Plant Pathology (2016) **65**, 1403–1410 Doi: 10.1111/ppa.12545

REVIEW

Possible origin of ratoon stunting disease following interspecific hybridization of *Saccharum* species

A. J. Young*

Centre for Crop Health, University of Southern Queensland, Toowoomba, QLD 4350, Australia

Ratoon stunting disease (RSD) is the most economically significant disease of sugarcane, and, although it was first discovered in 1945, surprisingly little is understood of the nature of the relationship between the host and the pathogen, *Leifsonia xyli* subsp. *xyli*. This review traces RSD to the release of modern commercial hybrids, and provides evidence that *Saccharum officinarum*, the major progenitor of modern sugarcane cultivars, is not the natural host for *L. xyli* subsp. *xyli*. Rather, it is proposed that the wild relative, *S. spontaneum*, is more likely to be the original host, and that *L. xyli* subsp. *xyli* was acquired during interspecific hybridization work undertaken in Java during the 1920s. The release of the universally adopted variety POJ2878 then facilitated the dissemination of a single, worldwide clone of *L. xyli* subsp. *xyli*. The implications of the hypothesis are discussed in relation to plant improvement and the potential for new diseases to emerge through further attempts at broadening the genetic base of commercial sugarcane.

Keywords: epidemiology, plant–bacterium interaction, RSD

First Detection and Field Presentation of RSD

Ratoon stunting disease (RSD) is the most economically significant disease of sugarcane (Hughes, 1974; Young & Brumbley, 2004), and has impacted sugarcane for at least 70 years. It was first recognized in Mackay, Australia, in the summer of 1944/5 following prolonged dry weather. Dramatic disparities in the performance of adjacent fields of the newly released hybrid variety Q28 were traced to different plant-cane sources (McDougall *et al.*, 1948). Shortly afterwards, 'Q28 disease' (Steindl, 1949) was identified in other cultivars and districts within Queensland and New South Wales, and the term RSD was coined (Mungomery, 1949; King, 1953).

Pathologists realized the presumed viral disease was widespread when it was diagnosed in imported canes growing in quarantine (King, 1953). It was later found in a wide range of the world's sugarcane industries (Hughes & Steindl, 1956). What initially appeared to be a disconcerting problem for a single variety at a single location became an issue of grave concern for the future of the sugar industry. It was soon apparent that the disease also had significant bearing on the past.

Ratoon stunting disease is caused by the xylem-limited bacterium *Leifsonia xyli* subsp. *xyli* (Davis *et al.*, 1984; Evtushenko *et al.*, 2000). The disease has no specific external symptoms, and the associated internal vascular discolouration can be ambiguous and cultivar variable (Hughes & Steindl, 1956). The bacteria extend systemically through the plant, with the highest concentrations at the lower nodes, where the xylem vessels are largest and most numerous (Bailey, 1977a). Generally, the more vascular bundles that are infected, the greater the susceptibility, and the higher the bacterial titre of expressed fibrovascular sap (Bailey, 1977a; Teakle *et al.*, 1978; Davis *et al.*, 1988; Roach, 1992; Roach & Jackson, 1992; Comstock *et al.*, 1995; Croft, 2001; McFarlane, 2002). Infected plants attempt to minimize vascular colonization by exuding a gummy substance, which occludes the xylem vessel (Kao & Damann, 1978). Thus, infection with *L. xyli* subsp. *xyli* interferes with water mobility in the plant, so water use efficiency and nutrient balance are impaired (Teakle *et al.*, 1978). Stunting is more pronounced in droughts, where infected plants are the first to show wilting and death of the leaf-tips (Steindl & Hughes, 1953).

Ratoon stunting disease typically results in lower yields through reductions in stalk weight and number (Steindl, 1950), although not all stalks within a stool, nor stools within a crop, are infected, so a general patchiness is usually observed. Numerous reports of RSD-associated losses have been published, but a few will suffice to demonstrate the direct impacts of the disease. Initial reports of RSD on the variety Q28 indicated losses of between 12% and 37% tonnage in the plant crop, and between 41% and 67% in the ratoon crops (that is, new crops sprouting from subterranean buds of harvested crops; Steindl & Hughes, 1953). It is now known that Q28 was particularly susceptible, and the losses were extreme. For many modern cultivars, estimates of yield

*E-mail: anthony.young2@usq.edu.au

Published online 11 May 2016

Plant Pathology

loss exceed 30%, and few are lower than 5% (Steib & Chilton, 1968; Koike *et al.*, 1982; Grisham, 1991; Bailey & Bechet, 1997). Even when assessing the same variety, yield loss estimates may vary due to the effects of different climatic conditions on plant growth, low levels of infection in control 'healthy' plants and also inconsistencies in inoculation, resulting in differences in the level of infection between experiments. Even under good growing conditions yield loss is usually significant (Bailey & Bechet, 1997). Under extreme dry conditions, affected crops can completely die out while adjacent healthy crops stay green (Steindl, 1950). The indirect losses associated with RSD, such as increased weed competition and reduced number of ratoon crops, are significant (Gillaspie & Teakle, 1989).

Leifsonia xyli subsp. *xyli* is physically transmitted during mechanical harvesting, so ratoon crops are most often more severely affected than plant crops. However, the bacteria are also spread vegetatively through planting infected material, eventually leading, in the absence of adequate control measures, to the complete infection of all planting stocks. There is no evidence of seed transmission or specific vectors (Bourne, 1965; Barbehenn & Purcell, 1993).

Ratoon stunting disease control measures include sterilization of harvesting and planting equipment, thermotherapy of seedcane and timely diagnosis of planting stocks. However, as all of these measures have limited efficacy, RSD persists in the world's industries, and has a high likelihood of increasing in incidence whenever control measures are relaxed (Koike *et al.*, 1982; Damann & Benda, 1983; Victoria *et al.*, 1986; Roach, 1987; Taylor *et al.*, 1988; Young *et al.*, 2012).

Having no specific external symptoms, and a field presentation that can readily be attributed to peripheral exacerbating factors such as drought, poor nutrition, inadequate soil preparation or root problems, the disease is generally under-recognized. However, in the absence of mitigating factors, it is possible that RSD was responsible for many historic unexplained growth problems of sugarcane.

