2017 International Symposium on Cocoa Research (ISCR), Lima, Peru, 13-17 November 2017

Safe Movement of Cocoa Germplasm

A.J. Daymond[1], M.J. End[2], C.J. Turnbull[1], A.C. Wetten[3] and P. Hadley[1]

[1]School of Agriculture, Policy and Development, the University of Reading, Whiteknights, Reading, UK, RG6 6AR

[2]Cocoa Research Association Ltd.

[3]Department of Applied Sciences, University of the West of England, Coldharbour Lane, Bristol, UK, BS16 1QY

Abstract

In order to access sufficient genetic diversity for cocoa breeding it is often necessary to import new genotypes. However, it is vital that such movement takes place within a quarantine framework to avoid introduction of pests and diseases. Safe movement procedures for cocoa germplasm are particularly important as many pests and pathogens of cocoa are confined to particular geographical regions.

The International Cocoa Quarantine Centre at the University of Reading (ICQC,R) is the main hub for global movement of cocoa germplasm. The centre has been in operation since 1985 and has worked closely with the international genebanks to provide a safe transit route for germplasm to institutes working on cocoa in over thirty different countries. Rigorous testing procedures include virus indexing through a combination of graft testing and laboratory screening using a suite of PCR primers. Approximately 400 accessions are currently held at the ICQC,R, with current lists and further information available on the centre's website (www.icgd.reading.ac.uk/icqc/), which has links to the International Cocoa Germplasm Database (ICGD) enabling recipients of material to access associated data.

The *Technical Guidelines for the Safe Movement of Cacao Germplasm* document, based on the original FAO/IPGRI Technical Guidelines, was updated extensively in 2010 by the CacaoNet Safe Movement Working Group to take into account a broader range of pest and disease threats. Subsequent revisions were made in 2014 and 2017. The guidelines serve as a reference point for the cocoa community and include information on the geographical spread of pests and diseases and sub-sections on particular pests and diseases that have been written by experts within those fields. Information includes physical symptoms, biology of the pathogen or insect pest and recommended quarantine measures.

The guidelines have been published on-line and in physical form in English, French and Spanish under the umbrella of CacaoNet and will continue to be updated to incorporate new knowledge as it becomes available.

Introduction

In order to develop improved varieties, cocoa researchers and breeders require access to a broad range of genetically diverse cocoa germplasm, with desirable agronomic traits such as high yield potential and resistance to particular diseases. Since a large proportion of cocoa production takes place away from the centre of origin of cocoa, it is often necessary for researchers engaged in cocoa improvement programmes to import cocoa germplasm. However, such movement of plant material carries a concurrent risk of inadvertent spread of pests and diseases. This risk is particularly significant given the fact that many pests and diseases of cocoa are confined to particular geographical regions. For example, the fungal disease frosty pod rot (caused by *Moniliophthora roreri*) is only found in parts of the Americas whilst the insect pest, cocoa pod borer (*Conopomorpha cramerella*) is confined to large parts of South-East Asia. Specific incidences of the spread of cocoa diseases, such as witches' broom into the Brazilian state of Bahia (Pereira *et al*., 1989), frosty pod rot into Mexico (Phillips-Mora *et al*., 2006) and recently into Jamaica (Johnson *et al*., 2017) and the movement of cocoa pod borer into Papua New Guinea (Yen *et al*., 2010) are likely to have been a result of human movement of cocoa planting material. It is therefore vital that researchers and others involved in breeding and dissemination of cocoa planting material are aware of the risks involved with plant movement and are taking adequate precautions. For example, all movement of cocoa vegetative material between countries/ regions should take place via an intermediate quarantine centre. The principal route for such intermediate quarantine is the International Cocoa Quarantine Centre, at the University of Reading (ICQC,R), whose operation is described here. We also describe safe movement guidelines for cocoa germplasm, which provide technical procedures and information to minimise the risk of the introduction of pests or diseases with movement of germplasm.

