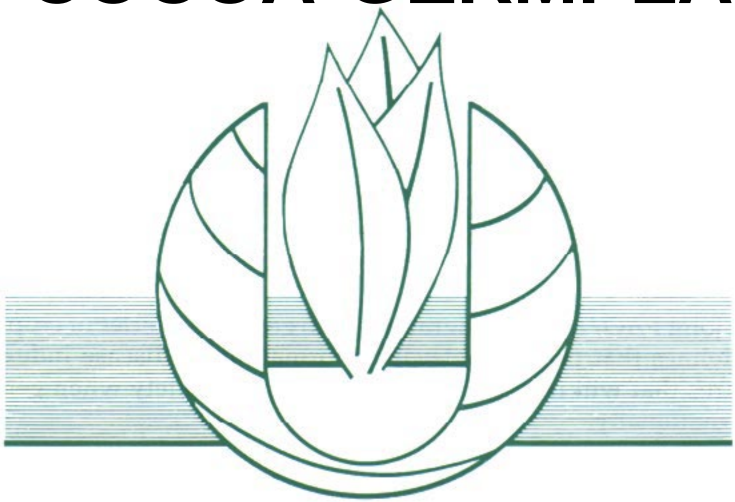
FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS

INTERNATIONAL BOARD FOR
PLANT GENETIC RESOURCES

# FAO/IBPGR TECHNICAL GUIDELINES
## FOR THE
# SAFE MOVEMENT OF COCOA GERMPLASM



**Edited by**
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In collaboration with

***American Cocoa Research Institute***

# 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 quarantine pests along with the host plant material; in particular, cryptic pathogens such as viruses pose a special risk. In order to minimize this risk, effective testing (indexing) procedures are required to ensure that distributed material is free of pests that are of quarantine concern.

The ever increasing volume of germplasm exchanged internationally, coupled with recent rapid advances in biotechnology, has created a pressing need for crop specific overviews of the existing knowledge in all disciplines relating to the phytosanitary safety of germplasm transfer. This has prompted FAO and IBPGR to launch a collaborative programme for the safe and expeditious movement of germplasm reflecting the complementarity of their mandates with regard to the safe movement of germplasm. FAO has a long-standing mandate to assist its member governments to strengthen their Plant Quarantine Services, while IBPGR's mandate - *inter alia* - is to further the collecting, conservation and use of the genetic diversity of useful plants for the benefit of people throughout the world.

The aim of the joint FAO/IBPGR programme is to generate a series of crop-specific technical guidelines that provide relevant information on disease indexing and other procedures that will help to ensure phytosanitary safety when germplasm is moved internationally.

The technical 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 elaboration of the guidelines in their private capacities and do not represent the organizations to whom they belong. FAO, IBPGR and the contributing experts cannot be held responsible for any failures resulting from the application of the present guidelines. By their nature they reflect the consensus of the crop specialists who attended the meeting, based on the best scientific knowledge available at the time of the meeting.

The technical guidelines are written in a short, direct, sometimes 'telegraphic' style, in order to keep the volume of the document to a minimum and to facilitate updating. The guidelines are divided into two parts: The first part makes general recommendations on how best to move germplasm of the crop concerned and mentions available intermediate quarantine facilities when relevant. The second part covers the important pests and diseases of quarantine concern. The information given on a particular pest or disease does not pretend to be exhaustive but concentrates on those aspects that are most relevant to quarantine. In general, references are only given on the geographical distribution of the diseases and pests.

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# GENERAL RECOMMENDATIONS

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

- Shipping pods or rooted plants is not recommended.

- Material should be moved as seeds whenever possible, according to the following procedure:
  1. Collect apparently healthy seed pods.
  2. Open pods and discard all immediately suspect pod and seed material.
  3. Remove pulp from seeds and reject all seeds from a pod if any seeds appear defective.
  4. Remove surface moisture from the seeds and treat them with appropriate fungicides and insecticides (see specific guidelines) according to manufacturers' instructions.
  5. Pack in a suitable medium, such as sterile sawdust. Outer packing material should be sterilized and treated with a contact insecticide. Packing should be carried out in a clean, covered area out of the wind.
  6. Seeds should be subjected to strict post-entry quarantine following arrival in the recipient country for the duration of at least three growth flushes

- All vegetative material should be imported only through intermediate quarantine using the following procedures:
  1. Budwood should be selected from healthy plants and washed in soapy water. It should then be dipped in a water solution or suspension of a combination of an appropriate fungicide and insecticide according to the manufacturers' instructions.
  2. The ends of the budsticks should then be dipped in paraffin wax to prevent dessication.
  3. Each budwood stick should be labelled clearly so that no confusion may occur, and each accession should be packed separately.
  4. Budsticks should be incinerated in intermediate quarantine after removal of the buds.
  5. Following arrival in the recipient country the budded material should be kept under close observation for at least three growth flushes.
  6. Material originating in countries where virus diseases are known to occur should be indexed during intermediate quarantine (see specific guidelines for swollen shoot virus).
  7. Material sent from intermediate quarantine to the recipient country should undergo post-entry quarantine for at least three growth flushes.

