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Effective quarantine: Interception of sugarcane diseases in the last 20 years has protected the industry from exotic threats

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Abstract Sugar Research Australia Indooroopilly has a post-entry quarantine facility that imports foreign clones per year. The imported clones are used for germplasm improvement in the SRA breeding program, with about 50% of released cultivars having at least one foreign clone as a parent. Quarantine of clones prior to their entry into the Australian sugarcane industry is important to keep out exotic pests and diseases. There have been many changes to quarantine since the industry began over 100 years ago: from no precautions at all, to the comprehensive system that we have today. This paper discusses the major milestones in quarantine, the evolution of the post-entry quarantine facility, the diseases tested for in post-entry quarantine, and the diseases that have been intercepted. We conclude with a summary of improvements that could be made to minimise the risk of importing diseases when achieving effective variety exchange.

Key words Quarantine, import, disease, intercept

INTRODUCTION

The Australian sugar industry through the former Bureau of Sugar Experiment Stations (BSES), then BSES Limited and now Sugar Research Australia (SRA) has a long history of importing sugarcane for germplasm improvement. In the early days, prior to a crossing program in Australia, all sugarcane clones were imported. More than 100 years later, foreign germplasm still plays an important role with ~50% of released varieties having at least one foreign parent (Piperidis *et al.* 2008). The earliest importations into Australia of sugarcane remain largely undocumented and were done without much consideration of what diseases and pests were also being imported. This has changed radically over time, as the understanding of the impacts of exotic pests and diseases and the methods for controlling unregulated importation were put into place.

A BRIEF HISTORY OF QUARANTINE FROM 1885–1999

The first recorded commentary on quarantine of sugarcane in Australia is that of two open state nurseries: one at "The Lagoons" in Mackay (1889) and "Kamerunga" in Cairns (1891). In these nurseries, collections made by Cowley and Tryon were housed, but there is no record of inspections or limitations on the import (King 1970). In 1895, Henry Tryon brought back 66 clones from New Guinea, including Badila and first planted this at Kamerunga (Anonymous 1975).

King (1970) also notes that prior to 1938 there was "…uncontrolled importation of cane varieties from all over the world since the start of the industry, and because of the absence of adequate quarantines – or trained personnel to recognize diseases – (Australia) had become the repository for most of the world's major diseases." Hughes (1960) notes that during this time of unrestricted importation smut, streak and sereh were the only diseases not also imported (Hughes 1960). King 1970 laments the general lack of recognition of disease symptoms, with one exception being Henry Tryon (of the Tryon collection), so it can be assumed that this collection at least was

inspected for diseases. King further notes that BSES staff had not personally played a part in cane collecting until 1951, but had sponsored and organised several collections, including the Wells 1912 collection from New Guinea. Wells was a Childers cane grower who collected 158 clones from New Guinea that were planted in Mackay (King 1970).

1935–1958

In 1935 the first sugarcane quarantine house was established by Arthur Bell on the grounds of the Department of Agriculture and Stock in Brisbane, on the Brisbane waterfront (Bell 1935; King 1970). The standard treatments for incoming cane have remained remarkably similar since 1935 – albeit, with some notable differences. The incoming cane was given a short hot-water treatment on arrival (20 mins at 52°C) in an organic mercurial solution before planting (Hughes 1960). Nowadays, we have modified the hot-water treatment (30 minutes at 50°C) and do not use any mercurial solutions! All imported canes were germinated and grown to maturity under a regular observation schedule. Any insects were promptly treated with insecticide and it was noted that the larger insects were kept out by the use of insect-proof screening on the quarantine house (Hughes 1960). After approximately 12 months (in the spring), the cane was harvested and given a hot-air treatment of 16 hours in a water-jacketed oven at 54°C as an RSD treatment, or a standard 2 hour 52°C treatment at the end of the following growth cycle. Care was taken to ensure no knife-contamination occurred while the cane was in quarantine. The treated setts were then distributed to the experiment stations for evaluation (Hughes 1960; King 1970).

