial crops and older ratoons that have had the highest potential exposure are rarely if ever tested. **B**, As xylem sap can be difficult to extract, industry guidelines recommend sampling the biggest stalk in the smallest stool. This introduces a bias against smaller stalks that are more likely to be infected. **C**, Xylem sap expression is easier from higher nodes that support fewer bacteria, while it is more difficult to collect sap from infected, and thus occluded, vascular vessels and stalks than uninfected. **D**, This can be more of a problem when pooled xylem sap samples come from both infected and uninfected stalks, leading to a higher proportion of sap being contributed by uninfected xylem vessels. An infinitesimally small number of stalks per field are ever tested, and some regions conduct limited to no testing at all. With these biases and technical shortcomings, it is difficult to see how the Australian industry can claim that the general field incidence of RSD is less than 5%.

Roach 1987; Steib and Forbes 1959). The continuous replacement of varieties throughout the 1930s and beyond contrasts with the longevity of the older varieties, which, regardless of soil or environmental conditions, sustained sugar industries for more than 400 years (Deerr 1949; Rosenfeld 1956). When the newly discovered RSD was linked to “varietal yield decline,” the latter phenomenon disappeared from the literature.

The “varietal yield decline” of yesteryear should not be confused with what is termed “yield decline” today, although the similarities between the presentations have not been previously addressed. The Sugarcane Yield Decline Joint Venture (SYDJV), which ran from 1993 to 2005, was initiated in the face of a plateau or actual decline of Australian sugarcane yields in the 1970s to 1990s (Troedson and Garside 2005). The copious literature emanating from the SYDJV focuses on soil biology as the major factor responsible for “yield decline;” however, none of it, to the best of the author’s knowledge, considers the possible role of RSD. That RSD was not considered as a possible factor is clearly accounted for by the general industry stance toward the disease outlined earlier. However, a possible link to the role of RSD in “yield decline” comes from the same seminal paper that is cited by a significant proportion of the SYDJV literature. This paper, entitled “Sick Soils,” was written by Arthur Bell (Bell 1935a), who was later to become the director of the BSES, and is perhaps best known for being instrumental in the introduction of the cane toad (*Bufo marinus*) into Australia (Mungomery 1935). Bell’s investigations into soil biology were aimed at addressing the following problem:

“...it has become increasingly apparent that, at least with certain varieties in certain soil types, there is present some unknown factor which limits the magnitude of the yields obtained” (Bell 1935a).

Bell went on to show that yields could be improved by fumigation of the soil with metham bromide, and concluded, with reservations, that there may be soil factors involved in the deterioration of yields.

What is less well known is that Bell’s work on “sick soils” was part of a larger program aimed at addressing a broader issue that was then emerging. The same year that his “Sick Soils” paper was published, Bell was seeking an explanation for “variation in a clonal population” (Bell 1935b). Variation in performance among stools within a crop was irreconcilable with a clonally propagated plant, but no satisfactory explanation was made. Although RSD was first recognized as a distinct pathology some 10 years later, these field presentations, like other phenomena such as “stubble deterioration” and “varietal yield decline,” bear the hallmarks of RSD (Young 2016a). In particular, differences among varieties and growing conditions, inconsistent stool sizes, and general absence of mitigating factors. Prominent among the varieties exhibiting such variation in stool size was Q813, a variety that was highly susceptible to RSD. Of interest is the fact that Bell’s efforts to reduce variation in the plant selection process led to the 4 × 10 m row conformation still used today in Australian plant improvement trials (Bell 1938). It is probable that, like others in the period immediately prior to the discovery of RSD, Bell was actually investigating the symptoms of this new phenomenon that had recently become established throughout the world’s industries (Young 2016a).

It may be argued that growth responses following soil fumigation excludes RSD as a factor. However, this is countered by observations by Steib et al. (1965), which demonstrated that cane with RSD made larger yield gains than healthy cane when planted into fumigated soil. Likewise, eminent Australian sugarcane pathologist Brian Egan observed similar enhanced growth from sterilized soil with cane infected with chlorotic streak (Steib et al. 1965, Discussion). Thus, although destruction of minor deleterious pests by fumigation may lead to increased yields, it does not follow that the soil biota itself was the primary cause of the yield variations. And while significant advances have been made in our understanding of soil health through the SYDJV initiative, any research on putative “yield decline” that has not accounted for the potential influence of RSD has to be considered incomplete, particularly in light of clear evidence of industry-wide underdiagnosis of the disease. It may be that the yield increases expected to flow from the SYDJV work may not yet have been realized because the underlying impediment was never addressed. Without clear evidence, it is not claimed here that RSD is the positive

cause of sugarcane yield decline. However, why the disease has never been satisfactorily discounted as a possible factor remains a mystery. As was shown with “varietal yield decline,” undiagnosed RSD may be the most important factor in modern “yield decline,” but not being considered a possibility, has never been investigated.

