Abstract

The South African Sugarcane Research Institute (SASRI) exchanges sugarcane varieties with many different countries, including Australia, United States of America, Colombia, Brazil, Barbados and Zimbabwe, mainly to increase the genetic pool of parents for breeding new varieties. Imported varieties are also evaluated as potential commercial varieties. The movement of sugarcane between countries carries a risk of introducing potentially serious diseases and therefore requires stringent quarantine procedures. The current quarantine facility at SASRI, Mount Edgecombe, has been in use since 1984. It replaced the sugar industry’s original quarantine glasshouse that was built in the Botanic Gardens, Durban, in the mid-1920s. Twenty-one years after its opening, the current facility at Mount Edgecombe can be described as a world-class laboratory where molecular techniques are used for the accurate detection of the most important sugarcane pathogens.

The release of imported varieties from quarantine over the past two years has been seriously hampered by the frequent presence of the sugarcane yellow leaf virus (SCYLV). A new tissue culture facility for the ‘cleaning’ of diseases such as SCYLV, sugarcane mosaic virus (SCMV) and unknown viral diseases from varieties was recently added to the quarantine building. This enables SASRI to eliminate most pathogens from imported sugarcane varieties, so that disease-free plants can be used in the breeding programme. It also ensures that SASRI will soon be in a position to export healthy, tissue culture-derived plants of South African bred (N) varieties, instead of the conventional sets. The history of, and new developments in, sugarcane quarantine in South Africa are discussed in this paper.

Keywords: sugarcane, quarantine, import, export, disease diagnostics, tissue culture

Objectives and history of the quarantine glasshouse

Early history of the sugar industry

Shipwrecked mariners found sugarcane growing in South Africa in 1635 near Umzimkulu, in what is now southern KwaZulu-Natal. The sugarcane was grown in small quantities amongst the local people’s other crops (Schrire, 1983). It was originally thought that this early sugarcane was indigenous to South Africa, but according to Dr A McMartin, a previous director at SASRI (formerly SASEX), Arab slave traders in the Lake regions and early Portuguese explorers along the Zambezi River introduced the sugarcane plants. It appeared that the cane was Indonesian in origin and entered Natal via Mozambique (Schrire, 1983). Edmund Morewood was the first person to grow sugarcane on a commercial basis, and the original introductions, together with other canes that were imported from Mauritius in 1847, provided the foundation for the sugar industry (Schrire, 1983). To distinguish the local cane...
from the imports, Morewood called the ‘local cane’ Green Natal; this variety was to form the basis of the industry until 1870. The sugar industry flourished along the Natal coast, and by 1870 a variety called China Cane had taken over from Green Natal as the major variety. However, in 1887, the smut fungus (*Ustilago scitaminea*) caused severe damage to China Cane (personal communication) leading to the variety Uba becoming the backbone of the industry (Schrire, 1983).

In 1913/14 the Natal Sugar Association obtained a piece of land on the Eastern vlei (where Kingsmead stands today) to be used as a quarantine/experimental station. Planting began in 1914 under the supervision of the sugar industry, the Government pathologist and entomologist, and a sanitary inspector. The first batch of canes released from the quarantine station included varieties from Argentina. It was these canes that were identified in 1919-20 as being infected with the sugarcane mosaic virus (SCMV). All commercial varieties eventually became infected with SCMV except Uba, which for a number of years was the major variety grown in the industry. From the late 1920s another viral disease, sugarcane streak, spread rapidly in Uba to the extent that almost all crops were diseased (Bock and Bailey, 1989; Schrire, 1983). Uba was replaced by POJ 2785, POJ 2878 and Co 281, imported from India in 1926.

**A quarantine glasshouse for the sugar industry**

Because of the serious diseases (smut, mosaic and streak) troubling the sugar industry in the mid-1920s, SASRI built a quarantine glasshouse in the Botanic Gardens in Durban. (Figure 1). This glasshouse was financed by Mr David Fowler, President of the South African Sugar Association (SASA), and was officially opened on 14 April 1926 by Mr F Piccione, Vice-President of SASA. Custody of the glasshouse was then handed over to the Government, with quarantine supervision by the Mycologist and Entomologist, Dr HH Storey (Storey, 1926).