Pre-history of RSD

Given its apparent industry-wide distribution, it was thought that RSD was present during the early years of sugarcane agriculture, and must have been associated with the original source of sugarcane germplasm, the noble cane, *Saccharum officinarum*. These canes were domesticated from *S. robustum* in prehistoric times in New Guinea (Artschwager & Brandes, 1958). During the period of colonial agricultural expansion in the 19th and early 20th centuries, sugarcane pioneers and entrepreneurs collected thousands of clones to augment many of the early industries (Artschwager & Brandes, 1958). In 1951, Australian scientists collected over a hundred clones of *S. officinarum* and other *Saccharum* species from across New Guinea, but using the internal diagnostic symptoms, cross inoculations with Q28 and observation plots failed to find RSD (King, 1953; King & Steindl, 1953; Hughes, 1955). Surveys conducted over the next 50 years, most recently in 2001 (Magarey *et al.*, 2002), also failed to identify the disease.

It was not until 2002 that RSD was first positively identified in Papua New Guinea (Kuniata *et al.*, 2005). Initially, the disease was found in 40% of the samples taken from hybrid varieties growing at Ramu. Since then, its spread has been rapid. As had been previously postulated (Magarey *et al.*, 2002), the frequent use of bush knives would see the rapid spread of the mechanically transmitted disease if ever it were introduced. Follow-up survey work in 2004 found that more than 85% of samples from commercial hybrids were positive for RSD, and that the disease could now be found in wild *Saccharum* growing nearby and in immediately neighbouring provinces. However, RSD was not found in native canes growing on the neighbouring island of New Britain (Kuniata *et al.*, 2005). While there is a remote possibility that, despite intensive surveys, RSD had been present but undetected among *S. officinarum* clones of New Guinea for many years, this is unlikely, as it has not yet been detected in areas remote from commercial sugarcane plantations, and the spread from the commercial plantations of Ramu could not have been noticed if the disease was always present. If RSD was not originally present in the centre of origin of *S. officinarum*, it is difficult to reconcile a long relationship between this plant and the bacterium *L. xyli* subsp. *xyli*.

The original detection of RSD was facilitated by the high susceptibility of the commercial hybrid Q28; however, many other varieties experienced abnormal growth reductions in the period immediately prior to the discovery. While older varieties were typically grown over extended periods, and were replaced only after severe reactions to diseases such as mosaic and (the still unidentified) sereh disease, the new hybrids experienced much shorter commercial lifetimes (Deerr, 1949; King, 1951; King & Steindl, 1953; Rosenfeld, 1956; Abbott, 1959). This circumstance was recognized by Australian scientists King & Steindl (1953), when their interests converged on seemingly different problems: Steindl on the newly discovered RSD, and King on the unexplained phenomenon of varietal yield decline.

Varietal yield decline, the 'running out' or senescence of varieties, was the observation that new varieties needed to be replaced as yields dropped far below what was initially expected. Without treatment, the incidence of RSD increases until ratoons are unprofitable and all available planting stocks are infected. For a susceptible variety, 10–12 years is typically the timeframe required for RSD to infiltrate crops and contaminate planting stocks (Steib & Chilton, 1968). This readily accounts for the observed timeframe for 'decline' of a variety.

Comparison of yield records and varietal susceptibility has shown that RSD was probably involved in the downfall of some older varieties (Hughes & Steindl, 1956; Steib & Forbes, 1959). Significantly, Badila, a New

Guinea *S. officinarum* clone commercially grown in Australia for over 40 years at the time RSD was discovered, never suffered varietal yield decline (King, 1951); it was also found to be highly resistant to RSD (King, 1953; Roach, 1992). Subsequently, it has been shown that cultivars not affected by varietal yield decline were resistant to RSD (King, 1951; Abbott, 1959).

Early records on varietal yield decline are fragmentary, anecdotal and commentary in nature. However, a range of 'unexplained' growth problems has been uncovered in several key industries throughout the 1930s, including 'variations in primary vigour' (Bell, 1935a), 'variations in clonal populations' (Hill, 1935), 'sick soils' (Bell, 1935b), 'ratooning problems' (Tapiolas, 1934), 'stool disparity' (Anonymous, 1934), 'variety deterioration' (Stevenson, 1947), 'root trouble' (Anonymous, 1935) and 'stubble deterioration' (Denley, 1938; Edgerton, 1939). What links these reports is a pattern where varieties were released and enjoyed good success for 5–10 years, but diminishing returns, especially in the ratoons or through drier periods, resulted in their abandonment. These presentations are identical to RSD. Given the disease could not have spontaneously appeared around the world sugarcane industry the moment it was discovered, it is not unreasonable to suppose that it was the cause of these previously unexplained disorders. If these problems had always existed, or if they were explained by other factors, it is unlikely that they would be noticed as something out of the ordinary, let alone reported. Whatever was the cause of these unexplained growth problems, it was new, and of sufficient concern to warrant reporting and broader discussion.

Genetics of *L. xyli* subsp. *xyli*

Leifsonia xyli subsp. *xyli* is remarkably genetically uniform. An examination of isolates from nine countries revealed no DNA sequence variation at the 16S rRNA and intergenic spacer loci, nor any genomic rearrangements based on DNA fingerprinting profiles that are useful for determining genetic variation across a wide range of taxa (Gillings & Holley, 1997; Young *et al.*, 2006). These results are consistent with other sequencing results that show no genetic variation for this pathogen (Fegan *et al.*, 1998; Taylor *et al.*, 2003; Li *et al.*, 2013). A total of 11 kb of the flanking regions from 461 transposon mutation sites of an Australian isolate had 100% sequence identity to the genome sequence of a Brazilian isolate, CTCB07 (Monteiro-Vitorello *et al.*, 2004; Brumbley *et al.*, 2006; Young *et al.*, 2006). Further confirmation of the apparent clonal nature of *L. xyli* subsp. *xyli* comes from recent genome sequencing of a Chinese isolate, GXBZ01 (X. Q. Zhang *et al.*, Agricultural College, Guangxi University, Nanning, China unpublished data, accessed from the National Center for Biotechnology Information (NCBI) on 09/02/2016). Comparison of 2.2 Mb of sequence, comprising 2725 contigs from the unassembled shotgun sequence of GXBZ01, showed 100% sequence identity to homologous sequences of the genome of isolate CTCB07. This extraordinary degree of genomic conservation, if confirmed, adds further evidence to the postulated recent host jump of *L. xyli* subsp. *xyli*, and is further evidence for extremely strong stabilizing selection (Young *et al.*, 2006).

