Technical Guidelines for the Safe Movement of Cacao Germplasm

Revised from the FAO/IPGRI Technical Guidelines No. 20

(Fifth Update, 2024)

Michelle J End, Andrew J Daymond and Paul Hadley, editors

CacaoNet
Global Network for Cacao Genetic Resources

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CacaoNet would like to thank all those who have contributed to the revision of the Guidelines for the Safe Movement of Cacao Germplasm as well as those who contributed to the original FAO/IPGRI Technical Guidelines No. 20, on which this fifth update is based (See section 2 for contact details). We are indebted to those who have written or revised sections relating to specific pests and diseases and are also grateful to those members of the CacaoNet Safe Movement Working Group who have supplied additional information, and who have made comments and suggestions to improve this revision, and previous revisions, of these Guidelines. We thank the many cocoa research institutes and organisations which have allowed their staff to contribute and to organisations such as COPAL and ICCO for providing the opportunities and facilities which have enabled these scientists to meet and share their knowledge. The publication of this revision has been supported by financial and in-kind contributions from the Cocoa Research Association Ltd., UK (CRA Ltd., a UK-based organization managing scientific cocoa research on behalf of Mars-Wrigley, Mondelēz International and the London Cocoa Trade [ICE Cocoa Futures Europe]) and the University of Reading. We gratefully acknowledge the additional financial and in-kind contributions Bioversity International and the CGIAR Research Programme on Forests, Trees and Agroforestry (FTA) that allowed the publication of the previous revisions. CacaoNet has received additional financial support from Mars-Wrigley, the U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS) and the World Cocoa Foundation (WCF).

The Secretariat for CacaoNet, hosted by Bioversity International, is responsible for providing coordination and administrative support for the network. Jan Engels was CacaoNet Coordinator from its initiation in 2006 to 2010 when this role was taken over by Stephan Weise. Brigitte Laliberté acted as Scientific Advisor to CacaoNet from 2010 to 2023.

The design, layout and editing of this document were originally done by Claudine Picq of Bioversity International. Spanish and French versions are also available.

Disclaimer

While every effort is made to ensure the accuracy of the information reported in this publication, CacaoNet, Alliance of Bioversity International and CIAT, and any contributing authors and editors and their affiliated organisations cannot accept any responsibility for the consequences of the use of this information. Any views expressed herein are the authors' and may not coincide with those of their institutions or the sponsor.

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1. Introduction

These guidelines describe technical procedures that minimize the risk of pest introductions with movement of germplasm for research, crop improvement, plant breeding, exploration or conservation. It is important to emphasize that these guidelines are not meant for trade and commercial consignments of planting materials or cocoa beans (see IPPC - International Plant Protection Convention for information on the International Plant Protection Convention which aims to protect the world's plant resources from the spread and introduction of pests, and promotes safe trade).

The collection, conservation and utilization of plant genetic resources and their global distribution are essential components of research activities underpinning the implementation of international crop and tree 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 minimize such risks, preventive measures and effective testing procedures are required to ensure that distributed material is free of pests of potential phytosanitary importance.

The international, and inter-regional, movement of plant germplasm for research (including plant biotechnology), conservation and basic plant breeding purposes requires complete and up to date information concerning the phytosanitary status of the plant germplasm. In addition, the relevant and current national regulatory information governing the export and importation of plant germplasm in the respective countries is essential.

The recommendations made in these guidelines are intended for small, specialized consignments used in research programmes, e.g. for collection, conservation and utilization for breeding of plant genetic resources. When collecting and transporting germplasm, standard phytosanitary measures, for example pest risk assessment (IPPC 2021), should be considered.

This revision of the technical guidelines for cacao has been produced by the Safe Movement Working Group of CacaoNet, an international network for cacao genetic resources[2]. The experts on cacao pests contribute to the elaboration of the technical guidelines in their personal capacity and do not represent or commit the organizations for which they work. The guidelines are intended to provide the best

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[1] The word 'pest' is used in this document as defined in the FAO Glossary of Phytosanitary Terms (2023): 'Any species, strain or biotype of plant, animal, or pathogenic agent, injurious to plants or plant products'.

[2] CacaoNet (www.cacaonet.org) is an international network for cacao genetic resources coordinated by Bioversity with a steering committee and working groups composed of representatives from various cocoa research institutes and organizations supporting cocoa research.

possible phytosanitary information to institutions involved in small-scale plant germplasm exchange for research purposes. The Alliance of Bioversity International and CIAT and the contributing experts cannot be held responsible for any problems resulting from the use of the information contained in the technical guidelines. These reflect the consensus and knowledge of the specialists who have contributed to this revision, but the information provided needs to be updated regularly. The experts who contributed to the production of these technical guidelines are listed in this publication. Correspondence regarding this publication should be addressed to the editors or to the relevant section authors.

The guidelines are written in a concise style to keep the volume of the document to a minimum and to facilitate updating. Suggestions for further reading are provided, in addition to specific references cited in the text (mostly for geographical distribution, media and other specific information).

The guidelines are divided into two parts.

- The first part makes general and technical recommendations on safe procedures to move cacao germplasm and mentions available intermediate quarantine facilities when relevant.
- The second part covers pests of phytosanitary concern for the international or regional movement of cacao genetic resources. The information given on a particular pest is not exhaustive but rather concentrates on those aspects that are most relevant to the safe movement of germplasm. Because eradication of pathogens from a region or country is extremely difficult, and even low levels of infection or infestation 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 (PRA) will produce information on which management options are appropriate for the case in question. General precautions are given in the General Recommendations.

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 may require a review of the guidelines such as new records, detection methods or control methods.

References

FAO. 2024. Glossary of Phytosanitory Terms (as adopted by CPM-18, 2024). International Standards for Phytosanitary Measures. FAO, Rome. Available from https://www.ippc.int/en/publications/622/

IPPC. 2021. Framework for pest risk analysis ISPM 2 (Rev. 2021) https://www.ippc.int/en/publications/592/

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3. Intermediate and regional quarantine centres

3.1 Intermediate quarantine centres

The role of intermediate quarantine centres is to prevent the spread of pests and diseases when moving planting material from one region to another by subjecting the material to a quarantine process in a country where cacao is not cultivated (thus minimising the risk of pest/pathogen entry into the system). Intermediate quarantine is particularly important when plant material is moved as budwood, as such material has the potential to harbour latent viruses and endophytic pathogens such as fungi.

The following intermediate quarantine centres are in operation:

International Cocoa Quarantine Centre (ICQC, R)
School of Agriculture, Policy & Development
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Reading
RG6 6AR
United Kingdom
Email: a.j.daymond@reading.ac.uk
Tel: +44 118 378 6628/ + 44 118 9760355
The Operational Manual for ICQC, R can be found at: http://www.icgd.reading.ac.uk/icqc/documents.php

United States Department of Agriculture (USDA)
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3.2 Regional (post-entry) quarantine centres

Post-entry quarantine stations are present in some cocoa-producing countries and are used primarily for material newly imported into the country in question. The length of time in post-entry quarantine can vary from six months to two years. In some cases, post-entry facilities are also used for within-country movement of germplasm.

The following post-entry quarantine centres are in operation for cacao:

Pusat Penyelidikan dan Pembangunan Koko Hilir Perak
(Cocoa Research and Development Centre of Hilir Perak),
Lembaga Koko Malaysia (Malaysian Cocoa Board),
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Email: nura@koko.gov.my

Cenargen Quarantine Facility
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Caixa Postal 02372 – Brasília, DF – CEP 70770-917,
BRAZIL
Email: cenargen.nig@embrapa.br

4. General recommendations

Whilst specific guidelines are given in subsequent sections in relation to particular pests/diseases the following general recommendations apply:

- Pest risk analysis should precede the movement of germplasm (see individual pest sections).
- Germplasm should be obtained from the safest source possible, e.g. from a pathogen–tested intermediate quarantine collection.
- Shipping of whole pods[3] is NOT recommended.
- The movement of whole plants in soil, or even bare-rooted plants, carries a very high risk of transferring soil-borne organisms and pests associated with the roots and aerial parts of the plant. Extreme caution must therefore be exercised when considering moving any whole plants, and the transfer of germplasm between regions as whole plants is NOT recommended unless the material can be transferred through a quarantine facility.
- When transferring material as seed, a sterile inorganic packing material such as vermiculite or perlite is preferable to an organic material such as sawdust. Used packaging material should be incinerated or autoclaved prior to disposal.
- Region to region transfer of budwood should usually take place via a quarantine centre.
- Budwood for international exchange should be treated with an appropriate fungicide/ pesticide mixture in cases where this is specified on the import certificate of the recipient country.
- After grafting the budwood in the recipient country, any waste plant material should be incinerated or autoclaved prior to disposal.
- The transfer of germplasm should take place in consultation with the relevant plant health authorities in both the importing and exporting countries. International standards for phytosanitary measures as published by the Secretariat of the International Plant Protection Convention (IPPC) should be followed (https://www.ippc.int/).
- In accordance with IPPC regulations, any material being transferred internationally must be accompanied by a phytosanitary certificate.

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[3] The fruits of *Theobroma cacao* are commonly known as "pods", with young fruits sometimes referred to as "cherelles", and the seeds are sometimes referred to as "beans".

5. Options for the movement of cacao germplasm in relation to the risk of moving pests

5.1 Seed

This is the safest way of moving cacao germplasm. However, care should be taken to ensure that only healthy pods are selected and appropriate fungicidal treatments given to avoid concomitant contamination. Samples should be examined using a hand lens or microscope. It should be noted that some pests may be transmitted by seed (Table 5.1).

Table 5.1. Seedborne pathogens in cacao.

Pathogen	Disease	Internally seed borne	Externally seed borne	Concomitant contamination
Cacao necrosis virus	Cacao necrosis	Reported in other species, but not in cacao	Not possible	Not possible
Cacao mild mosaic virus	CaMMV	Reported	Not possible	Not possible
Moniliophthora perniciosa	Witches' broom disease	Reported	Possible	Possible
Moniliophthora roreri	Frosty pod rot	No natural infection of seeds	Possible	Possible
Phytophthora spp.	Black pod rot	Reported	Possible	Unlikely
Ceratobasidium theobromae	Vascular streak dieback	Not reported	Possible	Unlikely

5.2 Budwood

Movement of cacao germplasm as budwood is practiced when a genetically identical copy of a particular genotype is required by the recipient (for example, if the genotype in question has particular useful traits for breeding purposes).

Since budwood may be infected with a number of viruses, e.g. *Cacao swollen shoot virus* (CSSV), budwood should only be moved via an intermediate quarantine station in which virus indexing procedures are conducted. The current recommended virus-indexing procedure is as follows (see also Thresh 1960):

1. Budwood is taken from a given plant in quarantine and buds grafted onto seedlings of Amelonado cacao. These show conspicuous symptoms when infected with viruses such as CSSV. It is recommended that at least three successful budded seedlings are needed per plant being tested.

2. Once the bud has formed a union with the seedling, the leaves and stems arising from both the rootstock and the scion of these test plants should then be inspected weekly over a period of two years for characteristic leaf symptoms and swellings (see the individual sections on cacao viruses).
3. Should viral symptoms be observed then the test plants along with the mother plant should be destroyed by incineration or autoclaving.

While the efficacy of molecular monitoring for viruses such as CSSV continues to improve, to date no fully isolate-independent detection technique has been produced and for this reason visual indexing is still recommended in combination with PCR-based screening.

Other pests that can be transferred via budwood include insects, such as mealybugs and endophytic pathogens e.g. *Ceratobasidium (*formerly *Oncobasidium theobromae*) and *Ceratocystis cacaofunesta*.

General recommendations when cutting budwood are:

1. Material should be taken from plants that show no visible signs of pest or disease activity
2. Cutting tools should be sterilized (e.g. using 70% ethanol) between cuts.
3. The budwood should be examined under a microscope or with a hand lens for the presence of insects/ mites or insect bore holes.

5.3 Whole plants

The movement of whole plants in soil between countries/ growing areas is **NOT RECOMMENDED** due to the high risk of transferring invertebrate pests and soil-borne organisms. Extreme care must be exercised when moving plant material as bare-rooted plants due to these same risks. Consequently, movement of bare-rooted plants is not recommended unless the material is transferred through a quarantine facility.

The exporting institute should raise the plant material in an insect-proof cage and an inert medium, such as perlite, should be used to minimise the chances of soil organisms being transferred. It is recommended that the material be treated with an appropriate pesticide before it is moved.

The receiving quarantine station should maintain the plants in a separate insect-proof area for a period of three months. During this period, daily inspections need to be made for insect pests. If a plant is found to be infected with a pest it should be destroyed by incineration or autoclaving.

5.4 *In vitro*

In vitro material should be shipped in sealed, transparent containers with sterile media. It should be inspected before dispatch and immediately upon receipt at destination. Ideally, *in vitro* material (or the material used to produce it) should be indexed for the presence of systemic pathogens, including viruses, in a quarantine facility. Infected or contaminated material should be destroyed.

5.5 Pollen and open flowers

Movement of pollen is NOT recommended out of areas in which *Moniliophthora* is present due to the possible contamination of pollen samples with fungal spores.

When moving pollen from other regions it should be examined by light microscopy for the presence of visible pests. Contaminated pollen should be discarded.

5.6 Flower buds

Flower buds may be transferred for use in tissue culture. These should be surface-sterilized before despatch. The precautions to prevent distribution of material with systemic pathogens described in 5.4 for *In vitro* materials should be taken.

5.7 Reference

Thresh JM. 1960. Quarantine arrangements for intercepting cocoa material infected with West African viruses. *FAO Plant Protection Bulletin* 8:89-92.

6. Summary of pest risks

Table 6.1. Summary of the principal pests of cacao, their distribution and the level of precaution needed when exporting plant parts.

Pest	Geographical spread[1]	Special precautions
7.1 *Cacao necrosis virus* (CNV): genus *Nepovirus*	Ghana, Nigeria	
7.2 *Cacao swollen shoot virus* (CSSV): genus *Badnavirus*	Benin, Côte d'Ivoire, Ghana, Liberia, Nigeria, Sierra Leone, Togo Reports also in Sri Lanka	Pod: Potential risk Seed: Low risk Budwood: High risk Quarantine advisable See: 5.2 Budwood SPECIAL RISK FACTOR: LATENT INFECTION UP TO TWO YEARS
7.3 *Cacao yellow mosaic virus* (CYMV): genus *Badnavirus*	Sierra Leone	
7.4 *Cacao yellow vein-banding virus* (CYVBV) (formerly known as Trinidad Cacao Virus B)	Isolated occurrences in Trinidad	Budwood: potential risk Use of molecular tests advocated since infections are often asymptomatic
7.4 *Cacao mild mosaic virus* (CaMMV) (formerly known as Trinidad cacao virus A)	Isolated occurences in Trinidad, Brazil, Puerto Rico, Hawaii. Recently reported to be widespread in Indonesia	Budwood: potential risk Seed: potential risk Use of molecular tests advocated since infections are often asymptomatic
7.5 Cacao Leafroll Virus (CaLRV) and Cacao Polerovirus (CaPV)	Recently very similar poleroviruses have been identified in isolated occurences in Brazil, USDA Miami and Mayaguez (CaLRV), ICQC,R and IC3 Costa Rica (CaPV)	Budwood: potential risk Use of molecular tests advocated since infections are often asymptomatic
8.1. Witches' broom disease (*Moniliophthora perniciosa*)	Angola (recent report, although not associated with cacao),Brazil (Bahia, Espirito Santo, Amazonian regions), Bolivia, Colombia, Ecuador, French Guiana, Grenada, Guyana, Panama, Peru, St. Lucia, St. Vincent, Suriname, Trinidad and Tobago, Venezuela	Whole pods: High risk, not recommended Seed: Moderate risk Budwood: Moderate risk See: 8.1.6 Quarantine measures

[1] **Note:** Information on the distribution of pests is based on available published information at the time of compilation. Pest distributions are liable to change over time.

Table 6.1. Summary of the principal pests of cacao, their distribution… (cont'd).

Pest	Geographical spread	Special precautions
8.2 Moniliophthora pod rot (frosty pod rot or moniliasis disease)	Belize, Bolivia, Brazil (Acre and Amazonas State), Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Jamaica, Mexico, Nicaragua, Panama, Peru, and western Venezuela	Pod: High risk, not recommended Seed: Moderate risk Budwood: Moderate risk Quarantine recommended SPECIAL RISK FACTOR: LONG LIVED SPORES See: 8.2.6 Quarantine measures
8.3 Phytophthora pod rot *Note that Phytophthora species are widespread and sometimes difficult to distinguish*		Whole pods: High risk, not recommended Seed: Low risk Budwood: High risk intermediate quarantine recommended SPECIAL RISK FACTOR: PRESENCE IN SOIL See 8.3.6 Quarantine measures
P. palmivora (syn. P. arecae)	Most cocoa-producing countries worldwide	
P. megakarya	Bioko (Fernando Po), Cameroon, Côte d'Ivoire, Gabon, Ghana, Nigeria, São Tomé and Principe,Togo	
P. capsici/P. tropicalis[4]	Brazil, Cameroon, Costa Rica, Côte d'Ivoire, Dominican Republic, El Salvador, French Guiana, Guatemala, India,Indonesia, Jamaica, Mexico, Panama, Peru, Trinidad, Venezuela	
P. citrophthora	Brazil, Cuba, Malaysia,India, Mexico, Philippines	
P. hevea	Brazil, Cameroon, Cuba, India, Malaysia, Mexico, Philippines	
P. megasperma	Brazil, Cuba, India, Malaysia,Venezuela, Philippines	

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[4] Prior to 2001 *Phytophthora capsici* and *P. tropicalis* were thought to be conspecific and historical references in the literature to *P. capsici* isolated from cacao must be considered possibly, if not likely, to be *P. tropicalis* since this species is more commonly recovered from woody perennials than *P. capsici* (Surujdeo-Maharah et al. 2016 – see section 8.3).

Table 6.1. Summary of the principal pests of cacao, their distribution… (cont'd).

Pest	Geographical spread	Special precautions
P. nicotianae var. parasitica	Brazil, Colombia, Cuba, India, Malaysia, Philippines	
P. theobromicola	Brazil	
8.4 Vascular streak dieback (*Ceratobasidium theobromae*)	Most cacao-growing areas in South and Southeast Asia: China (Hainan Island), India, Indonesia, West Malaysia and Sabah, Myanmar, PNG (islands of New Guinea, New Britain, New Ireland), southern Philippines, Thailand, and Vietnam	Whole pods: High risk, not recommended Seed: Low risk Budwood: High risk- intermediate quarantine recommended See 8.4.6 Quarantine measures
8.5 Verticillium wilt of cacao	Worldwide, especially Brazil, Colombia, DRC, Uganda	Whole pods: Low risk Seeds: Low risk Budwood: Moderate risk See: 8.5.6 Quarantine measures
8.6 Ceratocystis wilt	Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Haiti, Mexico, Peru,Trinidad & Tobago, Venezuela	Pod: High risk Seed: Low risk Budwood: Moderate risk See: 8.6.6 Quarantine measures
8.7 Rosellinia root rot *R. bunodes, R. pepo* *R. paraguayensis*	Widespread in Central and South America. Also in West Africa, India, Indonesia, Malaysia, Philippines	Pod: Low risk Seed: Low risk Budwood: High risk See: 8.7.6 Quarantine measures
8.8 Other fungal pathogens	Widespread	See section 8.8 for details
9.2 Cocoa pod borer	Southeast Asia including India, Indonesia, Malaysia, Papua New Guinea, the Philippines and Sri Lanka, Taiwan, Thailand	Pod: High risk, not recommended Seed: High risk Budwood: Moderate risk See: 9.2.6 Quarantine measures
9.3 Cocoa fruit borer (*Carmenta spp.*)	Brazil, Colombia, Ecuador, Panama, Peru, Trinidad & Tobago and Venezuela	Pod: Moderate risk Seed: Low risk See 9.3.6 Quarantine measures
9.4 Other Lepidopteran pests	Widely distributed	
9.5 Mirids (and other heteropterous plant sucking bugs)	All cacao-growing regions except Caribbean	Pod: Moderate risk Seed: Low risk Budwood: Moderate risk See: 9.5 mirids

Table 6.1. Summary of the principal pests of cacao, their distribution… (cont'd).

Pest	Geographical spread	Special precautions
9.6 Mosquito bug	Widely distributed	Pod: Moderate risk not recommended Seed: Low risk Budwood: Moderate risk 9.6.6 Quarantine measures
9.7 *Pseudotheraptus devastans*	Widely distributed in Africa	Pods: High risk See 9.7.5 Quarantine measures
9.8 Mealybug	All cacao-growing regions	Pod: Moderate risk Seed: Low risk Budwood: Moderate risk See 9.8 Mealybugs
9.9 Ambrosia beetles	Widely distributed	Budwood: Moderate risk See 9.9.6. Quarantine measures:
9.10 Phytophagous mites	Widely distributed	Budwood: High risk See 9.10.6 Quarantine measures
10. Parasitic nematodes	Widely distributed	See 10.6 Quarantine measures

Table 6.2. Summary of pest risk by country (*Phytophthora palmivora* is widespread as are a number of insect and other invertebrate pests, and the table does not include some pathogens of only limited/local importance. Users are recommended to check the individual chapters of this document and other reports of pest/ disease outbreaks in the country in which they are working for further details).

Country	Pest risk
Belize	*Moniliophthora* pod rot
Benin	*Cacao swollen shoot virus* (CSSV)
Bioko (Fernando Po)	*Phytophthora megakarya*
Bolivia	Witches' broom disease Moniliophthora pod rot
Brazil	*Cacao mild mosaic virus* (CaMMV) Cacao polerovirus (CaPV) Moniliophthora pod rot (Acre and Amazonas States) Witches' broom disease *Phytophthora capsici/P. tropicalis* *P. citrophthora* *P. heveae* *P. megasperma* *P. nicotianae* *P. theobromicola* Verticillium wilt of cacao Ceratocystis wilt Rosellinia root rot Anthracnose *Lasiodiplodia theobromae*
Cameroon	*Phytophthora megakarya* *Phytophthora capsici/ P. tropicalis* *Ceratocystis spp.* (*C. ethacetica and C. paradoxa)* *Lasiodiplodia theobromae*
Colombia	Witches' broom disease Moniliophthora pod rot Verticillium wilt of cacao Ceratocystis wilt *Phytophthora nicotianae* Rosellinia root rot

Table 6.2. Summary of pest risk by country (cont'd).

Country	Pest risk
Costa Rica	Moniliophthora pod rot Ceratocystis wilt Rosellina root rot *Phytophthora capsici/P. tropicalis* Cacao polerovirus (CaPV)
Côte d'Ivoire	*Cacao swollen shoot virus* (CSSV) *Phytophthora megakarya*
Cuba	*Phytophthora citrophthora* *Phytophthora heveae* *Phytophthora megasperma* *Phytophthora nicotianae*
Democratic Republic of Congo	Verticillium wilt
Dominican Republic	*Phytophthora* spp.
Ecuador	Witches' broom disease Moniliophthora pod rot Ceratocystis wilt
El Salvador	*Phytophthora capsici* Moniliophthora pod rot
French Guiana	Witches' broom disease *Phytophthora capsici*
Gabon	*Phytophthora megakarya*
Ghana	*Cacao necrosis virus* (CNV) *Cacao swollen shoot virus* (CSSV) *Phytophthora megakarya*
Grenada	Witches' broom disease
Guatemala	Moniliophthora pod rot *Phytophthora capsici* Ceratocystis wilt
Guyana	Witches' broom disease
Haiti	*Phytophthora spp.* Ceratocystis wilt

Table 6.2. Summary of pest risk by country (cont'd).