- Unpacking of seeds and budwood should be carried out in an enclosed area to contain any escaping insects. Appropriate protective clothing should be worn to prevent skin/eye contact and breathing of chemicals from the packing material. The packing material should be destroyed.

## Intermediate quarantine stations available for cocoa*

IRCC
BP 5035
34032 Montpelier Cédex
France
Tel. 67615800
Telex 480762F

USDA/ARS
Sub-Tropical Horticulture Research Station
13601 Old Cutler Road
Miami
Florida 33158 USA
Tel. 305-2389321

Cocoa Research Unit Quarantine Station
Graeme Hall
Christ Church
Barbados
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Tel. 4285939

Cocoa Quarantine Project
Department of Horticulture
University of Reading
Reading, Berks RG6 2AS
UK
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Telex 847813 RULIB G

* This list is not exclusive but was developed by the meeting based on information given by the participants.

# PESTS OF QUARANTINE IMPORTANCE

## Virus diseases

### 1. Cacao swollen shoot

**Cause**
Cacao swollen shoot virus (CSSV), of which numerous strains are known. Cacao mottle leaf virus is serologically distantly related to CSSV and therefore is considered to be a strain rather than a distinct virus.

**Geographical distribution**
The disease is limited to the cocoa-growing countries of West Africa - Ghana, Ivory Coast, Nigeria and Togo (Partiot *et al.*, 1978; Posnette, 1940; 1947). Similar diseases have been observed in Sri Lanka and Indonesia, although it has not been established whether CSSV is involved (Brunt, 1970a; Peiris, 1953; Kenten and Woods, 1976).

**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 cocoa cotyledons. Natural infection with CSSV has been reported in *Adansonia digitata*, *Bombax* sp., *Ceiba pentandra*, *Cola chlamydantha.* and *C. gigantea.* Corchorus spp., *Hildegardia barteri* and other species have been infected experimentally.

**Particle morphology**
Particles are bacilliform and measure 121-130 x 28 nm (Fig. 5) (possibly a member of the rhabdovirus group).

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

**Indexing**
***Seed.*** Since the virus is not seed-transmitted, indexing is not required.

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

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

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

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

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

***Budwood.*** Budwood is 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. Serological and RNA test methods are being investigated but are not yet ready to determine with confidence the absence of the virus in cocoa sap.

Procedures to be followed in testing budwood:

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. Amelonado indicator plants are observed for symptoms for a minimum of three flushes of growth.
3. 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 stick until a successful graft has been achieved.
4. 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.
5. Budwood can be released to the recipient after the Amelonado indicator plant has remained symptomless through three flushes of growth.

## 2. Cacao necrosis

**Cause**
Cacao necrosis virus (CNV), a serotype of tomato blackring virus (nepovirus group).

**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 die-back of shoots. No swellings develop in the stems or roots.

**Transmission**
No known vector. CNV is not seed transmitted and is not mechanically transmissible directly from cocoa leaves. Purified preparations infect a wide range of herbaceous species.

**Particle morphology**
Particles are isometric, ca. 25 nm in diameter

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

**Indexing**
Refer to cacao swollen shoot disease above.

## 3. Cacao yellow mosaic

**Cause**
Cacao yellow mosaic virus (CYMV), which is serologically related to wild cucumber mosaic virus and to turnip yellow mosaic virus.

**Geographical distribution**
The disease is known only to occur in Sierra Leone (Blencowe *et al.*, 1963; Brunt, 1970b).

**Symptoms**
Conspicuous yellow areas on leaves. No swellings occur on stems or roots.

**Transmission**
Not seed-borne. Readily transmitted by sap inoculation to many herbaceous species.

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

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

### Indexing
Refer to cacao swollen shoot disease above.

## 4. Other virus-like diseases

### Trinidad virus disease
Two variations of this disease, characterized by red mottling and vein-clearing symptoms, are similar to those described for cacao swollen shoot disease but without swelling in plant parts (Posnette, 1944). Like CSSV, the causal agent is transmitted by mealybugs. There is no evidence that the disease exists today in Trinidad because all affected areas were replaced by housing development. (For indexing, refer to cacao swollen shoot disease above.)