The genetic and genomic conservation of *L. xyli* subsp. *xyli* is unusual for many populations, particularly plant pathogens, where participants in host–pathogen systems normally exhibit inherent variation. Where long interactions between hosts and pathogens have occurred, particularly under strong selection pressures, there is ample scope for generation of variation among the host and pathogen populations. If *L. xyli* subsp. *xyli* was originally associated with *S. officinarum*, it may be expected that repeated translocation of clones of this cane from its centre of origin and diversity (Janoo *et al.*, 1999) over many years would have facilitated the transmission of multiple strains of the bacterium. However, instead, it may be concluded that *L. xyli* subsp. *xyli* has undergone a recent population bottleneck.

Leifsonia xyli subsp. *xyli* is a highly evolved plant endosymbiont that does not fit the usual model of a plant pathogenic bacterium. The small genome sequence of 2.6 Mb has fewer predicted pathogenicity genes than most plant-pathogenic bacteria (Monteiro-Vitorello *et al.*, 2004; Brumbley *et al.*, 2006). It contains only one ribosomal RNA operon and has apparently lost many of the genes that were necessary to support its free-living ancestors, particularly those involved in amino acid biosynthesis pathways and flagellum assembly. *Leifsonia xyli* subsp. *xyli* does not appear to break down the host tissues, and cannot metabolize sucrose (Davis *et al.*, 1980; Monteiro-Vitorello *et al.*, 2004). The red gum, which occludes vascular bundles and constitutes the sole internal symptom of infection, appears to be associated with a generic host defence response targeted at restricting vascular colonization (Kao & Damann, 1978). The relatively reduced genome size, loss of free-living genes, and the low number of genes associated with pathogenicity all suggest that *L. xyli* subsp. *xyli* has had a long evolutionary interaction with its plant host, but not necessarily as a plant pathogen.

The Genus *Leifsonia*

The genus *Leifsonia* offers few clues as to the nature of the relationship between *L. xyli* subsp. *xyli* and its host. The closest known relative of *L. xyli* subsp. *xyli* is the xylem-limited *L. xyli* subsp. *cynodontis* (Davis *et al.*, 1984; Evtushenko *et al.*, 2000). This species was originally isolated from Bermuda grass (*Cynodon dactylon*) (Davis *et al.*, 1980), but has since been identified in several other grass species (Mills *et al.*, 2001). Other *Leifsonia* species have been described from soils in east Asia (Suzuki *et al.*, 1999; Dastager *et al.*, 2008), glaciers in India (Reddy *et al.*, 2008; Pindi *et al.*, 2009), Antarctic ponds and sediments (Reddy *et al.*, 2003; Pindi *et al.*, 2009), snow (Schuerger & Lee, 2015), insects (Nishiwaki *et al.*, 2007), and distilled water in Russia (Leifson,

1962). Another species is associated with plants (Evtushenko *et al.*, 2000), and one with lichens (An *et al.*, 2009), but these are not known to be pathogenic.

There have been numerous molecular detections of *Leifsonia* (and bacteria erroneously attributed to the genus) from a range of environmental samples, as revealed by searching the GenBank database of the NCBI. It is possible that, being generally fastidious and perhaps of low environmental abundance, it is only with advances in culturing techniques and molecular profiling that they are being revealed (Ferrari *et al.*, 2005). Other than their identification in varied habitats, little is known of their evolutionary history or environmental functions.

Given its specialization to the xylem habitat, and that it has been artificially inoculated into at least 14 other grasses (Steindl, 1957; Rao *et al.*, 1990), it is clear that *L. xyli* subsp. *xyli* has had an evolutionary association with one or more grass species. RSD susceptibility is generally determined by the number of colonized vascular bundles in infected plants, and, thus, *L. xyli* subsp. *xyli* titres in expressed xylem fluid (Bailey, 1977a; Teakle *et al.*, 1978; Davis *et al.*, 1988; Roach, 1992; Roach & Jackson, 1992; Comstock *et al.*, 1995; Croft, 2001; McFarlane, 2002). In general, varietal differences in the severity of stunting correspond with xylem anatomy. The highest resistance is observed in varieties with highly branched vessels, and with fewer vessels that pass uninterrupted through the nodes (Teakle *et al.*, 1978). This inherent structural 'resistance' is presumed to operate by restricting colonization of new vascular bundles. Thus, highly susceptible varieties have higher numbers of colonized, and consequently occluded, vascular bundles, which, therefore, support greater numbers of *L. xyli* subsp. *xyli* in expressed xylem fluid. No other mechanism of resistance is known.

When inoculated into other hosts, *L. xyli* subsp. *xyli* does not attain the high cell densities observed in sugarcane cultivars (Davis *et al.*, 1980; Rao *et al.*, 1990). Likewise, when *L. xyli* subsp. *cynodontis* is inoculated into sugarcane, it does not reach the same population densities as *L. xyli* subsp. *xyli*, nor does it induce stunting or the internal symptoms characteristic of RSD (Davis *et al.*, 1980; Mills *et al.*, 2001). Unless it can be reasoned that disparate hosts independently evolved specific and highly efficient defences against this particular, clonal endosymbiont, then the low *L. xyli* subsp. *xyli* titres supported by other grasses must reflect the suboptimal nature of these plants as hosts. It follows that it may be expected that the natural hosts will support the highest numbers of the endosymbionts.

