Current cocoa quarantine facilities of the University of Reading

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Intermediate cocoa quarantine facilities were established at Reading in 1987 to ensure maintained availability of cocoa germplasm after intermediate quarantine operations ceased at the Royal Botanic Gardens, Kew. Following recent expansion, operations are carried out in a single span and a three-span tunnel giving a total working area of approximately 480 m^2. The tunnels are covered with two layers of polyethylene to enable maintenance of high humidities and a nominal day temperature of 28°C and a night temperature of 19°C with a 12-hour thermoperiod under U.K. winter conditions. Temperatures are maintained by independently controlled oil-fired heaters and fan ventilators in each greenhouse section, with solid state controllers. Plants are grown in pots containing a completely inorganic medium of vermiculite, sand and gravel irrigated with a nutrient solution, the concentration and pH of which are controlled automatically. This reduces the incidence of nutrient disorders often encountered in conventional pot culture of cocoa. Quarantine procedures adopted at Reading follow guidelines laid down in the FAO/IBPGR Technical Guidelines for the Safe Movement of Cocoa Germplasm, although the quarantine period has now been extended to 24 months. Germplasm for quarantine is obtained initially as bud sticks and grafted on to West African Amelonado seedlings. Grafted material is used either for virus checking, where root stock and scion are examined regularly for evidence of virus transmission from the bud patch to the mother, or for clonal propagation. As well as checking for virus transmission, all material is checked regularly for pest and disease problems. The collection consists of approximately 150 clones from Costa Rica, Ecuador and Venezuela, and from the International Cocoa Genebank, Trinidad.

Introduction

Up until the early 1980's, intermediate cocoa quarantine facilities in the U.K. were provided by the Royal Botanic Gardens, Kew. However, in 1983 quarantine operations ceased at Kew and the collection of approximately 90 clones was transferred to the newly established cocoa growing facilities at the University of Reading. For a period after this transfer, budwood continued to be distributed from this original collection under the umbrella of the Royal Botanic Gardens, although no quarantine operations were carried out at Reading.

In 1987 the Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA) initiated quarantine operations at Reading. Under this arrangement funding for carrying out a quarantine operation was provided by cocoa producing countries wishing to receive cocoa germplasm. Funding is underwritten by BCCCA.

Facilities

Initially, quarantine operations began at Reading in a single span, twin-clad polyethylene covered tunnel (greenhouse). In 1989, the facilities were expanded with the construction of a similar twin-span tunnel which enabled the area of cocoa under quarantine to be increased from 120m^2 to 360m^2. This was expanded to a three-span tunnel in 1991, increasing the area to 480m^2. The double cladding enables high temperatures and humidities to be maintained even when external air temperatures fall below freezing. The tunnels utilize much of the technology developed during the construction of the main cocoa research glasshouse on the University Campus. Heating is provided by an oil-fired air heater which distributes warm air evenly around the tunnel through a perforated polyethylene tube. Cooling, when required, is

provided by a single fan at the end of each house which draws air through a vent at the opposite end of the house. Heating and cooling are maintained by solid state controllers to provide a nominal day temperature of 28°C and a night temperature of 19°C, with a 12-hour thermoperiod. Relative humidity is usually maintained between 75% and 85%. Fans, vents and doors are all netted to provide insect proofing. Plants in pots are placed either at ground level in the case of the collections or on benches positioned about 30 cm above the floor of the house in the case of material passing through quarantine.

Cocoa is sensitive to nutrient disorders and does not grow well for long in conventional potting composts. For this reason an advanced growing system has been developed to ensure optimal growing conditions. Briefly, the cocoa is grown hydroponically in a free draining inorganic growing medium containing sand, gravel and vermiculite which is watered frequently (up to 9 times per day) with a defined nutrient solution through a drip irrigation system. Nutrient concentration and pH are maintained by a microprocessor controller, as are all irrigation operations. The system is entirely automatic and requires only periodic replenishment of the acid and nutrient stock solutions and checks of drip lines for blockages.

Quarantine Procedures

The quarantine procedure adopted at Reading follows the guidelines outlined by Thresh (1960) and more recently by Frison and Feliu (1989). The procedure is based on experience gained with cocoa swollen shoot virus (CSSV) and other viruses of cocoa in West Africa. New molecular and serological detection methods are currently under development at a number of centres and it is hoped that additional tests can be incorporated as techniques become available.

