RECENT IMPROVEMENTS IN QUARANTINE PROCEDURES FOR SUGARCANE IN SOUTH AFRICA

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Abstract

Sugarcane clones imported into South Africa undergo a period of closed quarantine before being released for further propagation. Since the opening of a new, specially designed quarantine glasshouse at Mount Edgecombe in 1984, the average duration of quarantine (two plantings) has been reduced from 20 to 15 months. Routine procedures include thermotherapeutic treatments at the start of, midway through, and at the end of the quarantine cycle, frequent inspections for disease symptoms, and the examination of the clones by immunofluorescence microscopy to detect ratoon stunting disease and leaf scald, and by ELISA to detect mosaic. Some of the features of the glasshouse and the operation of quarantine procedures are described.

Introduction

The importation of clones of sugarcane and related species is a necessity for any sugarcane industry engaged in a continuing search for improved productivity. Foreign clones may be useful as released cane varieties, and in South Africa these have included Co301, J59/3 and, most recently, CP66/1043; but a more important use of imported material in countries with active breeding programmes is to broaden the genetic base of the parental breeding stock.

The multiplication of sugarcane clones depends on the propagation of vegetative plant material, and this usually involves the planting of setts cut from cane stalks. This method of propagation is still the usual practice for the international distribution of varieties and it inevitably carries with it the risk of distributing seedcane-borne diseases from country to country. It is likely that the present widespread occurrence of ratoon stunting disease (RSD) and leaf scald, among other diseases which hitherto have been difficult to identify with certainty, is largely due to their having been unintentionally distributed by the traffic in clones among cane producing countries.

Introduced pathogens may already occur in the receiving country, and if so are of little significance, but the risk of exotic pathogens being introduced is a serious hazard. Exotic diseases in the South African context include Fiji disease, grassy shoot disease and striate mosaic, while exotic pathogens also include new strains or pathotypes of the causal agents of diseases that already occur here, such as mosaic and smut. Most countries that import foreign clones operate a system of quarantine procedures that is intended to minimise the risk of introducing new pathogens.

The application of quarantine to sugarcane in South Africa commenced in 1925, when a specially designed glasshouse was erected in the Botanic Gardens in Durban (Figure 1). At that time the industry was dependent on imported varieties and urgently needed new introductions to combat a serious outbreak of sugarcane mosaic virus (SCMV). The teak structure had a number of features that suited its use for quarantine, including fine-mesh screens over all opening windows and double-door air-locks leading to the compartments. Early procedures used in this glasshouse were described by Thomson.[1] The first glasshouse served the industry for almost 60 years, during which more than 1 200 clones were imported, but increasing maintenance problems led to its closure in 1984.

FIGURE 1 The old quarantine glasshouse, built in 1925.

The need for a new quarantine glasshouse introduced the problem of selecting a suitable site. In most countries sugarcane quarantine is conducted in areas remote from commercial cane production. Although isolated quarantine minimises the hazard of accidental escapes, in South Africa this would have caused problems in the provision of close supervision by staff skilled in the diagnosis of sugarcane diseases. The policy adopted by the South African Sugar Association was to locate the new quarantine facility at the Experiment Station, Mount Edgecombe, ie in the heart of a cane producing area. The risks inherent in this decision are minimised by having experienced diagnosticians at hand, by adopting stringent quarantine procedures and by building a facility with a number of innovative features specifically for secure quarantine. The new building (Figure 2) was opened in October 1984 and named the 'A McMartin Quarantine Glasshouse' in honour of Dr A. McMartin, Director of the Experiment Station from 1950 to 1958.

Quarantine principles for sugarcane

The causal agents of sugarcane diseases may be broadly described as being either fungi, bacteria or viruses. Fungal pathogens present within the imported setts or carried as external contaminants can readily be eliminated by fungicidal and thermotherapeutic treatments, and so pose little or no risk. Thermotherapy also provides some, but not complete control of bacterial pathogens of sugarcane, but has little or no useful effect on virus diseases of the crop. Therefore the main risks from importing sugarcane clones in the form of conventional setts lie in the possibility of introducing viral and bacterial pathogens.