In research examining the distribution of RSD susceptibility within the *Saccharum* complex, Roach (1992) made bacterial counts from expressed xylem fluid of over a hundred 'naturally infected' *Saccharum* clones (*S. officinarum*, *S. robustum*, *S. sinense*, *S. barberi*, *S. edule*, *S. spontaneum*) and some allied genera (*Coix*, *Erianthus*, *Miscanthus*, *Narenga*, *Pennisetum*). The canes were sampled from the breeding collection where they had been grown for many years without application of RSD control measures. There was great variation in the number of *L. xyli* subsp. *xyli* cells present in samples of expressed xylem sap, with clones of *S. spontaneum* supporting on average more than ten times as many as *S. officinarum* clones. This suggests that *L. xyli* subsp. *xyli* is more adapted to *S. spontaneum* than it is to *S. officinarum*; therefore, it is probable that *S. spontaneum* is the natural host of *L. xyli* subsp. *xyli*, and that susceptibility to RSD comes from genetic introgression of this species. If *S. spontaneum* was the original host of *L. xyli* subsp. *xyli*, then it is necessary to determine how the bacterium entered into modern sugarcane.

Origins of Sugarcane

Like many other agriculturally important crops, modern sugarcane is the result of a complex genetic interaction among multiple taxa. The key progenitor, *S. officinarum*, was originally derived from *S. robustum* in New Guinea, which has the same basic chromosome number (2n = 80) and similar morphology, but does not have a high sugar content (Price, 1957; Artschwager & Brandes, 1958). The origins of *S. robustum* are less clear, but are thought to involve ancient interactions between locally evolved taxa and the radiating *S. spontaneum* species complex (Panje & Babu, 1960). *Saccharum spontaneum* has played several roles in the later history and expansion of sugarcane agriculture.

The *S. spontaneum* species complex is a diverse assemblage of tough, perennial plants with a native range that extends from Africa to Melanesia. They are thought to have emerged in India, where clones with the lowest chromosome numbers (2n = 40) are found (Panje & Babu, 1960). They radiated widely, evidently hybridizing with locally endemic forms to the extent that karyotypes are observed with the range of 2n = 40 through to 2n = 128 (Panje & Babu, 1960; Daniels & Roach, 1987). In Melanesia, it is thought that ancestral clones of *S. spontaneum* hybridized with local forms to give rise to *S. robustum* (2n = 80), from which *S. officinarum* was progressively selected for high sugar content by human and animal agency (Artschwager & Brandes, 1958).

Since the advent of *S. officinarum*, clones of *S. spontaneum* have made at least two additional and very different contributions to sugarcane agriculture. When *S. officinarum* came through trade to the Asian mainland, these are thought to have hybridized with endemic *S. spontaneum* clones, leading to the establishment of the canes originally described as *S. barberi* and *S. sinense* (Jeswiet, 1927; Deerr, 1949; Rosenfeld, 1956; D'Hont *et al.*, 2002). The hybridization is presumed to have been natural, because spontaneous hybridization between *S. officinarum* and *S. spontaneum* has long been established (Jeswiet, 1927).

It may be expected that wherever humans transplanted *S. officinarum* outside of its centre of origin, any natural hybrids that arose would have enjoyed a degree of climatic pre-adaptation conferred by the endemic parent. Therefore it is probable that these agronomically

superior spontaneous hybrids and natural backcrosses rapidly supplanted the introduced *S. officinarum* clones originally propagated for their sugar content. Thus *S. barberi* and *S. sinense* (now considered too similar for separate species status) were derived from *S. officinarum* and *S. spontaneum* hybrids (D'Hont *et al.*, 2002). These became the basis for the ancient Chinese, Indian and Persian sugar industries, and later accompanied the modern expansion of the sugar industry throughout the rest of the world (Rosenfeld, 1956).

Artificial Hybridization

The second major contribution made by *S. spontaneum* is the role it played in resurrecting sugarcane agriculture throughout the 1920s and 1930s. A combination of pests and diseases threatened the collapse of several key industries that were largely based on *S. officinarum* clones. Briefly, these were Fiji leaf gall virus and gumming in Australia (North, 1935), mosaic disease in the USA (Rosenfeld, 1929), and, in Java, mosaic and the still mysterious sereh disease (Jeswiet, 1927). The rediscovery of sugarcane fertility in the late 19th century facilitated attempts at resistance breeding, which initially failed due to the focus on intraspecific crosses of *S. officinarum* (Jeswiet, 1927). Furthermore, the production of hybrids was haphazard, with workers relying on the close field proximity of the parent canes to achieve the crosses, and therefore not being able to confidently ascribe the male parent.

Leading to what was a major and revolutionary breakthrough was Jeswiet's observations that the wild *S. spontaneum* clones growing in Java were resistant to sereh. Jeswiet implemented a hybridization programme and pioneered techniques, still used today, whereby the male flower was cut and placed in a pollen-proof bag with the female flower, and later the seed was collected for trial. In a process termed 'nobilization', by backcrossing the original interspecific hybrids with other *S. officinarum* (noble) clones, canes that were resistant to sereh but retained commercially acceptable sugar levels were produced. It was also found that these POJ canes (known from the acrostic of where they were bred, the Proefstation Oost Java), showed good resistance to the other known sugarcane pathogens (Jeswiet, 1927).

Of the POJ series of canes, the most important was POJ2878, known colloquially as the Javan Wondercane, Javan Wonder, or simply Java. This cultivar came from a 1921 cross, and was so successful that it traversed the world, and was adopted wherever cane was commercially grown. The success of POJ2878, and varieties bred from it, eventually led to the complete replacement of the original *S. officinarum* clones. Likewise, they formed the basis of all subsequent sugarcane breeding work around the world. There can be very few, if any, existing commercial cultivars that do not find one or more of Jeswiet's hybrids in their pedigree, and thus a bottleneck has long been in existence in sugarcane improvement programmes (Jackson, 2005). At a distance of nearly a century, there can be no proof, but it is possible that the physical cutting of parent flowers during breeding operations presented a pathway for the introduction of *L. xyli* subsp. *xyli* into the breeding collection, and that dissemination of the seminal POJ2878 around the world facilitated the establishment of a single worldwide clone of *L. xyli* subsp. *xyli*. This proposed scenario is illustrated in Figure 1.