All cane imported into Queensland from 1935 onwards has come through quarantine, excepting the New Guinea expedition collections of 1951 and 1957 (Hughes 1960). For large collections, such as BSES's 1951 cane collection expedition to New Guinea, an open quarantine was done. In this case, the 162 clones were quarantined at Redlands near Brisbane and were under observation for a full growth cycle, as well as being treated with an insecticide to prevent insect colonisation (Hughes 1960; King 1970). The 1957 New Guinea collection of canes were quarantined on Magnetic Island near Townsville (Hughes 1960), and in addition to the observation an intensive insecticide treatment used Metasystox by spraying and injecting into the soil (Hughes 1960). Setts from the plant crop were harvested, hot-water treated at 52°C for 2 hours and distributed to stations for propagation.

From 1935-1958, 240 clones were imported from many sugar-producing areas in the world. Diseases and pests intercepted included Fiji disease from New Britain (Hughes 1948), mosaic, ratoon stunting, wireworm in packaging and one instance of freckling with no known cause (Hughes 1948, 1960).

1958–1980

In 1958 a glasshouse was built on the top of the BSES building in Brisbane to conduct international quarantine. There was still some local quarantine done in Beerwah, but all international quarantine was carried out at in this facility (King 1958; Steindl 1966). Unfortunately, information about intercepts and procedures could not be found.

1980–1995

In 1980 there all quarantine activities were centralized at BSES Indooroopilly. At the time there were eight chambers: five small ones used for international quarantine were attached to one building, and three large ones that were used for local (domestic) quarantine. This was the first time that all sugarcane quarantine was centralised (Anonymous 2000), and apart from a few exceptions, this has remained the same. During this time the quarantine procedures were also modernised, with the Australian Quarantine and Inspection Service (AQIS) working with BSES to formalise an agreement for importation of sugarcane, to determine procedures for sugarcane, and to have this reflected in import permits.

This new approach was supported by the development of many modern diagnostic tests, as well as more widespread treatments: cane was kept for two complete growth cycles under inspection with cuttings from the first cycles being treated by immersion for 40 hours in cold/tepid running water followed by 3 hours at 52°C water (CS-LHWT) prior to replanting for the second growth cycle. It was always recognised that the "high risk" activity was the first growth cycle and, therefore, incorporated the new diagnostic tests, whilst the second cycle was used as a propagation stage.

In January 1994 there was a large consignment of 200 *S. officinarum* imported, and symptoms of the newly discovered disease 'Yellow leaf syndrome' (YLS) were found by BSES and AQIS. The continuation of this cane

into Australia was deemed to be too risky, and the cane was destroyed, and importation halted until a solution could be found (Anonymous 1994, 1995). This cane was likely to be infected with Sugarcane yellow leaf virus (SCYLV), but infection with Sugarcane Bacilliform Virus (SCBV) was also found. This finding initiated a resurgence into the development of molecular testing, and in 1993-1994 BSES researchers developed molecular assays for Sugarcane mosaic virus (SCMV), Fiji disease virus (FDV) (Smith and Van de Velde 1994) and SCBV (Braithwaite *et al.* 1995). A good test for SCYLV was years away, and at that time there were conjectures as to the cause: phytoplasma (Cronje *et al.* 1998) or virus (Scagliusi and Lockhart 2000).

In 1994 a dedicated quarantine glasshouse was funded by the Sugar Research and Development Corporation (SRDC) and this was built in 1995 (Anonymous 1994, 1995). This glasshouse is located on the same site at Indooroopilly, and the first consignment of canes went in later that year. This glasshouse complex has six chambers and at the time, four chambers were approved by AQIS for quarantine imports.

1995-current

From 1995 the Post-Entry Quarantine (PEQ) was done at Indooroopilly using three or four of the chambers in the international quarantine glasshouse. The remaining two chambers were used for pre-export quarantine collections that are screened prior to dispatch to exchange partners overseas.

