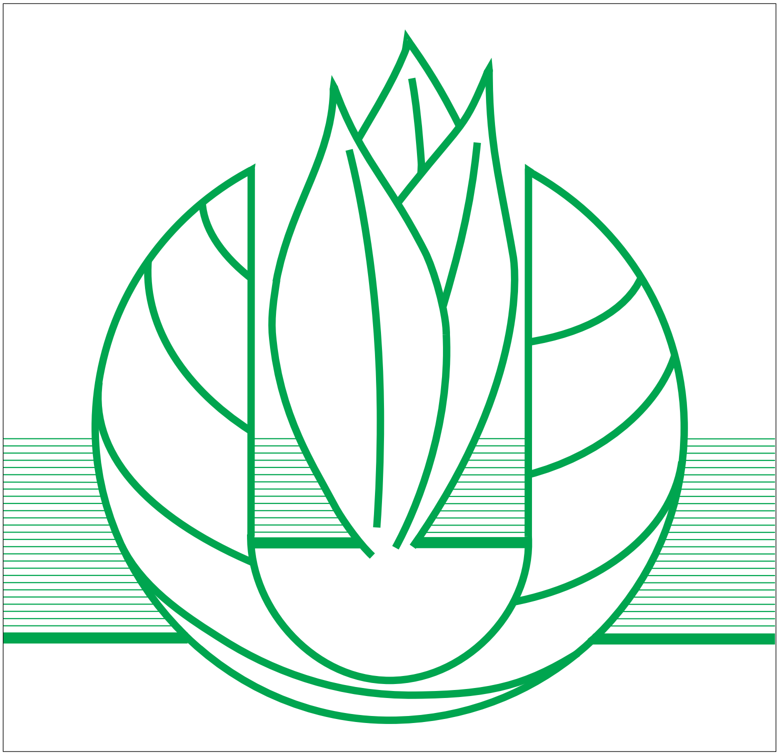
FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm No. 20

Cacao

edited by **E.A. Frison, M. Diekman** *and* **D. Nowell**



FAO FIAT PANIS

International Plant Genetic Resources Institute IPGRI

in collaboration with
the American Cocoa Research Institute

Previously published Technical Guidelines for the Safe Movement of Germplasm

These guidelines describe technical procedures that minimize the rist of pest introductions with movement of germplasm for research, crop improvement, plant breeding, exploration or conservation. The recommendations and information in these guidelines are intended for germplasm for research, conservation and plant breeding programmes. Recommendations for commercial consignments are not the objective of these guidelines.

Cocoa	1989
Edible aroids	1989
Musa (1st edition)	1989
Sweet potato	1989
Yam	1989
Legumes	1990
Cassava	1991
Citrus	1991
Grapevine	1991
Vanilla	1991
Coconut	1993
Sugarcane	1993
Small fruits (*Fragaria, Ribes, Rubus, Vaccinium)*	1994
Musa spp. (2nd edition)	1996
Stone Fruits	1996
Eucalyptus spp.	1996
Allium	1998
Potato	1998

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INTRODUCTION

Collecting, conservation and utilization of plant genetic resources and their global distribution are essential components of international crop improvement programmes.

Inevitably, the movement of germplasm involves a risk of accidentally introducing plant pests[1] along with the host plant. In particular, pathogens that are often symptomless, such as viruses, pose a special risk. To manage this risk, effective testing or indexing procedures are required to ensure that distributed material is free of pests that are of concern.

The exchange of germplasm internationally for research, conservation and plant breeding purposes requires specific information relating to the phytosanitary safety of germplasm transfer. As the depository of the International Plant Protection Convention (IPPC), FAO collaborates with IPGRI in programmes reflecting the complementarity of their mandates with regard to the safe movement of germplasm.

The IPPC is recognized as the primary instrument for international cooperation in the protection of plants from injurious pests and the harmonization of phytosanitary measures. IPGRI's mandate - *inter alia* - is to further collecting, conservation and evaluation of germplasm to determine opportunities to use the genetic diversity of useful plants for the benefit of people throughout the world. The objective of the cooperative relationship between IPGRI and FAO is to facilitate the safe movement of germplasm by identifying technically sound practices that safeguard against the introduction and establishment of pests.