A previously unsuspected factor that may have contributed to an underestimate of the incidence of RSD is the significant change in field sampling that occurred in the 1970s. Until the discovery of the bacterial nature of the disease, the main diagnostic method for RSD was slicing cane to observe the internal symptoms (Fig. 1). This would involve slicing stalks from numerous stools of cane to determine if the characteristic red “commas” were present. Sufficient stalks were sliced to satisfy the confidence level required (Brian Egan, personal communication). However, following the discovery of the RSD-associated bacterium, the diagnostic platforms in Australia all articulated upon analysis of expressed xylem sap. As has been demonstrated, expressed xylem sap platforms have multiple inherent biases that operate against identification of the bacterium. Coupled with small sample sizes and a weak diagnostic method, the very nature of xylem sap-based RSD diagnostics may have led to industry complacency in relation to the incidence and impacts of the disease. Could it be that the advent of the modern “yield decline,” so soon after the move to expressed xylem sap diagnostics, may be explained by an increased incidence of undiagnosed RSD?

The insidious effects of RSD may also be apparent in the general consensus regarding the need for better ratooning varieties, as highlighted as an SRA priority in the 2016 Project Funding Call. Ratoons are typically only plowed out when no longer profitable. As implied by its name, RSD has a higher relative impact on ratoon crops, and from the foregoing it can be seen that commercial fields are never tested for RSD. Thus, it is entirely possible that RSD itself is a factor in poor ratooning performance. This is further evidenced by the fact that there is a higher RSD incidence in crops arising from replanting immediately after plow-out than crops planted after a fallow (McGuire et al. 2009; Young et al. 2012). If there is a statistically significant higher incidence in replant versus fallow crops, the question is, how did the replant crops become infected?

L. xyli subsp. *xyli* does not survive in soil. Through its long association with a plant host, it has a highly reduced genome that lacks the genes required for survival outside the host (Monteiro-Vitorello et al. 2004). However, *L. xyli* subsp. *xyli* can be detected in plant debris months after plow-out (Young 2003), and it may be possible that new plants can actively uptake the bacteria through their roots (Paungfoo-Lonhienne et al. 2010; Young et al. 2012). The infection can also be transmitted from “volunteers,” resprouting cane that was not effectively destroyed during plow-out. Notwithstanding the possibility of infected propagation material, which is at least as likely to impact fallow plant as replant, whether the disease was acquired from plant debris or transmitted at harvest through volunteers, it had to be present in the previous crop in order to infect the new one. Yet somehow the connection between RSD reaction and the decision to plow out the previous crop has escaped the attention of the industry. Such is the strength of the long-standing meme about the success of RSD management in Australia, it appears that any number of possible causes can be investigated before this insidious disease is considered a possibility. As will be seen, as the Australian plant improvement program has never actively targeted RSD resistance, there is a high average susceptibility of Australian varieties that exacerbate high undetected RSD levels throughout the Australian industry.

Management of RSD: Past and Future

Former Colonial Sugar Refinery (CSR) plant breeder Brian Roach commented that RSD is unique among sugarcane diseases in that no consideration is given for tolerance or resistance in the breeding program (Roach 1987). Although Roach conducted important RSD susceptibility screening and heritability analysis that laid the foundation for breeding resistant varieties (Roach 1988, 1992a, b; Roach and Jackson 1992), this work was not further pursued, and RSD resistance has never been considered a priority in Australia. Instead, it is argued that RSD is economically managed, and there is no need

for resistant varieties (Croft and Johnson 2013). However, as there has apparently never been a cost-benefit analysis conducted into the management of RSD, it is again impossible to determine the foundation upon which this assertion is based.