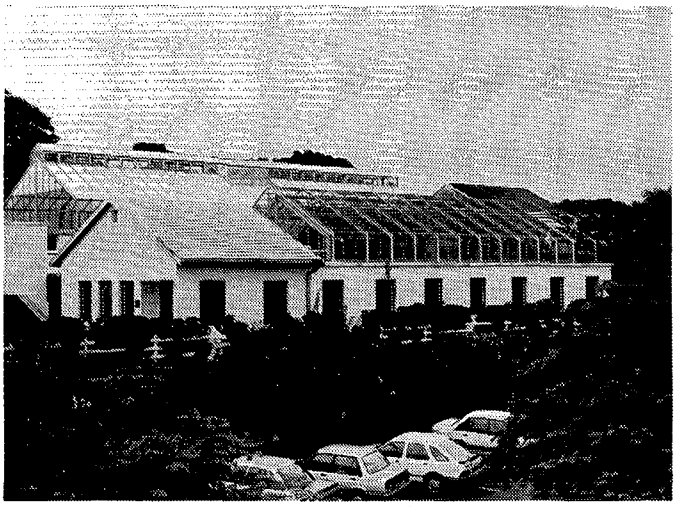

FIGURE 2 The new quarantine building at Mount Edgecombe was opened in 1984.

Safe quarantine procedures must provide for the routine elimination of pathogens, even if undiagnosed, by appropriate means and for the identification of all other diseases caused by pathogens that cannot be routinely eliminated, so that infected plants can either be destroyed or given specific treatment. The procedures followed when a disease is diagnosed depend on the importance attached to that disease in the local situation and the probability of successful control if treatment is applied. RSD, for example, is amenable to control by thermotherapy and already occurs in most if not all cane producing countries. However, a 'safety first' policy is usually adopted by importing countries, by which clones with symptoms of serious diseases or with unknown but unusual symptoms are destroyed. In particularly serious cases, such as would be presented by Fiji disease if detected in quarantine in South Africa, all the clones in that particular consignment might be destroyed.

Frequent inspections by a diagnostician skilled in recognising local and exotic disorders of sugarcane are the most important requirement for safe quarantine. However, recently developed diagnostic techniques based on immunological reactions, such as the enzyme-linked immunosorbent assay (ELISA) and immunofluorescence microscopy, can be usefully included in routine quarantine procedures. These techniques are particularly valuable for detecting diseases which may remain latent or have inconspicuous symptoms, provided that suitable antisera can be obtained.

A number of systemic sugarcane diseases are more likely to exhibit symptoms in ratoon crops than in the plant crop. For this reason and as an added precaution, quarantine procedures in many importing countries require that imported material is grown through two planting cycles: setts obtained from the first crop are used to establish the second planting, and the first planting, after being cut back, is retained for disease inspections of the young regrowth. The second planting has to be successfully established before the stubble and regrowth of the first planting are destroyed.

The escape of diseases through or from quarantine may be caused by the release of varieties with unidentified problems, which can be countered only by diagnostic skills and suitable procedures, or by an accidental breach of quarantine security. The most likely ways in which the latter can occur are: the escape of pathogens in drainage water; infective plant material, debris or soil being taken out of quarantine; and infective vectors escaping through doors, windows or cracks and breaks in the structure. These possibilities can be avoided by the appropriate design of the building and by adopting safe procedures, and all were considered in the design and construction of the new facility at Mount Edgecombe.

A further factor to be considered when quarantine is conducted in a cane producing area is the possibility of infection by local pathogens: precautions must be taken to avoid this.

Design and features of the quarantine building

The quarantine building (Figures 2, 3 and 4) comprises 6 glasshouse compartments, each with 6 x 2,5 m of floor area, linked by a common corridor to a laboratory and sterilising room. Entry into the building is through an 'air-lock' vestibule into the laboratory. The laboratory, corridor, and glasshouse compartments are all within the quarantine area and are separated by doors, so that there is effectively a 3-stage system of air-locks before entry is gained to the compartments.