The recommendations in these guidelines are intended for germplasm conservation, research and plant breeding programmes. When collecting and transporting germplasm, standard phytosanitary measures, for example pest risk assessment (FAO 1996), have to be considered.

The technical guidelines are intended to be the best possible advice to institutions involved in germplasm exchange. The guidelines are produced by meetings of panels of experts on the crop concerned, who have been selected in consultation with the relevant specialized institutions and research centres. The experts contribute to the

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[1] The word 'pest' is used in this document as it is defined in the FAO Glossary of Phytosanitary Terms (1996): 'any species, strain or biotype of plant, animal, or pathogenic agent, injurious to plants or plant products'.

elaboration of the guidelines in their private capacities and do not represent the organizations to which they belong. The guidelines reflect the consensus of the specialists who attended the meeting, based on the best scientific knowledge available at the time of the meeting. The experts who have contributed to this document are listed after this introduction. FAO, IPGRI and the contributing experts cannot be held responsible for any failures resulting from the application of the present guidelines.

Because eradication of pathogens is extremely difficult, and even low levels of infection / contamination may result in the introduction of pathogens to new areas, no specific information on treatment is given in the pest descriptions. A pest risk analysis will produce information on which management options are appropriate for the case in question. General precautions are given in the **General Recommendations** section.

The technical guidelines are written in a concise style. They are divided into two parts. The first part makes general recommendations on how best to move the germplasm and mentions available intermediate quarantine facilities when relevant. The second part describes important pests. The information given on a particular pest does not pretend to be exhaustive but concentrates on those aspects that are most relevant to germplasm health. In general, references are only given on the geographical distribution of the diseases and pests.

Guideline update

In order to be useful, the guidelines need to be updated when necessary. We ask our readers to kindly bring to our attention any developments that possibly require a review of the guidelines such as new records, new detection methods or new control methods.

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[2] Dr Prior kindly supplied many of the illustrations in the guidelines published in 1989, while employed at CIBC. His new address is given here.

INTERMEDIATE QUARANTINE

As cacao swollen shoot virus infection may be latent for up to 20 months, the indicator plants have to be observed for 24 months before the budwood can be released, not just for three flushes of growth (Prof. P. Hadley, University of Reading, pers. comm.).

Intermediate quarantine stations available for cacao

Cocoa Programme
CIRAD-CP
BP 5035
34032 Montpellier Cédex
FRANCE
Tel: +33-467615800

Dept. of Horticulture
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Only for material from Central America, South America, the Caribbean, or another Cocoa Quarantine Station:

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GENERAL RECOMMENDATIONS

The guidelines set out below should be followed when transferring cacao germplasm:

- Germplasm should be obtained from the safest source possible, e.g. from a pathogen-tested intermediate quarantine collection.
- Shipping of pods or rooted plants is **NOT** recommended.
- If available, seed is preferred for the movement of cacao germplasm since seed poses a relatively low risk of moving and introducing pests. A sterile inorganic packing material such as vermiculite or perlite is preferable to an organic one such as sawdust.
- Budwood for international exchange should be collected from healthy plants and treated by dipping for 30 seconds in each of the following solutions (ensuring that the use of these chemicals complies with local pesticide regulations):
  - 0.1% active ingredient malathion
  - 0.1% active ingredient carbaryl
  - 1% mineral oil (white oil)
  - 0.2% sodium hypochlorite

  then the ends are dipped in paraffin wax to prevent desiccation.
- Indexing procedures and results should be documented in detail, and the description should accompany the shipment **IN ADDITION TO** a phytosanitary certificate if required by the plant protection organizations of importing and/or exporting country.
- The transfer of germplasm should be carefully planned in consultation with quarantine authorities, in both the importing and exporting countries, and the relevant indexing laboratory. International standards for phytosanitary measures as published by the Secretariat of the International Plant Protection Convention (IPPC) should be followed. Particular attention should be given to the Pest Risk Analysis procedure (FAO 1996).