Material for quarantine is obtained as bud sticks with phytosanitory certificates. On arrival the material is inspected by Ministry of Agriculture, Fisheries and Food plant health inspectors for presence of pests and disease and any material found to be contaminated is destroyed. The material is first budded on to approximately 3 month old seedlings of the variety West African Amelonado (this variety should be distinguished from other varieties called Amelonado). When infected with CSSV, West African Amelonado develops conspicuous leaf and stem symptoms and these are well documented (Thresh, 1960 and many other publications).

A widely-used patch bud method of grafting is used. A small rectangular piece of budwood containing a dormant bud is grafted on to a seedling below the cotyledonary node and secured with grafting tape. The tape is removed after approximately 14 days, by which time it is usually apparent whether or not the graft has formed a successful union. Successfully grafted plants are divided into two groups. The first group is regularly inspected for virus transmission from the bud patch to the mother plant by regular observation over a period of approximately 24 months. The other group is used for propagation and, for these plants, the shoot of the mother plant is removed. As well as checking for virus transmission, all the material is checked regularly for pest and disease problems. Since the majority of the material in quarantine is of South American origin, the main disease threat is Witches' Broom disease (*Crinnipellis perniciosa*). Any material suspected of being infected (either with virus or fungus) is immediately destroyed by incinerating the affected plants and any derived from them. Clones in the propagation, quarantine and maintenance phases of the operation are kept in different areas.

In addition to regular inspection by Reading staff, all material within the facilities is inspected approximately once every 6 months by a virologist and a pathologist, authorities in detecting cocoa virus and fungal pathogens.

Dispatch of Material

Budwood is normally supplied as 2-3 budsticks, 7-10 mm in diameter, each with 3-5 buds. An import permit from the destination country is required before material can be dispatched. Treatment of budsticks prior to dispatch varies and depends on the phytosanitary requirements stated on the import permit, but includes dipping in insecticide and fungicide solutions. Each consignment of budwood is examined by inspectors of the Plant Health Division of the Ministry of Agriculture, Fisheries and Food, who then supply a Phytosanitary Certificate, which will accompany the budwood together with a quarantine certificate from the University of Reading. The ends of the budsticks are then coated in paraffin wax, wrapped in damp paper towels and placed in a polythene bag to aid retention of moisture during transit.

Quarantine Management Committee

The operation and management of the Reading Quarantine Facility is overseen by a committee which meets regularly to advise on quarantine operations. Members of the Committee include a virologist, a pathologist, a breeder and representatives from the Plant Health Division of MAFF, BCCCA and a chairman (currently Mr. R.A. Lass, Cadbury Ltd.).

Health Problems Observed

Fungal pathogens have only rarely been observed during the quarantine operation. The only incident of note has been the presence of *Verticillium*, insects, usually mealy bugs, have been occasionally observed on imported budwood on a single clone. Galls have occassionaly been noted but it has been concluded that this has been due to local infection from soil-borne bacteria. In all cases, the infected clones were destroyed.

Viral symptoms were noted in a clone of West African origin after 23 months in quarantine. Following this, our quarantine procedures were altered to include a 24-month observation period.

Swellings accompanied by death of the apical bud have been observed in one or two clones originating from Guyana. These were not accompanied by leaf symptoms and did not give a positive reaction in serological tests for CSSV.

Even with the nutrient supply system, nutrient deficiencies can occur and can interfere with virus detection procedures.

Conclusion

The facilities currently house a collection of some 150 clones which have passed through intermediate quarantine. These contain representatives from ICG,T in Trinidad, CATIE in Costa Rica, Pichulinque in Equador and Venezuela. Selection of clones for quarantine is based primarily on requests from breeding and improvement projects although material may also enter through specific recommendations from curators of genebanks. The collection is also used for research purposes by other institutions both in the U.K. and abroad and clones may enter the collection as a result of specific requests from research workers. The collection is not permanent and individual clones can be deleted when transfer has been completed.

Intermediate quarantine of cocoa germplasm is a vital step in the safe movement of material from germplasm collections to producing countries and can only be carried out if reliable procedures are employed. At present these techniques are time consuming. When combined with post-entry quarantine procedures approximately five years can elapse between the original request and material becoming available to a breeding programme. However, modern molecular techniques for virus detection hold the prospect of not only improving the reliability of quarantine measures but also in sigificantly reducing the quarantine period that is currently employed. The development of such methods is urgently required.

References

Frison, E.A. and Feliu, E. (Eds.) (1989) *FAO/IBPGR Technical Guidelines for the Safe Movement of Cocoa Germplasm.* Food and Agricultural Organisation of the United Nations, International Board for Plant Genetic Resources, Rome

Thresh, J.M. (1960) Quarantine arrangements for intercepting cocoa material infected with West African viruses. *FAO Plant Prot. Bull.* **8** 89-92