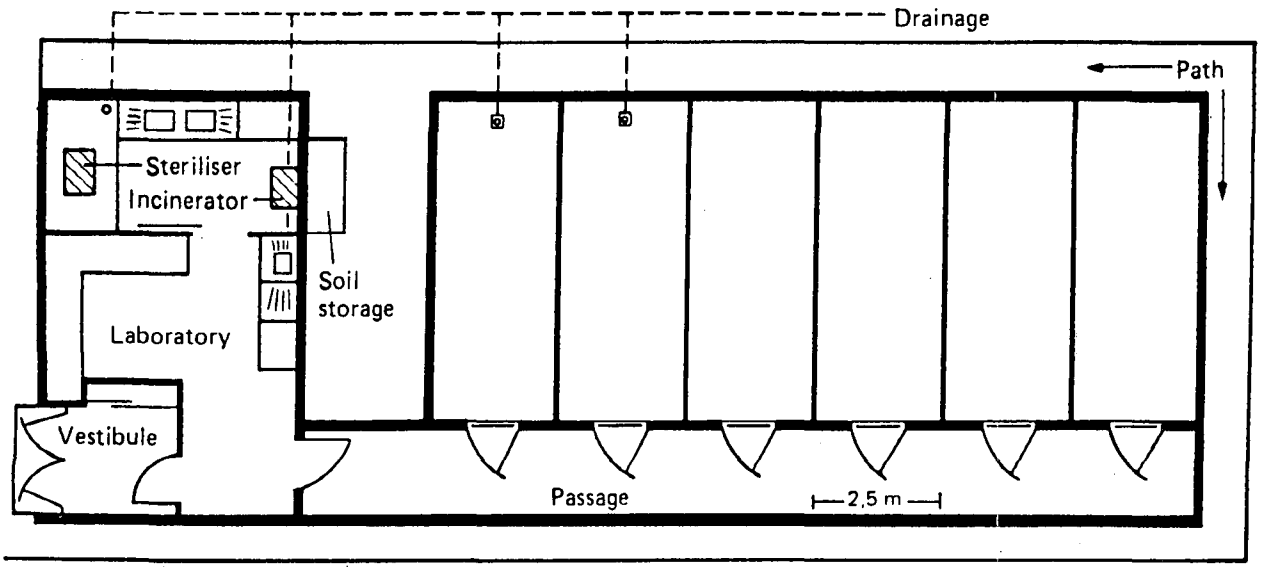

FIGURE 3 Floor plan of the quarantine building.

The glasshouse superstructure consists of an aluminium framework to which the glass is clamped onto rubber seals. The glazing is butt-jointed and all glass-to-glass, aluminium-to-aluminium, and framework-to-substructure joints are sealed with a flexible silicon sealing compound. All windows are non-opening and all doors seal onto rubber strips. Except for filtered vents (discussed later), the entire building is effectively airtight.

Each compartment (Figure 4) is fitted with a bank of 3 thermostatically controlled stainless steel tubular bar heaters (each 5 m long, total of 3 kW) and is individually cooled by means of a thermostatically controlled closed-circuit air-conditioner (cooling capacity of 4 000 kcal h^{-1}) mounted in the roof and linked to an exterior motor and compressor. The power supply to the entire building is controlled from a central distribution board located in the corridor. The total power supply to the building is 120 kW.

