Sugarcane Quarantine

in South Africa

SOUTH AFRICAN SUGARCANE RESEARCH INSTITUTE

Sugarcane Quarantine

in South Africa

Published by:

South African Sugarcane Research Institute (SASRI).

March 2006

This booklet has been compiled by
SASRI Plant Pathologist, **Tania van Antwerpen**, using information provided by various SASRI specialists.

ISBN: 1-874903-33-6

Introduction

Sugarcane clones from foreign countries are imported into South Africa to broaden the genetic base of the parental breeding stock. 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. These pathogens are comprised mainly of viruses, bacteria and phytoplasmas.

Imported sugarcane varieties are frequently inspected for disease symptoms.

History

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 at the Botanic Gardens, Durban, in the mid-1920s. The original glasshouse was built because of the serious diseases (smut, mosaic and streak) affecting the sugar industry during those years. The facility at Mount Edgecombe is a world-class laboratory where molecular techniques are used for the accurate detection of the most important sugarcane pathogens.

The first quarantine glasshouse of the sugar industry was built at the Botanical Gardens, Durban, in the mid-1920s.

The opening of the quarantine glasshouse at Mount Edgecombe in 1984.

The quarantine glasshouse and growing conditions

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 standards that are adhered to in the quarantine glasshouse are based on the FAO/IBPGR Guidelines for the Safe Movement of Sugarcane Germplasm with some more recent improvements. Although the national government's Directorate SAAFQIS (South African Agricultural Food, Quarantine and Inspection Services) is ultimately responsible for plant quarantine, including sugarcane, for many years the technical management of the sugarcane quarantine has been delegated to SASRI's pathologists.

The current quarantine glasshouse at Mount Edgecombe.

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. Four of the ten cubicles were added in 1999. Six cubicles are used mainly for imported varieties and four mainly for growing local varieties in quarantine isolation before export. Seventy-two foreign varieties can be accommodated at any one time. The glasshouse has a number of special features to ensure secure quarantine and to provide good conditions for plant growth:

In this quarantine cubicle, hardened-off micropropagated plants are inspected before planting in post quarantine.

Sterilising facilities in quarantine (such as autoclaves) are used to sterilise soil and plant material.

- Only sterilised soil and imported setts (sealed in the original packaging) are brought into the building. Sterilising facilities are provided within the area of quarantine security.
- The only live plant material taken out of the quarantine glasshouse are setts of healthy varieties or micropropagated plants to be used for subsequent propagation. All other plant material and all soil used in the glasshouse are sterilised before being removed from the quarantine area for disposal.
- A slightly negative air pressure is maintained in the building by means of extractor fans mounted in the exterior walls of the glasshouse. These draw air through ventilation ports, which are protected by fine

filters (c.10 µm). The filters are cleaned and replaced monthly.

- Access to the growth compartments is through a three-stage system of airlocks. These minimise the risk of any potential insect vector of disease organisms entering or escaping from the building.
- Drainage from the building, including the growth compartment cubicles, the laboratory and the sterilising room, is effected through a contained drainage system.
- The compartments are fumigated (insecticidal fog) and sprayed with an insecticide between consignments. The compartments are also cleaned weekly and sprayed with insecticides monthly for the control of insects. Chlorpyrifos is sprayed on the outside of the glasshouse to control ants.

Three stage system of airlock doors and disinfectant footbath mats are in place to prevent the escape of pathogens.

- Temperatures within the compartments are maintained close to the optimum for cane growth by means of thermostatically controlled heaters and air conditioners. Maximum and minimum temperatures are recorded daily in each compartment.
- A dripper irrigation system provides water to each plant container.
- All persons entering the building are required to wear dustcoats and to wear overshoes or clean their footwear in a disinfectant footbath when entering and leaving the building. Windows, floors and all apparatus in the laboratory are cleaned weekly.

Importing and quarantine procedures

Sugarcane varieties are imported from countries such as Australia, Brazil, Colombia, Barbados and Zimbabwe.