Country	Pest risk
Hawaii	*Phytophthora spp.* *Cacao mild mosaic virus* (CaPV)
Honduras	Moniliophthora pod rot
India	*Phytophthora capsici* *Phytophthora citrophthora* *Phytophthora heveae* *Phytophthora megasperma* *Phytophthora nicotianae* Vascular streak dieback Rosellinia root rot *Lasiodiplodia theobromae*
Indonesia	Vascular streak dieback Rosellina root rot Cocoa pod borer *Phytophthora capsici* *Lasiodiplodia theobromae* *Cacao mild mosaic virus* (CaMMV)
Jamaica	*Phytophthora capsici* Rosellinia root rot Moniliophthora pod rot *Thielaviopsis* [*Ceratocystis*] *paradoxa*
Liberia	*Cacao swollen shoot virus* (CSSV)
Malaysia	*Phytophthora citrophthora* *Phytophthora heveae* *Phytophthora megasperma* *Phytophthora nicotianae* Vascular streak dieback *Lasiodiplodia theobromae* Rosellina root rot Cocoa pod borer
Mexico	Moniliophthora pod rot *Phytophthora capsici* *Phytophthora citrophthora* *Phytophthora heveae*
Nicaragua	Moniliophthora pod rot

Table 6.2. Summary of pest risk by country (cont'd).

Country	Pest risk
Nigeria	*Cacao necrosis virus* (CNV) *Cacao swollen shoot virus* (CSSV) *Phytophthora megakarya*
Panama	Witches' broom disease Moniliophthora pod rot *Phytophthora capsica*
Papua New Guinea	Vascular streak dieback Cocoa pod borer
Peru	Witches' broom disease Moniliophthora pod rot Ceratocystis wilt Rosellinia root rot Verticillium wilt
Philippines	*Phytophthora citrophthora* *Phytophthora heveae* *Phytophthora megasperma* *Phytophthora nicotianae* Vascular streak dieback Rosellinia root rot Cocoa pod borer
Puerto Rico	*Cacao mild mosaic virus* (CaMMV) *Lasiodiplodia theobromae*, *Colletotrichum siamense* *Phytophthora palmivora*, *Diaporthe tuliensis*
São Tomé and Principe	*Phytophthora megakarya*
Sierra Leone	*Cacao swollen shoot virus* (CSSV) *Cacao yellow mosaic virus*
Sri Lanka	*Cacao swollen shoot virus* (CSSV) [reported but likely to be CBSLV] Rosellinia root rot
St Vincent	Witches' broom disease
Suriname	Witches' broom disease
Thailand	Vascular streak dieback
Togo	*Cacao swollen shoot virus* (CSSV) *Phytophthora megakarya*

Table 6.2. Summary of pest risk by country (cont'd).

Country	Pest risk
Trinidad and Tobago	Witches' broom disease *Phytophthora tropicalis* Rosellinia root rot Ceratocystis wilt *Cacao mild mosaic virus (CaMMV) and Cacao yellow vein-banding virus* (CYVBV) (formerly referred to as Trinidad Cocoa Virus A and B)
Uganda	Verticillium wilt
Venezuela	Witches' broom disease Moniliophthora pod rot (Western Venezuela) *Phytophthora capsici* *Phytophthora citrophthora* *Phytophthora heveae* *Phytophthora megasperma* *Phytophthora nicotianae* Ceratocystis wilt
Vietnam	Vascular streak dieback

Description of pests of cacao

7. Virus diseases

7.1 *Cacao necrosis virus* (CNV)*:* genus *Nepovirus*

Update by George A. Ameyaw and Owusu Domfeh

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Cacao necrosis virus: genus *Nepovirus* (CNV) is serologically distantly related to *Tomato black ring virus*.

7.1.1 Symptoms

Infected plants show veinal necrosis 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.

7.1.2 Geographical distribution

The disease is reported in Nigeria and Ghana (Owusu 1971, Thresh 1958).

7.1.3 Transmission

Possibly through a nematode vector (Kenten 1977). The same author reported seed transmission of up to 24% in the herbaceous hosts *Glycine max*, *Phaseolus lunatus* and *P. vulgaris*. Successful sap or mechanical transmission has also been reported by Adomako and Owusu (1974) using the technique developed for *Cacao swollen shoot virus*.

7.1.4 Particle morphology

Particles are isometric and of 25 nm diameter.

7.1.5 Therapy

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

7.1.6 Indexing

As for *Cacao swollen shoot virus*: Genus: *Badnavirus*. Graft onto Amelonado rootstock (sensitive cacao cultivar) and examine all parts of resulting plants for symptoms (See Section 5.2 Budwood).

7.1.7 References and further reading

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Figure 7.1.1. Veinal necrosis along midrib and main veins in a cacao leaf (G.A. Ameyaw; O. Domfeh, unpublished)

7.2 *Cacao swollen shoot virus* (CSSV): genus *Badnavirus*

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Many isolates of CSSV have been collected and maintained at the Virus Museum of the Cocoa Research Institute of Ghana. The isolates were previously named by capital letters or the name of the locality where they were first collected. Further analysis of CSSV molecular variability through advanced sequencing techniques have revealed at least eight species of the virus to be present across West Africa when using the International Committee on Taxonomy of Viruses recommendations, which consider nucleotide diversity in the RT/RNaseH region (Kouakou et al. 2012, Oro et al. 2012, Abrokwah et al. 2016, 2022, Chingandu et al. 2017, Muller et al. 2018, Muller et al. 2021, Ramos-Sobrinho et al. 2021, Ameyaw et al. 2024). *Cacao mottle leaf virus* is a synonym of the *Cacao swollen shoot Ghana M virus*

(CSSGMV) species (Brunt et al. 1996, Ramos-Sobrinho et al. 2021, Ameyaw et al. 2024).

7.2.1 Symptoms

Symptoms of the disease are highly variable and depend on the virus species and strain and the stage of infection. The most common symptoms on expressed sensitive cocoa varieties (e.g. West African Amelonado) include a characteristic red vein banding of the young (flush) leaves (Fig. 7.2.1), yellow vein banding, interveinal flecking and mottling of mature leaves (Fig. 7.2.2), vein clearing on leaves and stem swellings (Fig. 7.2.3). Some strains of the virus (e.g. some mild isolates and mottle leaf types) do not induce swellings in infected plants.

7.2.2 Geographical distribution

Benin, Côte d'Ivoire, Ghana, Liberia, Nigeria, Sierra Leone, Sri Lanka (see also section 7.6), Togo (Brunt et al. 1996, Kouakou et al. 2012, Oro et al. 2012, Abrokwah et al. 2016, Ameyaw et al. 2024).

7.2.3 Hosts

Natural infection with CSSV has been reported in *Adansonia digitata, Bombax* spp., *Ceiba pentandra, Cola chlamydantha, Cola gigantea, Theobroma cacao* and other tree species of the Malvaceae. *Corchorus* spp. have been infected experimentally.

7.2.4 Transmission

CSSV is transmitted by at least 14 species of mealybugs (Hemiptera: Pseudococcidae).

Whilst positive DNA PCR results using CSSV specific primers have been found in seedlings from self-pollinated infected trees, no expression of CSSV was found in such seedlings either visually or through reverse transcription (RT) PCR screening (Ameyaw et al. 2013). Subsequent screening of cocoa seeds harvested from infected plants have supported non-transmission of the virus through seeds. While there has been the recent discovery of integrated badnaviral sequences in most of the cacao genetic groups (Muller et al. 2021), there is no evidence of the integrated sequences resulting in infection of the cocoa plants. However, deliberate inoculation of cocoa seeds using viruliferous mealybugs or by sap/mechanical with purified viral particles results in high infection success in the resultant cocoa plants. These techniques are mainly utilised for laboratory transmission and investigation on the virus.

7.2.5 Particle morphology

Particles are bacilliform and measure 121-130 x 28 nm.

7.2.6 Therapy

There has not been any effective cure for the virus in infected cocoa plants over the years. Once a cocoa plant becomes infected it cannot be cured but remains systemically infected unless it is removed or dead. However, passage through somatic embryogenesis has been shown to reduce the viral load though further research is needed to establish whether or not this technique can be used to completely eliminate CSSV (Quainoo et al. 2008, Edward and Wetten 2016). Like most plant viral diseases, the disease can be largely contained or prevented if healthy plants are isolated within barriers of CSSV-immune crops.

7.2.7 Quarantine and detection measures

A variety of techniques have been successfully developed to detected CSSV including, ELISA, ISEM, LAMP and PCR (Sagemann et al. 1985, Muller 2008, Abrokwah et al. 2016, Ameyaw et al. 2021); also virobacterial agglutination has been utilized (Hughes and Ollennu 1993). Various other successful detection methods have been reported, and these have been reviewed (Dzahini-Obiatey 2008, Dzahini-Obiatey et al. 2008). While the efficacy of molecular monitoring for CSSV continues to improve, to date no fully isolate-independent detection technique has been produced and for this reason visual indexing is still recommended in combination with molecular -based screening. 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.). See Section 5.2.

7.2.8 References and further reading

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Figure 7.2.1. Red vein banding on young flush leaf. Note the fern-like pattern of the red vein banding. (H Dzahini-Obiatey and Y Adu-Ampomah, G.A. Ameyaw and Owusu Domfeh, unpublished)

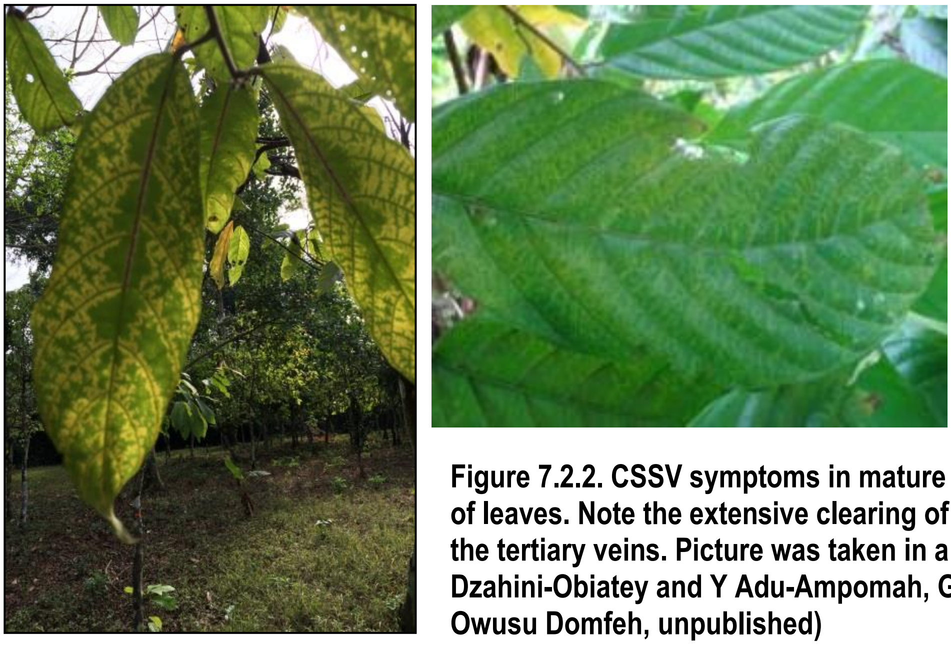

Figure 7.2.2. CSSV symptoms in mature leaves. Vein clearing of leaves. Note the extensive clearing of chlorophyll along the tertiary veins. Picture was taken in a farmer's field (H Dzahini-Obiatey and Y Adu-Ampomah, G.A. Ameyaw and Owusu Domfeh, unpublished)

Figure 7.2.3. Stem swellings. Note the club-shaped swelling on the basal chupon of an old tree. Picture was taken in an infected cocoa field (H Dzahini-Obiatey and Y Adu-Ampomah, GA Ameyaw and O Domfeh, unpublished)

7.3 *Cacao yellow mosaic virus*: genus *Tymovirus*

7.3.1 Geographical distribution

The virus is reported only in Sierra Leone (Blencowe et al. 1963, Brunt et al. 1965).

7.3.2 Symptoms

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

7.3.3 Transmission

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

7.3.4 Particle morphology

Particles are isometric and measure about 25 nm in diameter.

7.3.5 Therapy

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

7.3.6 Indexing

Refer to *Cacao swollen shoot virus* above and Section 5.2.

7.3.7 References and further reading

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7.4 *Cacao mild mosaic virus* (CaMMV) and *Cacao yellow vein banding virus* (CYVBV): genus *Badnavirus*

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Previously used names (Posnette 1944; Baker and Dale 1947)

CaMMV- *Red Mottle Virus; Cacao Trinidad Virus Strain A*

CYVBV- *Vein-Clearing Virus; Cacao Trinidad Virus Strain B*

7.4.1 Geographical distribution

Viruses on cacao were reported in Trinidad in 1943 (Posnette 1944) and named *Cacao Trinidad Virus Strain A* and *Strain B* (Baker and Dale 1947). They were present throughout the island until the 1950s, when the government initiated a tree removal programme targeting virus-infected cacao. After decades with no reports of symptomatic material, both viruses were found in cacao plants in 2007 (Sreenivasan

2009). CaMMV was confirmed in commercial production areas in Puerto Rico (Puig et al. 2020) and Brazil (Ramos-Sobrinho et al. 2021) and germplasm collections in USA and UK (Puig 2021, Ullah et al. 2021, Brill et al. 2024), indicating it may be widely distributed globally. In addition, a closely related virus was reported in Indonesia (Kandito et al. 2022), which was found to be widespread in the country (Kandito et al. 2024) Indonesian isolates shared 90-95% homology with those from the Americas based on the movement protein-coat protein (MP-CP) region of the genome. This is lower than the homology shared between known CaMMV isolates and sequencing of the RT-RNase H region is needed to determine if it meets the minimum 80% similarity used by ICTV to demarcate Badnavirus species. Virus-like symptoms have been reported in other cocoa growing areas in the region, including Colombia, Dominican Republic, and Venezuela but those have not yet been characterized. To date, reports of CYVBV have been confined to Trinidad.

7.4.2 Hosts

Numerous alternative hosts have been identified for CaMMV and could be serving as inoculum reservoirs. These include relatives in the Malvaceae family such as *Theobroma grandiflorum, Hibiscus sp.,* and *Thespesia grandiflora* (Puig et al. 2024). No alternative hosts are known for CYVBV.

7.4.3 Symptoms

Although CaMMV and CYVBV cause less damage than some CSSV strains in West Africa, early researchers in Trinidad reported reduced yield and branch dieback on infected trees (Baker and Dale 1947, Cope 1953). A preliminary study recently found that CaMMV-infected seedlings were more susceptible to disease caused by *Lasiodiplodia theobromae*, one of the most common pathogens causing dieback on cacao (Puig 2022). No stem swelling has been observed, but infected plants develop a range of leaf and pod symptoms.

Pods on trees infected with CaMMV can develop mosaic, mottling, chlorotic islands, and abnormal shape (Fig 7.4.1). Common leaf symptoms include red vein banding, pink pigmentation near veins and margins, mosaic, and yellow vein banding (Fig 7.4.2). Red mottling, the symptom this virus was originally named for, can develop on both leaves and pods.

In contrast, CYVBV is characterized by persistent yellow vein-banding in major and minor veins of the mature leaves that may be accompanied by red vein-banding.

7.4.4 Transmission

Both viruses are transmitted by several mealybug species and the use of infected material during grafting, even from asymptomatic tissue. *Planococcus citri*, is considered the primary vector in Trinidad due to its abundance, mobility, and ability to transmit both CaMMV and CYVBV. Four additional species were confirmed as vectors of CaMMV: *Dysmicoccus brevipes, D. sp. near brevipes, Ferrisia virgata*, and *Pseudococcus comstocki*. However, infections were characterized by longer latent periods than observed for CSSV. When infectious mealybugs were allowed to feed on cacao beans prior to planting, latent periods ranged from 40 to 178 days in CaMMV transmission studies (Kirkpatrick 1950, Kirkpatrick 1953). Few transmission studies have been done with CYVBV, so only two species (*Pl. citri* and *D. sp. near brevipes*) have been confirmed as vectors. In those studies, symptoms appeared 41-91 days after feeding. No transmission of CYVBV was observed with *D. brevipes* (Kirkpatrick 1950).

Following graft transmission, virus symptoms appear when new leaves (flush) are produced. Transmission experiments showed incubation periods of 34-125 days with CaMMV, and 45-136 days with CYVBV (Posnette 1944; Baker and Dale 1947). Since these viruses are unevenly distributed in cacao trees, not all budwood taken from infected plants will transmit the virus. Early transmission tests showed that approximately 50% of grafted trees developed virus symptoms when budwood from infected trees was used in propagation (Posnette 1944).

In Florida, Puig et al. (2021) found *Pseudococcus jackbeardsleyi*, *Maconellicoccus hirsutus, Ps. comstocki,* and *F. virgata* feeding on cacao trees infected with CaMMV (listed in decreasing order of abundance). Although *P. jackbeardsleyi* and *M. hirsutus*, have been reported on CSSV-infected cacao in Cote d'Ivoire (N'Guessan et al. 2019), their ability to transmit cacao viruses has not been assessed. Virus acquisition was estimated from mealybug DNA using a recently developed nested PCR (Puig 2021b), and CaMMV sequences were obtained from a subset (34.6 to 44.6%) of all four species. Additional tests are needed to determine whether *P. jackbeardsleyi* and *M. hirsutus* can transmit the virus.

Recently, seed transmission was reported from mother plants infected with CaMMV (Puig 2021a). In transmission studies conducted with seed from highly symptomatic pods from Florida (USA) 57.6 and 64.3% of seedlings tested positive for CaMMV six and twelve weeks after planting, respectively. Although most plants developed symptoms such as leaf mosaic and vein banding, these were often only present on a subset of leaves (Puig 2021a). A subsequent study using only mildly symptomatic pods from Hawaii and Puerto Rico, showed transmission rates of 17.5 and 41.7% for pods from each location, respectively (Puig et al. 2023). No information is available on seed transmission of CYVBV.

Integrated badnaviral sequences, either as single or multiple copies, were recently reported in asymptomatic cacao plants belonging to multiple genetic groups (Muller et al. 2021, Ullah and Dunwell 2023). These integrated sequences, which vary from a partial to a whole genome, are significantly different from those known to cause disease and are referred to as eTcBV1 and eTcBV2 for endogenous *Theobroma cacao* bacilliform virus 1 and 2. Although they are not believed to be infective, these integrated sequences have been shown to affect the expression of a host gene. The sequences detected so far are most similar to a region of the CYVBV genome (up to 72.5% nucleotide identity). However, there is no evidence specifically of CaMMV or CYVBV integrating into the genome of T. cacao (Chingandu et al. 2017).

7.4.5 Particle morphology

Virus particles have not been visualized in CaMMV or CYVBV-infected tissue. They are assumed to have morphology characteristic of the Badnavirus genus.

7.4.6 Therapy

None. Infected plants cannot be cured. Virus elimination from infected budwood was attempted using high temperature treatments (Posnette 1944) but was not successful.

7.4.7 Quarantine and Detection Methods

Multiple primer pairs are available for PCR detection of CaMMV, including a nested PCR capable of detecting multiple different strains. Results from leaf tissue assays indicate that the virus is unevenly distributed, and that petiole tissue should be used in molecular diagnostics (Puig 2021b). However, due to the high genetic variability found in CaMMV, some strains may not be detectable with currently available primers.

To avoid false-positives due to the presence of integrated badnaviral sequences, screening should be done with primers specifically designed for CaMMV and CYVBV. Amplicon identity can be confirmed through Sanger sequencing.

The current bioassay, where budwood is grafted onto a susceptible indicator plant (ICS 6 or Amelonado), should still be used alongside molecular tools. In addition to the leaf symptoms described above, Amelonado plants may also produce nearly white leaves following grafting with infected budwood (Puig, unpublished). A novel calorimetric Loop-mediated isothermal amplification (LAMP) assay for detection of CYVBV has been developed (Ullah et al. 2021).

Due to evidence of seed transmission of CaMMV, care must be taken when transporting pods. In areas where CaMMV is present, seeds grown for rootstock must only be taken from trees that have been screened for the virus. No studies exist with regards to the seed transmission of CYVBV.

Figure 7.4.1. Pods on trees infected with CaMMV display a range of symptoms, such as (a) red mosaic, (b) mottling, (c) chlorotic islands, and (d) abnormal shape (AS Puig, unpublished)

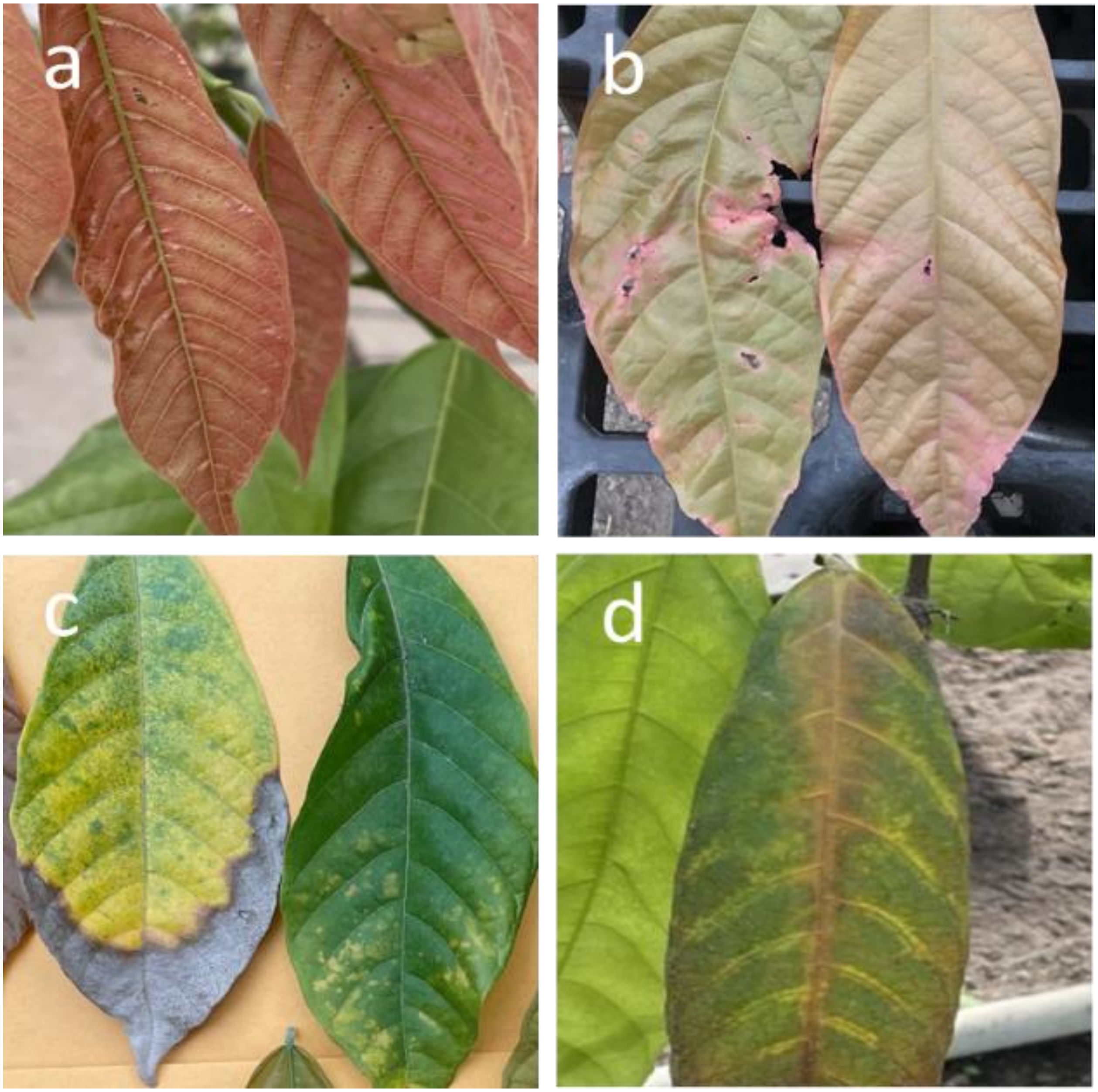

Figure 7.4.2. Foliar symptoms on trees infected with CaMMV: (a) red vein banding on young leaves, (b) pink pigmentation near veins and leaf margins, (c) mosaic on mature leaves, and (d) yellow vein banding and necrosis on midrib (AS Puig, unpublished)

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7.5 *Cacao polerovirus* (CaPV), Cacao Leaf Roll Virus (CaLRV): genus *Polerovirus*

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Recently, several studies based on molecular techniques and bioinformatics approaches have reported the detection of species from a positive-strand RNA virus, a member of the genus Polerovirus, in cacao plants. Polerovirus is a diverse genus infecting a wide range of plants, from monocots to eudicots. Polerovirus-like partial sequences, designated as cacao leafroll virus (CaLRV),were first reported in four cacao plants within the USDA-ARS-SHRS cacao quarantine facilities (Adegbola et al. 2023). A cotton leafroll dwarf virus (CLRDV) was reported from cacao trees in Bahia, Brazil showing virus-like symptoms (Ramos-Sobrinho et al. 2022). Illumina sequencing and RT-PCR of the cacao leaf samples confirmed that the viruses detected are closely related. A further detailed study of the occurrence of this virus in cacao was based on a combination of bioinformatic analysis of the Sequence Read Archive (SRA) database (samples originally from IC3 CATIE) and molecular analysis of leaf samples of plants within the ICQC,R (Ullah et al. 2023). This analysis identified virus-positive plants from a wide range of diverse germplasm. The main conclusions from this study, in terms of molecular characterization, were subsequently confirmed by Adegbola et al. (2024) who studied samples from symptomatic plants of five accessions held at USDA-ARS-SHRS, and repeated the designation of CaLRV.

