THE UNIVERSITY OF READING QUARANTINE PROJECT

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INTRODUCTION

In little more than two centuries, the commercial growing of cocoa has extended from its centre of origin in South America to West Africa, the Far East and Oceania. Now, cocoa has become an important crop throughout the Humid Tropics. However, much of the material that has been used for commercial plantings in each of these areas has been drawn from a very narrow genetic base. It has long been recognised that there is a need to improve the genetic diversity of material available to cocoa breeders and this has led to scientific expeditions to collect wild cocoa over the last 50 years (Soria, 1970; Allen & Lass, 1983, 1987; Almeida et al., 1987). Much of the material collected in these expeditions is now maintained in national and international germplasm collections in Central and South America, and in the Caribbean (Table 1). However, because these collections are situated in cocoa producing areas, it is not appropriate to transfer this material directly for use elsewhere. This is because of the risk that pests and particularly diseases, that are endemic to cocoa in South America will be transferred to other areas which are presently free of such problems. The principal

TABLE 1: NATIONAL AND INTERNATIONAL GERMPLASM COLLECTIONS IN CENTRAL AMERICA, SOUTH AMERICA, AND THE CARIBBEAN MAINTAINING PRIMITIVE COCOA GENOTYPES COLLECTED FROM THE WILD

STATION	LOCATION	COUNTRY
Centro Agronomica Tropical de Investigacion y Ensenanza (CATIE)	Turrialba	Costa Rica
Departamento Especial da Amazonia, CEPLAC	Belem	Brazil
Instituto Colombiano Agropecuaria	Palmira	Colombia
Estacion Experimental Tropical	Pichilingue	Ecuador
International Cocoa Genebank, Trinidad (ICG,T) at University of West Indies	St Augustine	Trinidad
IRCC	Sinnamary	French Guiana
U.S.D.A. Agricultural Research Station	Mayaguez	Puerto Rico

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threat comes from witches' broom disease (caused by Crinipellis perniciosa) which occurs only in South and Central America. The risk of the spread of this disease is well illustrated by its recent identification from cocoa plantings in the State of Bahia.

It should be noted that the collections at CATIE and ICC,T have been designated as primary collections by the International Board for Plant Genetic Resources (IBPGR) and the germplasm from these centres is therefore freely available to all who request it.

Similar threats also arise when cocoa is transferred between newer regions of cocoa cultivation. For example, authentic Cocoa Swollen Shoot Virus is only known to be present in West Africa and vascular streak dieback caused by (Oncobasidium theobromae) is only found in the Far East. For these reasons, cocoa germplasm should not be transferred from one cocoa producing region to another unless the material first passes through a period of intermediate quarantine in a non-cocoa producing country where the material can be safely screened for pests and diseases (Frison and Feliu, 1989).

In recent years, the major intermediate cocoa quarantine centre was at the USDA Sub-Tropical Research Station at Miami, Florida. However, over the last two and a half years a new quarantine facility has been established at the University of Reading, UK to increase the availability of cocoa germplasm. The Department of Horticulture at Reading University has had a large cocoa research programme underway for some years and as part of its work it had maintained a collection of cocoa clones, which had been previously held at the Royal Botanic Gardens, Kew until cocoa quarantine activity there ceased some years ago.

GROWING CONDITIONS AT READING

The first phase of the project began in Spring, 1987 and was devoted to establishing the growing facilities required. These are located at the School of Plant Sciences Field Unit, about 5km from the main University of Reading Campus which is 35km from Heathrow airport, London. Initially, a tunnel covered with polyethylene having a floor area of approximately 100m^2 was used to house the cocoa. This has now been expanded to two tunnels. This utilised much of the technology developed earlier during the construction of the main cocoa research glasshouse on the University Campus. To enable both high temperatures and high humidities to be maintained, even when external air temperatures fall below freezing, the house is covered with two layers of a novel polyethylene film (Thermofilm IR). This film contains an infra-red filter to increase its insulating properties whilst retaining a high transmittance to visible light. The two layers of film are separated by an air gap which is maintained by blowing air between the two plastic films using an internal fan. The inner skin is warmer than the outer due to the insulating effect of the air gap and this reduces condensation, allowing high humidities to be maintained even under very cold external conditions. Heating is provided by an oil-fired air heater which distributes warm air evenly around the tunnels through a perforated polyethylene pipe. This air heater has been modified so that its main fan is switched on continuously to ensure that the air within

within the tunnels is uniformly stirred. Cooling, when required, is provided by a single fan at one end. When this is turned on, cool air from outside is drawn through vents at the opposite end of the tunnels. Heating and cooling are controlled by solid state controllers. A high pressure misting system provides additional humidification as required. The houses are maintained at 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% (see Figure 1 for layout of facility).