OPTIONS FOR MOVEMENT OF CACAO GERMPLASM IN RELATION TO THE RISK OF MOVING PESTS

1. Can the germplasm be moved as seed?

This is the safest method of moving cacao germplasm. To date, only the pests listed in Table 1 may be seed-transmitted in cacao. Seeds should be collected from apparently healthy pods. Treatment of seeds with copper fungicides or metalaxyl further reduces the risk of pathogen transmission.

If the germplasm can definitely not be moved as seed, the next decision is to determine if it can be shipped as *in vitro* material.

Table 1. Seedborne pathogens in cacao

Pathogen	Disease	Internally seedborne	Externally seedborne	Concomitant contamination
Cacao necrosis *nepovirus*	cacao necrosis	reported in other species, but not in cacao	not possible	not possible
Crinipellis perniciosa	witches' broom	reported	possible	possible
Moniliophthora roreri	pod rot	no natural infection of seeds	possible	possible
Phytophthora spp.	pod rot	not reported	possible	unlikely

2. Movement of *in vitro* cultures

Although no standardized technique can be recommended yet, it is possible to introduce budsticks *in vitro*, to induce growth of the axillary bud, and to transfer *in vivo* the new shoot after a rooting treatment. Information on techniques may be found in Flynn *et al.* (1990), Esan (1992), Figueira and Janick (1993), Duncan (1993) and Gotsch (1997).

In vitro material may be infected with any or all of the following viruses: cacao necrosis *nepovirus*, cacao swollen shoot *badnavirus*, cacao yellow mosaic *tymovirus*.

Other viruses that have not been characterized yet also may be present and can be detected by electron microscopy.

The virus indexing should be done on the source material **BEFORE** tissue culture.

3. Movement of budwood

If the germplasm cannot be sent as seed or *in vitro*, the next safest option is the movement of budwood. Pests that may move with budwood include, in addition to the viruses listed above, systemic fungi (such as *Oncobasidium theobromae*), insects (in particular mealybugs) and mites.

DESCRIPTIONS OF PESTS

Viruses

Cacao necrosis *nepovirus* (CNV)

Cacao necrosis *nepovirus* (CNV) is serologically distantly related to tomato black ring virus.

Geographical distribution
The disease is reported from Nigeria and Ghana (Owusu 1971; Thresh 1958).

Symptoms
Infected plants show translucent and necrotic spots along the midrib and main veins of the leaves and, in the early stages of infection, a terminal dieback of shoots. No swellings develop in the stems or roots.

Transmission
Possibly through a nematode vector (Kenten 1977). The same author reported seed transmission of up to 24% in *Glycine max*, *Phaseolus lunatus* and *P. vulgaris*.

Particle morphology
Particles are isometric, about 25 nm in diameter.

Therapy
None. Once a plant is infected it cannot be cured.

Indexing
Refer to cacao swollen shoot virus *badnavirus*.

Cacao swollen shoot *badnavirus* (CSSV)

Many isolates of CSSV have been collected and are known by the locality of their origin. There is serological heterogeneity between the isolates, which has led to the differentiation of the isolates into 8 serogroups by Hughes *et al*. (1995). According to Brunt *et al.* (1996), cacao mottle leaf virus is a synonym of cacao swollen shoot *badnavirus*.

Geographical distribution
Côte d'Ivoire, Ghana, Nigeria, Sierra Leone, Sri Lanka, Togo (Brunt *et al.* 1996).

Symptoms
Symptoms of the disease are highly variable and depend on the virus strain and the stage of infection. The most characteristic symptoms on sensitive types (e.g. West African Amelonado) include red vein banding of the young leaves (Fig. 1), yellow vein banding (Fig. 2), interveinal flecking and mottling of mature leaves (Fig. 3) and pronounced swellings of the stems and roots (Fig. 4). Some strains of the virus (e.g. mottle leaf) do not induce swellings.

Transmission
CSSV is transmitted by at least 14 species of mealybugs (Homoptera: Coccidae). There is no transmission through seed or by leaf inoculation with sap, except to cacao cotyledons. Natural infection with CSSV has been reported in *Adansonia digitata, Bombax* sp., *Ceiba pentandra* and *Cola gigantea*. *Corchorus* spp. and other species have been infected experimentally.