7.5.1 Geographical distribution

Although the virus has only been identified in samples originating from the Americas (Costa Rica, Brazil) to date, its occurrence in other countries has not yet been investigated.

7.5.2 Hosts

Alternative hosts have not yet been reported though the closely related CLRDV was found in 23 species of weeds growing adjacent to cotton fields in Georgia, USA (Sedham et al. 2021).

7.5.3 Symptoms

Some plants testing positive for the Polerovirus have exhibited virus-like symptoms such as leaf mosaicism, vein clearing and yellow spots (Ramos-Sobrinho et al. 2022), or leaf distortion with downward rolling of leaves, leaf discoloration and malformation (Adegbola et al. 2023, 2024). However, there is no evidence as yet that definitively links the presence of the virus to any specific symptoms, since the CaPV virus has been detected in asymptomatic plants as well as in those with vein clearing (Ullah et al. 2023) and, moreover, some leaves showing similar symptoms do not test positive for the virus (Ramos-Sobrinho et al. 2023, Ullah et al. 2023). It is possible that symptomology may be caused by a combination of virus(es), cacao genotype and nutritional or other environmental stress(es). To date, it is not known what impact (if any) the virus has on cacao growth and yield.

7.5.4 Transmission

Poleroviruses such as cotton leafroll dwarf virus (CLRDV) are known to be transmitted by aphids (Edula et al. 2023, Hoffman et al. 2001), though there is no such experimental evidence from cacao. There is no information concerning graft or seed transmissibility.

7.5.5 Particle morphology

No studies have yet been undertaken to visualize virus particles in CaPV infected tissue. They are assumed to have morphology characteristic of the Polerovirus genus.

7.5.6 Therapy

No studies on methods to eliminate the virus from infected plants have been reported

7.5.7 Quarantine and Detection Methods

The variability in, or indeed lack of, visual symptoms in infected materials makes this virus difficult to detect using standard virus indexing procedures. Multiplex RT-PCR assays that include a CaPV specific primer set and a cacao Acyl Carrier Protein1 (ACP1) primer set as an internal control are described in Ullah et al. (2023).

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7.6 Other viruses and virus-like diseases

Update by Alina S. Puig

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Mosaic virus was reported in Indonesia in 1962 and was thought to be similar to the Cacao swollen shoot viruses in West Africa. Early work by H. Semangun showed it was transmitted through grafting and mealybug vectors. The virus particles visualized in infected trees were bacilliform, which is typical for the Badnavirus genus (Kenten and Woods 1976, Probowati 2019). Symptoms include red vein banding, mosaic, and chlorotic feathering on leaves; however, no stem swelling has been observed on infected trees in Indonesia. Probowati et al. (2019) showed that sequences obtained from infected plants in Indonesia closely resembled virus sequences from West Africa such as Cacao swollen shoot Togo A virus (AJ781003) and the New Juaben isolate of CSSV (AJ608931).

In Sri Lanka (formerly known as Ceylon), cacao trees with leaf mosaic and stem swelling symptoms have been documented (Peiris 1953, Orellana and Peiris 1957). Laboratory assays found that *Planococcus citri* and *Planococcus lilacinus*, the most prevalent mealybugs in the area, could transmit the virus (Carter 1956). In 2018, a complete virus genome (7215bp) was obtained from a symptomatic leaf from Sri Lanka (Muller et al. 2018). This new species was named cacao bacilliform Sri Lanka virus (CBSLV) and shared 65.9% nucleotide identity with the genome of the Gha25-15 isolate of Cacao swollen shoot Togo A (MF642716).

Virus-like diseases have been reported on cacao in Venezuela, Colombia, and the Dominican Republic (Posnette and Palma 1944, Ciferri 1948). Transmission tests were conducted in the Dominican Republic, and the disease was shown to be graft transmissible (Ciferri 1948). However, no additional studies have been conducted.

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8. Fungal and oomycete diseases

Of the different diseases affecting cacao crops, fungal and oomycete diseases pose a major constraint. Some have a worldwide distribution and others are restricted to cacao-growing regions of the Americas, Africa and Southeast Asia. In the following sections, different experts have summarized basic information on different diseases considered of economic importance. A summary of research results for black pod, *Moniliophthora* pod rot and witches' broom diseases was published by Fulton (1989) and a comprehensive review of cocoa pathogens is available in Bailey and Meinhardt (2016).

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8.1 Witches' broom disease of cacao

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8.1.1 Causal agent

Moniliophthora perniciosa (Stahel) Aime & Phillips-Mora (Syn. *Crinipellis perniciosa*) causes a destructive disease known as witches' broom disease of cacao (WBD). WBD has led to significant declines in cacao production and, in some cases, has resulted in the complete abandonment of plantations in the regions it has affected. Although variability exists with the fungus, there are two main biotypes, the C (on cacao and its relatives) and S-biotype (on Solanaceae). Within the C-biotype, variants seem to occur according to their country of origin (e.g. Ecuador, Peru, Brazil, Bolivia).

8.1.2 Symptoms

Moniliophthora perniciosa can infect all actively growing tissues (shoots, flower cushions, pods (fruits)), inducing various symptoms that depend on the infected plant organ. The fungus has a long incubation period (usually 4-6 weeks) from initial penetration to the appearance of symptoms; shorter for systemic flower infections. The typical symptoms are the vegetative brooms (plagiotropic/

orthotropic broom) that develop following infection of an active apical bud on an elongating shoot giving rise to terminal and axillary brooms. Stem, petiole or pulvinus swellings are formed following infection of the main axis at an internode or node involved. Death of the pulvinus tissue causes the leaf to die without abscising, giving a necrotic leaf. Brooms are initially green and become necrotic after several weeks. Necrotic brooms may remain attached, or they may fall into the canopy or to the soil surface. Witches´ broom symptoms are shown in Fig. 8.1.1 and Fig. 8.1.2.

Infection of flower cushion may remain asymptomatic or produce vegetative brooms, abnormal flowers, carrot- and strawberry-shaped fruit (chirimoya; seedless fruit) (Fig. 8.1.2 C).

Pods can be infected at any stage, being most susceptible when young (0- to 2-months old). Infected pods suffer hypertrophy, distortion, early ripening, and external necrosis, which sets in before the pod reaches full size, and spreads rapidly over the whole surface. External necrosis develops on directly infected mature pods from 11-13 weeks after penetration by the fungus. The form of this necrosis depends on the age of the fruit when infected and the genotype of the tree. The infected areas may remain as swellings as the rest of the fruit dies. Levels of internal damage depend on when the infection occurs and can vary from watery rot to a dry compacted bean mass. Although, in most cases, the seeds become partly/completely cemented to each other and the fruit wall, infections of maturing pods can result in localized necrotic areas on the fruit walls with some seed retaining viability. For details on disease symptomatology, see Purdy and Schmidt (1996) and Silva et al. (2002).

8.1.3 Geographical distribution

Originally from the Amazon Basin, WBD was first reported in 1895 in Suriname and rapidly spread over the next 30 years to the producing regions near the Amazon Basin. The disease is currently present in Bolivia, Belize (unsubstantiated report), Brazil (Bahia, Pará, Rondônia, Espirito Santo, Amazonian regions, Mato Grosso, Minas Gerais (S-biotype, see below), Colombia, Ecuador, French Guiana, Grenada, Guyana, Panama (South of Panama canal), Peru, St. Lucia (Kelly et al. 2009), St. Vincent, Suriname, Trinidad and Tobago and Venezuela. In 2019, a pink-pigmented mushroom was found fruiting on unidentified branches in Angola’s Serra Vamba which was subsequently identified as *Moniliophthora perniciosa*, allied within the C-biotype of the species and closely related to the types found in Latin America (Aime et al. 2024). Although Angola is not a major cocoa producer, the presence of the fungus in the Eastern Hemisphere is a concern, pointing to the need for awareness raising of the potential threat to the developing cocoa growing industry in Angola and neighbouring countries, further surveillance and introduction of measures to prevent its spread.

8.1.4 Hosts

The fungus is endemic to the Amazon Region, not only in native but also in cultivated cacao. The species *M. perniciosa* consists of geographically separated populations (Ploetz et al. 2005, Patrocinio et al. 2017*)* that infect a broad range of hosts. Based on host specificity, the fungus has been grouped into four biotypes according to their host range: C (Malvaceae); H (Malpighiaceae); L (Bignoniaceae), and S (Solanaceae). The most important hosts are species from the **Malvaceae Family:** *Theobroma cacao* (cacao), *T. grandiflorum* (cupuaçu), *T. sylvestris*, *T. obovata*, *T. bicolor*, *Herrania* spp.

Alternative hosts include *Bixa orellana* (Family: **Bixaceae),** *Solanum cernuum, S. grandiflorum* var. *Setosum*, *S. paniculatum L. (jurubeba), and S. stipulaceum*, (caiçara), *S. lasianterum*, *S. rugosum*, *S. lycocarpum* (tomato), *S. melongena* (eggplant), *Capsicum annuum L. (pepper)*, *C. frutescens* (hot pepper), *Athenaeum pogogena* (Family: **Solanaceae**); *Banisteriopsis caapi*, *Mascagnia* cf. *Sepium*, *Stigmaphyllon blanchetti*, (Family: **Malpighiaceae);** *Arrabidaea verrucosa (*Family: **Bignoniaceae).**

For a review of the occurrence of *M. perniciosa* on putative hosts, see De Souza et al. (2018), Evans (2016), Lisboa et al. (2020), Patrocínio et al. (2017).

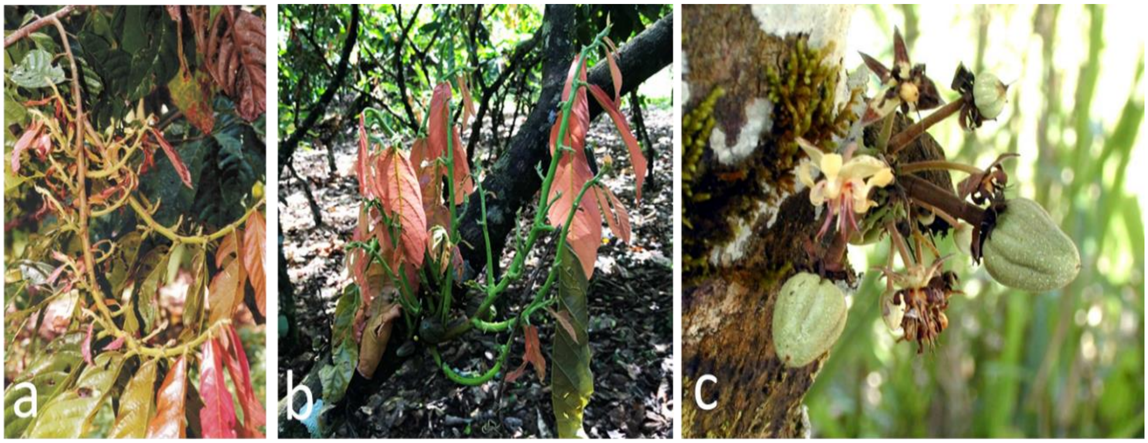

Figure 8.1.1. Field symptoms of WBD: a) canopy vegetative broom, b) vegetative broom developed from a flower cushion, c) strawberry fruits. Source: Ceplac/Cepec/Fitomol-Lab

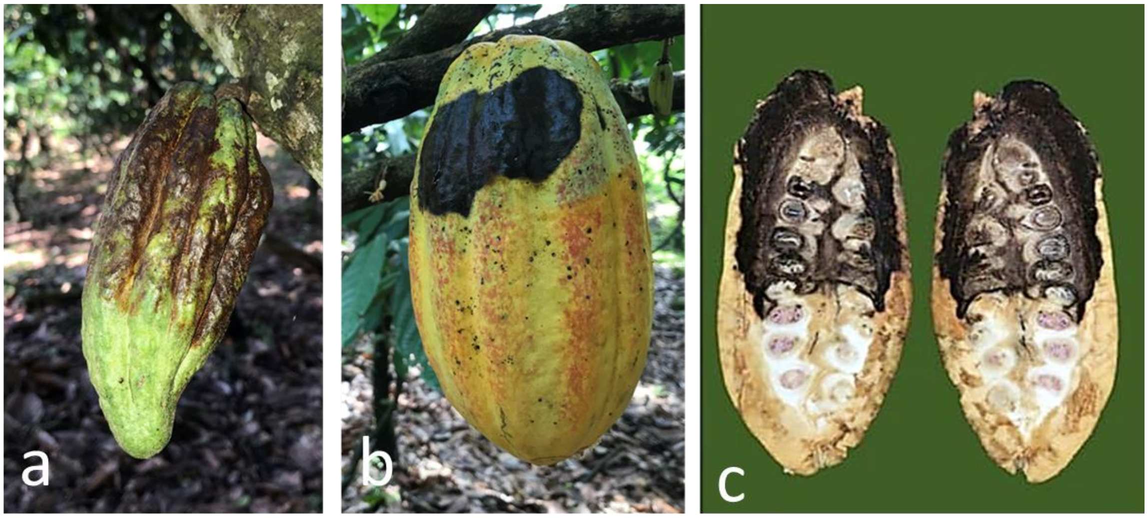

Figure 8.1.2. Witches' broom symptoms on fruits: a) swellings and distortion, b) external necrotic lesion, and c) internal necrosis with watery beans. Source: Ceplac/Cepec/Fitomol-Lab

8.1.5 Biology

M. perniciosa is a hemibiotrophic, homothallic fungus with a long biotrophic phase (45–60 days) (Purdy and Schmidt 2006). Basidiospores, the only infective propagules, are produced on basidia located on the lower side of caps of pink/reddish mushroom-like Basidiocarps (basidiomata) about 4–8 weeks after the onset of rain. Basidiocarps can only form on necrotic tissues, such as stem, seeds/beans, leaf vein, or fruit tissue that has undergone necrosis (Fig. 8.1.3 A-C).

Wind is the primary mode of spore dissemination, although dispersal can also occur by water and human beings. Since the basidiospores are sensitive to ultraviolet light and are easily desiccated, they quickly lose their germination capacity (Purdy and Schmidt, 1996). Therefore, the pathogen is only likely to be dispersed over long distances by introducing diseased material through anthropogenic action since the fungus can survive as hidden infections in parts of the plant (See Quarantine Precautions section below). Spores have multiple penetration modes and can infect directly through the epidermis, base of trichomes, and/or stomata (Sena et al. 2014, Meraz-Pérez et al. 2021). Soon after infection, the pathogen establishes a biotrophic phase. However, the infection may become latent, and symptoms will develop when the plant restarts growth under suitable conditions of humidity (80%) and temperature (25°C) (Purdy and Schmidt 1996, Silva et al. 2002). The length of the biotrophic phase will vary according to factors such as the WBD strain, host genotype, plant nutrition, and environmental conditions. Following the switch to the necrotrophic phase, *M. perniciosa* survives as a saprophyte in dry brooms, mummified pods, flower cushions, and infected dormant buds. Such infections are of epidemiological importance as they allow the survival of the fungus between successive periods of plant growth and fruiting. Although chlamydospores have been reported in dry brooms, their role in the life cycle is not well understood. However, they may represent a dormant phase following host infection (Meinhardt et al. 2008).

Figure 8.1.3. Basidiocarp production on (a) cacao necrotic fruit, (b) cacao leaf vein and (c) cacao stem (dry brooms) of cacao.

8.1.6 Detection methods

In some cases, symptoms related to WBD are very similar to other cocoa diseases, such as black pod rot (*Phytophthora* spp.) and frosty pod rot (*M. roreri*) in cocoa. Isolation and identification of pathogens from diseased samples require specialized knowledge.

The species is identified through a combination of morphological and molecular analyses. The molecular diagnostics target three specific loci—the 28S ribosomal RNA gene (28S), the internal transcribed spacer region (ITS) of the rDNA repeat, and the translation elongation factor 1-α (EF1α) specifically designed for members of the Marasmiaceae family (Aime and Phillips-Mora 2005). Species-specific PCR for identifying *M. perniciosa* from pure culture is also available (da Silva et al. 2022).

8.1.7 Quarantine measures

The following plant parts are likely to carry the pathogen in trade and transport:

- Fruits (Pods): Presenting fruiting bodies (externally) and/or hyphae (internally).

- Leaves: Presenting hyphae (internally).
- Stems (above ground)/shoots/trunks/branches: Presenting fruiting bodies (externally) and/or hyphae (internally); usually invisible to naked eye.
- Seeds: Presenting hyphae (internally), invisible to naked eye.

Movement of these plant parts into disease-free areas within a country or region is not recommended unless the material can be transferred through a quarantine facility. The occurrence of sub-populations within the C biotype (e.g., according to their country: Ecuador, Peru, Brazil, Bolivia) with different levels of virulence, plus the potential for cross-pathogenicity between biotypes, make quarantine precautions essential even when moving plant material between areas where WBD is already present. For the same reason, the exchange of diseased material and isolates of the fungus for research between regions/countries is not recommended.

Moniliophthora perniciosa may be seed-transmitted; however, germplasm movement as seed is the safest method of moving germplasm. Seeds should be collected from apparently healthy pods and treated with copper fungicide or a recommended fungicide to reduce the risk of pathogen transmission.

It is recommended that newly introduced material is grown in isolation glasshouses under strict supervision in a quarantine station for at least a year to ensure that plants are disease-free before being released for general use.

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8.2 *Moniliophthora* pod rot (frosty pod rot or moniliasis disease)

Update by Wilbert Phillips-Mora

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8.2.1 Causal agent

Moniliophthora roreri (Cif.) H.C. Evans, Stalpers, Samson & Benny.

8.2.2 Symptoms

Under natural conditions the disease affects only the pods, which are often infected when they are young (0-3 months old) and become less susceptible as they mature. Fruits that are infected very early in their development promptly die. The fungus has a long incubation period (3-4 weeks) from initial penetration to the appearance of symptoms.

External fruit symptoms: may include small water-soaked lesions, which enlarge into necrotic areas with irregular borders; one or more swellings (Fig. 8.2.1) and premature ripening showing different patterns of green and yellow mosaics. A white fungal stroma (Fig. 8.2.2) covers the necrotic area within 3-5 days, with profuse formation of cream to light brown spores. This is the most characteristic stage of the disease in the field. After a period of approximately three months, the infected pods become dry and mummified on the trees and remain attached to the trunk for long periods (Fig. 8.2.3). These pods are a major source of inoculum responsible for new waves of infection of the disease over a considerable period of time.

Internal fruit symptoms: Infected cherelles fail to develop seeds and are filled with gelatinous, disorganised tissues. When the infection occurs at a later stage, fruit tissues including parts of the husk, placenta, pulp and the beans appear to form a compact, homogenous mass, in which it is difficult to distinguish the component parts. These tissues are surrounded by a decayed watery substance as a result of tissue maceration, which makes the pods weigh more than healthy ones. The beans may be partially or completely destroyed, depending on the stage of maturation when infection occurs.

8.2.3 Geographical distribution

M. roreri was confined to northwestern South America until the 1950s. Its appearance in Panama in 1956 signalled a change in its geographic distribution. Now, it is found in 14 countries in tropical America. The disease is present in Colombia and Ecuador on both sides of the Andes, western Venezuela, Peru, Panama, Costa Rica, Nicaragua, Honduras, Guatemala, Belize, Bolivia, Mexico (Phillips-Mora et al. 2007) and El Salvador (Phillips-Mora et al. 2010). It was first detected in the Caribbean in Jamaica in 2016 (IPPC 2016, Johnson et al. 2017). In Brazil, it was first reported in an urban area of Acre State, close to the Peruvian border in 2021 (CEPLAC 2021). In 2022, the pathogen was detected attacking *Theobroma* and *Herrania* species in other cities, such as Tabatinga and Benjamim Constant, in Amazonas State, close to the Peruvian and Colombian borders (Personal communication, Ana Francisca Tiburcia, Universidad Federal de Amazonas).

8.2.4 Hosts

Apparently, all species of the closely related genera *Theobroma* and *Herrania*, the most important being the cultivated species *T. cacao* (cacao) and *T. grandiflorum* (cupuaçu) and *T. bicolor* (pataxte).

8.2.5 Biology

M. roreri is most commonly believed to be an anamorphic fungus. However, a cytological mechanism that enables it to undergo sexual reproduction has been described (Evans et al. 2002), which apparently is not very active in nature.

M. *roreri* is a hemibiotroph with a long biotrophic phase (45–90 days) (Bailey et al. 2018). Spores, which are produced in great abundance on diseased pods, are the only infective propagules of *M. roreri*, and natural infections have only been observed on fruits. Spores are viable for several weeks and can withstand exposure to sunlight. The dry powdery masses of spores are efficiently dispersed into the air by any physical contact with the infected pod (Evans 1981). Wind is the main mode of spore dissemination, although dispersal can also occur by water, insects, human beings and other animals. Disease transmission by infected seeds has not been observed and is most unlikely. Spores germinate and penetrate the pod at all stages of development, directly through the epidermis or via stomata, without the presence of wounds (Suárez 1972).

8.2.6 Quarantine measures

The following plant parts are likely to carry the pathogen in trade and transport:

- Fruits (inc. Pods): external hyphae and spores visible to the naked eye; borne internally
- Stems (above ground)/shoots/trunks/branches: Spores.
- Seeds: not normally seedborne but spores may be carried on surface.

The aggressiveness of *M. roreri*, its capacity to survive different environmental conditions, its rapid natural dispersal, its propensity for man-mediated dispersal, and the susceptibility of most commercial cacao genotypes, all indicate that the fungus presents a substantial threat to cacao cultivation worldwide (Phillips-Mora and Wilkinson 2007).

Human beings are responsible for disease dispersal over significant distances and geographical barriers and hidden infections can have a very important role in disseminating the disease into new areas. In addition to the precautions that should be taken when moving plant material described below, it should be noted that spores can also survive on clothing, footwear and on the human body. Therefore, after visiting an infected area, appropriate measures need to be taken before entering an uninfected region (discarding or appropriate washing of the clothes, footwear and equipment used, avoiding visiting disease-free areas for a period of time, etc.).

Since the fruits are the only parts of the cacao plant to be infected by *M. roreri* under natural conditions, most quarantine efforts have to be concentrated on preventing the movement of fruits from affected places into new farms, territories and countries.

The disease is not internally seed borne. However, the long-lived spores can be transported on entire plants or their parts (seeds, leaves, budwood, etc.). The powdery spores would readily adhere to such tissues and remain viable in this situation for many months. Consequently, movement of these parts into disease-free areas should only be carried out following a disinfection protocol. Fungicide treatment would certainly reduce the inoculum and considerably limit the chances of an unwanted introduction.