![Figure 1. The opening of the first sugarcane quarantine glasshouse in the Botanical Gardens in Durban.](image)

The National Government’s Directorate of Plant Health is ultimately responsible for plant quarantine, including sugarcane, but for many years the technical management of sugarcane quarantine has been delegated to SASRI pathologists. More than 1200 sugarcane varieties

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1 Roger Bailey, Assistant Director, SASRI, P/Bag X02, Mount Edgecombe, 4300, South Africa
were imported through the old glasshouse up to 1984. However, a new structure was eventually required and it was decided that a modern quarantine facility would be situated at the Sugarcane Research Institute, Mount Edgecombe (Bailey and Bechet, 1988).

**A modern sugarcane quarantine facility**

In most countries sugarcane quarantine is conducted in areas remote from commercial cane production; for example, the quarantine glasshouse at CIRAD in France serves countries such as Guadeloupe, Barbados, certain regions of the Antilles, Reunion Island and Mauritius. Although isolated quarantine minimises the hazard of accidental escapes, in South Africa this would have caused problems because of the essential need for close supervision by staff skilled in sugarcane disease diagnosis. The South African Sugar Association decided to locate the new quarantine facility close to the Research Institute in Mount Edgecombe, in the heart of a cane producing area (Bailey and Bechet, 1988). Quarantine risks were minimised by the presence of experienced diagnosticians (Roger Bailey and Roger Bechet), by designing a facility with a number of innovative features specifically for secure quarantine and by adopting stringent quarantine procedures (Bailey and Bechet, 1988). The new building was opened in October 1984 and named the A McMartin Quarantine Glasshouse in honour of Dr A McMartin, a former pathologist and the director of SASRI from 1950 to 1958 (Bailey and Bechet, 1988).

The standards that are adhered to in the quarantine glasshouse are based on the FAO/IBPGR Guidelines for the Safe Movement of Sugarcane Germplasm (Frison and Putter, 1993) with some more recent improvements. The quarantine glasshouse has ten growth cubicles, each with a floor area of 6 m x 2.5 m, linked by a corridor to a laboratory (Bailey and Bechet, 1988). Four of the ten cubicles were added in 1999 (Figure 2).

![Figure 2. Excavations and addition of four extra growth compartments to the quarantine glasshouse at SASRI.](image)

Some of the special features of the glasshouse include sterilising and incinerating facilities inside the quarantine building. The only live plant material that ever leaves quarantine are sugarcane setts of healthy varieties for subsequent propagation, or plants derived from single-budded setts that have germinated in the quarantine facility. All other plant material, and all the soil used in the glasshouse, are incinerated or sterilised before they are removed from the quarantine area. A slightly negative air pressure is maintained within the building by means of extractor fans and a series of very fine filters. In addition to the fans and filters there is a three-stage system of airlocks in the entry area to the growth cubicles. This minimises the risk of any potential insect vector or pathogen leaving the building. Apart from the extractor vents, which are protected by the filters, the entire building is effectively airtight. All water
draining from the building, including the growth cubicles, the laboratory and the sterilising rooms, runs through a specially contained and protected drainage system. Optimum temperatures are maintained within the growth cubicles by means of thermostatically controlled heaters and coolers. Compartments are also fumigated and sterilised between consignments. A picture of the quarantine glasshouse today is shown in Figure 3.

![Figure 3. The current quarantine facility at SASRI. The tissue culture laboratory is on the left.](image)

The release of imported varieties from quarantine over the past two years has been seriously hampered by the frequent presence of the sugarcane yellow leaf virus (SCYLV) in imported germplasm. A new tissue culture facility for the ‘cleaning’ of most pathogens (SCYLV, SCMV, and at least some unknown pathogens) from imported varieties was added to the quarantine building in 2004. This enables SASRI to use disease-free plants in breeding programmes. The facility also ensures that SASRI will soon be in a position to export healthy, tissue culture-derived plants of South African (N) varieties instead of conventional setts.

**Purpose of the quarantine glasshouse**

The main purpose of the quarantine glasshouse is to enable the safe importation of sugarcane varieties to increase the genetic material available for crossing. The crossing programme is the basis for breeding improved, higher sucrose-yielding and disease-resistant varieties for the industry. Pest and disease risks are minimised by growing the imported canes in the quarantine glasshouse for at least 15 months, and subjecting the canes to a wide range of diagnostic assays.