Implications of the Hypothesis

As the interspecific hybrids and backcrosses had good resistance to sereh and mosaic disease, any effects of RSD would be relatively masked. That unexplained crop abnormalities identical to the presentation of RSD in the field appeared within 10 years of the release of POJ2878 and other hybrids is not irrelevant. Either the hybrids carried the disease with them, or the increased susceptibility of the hybrid varieties accentuated the symptoms of a pre-existing disease, whose emergence, apart from not being associated with *S. officinarum*, must otherwise remain a mystery.

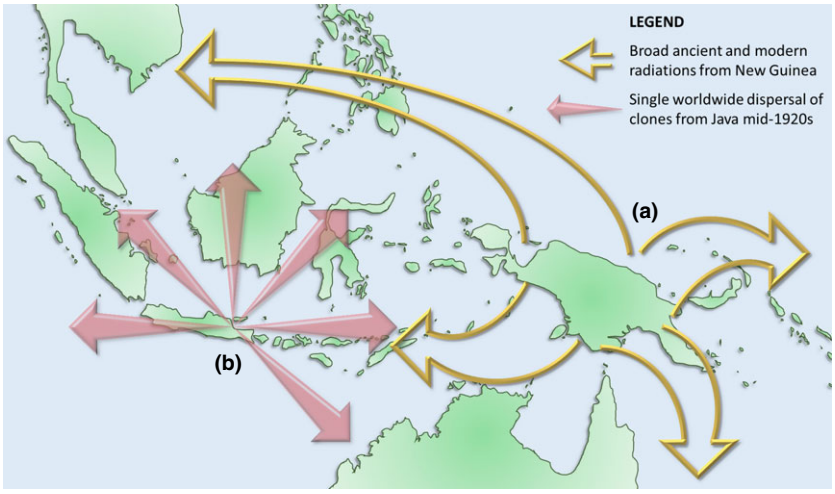

Figure 1 Proposed route for the advent of RSD following the release of commercial interspecific hybrids in the 1920s. (a) Sugarcane was transported out of its centre of origin, New Guinea, over thousands of years, but RSD was not found there until 2002, when it was discovered in commercial plantations based on hybrid varieties. (b) The release of the Javan Wondercane, POJ2878, in the 1920s may have facilitated the worldwide dissemination of a single clone of the RSD pathogen, *Leifsonia xyli* subsp. *xyli*.

If *S. spontaneum* is the natural host of *L. xyli* subsp. *xyli*, it could be expected that natural populations of this cane may support diverse assemblages of *L. xyli* subsp. *xyli* from which the widespread strain could have been drawn. Of particular interest would be *S. spontaneum* populations from Java and southeast Asia, whence arose modern sugarcane cultivars, or India, whence arose *S. spontaneum* and some of the *S. spontaneum* material used by Jeswiet and his predecessors (Jeswiet, 1929).

If *L. xyli* subsp. *xyli* was originally associated with *S. spontaneum*, a broader phenomenon may be involved. As canes and related grasses became established throughout different regions, they have been exposed to different pathogens and microbial consortia, which have adapted to the host resources. Although *S. spontaneum* germplasm introgression was overwhelmingly beneficial, it is probable that the resulting hybrids would be more suitable hosts for *S. spontaneum* pathogens than the unhybridized *S. officinarum* canes. This may also be the case with sugarcane smut, caused by the fungus *Sporisorium scitamineum*, which has long been associated with *S. spontaneum* in India, but was historically absent from New Guinea (Chona & Gattani, 1950; Srinivasan & Chenulu, 1956; Magarey *et al.*, 2002). Evidence that *L. xyli* subsp. *xyli* inhibits smut (James, 1976; Bailey, 1977b) may suggest a selective advantage for clones of *S. spontaneum* that harbour a bacterium that clearly does not exhibit properties that are characteristic of most plant-pathogenic bacteria. This possibility requires further exploration.

It is possible that undiscovered strains related to *L. xyli* subsp. *xyli* may cross into commercial hybrids and have an impact on sugarcane productivity. For example, Young *et al.* (2006) revealed that a bacterium isolated from putatively RSD-affected sugarcane in Colombia was a related actinomycete distinct from *L. xyli* subsp. *xyli*. Furthermore, the bacterium on which Venezuelan researchers conducted Koch's postulates was clearly not *L. xyli* subsp. *xyli*, based on growth rate, culture medium and biochemical profile (Contreras *et al.*, 2008), and, like the Colombian strain, may represent a distinct sugarcane pathology. As plant breeders look to broaden the germplasm basis for plant improvement (Piperidis *et al.*, 2000), the endophytic composition of canes and grasses of interest should be examined not just for known pathogens, but also for other endophytes that may potentially have deleterious impacts.

Conclusion

The hypothesis presented is that unidentified clones of *S. spontaneum* were the natural host of *L. xyli* subsp. *xyli*, and that the release of POJ2878 in the mid-1920s facilitated the dissemination of a single worldwide clone of the bacterium. Consistent with this is the historic absence of RSD in the centre of origin for *S. officinarum*, and the period of emergence of RSD, which appears to coincide with the release of the artificial commercial hybrid varieties. That *S. spontaneum* clones support many more *L. xyli* subsp. *xyli* in their vascular fluid than *S. officinarum* clones indicates that they are more likely to be the natural hosts, and that susceptibility to RSD among commercial hybrids is derived from the *S. spontaneum* contribution. This hypothesis, that *L. xyli* subsp. *xyli* was naturally associated with clones of *S. spontaneum*, is consistent with the available evidence and is more likely than the alternative that RSD was derived from *S. officinarum*.

Acknowledgements

The author wishes to thank Stevens Brumbley and Michael Gillings, who were the supervisors when his research commenced, and provided comments on the PhD thesis, in which this hypothesis was first presented. The author also wishes to thank Ken Pegg, Andre Drenth, John Thompson and Julie Harris for reviewing and providing critical input into this work and Kiruba Arun Chinnappa for bioinformatic assistance.

References

Abbott EV, 1959. Relation of ratoon stunting disease to varietal yield decline in Louisiana. *Proceedings of the International Society of Sugar Cane Technologists* **10**, 66–71.