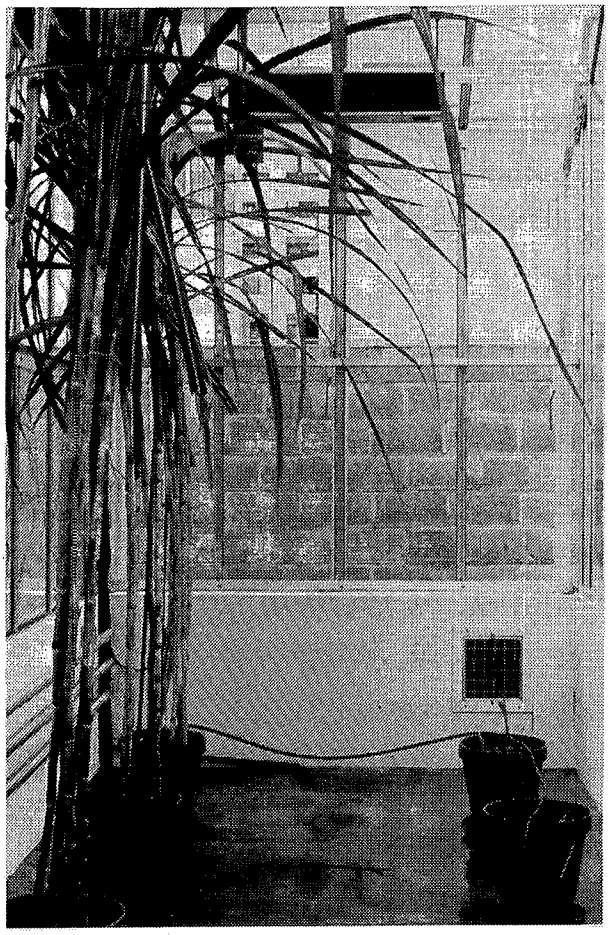

FIGURE 4 Interior view of a quarantine compartment, showing heaters, airconditioner, filter-protected vent, and automatic watering system.

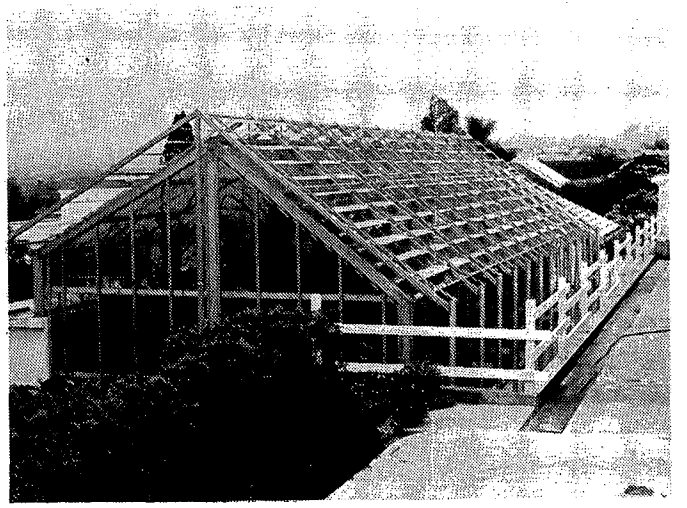

FIGURE 5 Shade louvres are mounted over the glasshouse compartments.

A system of adjustable, lever-operated louvres mounted over the compartments (Figure 5) provides up to 70% shade as a safeguard against overheating in the event of prolonged power failure or the breakdown of an airconditioner during hot weather. Because the compartments are cooled individually, the effect of any breakdown of equipment is limited.

All drainage and waste water from the compartments and laboratory is disposed of in a deep soak-pit via a sealed system of pipes and drains protected by water traps. All interior non-glass walls are either tiled or smoothly plastered and floors are finished with smooth grano-cement (compartments), vinyl (corridor and laboratory), or tile (sterilising room) to facilitate the maintenance of cleanliness.

The laboratory is equipped for the heat treatment of setts, the investigation of disease symptoms, and the isolation and culture of pathogens within the bounds of quarantine.