In 1996 quarantine procedures were formalized with AQIS and these form the backbone of the current procedures. The procedures were based on previous experience: this included a 12-month quarantine with regular inspections, followed by a CS-LHWT after 12 months. The treated cane was replanted into a separate chamber and the ratoon kept for observation for approximately 6 months. The replanted cane was released from quarantine restriction by AQIS, grown to maturity and the stalks distributed to BSES stations for evaluation. At this time, BSES were already doing more than the minimum requirements, with all cane being tested by ELISA for RSD (Croft *et al.* 1994) and potyviruses (Agdia commercial kit). YLS testing for SCYLV using an experimental tissue blot immuno-assay (TBIA) was done on a small number of imports as part of a research project (Scagliusi and Lockhart 2000; Fitch *et al.* 2001).

To complement the new procedures, a project was funded by BSES and SRDC to investigate and expand on the number of disease tests that were done. At the end of this project in 1998-1999, additional tests for Fiji disease virus (FDV), Phytoplasma, Sugarcane streak viruses and Leaf scald were implemented and have been further developed over time.

From 1995 to 2014 there was a targeted approach taken to imports. This meant that the diseases tested for were those only known to be in the country of origin. All imports were tested for RSD, mosaic (using a general potyvirus ELISA by Agdia) and leaf scald (bio-assay or ELISA (Wang *et al.* 1999). For clones from Africa, the addition of the Sugarcane streak virus and Maize streak virus assays (Rybicki and Hughes 1990). For clones from Asia, there was the addition of Sugarcane streak mosaic virus (SCSMV; (Chatenet *et al.* 2005; Damayanti and Putra 2011) and phytoplasma (Thompson *et al.* 2012).

Sugarcane yellow leaf virus was an interesting case study. Once YLS was determined to be caused by SCYLV in most parts of the world (Lockhart and Cronje 2000), both an antisera-based test (Scagliusi and Lockhart 2000; Fitch *et al.* 2001) and a PCR-based test was developed (Chatenet *et al.* 2001). It was also found to be present in Australia, so the fear of importation was lessened. Therefore, testing was done only as part of research projects and TBIA was implemented partially in 2005. There have been worldwide shortages of antisera, but in the same time great improvements in the RT-PCR methods. The first “good” primers were implemented in our quarantine program in 2005 (Chatenet *et al.* 2001) and these were replaced by a superior primer set in 2012 (Girard *et al.* 2012).

Molecular tests for Fiji disease virus (Smith and Van de Velde 1994) were implemented in a targeted approach in 2002, and targeted testing for phytoplasma (Tran-Nguyen *et al.* 2000) started in 2003. The phytoplasma firstly targeted are White leaf phytoplasma and Grassy shoot phytoplasma in a specific assay, however a generic assay was implemented in 2011 (Thompson *et al.* 2012) to enable the detection of Green grassy shoot and other phytoplasma. Ramu stunt testing began when the first canes from PNG were imported in 2010, and this test refined as the virus sequence was discovered (Mollov *et al.* 2016; Braithwaite *et al.* 2019). Testing for Peanut clump virus (causing red leaf mottle) was implemented in 2014 after a travel and learning scholarship to CIRAD in France was completed and this brought all target organisms into alignment with CIRAD’s protocols.

Testing for RSD has been consistently by EB-EIA until 2017, where all samples were tested by both ELISA of the xylem extract as well as qPCR of the xylem extract (Grisham *et al.* 2007). In 2018 xylem qPCR was done exclusively and in 2019 a LSB method was trialled alongside the qPCR of xylem.

In 2011 the Post Entry Quarantine facilities begun further segregate imported clones by using only two glasshouses for the high-risk work. This was in preparation of upcoming legislative and biosecurity requirements. Since 2014 there has been a blanket approach to the testing, and there has been subsequent updating of the import permit conditions to reflect the tests required. The number of imports and disease intercepts are shown in Figure 1.

In 2013/14 there was a change in Commonwealth legislation with the repeal of the *Quarantine Act, 1908* and the enactment of the *Biosecurity Act, 2014*. At the same time, there were underlying changes to the quarantine requirements for approved facilities, which required greater separation of imported items and thus a reduced capacity of imports to two import chambers. The import permits likewise changed to include all the diseases that were required to be tested for, all the inspections and all treatments as well as day-to-day logging. For the most part, this was in line with the procedures already in place at SRA and required some upgrading to the facility to ensure it meets the criteria for importation of sugarcane.