An SY, Xiao T, Yokota A, 2009. *Leifsonia lichenia* sp. nov., isolated from lichen in Japan. *Journal of General and Applied Microbiology* **55**, 339–43.

Anonymous, 1934. In: *BSES Annual Report*. Queensland, Australia: Bureau of Sugar Experimentation Stations, 58.

Anonymous, 1935. Darnall planter's field day – soil conditions and environment suitable for new variety canes. *South African Sugar Journal* **14**, 225–31.

Artschwager E, Brandes EW, 1958. Sugarcane (Saccharum officinarum *L.). Origin, Classification, Characteristics, and Descriptions of Representative Clones*. USDA Handbook no. 122. Washington, DC, USA: USDA.

Bailey RA, 1977a. The systemic distribution and relative occurrence of bacteria in sugarcane varieties affected by ratoon stunting disease. *Proceedings of the South African Sugar Technologists Association* **51**, 55–6.

Bailey RA, 1977b. The effect of hot water treatment, ratoon stunting disease and moisture stress on the incidence of smut in sugarcane. *Proceedings of the International Society of Sugar Cane Technologists* **16**, 327–35.

Bailey RA, Bechet GR, 1997. Further evidence of the effects of ratoon stunting disease on production under irrigated and rainfed conditions. *Proceedings of the South African Sugar Technologists Association* **71**, 97–101.

Barbehenn RV, Purcell AH, 1993. Factors limiting the transmission of a xylem-inhabiting bacterium *Clavibacter xyli* subsp. *cynodontis* to grasses by insects. *Phytopathology* **83**, 859–63.

Bell AF, 1935a. Variation within a clonal population. *Proceedings of the International Society of Sugar Cane Technologists* **5**, 557–62.

Bell AF, 1935b. Sick soils. *Proceedings of the Queensland Society of Sugar Cane Technologists* **12**, 9–18.

Bourne BA, 1965. The failure of a species of rabbit to transmit the ratoon stunting disease virus of sugar cane. *Proceedings of the International Society of Sugar Cane Technologists* **12**, 1071–7.

Brumbley SM, Petrasovits LA, Hermann SR, Young AJ, Croft BJ, 2006. Recent advances in the molecular biology of *Leifsonia xyli* subsp. *xyli*, causal organism of ratoon stunting disease. *Australasian Plant Pathology* **35**, 681–9.

Chona BL, Gattani ML, 1950. Kans grass (*Saccharum spontaneum* L.) a collateral host for sugarcane smut in India. *Indian Journal of Agricultural Science* **20**, 359–62.

Comstock JC, Miller JD, Shine JM, Tai PYP, 1995. Screening for resistance to ratoon stunting disease. *Proceedings of the International Society of Sugar Cane Technologists* **22**, 520–6.

Contreras N, Jimenez O, Bonilla M, Nass H, 2008. Identification and characterization of *Leifsonia xyli* subsp. *xyli* as sugarcane pathogen (*Saccharum* sp.) in the Centro Occidental region of Venezuela. *Bioagro* **20**, 111–8.

Croft BJ, 2001. A method for rating sugarcane cultivars for resistance to ratoon stunting disease based on an enzyme-linked immunoassay. *Australasian Plant Pathology* **31**, 63–6.

Damann KE, Benda GTA, 1983. Evaluation of commercial heat-treatment methods for control of ratoon stunting disease of sugarcane. *Plant Disease* **67**, 966–7.

Daniels J, Roach BT, 1987. Taxonomy and evolution. In: Heinz DJ, ed. *Sugarcane Improvement Through Breeding*. Amsterdam, Netherlands: Elsevier, 7–84.

Dastager SG, Lee JC, Ju YJ, Park DJ, Kim CJ, 2008. *Leifsonia bigeumensis* sp. nov., isolated from soil on Bigeum Island, Korea. *International Journal of Systematic and Evolutionary Microbiology* **58**, 1935–8.

Davis MJ, Gillaspie AG Jr, Harris RW, Lowson RH, 1980. Ratoon stunting disease of sugarcane: isolation of the causal bacterium. *Science* **210**, 1365–7.

Davis MJ, Gillaspie AG Jr, Vidaver AK, Harris RW, 1984. *Clavibacter*: a new genus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. nov., subsp. nov. and *Clavibacter xyli* subsp. *cynodontis* subsp. nov., pathogens that cause ratoon stunting disease of sugarcane and Bermuda grass stunting disease. *International Journal of Systematic Bacteriology* **34**, 107–17.

Davis MJ, Dean JL, Harrison NA, 1988. Quantitative variability of *Clavibacter xyli* subsp. *xyli* populations in sugarcane cultivars differing in resistance to ratoon stunting disease. *Phytopathology* **78**, 462–8.

Deerr N, 1949. *The History of Sugar, Volume 1*. London, UK: Chapman and Hall Ltd.

Denley CL, 1938. Yield trends in Louisiana as affected by varieties. *Proceedings of the International Society of Sugar Cane Technologists* **6**, 714–8.

D'Hont A, Paulet F, Glaszmann JC, 2002. Oligoclonal interspecific origin of 'North Indian' and 'Chinese' sugarcanes. *Chromosome Research* **10**, 253–62.

Edgerton CW, 1939. Stubble deterioration. *Proceedings of the International Society of Sugar Cane Technologists* **6**, 334–41.

Evtushenko LI, Dorofeeva LV, Subbotin SA, Cole JR, Tiedje JM, 2000. *Leifsonia poae* gen. nov., sp. nov., isolated from nematode galls on *Poa annua*, and reclassification of '*Corynebacterium aquaticum*' Leifson 1962 as *Leifsonia aquatica* (*ex* Leifson 1962) gen. nov., nom. rev., comb. nov. and *Clavibacter xyli* Davis *et al.* 1984 with two subspecies as *Leifsonia xyli* (Davis *et al.* 1984) gen. nov., comb. nov. *International Journal of Systematic and Evolutionary Microbiology* **50**, 371–80.