Sugarcane varieties are imported under the terms and conditions of a specific import permit issued by the government Directorate SAAFQIS. Upon arrival, parcels of imported sugarcane are unpacked in the laboratory. All packaging materials are sterilised by autoclaving. The setts are treated in water at 50°C for 30 minutes and are then dipped in a fungicide to eliminate any fungi, and an insecticide for the elimination of insects.

Imported sugarcane setts are hot-water-treated before being planted.

The treated setts are planted in sterilised potting medium in 25-litre pots. Up to four setts of one variety are planted in each pot and up to 12 varieties can be grown in one compartment. Each consignment is grown in a separate compartment.

The temperature is initially adjusted to 35-37°C to facilitate germination of the setts and, after the plants are established, the temperature is adjusted to a regime of approximately 30°C (days) and 25°C (nights).

Superphosphate (50 g) and Curaterr (carbofuran, 1 g/pot) are added to the upper layer of medium in each pot at planting. Hydroponic nutrients are mixed with nitrogen and applied to the pots weekly.

The first planting is grown for 9 to 12 months. The plants are inspected weekly for symptoms of diseases and other problems. Towards the end of

this period, leaf samples are taken from each plant and subjected to several diagnostic tests.

After the first 9 to 12 months, setts are cut from each plant, soaked in cold water for 48 hours and treated in hot water for 2 or 3 hours at 50°C. The setts are then dipped in fungicide and insecticide and replanted into sterilised soil in clean pots.

The 'mother plants' from the first planting are kept in the glasshouse until the second planting has become established. The original plants are ratooned, inspected for disease symptoms and destroyed once the new planting is established. The second planting is grown for a further 9 to 12 months. If these plants do not express any disease symptoms, single-budded setts obtained from these plants are treated for 40 minutes at 52°C in a hot water tank to which a fungicide has been added. The transplants obtained from the single-budded setts are then planted in a post-quarantine area. Once the transplants are established, the 'mother plants' in quarantine are destroyed.

Imported varieties in pots are placed in the quarantine cubicle.

Inspection of sugarcane plants for diseases.

Diseases

Disease diagnostics

Molecular tests such as RT-PCR and electrophoresis are used to detect pathogens.

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 sugarcane mosaic virus (SCMV) and sugarcane yellow leaf virus (SCYLV). Current diagnostic tests include immunofluorescence microscopy and PCR for the detection of ratoon stunting disease (RSD) (*Leifsonia xyli* subsp *xyli*), RT-PCT for the detection of SCMV, Fiji disease virus (FDV) and SCYLV, and RT-PCR, PCR and RFLPs for the detection of sugarcane yellows phytoplasma (SCYP), sugarcane white leaf and grassy shoot. PCR and selective plating are used for the detection of leaf scald (*Xanthomonas albilineans*). PCR can also be used for the detection of smut (*Ustilago scitaminea*).

Research on improving molecular techniques 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.

Symptoms of pokkah boeng.

Diseases intercepted in quarantine

The main diseases intercepted in quarantine are SCYLV, SCYP, pokkah boeng and RSD.

Exporting sugarcane varieties

Since 1984 hundreds of 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, followed by N16, N19 and N23. Infection of some newer South African varieties, including N32, N38, N40 and N42, with SCYLV and SCYP has hampered the export of these varieties. This problem will be overcome by the production of disease-free micropropagated plants.

Popular and high potential South African varieties are grown in isolation in the quarantine glasshouse and subjected to diagnostic tests before being dispatched. The procedures and diagnostic tests used are identical to those used when sugarcane varieties are imported into South Africa, with the same high degree of security.

For each variety, three 3-budded setts are collected from the plants in the quarantine glasshouse. The setts are washed in soapy water using a brush, and the leaf sheath material is removed with a disinfected scalpel. The setts are treated in hot water at 50°C for 30 minutes, and then dipped in fungicide and insecticide.