### Yellow vein-banding and watermark diseases
Two suspected virus diseases have been described from Sabah, Malaysia. One disease, referred to as yellow vein-banding disease (Liu and Liew, 1975), is characterized by a yellowing that develops along the midrib and secondary veins in mature leaves. The second disease, called watermark disease, can be distinguished by the appearance of a translucent blotch near the midrib that extends across the leaf blade at the basal portion of the leaf (Liu, 1979).

Virus particles were not associated with these diseases, but the agents were graft-transmitted to healthy rootstocks. These diseases occurred in only a few trees which were subsequently destroyed. (For indexing, refer to cacao swollen shoot disease above.)

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# Fungal pathogens

## 1. 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 cherimoyas (strawberry-shaped pods) (Fig. 7). 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. 8) under conducive environmental conditions, usually 4-6 weeks from the onset of a wet period.

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

Fig. 7. Witches' broom: infected flower cushion with a small cherimoya-like pod, a small flower broom, a cushion broom and infected flowers. (Dr. L.H. Purdy, University of Florida, Gainesville)

Fig. 8. Witches' broom: basidiocarps of *Crinipellis perniciosa* on the peduncle of a cherimoya-like pod. (Dr. L.H. Purdy, University of Florida, Gainesville)

### Geographical distribution
Witches' broom was first reported from Surinam and is now found in Bolivia, the Amazon region of Brazil (not in Bahia), Colombia, Ecuador, Grenada, Panama, Peru, St. Vincent, Trinidad and Tobago and Venezuela (Wood and Lass, 1985).

### 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.

### Quarantine measures
***Seed.***
- Seeds should be collected only from apparently disease-free pods, removed from the pods and treated with a suitable fungicide.
- Seeds should be subjected to strict post-entry quarantine procedures in the recipient country. The germinated seeds should remain in the post-entry quarantine nursery for at least three growth flushes.

***Budwood.***
- Budwood should be selected from healthy plants and treated with a suitable fungicide.
- Budwood should always be sent to an Intermediate Quarantine Station and grown there for up to three growth flushes. Budwood proven free of the disease may be dispatched to the recipient country.

## 2. *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. 9), or small water-soaked lesions which enlarge into necrotic areas with irregular borders. A white fungal stroma (Fig. 10) 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. 11).

Fig. 9. *Moniliophthora* pod rot: swellings characteristic of infection on young pods. (Dr. J.J. Galindo, CATIE, Turrialba)

Fig. 10. *Moniliophthora* pod rot: mature pod showing necrosis and white fungal stroma. (Dr. J.J. Galindo, CATIE, Turrialba)

Fig. 11. *Moniliophthora* pod rot: seed necrosis and early ripening of infected pods. (Dr. J.J. Galindo, CATIE, Turrialba)

**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 have produced infected seedlings. Under natural conditions disease transmission by infected seeds has not been observed and is most unlikely.

**Quarantine measures**
***Seed.***
Seeds originating from countries known to have the disease should be handled as follows:
- Seeds should be collected only from apparently disease-free pods.
- Remove the seeds from the pods, preferably inside a building.
- Discard all the seeds from any pod that shows any signs of infection.
- Treat the seeds with a copper fungicide.

***Budwood.***
- Treat budwood with a copper fungicide before dispatch to an Intermediate Quarantine Station where non-viable materials should be incinerated.

## 3. Vascular streak dieback

**Cause**
*Oncobasidium theobromae.*

**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. 12). 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 swollen lenticels on the bark in the area of leaf fall (Fig. 14), discolouration of the vascular traces on the scars of freshly fallen leaves (Fig. 13) and sprouting of axillary buds, which subsequently die. Interveinal necrosis of the terminal leaves similar to calcium deficiency can be observed (Fig. 12). The wood of the infected stem shows brown streaking which can be seen when the wood is split longitudinally (Fig. 15). See Keane *et al.* (1972) and Prior (1980) for more detailed descriptions.