Resistant clones have been identified from different genetic and geographic origins, some examples are EET-233, GU-133 C, NA-807, PA-169, RB-33/3, UF-273, and UF-712 (Phillips-Mora et al. 2017). Hybrids among some of these clones, such as CATIE-R1, CATIE R-4 and CATIE-R6 were released for farmer use in Central America in 2007 with very good success, confirming that the use of resistant cultivars is the most appropriated and cost-effective means of managing the disease.

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Figure 8.2.1. *Moniliophthora* pod rot: swellings characteristic of infection on young pods (W Phillips-Mora and A Mora, CATIE, Costa Rica)

Figure 8.2.2. Left: premature ripening, necrosis and white, young pseudostroma on large pod infected by *M. roreri*. Right: healthy green pod (W Phillips-Mora and A Mora, CATIE, Costa Rica)

Figure 8.2.3. (left) *Moniliophthora* pod rot: seed necrosis and early ripening of infected pods (W Phillips-Mora and A Mora, CATIE, Costa Rica)

Figure 8.2.4. *Moniliophthora* pod rot: evolution of the disease from a necrotic spot to a sporulated lesion, and a dried mummified pod.
(W Phillips-Mora, CATIE, Costa Rica)

8.3 *Phytophthora* spp.

Update by G Martijn ten Hoopen[1], S Nyassé[2] and R Umaharan[3]

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8.3.1 Causal agents

Phytophthora palmivora, P. megakarya, P. citrophthora. P. tropicalis (*P. capsici*) and occasionally other *Phytophthora* species such as *P. heveae*, *P. megasperma*, *P. nicotianae* var *parasitica. P. katsurae, P. meadii, P. botryosa* (Surujdeo-Maharaj et al. 2016) and *P. theobromicola* sp. nov (Decloquement et al. 2021). However, only the first four species are currently considered of commercial importance.

8.3.2 Alternative hosts

Phytophthora palmivora – a very large number and wide variety of plant species, including coconut, papaya, *Citrus* spp., *Hevea, m*ango, pepper (*Capsicum* spp.) oil palm, and tomato.

P. tropicalis, previously thought to be conspecific with *P. capsici*, it seems that *P. tropicalis* is more commonly recovered from woody perennials, including cacao, than *P. capsici* (Surujdeo-Maharaj et al. 2016).

P. capsici – among other peppers, cucurbit crops and tomato (see e.g. Tian & Babadoost, 2004).

P. citrophthora – among others *Citrus* spp., cucurbit crops, rubber (*Hevea*)

P. megakarya – putative alternative hosts – *Cola nitida* (Nyassé et al., 1999), *Irvingia* spp. (Holmes et al., 2003) *Funtumia elastica*, *Sterculia tragacantha*, *Dracaena mannii* and *Ricinodendron heudelotii* (Opuku et al. 2002, Bailey et al. 2016). Recently Akrofi et al. (2015) recovered the pathogen from asymptomatic roots of numerous other species in cacao plantations, including Pineapple, *Athyrium nipponicum*, Papaya, Mango, Avocado, Cocoyam (*Xanthosoma sagittifolium*), Taro (*Colocasia esculentum*), Oil palm and even banana.

Many of the alternative hosts of the above-mentioned *Phytophthora* species are often found in close association with cacao.

For a general overview of *Phytophthora* spp. affecting cacao see also Surujdeo-Maharaj et al. (2016) and Bailey et al. (2016). For more information on crops affected by different *Phytophthora* spp. see e.g. Erwin and Ribeiro (1996), the CABI Crop Protection Compendium (https://www.cabi.org/cpc/) and the USDA-ARS fungal database (https://nt.ars-grin.gov/fungaldatabases/).

8.3.3 Symptoms

Phytophthora spp. can attack all parts of the cacao plant (although this is somewhat species dependent) but the main manifestations of infection are:

- Pod rot – a firm brown rot of the pod (Fig. 8.3.1) (economically speaking the most important aspect of *Phytophthora* induced disease). Pods of all stages of development can be affected. Infections can be initiated by sporangia, chlamydospores and zoospores and disease symptoms normally appear within 3-4 days after infection.
- Stem canker – dark sunken lesions on the stem (Fig. 8.3.2). Stem canker often develops as a result of mycelial spread from pods into flower cushions and further along the stem or directly through wounds.
- Leaf and seedling blight – extensive necrosis of leaves and shoots of seedlings (Fig. 8.3.3).
- Flower cushion infection
- Root infection

8.3.4 Geographical distribution

Phytophthora is present in all cocoa growing countries/regions in the world although the different species attacking cocoa mostly have restricted distributions. At least eleven species of *Phytophthora* have been identified on cacao (Surujdeo-Maharaj et al. 2016 and references therein). *Phytophthora palmivora* has a pantropical distribution. *Phytophthora megakarya* is the only known *Phytophthora* species attacking cocoa, originating from Africa. It is present in Gabon, São Tomé and Principe, Bioko (formerly Fernando Po), Cameroon, Nigeria, Togo, Ghana and Côte d'Ivoire. However, in Ghana and Côte d'Ivoire, the two biggest cacao producers worldwide, *P. megakarya* is still in an invasive phase. For more information on *Phytophthora* species present in Sub-saharan Africa see Bose et al. (2023). *P. tropicalis*/*P. capsici* is found in the Americas, Caribbean, Asia and Africa (e.g. Brazil, Cameroon Dominican Republic, El Salvador, Guatemala, India, Jamaica, Mexico, Trinidad, Venezuela), whereas *P. citrophthora* is present on cacao in the Americas and Asia (e.g. Brazil, Mexico, India, Indonesia). *P. megasperma* has been found in Venezuela, *P. nicotianae* var. *parasitica* in Cuba, *P. heveae*, in Malaysia and *P. theobromicola* sp. nov. has recently been described from Brazil.

8.3.5 Biology

The activity of *Phytophthora* spp. is very much associated with wet and humid conditions, although the soil often 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. Major human activities that may spread *Phytophthora* spp. are road building, timber harvesting, mine exploration, nursery trade and hiking/bushwalking.

8.3.6 Quarantine measures

The following plant parts are likely to carry the pathogen in trade and transport:

- Fruits (pods) – Infection is invisible during early stages of pod infection but later stages are easily recognizable due to pod lesions (firm, dark brown spots) and zoospore production on lesions (Fig. 8.3.1).
- Roots (*Phytophthora* is often found associated with roots of cacao) – infection is invisible to the naked eye.
- Budwood
- Trunk/branches - especially when cankers are present (Appiah et al. 2004).
- Leaves
- Growth media accompanying plants, especially soil, can carry *Phytophthora* inoculum.

Pods: Generally speaking, pods should not be used for germplasm transfer. However, if pods are used they should be quarantined for the duration of at least one week before shipping and distribution. Since *Phytophthora* symptoms appear after only a few days, diseased pods should be easily recognizable within this one-week period and can subsequently be destroyed. To reduce risk further, pods should be put into a pesticide bath (e.g. a mix of Metalaxl-M and a Copper compound) before distribution.

Whole plants (with soil): Whole plants (with soil) - the transfer of whole plants represents an extremely high risk, particularly if they are in soil. Movement of whole plants (even symptomless plants) within a country or region where *Phytophthora* spp. are still in an invasive phase, is NOT recommended unless the material can be transferred through a quarantine facility.

Budwood: Only budwood from (apparently) healthy trees should be used. No collection should be done from trees with cankers or any other signs of disease. Since *Phytophthora* zoospores are relatively short-lived and susceptible to pesticides and drought, the risk of dispersal of *Phytophthora* propagules possibly present on budwood can be further reduced with a pesticide application/bath (e.g. a mix of Mefenoxam and a Copper compound) (Opoku et al. 2007).

Leaves: *Phytophthora* can be present on leaves. Leaves and plants showing symptoms of blight (Fig. 8.3.3) should not be used for transfer. *Phytophthora* propagules may survive for short periods of time on top of leaves. Pesticide treatments and storage under dry conditions should be sufficient to eliminate this risk.

Transport by Humans: Human beings are the most likely culprits for long range dispersal of *Phytophthora* either by not taking care when transporting plant materials (pods, budwood etc), food crops such as cocoyam, corms and plantain suckers, soil, or by human activities such as road building, and hiking.

NB Since *P. megakarya* is more aggressive and causes higher yield losses than *P. palmivora* (Appiah 2001, Ali et al. 2016) special care should be taken when moving plant/soil materials within Ghana, Togo and Côte d'Ivoire where both *P. palmivora* and *P. megakarya* are not uniformly present. Some production areas in these three countries are not yet affected by *P. megakarya.*

The following plant parts are **unlikely** to carry the pest in trade and transport

- Seeds originating from pods without any obvious signs of infection

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Figure 8.3.1. Pods attacked by *Phytophthora megakarya*. Notice the abundant sporulation (GM ten Hoopen, CIRAD)

Figure 8.3.2. (A) Cacao tree trunk with canker symptoms (black discoloration) (B) discoloration of the sapwood (T Sreenivasan, CRC).

Figure 8.3.3. Cacao leaves attacked by *P. palmivora*. (V Singh, CRC)

8.4 Vascular Streak Dieback (VSD)

Update by Julie Flood

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8.4.1 Causal agent

Ceratobasidium theobromae (P.H.B. Talbot & Keane) Samuels & Keane (2012)

Synonym: *Rhizoctonia theobromae* (P.H.B. Talbot & Keane) Oberq., R. Bauer, Garnica, R. Kirschner (2013)

Previously described as: *Oncobasidium theobromae* (P.H.B. Talbot & Keane 1971)

8.4.2 Symptoms

The most characteristic initial symptom is the general chlorosis of one leaf, usually on the second or third flush behind the tip, with scattered islets of green tissue 2–5 mm in diameter (Keane and Prior 1991) (Fig. 8.4.1a,b). This leaf is shed within a few days and symptoms progressively develop in adjacent leaves. Lenticels usually become noticeably enlarged, causing roughening of the bark on the affected branches. Three blackened vascular traces are visible when the dry surface is scraped off the leaf scars which remain on the stem following the fall of diseased leaves (Fig. 8.4.2a). This is a useful way of distinguishing between leaf scars resulting from vascular streak dieback and those arising from leaf fall due to normal leaf senescence. Blackened vascular traces are also seen on detached petioles of infected trees (Fig. 8.4.2b). Another characteristic of diseased stems is the rapid discoloration of the cambium to a rusty-brown colour when the bark is removed and the tissue is exposed to air. The presence of this brown streaking in the wood of still-living branches is another diagnostic for the disease. Infection hyphae of the pathogen can be observed within xylem vessels of stems and leaves and the infected xylem is discoloured by brown streaks which are readily visible when stems are split (Fig. 8.4.3a). Infection hyphae have been observed in the stem usually up to 1 cm, and never more than 10 cm, beyond regions of obvious vascular streaking. Pods are occasionally affected to the extent that the fungus can colonize the central vascular system of the pod but infected pods show no external symptoms. Eventually, leaf fall occurs right to the growing tip, which then dies. Lateral buds may proliferate then die, causing ‘broomstick’ symptoms. The fungus may spread internally to other branches or the trunk; if it spreads to the trunk, it usually kills the tree.

When an infected leaf falls during wet weather, hyphae may emerge from the leaf scar and develop into a basidiocarp (basidioma) of the pathogen, evident as a white, flat, velvety coating over the leaf scar and adjacent bark. Presence of these basidiocarps (basidiomata) is also diagnostic for the disease (Fig. 8.4.3b).

In addition to the symptoms described above, over the last 10 years or so, other symptoms have been seen which involve more leaf necrosis and these infected leaves remain attached to the branch for a period of weeks (McMahon and Purwantara 2016). Interestingly, all symptoms can be seen on the same genotype and even on the same branch. The factors leading to these changes in symptoms are not yet known though it has been suggested that they could include an enhanced resistance response, perhaps associated with climate change (e.g. raised temperatures or increased CO_2 levels) or associated with the lack of essential nutrients, such as potassium (K), reaching the canopy since there is little evidence of an alternative strain of the pathogen being responsible for the necrotic symptoms (McMahon and Purwantara 2016).

8.4.3 Geographical distribution

The disease has been observed in most cacao-growing areas in South and Southeast Asia and PNG (Islands of New Guinea, New Britain, New Ireland) in the East to Hainan Island (China) in the North and Kerala State (India) in the West. It has been a major problem in the large commercial plantations in West Malaysia and Sabah and is widespread in Indonesia, including in the fine flavour cacao plantations in East, Central and West Java, in Sumatra, in Kalimantan, the Moluccas and in the large areas of new cacao plantings in Sulawesi. Junaid et al. (2020) reported VSD in several villages in Barru district of South Sulawesi. It has also been reported from southern Thailand, Myanmar, Vietnam and the southern Philippines (Keane and Prior 1991, Flood and Murphy 2004, McMahon and Purwantara 2016). There is strong evidence that the fungus evolved on an indigenous host, as yet unidentified, in Southeast Asia/Melanesia and has adapted to cacao when the crop was introduced to the region.

With the exception of a single record from avocadoes in Papua New Guinea (Keane and Prior 1991), the fungus has usually been associated with cacao so the geographical distribution generally reflects the occurrence of cacao in South and Southeast Asia and Melanesia.

For cacao, the most easterly natural limit of the disease is probably New Britain (PNG) and its discovery in New Ireland almost certainly represents a quarantine breach. This is most likely due to “unofficial” movement of cacao material from heavily infected areas such as the Gazelle Peninsular in New Britain, despite the awareness-raising campaign at ports and airports of the risks involved, since all official movement of germplasm follows stringent quarantine procedures. The disease is not found on Manus or the North Solomons which are further east despite the fact that there is widespread cacao planting there. This distribution suggests that either the hypothesized indigenous host may not occur further out into the Pacific than New Britain or that the pathogen has not reached the limits of distribution of its indigenous host (which seems unlikely). Even on the main island of PNG and on New Britain, disease incidence is patchy, with isolated plantations being free of disease (Prior 1980).

The most southerly limit is the Papuan coast of Papua New Guinea, but the unknown original host(s) may occur in northern Australia. There appears to be very little morphological variation between strains collected in the region, though a phylogenetic survey conducted by Samuels et al. (2012) indicated some regional genetic variability with three haplotypes identified from Vietnam, Malaysia/Indonesia and Papua. There are no records from Africa but recently, *Ceratobasidium theobromae* has been associated with a disease of cassava in SE Asia and in Brazil and with a disease syndrome of woody perennials in the US (see below) so the distribution of the disease could change as more investigation is conducted.

8.4.4 Alternative hosts

Avocado has long been established as an alternative host (Keane and Prior 1991) but recently, Leiva et al. (2023) reported that a fungus of the *Ceratobasidium* genus and sharing more than 98.3–99.7% nucleotide identity at the Internal Transcribed Spacer (ITS), with *Ceratobasidium theobromae* has been shown to be strongly associated with Cassava witches' broom disease in SE Asia. Gil-Ordóñez et al. (2024) continuing the study of *C. theobromae* from cassava in SE Asia, reported tissue localization of the fungus in the xylem and epidermis along the cassava stem. They also conducted further genomic analysis providing more molecular evidence that the fungus is *C. theobromae* but culturing of the fungus proved difficult due to contamination during isolation and sub-culturing. Due to these problems of maintaining the fungus *in vitro,* Koch's postulates have not yet been achieved for the disease and this needs to be confirmed. Cassava, a member of the Euphorbiaceae, is native to South America (as is cacao) while avocado originates from Central America. Gil-Ordóñez et al. (2024) have suggested that Cassava witches' broom disease in SE Asia could be an example of a new encounter disease in SE Asia (as is VSD in cacao). However, a first report has also been made of the fungus being associated with Cassava witches' broom disease in indigenous lands in the municipality of Oiapoque, Amapá, Brazil, posing a significant threat to cassava productivity there (Embrapa 2024) so the situation in Brazil remains unclear.

In addition to the above, recently in North America, there have also been reports of a similar syndrome on various woody perennials including *Cercis* species (Beckerman et al. 2022) while Bily and Bush (2023) reported wilting and severe die-back in maples, redbud, dogwoods and many other woody perennials. The disease, which has been referred to in the literature as VSD and *Ceratobasidium theobromae* (synonym: *Rhizoctonia theobromae*), has been consistently associated with vascular tissue of nursery stock showing the symptoms described above. Liyanapathiranage et al. (2024) also reported the fungus was consistently associated with VSD-symptomatic eastern redbuds but the causal agent has not yet been conclusively confirmed. Determination of pathogenicity is crucial but Koch's postulates have not been fully achieved with these hosts (as with cassava) due to the fastidious nature of the fungus. A Rhizoctonia-like fungus was isolated from redbud wood with vascular streaking symptoms and inoculation of the stems of test plants with mycelial plugs produced similar symptoms so Koch's Postulates were partially achieved but attempts at re-isolation of the fungus from inoculated plants failed (Liyanapathiranage et al. 2024). Much more investigation is required to ascertain the exact relationship of the pathogen of woody perennials in the US with the pathogen affecting cacao in Asia before implications for quarantine can be ascertained.

8.4.5 Biology

Formation of basidia and forcible discharge of basidiospores occurs mainly at night after the basidiocarps (or fungal fruit bodies) have been wetted by rain (Keane et al. 1972). Prior (1982) showed that onset of darkness is also a stimulus for sporulation. Basidiospores were produced 8-12 h after basidiocarps were subjected to darkness, whereas those exposed to continuous artificial light during the night did not sporulate. There was some evidence that a temperature drop of 5°C also stimulated sporulation brought into the laboratory (Prior 1982). Basidiocarps remain fertile for an average of only ten days on attached branches; on detached branches they cease shedding spores after only two days. Basidiospores are large (15-25 µm x 6.5-8.5 µm), are hyaline, smooth and thin walled and are *circa* twice the length of the sterigmata (McMahon and Purwantara 2016). The hyphal cells are binucleate which is characteristic of the genus *Ceratobasidium* but this characteristic for taxonomic purposes has been questioned by Oberwinkler et al. (2013).

Basidiospores are dispersed by wind at night and are rapidly destroyed by sunlight. Exposure to the normal, shaded atmosphere in a plantation for only 20 min was sufficient to reduce germination by 80% (Keane 1981). Exposure of spores to direct sunlight for 12 min reduced germination by 95%. Because spores are rapidly killed by exposure to normal day-time conditions in the tropics and require free water for germination, effective spore dispersal is probably limited to the few hours of darkness and high humidity following their discharge.

Spore dispersal is probably further limited by the dense canopy of cacao and shade trees in plantations. As a result, disease spread from older, infected cacao into adjacent younger, healthy populations is limited with very few primary infections occurring beyond 80 m from diseased cacao.

The rate of disease spread is also limited by the relatively low sporulation rate of the fungus. Each infection only produces basidiocarps when leaf fall occurs during wet weather and these basidiocarps are short lived so consequently less than 10% of leaf abscission induced by the disease results in basidiocarp (and hence basidiospore) production. Epidemiological aspects of the disease are discussed in more detail by Keane (1981), Keane and Prior (1991) and more recently by McMahon and Purwantara (2016).

Basidiospores have no dormancy and free water is required for spore germination and infection. When a spore suspension was placed on young leaves, spores germinated within 30 minutes if leaves remained wet, but did not grow further once the water had evaporated (Prior 1979). The first sign of penetration occurred after 12 h, with swelling of the germ tube tip to form an appressorium which became attached to the leaf surface. Adjacent epidermal cells showed a browning reaction to the presence of the fungus. Often infection progressed no further, but occasionally penetration pegs were formed below appressoria. Hyphae have not been observed penetrating into the xylem elements of veins, although Prior (1979) observed trails of discoloured mesophyll cells leading from the surface to the bundle sheath surrounding the xylem. In cleared and stained leaves, hyphae were observed growing within the inoculated leaf in the vicinity of the veins (Keane 1972, Prior 1979), but these could not be traced back to empty spore cases on the leaf surface. There is evidence (Prior 1979) that dew forms first on the hairs and glands that are concentrated directly above the veins of young cacao leaves. These may form a trap for deposited spores and may explain the occurrence of penetrations directly above veins as observed by Keane (1972).

The fungus can be isolated from infected plant material and transferred to Corticium Culture Medium (CCM) (Kotila, 1929) but cannot be maintained in subculture as other faster growing fungi will rapidly overgrow it. Surface sterilization using 10% sodium hypochlorite with 70% ethanol (Keane et al. 1972) increases the likelihood of obtaining pure cultures (McMahon and Purwantara 2016). However, sporulation is not induced routinely on artificial media and even if basidiospores are produced, they are produced in insufficient numbers for use in pathogenicity tests.

To date, pathogenicity tests have been successful only when inoculated plants have been exposed to natural conditions of temperature and dew deposition under the open sky at night. It appears that, as with sporulation, infection requires very particular conditions which are difficult to simulate in the laboratory. In these tests, symptoms developed in 3-week-old seedlings about 6-9 weeks after basidiospores had been shed onto them during overnight dew periods (Keane 1981) or after they had been inoculated with a basidiospore suspension (Prior 1978); in 6-month-old seedlings, symptoms developed after 10-12 weeks (Keane et al. 1972).

Peaks in disease occurrence in the field are often observed to occur several months after seasonal rainfall peaks (Prior 1980, 1981). The fungus infects young leaves which then start to grow after the onset of the rains. The branch or seedling continues to grow for another 3-5 months before the fungus has ramified sufficiently to induce disease symptoms in the penetrated leaves which accounts for the occurrence of the first symptoms on the second or third flush behind the growing tip.

Ceratobasidium theobromae can colonize the vascular system of pods: this had some potential importance for quarantine and the possibility of transmitting the disease via infected pods distributed for seed. However, no infection was ever detected in seed and Prior (1985) discounted the possibility of seed transmission.

Problems with culturing and maintenance of the fungus in culture, have restricted studies of genetic diversity and the genome. However, Ali et al. (2019) described a

33.90Mbp *de novo* assembled genome. *Ab initio* gene prediction identified 9264 protein-coding genes, of which 800 are unique to *C. theobromae* when compared to *Rhizoctonia* spp., a closely related group. The genome presented supported a typical pathogenesis model, where the fungus secrets effector proteins involved in plant defence suppression along with enzymes required for degradation of cell walls and other cell components. The authors believed these findings provide a model for testing and comparison in the future.

8.4.6 Quarantine measures

The following is a list of plant parts liable to carry the pest in trade/transport:

- Fruits (inc. Pods): Hyphae; borne internally; invisible.
- Leaves: Hyphae; borne internally; visible to naked eye.
- Roots: Hyphae; borne internally; invisible.
- Stems (above ground)/shoots/trunks/branches: Hyphae, fruit bodies; borne internally; borne externally; visible to naked eye.

Plant parts not known to carry the pest in trade/transport

- Growing medium accompanying plants
- Seeds.

Whole plants or cuttings should not be sent from areas that are infested with *C. theobromae*. Where clonal material is required, it should be supplied as budwood from disease-free areas where possible. Budwood from plants grown in infested areas should be sent to an intermediate quarantine station in a disease-free area and budded onto rootstocks raised from seed collected from a disease-free area. The scion should be maintained for three growth flushes and confirmed as free from *C. theobromae* before cutting and sending to the final destination. In countries such as Papua New Guinea, it has been found that a post-entry quarantine period of six months in an isolated screened shade house provides adequate opportunity for the detection of VSD and this treatment has replaced the former recommendation of a post-entry quarantine period on an isolated island.

Microscopic examination of transverse sections of budwood sticks and pod stalks provides a further very thorough precaution against disease transmission because hyphae of the pathogen are large and easily detected. Hyphae were found within the stalks and placentae of pods from diseased branches but seeds from these pods germinated normally and there was no evidence of seed transmission. Dipping seeds in 1g/L propiconazole + 5g/L metalaxyl M caused a small but statistically significant reduction in seedling stem height. However, root length and percentage germination were not affected and this prophylactic seed treatment may be useful in situations where quarantine authorities require additional precautions.