Particle morphology
Particles are bacilliform and measure 121-130 x 28 nm (Fig. 5).

Therapy
None. Once a plant is infected it cannot be cured.

Detection
ELISA and ISEM have been used successfully (Sagemann *et al*. 1985) for detection of CSSV; also virobacterial agglutination (Hughes and Ollennu 1993). It is important to note that infection with cacao swollen shoot virus may be latent for up to 20 months (Prof. P. Hadley, University of Reading, pers. comm.).

Fig. 1. CSSV: red vein banding of the young leaves. (Dr J. Amponsah, Cocoa Research Institute)

Fig. 2. CSSV: yellow vein banding of the young leaves. (Dr J. Amponsah, Cocoa Research Institute)

Fig. 3. CSSV interveinal flecking and mottling of mature leaves.
(Dr J. Amponsah, Cocoa Research Institute)

Fig. 4. CSSV: stem swelling.
(Dr J. Amponsah, Cocoa Research Institute)

Fig. 5. CSSV: electron micrograph of viral particles. (Dr R.A. Muller, IRCC, Montpellier)

Budwood should be tested for the presence of virus by grafting to West African Amelonado seedlings, which express conspicuous symptoms when infected. Seed of West African Amelonado can be obtained from the Department of Horticulture, University of Reading, UK. Procedures to be following in testing budwood are:

1. One bud is sampled from each stick and grafted onto an Amelonado seedling. The remaining buds are grafted on to seedling rootstocks with their origins clearly labelled for future reference.
2. When grafts fail to unite with the Amelonado indicator, the test must be repeated with a bud of a plant derived from the same bud stock until a successful graft has been achieved.
3. If foliar symptoms or swellings are observed on the Amelonado indicator plants, then this plant and all plants derived from the same mother plant must be destroyed by incineration or autoclaving.

Cacao yellow mosaic *tymovirus*

Geographical distribution
The virus is reported only from Sierra Leone (Blencowe *et al*. 1963; Brunt 1970b).

Symptoms
Conspicuous yellow areas on leaves. No swelling occurs on stems or roots.

Transmission
Not seedborne. Readily transmitted by sap inoculation or many herbaceous species.

Particle morphology
Particles are isometric and measure about 25 nm in diameter.

Therapy
None. Once a plant is infected it cannot be cured.

Indexing
Refer to cacao swollen shoot virus above.

Other virus-like diseases

Trinidad virus disease, yellow-vein banding and watermark diseases were listed in the 1989 guidelines. However, as the original text suggests that they have disappeared, and as they are not mentioned in the recent literature, they have been deleted from this update.

Fungi

A summary of research results for black pod, Monilia pod rot and witches' broom diseases was published by Fulton (1989).

Witches' broom

Cause
Crinipellis perniciosa.

Symptoms
The pathogen infects all actively growing aerial meristematic tissue, resulting in hypertrophy. Vegetative brooms develop from infection of terminal and axillary buds (Fig. 6). Stem cankers result from infection of leaves, pulvini and petioles. Flower cushion infection causes cushion brooms or cherimoya-like pods (strawberry-shaped pods). Early infection of pods destroys developing beans. Late infection of pods gives rise to some usable beans while others may be infected. After 5-6 weeks the infected plant parts become necrotic. The fungus changes to a saprophytic phase and may form mushroom-like basidiocarps (Fig. 7) under conducive environmental conditions, usually 4-6 weeks from the onset of a wet period.

Geographical distribution
Purdy and Schmidt (1996) listed the following countries: Bolivia, Brazil, Colombia, Ecuador, Grenada, Guyana, Panama, Peru, St. Vincent, Surinam, Trinidad and Tobago, Venezuela. In 1989, the disease was reported for the first time to occur in Bahia, Brazil (Periera *et al.* 1989).

Biology
Infection is caused only by basidiospores, which are killed by exposure to sunlight and desiccation. The viability of the spores is limited to 6 hours. The hyphal growth of the fungus is limited to the meristematic tissue. The fungus can infect seeds, giving rise to infected seedlings.