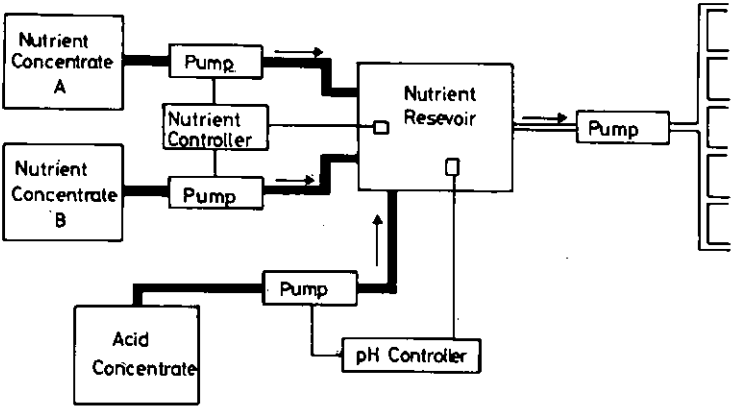

FIGURE 1: Schematic Diagram of Nutrient Control & Distribution System of Cocoa Quarantine Glasshouse at Reading University

Plants are arranged on benches positioned about 30cm above the floor of the houses. Cocoa is very sensitive to nutrient disorders and does not grow well for long in conventional potting composts. This is because nutrient imbalances build up quickly and soon affect plant growth. At Reading, the problem has been overcome by growing plants in a completely inorganic medium of vermiculite, sand and gravel, which is irrigated with a modified "Long Ashton" nutrient solution. The growing medium is free-draining so that frequent irrigation is possible without any danger of waterlogging, enabling unused salts to be washed out of the rooting medium with the drainage from each pot. The nutrient solution is held in a recirculating central tank (Figure 1) which is fed with tap water through a float valve. When required, nutrient solution is pumped through a network of drip lines enabling each pot to be irrigated

with the same volume of solution. The timing and duration of each irrigation is controlled by a small microprocessor controller. As nutrient is pumped out of the central tank, it is replaced with tap water. A conductivity probe immersed in this central tank is connected to a controller which pumps concentrated nutrient from two stock tanks to maintain nutrient concentration within the central tank.

It is very important to maintain the medium at around pH 5.8-6.5 since either too high or too low a pH can also lead to nutrient imbalances due to certain elements being rendered unavailable under these conditions. To achieve this, a probe senses the pH of the nutrient solution. This is connected to a controller which pumps a mixture of dilute nitric and phosphoric acids into the nutrient solution when the pH rises to maintain the slightly acid conditions required for optimal growth.

The system is set to irrigate the plants on up to seven occasions per day depending on the season (fewer irrigations are required during the winter months). The system has the added advantage that it is automatic and requires only periodic replacement of the acid and nutrient stock solutions and checks of drip lines for blockages (see Figure 2 for schematic diagram of nutrient control and distribution system).

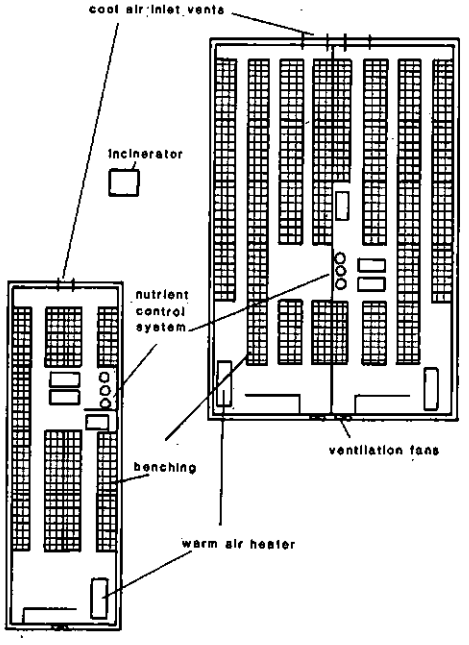

FIGURE 2: Schematic Diagram of Cocoa Quarantine Glasshouse at Reading University

QUARANTINE PROCEDURES

The quarantine procedure adopted at Reading follows the guidelines outlined by Thresh (1960) and more recently by Frison and Feliu (1989). In the absence of any information on the occurrence of cocoa viruses in South America, the procedure is based on experience gained with swollen shoot and other viruses of cocoa in West Africa. Additional tests may become necessary as further information becomes available or as new methods of virus detection are introduced.