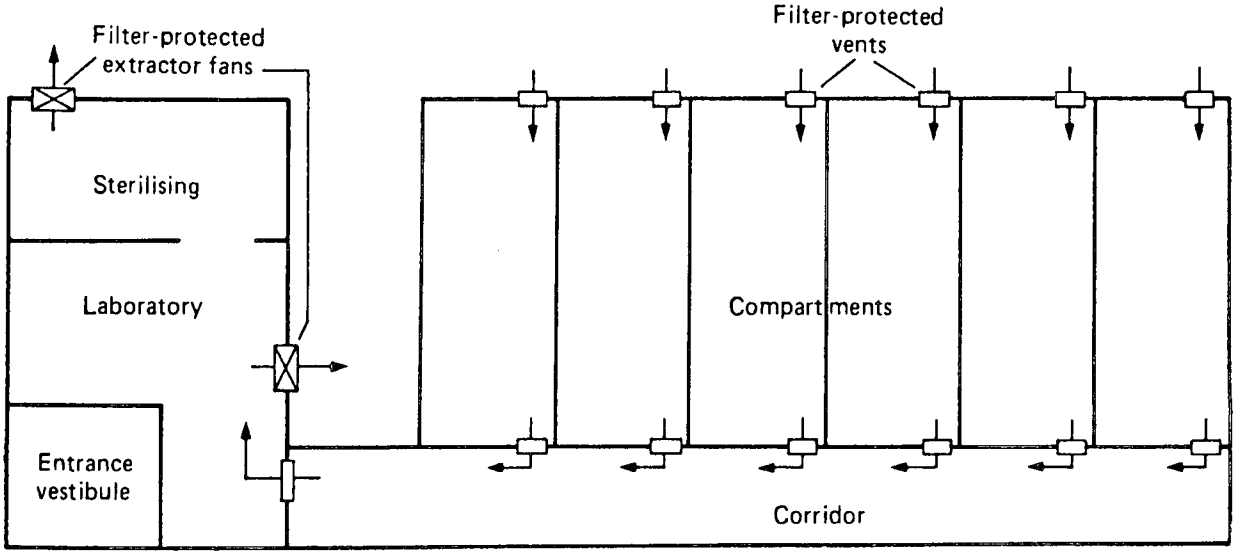

FIGURE 6 System for maintaining negative air pressure in quarantine. Air is drawn through filter-protected (5 μ) vents through the compartments, corridor, and laboratory by filter-protected extractor fans in the laboratory walls.

A novel feature of the design is the provision of a slightly negative air-pressure within the building (Figure 6). This is maintained by low-pressure, filter-protected (5 μ) extractor fans mounted in the exterior walls of the laboratory and drawing air through a system of filter-protected (5 μ) vents successively through the laboratory, corridor, and compartments. Fresh air enters each compartment via a filter-protected vent from the exterior. The purposes of this system are to prevent unfiltered air (possibly containing insects and pathogens) from entering the compartments when staff open doors, to prevent insects and pathogens escaping from the building in the event of glass being broken, and to provide ventilation.

The sterilising room is equipped with an electrically powered incinerator (3 kW, 33 ℓ capacity) for the disposal of plant material (the flue is capped by an air-tight cover when the incinerator is not operating), electrically powered steam sterilisers (80 ℓ capacity) for treating used soil, and deep sinks for cleaning pots. All soil is sterilised with methyl bromide before being brought into the building.

Because of the high humidity generated in the building, two 0,3 kW dehumidifiers are mounted in the corridor to dry exhaust air from the compartments. This has proved to be the most effective way of preventing microfungi growing on the corridor walls when moisture condenses on the cooler surfaces during cold weather.

As far as possible, watering is automated, using drip irrigation in each pot and controlled by a time-clock in each compartment. Nutrients are supplied in a proprietary hydroponics mixture (6,5% nitrogen, 2,7% phosphorus, 13% potassium, 7% calcium, 7,5% sulphur, 2,2% magnesium and iron, manganese, copper and molybdenum) applied at a rate of 1,0 g together with 1,0 g ammonium sulphate in 1 ℓ water per pot every 2 weeks. The temperature regime within the glasshouse compartments is maintained within a daily range of approximately 24°C minimum and 30°C maximum. This promotes good germination and rapid growth.

Quarantine procedures

National responsibility for the importation of live plant material into South Africa lies with the Directorate of Plant and Seed Control of the Department of Agriculture, Economics and Marketing. Technical responsibility for the importation of sugarcane is delegated to the South African Sugar Association Experiment Station by Plant and Seed Control, who authorise the procedures used. Good liaison exists between the Experiment Station and the governmental authorities, who are routinely kept informed of developments in quarantine.