Fig. 6. Witches' broom: green terminal vegetative broom. (Dr L.H. Purdy, University of Florida, Gainesville)

Fig. 7. Witches' broom: basidiocarps of *Crinipellis perniciosa* on the peduncle of a cherimoya-like pod. (Dr L.H. Purdy, University of Florida, Gainesville)

Moniliophthora pod rot

Cause
Moniliophthora roreri.

Symptoms
Under natural conditions the disease affects only the pods. Infection can occur at very early stages of development and susceptibility decreases with increasing pod age. Initial symptoms are characterized by one or more swellings appearing on the pod (Fig. 8), or small water-soaked lesions which enlarge into necrotic areas with irregular borders. A white fungal stroma (Fig. 9) covers the area within 3-5 days, with profuse formation of cream to light brown conidia. Late infection of pods results in premature ripening showing a green and yellow mosaic pattern. In the infected pods the seeds become necrotic and compact into a mass (Fig. 10).

Geographical distribution
The disease is presently found in Colombia, Costa Rica, Ecuador (on both sides of the Andes), Nicaragua, Panama and Venezuela (Wood and Lass 1985).

Biology
Pods are infected by conidia which are viable for several weeks and can withstand exposure to sunlight. Dissemination is by wind. Natural infections have only been observed on pods, although artificial inoculation of seeds with conidia has produced infected seedlings. Under natural conditions disease transmission by infected seeds has not been observed and is most unlikely.

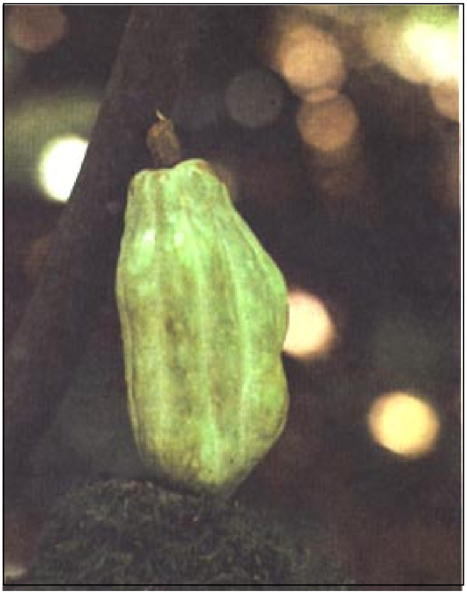

Fig. 8. *Moniliophthora* pod rot: swellings characteristic of infection on young pods. (Dr J.J. Galindo, CATIE, Turrialba)

Fig. 9. Severely damaged large pod. Left: premature ripening, partial necrosis and white, young pseudostroma on large pod infected by *M. roreri*. Right: healthy green pod. (CABI Crop Protection Compendium; copyright Luis C. Gonzalez)

Fig. 10. *Moniliophthora* pod rot: seed necrosis and early ripening of infected pods. (Dr J.J. Galindo, CATIE, Turrialba)

Vascular streak dieback

Cause
Oncobasidium theobromae.

Hosts
Keane and Prior (1991) reported that avocado (*Persea americana*) is also a host for *O. theobromae.* The pathogen may therefore move with vegetative avocado material from areas where the pathogen occurs.

Symptoms
Vascular streak dieback affects shoots and branches, but symptoms manifest themselves in the leaves which become chlorotic and may develop a characteristic green mottling on a yellow background (Fig. 11). Infected leaves are often found in the middle of the branch in mature plants. In seedlings, symptoms can occur in any of the leaves. Accompanying symptoms are discolouration of the vascular traces on the scars of freshly fallen leaves (Fig. 12), swollen lenticels on the bark in the area of leaf fall (Fig. 13) and sprouting of axillary buds, which subsequently die. Interveinal necrosis of the terminal leaves similar to calcium deficiency can be observed (Fig. 11). The wood of the infected stem shows brown streaking which can be seen when the wood is split longitudinally (Fig. 14). See Keane *et al.* (1972) and Prior (1980) for more detailed descriptions.

Geographical distribution
Reports from Burma, China (Hainan Island), India (southern part), Indonesia, Malaysia, Papua New Guinea (main island, New Britain and New Ireland), the Philippines and Thailand have been confirmed (Keane and Prior 1991).