Fig. 12. Vascular streak dieback: green mottling on yellow leaves and 'calcium deficiency' symptom on younger leaves. (Dr. C. Prior, CIBC, Ascot)

Fig. 13. Vascular streak dieback: discolouration of the vascular traces on the leaf scars. (Dr. C. Prior, CIBC, Ascot)

Fig. 14. Vascular streak dieback: seedling showing cessation of growth, leaf chlorosis and bark roughening. (Dr. C. Prior, CIBC, Ascot)


Fig. 15. Vascular streak dieback: brown streaks in infected wood, and healthy wood in comparison. (Dr. C. Prior, CIBC, Ascot)

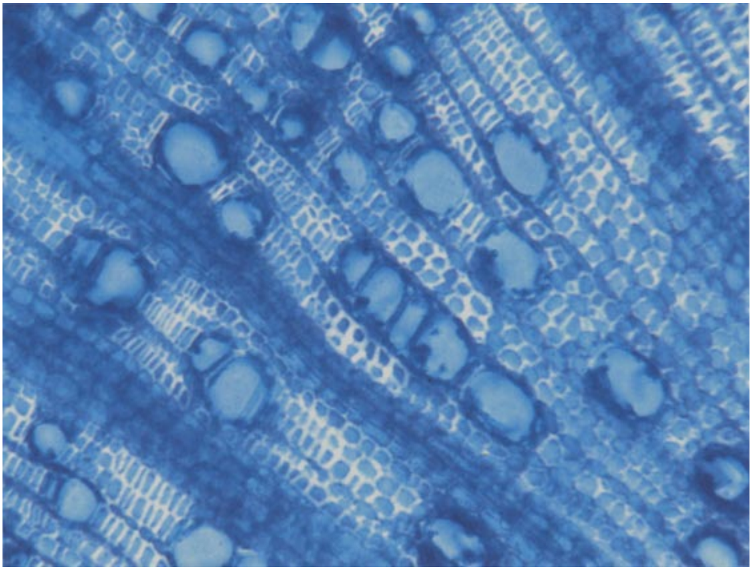

Fig. 16. Vascular streak dieback: hyphae of *O. theobromae* in infected xylem, stained with lactophenol cotton blue. (Dr. C. Prior, CIBC, Ascot)

Fig. 17. Vascular streak dieback: vegetative and monilioid hyphae of *O. theobromae* in culture. (Dr. M.A. Zainal Abidin, Universiti Pertanian Malaysia)

**Geographical distribution**

The disease can occur in Burma, China (Hainan Island), southern India, Indonesia, Malaysia, Papua New Guinea (the main island, New Britain and New Ireland), the Philippines and in Thailand (Keane, 1981; Prior, 1985; Wood and Lass, 1985).

**Biology**

The fungus is systemic within the xylem (Fig. 16) and growth in culture is limited (Fig. 17). 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.

**Quarantine measures**

***Seed.***

- Seeds have not been demonstrated to transmit the disease. However, a precautionary treatment with a systemic triazole fungicide solution is recommended (Prior, 1985).

***Budwood.***

- Budwood should be sent to an Intermediate Quarantine Station and maintained and observed for three growth flushes before being sent to the recipient country.
- Microscopic examination of budwood for hyphae in the xylem (Fig. 16) can also be carried out as a further check in the Intermediate Quarantine Station (Prior, 1985).

## 4. Phytophthora disease

**Cause**

*Phytophthora palmivora; P. megakarya; P. capsici* and *P. citrophthora*

**Symptoms**

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

- Pod Rot - a firm brown rot of the pod (Fig. 18) (the main disease).
- Stem Canker - dark sunken lesions on the stem (Fig. 19).
- Seedling Blight - extensive necrosis of leaves and shoots of seedlings (Fig. 20).

**Geographical distribution**

*P. palmivora* is widely distributed in all tropical areas.
*P. megakarya* is presently known to occur in Africa - Cameroon, Gabon, Ghana, Equatorial Guinea, Nigeria, Sao Tome and Togo.
*P. citrophthora* occurs in Brazil and West Africa.
*P. capsici* occurs in Brazil, Cameroon and Trinidad.
The species can be differentiated by morphological characters of sporangia, their behaviour in culture and by enzymatic electrophoresis (Blaha, 1987; Djiekpor, Partiot and Lucas, 1982; Wood and Lass, 1985).

**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.

**Quarantine measures**

***Seed.***

- Seed should be collected from visibly healthy pods.
- Seeds should be treated with an appropriate fungicide (e.g. metalaxyl).

***Budwood.***

- Budwood should be free of any visible lesions and it should be treated with an appropriate fungicide (e.g. metalaxyl).