Microscopic examination of cross sections of the budwood sticks, to check for the presence of *C. theobromae* hyphae in the xylem, can be used as an additional precaution to ensure freedom from infection at the Quarantine Station and is recommended (Prior 1985).

Although seeds have not been demonstrated to transmit the disease a precautionary dip in a triazole fungicide has been advocated (Prior 1985). Quarantine authorities in Malaysia currently require seed to be treated with thiram.

Management methods have been reviewed (McMahon and Purwantara 2016) and include cultural methods, attempts at chemical management and selection for host resistance which is considered the most promising strategy for management of VSD. Guest and Keane (2018) state that integrated management including the production of disease-free plants in covered nurseries, canopy management and regular pruning of infected branches, maintaining only low levels of shade, and use of partially resistant genotypes of cacao, provides adequate control of the disease in the areas currently affected, though they advocate the development of resistant varieties suitable for use in Latin America and Africa in case the disease spreads outside of Southeast Asia. Biocontrol strategies, such as the use of endophytic fungi or bacterial elicitors also show some promise as part of an integrated management strategy (Vanhove et al. 2016, Asman et al. 2018, Rosmana et al. 2015, 2019).

8.4.7 References and further reading

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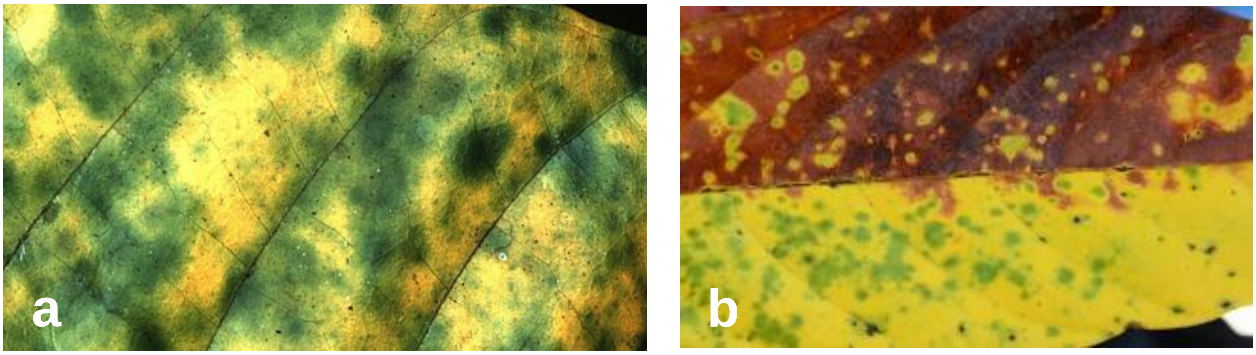

Figure 8.4.1. a) Vascular streak dieback: chlorotic leaf (M. Holderness, CABI) and b) Leaf showing necrosis and scattered islets of green tissue (AJ Daymond, University of Reading)

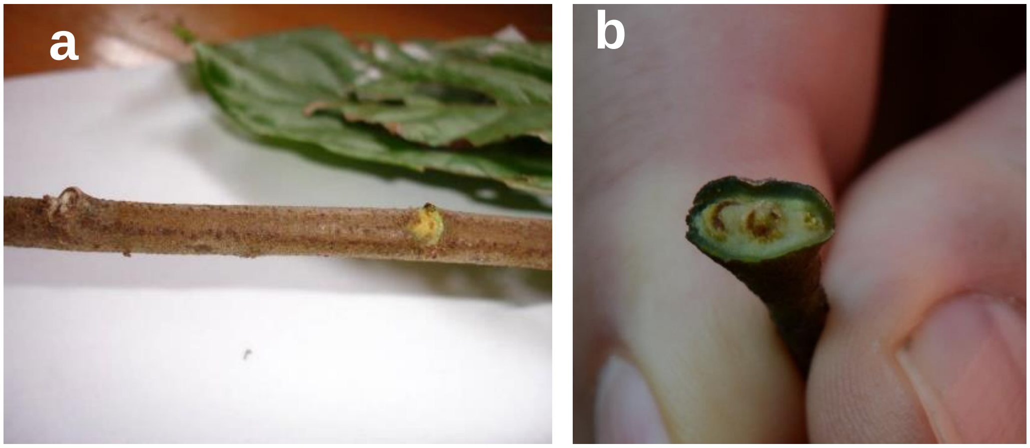

Figure 8.4.2. a) VSD Infected stem showing enlarged lenticels and blackened vascular traces in leaf scar (J Flood, CABI) and b) VSD infected petiole (AJ Daymond, University of Reading).

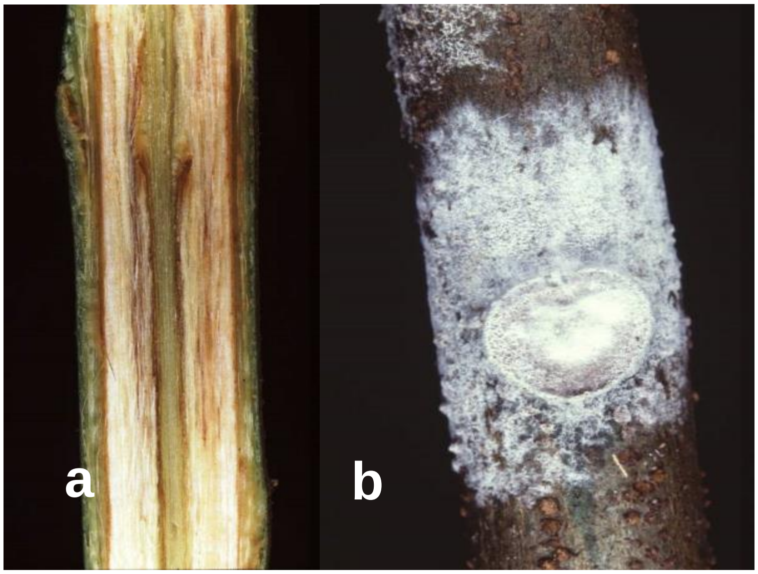

Figure 8.4.3. a) VSD infected stem section showing brown streaking (CABI) and b) VSD fruiting body (CABI).

8.5 *Verticillium* wilt of cacao

Updated in 2021 by Mário Lúcio Vilela de Resende[1], Anne-Sophie Bouchon[2]. Adriano Augusto de Paiva Custódio[1] and Fernanda Carvalho Lopes de Medeiros[1]

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8.5.1 Causal agent

Verticillium dahliae Klebahn (Ascomycota, in the family Plectospharellaceae)

8.5.2 Symptoms

General symptoms of *Verticillium* wilts include epinasty (Fig. 8.5.1 A), yellowing, necrosis, and wilting or abscission of leaves (Fig. 8.5.1 B-D), followed by stunting or death of the plant (Resende et al. 1996). According to Fradin and Thomma (2006), typically wilting starts from the tip of an infected leaf, usually in the oldest shoots as invasion is acropetal (from base to apex). In cacao, infected plants generally exhibit sudden wilting and subsequent necrosis of leaves and flushes.

Similar defoliating (Fig. 8.5.1 B) and non-defoliating (Fig. 8.5.1 C) types of symptom development can occur on cacao and other hosts. For example, *V. dahliae* pathotypes were described as defoliating or non-defoliating on cotton and olive (Schnathorst and Mathre 1966, Bejarano-Alcázar et al. 1996), but a continuum of symptoms related to the relative aggressiveness amongst strains of *V. dahliae*, rather than the occurrence of distinct pathotypes was suggested by other authors (Ashworth Jr 1983, Dervis et al. 2010). In olive and cotton, the resistance of certain cultivars may vary according to *V. dahliae* pathotype or vegetative compatibility groups (VCGs) (López-Escudero et al. 2004, Göre et al. 2014).

Generally, wilt symptoms are thought to be due to water stress caused by vascular occlusion, whilst defoliation may also involve imbalances in growth regulators. Thus, Talboys (1968) suggested that defoliation was related to the level of water stress, while Tzeng and DeVay (1985) and Resende et al. (1996) demonstrated enhanced production of ethylene, respectively, from cotton and cacao plants inoculated with defoliating isolates compared to those infected with non-defoliating isolates.

In stem sections, a brown discoloration of the vascular tissues (Fig. 8.5.1 E, F) can be seen. Browning, tyloses (Fig. 8.5.1 G), and deposition of gels and gums (Fig. 8.5.1 G) may be observed internally in the vessels.

Symptom levels depend mainly on the concentration of inoculum, pathotype or VCG of *Verticillium*, plant variety and stage of plant development, temperature, soil moisture, and nutrition, particularly potassium content (Trocmé 1972, Emechebe

1975, Resende 1994, Bouchon 2020). Infestation of plant roots by parasitic nematodes can enhance the occurrence and severity of diseases caused by soil-borne fungi such as *V. dahliae* (Johnson and Santo 2001, Bae et al. 2011). *Verticillium dahliae* attacking cacao appears to be favoured by temperatures between 20°C and 28°C, though different VGCs have different optimal temperatures (Resende 1994, Bouchon 2020).

In cacao fields, symptoms of *V. dahliae* infection appear at the time when the tree begins to produce pods, i.e. 2 to 3 years after planting (Matovu, 1973). Severe attacks, following especially dry conditions or waterlogging, can cause the death of a cacao tree one week after a situation of apparent health and vigour (Leakey, 1965). In other cases, natural recovery from the tree is observed, depending mainly on the genotype (Resende, 1994).

In Brazil, an increased incidence of Verticillium wilt was noted in dry areas in combination with a lack of shade (de Almeida et al. 1989). Shading cacao has been shown to reduce both the incidence and severity of Verticillium wilt of cacao in Uganda (Trocmé 1972, Matovu 1973).

8.5.3 Geographical distribution

Verticillium spp. are soil-borne fungi with worldwide distribution, causing vascular disease that results in severe yield and quality losses in several crops (Inderbitzin et al. 2011).

In Brazil, Verticillium wilt is a serious problem in the States of Bahia and Espírito Santo (Resende et al. 1995, Agrianual 2009). In Uganda, Verticillium wilt was consistently reported to be the principal disease affecting cacao (Emechebe et al. 1971, Matovu 1973, Bouchon 2020) with losses of up to 30% in some farms (Matovu 1973). Verticillium wilt has recently been reported in the Province of North Kivu in the Democratic Republic of Congo (Bouchon 2020). *Verticillium dahliae* has also been found on cacao in Colombia (Granada 1989, Resende et al. 1995) and in Peru (Bouchon 2020, Leon-Ttacca et al. 2019). In Ecuador, a pathogen causing wilt of cacao was also identified as being in the genus *Verticillium* but was not identified to the species level (Zavala et al. 2010). The disease was also reported in other cocoa-producing countries, including São Tomé and Príncipe, Gabon, and Sri Lanka (Chalot and Luc 1906, Kaden 1933, Navel 1921, Park 1933, 1934 cited by Oliveira and Luz 2005).

8.5.4 Alternative hosts

Over 400 dicotyledonous species are host to *V. dahliae*, including other members of the Malvaceae family such as cotton (Malcolm et al. 2013).

8.5.5 Biology

The vegetative mycelium of *V. dahliae* is hyaline, usually branched, septate, and multinucleate (Fig. 8.5.2 A). The appearance of the conidiophore is characteristic: it is verticillate due to the production of conidia at the tips of conidiogenous cells; between 2 to 3 conidiogenous cells per node are produced in whorls. Conidia are ellipsoidal to ovoid (Fig. 8.5.2 A) (Inderbitzin et al. 2011). Microsclerotia, considered resting structures, are commonly observed. Conidia and microsclerotia are commonly used to identify *V. dahliae* at a species level.

Distinct morphological variations (culture aspects, size of conidia and microsclerotia) were found to be discriminative to the different vegetative compatibility groups of *V. dahliae* attacking cacao in Uganda and Peru (Bouchon 2020) (Fig. 8.5.2 B).

The life cycle of *V. dahliae* can be divided into a dormant, a parasitic, and a saprophytic phase. A unique adaptation of these organisms is that until the advanced stages of vascular colonization, the pathogen is exclusively confined in the xylem, which contains fluids with only low concentrations of sugars, amino acids, and various inorganic salts (Resende 1994). The germination of microsclerotia in infested soils is stimulated by root exudates and the germ tube penetrates the host through the roots, proceeds to grow both inter-and intracellularly in the cortex, and spreads into the xylem. Systemic invasion occurs when successive generations of conidia are produced and then transported through the xylem transpiration stream to the aerial parts of the plant (Veronese et al. 2003). It has been reported that colonization of the plant at this stage appears to occur in cycles of fungal proliferation and fungal elimination, with elimination probably driven by plant defence responses (Fradin and Thomma 2006). During tissue necrosis or plant senescence, the fungus enters a saprophytic stage. Apart from the vascular tissues, shoots, and roots of the plant also become colonized. In *V. dahliae* infection, large amounts of microsclerotia are produced (Fig. 8.5.2 C and 8.5.2 D).

8.5.6 Quarantine measures

The following is a list of plant parts liable to carry the pest in trade/transport (information from various crops):

- Fruits (inc. Pods): Hyphae; borne internally; invisible.
- Leaves: Hyphae; borne internally; invisible to naked eye.
- Roots: Hyphae; borne internally; invisible.
- Stems (above ground)/shoots/trunks/branches: Hyphae, sclerotia; borne internally; borne externally; invisible to naked eye.
- Seeds: hyphae, sclerotia, spores; invisible to naked eye
- Growing medium accompanying plants

Although *V. dahliae* is very widespread, it is important to prevent the spread of different strains between cocoa growing areas. Special care is needed due to the long-lived nature of the microsclerotia, which can survive in soil, for example, for over 10 years. It is necessary to restrict the movement of germplasm into areas where the disease does not occur, and to collect branches for bud grafting from areas free of the pathogen. When coming from infected areas, the plant material must be placed in a quarantine station, for observation and analyses since the fungus can remain dormant inside the plant tissue.

Verticillium dahliae can be isolated from the xylem of roots, stems, branches, twigs and even leaves and seeds of many commercial crops. Diagnostic protocols have been published for several crop/*Verticillium* species combinations (for example, EPPO, 2020). Diagnosis is often carried out following isolation of the fungus from excised vascular tissue on streptomycin sulphate-alcohol-agar (SAA) medium or NP-10 semi-selective medium (Kabir et al. 2004). Although serological tests have been developed to certify planting materials, recent efforts to detect and identify *Verticillium* species are mainly concentrated on the use of molecular diagnostic techniques using PCR amplification (for example, Maurer et al. 2013) and *in planta* tests have been developed for crops such as olive (Mousavi et al. 2020). Bouchon (2020) has used a PCR technique to identify the VCGs of *V. dahliae* attacking cacao.

For controlling Verticillium wilt on trees, an integrated management strategy including combinations of biological, chemical, physical, and cultural control measures, is needed to reduce losses due to *V. dahliae* and to prevent its spread to new planting areas. Clean planting materials are an important starting point, and for other crops, the European and Mediterranean Plant Protection Organization (EPPO) recommends that planting material should come from a field where Verticillium wilt has not occurred in the last five years and that consignments and their mother plants should have been found free from the disease in the last growing season. Moreover, solarization can eradicate pathogens potentially present in the soil associated with the planting material (Kanaan et al. 2015). Even though genetic resistance is desirable, cacao planting materials with satisfactory level of resistance are not yet available though some clones have been shown to be partially resistant to the disease (Resende 1994, Oliveira and Luz 2005, Pereira et al. 2008, Bouchon 2020). Integrated management measures have been recently reviewed (Bouchon and ten Hoopen 2022) and include removal of infected crop residues and elimination of dead trees and their root systems (Oliveira and Luz 2005), preventing damage to cacao roots when handling seedlings or during weeding (Emechebe 1975) and the use of appropriate shade and fertiliser which can improve disease management and extend the life of the plants (Oliveira and Luz 2005, Pereira et al. 2008). The importance of weed management in cacao-growing areas has been stressed by Resende (1994) because weeds can act as a reservoir of *V. dahliae* (Resende 1994). Research on biological control agents for *V. dahliae* is showing promising results, but these studies are mostly conducted under controlled environment conditions (Deketelaere et al. 2017, Leon-Ttacca et al. 2019, Montes-Osuna & Mercado-Blanco, 2020). Organic or biological soil amendments can be effective in reducing *Verticillium* wilt disease in some cropping systems (Montes-Osuna and Mercado-Blanco 2020).

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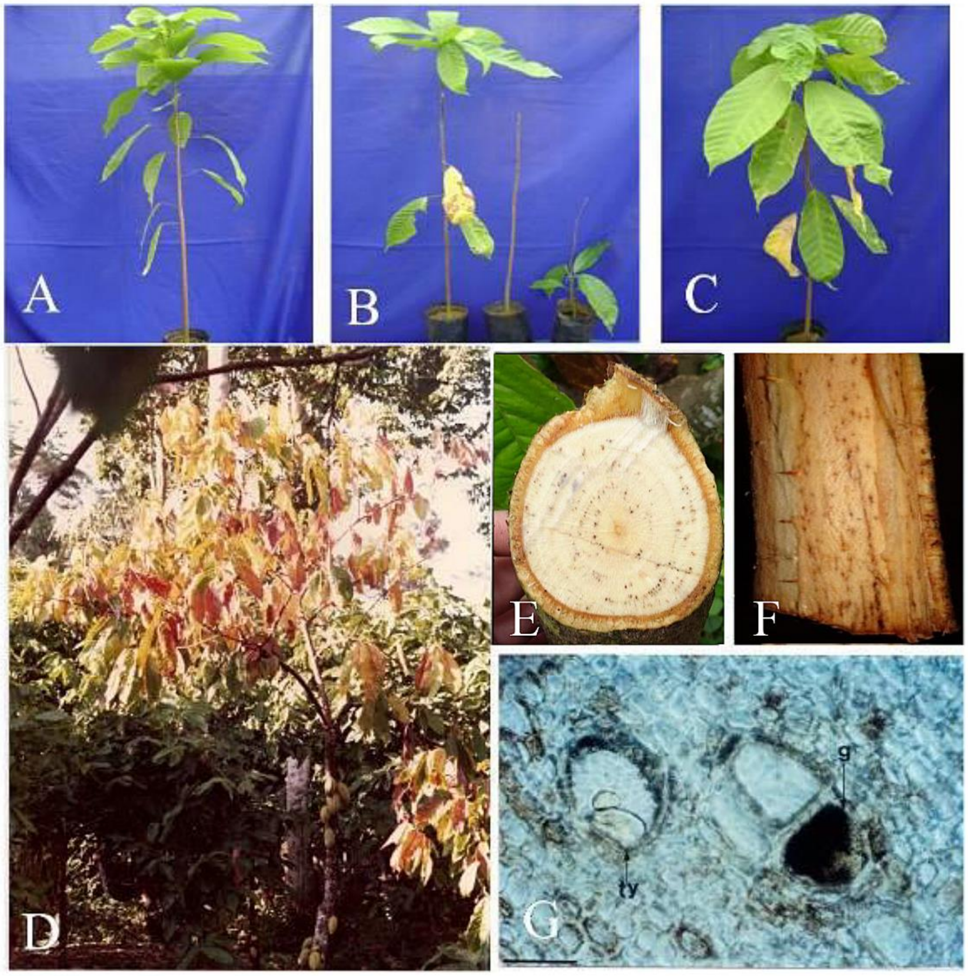

Figure 8.5.1. External (A-D) and internal (E-G) symptoms of *Verticillium dahliae* – cocoa interactions (MLV Resende, Univ. Federal de Lavras, Brazil):

A Epinasty (from base to apex – acropetal direction)
B Defoliating
C Nodefoliating
D General wilting of the leaves in field
E Transverse section of a cacao branch showing vascular discolorations
F Longitudinal section showing vascular streak
G Transverse section of an infection cacao stem under light microscopy: dark brown gum deposits (g) and tylosis (ty), produced in response to infection

(Bar markers represent 50 µm).

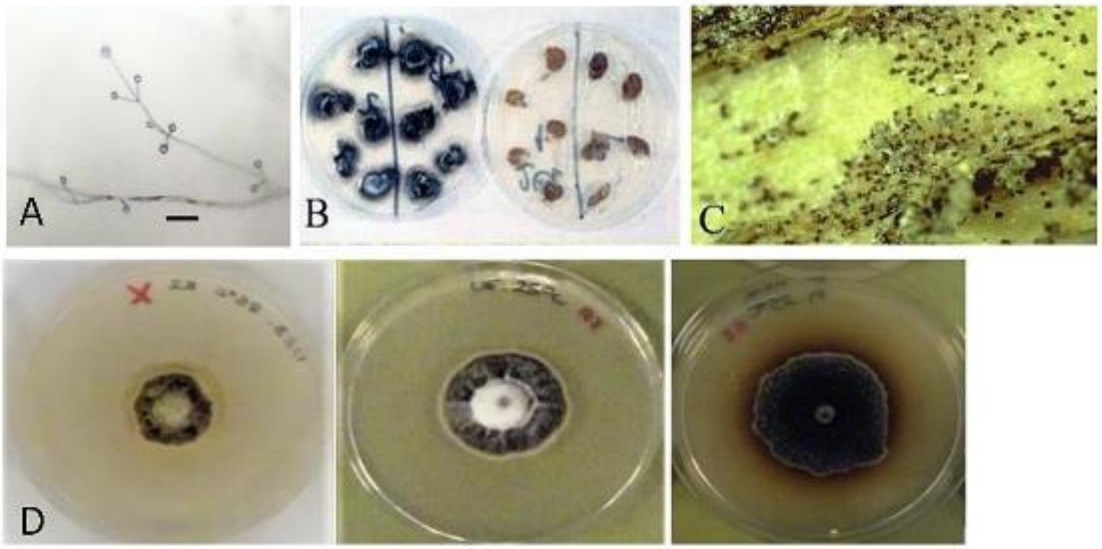

Figure 8.5.2. Biological cycle of *Verticillium dahliae*:

A . Isolate of *V. dahliae* (bar marker represents 160µm; AS Bouchon)

B. Typical colony morphology of *V. dahliae* reisolated from cross-sections of cacao stems on an alcohol agar medium. (Petri dishes containing samples from infected plants in the left side and non-infected in the right side) (MLV Resende, Univ. Federal de Lavras, Brazil)

C. Microsclerotia in infected cotton stem (Gómez-Alpízar 2001)

D. *V. dahliae* colonies after 14 days of incubation at 25°C on potato dextrose agar medium (left: VCG4A, middle: VCG4B, right: Peru; AS Bouchon)

8.6 *Ceratocystis* wilt of cacao or mal de machete

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8.6.1 Causal agent

Ceratocystis cacaofunesta Engelbr. & T.C. Harr.

The cocoa pathogen is a member of the Latin American clade of the *Ceratocystis fimbriata* species complex, which has a substantial genetic variation and a wide range of hosts. An extensive review of the genus has been published by Marin and Wingfield (2006), and a recent update for *C. cacaofunesta* is included in the CABI Invasive Species Compendium (CABI, 2021).

“Mal de machete,” or *Ceratocystis* wilt of cacao, is caused by a species specialized in infecting *Theobroma cacao* known as *C. cacaofunesta* (Engelbrecht and Harrington 2005). Earlier studies suggested that *C. fimbriata* was a complex of cryptic species showing host specialization (Baker et al. 2003, Engelbrecht and Harrington 2005). Modern molecular techniques and morphological differences among isolates from cacao (*Theobroma cacao*), sweet potato (*Ipomoea batatas*), and sycamore (*Platanus* spp.) allowed the cacao-specific species to be reclassified as *Ceratocystis cacaofunesta* (Engelbrecht and Harrington 2005).