In 2017 a Rural Research and Development for Profit project was funded by the Australian Government to modernise sugarcane diagnostics: this project is ongoing and investigating high-throughput sequencing methods for addition to quarantine disease testing, as well as modernising tests that are over 10 years old.

In 2019 SRA formalised a new compliance arrangement with Australian Government Department of Agriculture to run post-entry quarantine for sugarcane.

DISCUSSION

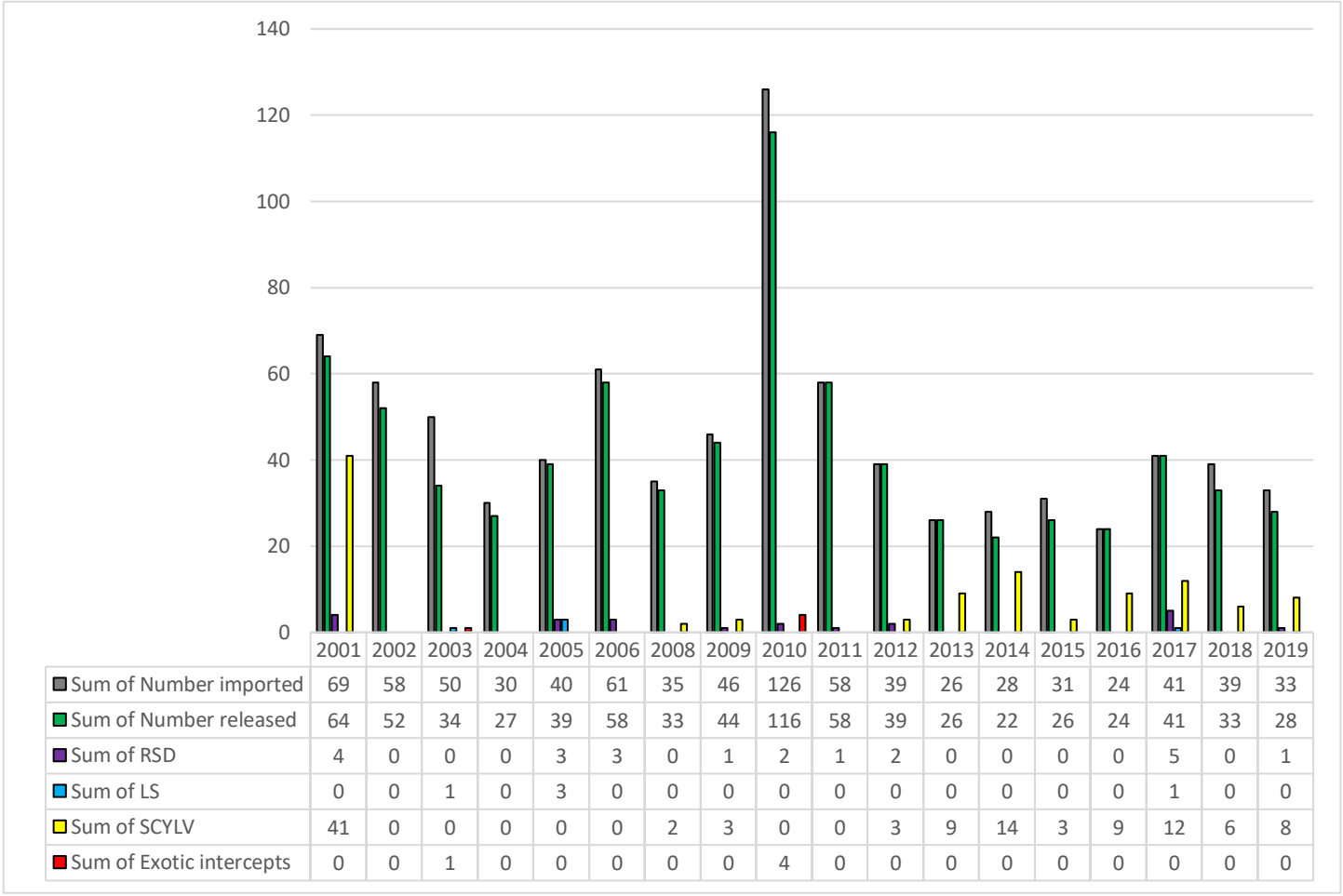
Figure 1 shows the results for imports over the last 20 years: the number of clones imported, the number of clones released and the disease intercepts for diseases already present in Australia (RSD, Leaf Scald (LS) and SCYLV) and the number of exotic pathogens intercepted. It should be noted that the current protocol is that no diseased cane will pass SRA quarantine, and our standard operating procedures ensure that there is no disease spread within quarantine. In 2010 there was a large number of imports for an ACIAR project, and this stretched the facility to its limit as this also had a number of disease intercepts. Apart from this outlier, the number hovers between 20 and 40 per year, and the number of intercepts is rising.