Suitable germplasm for quarantine is obtained initially as budsticks, the condition of which can vary enormously on arrival. Factors influencing condition include the time spent in transit, the conditions of the donor tree and the treatment of the budwood whilst in transit. On receipt, material is first budded onto approximately 3-month-old seedlings of the variety West African Amelonado. This variety should be distinguished from others also referred to as Amelonado. When infected with Cocoa Swollen Shoot Virus (CSSV) it develops conspicuous leaf and stem symptoms and these are well documented (Thresh, 1960 and many other publications).

A patch bud method of budding has been adopted which is widely used for cocoa propagation. A small rectangular piece of budwood containing a dormant bud is grafted onto a seedling where an approximately equivalent sized piece has been removed from below the cotyledonary node. The patch is then secured with grafting tape. This is removed after approximately 14 days when it is usually apparent which grafts have formed a good union. Provided sufficient material is available, the successfully grafted plants are divided into two groups. The first group is retained for quarantine procedures. Here evidence of virus transmission from the bud patch to the mother plant is checked by regular observations of root stock and sion for symptoms over a period of approximately 18 months. The other group is used for propagation and for these plants, the shoot of the mother plant is removed. The latter procedure may surprise many who are used to gradual cutting and bending of the shoot to encourage the development of the bud before the shoot is finally removed when the bud is growing vigorously. However, immediate removal of the shoot appears to work effectively, at least under Reading conditions, and the bud usually grows out rapidly. As well as checking for virus transmission all the material is checked regularly for pest and other disease problems. Since the majority of the material in quarantine is of South American origin, the main potential disease threat comes from witches' broom disease. Any material suspected of being infected (either with virus or fungus) is immediately destroyed by incinerating the affected plants and any derived from them.

Where difficulty has been experienced in grafting material, priority is given to propagation rather than quarantine tests. The latter are then begun later once the propagated material can itself be a source of budwood for grafts to West African Amelonado. This normally takes between 8 - 10 months.

Clonal material that has passed through quarantine can be supplied

as budwood. Material is freely available to those who contribute to the funding of the quarantine work, which is coordinated by the Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA). The contact address is BCCCA, 11 Green Street, London W1, Telephone: 01.629.8971, Telex: 24738, Fax: 01.493.4885. The consignee is expected to meet the transport costs. Budwood is normally supplied as 2-3 budsticks, each of 7-10 mm in diameter and containing 3-5 buds. An import permit from the destination country is required before material can be dispatched. Treatment of the budsticks prior to dispatch varies and depends on the phytosanitary requirements stated on the import permit, but includes dipping in an insecticide and a fungicide solution. Each consignment of budwood is examined by inspectors of the Plant Health Division of the Ministry of Agriculture, Fisheries and Food of the UK 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.

Cocoa budwood is valuable fragile material, which needs to be transported as quickly as possible. Although material has been sent successfully via air freight, it is preferable to transport it via personal couriers. This avoids problems such as exposure to adverse temperatures and the delays at customs likely to arise during unaccompanied transit.

Since the beginning of the Reading project the size of the cocoa collection has increased to a current figure of some 150 clones. The rapid increase in the size of the collection has led to an accommodation problem in arranging both propagation and quarantine. Hence, a new facility consisting of a twin span, polythene-clad houses with a total floor area of 300m^2 has been commissioned recently. This is separated into two compartments and uses the same heating, ventilation and nutrient/irrigation control systems described above. The new house will form the main area for maintaining the collection whilst plant propagation and quarantine procedures are carried out in the smaller of the two houses (Figure 2).

Intermediate quarantine of cocoa germplasm is a key step in the safe movement of the genetic material required for crop improvement programmes. Without such procedures to ensure that only healthy material is distributed, cocoa production is threatened by the inadvertent introduction of novel pests and diseases. The object of the quarantine facilities at Reading is to provide an essential step in the global distribution of cocoa germplasm. It is not, however, concerned directly with the conservation of cocoa genetic resources. This latter role is taken up by both national and international germplasm collections (Table 1).

A list of clones available at Reading can be obtained from the authors at the address above. Requests are welcome from any group interested in receiving material from Reading and material can be made available once suitable financial arrangements have been agreed.

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