8.6.2 Symptoms

Infected trees show limp, brown foliage on a single branch or across the whole tree, depending on whether only a branch or the main stem has been infected; the first symptom is a general yellowing of the leaves (chlorosis), followed by darkening of stems and wilting and desiccation of the leaves for 2 to 4 weeks. However, the leaves usually remain attached to the plant for long periods (Delgado and Suárez, 2003). Internal vascular darkening occurs due to the entrapment of conidia in the parenchyma cells surrounding the xylem vessels (Santos et al. 2013). Thus, a large number of vessels can be simultaneously colonized and blocked, preventing water transport through the plant and resulting in wilting (Talboys 1972). The fungus will also kill the cells of the cambium and bark tissue, creating a canker on the stem or branch, usually associated with weakening of the tree. *Ceratocystis* cankers are only visible at a very late stage of the infection process on mature trees.

Typically, Ceratocystis wilt is recognized through limp brown foliage that hangs from the tree without falling, even when the branch is shaken (Fig. 8.6.1.a). Another typical symptom well recognised is the odour of ripe fruit in the infected area. Ambrosia beetles of the genus *Xyleborus* are attracted to the diseased trees and bore into the branches or main stem (Fig. 8.6.1.b) (Saunders 1965). The frass from the beetles is pushed to the outside of the infected stem or branches and is seen on the base of the trees as light, powdery masses (Fig. 8.6.1.d). This is recognized as the first positive sign of Ceratocystis wilt, frequently the frass is seen even before the yellowing of the leaves is visible.

8.6.3 Alternative hosts

This specialized form of the *Ceratocystis* complex apparently has *Theobroma cacao* and the related genus *Herrania* as hosts; other *Theobroma* species have not been reported as susceptible (Engelbrecht et al. 2007, CABI 2021).

8.6.4 Geographical distribution

Ceratocystis wilt of cacao (as *Ceratocystis fimbriata* Ellis & Halstead) has a broad geographical distribution in Central America, South America, and the Caribbean. This disease significantly affects cacao trees and was first reported in western Ecuador in 1918 (Rorer 1918). The disease caused serious losses in Colombia after 1940, and in the late 1950s, it reached Venezuela (Thorold 1975), Costa Rica (Thorold 1975), and Trinidad (Spence and Moll 1958). Subsequently, the disease has been reported in many countries in South America, including Peruvian Amazon (Soberanis et al. 1999), Ecuador, Colombia, and Venezuela (Thorold 1975); in Central America: Costa Rica, Guatemala (Schieber and Sosa 1960), and Mexico, and in the Caribbean island of Haiti (CABI 2021). The disease is also found in French Guiana (M Ducamp, pers. comm.). In Brazil, the disease was reported in the southwestern Amazon (Rondônia) in 1978 (Bastos and Evans 1978). In the 1990s, it was reported in Bahia (Bezerra 1997) and has become an important emerging disease. It is widespread in the cacao-producing region of Bahia State (Luz et al. 2013) and, in 2022, stretched to the new cocoa plantations in the Cerrado region of Western Bahia.

Two closely related sub-lineages exist within this species, one centred in western Ecuador and the other containing isolates from Brazil, Colombia, and Costa Rica. The two sub-lineages differ little in morphology but are inter-sterile and have unique microsatellite markers (Engelbrecht et al. 2007). Engelbrecht and Harrington (2005) differentiate the host specialized species *C. cacaofunesta* by its pathogenicity in cacao and locate it in western Ecuador and Brazil, Costa Rica, Colombia. Isolates from Bahia, in particular, have been shown to be more aggressive than other isolates from Latin America (Silva et al. 2004).

8.6.5 Biology

Ceratocystis cacaofunesta infects cacao trees through fresh wounds, generally caused by agricultural practices such as pruning and harvesting wounds, boring insects, and other mechanical damage (Malaguti, 1952).

Inside the plant, the fungus causes necrosis of the parenchyma cells of the rays, compromising the xylem and moving through the secondary xylem, advancing towards the plant's apex and colonizing transversely (Fig. 8.6.1.c) (Harrington, 2004). The fungus also will kill the cambium and bark tissue, creating a canker on the stem or branch, usually associated with weakening the tree. *Ceratocystis* cankers are only visible at a very late stage of the infection process on mature trees; on six-month-old seedlings inoculated with the fungus, the disease may take six to eight months to show symptoms, depending on the degree of resistance in the plant.

Resistant genotypes exhibit minimal and restricted colonization, predominately with ungerminated conidia. In contrast, susceptible genotypes undergo extensive colonization with the production of numerous perithecia and conidiophores (Santos et al. 2013). These reproductive structures are disseminated to new plants, initiating new cycles of infection. In breeding efforts to improve resistance, CCN 51 is used as susceptible standard and TSH 1188 as resistant one.

Fungal propagules may be dispersed via insect vectors, particularly Ambrosia beetles of the genus *Xyleborus* which are attracted to infected trees by the characteristic banana odour produced by the fungus. On the cut surfaces of diseased plants, the fungus produces sporulating mats with perithecia (fruiting bodies) (Fig. 8.6.2) that exude sticky masses of spores for insect dispersal. As well as sticking to the insects bodies externally, the pathogen's spores can remain viable as they pass through the beetles' intestinal tract (Iton 1966, Paladines-Rezabala et al. 2022) and thus can be spread as the beetles fly to neighbouring trees. Moreover, as the beetles excavate their galleries, they help to spread the fungus within the cocoa tissue and the sawdust and excrement containing viable spores are pushed out, contaminating the soil. They can then be spread by wind, rain splashes, and human activities. Although frass can transmit the infection to other plants (Iton 1960, Hughes et al. 2023), pruning tool, especially machete blades (Malaguti, 1952), are highly effective in spreading the disease. In new areas, the primary means of spreading the fungus is the introduction of contaminated asymptomatic seedlings and infested soil.

The fungus' main mechanism of survival is infected plants left in the area, including roots, trunks, and branches. In soil, the fungus can survive mainly as chlamydospores, resistant structures that allow it to persist in adverse conditions until it finds a new host.

8.6.6 Quarantine measures

- The following is a list of plant parts liable to carry the pest in trade/transport:-
- Roots: Hyphae; borne internally; invisible
- Stems (above ground)/shoots/trunks/branches: Hyphae, fruit bodies; borne internally and externally; visible to the naked eye
- Growing medium accompanying plants
- Viable infective structures of the fungus can be transferred via insects.
- Plant parts not known to carry the pest in trade/transport: seeds

The disease can be spread by mycelium, asexual spores (endoconidia and aleurioconidia), and sexual spores (ascospores). Aleurioconidia are thick-walled spores that allow long-term survival of the fungus in wood or soil; survival of *Ceratocystis* in wood for up to five years has been reported. Thus, untreated wood-based packaging and soil are high-risk factors for the long-distance spread of Ceratocystis diseases (CABI 2021).

Once infection occurs, an extensive growth of mycelium is produced within the cacao tissue well before any symptoms are visible. All these facts should be considered when dealing with the movement of plants or plant parts since unrestricted movement of cuttings or other propagative material is potentially dangerous. Consequently, transporting whole plants or cuttings from areas where *C. cacaofunesta* is present should be avoided. It is recommended that where material for vegetative propagation is required, it should be treated with insecticide and fungicide before dispatch to an intermediate quarantine station in a disease-free area. Budded material should be kept in isolation for several successive growth flushes to confirm that it is free from *C. cacaofunesta*.

Currently, identification is recommended based on culture and morphological characteristics as outlined by Engelbrecht and Harrington (2005). While molecular or serological diagnostic techniques for *C. cacaofunesta* have not yet been reported, DNA sequences of ITS-rDNA and other genes unique to Ceratocystis species could be developed for diagnosis (CABI 2021). Molecular identification can be enhanced through phylogenetic analyses that utilize multiple gene regions, including ITS-rDNA sequences. Furthermore, population studies have identified unique microsatellite markers that can be used for pathogen identification (Steimel et al. 2004, Engelbrecht et al. 2007b). Host specialization appears to be a major factor defining groups of closely related, morphologically indistinguishable species of Ceratocystis (Engelbrecht 2004; Baker et al. 2003). Recognition of these unique populations as species would facilitate disease management and the development of more effective quarantine measures to minimize the risk of introducing specialized forms of the pathogen to new regions.

8.6.7 References and further reading

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Fig 8.6.1. a) A young tree with ceratocystis wilt, b) Ambrosia beetles causing gallery on the branch of an infected tree, c) Internal necrosis, and d) Abundant frass from Ambrosia beetles at the base of an infected tree (Assunção, Ilhéus, Bahia, Brazil)

Figure 8.6.2. Perithecia of *Ceratocystis cacaofunesta* growing over the xylem of cocoa branches inoculated with the pathogen (C Suárez-Capello, UTEQ, Ecuador)

8.7 *Rosellinia* root rot

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8.7.1 Causal agents

Rosellinia bunodes (Berk. et Br.) Sacc

Rosellinia pepo Pat.

Rosellinia paraguayensis Starb, only once described from cacao in Grenada (Waterston 1941)

8.7.2 Symptoms

Pathogenic soil-borne *Rosellinia* spp. cause aerial disease symptoms not unlike those caused by many other root diseases. In cacao and coffee, the first symptoms include yellowing and drying up of the leaves, defoliation, drying up of tree branches, and finally the bush or tree dies (Fig. 8.7.1). Immature fruits tend to ripen prematurely, remain empty of beans and, when not harvested, turn black and dry out (Merchán 1989 and 1993, Mendoza 2000, Ten Hoopen and Krauss 2006).

Although both *R. bunodes* and *R. pepo* cause similar external disease symptoms, differences exist with respect to the form of the mycelium on the roots. On roots, *R. pepo* is present as greyish cobweb-like strands that become black and coalesce into a woolly mass. Beneath the bark, white, star-like fans can be observed (Fig. 8.7.2). *Rosellinia bunodes* shows black branching strands that are firmly attached to the roots and may thicken into irregular knots (Fig. 8.7.3). *Rosellinia bunodes* can be seen on the exterior as well as interior of the root bark (Fig. 8.7.4) and may extend well above the soil surface in humid conditions (Sivanesan and Holliday 1972).

In the Americas, it seems that *Rosellinia* and *Ceratocystis cacaofunesta* (formerly *C. fimbriata*; see also Chapter 8.6 of this guide) act together as they are often found together on cacao (Aranzazu et al. 1999, Ten Hoopen and Krauss 2006). Symptoms of one of the pathogens might conceal the presence of the other.

8.7.3 Geographical distribution

Rosellinia bunodes and *R. pepo* occur in tropical areas in Central and South America, West-Africa, the West Indies and Asia. The distribution of *R. pepo* is probably more restricted than that of *R. bunodes* (Waterston 1941, Saccas 1956, Sivanesan and Holliday 1972, Holliday 1980). For more information check also https://nt.ars-grin.gov/fungaldatabases/ and the CABI Crop Protection Compendium (http://www.cabi.org/cpc/).

8.7.4 Hosts

Rosellinia bunodes and *R. pepo* attack numerous cash crops and tree species like avocado (*Persea americana*), plantain (*Musa* AAB), coffee, cacao, lime (*Citrus aurantifolia*), nutmeg (*Myristica fragrans*), *Inga* spp., *Leucena* spp., *Erythrina* spp. and *Populus deltoides* among others (Waterston 1941, Saccas 1956, Booth and Holliday 1972, Sivanesan and Holliday 1972, Aranzazu et al. 1999, Ten Hoopen and Krauss 2006, Kleina et al. 2018).

Many of these hosts are often associated with cacao.

8.7.5 Biology

Outbreaks of *Rosellinia* root rots are often characterized by their occurrence in patches that extend in a circular pattern due to the way in which the pathogen infests neighbouring plants. It is generally believed that *Rosellinia* spp. spread through direct root contacts between host plants (Aranzazu et al. 1999) and to date it is not clear which role ascospores or sclerotia, play in the epidemiology. No evidence exists that tools used by farmers play a role in disease propagation.

Initial infection points are often associated with dying or already dead shade trees. The decomposing root system allows the infection with *Rosellinia* which subsequently builds-up enough inoculum potential to infect healthy trees (Ten Hoopen and Krauss 2006). The economic impact of *Rosellinia* is due to the progressive loss of productive trees, the removal of infected trees and the direct costs of control but also because a farmer will not be able to replant for several years in infected soil.

Both *R. bunodes* and *R. pepo* have similar requirements in terms of soil, and climatic conditions. Both species are often associated with acid soils, rich in organic matter (Waterston 1941, López and Fernández 1966, Mendoza et al. 2003). In those areas where both species are present, it is not uncommon for both to infect a plant at the same time.

8.7.6 Quarantine measures

The following parts could carry the disease:

- Roots
- Trunks/branches
- Growing media accompanying plants could carry *Rosellinia* inoculum.

Parts of the plant unlikely to carry the disease:

- Pods
- Seeds have not been demonstrated to transmit the disease
- Leaves

Whole plants or cuttings should not be sent from areas that are infested with *Rosellinia*. Clonal material should be supplied as budwood from disease-free areas where possible. Budwood from plants grown in infested areas should be sent to an Intermediate Quarantine Station in a disease-free area and budded onto rootstocks raised from seed collected from a disease-free area. When obtaining budwood from plants growing in an infested area, care should be taken that the tree that provides the budwood and all its neighbours do not show symptoms of the disease.

**Figure 8.7.1. Tree infected with *Rosellinia* sp.
F Aranzazu, FEDECACAO)**

Figure 8.7.2. Star-like fans of *Rosellinia pepo* on roots (F Aranzazu, FEDECACAO)

Figure 8.7.3. Black strands and irregular knots due to *Rosellinia bunodes* (here shown in coffee) (BL Castro, Cenicafé)

Figure 8.7.4. Grey coloured mycelium of *Rosellinia* growing on the bark of a root (F Aranzazu, FEDECACAO)

8.7.7 References

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8.8 Other Fruit and Canopy Pathogens

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Introduction: In addition to the major diseases covered in the previous sections, there are a number of emerging fungal species which can also have severe effects on cocoa production in local outbreaks, particularly with changes in the environmental conditions due to global warming and cocoa cultivation practices. Moreover, there are a number of species with widespread distribution and host ranges which can be associated with various symptoms in cocoa such as dieback, galls and cankers, though in some cases it is not clear whether these are opportunist pathogens entering through existing wounds, latent infections or pathogenic strains of endophytic species. A brief description of some of the causal organisms is provided below but further details can be found in Akrofi et al. (2016).

General Reference:

Akrofi AY, Amoako-Atta I, Acheampong K, Assuah MK, Melnick RL. 2016. Fruit and Canopy Pathogens of Unknown Potential Risk. In B. A. Bailey & L. W. Meinhardt (Eds.), *Cacao Diseases: A History of Old Enemies and New Encounters*. Springer International Publishing. http://link.springer.com/10.1007/978-3-319-24789-2

8.8.1 Pink Disease

Erythricium salmonicolor (Berk. & Broome) Burdsall (Syn. *Corticium salmonicolor* Berk. & Broome) (syn. *Phanerochaete salmonicolor* Berk. & Broome, Julich). Known as “maladie rose” in French, and “mal rosado” in Spanish and Portuguese.

8.8.1.1 Alternative hosts

Found on many plant species including crops such as rubber, tea, coffee, citrus, mango and kola, cover crops such as *Cajanus cajan, Crotolaria* and shade trees such as *Leucaena* and *Gliricidia* (Smith 1985, Wood and Lass 1985), Eucalyptus (Seth et al. 1978).

8.8.1.2 Distribution

Widely distributed (reported on cocoa in Brazil, Cameroon, Colombia, Ghana, India, Nigeria, Malaysia, Papua New Guinea, Western Samoa and Trinidad). Although it was first reported in Ghana as a minor disease in 1962, it appears to be spreading and is emerging as an important cocoa disease (Akrofi et al. 2014, 2016) with several genetically distinct strains being reported (Kwarteng et al. 2018).

8.8.1.3 Symptoms

The disease appears as a sparse white mycelium (threads) in the form of cobwebs over the bark, which spread mainly along the underside of the branch. Pinkish white pustules appear through cracks in the bark and through natural openings, about 1-8 cm behind the leading edge of the infection. Hyphae penetrate the branch, causing death of distal tissues and subsequently, progressive death of leaves distal to the infection. A coating of pinkish to orange coloration of fruiting bodies (conidia) is observed on infected branches with dead leaves remaining attached for several weeks. Four distinct growth forms have been observed on the bark of infected trees: cobweb stage with white/light pink vegetative mycelia which can be easily overlooked when the bark is wet (Fig. 8.8.1a), pink to salmon encrustation/pustules on any part of the branch (Fig. 8.8.1b), creamy pustules which are more conspicuous on the underside of infected branches (Fig. 8.8.1c) and orange fruiting bodies which develop from the creamy pustules on dying infected stems (Fig. 8.8.1d). This is followed by dieback in infected branches with dead leaves hanging (Fig. 8.8.1e). All the growth forms may be found together on the diseased bark at the same time, but the most conspicuous and distinctive are the salmon-pink encrustations formed by hyphal fruiting bodies on branches and stems of the tree (Akrofi et al. 2016).

8.8.1.4 Biology

The fungus can spread by basidiospores (broadly ellipsoidal with a prominent apiculus) which are produced in basidiocarps (basidiomata) in the pink/orange crust mostly found on the underside of infected branches. The basidiospores are released shortly after rainfall and must settle on moist brown bark for successful germination and penetration. The fungus can also be spread from conidia produced from the orange/red pustules. These can remain viable for approximately 20 days under dry conditions but high humidity is required for germination. Most spores are spread by wind, rainsplash, ants and other insects though it has been suggested that the discontinuous distribution of the disease on farms in Ghana could be a result of human involvement (Akrofi et al. 2014, Kwarteng et al. 2018).

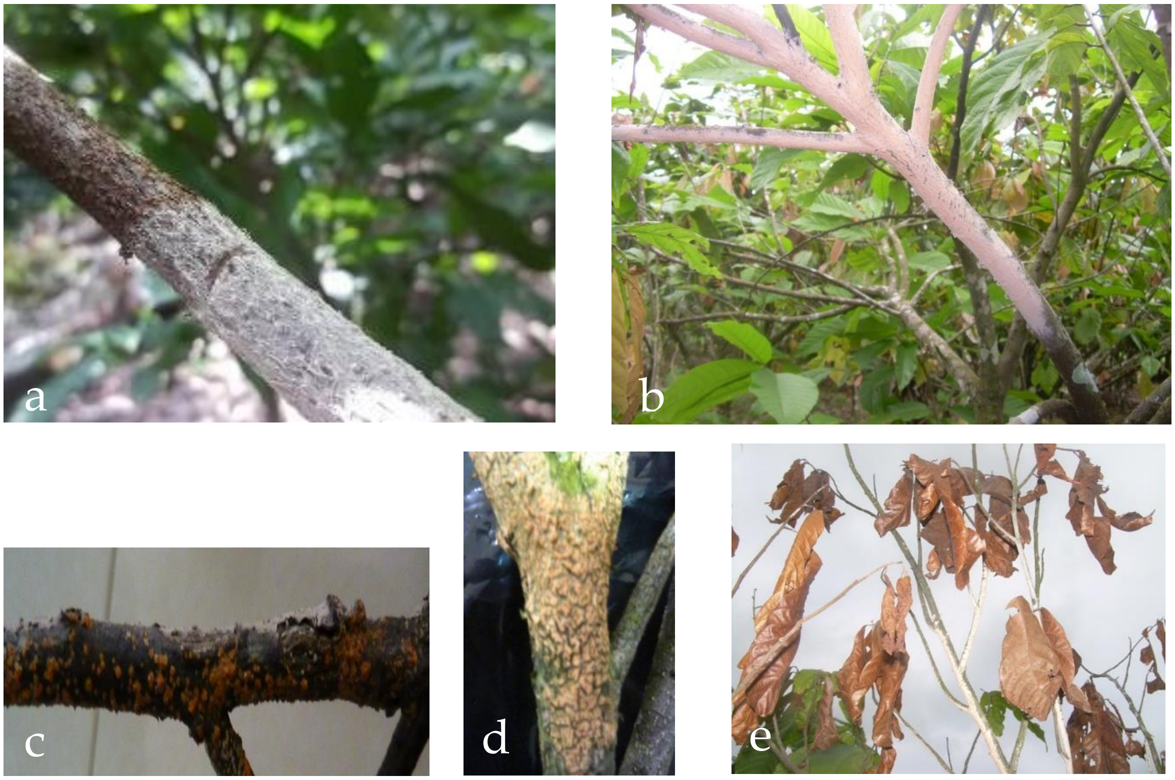

Fig. 8.8.1 Symptoms of pink disease on cacao: (a) white/light pink vegetative mycelia which can be easily overlooked when the bark is wet; (b) pink to salmon encrustation/pustules on the branch; (c) creamy pustules which are more conspicuous on the underside of infected branches; (d) orange fruiting bodies which develop from the creamy pustules on dying infected stems and (e) dieback in infected branch with dead leaves hanging (Source: AY Akrofi).

8.8.1.5 Quarantine measures

The following parts could carry the disease:

- Trunks/branches/stems/young shoots
- leaves

Parts of the plant unlikely to carry the disease:

- Pods
- Seeds have not been demonstrated to transmit the disease

The pathogen has not been shown to be seed borne but the conidia can survive for 20 days on shoots and branches. Where clonal material is required, it should be supplied as budwood from disease-free areas where possible. When obtaining budwood from plants growing in an infested area, care should be taken that the tree that provides the budwood and all its neighbours do not show symptoms of the disease. A range of fungicides, including copper formulations, have been shown to show activity against *E. salmonicolor* and should be used as fungicide dip for budwood.

8.8.1.6 References

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8.8.2 Anthracnose of Cacao

Species such as *Colletotrichum theobromicola* and C. *siamense*, within the *Collectotrichum gloeosporioides* complex and *C. aeschynomenes* have been associated with Colletotrichum disease of cacao. Although some *Colletotrichum* species cause disease, some such as *C. tropicale* are the major foliar endophytic fungi in healthy cocoa plants and have potential use as biological control agents due to their ability to reduce disease incidence.

8.8.2.1 Alternative hosts

Colletotrichum species cause anthracnose in many crops including mango, avocado, almond and passion fruit (Freeman et al. 1996, Nelson 2008, Anaruma et al. 2010). *C. gloeosporioides*, a complex of *Colletotrichum* species including *C. theobromicola* (Roljas et al. 2010) and C. *siamense*, have been reported to cause anthracnose disease of cocoa (Suryanto et al. 2014, James et al. 2014, Asare et al. 2021). *C. aeschynomenes* was responsible for a recent report of anthracnose disease of cocoa in Brazil (Nascimento et al. 2019).

8.8.2.2 Distribution

Colletotrichum disease is widely distributed and it is reported to be of particular concern for areas growing the susceptible “Porcelana” variety in areas of South

America, in some cocoa growing regions of India, Malaysia, Brazil and recently in Ghana (references cited in Akrofi et al. 2014, Akrofi et al. 2016, Asare et al. 2021).

8.8.2.3 Symptoms

Foliar symptoms (noted particularly on young leaves exposed to high light levels) include brown necrotic lesions surrounded by a chlorotic yellow halo. In severe infections, large areas of the leaves can be blighted and this can lead to defoliation and branch dieback (Fig. 8.8.2A). On cocoa pods, the pathogen causes soft brown lesions covered with orange spore masses or acervuli, often in concentric rings. (Fig. 8.8.2 B).