Interceptions of diseases already present in Australia

Ratoon stunting disease is intercepted nearly every year. Care is taken with any intercepts of RSD, with any cane testing positive being subjected to a third year of quarantine and at least one round of CS-LHWT. It is expected that the LHWT will eliminate RSD, and only once we have a negative result will the cane be released the subsequent year (i.e. after 2 consecutive LHWT and negative RSD testing result).

Leaf scald is periodically intercepted, with all recent intercepts being from the USA. This is likely due to there being no US standardised quarantine of canes prior to export. Once LS is intercepted, we subject the cane to the same protocol as for RSD. Occasionally, imported cane that is infected with LS (or RSD) is also infected with SCYLV, so these canes are destroyed as described below rather than put through a third year of quarantine. There is evidence for aerial spread of LS (Autrey *et al.* 1995), so any positive canes that are found should be isolated if not destroyed.

Sugarcane yellow leaf virus is the most problematic disease we encounter in quarantine. This is because of the increasing number in recent years as well as the inability for any in-house treatment to eliminate the virus. Overseas, this virus can cause from 2-20% yield losses depending on variety (Lockhart and Cronje 2000), whilst in Australia it is generally seen as a “minor disease”. The reason behind this difference is unknown, but it could be due to a greater general resistance of the Australian varieties, a less favourable environment in Australia (less prevalent or a different vector) or the absence of an aggressive strain in Australia. In any case, the importation of an exotic strain could be devastating to the industry, so it has been the intent of quarantine to limit the introduction of new strains of SCYLV. This means any cane infected with SCYLV is destroyed and not cleared to leave SRA quarantine. In recent years, SCYLV has spread throughout South America, and the control is very difficult. Elimination of the virus through tissue culture is possible, but it requires a very skilled meristematic tissue culture to eliminate. We do not have the ability to clean up clones at this time, so we are asking all countries to ensure they are sending clean material to Australia, either by their own testing, tissue culture, or by sending leaf samples in advance of the cane so that SRA can test prior to sending.



	2001	2002	2003	2004	2005	2006	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Sum of Number imported	69	58	50	30	40	61	35	46	126	58	39	26	28	31	24	41	39	33
Sum of Number released	64	52	34	27	39	58	33	44	116	58	39	26	22	26	24	41	33	28
Sum of RSD	4	0	0	0	3	3	0	1	2	1	2	0	0	0	0	5	0	1
Sum of LS	0	0	1	0	3	0	0	0	0	0	0	0	0	0	0	1	0	0
Sum of SCYLV	41	0	0	0	0	0	2	3	0	0	3	9	14	3	9	12	6	8
Sum of Exotic intercepts	0	0	1	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0

Figure 1. Number of clones imported, released and diseases intercepted per year.

Interception of exotic pests and diseases

Records of early interceptions are few and brief. There are reports of interception of Fiji leaf gall in canes from new Britain prior to 1948, as well as some insects (wireworm) and other diseases (not specified). All these early interceptions were identified via close observation of trained and experienced staff; however, we assume that symptomless or latent diseases would have been near-impossible to identify until molecular assays became available. Once molecular assays, such as ELISA, TBIA and later PCR and RT-PCR, were used routinely the records became more consistent. The data for the last 20 years is in Figure 2.