**Methodology in the quarantine glasshouse**

**Planting cycles and post-quarantine procedure**

Varieties are usually sent and received as three-budded setts. On arrival at the quarantine glasshouse, the setts are hot water treated (HWT, 30 min at 50°C) and dipped in an insecticide and fungicide. Each batch of imported setts is then planted into sterilised soil in a designated compartment in the glasshouse. After approximately 10 months, diagnostic tests are carried out and the stalks are cut into setts and replanted after hot water treatment for 2 hours at 50°C. The setts are then again dipped in insecticide and fungicide. The plants are continually inspected for disease symptoms during growth in the quarantine glasshouse. After the second growth cycle, transplants are made from healthy plants and planted in the field in a post-quarantine bulking area at SASRI, Mt Edgecombe. They remain there for a minimum of 10 months and are again inspected frequently for diseases. After post-quarantine bulking,
the imported varieties are introduced into the variety selection programme at SASRI’s Pongola research station.

**Disease diagnostics**

Prior to 1998 the main methods for diagnosing diseases were recognition of visual symptoms and isolation of pathogens on selective growth media. The enzyme-linked immunosorbent assay (ELISA) was used for the detection of SCMV and SCYLV and immunofluorescence microscopy for the detection of *Leifsonia xyli* subsp. *xyli*. Since 1998 the main diagnostic tests used are tissue blots for SCYLV, RT-PCR for SCYLV, FDV (Fiji disease) and SCMV, and PCR for the detection of phytoplasmas such as the sugarcane yellows phytoplasma (SCYP), grassy shoot and white leaf. PCR can also be used for the detection of smut. Research on improving molecular methods for disease detection is ongoing to make diagnostic tests faster, cheaper and more reliable. Plants are still inspected weekly for any visible disease symptoms or nutritional problems.

**Disease elimination in sugarcane by in vitro culture**

A meristem tissue culture laboratory was added to the quarantine glasshouse in 2004. The intention is that all imported varieties will be processed through this laboratory to eliminate most pathogens from plants. In this process the growing point is dissected out under a stereomicroscope and transferred to growing media in Magenta jars (Chatenet *et al.*, 2001). After several months on a shoot proliferation medium, the plants are transferred to a rooting medium and eventually to pots. All meristem-derived plants are indexed for diseases before they are exported or moved to post-quarantine.

**Results and discussion**

**Imports**

Since the new glasshouse was opened at SASRI in 1984, 458 varieties have been imported from 14 different countries. Most importations have been from Australia, Barbados, Brazil, Colombia, the USA and Zimbabwe.

Until 1997, the main diseases intercepted in the quarantine glasshouse were SCYLV, SCYP, pokkah boeng and RSD. Thirty-one per cent of imported varieties that are currently (March 2005) in the quarantine glasshouse are infected by SCYLV. These include 10% of varieties from Colombia, 46% of varieties from Brazil and 92% from the USA. Varieties from Australia have been mainly infected by SCYP. Because of the incidence of SCYLV and SCYP, not many varieties have recently been released from quarantine. Varieties held back include LCP85-384, the ‘wonder variety’ from the USA. SCYLV in particular is significant because it exists as a number of different strains and emerging evidence suggests it has a significant effect on yield. The need to deal with these diseases in imported germplasm was the ‘trigger’ behind the decision to introduce meristem tissue culture.

**Tissue culture**

All imported varieties in the quarantine glasshouse, as well as local varieties, were put into meristem culture (approximately 100 varieties/clones) in late 2004. Meristem culture effectively eliminates diseases such as SCLV and mosaic (Rott *et al.*, 1998). The passage of all imported varieties through the tissue culture system will eliminate most diseases...
(including some unknown ones). Meristem tip culture is used because no somaclonal variation occurs in these micropropagated plants.

Exports

Since 1984, 271 batches of South African varieties have been exported to various countries, mainly Australia, Brazil, Colombia, Mauritius, Reunion and Zimbabwe. True seed from sugarcane crosses has been exported to Japan, Pakistan and Zimbabwe. Variety N27 has been exported most frequently (12 batches), followed by N16, N19 and N23 (each 10 times). Infection of some newer South African varieties, including N32, N38, N40 and N42, with SCYLV and SCYP has hampered the export of these varieties. Meristem tissue culture offers a means of overcoming this problem.

Conclusions

Imported varieties or clones that are infected with pathogens are destroyed and removed from the quarantine glasshouse or put through the tissue culture system. The main diseases observed in quarantine were SCYLV, SCYLP and SCMV, as well as pokkah boeng. If cane varieties are imported illegally, they may not only be infected with diseases common to the country of origin, but may also be susceptible to local diseases. The outbreak of a foreign disease in our local susceptible varieties would be extremely serious. In this light, quarantine must be regarded as one of the most important entities of any sugarcane research institute. Research regarding the improvement of quarantine procedures and disease diagnosis and strains of pathogens is a high priority and is ongoing at SASRI.

REFERENCES