Fegan M, Croft BJ, Teakle DS, Hayward AC, Smith GR, 1998. Sensitive and specific detection of *Clavibacter xyli* subsp. *xyli*, causal agent of ratoon stunting disease, with a polymerase chain reaction-based assay. *Plant Pathology* **47**, 495–504.

Ferrari BC, Binnerup SJ, Gillings M, 2005. Microcolony cultivation on a soil substrate membrane system selects for previously uncultured soil bacteria. *Applied and Environmental Microbiology* **71**, 8714–20.

Gillaspie AG Jr, Teakle DS, 1989. Ratoon stunting disease. In: Ricaud C, Egan AG, Gillaspie AG Jr, Hughes CG, eds. *Diseases of Sugarcane. Major Diseases*. Amsterdam, Netherlands: Elsevier, 59–80.

Gillings M, Holley M, 1997. Repetitive element PCR fingerprinting (rep-PCR) using enterobacterial repetitive intergenic consensus (ERIC) primers is not necessarily directed at ERIC elements. *Letters in Applied Microbiology* **25**, 17–21.

Grisham MP, 1991. Effect of ratoon stunting disease on yield of sugarcane grown in multiple three-year plantings. *Phytopathology* **81**, 337–40.

Hill AG, 1935. The maintenance of first-year characters in new sugar cane clones. *Proceedings of the International Society of Sugar Cane Technologists* **5**, 563–7.

Hughes CG, 1955. Ratoon stunting disease of sugar cane. *Journal of Australian Institute of Agricultural Science* **21**, 3–9.

Hughes CG, 1974. The economic importance of ratoon stunting disease. *Proceedings of the International Society of Sugar Cane Technologists* **15**, 213–7.

Hughes CG, Steindl DRL, 1956. Some further developments in the study of ratoon stunting disease in Queensland. *Proceedings of the International Society of Sugar Cane Technologists* **9**, 1012–22.

Jackson PA, 2005. Breeding for improved sugar content in sugarcane. *Field Crops Research* **92**, 277–90.

James GL, 1976. The effect of ratoon stunting disease on the expression of smut symptoms. *Proceedings of the South African Society of Sugar Cane Technologists* **51**, 69–72.

Janoo N, Grivet L, Seguin M *et al.*, 1999. Molecular investigation of the genetic base of sugarcane cultivars. *Theoretical and Applied Genetics* **99**, 171–84.

Jeswiet J, 1927. The history of sugar cane selection work in Java. *Proceedings of the International Society of Sugar Cane Technologists* **2**, 115–22.

Jeswiet J, 1929. The development of selection and breeding of the sugar cane in Java. *Proceedings of the International Society of Sugar Cane Technologists* **3**, 44–57.

Kao J, Damann KE, 1978. Microcolonies of the bacterium associated with ratoon stunting disease found in sugarcane xylem matrix. *Phytopathology* **68**, 545–51.

King NJ, 1951. Varietal deterioration in Queensland. *Cane Growers' Quarterly Bulletin* **14**, 122–6.

King NJ, 1953. The ratoon stunting disease problem. *Cane Growers' Quarterly Bulletin* **17**, 10–3.

King NJ, Steindl DRL, 1953. The relationship between varietal yield deterioration and ratoon stunting disease. *Cane Growers' Quarterly Bulletin* **17**, 64–73.

Koike H, Gillaspie AG Jr, Benda GTA, 1982. Cane yield response to ratoon stunting disease. *International Sugar Journal* **84**, 131–3.

Kuniata LS, Rauka GB, McFarlane SA, Magarey RC, 2005. The impact of ratoon stunting disease at Ramu Sugar, Papua New Guinea. *Proceedings of the South African Society of Sugar Cane Technologists* **79**, 124–31.

Leifson E, 1962. The bacterial flora of distilled and stored water. III. New species of the genera *Corynebacterium*, *Flavobacterium*, *Spirillum* and *Pseudomonas*. *International Bulletin of Bacteriological Nomenclature and Taxonomy* **12**, 187–9.

Li W-F, Shen K, Huang Y-K *et al.*, 2013. PCR detection of ratoon stunting disease pathogen and natural resistance analysis in sugarcane core germplasms. *Crop Protection* **53**, 46–51.

Magarey RC, Suma S, Irawan Kuniata LS, Allsopp PG, 2002. Sik na binatang bilong suka – diseases and pests encountered during a survey of *Saccharum* germplasm 'in the wild' in Papua New Guinea. *Proceedings of the Australian Society of Sugar Cane Technologists* **24**, 219–27.

McDougall WA, Steindl DRL, Elliot JT, 1948. Variations in primary vigour in the variety Q28. *Cane Growers' Quarterly Bulletin* **12**, 31–4.

McFarlane SA, 2002. The relationship between extent of colonisation by *Leifsonia xyli* subsp. *xyli* and yield loss in different sugarcane varieties. *Proceedings of the South African Society of Sugar Cane Technologists* **76**, 281–4.

Mills L, Leaman TM, Taghavi SM *et al.*, 2001. *Leifsonia*-like bacteria are endophytes of grasses in eastern Australia. *Australasian Plant Pathology* **30**, 145–51.

Monteiro-Vitorello CB, Camargo LEA, Van Sluys MA *et al.*, 2004. The genome sequence of the Gram-positive sugarcane pathogen