Biology
The fungus is systemic within the xylem (Fig. 15) and growth in culture is limited (Fig. 16). Infection through young leaves originates from basidiospores. Basidiomata are found on abscised leaf patches on the stem under very wet conditions. Basidiospore production occurs after midnight and lasts until dawn. Dissemination is by wind. Spores are viable for a few hours only. Incubation in seedlings takes approximately 6 weeks whereas in mature plants 8 to 16 weeks may elapse before symptoms appear.

Fig. 11. Vascular streak dieback: green mottling on yellow leaves and 'calcium deficiency' symptom on younger leaves. (Dr C. Prior, Royal Horticultural Society's Garden, Surrey)

Fig. 12. Symptoms on seedling leaves. Leaf yellowing with green islands (right); interveinal necrosis on younger leaves (left); sporophores on scars of fallen leaf (right: white growth on stem above lowest petiole). (CABI Crop Protection Compendium; copyright Chris Prior)

Fig. 13. Vascular streak dieback: seedling showing cessation of growth, leaf chlorosis and bark roughening. (Dr C. Prior, Royal Horticultural Society's Garden, Surrey)

Fig. 14. Vascular streak dieback: brown streaks in infected wood, and healthy wood in comparison. (Dr C. Prior, Royal Horticultural Society's Garden, Surrey)

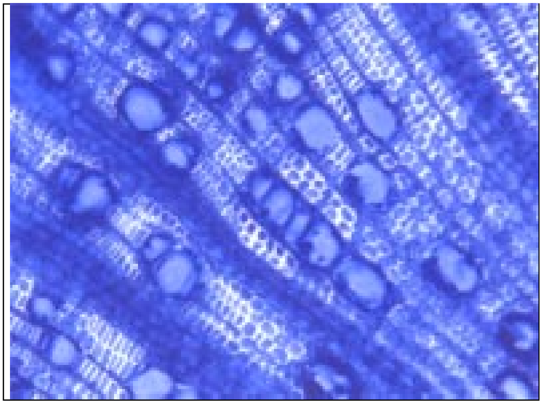

Fig. 15. Vascular streak dieback: hyphae of *O. theobromae* in infected xylem, stained with lactophenol cotton blue. (Dr C. Prior, Royal Horticultural Society's Garden, Surrey)

Fig. 16. Vascular streak dieback: vegetative and monilioid hyphae of *O. theobromae* in culture. (Dr M.A. Zainal Abidin, Universiti Pertanian Malaysia)

Phytophthora spp.

Cause
Phytophthora palmivora: P. megakarya, P. capsici and *P. citrophthora.*

Symptoms
Phytophthora spp. can attack all parts of the cacao plant but the main manifestations of the fungus are:

- pod rot – a firm brown rot of the pod (Fig. 17) (the main disease)
- stem canker – dark sunken lesions on the stem (Fig. 18)
- seedling blight – extensive necrosis of leaves and shoots of seedlings (Fig. 19).

Geographical distribution
Seven species of *Phytophthora* have been identified on cacao (Montes-Belmont and De los Santos 1989; Zentmyer 1990; Nyassé 1992; Chowdappa *et al.* 1993; Chowdappa and Mohanan 1996; Mr A.D. Iwaro, pers. comm.). See summary in Table 2.

Table 2. Geographical distribution of *Phytophthora* spp. reported in cacao

***Phytophthora* spp.**	**Geographical distribution**
P. arecae	Philippines and Vanuatu
P. capsici	Brazil, El Salvador, Guatemala, India, Jamaica, Mexico, Trinidad, Venezuela
P. citrophthora	Brazil, India, Mexico
P. megakarya	Cameroon, Gabon, Ghana, Nigeria
P. megasperma	Venezuela
P. nicotianae var. *parasitica*	Cuba
P. palmivora	cosmopolitan

Biology
The activity of *Phytophthora* spp. is very much associated with wet and humid conditions, although the soil serves as a permanent reservoir and the most frequent source of primary inoculum. Infection of plant parts is caused by spores (zoospores, sporangia) which are carried by water, rain splashes, ants and animals.