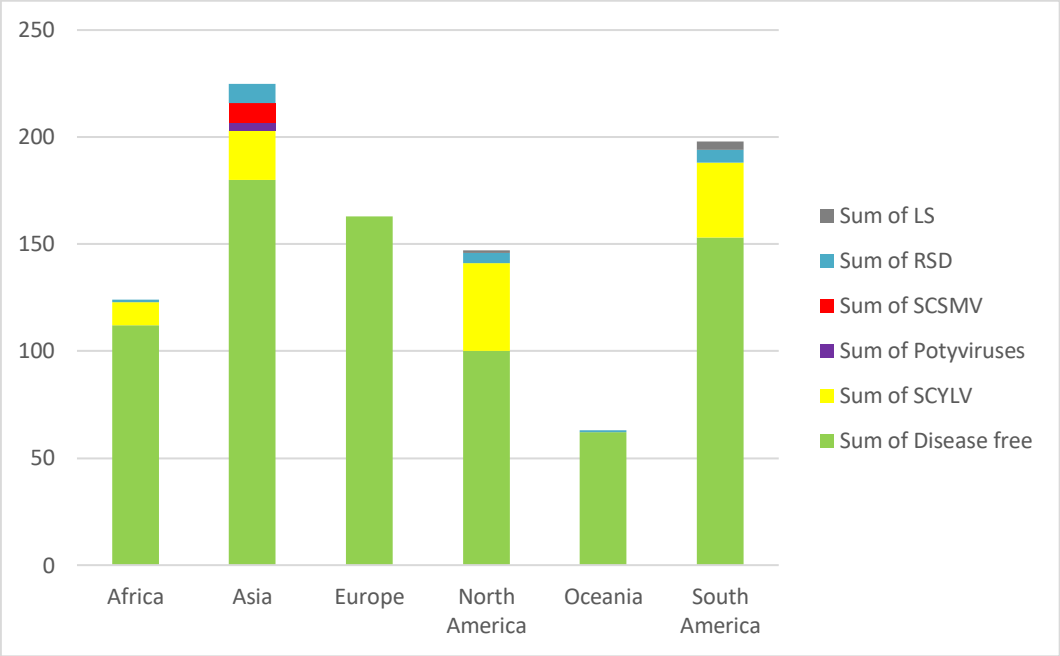


Figure 2. Disease intercepts and total number of imports from 1999-2019 by continent.

Occasionally there are stalks intercepted with borer holes and these are either intercepted by the Department of Agriculture on arrival or found by SRA. Australia is free of major moth borer pests, so all cane with borer holes are split to identify if a borer is present. In 2001 there was a *Diatraea* moth borer intercepted in consignment from South America. The consignment had borer holes on the stalk, and inside was a larva which was immediately placed in alcohol and its identity confirmed to be *Diatraea*. This one instance is the only account of a borer being inside the cane.

In 2010 a large consignment of cane from Asia was imported and we intercepted Sorghum mosaic virus (three clones infected and an exotic strain of Sugarcane mosaic virus (one clone infected). The interception was first noted during an inspection shortly after germination of the cane, with confirmation by ELISA in the BSES laboratory. The interception was reported to AQIS (now Department of Agriculture) and the four clones destroyed under their supervision. The remainder of the consignment was tested every three months by ELISA and RT-PCR until its eventual release from quarantine. No further disease was found in this consignment and no spread of the disease was found to other plants in quarantine.

During 2015 we intercepted Sugarcane streak mosaic virus in a small consignment of tissue culture plants from Asia. The tissue culture was attempted by the sender to ensure that the plants were disease free, but SCSMV is difficult to eliminate (CIRAD, personal communication) and the attempt was unsuccessful. Similar to the 2010 interception, we observed symptoms very early in the quarantine period, and finding mosaic symptoms on the tissue culture plantlets prior to them being de-flasked. The presence of SCSMV was confirmed in all plants by RT-PCR, reported to Department of Agriculture and Water Resources (now Department of Agriculture) and the nine clones destroyed under their direction. The plants were never de-flasked, and no other plants in the PEQ nursery tested positive for SCSMV.