Leifsonia xyli subsp. *xyli*. *Molecular Plant–Microbe Interactions* **17**, 827–36.
Mungomery RW, 1949. In: *BSES Annual Report. Report of the Division of Entomology and Pathology*. Queensland, Australia: Bureau of Sugar Experimentation Stations, 36–45.
Nishiwaki H, Ito K, Shimomura M, Nakashima K, Matsuda K, 2007. Insecticidal bacteria isolated from predatory larvae of the antlion species *Myrmeleon bore* (Neuroptera: Myrmeleontidae). *Journal of Invertebrate Pathology* **96**, 80–8.
North DS, 1935. The gumming disease of the sugar cane, its dissemination and control. *Agricultural Report. No. 10*. Sydney, Australia: Colonial Sugar Refining Co. Ltd.
Panje RR, Babu CN, 1960. Studies in *Saccharum spontaneum*. Distribution and geographical association of chromosome numbers. *Cytologia* **25**, 152–72.
Pindi PK, Kishore KH, Reddy GS, Shivaji S, 2009. Description of *Leifsonia kafniensis* sp. nov. and *Leifsonia antarctica* sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **59**, 1348–52.
Piperidis G, Christopher MJ, Carroll BJ, Berding N, D'Hont AM, 2000. Molecular contribution to selection of intergeneric hybrids between sugarcane and the wild species *Erianthus arundinaceus*. *Genome* **43**, 1033–7.
Price S, 1957. Cytological studies in *Saccharum* and allied genera II. Geographic distribution and chromosome numbers in *S. robustum*. *Cytologia* **22**, 40–52.
Rao GP, Singh M, Singh HN, 1990. Alternative hosts of sugarcane diseases. *Sugar Cane* Autumn (Supplement), 8–26.
Reddy GSN, Prakash JSS, Srinivas R, Matsumoto GI, Shivaji S, 2003. *Leifsonia rubra* sp. nov. and *Leifsonia aurea* sp. nov., psychrophiles from a pond in Antarctica. *International Journal of Systematic and Evolutionary Microbiology* **53**, 977–84.
Reddy GSN, Prabagaran SR, Shivaji S, 2008. *Leifsonia pindariensis* sp. nov., isolated from the Pindari glacier of the Indian Himalayas, and emended description of the genus *Leifsonia*. *International Journal of Systematic and Evolutionary Microbiology* **58**, 2229–34.
Roach BT, 1987. Observations on the incidence, effects and control of ratoon stunting disease. *Proceedings of the Australian Society of Sugar Cane Technologists* **9**, 109–16.
Roach B, 1992. Susceptibility to ratoon stunting disease in the *Saccharum* complex and feasibility of breeding for resistance. *Sugar Cane* **3**, 1–11.
Roach BT, Jackson PA, 1992. Screening sugar cane clones for resistance to ratoon stunting disease. *Sugar Cane* **2**, 2–12.
Rosenfeld AH, 1929. The decline and renaissance of Louisiana's sugar industry. *Proceedings of the International Society of Sugar Cane Technologists* **3**, 317–26.
Rosenfeld AH, 1956. *Sugar Cane Around the World*. Chicago, USA: University of Chicago Press.
Schuerger A, Lee P, 2015. Microbial ecology of a crewed rover traverse in the Arctic: low microbial dispersal and implications for planetary protection on human Mars missions. *Astrobiology* **15**, 478–91.
Srinivasan KV, Chenulu VV, 1956. A preliminary study of the reaction of *Saccharum spontaneum* variants to red rot, smut, rust and mosaic. *Proceedings of the International Society of Sugar Cane Technologists* **9**, 1097–107.
Steib RJ, Chilton SJP, 1968. The role of RSD in the determination of sugarcane varieties. *Sugar Journal* **30**, 10–2.
Steib RJ, Forbes IL, 1959. Effects of controlling ratoon stunting disease on yields of present and former commercial varieties of sugarcane in Louisiana. *Proceedings of the International Society of Sugar Cane Technologists* **6**, 1053–61.
Steindl DRL, 1949. Q.28 disease. *Cane Growers' Quarterly Bulletin* **12**, 191–3.
Steindl DRL, 1950. Ratoon stunting disease. *Proceedings of the International Society of Sugar Cane Technologists* **7**, 457–65.
Steindl DRL, 1957. Host range of the sugarcane ratoon stunting disease virus. *Journal of the Australian Institute of Agricultural Science* **23**, 238.
Steindl DRL, Hughes CG, 1953. Ratoon stunting disease. *Cane Growers' Quarterly Bulletin* **16**, 79–95.
Stevenson GC, 1947. Deterioration of sugar cane varieties. In: *Proceedings of the Meeting of British West Indies Sugar Technologists*. Barbados: British West Indies Sugar Association, 17–23.
Suzuki K, Suzuki M, Sasaki J, Park YH, Komagata K, 1999. *Leifsonia* gen. nov., a genus for 2,4-diaminobutyric acid-containing actinomycetes to accommodate '*Corynebacterium aquaticum*' Leifson 1962 and *Clavibacter xyli* subsp. *cynodontis* Davis 1984. *Journal of General and Applied Microbiology* **45**, 253–62.
Tapiolas B, 1934. Ratooning problems on the lower Burdekin. *Proceedings of the Queensland Society of Sugar Cane Technologists* **11**, 107–11.
Taylor PWJ, Ryan CC, Birch RG, 1988. Harvester transmission of leaf scald and ratoon stunting disease. *Sugar Cane* **2**, 11–4.
Taylor PWJ, Petrasovits LA, Van der Velde R *et al.*, 2003. Development of PCR-based markers for detection of *Leifsonia xyli* subsp. *xyli* in fibrovascular fluid of infected sugarcane plants. *Australasian Plant Pathology* **32**, 367–75.
Teakle DS, Appleton JM, Steindl DRL, 1978. Anatomical basis for resistance of sugarcane to ratoon stunting disease. *Physiological Plant Pathology* **12**, 83–91.
Victoria JF, Ochoa O, Cassale HC, 1986. Thermic control of ratoon stunting disease of sugarcane in Colombia. *Proceedings of the International Society of Sugar Cane Technologists* **19**, 325–31.
Young AJ, Brumbley SM, 2004. Ratoon stunting disease of sugarcane: history, management and current research. In: Rao GP, Salem Saumtally A, Rott P, eds. *Sugarcane Pathology, Vol. 3: Bacterial and Nematode Diseases*. Enfield, NH, USA: Science Publishers Inc., 97–124.
Young AJ, Petrasovits LA, Croft BJ, Gillings M, Brumbley SM, 2006. Genetic uniformity of international isolates of *Leifsonia xyli* subsp. *xyli*, causal agent of ratoon stunting disease of sugarcane (*Saccharum* interspecific hybrids). *Australasian Plant Pathology* **35**, 503–11.
Young AJ, Lokes S, Davis W, Aitken R, 2012. Reassessing RSD: insights from Harwood. *Proceedings of the Australian Society of Sugar Cane Technologists* **34**, 1–7.