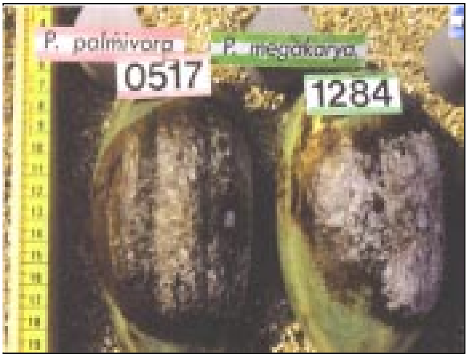

Fig. 17. *Phytophthora palmivora* and *Phytophthora megakarya* pod rot. (Dr R.A. Muller, IRCC, Montpellier)

Fig. 18. *Phytophthora palmivora* stem canker: outer bark cut away to show red, infected bark beneath. (Dr C. Prior, Royal Horticultural Society's Garden, Surrey)

Fig. 19. *Phytophthora palmivora*: seedling blight symptoms. (Dr C. Prior, Royal Horticultural Society's Garden, Surrey)

Insects

Following the recommendation of not moving pods or rooted plants will reduce the risk of the spread of insects with cacao germplasm. However, there is still a considerable risk when budwood is being moved, particularly with regard to mealybugs. For example, the introduction of the pink mealybug (*Pseudococcus sacchari*) to the Caribbean region caused severe damage to cacao (Mr A.D. Iwaro, pers. comm.).

While there are a large number of insects attacking cacao around the world, the danger of transferring insect pests with cacao germplasm is relatively low compared with transferring fungi and viruses. This is because insects are generally easier to see than pathogens, and the insects do not have long cryptic phases. Table 3 lists some regional pests of cacao which could be of economic importance. Descriptions of those and additional insects can be found in Entwistle (1972) and Wood and Lass (1985). Figures 20 to 23 show some of the insects and their damage. Table 4 lists some particular problems that could be encountered with each form of germplasm movement.

Table 3. Regional insect pest problems of cacao

Americas	Africa	Asia	Pacific
mirids	mirids	mirids	mirids
mealybugs	mealybugs	mealybugs	mealybugs
thrips	thrips	-	-
-	-	*Conopomorpha* pod borer	-
-	-	-	*Pantorhytes* weevil
termites	-	-	termites
-	*Cryptophlebia* husk borer	*Cryptophlebia* husk borer	*Cryptophlebia* husk borer
Conotrachelus borer	-	-	-
-	-	bagworms	-
leaf cutting ants	-	-	-
-	-	branch / stem borers	-

Table 4. Particular insect pest problems that may be encountered in germplasm

Type of material	Pest
Seeds	none likely
Budwood	mealybugs
Pods	pod borers, mealybugs, mirids, pod miners
Seedlings	mealybugs, mirids, scales, soil insects
Packing material	stray insects, not necessarily associated with cacao

Fig. 20. Pod borer, *Conopomporpha cramerella*: tunnels inside a pod. (Dr J.D. Mumford, Imperial College, Ascot)

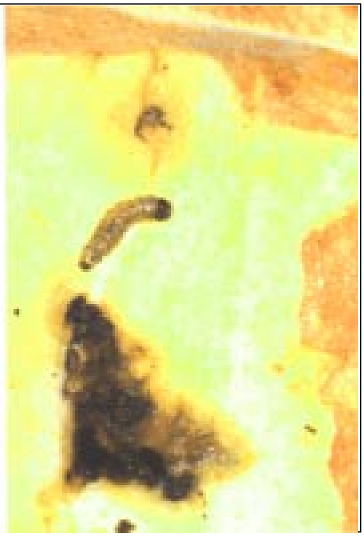

Fig. 21. Pod borer, *Cryptophlebia* sp. (Dr C. Prior, Royal Horticultural Society's Garden, Surrey)

Fig. 22. Mirid bug damage on pods. (Dr J.D. Mumford, Imperial College, Ascot)

Fig. 23. *Pantorhytes batesii.* (Dr C. Prior, Royal Horticultural Society's Garden, Surrey)

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