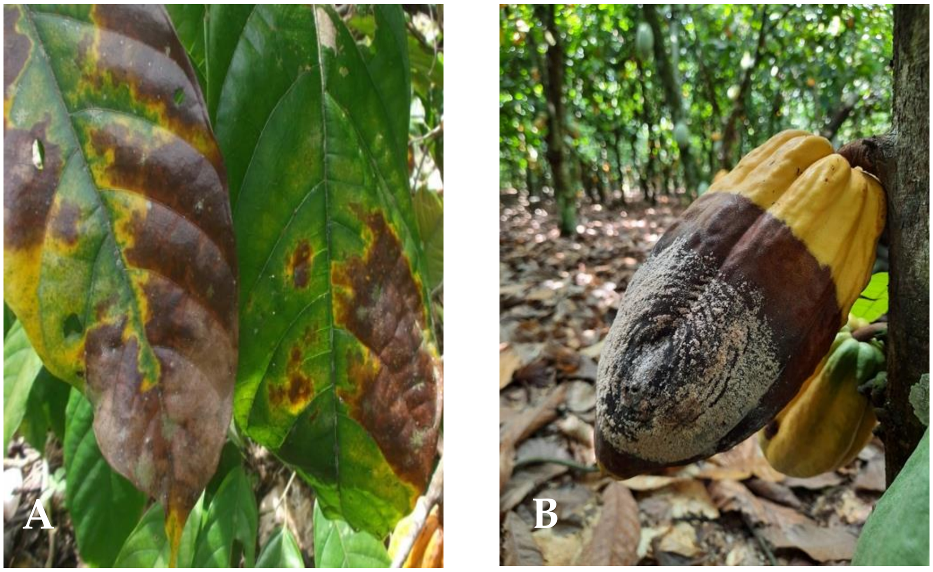

Fig. 8.8.2. Symptoms of anthracnose showing dark brown lesions on cacao leaves (A) and cacao pods covered with orange spore masses or acervuli in concentric rings (B) (Source: E Kumi Asare, CRIG, Ghana).

8.8.2.4 Biology and Spread

Colletotrichum infects plants by conidial germination and formation of appressoria with which the pathogen penetrates host tissues (Zakaria 2021). The spores are produced on the stem and fruit lesions when environmental conditions are humid. The spores are disseminated by the wind, rainwater or irrigation, insects and tools. Infection of the foliage occurs during the rainy season, often via wounds caused by insects. The disease can be controlled using effective phytosanitation. A number of fungicides, including copper-based formulations, have been shown to be effective.

8.8.2.5 Quarantine measures

The following parts could carry the disease:

- trunks/branches/stems/young shoots

- leaves
- pods

Parts of the plant unlikely to carry the disease:

- Seeds have not been demonstrated to transmit the disease

Where clonal material is required, it should be supplied as budwood from disease-free areas where possible. When obtaining budwood from plants growing in an infested area, care should be taken that the tree that provides the budwood and all its neighbours do not show symptoms of the disease. A range of fungicides, including copper formulations, have been shown to show activity against *Colletotrichum* spp. and copper fungicide dip could be used as budwood treatment.

8.8.2.6 References

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8.8.3 Lasiodiplodia Pod Rot, Cushion Gall and Dieback diseases

Lasiodiplodia theobromae (syn. *Botryodiplodia theobromae* (Pat.) Griff. and Maubl), the asexual state of the fungus *Botryosphaeria rhodina*. Also known as Charcoal Pod Rot, Diplodia rot, "Pourriture Noire", "Podredumbre de carbon". In Cushion Gall and Dieback diseases, *Lasiodiplodia* species are often found together with other species such as *Fusarium decemcellulare* and other *Fusarium* species.

8.8.3.1 Hosts

Widespread and known to cause various diseases on a range of tropical and sub-tropical tree crops including mango, cashew, *Jatropha podagrica,* and food crops such as yam and banana/plantain.

8.8.3.2 Distribution

Lasiodiplodia pod rot of cacao has been reported in areas of West Africa including Cameroon, Nigeria and Ghana (references cited in Akrofi et al. 2016), Bangladesh (Shamim et al. 2010) and it has also been reported as a constraint to cacao production in India (Kannan et al. 2010) and in the Philippines (Alvinda 2017).

8.8.3.3 Symptoms and Biology

Pod infection is usually via wounds caused by insects or other pests but the infection of undamaged pods in Hawaii has recently been reported (Puig et al. 2021). The first symptom is a brown lesion which eventually turns black. These lesions produce copious black conidia making the pod appear as if coated with a sooty powder (Fig. 8.8.3). The spores are easily dispersed in the wind.

L. theobromae, together with *Fusarium* species, are associated with dieback disease whereby leaves on the outer twigs turn yellow, then desiccate but remain attached to the twigs for several weeks. The fungi infect stems via mirid feeding wounds and pruning cuts and grow systemically spreading from the twig to the main branch. In severe cases, the infection extends to the trunk and can eventually result in tree death. Infected stems and branches show internal discoloration with brown streaks in the vascular tissues. White and yellowish exudates from infected trunks (gummosis) have also been reported. These symptoms resemble those of other diseases and there is speculation concerning associations of *L. theobromae* with other cacao pathogens, such as canker caused by *Phytophthora* species (Jaiyeola et al. 2014) and vascular streak dieback (VSD) (Alvindia and Gallema 2017, McMahon and Purwantara 2016) (references cited in Ali et al. 2019). *L. theobromae* has also been isolated from cushion galls in Cuba (Pérez et al. 2012) and Venezuela (Castillo et al. 2016). In the latter study, pathogenic strains of *L. theobromae*, together with strains of *Fusarium decemcellulare*, were shown to be capable of inducing galls in cocoa seedlings. Genetic variation and differences in pathogenicity of strains of *L.*

theobromae (and in some cases *L. pseudotheobromae*) have also been reported in isolates from Ghana, India, Indonesia. The Philippines and Puerto Rico (Adu-Acheampong 2009, Ali et al. 2019, Castillo et al. 2016, Puig et al. 2021).

Fig. 8.8.3 Cacao pod showing typical black conidia making the pod appear as if coated with a sooty powder (Source: E Kumi Asare, CRIG).

8.8.3.5 Quarantine measures

The following parts could carry the disease:

- trunks/branches/stems
- leaves
- pods
- roots

Parts of the plant unlikely to carry the disease:

- Seeds have not been demonstrated to transmit the disease

Where clonal material is required, it should be supplied as budwood from disease-free areas where possible. When obtaining budwood from plants growing in an infested area, care should be taken that the tree that provides the budwood and all its neighbours do not show symptoms of the disease.

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8.8.4. Thread blight:

Four *Marasmiellus* species distinguished from five morpho-types (based on unique mycelia strands' form and colour, presence and absence of rhizomorphs under field conditions and fruiting structures), ITS, LSU and mtSSU gene sequences have been reported on cocoa (Amoako-Atta et al. 2020). These species are :(a) *Marasmius crinis-equi* (F.Muell. ex Berk) Dennis (black, "horse hair" type mycelia strands); (b) *Marasmius tenuissimus* (Jungh.) Singer (brownish mycelia strands); (c) *Marasmiellus* *palmivorus* Sharples (whitish to brownish-white mycelia strands) and (d) *Marasmiellus scandens* Massee (faint cream or dull white mycelia strands).

8.8.4.1 Hosts

Found on many tropical crops including banana, plantain, taro, yam, cocoyam, coconut, maize, pineapple, ginger, tea, rubber and coffee (Dechassa 2019; Nelson and Javier 2001, Dutta and Archaya 2018, Farr and Rossman 2017); oil palm, coconut (Pong et al. 2012, Amoako Atta et al. 2020).

8.8.4.2 Distribution

Global distribution and is particularly common in humid tropical regions. It is widely distributed in Brazil and West Indies, and parts of Central America (Barros 1981, Ceresini et al. 2012), Ecuador, Peru, Belize and Surinam (Koch et al. 2018). Ghana, Papua New Guinea, Brazil, Trinidad & Tobago, India, Malaysia (Amoako-Atta et al. 2020).

8.8.4.3 Symptoms and biology

The fungus grows as a network of web-like dried strands (rhizomorphs), mostly on petioles and on the lower surfaces of leaves and branches, and may be predominantly either black in colour (black thread) (Fig. 8.8.4A) or white in colour (white thread) (Fig. 8.8.4B). The strands, up to 2 mm thick, always branch off from the petioles onto leaf blades and then spread out into numerous fine ones (1-4µm). These fine strands initiate dark-brown necrosis and later, the whole leaf dries up and becomes papery. Blighted leaves are distinctively brown to dark-brown in colour and these leaves cling to each other and remain suspended by the strands on the tree (Fig. 8.8.4C) (Opoku et al. 2007, Amoako-Atta et al. 2016). In severely infected trees, the thick mass of dead leaves suspended in the canopy (Fig 8.8.4D) obstructs new flushes and creates favourable environment for pests and diseases such as *Phytophthora* rot development (David 2005).

Dead leaves and branches with mycelia are major source of inoculum and are spread by wind, rain, insects, nesting birds and human activities (César et al. 2018). At certain points of the mycelia growth, the fungus forms irregular shaped hyphal clumps, concave in shape (1-3 mm high and 2-8 mm wide) on leaf edges or on veins (Fig 8.8.4E). The clumps serve as survival structures, but not fruiting bodies, and occur on both living and dead leaves but rarely on branches. The clumps absorb moisture readily and become sticky, under field conditions, enabling them to adhere to healthy host leaves and branches to start new infections within 24 hrs. The fungi generally grow faster on branches (4.9 – 49.7mm/day) than on leaves (0 – 37.6 mm/day). The disease may reach epidemic proportion when warm temperature, high humidity, shade and overhanging branches prevail.

Fig. 8.8.4. Signs and symptoms of thread blight disease on cocoa leaves: A: Strands of black thread pathogen hyphae on cacao branch; B: Strands of white thread pathogen hyphae on cacao branch; C: White rhizomorphs of white thread pathogen on detached and hanging infected leaf surface; D: Mass of dead leaves detached and hanging in canopy and E: Hyphal clumps on leaf margin (Source: I Amoako-Atta & E Kumi-Asare, CRIG).

8.8.4.4 Quarantine measures

The following parts could carry the disease:

- Trunks/branches/stems
- leaves

Parts of the plant unlikely to carry the disease.

- Pods
- Seeds

Where clonal material is required, it should be supplied as budwood from disease-free areas where possible. When obtaining budwood from plants growing in an infested area, care should be taken that the tree that provides the budwood and all its neighbours do not show symptoms of the disease. A range of fungicides, including copper and copper-mefenoxam formulations, have been shown to show activity against the leaf blight fungus. Fungicide treatment would reduce the inoculum and considerably limit the chances of an unwanted introduction.

8.8.4.5 References

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8.8.5 Trachysphaera Pod Rot: *Trachysphaera fructigena*

8.8.5.1 Hosts

Causes fruit rot of cacao, coffee, banana and avocado (Asare-Nyako and Dakwa 1974, Akrofi et al. 2016).

8.8.5.2 Distribution

Limited distribution but common in countries in West and Central Africa (UK, CAB International (1988).

8.8.5.3 Symptoms and biology

The fungus infects wounded pod tissue arising from human, insect, rodent and bird damage (Opoku et al. 2007) to cause brown spreading lesions on mature pods. Dense white conidial masses which later turn pinkish brown are produced on the surface of the lesions (Fig.8.8.5). The conidia have a mealy appearance and feel coarse when rubbed between the fingers due to echinulations on the conidial walls (Asare-Nyako and Dakwa, 1974). The symptoms on cacao pods are similar to those caused by *Phytophthora* (black pod), but unlike *Phytophthora* spores, the conidia of *Trachysphaera fructigena* can be blown around by wind.

8.8.5.4 Quarantine measures

The following parts could carry the disease:

- Pods

Parts of the plant unlikely to carry the disease.

- Trunks/branches/stems
- leaves

Mealy pod disease on cocoa caused by *Trachysphaera fructigena* is an insignificant component of pod diseases. Where clonal material is required, it should be supplied as budwood from disease-free areas where possible. When obtaining budwood from plants growing in an infested area, care should be taken that the tree that provides the budwood and all its neighbours do not show symptoms of the disease. Copper-based fungicides have been shown to show activity against the fungus.

8.8.5.5 References

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Figure 8.8.5. Mass of white conidia on *Trachysphaera fructigena* infected cacao pod (Source: Andrews Akrofi).

9. Insect and Mite pests

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A rich diversity of insects and mites are associated with the cocoa crop, often reflecting the composition of local forest fauna but also including pests associated specifically with shade species and other crops grown in the cropping system. Entwistle included around 1400 insect species in his 1972 list of species feeding on cocoa. The number of species found in the cocoa crop is expanded to nearly 3200 if natural enemies, pollinators and mites are included (Bigger 2012) though some of these species may be casual visitors. For example, *Conotrechalus humeropictus* Fiedler is one of nine species of polyphagous *Conotrechalus* spp weevils most often found infesting forest trees in Amazonia but is regarded as the most important pest of cacao in north western Brazil (Mendes et al. 1997) as, together with feeding damage to beans, and in common with other similar pests, the larval entry and exit holes on pods permit entry of fungal pathogens (Laker et al. 1993).

The main insect pests of cocoa include Cocoa Pod Borer (see section 9.2), Mirids (see sections 9.5 and 9.6) and Mealybugs (see Section 9.8). However, as with *C. humeropictus*, other pests can be of local significance, or population explosions can occur from time to time, necessitating vigilance on the part of those involved in any movement of germplasm to minimise the risk of transferring any pests on the plant material.

9.1 General recommendations to minimise the risk of spreading insect and mite pests

Extreme care should be taken in moving any whole pods due to the risk of pests and the eggs on the surface or inside the pods. Particular precautions are needed in areas infected by *Conotrechalus* spp., Cocoa Pod Borer or American Pod Borer (see sections 9, 9.2 and 9.3).

When transferring material as budwood, care should be taken to harvest budwood from branches that show no visual signs of either live insects or insect damage. The budwood should be treated with an appropriate pesticide according to local guidelines. However, since some insect eggs may not always be eliminated through a pesticide dip, it is recommended that on receipt of budwood, that grafted plants are then maintained in an insect proof cage and examined daily for the presence of insect activity, and wherever possible either autoclave or totally destroy all packaging by other means.

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9.2 Cocoa pod borer

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9.2.1 Causal agent

Conopomorpha cramerella (Snellen) (Lepidoptera: Gracillaridae).

9.2.2 Symptoms

Symptoms of Cocoa pod borer (CPB), *C. cramerella* infestation can be observed on cocoa pods, where immature pods show pre-ripened yellow patches and hardening of pods (Azhar 1988). In contrast, green patches are visible on mature pods (Fig. 9.2.1). These symptoms are due to larvae tunnelling inside the pod (Bakar et al. 2021). Heavy infestations occur with more than 3 to 4 larvae feeding and stop the pod from developing, inducing early or premature ripening. Larval entry holes on the pod surface are barely visible to the naked eye, but they can be detected by shaving the husk (Fig. 9.2.2). The larvae feed on the mucilage and placenta, leaving dark frass and burrowing signs (Fig. 9.2.3). As this entire stage of the life-cycle takes place inside the pods, larvae are almost entirely protected from any control approach. Larvae leave characteristic 1-2 mm diameter exit holes in pod walls (Fig. 9.2.4). The exit holes can be seen on the pod husk; meanwhile, black pinhole entry holes on the pod are observed when sliced. Cocoa beans in infected pods leads to the aggregation of beans where beans are hardened and clumped together (Fig. 9.2.5), making extraction from the pod husk and mucilage difficult (Azhar 1988, 1990, 1995, Azhar and Long 1993, 1996, Lee et al. 2013). The interruption in bean development and the solidification of pods in turn leads to challenges in harvesting and processing. Beans may also begin to germinate within pods that are infested when nearly ripe (Azhar 1986). Infested pods with clumped beans are unusable and the whole pod must be discarded when the infestation is severe (McMahon et al. 2015).

9.2.3 Geographical distribution

CPB was first detected in a cocoa plantation in Sulawesi, Indonesia, in the 1860s (Wardoyo 1980). The pest was recorded in the Philippines in 1936, in Malaysia in 1980 and in Papua New Guinea in 2006 (Ooi et al. 1987, Khoo et al. 1991, Azhar 1995, Yen et al. 2010, Saripah and Alias 2016). In 2011, this pest was reported in North Queensland, Australia; fortunately, the pest was successfully eradicated in Australia. It is also encountered in Sri Lanka, India, the Philippines, Taiwan and

Thailand (https://www.cabi.org/isc/datasheet/7017#todistribution). CPB continues to be the primary pest in Southeast Asia and the western Pacific archipelagos (Azhar et al. 2000, Shapiro et al. 2008, Sulistyowati 2015, Niogret et al. 2019, Iamba and Masu 2020, Niogret et al. 2023, Saripah et al. 2021).

Figure 9.2.1. Uneven yellowing of immature pods due to cocoa pod borer infestation (Saripah B, Malaysian Cocoa Board)

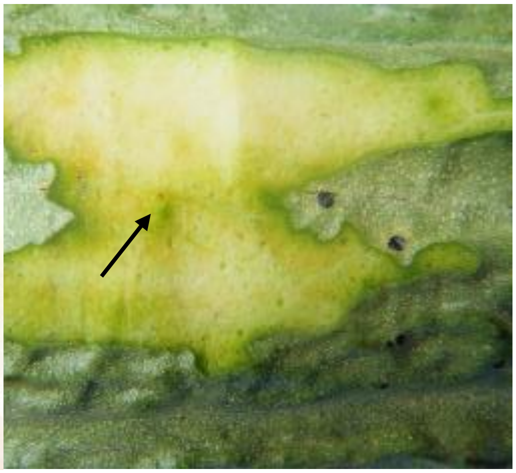

Figure 9.2.2. The entry hole is visible after the pod husk was shaved (Saripah B, Malaysian Cocoa Board)

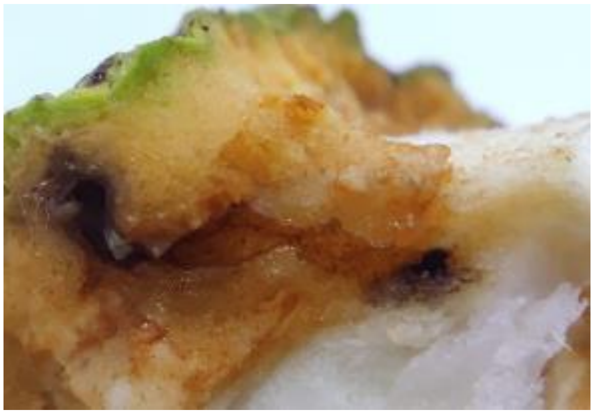

Figure 9.2.3. Galleries of larval infestation on the mucilage and pod husk (Saripah B, Malaysian Cocoa Board)

Figure 9.2.4. The exit holes on the pod surface (Saripah B, Malaysian Cocoa Board)

Figure 9.2.5. Beans clumped into a solid mass resulting from cocoa pod borer feeding at a heavy level of infestation (Saripah B, Malaysian Cocoa Board)

9.2.4 Host plants

CPB exhibits polyphagy, feeding on many host plants belonging to the Sapindaceae, Malvaceae and Fabaceae families (www.gracillariidae.net/species/2385). CPB attacks fruits from the Sapindaceae family including *Nephelium lappaceum* (rambutan) (Fig. 9.2.6), *Pometia pinnata* (Fijian longan), *Nephelium mutabile* (pulasan) and *Euphoria malaiense;* Leguminosae family, *Cynometra cauliflora* (nam-nam), *Cynometra cauliflora* as well as *Cola nitida* and *Lansium domesticum* (langsat) from Family: Malvaceae (Ooi et al. 1987). Another fruit reported to be associated with CPB is *Litchi chinensis*. *N. lappaceum* is believed to be the pioneer host, but since it has a short fruiting season (2 to 3 months) this is likely to have resulted in the spread to cocoa trees (Azhar and Long 1993, Posada and Vega 2005, Wardojo 1980). The Sapindaceae and Leguminosae species may be the original host of CPB as cacao is not indigenous to Southeast Asia. A recent study demonstrated clear preferences of female CPB for cocoa pods compared with its native host fruits (*N. lappaceum*, *P. pinnata* and *L. domesticum*) (Niogret et al. 2020).

Figure 9.2.6. Infestation of CPB on *N. lappaceum* (Saripah, B. Malaysian Cocoa Board)

9.2.5 Biology

The life cycle of CPB is relatively short, approximately 27 to 33 days as illustrated in Fig. 9.2.7. Gravid CPB females initiate flight at dusk and seek cocoa pods, laying their eggs directly on the outer husk (Niogret et al. 2020). Deposition of eggs can take place on pods at a relatively early stage of development (70 mm length), through to maturity. An adult female lays eggs singly or in groups of two or three on the cocoa pod surface and may lay 40-100 and up to 300 eggs during their maturity stage (Lee et al. 2013, Saripah et al. 2021). The ovipositional preference of CPB depends on the stage of pod development and egg-laying behaviour on full-size unripe pods and over-ripe pods (Niogret et al. 2020). Freshly laid eggs are orange in colour with a length of approximately 0.5-0.6 mm. The eggs are oval, strongly flattened, and usually laid singly near furrows on the pod surface. The egg stage lasts for 2-7 days.

The eggs typically hatch after *circa* three days, changing during maturation from an orange colour to nearly colourless. The first instar larvae usually tunnel through the eggshell (Niogret et al. 2023) and bore immediately through the pod walls (Fig. 9.2.8). Inside the pod, the larvae feed for 14-21 days on the mucilage, pulp, placenta, and sometimes the testas of the cotyledons. The feeding behaviour will disrupt the growth of the cocoa beans and affects their supply of nutrients. The entire larval stage takes 14-18 days to complete, with 4-6 instars (Lim et al. 1982). Once mature, larvae bore out through the pod wall (Fig. 9.2.9) and leave a sign of exit holes on the pod surface. The pre-pupa will spin the cocoon immediately, and pupation occurs outside the pod within the oval-shaped silken cocoon on another part of the canopy, on the furrow of the pod, green or dried leaves and other debris (Fig. 9.2.10).

Pupae change colour from an initial light green to dark grey as they mature. Completion of the pupation stage usually takes 6 to 8 days (Saripah et al. 2019). An adult emerges after completing the pupal stages and often rests transversely underneath the jorquette branches, especially in shady areas. The adults are *circa* 5 mm long with a 13 mm wingspan, and the forewings of newly emerged adults display a white zigzag stripe with a yellow-orange spot at the tip. Adult moths are active at night but rest during the day with wings, antennae, and legs tightly folded to the body and orient themselves crosswise on the undersides of horizontally inclined branches. Adult longevity usually is about one week and, exceptionally, up to 30 days. This multivoltine lepidopteran will continue to deposit their eggs, and the highest number of eggs and entry holes is usually recorded at pod lengths more than 150mm (Saripah 2019).

9.2.6 Quarantine measures

When transferring seed:

1. Whole unopened pods with signs of CPB symptoms, especially the exit holes and uneven ripening colours on the pod surface, should NOT be sent from infected areas.
2. Where movement of pods is required, they must be transferred in a container with a closed lid, or a gunny sack or plastic bag tied closed during the transportation process.
3. The source of the seeds should be clean pods with no signs of insect boring or fungus inside the pod.
4. The beans should be washed in water, treated with an appropriate insecticide/fungicide mix, and packaged in fresh packing material.