The International Cocoa Quarantine Centre

The International Cocoa Quarantine Centre at the University of Reading (ICQC,R) is the principal hub for the international movement of cocoa germplasm. The Centre has been in operation since 1985, when it acquired a number of accessions from the Royal Botanic Gardens, Kew, UK, which previously had a responsibility for quarantine and distribution of cocoa germplasm. The location of the ICQC,R in a temperate country is important as there are no endemic pests and diseases of cocoa that can enter the facility from the environs (it is a requirement of some cocoa-producing countries that when they receive germplasm from abroad it should have passed through quarantine in a temperate country). The facility has expanded over time and currently includes a 1000m^2 compartmentalised greenhouse in which tropical conditions are maintained. Plants within the facility are grown in an inert, soil free medium and fed with a nutrient solution. This system ensures optimum growth and minimises the chance of pests establishing within the growth medium.

Germplasm is received into the ICQC,R with particular desirable characteristics, such as resistance to diseases, high yield potential and bean quality traits. The majority of germplasm is received from the two international genebanks in Trinidad (ICG,T) (Iwaro *et al*., 2003) and Costa Rica, although other centres also periodically provide germplasm. Material is usually provided as budwood (although it has also been received as micrografted plants and as flower buds, the latter to be used to generate clonal plants via somatic embryogenesis) and is accompanied by a Phytosanitary Certificate and a Standard Material Transfer Agreement (effectively ensuring that it remains in the public domain). After receipt, the plant material is initially inspected in the laboratory for signs of insects, eggs or fungal spores, before being established in an insect-proof cage in a post-entry greenhouse compartment. Established material is indexed for viruses using two procedures, a PCR-based technique and visual virus indexing. For the former, leaf samples are taken from an actively growing flush on a recently established, imported plant. These are then tested in the laboratory using a suite of virus-specific PCR probes. Since PCR-based methods do not currently detect all viruses of cocoa, a visual indexing test is also utilised (Thresh, 1960). For this, budwood is taken from a recently imported established accession and buds are grafted onto seedlings of the Amelonado variety, which shows clear symptoms in the flush leaves when it is infected with viruses such as *Cacao swollen shoot virus*. A minimum of three such indicator plants are produced. Once the buds have formed a union with the Amelonado seedlings, the mother plant and indicator plants are moved to a greenhouse compartment specifically used for the purpose of virus indexing. Here, indicator plants are inspected on a weekly basis for characteristic leaf symptoms and stem swellings. The procedure currently continues for two years, as some viruses can remain latent for a long period of time. In the event that viral or other diseases symptoms are observed, the mother plant, along with the corresponding indicator plants are destroyed. After the two-year quarantine period has been completed for a given accession, and if it has a clean quarantine record, the mother plant is moved to the greenhouse compartment of the ICQC,R that houses the post-quarantine collection (see Figure 1 for a summary of the quarantine process).

In response to requests from institutes engaged in cocoa breeding and research, plant material is periodically exported from the ICQC, R, typically in the form of budwood. To facilitate such an export, an import permit must be provided by a relevant body in the importing country and the material is exported with a phytosanitary certificate (provided by the UK Plant and Animal Health Authority) and a Standard Material Transfer Agreement (SMTA) to ensure that it remains within the public domain. To date, cocoa germplasm has been provided to research institutes and cocoa growers in over 30 different countries, including all of the major cocoa-growing countries as well as smaller producers. The inventory of cocoa germplasm (available material and in quarantine) held at the ICQC,R is continuously updated on-line at www.icgd.reading.ac.uk/icqc/. The inventory has links to the International Cocoa Germplasm Database (ICGD) enabling users to assess information on germplasm such as its reported level of resistance to diseases and yield potential. The genetic fingerprints of the material held in ICQC,R are also maintained on the ICGD website.

Quality control measures within the ICQC,R include twice-yearly inspections by expert consultants in pathology, virology and entomology, who report to a Quarantine Advisory Board. The Board also reviews the activities of the ICQC, R and considers any necessary amendments to the *modus operandi* of the facility.

Safe Movement Guidelines

Technical Guidelines for the Safe movement of cacao germplasm were originally published by the FAO/IPGRI (Frison *et al*., 1999). A major revision of the guidelines took place in 2010 to incorporate advances in knowledge of pests and diseases, advances in detection techniques and to provide information on a greater range of pests and diseases. This update took place under the umbrella of the CacaoNet initiative (the Global Cacao Genetic Resources Network) thanks to the efforts of the CacaoNet Safe Movement Working Group. Subsequent more minor revisions were conducted in 2014 and 2017 (End *et al*. 2017).