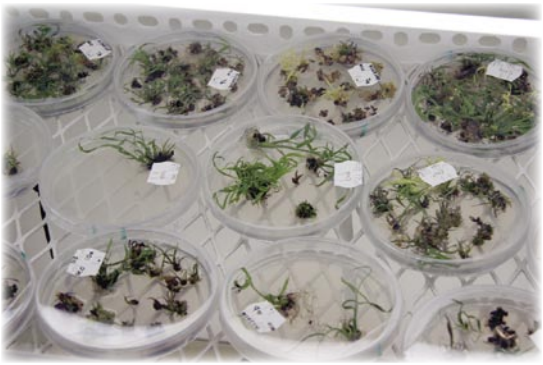
The variety name is written directly on each sett. The setts are then entirely dipped in low melting temperature paraffin wax before being wrapped in dry paper and suitably packed. A phytosanitary certificate from the Directorate SAAFQIS and the import permit from the importing country, are sent with the parcel.

Packaging of setts to be exported.

Micropropagated plantlets will also be exported in the future.

Tissue culture laboratory

The release of imported varieties from quarantine since 2002 has been seriously hampered by the frequent presence of SCYLV in imported germplasm. A new tissue culture facility for the 'cleaning' of varieties from diseases such as SCYLV, SCMV and unknown viral diseases was added to the quarantine building in 2004. This enables SASRI to clean imported sugarcane varieties from most pathogens so that disease-free plants can be used in the breeding programme. It also ensures that SASRI will be in a position to export healthy, tissue culture-derived plants of South African N varieties instead of conventional setts. All imported varieties will be processed through this laboratory to eliminate most pathogens from plants. Micropropagated plants are indexed for diseases before they are exported or moved to the post-quarantine area.

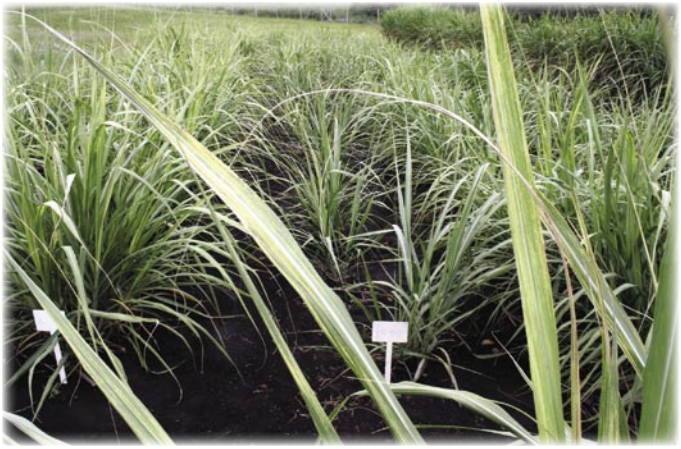


Micropropagation of imported and local varieties to make them disease free.

Light growth room for micropropagation of plants.

Post-quarantine

After the second growth cycle in the quarantine glasshouse, transplants are made from healthy plants and planted in the field in a post-quarantine bulking area at Mount 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 or Midlands research stations.



Imported varieties are planted in a post-quarantine area when released from the quarantine glasshouse.

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. 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 disease diagnosis and strains of pathogens is given a high priority at SASRI, and the improvement of quarantine procedures is ongoing.

Sugarcane leaves being prepared for testing for SCYLV.

Further reading...

Bailey RA and Bechet GR (1988). Recent improvements in quarantine procedures for sugarcane in South Africa. Proc S Afr Sug Technol Ass 67: 190-194.

Frison EA and Putter CAJ (Eds) (1993). FAO/IBPGR technical guidelines for the safe movement of sugarcane germplasm. Published by the International Society of Sugar Cane Technologists.

van Antwerpen T, McFarlane K and Subramoney DS. (2001). Recent improvements in procedures to improve security and efficiency of sugarcane quarantine in South Africa. Proc S Afr Sug Technol Ass 75: 255-256.

van Antwerpen T, Bailey RA, Subramoney DS, McFarlane K, Rutherford RS and Nuss KJ (2005). Eighty years of sugarcane quarantine in South Africa. Proc S Afr Sug Technol Ass 79: 114-119.

SOUTH AFRICAN SUGAR ASSOCIATION

South African Sugarcane Research Institute

170 Flanders Drive, Mount Edgecombe, 4300

Private Bag X02, Mount Edgecombe, 4300

Tel: (031) 508 7400 Fax: (031) 508 7597

Website: www.sugar.org.za