FUTURE DIRECTIONS

True seed import

The importation of true seed (aka “fuzz”) is an area of intense interest. True seed provides a diversity of germplasm from proven crosses overseas that could be selected under Australian conditions to produce elite varieties. To date, there has only been limited importation of true seed done in the early 2000s under an agreement with CSIRO, AQIS and BSES, but the possibility remains to incorporate this into a regular exchange. In the above seed import, a proportion of the seed was germinated, and the resulting plants inspected and tested in a closed quarantine facility, prior to transplanting to a larger open quarantine plot near Charters Towers. The open quarantine was located remote from the industry and testing and inspection for exotic diseases was rigorous. The greater question remains: what sugarcane diseases are seed transmitted? And can the previously used procedure be improved? Currently, quarantine for large seed imports is beyond the scope of the small existing quarantine facility, so these are important questions to be considered before seed import can begin.

Improvements for cane being sent to Australia

Post-entry quarantine will be an important pillar of variety exchange for the foreseeable future, and there is room for improvement in the system. Figure 2 shows that the disease intercepts come from all over the world: the only places that we have never received diseased cane from is CIRAD (the Visacane quarantine facility) and, more recently, Japan.

Below are three items which we have identified as areas for improvement:

1) Greater emphasis on exchanging clones that are free from disease.

In many countries, there is no procedure put in place to test for, treat or eliminate pathogens prior to sending the clones. The adoption of a standardised protocol for variety exchange is an important step and we propose the following minimum standards:

a) All cane should be from a source that is CS-LWHT
b) Cane should be kept free from insects, either in a shadehouse or preferably in a glasshouse.
c) All cane should be inspected regularly and tested using molecular techniques for known diseases.
    a. In many cases, molecular testing is not possible by the sender, either because of lack of expertise or laboratory availability. In these cases, the importer and exporter should work together, for example an exporter could send leaf specimens to SRA for pre-screening prior to sending the cane.
    b. Any disease positive clones samples should be either: eliminated from the export collection, or; “cleaned” via tissue culture or hot water treatment (or other) using appropriate methods
d) Any other phytosanitary requirements of the importing country should be adhered to.

2) Improvement, standardisation and collaboration for molecular disease testing.

Molecular testing for diseases is becoming more commonly used for import and export, but the number of validated tests remains low despite there being many published new tests every year. The added complication of adopting tests for exotic pathogens is the inherent reliance on protocols and specimens from overseas. Sometimes there are diseases that have been reported overseas, but it is near-impossible to get a specimen to develop or validate a test. The addition of HTS (high-throughput sequencing) as a diagnostic tool is on the horizon and will provide both an additional layer of biosecurity but also complication. SRA is involved in developing this tool to be added, but the question remains as for all diagnostic tests of appropriate validation, standardisation and collaboration to ensure a good outcome. The resources and expertise of sugar cane technologists vary wildly around the globe and this needs to be considered when new tests become standard, especially when advanced (and expensive) methods become more widely used.

3) Development of disease-recognition skills.

The underpinning of biosecurity and quarantine is observation and inspections by experienced personnel. This has been the standard since the first cane import to Australia, with the supplement of molecular tests over time. The molecular tests are invaluable for disease that are known to be asymptomatic or have a latent phase (such as RSD, leaf scald and SCYLV), but being able to recognise diseases in cane, especially exotic diseases, underpins quarantine. When we are completing our quarantine for imported canes, we are not just looking for the known diseases with a tick-box approach – we are looking for anything unusual. It is likely that not all diseases in sugarcane have been discovered from every country in the world, and so we proceed with caution if an unusual symptom is present, and do not discount an abiotic factor being involved. This skill of disease-recognition and investigation needs to be cultured and valued for its importance: when the next exotic pathogen arrives, we need to know how to identify it.

CONCLUSIONS

SRA successfully intercepts disease pathogens from overseas institutions every year. This is protecting the industry from exotic pathogens as well as arresting the spread of disease already known to be in Australia.

SRA is working towards constant improvement in the system, and is working closely with stakeholders, both in Australia and overseas, to reduce the risk of importing diseases and pests with the valuable germplasm.

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