The guidelines provide technical procedures and information to minimise the risk of the introduction of pests or diseases with movement of germplasm in connection with research, crop improvement/ breeding, collection of germplasm from the wild, or movement into genebanks for the purpose of conservation. General recommendations are provided regarding the movement of cocoa germplasm as well as current information on the distribution of particular pests and diseases. The relative risk associated with movement of different plant parts is also outlined. A description is then given of a range of key pests and diseases of phytosanitary concern, each authored by experts within the respective field, that includes the symptoms of the pest/ disease, its biology and particular quarantine measures required. The current edition includes sections on viral diseases (*Cacao necrosis virus*, *Cacao swollen shoot virus*), fungal and oomycete diseases (witches' broom disease, *Moniliophthora* pod rot, *Phytophthora* spp., Vascular Streak Die-back, *Verticillium* wilt of cacao, *Ceratocystis* wilt of cacao, *Rosellinia* root rot) and insect pests (mirids, cocoa pod borer, mealybugs, husk miners) and parasitic nematodes.

The Technical Guidelines for the Safe Movement of Cacao Germplasm are published online at http://www.cacaonet.org/ and are currently available in English, French and Spanish. They will continue to be updated regularly to incorporate new knowledge of pests and diseases and changes in their distribution.

Conclusions

Movement of cocoa germplasm is often a necessary step in breeding for new varieties and ultimately in providing new planting materials in farmers' fields. Quarantine procedures and facilities provide the necessary route for such movement to take place safely. Given the recent spread of particular diseases there is a continual need to raise awareness of risks associated with movement of cocoa germplasm and the consequent need for quarantine measures.

Acknowledgements

The International Cocoa Quarantine Centre is funded by the Cocoa Research Association (CRA- a UK-based non-profit scientific research organisation that is supported by Mars Chocolate, Mondelez International and ICE Futures Europe) and by the United States Department of Agriculture (USDA). The updating of the Technical Guidelines for Safe Movement of Cacao Germplasm was carried out as part of the CacaoNet initiative and the document has been published by Bioversity International. We thank all those organisations and individuals who contributed to this process through the CacaoNet Safe Movement Working Group.

References

End, M.J., Daymond, A.J. and Hadley, P. (Eds). 2017. Technical Guidelines for the Safe Movement of Cacao Germplasm. Revised from the FAO/IPGRI Technical Guidelines No. 20 (Third update, October 2017). Global Cacao Genetic Resources Network (CacaoNet), Bioversity International, Montpellier. http://www.cacaonet.org/

Frison, E.A., Diekmann, M. and Nowell, D. (Eds). 1999. FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm. No. 20. Cacao (1st revision). Food and Agriculture Organization of the United Nations, Rome/International Plant Genetic Resources Institute, Rome.

Iwaro, A.D., Bekele, F.L. and Butler, D.R. 2003. Evaluation and utilisation of cacao (*Theobroma cacao* L.) genotypes at the International Cocoa Genebank, Trinidad. Euphytica, 130, 207-21.

Johnson, E.S., Rutherford, M.A., Edgington, S., Flood, J., Crozier, J., Cafá, G., Buddie, A.G., Offord, L., Elliott, S.M. and Christie, K.V. 2017. First report of *Moniliophthora roreri* causing frosty pod rot on *Theobroma cacao* in Jamaica. New Disease Reports 36, 2. http://dx.doi.org/10.5197/j.2044-0588.2017.036.002.

Pereira, J. L., Ram, A., Figueredo, J. M. and Almeida, L. C. C. 1989. Primeira ocorrência de vassoura-de-bruxa na principal região produtora de cacao de Brasil. Agrotrópica, 1, 79–81.

Phillips-Mora, W., Coutiño, A., Ortiz, C. F., Lopez, A. P., Hernández, J. and Aime, M. C. 2006. First report of *Moniliophthora roreri* causing frosty pod rot (Moniliasis disease) of cocoa in Mexico. Plant Pathology, 55, 584.