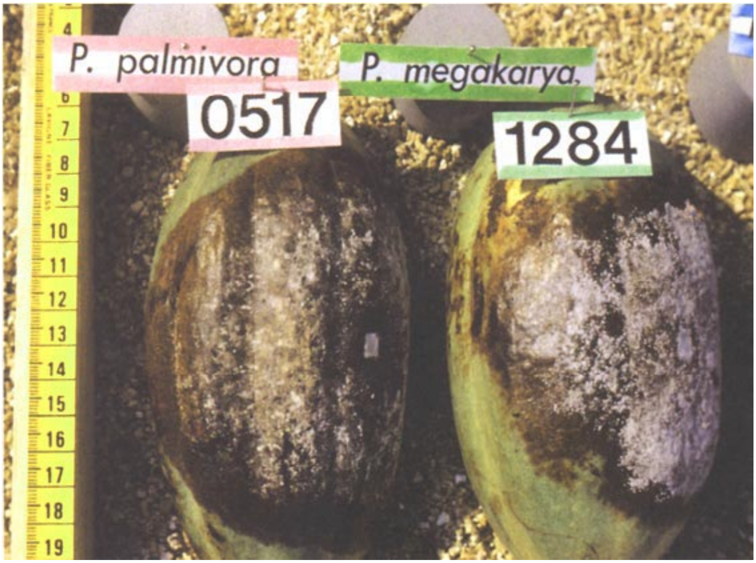


Fig. 18. *Phytophthora palmivora* and *Phytophthora megakarya* pod rot. (Dr. R.A. Muller, IRCC, Montpellier)

Fig. 19. *Phytophthora palmivora* stem canker: outer bark cut away to show red, infected bark beneath. (Dr. C. Prior, CIBC, Ascot)

Fig. 20. *Phytophthora palmivora:* seedling blight symptoms. (Dr. C. Prior, CIBC, Ascot)

## 5. Other fungal diseases of quarantine importance

**1. Mal de machete**

*Ceratocystis fimbriata*

This disease is a problem in Central and South America. Since infection results in the rapid death of the plants, transfer through Intermediate Quarantine Stations is a sufficient precaution.

**2. Cushion gall disorder**

a. Green point gall (Fig. 21)
   *Calonectria rigidiuscula (Fusarium decemcellulare)*

b. Flowery gall
   (causal agent not defined)

Since both the above disorders do not affect bud wood and seeds, normal quarantine procedures can effectively deal with these disorders.

Fig. 21. Green point gall: aborted flower buds. (Dr. C. Prior, CIBC, Ascot)

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# Insects

While there are a large number of insects attacking cocoa around the world, the danger of transferring insect pests with cocoa germplasm is relatively low compared to transferring fungi and viruses. This is because cocoa insects are generally easier to see than cocoa pathogens, and the insects do not have long cryptic phases. Under quarantine conditions, however, all insects, of any description, should be eliminated. Table 1 lists many of the regional pests of cocoa which could be of quarantine importance. Descriptions, and further insects, can be found in Entwistle (1972) and Wood and Lass (1985). Figures 22 to 25 show some of the insects and their damage.

The greatest risk of insect transfer comes from movement of pods, since insects could be hidden and protected inside the pods. Seedlings are also undesirable because of possible soil contamination. Table 2 lists some particular problems that could be encountered with each form of germplasm movement.

**References**

Entwistle. 1972. *Pests of Cocoa.* Longman, London.
Wood, G.A.R. and Lass, R.A. 1985. *Cocoa.* Longman, London.

**Table 1. Regional insect pest problems of cocoa**

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

Fig. 22. Pod borer *Conopomorpha cramerella:* tunnels inside a pod. (Dr. J.D. Mumford, Imperial College, Ascot)

Fig. 23. Pod borer *Cryptophlebia* sp. (Dr. J.D. Mumford, Imperial College, Ascot)

**Table 2. Particular pest problems that may be encountered in germplasm**

| Type of material | Pest problems |
|---|---|
| Seeds | none likely |
| Budwood | mealybugs |
| Pods | pod borers, mealybugs, mirids, pod miners |
| Seedlings | mealybugs, mirids, scales, soil organisms. |
| Packing material | stray insects, not necessarily associated with cocoa. |

Fig. 24. Mirid bug damage on pods. (Dr. J.D. Mumford, Imperial College, Ascot)

Fig. 25. *Pantorhytes batesii.* (Dr. C. Prior, CIBC, Ascot)

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