Cultivar variability in RSD susceptibility appears to be chiefly attributable to xylem architecture. Those cultivars with highly branching vessels, and fewer passing uninterrupted through the nodes, exhibit the highest resistance (Teakle et al. 1978). It is thought that this reflects an innate ability to limit colonization of uninfected adjacent vessels, thereby limiting the level of infection within the plant. This in turn reduces the number of plants cross-infected during harvesting operations, leading to relatively lower impacts (Comstock et al. 1996; Damann 1992; Harrison and Davis 1988). As resistant cultivars have fewer infected vascular bundles, they also have lower pathogen titers present in expressed xylem sap. Thus, in xylem fluid expressed from a number of different cultivars ranging from resistant to susceptible, the bacterial density ranged from 1.94×10^5 to 5.18×10^8 cells mL^{-1} (Davis et al. 1988). There is a high broad sense heritability associated with RSD resistance, which means that if resistant parents are used, the hybrids are likely to inherit the resistance (Roach 1992b).

In the absence of resistant varieties, the majority of the cost of RSD management is borne by the cane farmer. This involves direct production losses and plant re-establishment costs, phytosanitation and clean seed costs, and through funding local productivity services companies, whose staff are at the forefront of RSD control. Between establishing, maintaining, and distributing sugarcane from approved seed plots, and undertaking RSD diagnostics for grower seedbeds, RSD control is likely to be the single most time- and resource-significant function of many productivity services company staff (Jonathon Agnew, personal communication). In areas that employ the MP and ASP clean seed system, there are significant costs involving installing and maintaining hot water treatment infrastructure, running the systems, high time commitment of operating the treatment tanks, and then establishing and maintaining the plots. Additionally, damage to eyes in varieties sensitive to HWT results in poor strikes. These costs have been essentially borne by the growers for more than half a century.

Some regions, particularly those with existing irrigation infrastructure, have adopted tissue-cultured plants. The tissue-cultured plants are generated from undifferentiated meristematic tissue, so in theory they should be free from infection. However, if differentiated vascular tissue of an infected plant is accidentally intercepted, *L. xyli* subsp. *xyli* will be transmitted to the resulting progeny. It may be expected, therefore, that the greatest care possible will be taken to ensure the parent material is free from RSD. However, cane stalks selected for tissue culture propagation in Australia are tested using the EB-EIA method (Barry Croft, personal communication), despite this methodology having among the lowest assay sensitivities for RSD (Hoy et al. 1999; Young et al. 2016). It is surprising that for this critical step, an industry that promotes adoption of new technologies has not yet adopted highly sensitive molecular assays that have been available for 20 years. By not doing so, it increases the risk of RSD transmission to tissue cultured progeny.

Not including RSD resistance in the plant improvement program has resulted in the release of many varieties that are highly susceptible to the disease and suffer significant yield losses when infected. During variety trial propagations, all sugarcane clones are repeatedly hot water treated, meaning that they are selected under conditions where RSD infection must be minimal. However, with a projected high general RSD rate in many regions, when these promising varieties are released to growers, they rarely enjoy the success expected from their trial results. Many growers only purchase clean seed for new varieties they wish to propagate on their farms. This is typically a small amount, insufficient to establish a commercial crop until it is multiplied through several seasons. Each multiplication step represents at least two infection opportunities for bacteria transmitted through vegetative material and mechanical harvesting. Without ongoing control, this will lead eventually to the contamination of all available planting stocks. Thus, even very promising varieties are often considered obsolete after a small number of years. It is known that RSD can exacerbate the effects of other diseases, and it is therefore

not unlikely that the weakening effects of RSD may magnify the impacts of other diseases that, in the absence of RSD, may not have had such an impact. It may not be coincidental that Q200 and Q208, both by random chance highly resistant to RSD, have enjoyed such lasting success within the Australian industry.

The development of RSD-resistant varieties has been historically opposed for two reasons. The first was from the discoverer of the disease, who expressed a view that tolerant varieties would be a dangerous inoculum source for susceptible varieties (Steindl 1974). This view is not supported by the evidence that shows that resistant varieties harbor smaller titers of *L. xyli* subsp. *xyli* (Harrison and Davis 1988), resulting in reduced transmission rates (Damann 1992). Not surprisingly, it was stated before the correlation between bacterial titer and resistance was made. Steindl concluded that:

“It is, therefore, considered that the control of the disease by tolerant varieties should not be encouraged unless one is prepared to live with the disease and depend entirely on such varieties.”

Unfortunately, the adoption of this position has locked the industry into control measures incapable of eliminating the disease (Young et al. 2012). Thus, the industry as a whole is bound to live with RSD. The continued opposition to pursuing RSD resistance is based on a contestable view that adding another selective tier will cripple the output of the plant improvement program (Croft and Johnson 2013). Certainly other breeding programs have shown that elite but susceptible varieties can be retained (Comstock et al. 2001). A result of this policy is the release of varieties that are as likely as not to fail in the field owing to RSD.