Thresh, J. M. 1960. Quarantine arrangements for intercepting cocoa material infected with West African viruses. FAO Plant Protection Bulletin, 8, 89–92.

Yen, D.L., Water, E.K. and Hamilton, A.J. 2010. Cocoa pod borer (*Conopomorpha cramerella* Snellen) in Papua New Guinea: Biosecurity models for New Ireland and the Autonomous Region of Bougainville. Risk Analysis 30, 293- 309.

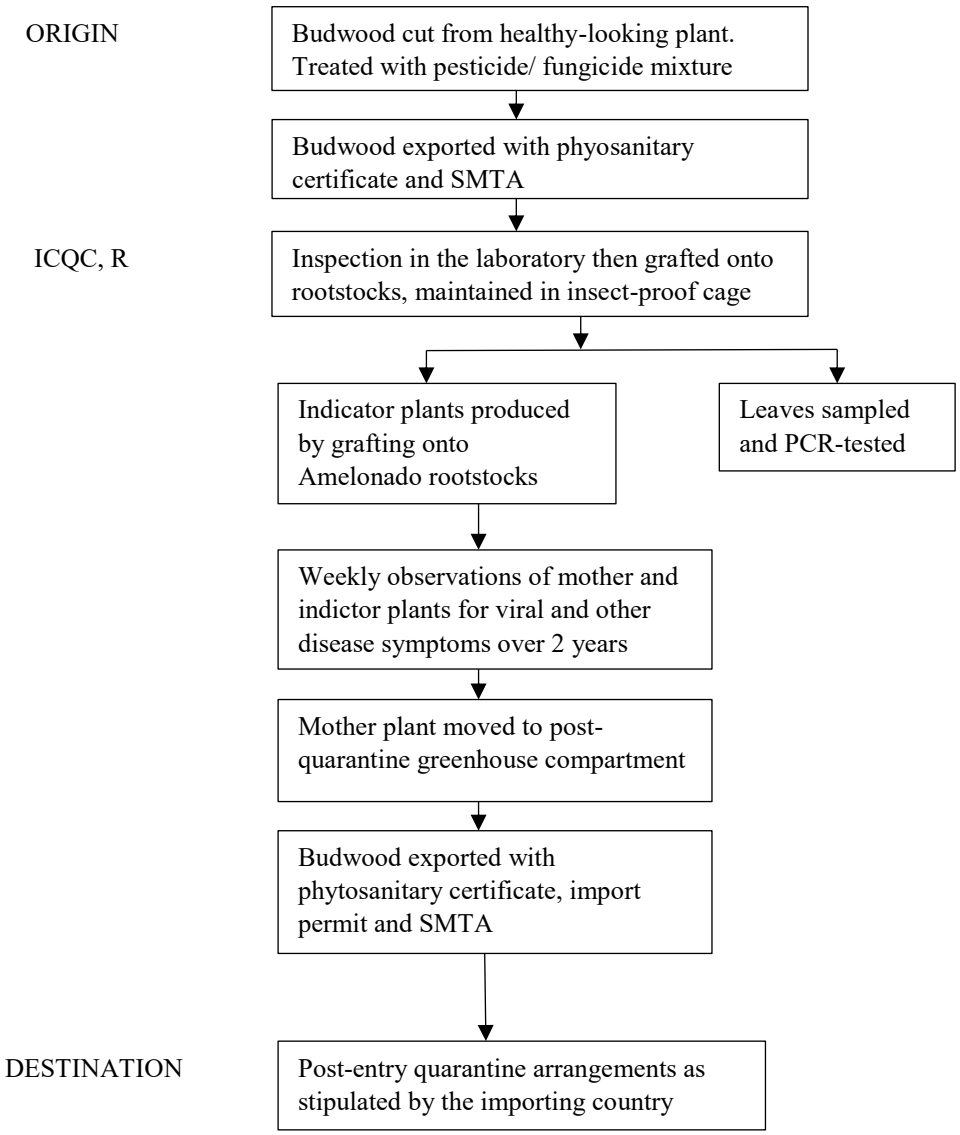

Figure 1: Summary of quarantine procedures at the International Cocoa Quarantine Centre, Reading (ICQC, R)