The operating principle is that only sterilised soil and imported setts (sealed in the original packing) are brought into the building and, except for healthy setts for further propagation, all plant material produced in the glasshouse and all used soil is incinerated or sterilised and all equipment is disinfected within the quarantine area.

All staff working in the building and visitors are required to wear dustcoats (kept in the vestibule) and to clean their footwear in a disinfectant footbath on entering and leaving the vestibule. The compartments are fumigated between the times that they are occupied by different consignments of imported clones.

Upon arrival, parcels of imported sugarcane are unpacked in the laboratory of the quarantine building and all packing materials are incinerated. The setts are treated in water at 50°C for 30 min and are then soaked for 5 min in benomyl (375 ppm ai) or guazatine (800 ppm ai) to eliminate fungi and insects. The treated setts are planted in sterilised potting medium (bagasse-based compost, river sand, and vermiculite in proportions of 8:2:1) in 25 ℓ rubber pots. One clone is planted in each pot and up to 12 clones can be grown in one compartment. Different consignments of clones are grown in separate compartments. Subsequent procedures can be summarised as follows:

1. First planting grown for 6 to 8 months. Twice-weekly inspections for symptoms of diseases and other problems (Figure 7). Samples tested by immunofluorescence microscopy for RSD and leaf scald (local antisera) and by ELISA for SCMV (antisera reactive to strains A, B, D, K, H, I, M).

FIGURE 7 Inspecting imported varieties in quarantine.

2. Setts are cut, serially treated in hot water (30 min at 50°C, 24 h soak at room temperature, and 2 h at 50°C) and planted in sterilised medium.
3. The original planting is ratooned, inspected for disease symptoms, and destroyed once the new planting is established.
4. The second planting is grown for a further 6 to 8 months, with procedures as in (1) above. Provided no problems arise, setts from this planting are released from closed quarantine.
5. The released clones are propagated outdoors in 'open quarantine' plots at Mount Edgecombe for approximately 15 months. Effectively this is a closely supervised bulking-up stage, with the clones exposed to low levels of locally occurring pathogens. Setts from this planting are treated again in hot water (2 h at 50°C) before being distributed further.

Some use has been made of the transplant process (growing small plants from one budded setts in multi-cell trays before planting into the field) in order to obtain the maximum possible rate of propagation of new clones after release from quarantine.

Discussion and Conclusions

To date 99 clones from 6 countries have been released from the quarantine building since October 1984. The duration of quarantine for these clones has varied from 13 to 18 months, with an average of less than 15 months. At this rate, approximately 50 clones can be processed through quarantine in one year. Diseases detected in the clones imported so far include RSD, leaf scald, striate mosaic, and severe stunting (not diagnosed further).

Growing conditions in the glasshouse have proved to be excellent. The heating and cooling systems easily accommodate extremes of weather and allow approximately uniform conditions to be maintained throughout the year. The stalks of some clones exceed a height of 4 m within 6 months and this rapid rate of growth is the main contributory factor to the rapid throughput of imported material.

Although the condition of imported material on arrival varies greatly, almost all the clones imported so far germinated well and were successfully established. Germination of the setts treated serially in hot water after the first period of growth is generally excellent and all the clones treated so far have been successfully re-established after this treatment.

A noteworthy feature of operations since the opening of the building has been the complete absence of symptoms of foliar pathogens and the general absence of insects of all types. This demonstrates that the filtered ventilation system and security procedures in general are proof against the ingress of local pathogens and insects. It is also a good indication that the quarantine facilities and procedures provide effective isolation of individual consignments and are secure against the escape of any imported pathogens.

The new building has proved to be highly functional, providing secure quarantine and a rapid throughput of imported varieties with very few operational problems. The ability to supervise quarantine operations easily and on a daily basis has proved most useful, and experience to date has justified the decision to locate the quarantine building at the Experiment Station.

Acknowledgements

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REFERENCE

1. Thomson, GM (1959). Sugarcane quarantine in South Africa. *Proc S Afr Sug Technol Ass* 33: 90–94.