Resistant varieties represent the most effective management for RSD. The mechanism of RSD resistance has long been established (Teakle et al. 1978), as has the heritability through the female parent (Roach 1992b). The pathogen population is highly uniform (Young et al. 2006; Zhang et al. 2016a), and as resistance is architectural, it may be expected to be efficacious across regions. While resistant varieties have inherent immediate value, they also represent the only way to safeguard the industry against the emergence of heat resistant strains of *L. xyli* subsp. *xyli*, or related bacteria that can infect the xylem vessels. The discovery of a range of other *Leifsonia* strains associated with sugarcane should be of significant concern to the sugar industry (Young and Nock 2017). Although their pathogenicity has not yet been established, there is evidence that they are resistant to thermotherapy and have been transmitted throughout the existing plant improvement and variety release program. The ill-defined yellow canopy syndrome (YCS) also bears symptoms that are consistent with bacterial pathogens of the vasculature, so the possible role of these novel strains needs to be investigated as a matter of urgency. RSD resistance based on xylem architecture offers the most sustainable means of ensuring productivity in the face of the possible emergence of new strains of *Leifsonia*.

Conclusions and Recommendations

There are large knowledge gaps in our understanding of the incidence and impacts of RSD in the Australian sugar industry. However, for many years the industry has exhibited what amounts to an ideological attitude, repeatedly claiming the disease is economically managed. This is despite the fact that there are serious deficiencies in the diagnostic framework, ranging from strong sample biases, small sample size and collection limitations, and technical processing. Furthermore, as there has never been a cost-benefit analysis conducted, claims of economic management are based purely on opinion. Likewise, because of the strength of the prevailing, but wholly untested, view that RSD is effectively managed, its involvement has never been suspected in otherwise unexplained phenomena such as “yield decline” and poor ratooning of modern varieties.

Given what are obvious shortcomings with the existing RSD diagnostic framework, some consideration must be given to the factors that underpin the stance of the Australian industry. For example, the EB-EIA diagnostic has been known to be a poor performer since 1999 (Hoy et al. 1999), so why has the Australian industry persisted with its use? How is it possible that the peak industry body can claim that RSD affects fewer than 5% of fields when only a fraction of the healthiest fields available are ever actually tested? Why is there a greater

incidence in replant versus fallow planted crops, and what does that say about the reasons why the original crop was plowed out in the first place? Why do productivity services companies need to request RSD resistance ratings for the variety selection tool QCANESelect, when they are automatically generated for other diseases of less importance? Why should growers not be reminded about this disease? Why is there investment in water and nutrient use efficiency traits when there is so little in resolving a disease that so obviously impacts these attributes? How can over a decade of research on “yield decline” not consider the possibility that RSD may be involved (Garside and Bell 2006)?

It defies reason to believe that these shortcomings would have escaped the attention of authorities for so long. However, either they have done, or their import has been ignored. Could it be possible that, after so long celebrating control of RSD, anything less than the near perfect control claimed will result in embarrassment for the industry? So entrenched is the belief in the success of RSD control that the Australian industry makes no reasonable attempt to assess its impact. Meanwhile, grower levies are spent trying to identify the factors involved in poor ratooning and mysterious yield declines. While it is theoretically possible that RSD occurs in fewer than 5% of fields, it is not supported by any evidence and no meaningful attempt has been made to establish this assertion. Delineating the impacts of RSD in Australia has not been deemed worth the time or money.

When the Australian industry eventually reassesses its stance on RSD, the first step is to determine the disease status of fields prior to plow out using the best available diagnostic platform. As these fields have had the greatest potential exposure to RSD through mechanical transmission, this would provide an estimate of the worst case scenario. If, as may be expected, there is a higher incidence, a stratified sample of different varieties and crop classes in the different regions would further delimit the disease incidence. It is then necessary to project relative yield losses by determining actual yield losses for different varieties under different management regimes. From this, a cost-benefit analysis must be undertaken to direct the next steps, which are likely to include incorporation of genetic resistance in the plant improvement program.

Record yields following implementation of unprecedented RSD control measures suggest that industry-wide gains can be made if more effective control is achieved. These gains will be enjoyed not just by the more progressive farmers, but would occur across the spectrum of farming skill. Based on 60 years of doing much the same thing, the next and most important step is to pursue RSD resistance in the plant improvement program. It is time for the Australian sugar industry to stop turning a blind eye to RSD.

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