When transferring budwood:

1. The source of the budwood should be trees that exhibit no signs of insect boring on the pods.
2. The budwood should be treated with an appropriate insecticide/fungicide mix and packaged in fresh packing material.
3. Budwood with CPB cocoon should NOT be sent to new area

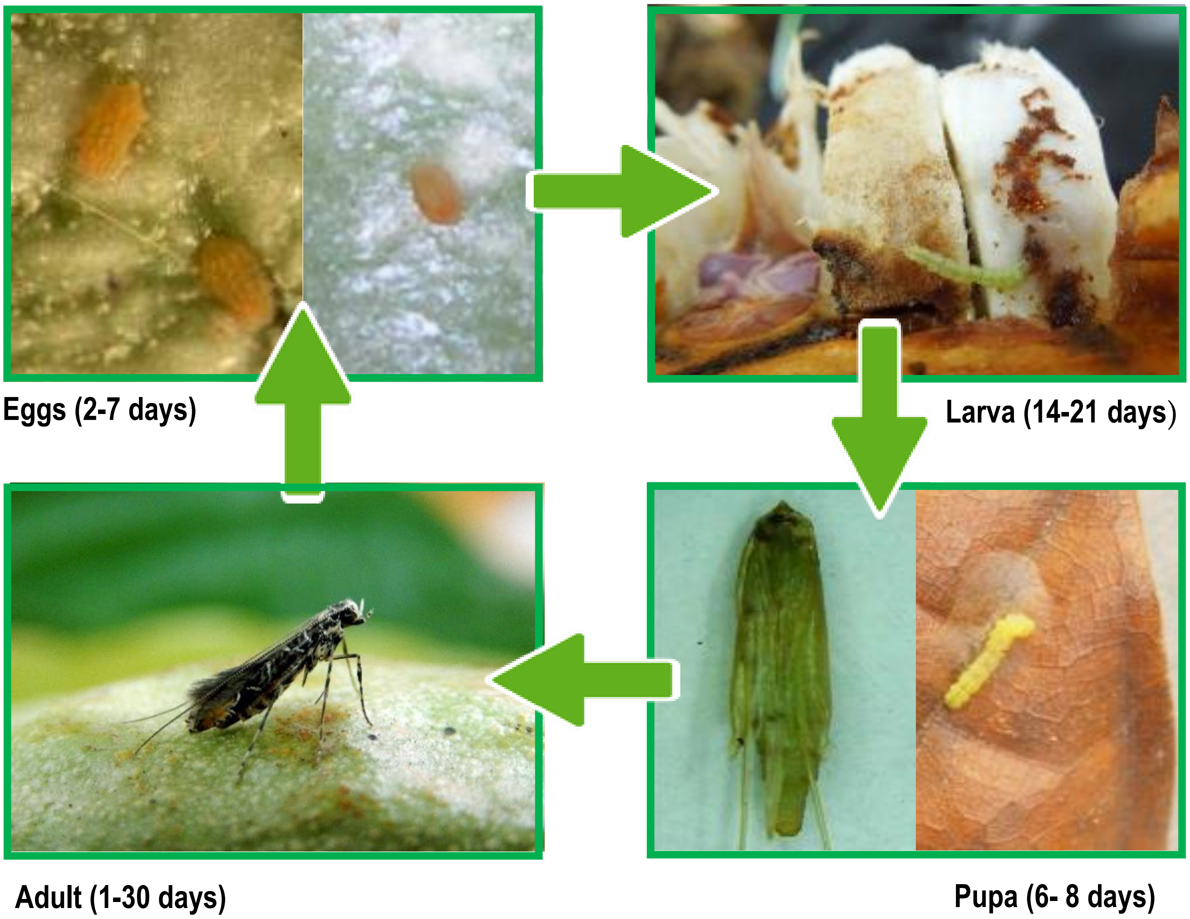

Figure 9.2.7. Life cycle and duration of the life stages of cocoa pod borer (Saripah B, Malaysian Cocoa Board)

Figure 9.2.8. Newly hatched cocoa pod borer larva tunneling into the pod wall (A Alias, Malaysian Cocoa Board)

Figure 9.2.9. Cocoa pod borer larva emerging from its exit tunnel in the pod wall (Saripah B, Malaysian Cocoa Board)

Figure 9.2.10. Cocoa pod borer pupa under its silk cocoon on a pod surface and leaf litter (Saripah B, Malaysian Cocoa Board)

Figure 9.2.11. CPB pupa after the silken cocoon were removed (Saripah B, Malaysian Cocoa Board)

9.2.7 References and further reading

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9.3 Cocoa Fruit Borer/ American Cocoa Pod Borer (*Carmenta spp.*)

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9.3.1. Causal agents:

Carmenta foraseminis Eichlin and *C. theobromae* (Busck) (Lepidoptera:Sesiidae) from the neotropics are morphologically similar species. Although slightly dissimilar in size (Delgado Puchi 2005), they can only be separated confidently by examining the male genitalia; *C.* spp. near *chrysophanes* (Meyrick) causes similar damage to cacao in Papua New Guinea (PNG).

9.3.2 Symptoms:

The damage caused by *C. foraseminis* in cacao pods resembles that caused by Cocoa Pod Borer, (*Conopomorpha cramerella* (Snellen), in Southeast Asia (Section 9.2). Newly laid eggs are reddish-brown, elongated-oval in shape (2.4-3.2 x 1.7-2.2 mm) with short longitudinal striae. *Carmenta theobromae* affects mainly the epicarp of the fruit so is less damaging. The eggs of *C. theobromae* are significantly shorter than those of *C. foraseminis* (2.4-3.3 vs 3.5-3.8 mm long). Larval entry and exit holes are similar in size to those of *C. cramerella* (Section 9.2) and the internal damage to beans within pods is also similar to that species (Fig. 9.3.1). Pupation occurs inside the pod, insects emerging as adults. In severe infestations around 60% of pods may be infested.

9.3.3 Geographical distribution:

Carmenta foraseminis has been recorded from cacao in Brazil, Colombia, Panama, Peru and Venezuela. Similarly, *C. theobromae* is reported as a cacao pest in Colombia, Panama, Peru, Trinidad and Venezuela. *Carmenta* spp. is also found in Ecuador.

9.3.4. Host plants other than *T. cacao*:

Larvae of *C. foraseminis* have been found in fruits of *Eschweilera* spp. and *Gustavia* spp. *C. theobromae* is an important pest of guava (*Psidium guajava*). *C. chrysophanes,* a stem-borer on cacao rather than a seed-feeder like *C.* sp. near *chrysophanes*, also feeds on Balsa (*Ochroma lagopus*) in PNG and *Alphitonia, Eucalyptus* and *Ficus* spp. in Australia.

9.3.5. Biology:

The biology of both Neotropical species is described by Delgado Puchi (2005). *Carmenta* spp. are day-flying clearwing moths. Adults are short-lived, dying within a week of emergence. Eggs, laid typically on 80-120 day old pods (Sotomayer-Parian and Soto-Cordova, 2018), hatch within 10-20 days whereupon the larvae bore through the pod wall and feed on developing beans and mucilage, causing damage similar to that caused by Cocoa Pod Borer (Section 9.2). The whole life-cycle is completed in between 90-110 days. The biology of *C.* sp. near *chrysophanes* and *C. chrysophanes* on cacao is unknown.

9.3.6. Quarantine measures:

Whole unopened pods should not be sent from infested areas as it is often difficult to assess pod infestation externally. Beans from pods found to be clean on opening should be washed and treated with an appropriate insecticide/fungicide mix prior to despatch.

9.3.7. References

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Figure 9.3.1. Larva of Carmenta (L. Bagnybeilhe)

9.4 Other Lepidopteran Pests

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9.4.1 Cocoa Stem Borer, *Eulophonotus myrmeleon* Felder (Lepidoptera: Cossidae)

The larvae of this moth bores into woody stems, branches and roots of cocoa in West and Central Africa (for example see CABI Digimap 2005), resulting in the death of affected limbs or young trees. Adult female moths lack mouthparts, but each may lay over 1600 eggs in their brief 4-day lifespan (Adu-Acheampong et al. 2004). The ovo-elongate 400 x 600 µm pale yellow to pink eggs, which may be laid on any part of the tree, hatch after about eleven days incubation whereupon the newly hatched larvae immediately burrow into fresh stems. However, stems below 1.5 cm diameter are unlikely to be attacked, so any shoots harvested for use as budwood above that size need careful inspection for tell-tale penetration holes, as larvae within their tunnels are protected from the effects of an insecticidal dip.

9.4.2 Husk miners

Transfer of Lepidopteran husk miners such as the Tortricids *Thaumatotibia Cryptophlebia encarpa* (Meyrick) from Malaysia and Papua New Guinea and *Gymnandrosoma* (*Ecdytolopha) aurantianum* (Lima) from Venezuela and *G. (E.).*

punctidescanum (Dyar) from Trinidad, the Gracillariids *Marmara* spp. from Brazil, Trinidad and Tobago, *Spulerina* spp. from West Africa and the Noctuid *Characoma stictigrapta* Hampson from Africa would be undesireable, but less disastrous than an accidental transference of CPB, as the damage these husk miners cause to cacao pods is mostly superficial, although susceptible to pathogen infection. The necrotic wandering galleries left by these species near the pod surface are unlikely to be overlooked during a visual inspection of pods prior to shipping.

9.5 Mirids (and other Heteropterous plant sucking bugs)

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The plant-sucking bugs in the Families Miridae and Pentatomidae are pests of cacao in every geographic region except the West Indies, while a few genera in these Families are predators of other pest insects. The most important pest species vary between cocoa growing areas and a separate section (9.6) is included to cover the Mosquito bug (*Helopeltis theobromae*) which is of particular concern in Southeast Asia.

9.5.1 Causal agents, geographic distribution and symptoms

Among the 56 species of Miridae so far recorded on cacao worldwide, 42 are plant feeders, four are predators and the status of the remaining species is unknown (Bigger 2012). About seven species of *Monalonion* feed on cacao shoots and fruits in South and Central America, together with a few less common genera. *Sahlbergella singularis* Haglund (Fig. 9.5.1) and *Distantiella theobroma* Distant (Fig. 9.5.2) are the commonest and most damaging species in West and Central Africa, often severely degrading the canopy while causing only superficial harm when they feed on pods. However, the resultant necrotic feeding lesions (Fig. 9.5.3 and Fig. 9.5.4) can function as entry points for pathogens such as black pod (*Phytophthora* spp.) and dieback caused by *Fusarium* spp. and *Lasiodiplodia* spp. (Adu-Acheampong and Archer 2011). *Monalonion* is replaced in West and Central Africa, India, Southeast Asia and Papua New Guinea by the similarly gracile *Helopeltis* of which about 21 species are recognised so far (Bigger 2012). Many of the *Helopeltis* that occur outside Africa cause serious damage to the fruit as well as degrading canopy shoots. Although those that occur in Africa feed mostly on fruits, often producing numerous necrotic feeding lesions in the pod walls, their mouthparts do not reach the beans and little economic damage is caused.

9.5.2 Biology

The biology of all of the plant-feeding species is quite similar and is discussed in detail by Entwistle (1972). In all genera, egg-laying females inject their eggs into the plant tissue with only two microscopically thin horns attached to the chorionic rim and a slight bulge from the domed operculum exposed. The eggs usually hatch in 11-16 days. The nymphs moult five times during their development, becoming an adult three-four weeks after hatching. Most species hide in dark refuges under pods and under branches during daylight hours, only emerging at night to feed. They also often either drop from the tissue on which they were feeding if disturbed, or rapidly move from sight. Eggs present in budwood and pods present the greatest quarantine risk, because not all are likely to be killed when the budwood or pod is dipped in an insecticide while egg incubation period is long enough to allow first instar nymphs to emerge undetected at night over a considerable period.

9.5.3 Other plant bugs

Other than mirids, over 150 Heteropterous plant sucking bugs from 14 Families have been recorded on cacao worldwide of which 55 species are reported as feeding on the crop (Bigger 2012). Most are mainly minor pests, but in the context of exported plant material, two Pentatomid species warrant special mention. *Antiteuchus tripterus* (Fabricius) in Latin America is a vector of a major fungal pod rot disease caused by *Moniliophthora roreri* (see Section 8.2), and the insect's presence may be indicative of a latent infection of the disease. In West and Central Africa, the pod feeder *Bathycoelia thalassina* (Herrich-Schäffer) has become increasingly prevalent owing to the increased planting of hybrid cacao which bear pods throughout the year. Both species are large conspicuous shield-shaped insects (> 1.5 cm long) whose females lay their eggs in batches externally on shoots and pods. Hence, neither eggs nor active stages are likely to be overlooked during a visual inspection of export material. In addition, females of *A. tripterus* actively guard their eggs and recently hatched nymphs, rendering them even more obvious.

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Figure 9.5.1. Adults of *Sahlbergella singularis* (KF N'Guessan, CNRA)

Figure 9.5.2. Adults of *Distantiella theobromae*

Figure 9.5.3. Mirids lesions (dark colour) on cacao pods (KF N'Guessan, CNRA)

Figure 9.5.4. Larvae of mirids on cocoa twig and Mirids lesions (dark colour) on cocoa pod

(KF N'Guessan, CNRA)

9.6 Mosquito bug

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9.6.1 Causal agent

Helopeltis spp. (Hemiptera: Miridae).

Common synonym *Helopeltis theivora* (Waterhouse) (Hemiptera: Miridae); *Helopeltis theobromae* (Miller) (Hemiptera: Miridae); *Helopeltis antonii* (Signoret) (Hemiptera: Miridae); *Helopeltis bradyi* (Waterhouse) (Hemiptera: Miridae).

9.6.2 Symptoms

Helopeltis spp. are present during both the fruiting and vegetative stages of the host plant. These true bugs feed by piercing and sucking on cocoa trees, particularly on cocoa branches and twigs during the period after the pods have been harvested, corresponding to the vegetative phase of the host plant (Etam et al. 2023). Both nymph and adult of *Helopeltis* spp. infest young shoots (Fig. 9.6.1), cacao pods and peduncles on which a single pest can produce approximately 25-35 lesions per day. An exudation of a resinous gummy substance results from the feeding punctures made by the suctorial mouth part of this insect (Thube et al. 2016). The fresh lesions on the pod are water-soaked and dark green in colour. The tissues around the point where the stylet enters become necrotized due to infection with secondary plant pathogens (Thube et al. 2019). The lesions will turn darker, slightly concave, and old lesions are dark in colour but are usually convex (Fig. 9.6.2). *Helopeltis* spp. begin attacking cacao pods at an early stage of pod development, and damage is clearly visible from when the pods are 70mm in length onwards (Saripah 2019). *Helopeltis* feed on the parenchymatous husk tissue of the cacao pod, and this usually induces abscission of young pods (cherelle wilt). Young pods, especially those less than three months old (Fig. 9.6.4), have little chance of surviving (Wan Ibrahim 1983). Therefore, early infestation may reduce the number of pods reaching maturity. Older pods are more likely to survive attacks, with pods from 85mm to 150mm long found to have the highest number of lesions (Saripah, 2019). Although the impact of infestations is reduced on older pods, which often tolerate direct damage unless the number of feeding lesions inflicted is high (Khoo et al. 1991), mirid damage may lead to invasion by secondary pests (Fig. 9.6.5) or disease organisms and severe infestations on the cacao pod can cause cracking or complete loss of the pod.

Thube et al. (2019) reported that *H. theivora* prefers to feed and oviposit on developing pods rather than on cacao leaves and shoots. Infestation on the shoots often occurs when only a few pods are available or as an alternative food source

(Alias 1983). The colour of fresh lesion on shoots is pale brown, oval shaped, and turns into black after 2-3 days. The lesion on shoots is approximately 4-7mm in length. In very serious infestations, the entire tree looks burnt. Mirids' feeding activities are marked by the presence of cankers (dark markings or lesions), black spots, withered leaves, and occasionally, cocoa trees with stunted growth due to the lack of leaves.

According to Entwistle (1972), the stunted growth was mostly observed or more widespread in areas where cocoa is cultivated without shade. Infestation usually increases particularly in the rainy season (MCB, 2013). The cocoa crop can have a significant decrease in yields due to severe attacks on shoots, with reductions ranging from 32 to 44% in Indonesia (Purwaningsih et al. 2014). The estimated yield loss in Indonesia has been estimated as 50-60% if the infestation is at a high level (Siswanto et al. 2020). Among this group of species, *H. theivora* is the most prevalent and influential, causing a potential output drop of over 40% in Indian cocoa farms (Thube et al. 2023).

Figure 9.6.1. ***Helopeltis*** **infestation on young shoots (B Saripah, Malaysian Cocoa Board)**

Figure 9.6.2. Old lesions on cherelles and cocoa pods are dark in colour (B Saripah, Malaysian Cocoa Board)

Figure 9.6.3. Symptoms of *Helopeltis* infestation at various size of cacao pods (B Saripah, Malaysian Cocoa Board)

Figure 9.6.4. *Helopeltis* infestation on a cherelle (B Saripah, Malaysian Cocoa Board)

Figure 9.6.5. Secondary pest and disease infestation (B Saripah, Malaysian Cocoa Board)

9.6.3 Geographical distribution

The pest is currently distributed widely throughout Asia including India (Thube et al. 2019), Malaysia (Saripah 2019), Indonesia (Siswanto et al. 2020) and the Philippines. India hosts four *Helopeltis* species: *H. antonii* Signoret, *H. theivora* Waterhouse, *H. bradyi* Waterhouse, and *H. cinchonae* Mann. Among them, *H. antonii* dominates, thriving on over 35 plant species from 24 different families (Srikumar and Bhat, 2012). Furthermore, three tea mosquito species, *Helopeltis antonii* Signoret, *H. theivora* Waterhouse, and *H. bradyi* Waterhouse, have been documented on cocoa plants in India (Thube et al. 2019, Thube et al. 2023). *H. theivora* is the primary pest responsible for harming young shoots, cherelles, and pods (Malhotra and Apshara, 2017). Nine species of *Helopeltis* species are reported be found in Indonesia; *H. bradyi, H. chinconae, H. bradyi, H. cuneata, H. fasciaticollis, H. insularis, H. sulawesi, H. sumatranus,* and *H. theivora* (Stonedahl, 1991). Two of these species have only been reported relatively recently, these being *H. bradyi* (Melina et al. 2016, Siswanto et al. 2020) and *H. antonii* Purwaningsih et al (2014). A few species of *Helopeltis* have been reported in the Philippines notably *H. bakeri* (Serrana et al. 2022, Pag-ong et al. 2024). In Malaysia, *H. clavifer, H. theobromae* and *H. theivora* had been reported either in Sabah state or Peninsula Malaysia (Azhar 1989, Lee et al. 2023, Saripah 2019). In West Africa region, *Helopeltis* spp. are frequently found in cocoa plantations in Southern Cameroon (Etam et al. 2023).

9.6.4 Host plants

Helopeltis spp. are a polyphagous insect, and the host plants for *Helopeltis* are cacao, mango, *Acalypha* spp. and Japanese Cherry (Khoo et al. 1991). Additionally, *Helopeltis* spp. also attacks flower buds and fruits of guava, cashew and apples. It also infests tea plantations in India (Sarmah and Phukan 2004, Sarmah and Bandyopadhyay 2009, Bhuyan et al. 2017) and Indonesia (Gusti Indriarti and Soesanthy 2014). There is also a report of *Helopeltis* spp. infesting crystal guava, *Psidium guajava* in Indonesia (Muhlison et al. 2023) and neem, *Azadirachta indica* in India (Chaitanya et al. 2024).

9.6.5 Biology

The life cycle of *Helopeltis* is between 21-35 days and up to 29 days for *H. theivora* (Thube et al. 2019). An adult female can lay approximately 80 eggs (Kalshoven 1980), which are oval in shape with two chorionic processes arising from this egg (Khoo et al. 1991). The female usually lays eggs in the outer layer of pods or beneath the bark of young shoots. The eggs hatch in 5-7 days and there are then 5 nymph stages (Entwistle 1965) with an incubation period of 2-17 days. The colour of the nymph changes from light green (Fig. 9.6.6) to dark green when it turns into an adult. The nymphs are smaller and have no wings. The adults are about 5-10 mm long (Fig. 9.6.7).

Figure 9.6.6 *Helopeltis* nymph which is light green colour (B Saripah, Malaysian Cocoa Board)

Figure 9.6.7. Helopeltis adult, usually up to 5.5mm in length (B Saripah, Malaysian Cocoa Board)

9.6.6 Quarantine measures

Transport of pods from areas infested with *Helopeltis* is not recommended due to the possible presence of eggs in fresh lesions. Any plant material should be inspected carefully before transit. The presence of eggs can be confirmed by staining the material using lactophenol blue and then examining under the microscope.

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9.7 *Pseudotheraptus devastans* (Dist.)

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9.7.1 Causal agent

Pseudotheraptus devastans (Dist.) (Hemiptera: Coreidae)

9.7.2 Symptoms and Biology

The nymphs and adults of *P. devastans* feed on pods by inserting their stylets through the husk into the beans, resulting in extensive deformation of the pods and agglutination or clumping of beans inside pods, leading to massive reduction in yields (Figures 9.7.1-9.7.4) (Lodos 1965). The feeding lesions caused by *P. devastans* are similar to those of mirids but those of *P. devastans* are larger on the pods (Lodos, 1965). On young shoots, feeding may result in dieback. Similar to the Pentatomid species, the increased planting of hybrid cocoa has enhanced their survival and development (Awudzi et al. 2019). This is attributed to the availability of pods all year round on hybrid cocoa which provide unlimited feeding sites for the pest. Populations reach their peak in March and April warranting pest control activities. Wounds created on fruits attacked by the bug are subsequently invaded by opportunistic fungi (e.g. *Fusarium decemcellulare* (anamorph of *Albonectria* (*Calonectria*) *rigidiuscula)* and other rot causing fungi (e.g. *Phytophthora spp*) to cause diebacks and fruit rots respectively (Akrofi et al. 2016).

9.7.3 Geographical distribution

Pseudotheraptus devastans has been recorded in West, Central Africa and East Africa where it is a pest of crops including coconut and cassava (CABI, 2021). In recent years, the incidence and damage caused by *P. devastans* on cocoa farms in Ghana has become increasingly important (Awudzi et al. 2024), most likely associated with the introduction of fruit crops into cocoa plantations. Recent pest survey and population studies indicate that the pest is now present in all the cocoa growing regions in *Pseudotheraptus devastans*, though it was previously mainly restricted to the Eastern region of Ghana (Lodos 1965), and is widely distributed in the cocoa growing regions of Côte d'Ivoire (Kouamé et al. 2023).

9.7.4 Host plants other than T. cacao

The pest is also known to attack other crops commonly grown on cocoa farms such as cassava, coconut, mango, guava, cashew, avocado pear and coconut (Yeboue et al. 2015).

9.7.5 Quarantine measures

Precautions should be taken when moving pods. Ensure pods are not deformed with deep feeding lesions extending into the cortex. Pod husks should be maintained in an enclosure for at least a week after pod breaking to contain eggs that may hatch.

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Figure. 9.7.1: *Pseudotheraptus devastans* nymph (G Awudzi)

Figure 9.7.2: Adult *Pseudotheraptus* devastans (G Awudzi)

Figure 9.7.3: Feeding lesions of *Pseudotheraptus devastans* on cocoa pods (G Awudzi)

Fig.9.7.4. Deformation of cocoa pods by *Pseudotheraptus devastans* (G Awudzi)

9.8 Mealybugs

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9.8.1 Causal agent

Various genera (Hemiptera: Pseudococcidae)

With few exceptions (e.g. *Planococcus lilacinus* (Cockerell), in Southeast Asia and the South Pacific which has phytotoxic saliva), mealybugs (Pseudococcidae) rarely damage cacao directly. Their main importance is as virus vectors. Not all species can transmit cacao viruses and those that do differ in their efficiency as vectors; only 14 of the 26 species recorded from cacao in West Africa are vectors of CSSV. More than 80 species have been recorded so far from cacao (Bigger 2012). Every conceivable feeding niche on a plant may be exploited by one species or more, but for plant quarantine considerations terminal buds and pods present the most vulnerable feeding sites. In Ghana, 22% of dissected terminal buds were infested mainly by nymphs, too small and too well hidden between the bud scales for detection by the unaided eye (Campbell 1983). Although most mealybug species feed from aerial tissues, 10% of species are specialist root feeders, some of which have now been re-classified to Family Rhizoecidae (Hodgson 2012).

9.8.2 Geographical distribution

Mealybugs are ubiquitous in the tropics and occur on cacao in all regions. A few highly polyphagous species have a worldwide distribution (e.g. *Ferrisia virgata* (Cockerell), *Planococcus citri* (Risso) and *Pseudococcus longispinus* (Targioni Tozzetti)), but most species have narrower host ranges and more localized regional distributions. Cacao is an introduced crop in most regions so in those regions mealybugs have adapted to cacao from indigenous hosts.

9.8.3 Biology

Mealybugs are small sap-sucking insects, rarely exceeding 4 mm in body length. Typically, the dorsal surface of adult females is covered in wax, the extent, distribution and colour of which is often species-specific and serves as an aid to identification in the field. Females are wingless. The body shape varies widely between species, but many of the commonest species on cacao are broadly oval and dorso-ventrally flattened. The mouthparts are located on the underside of the body almost level with the first pair of legs and consist of a short beak from which emerge needle like stylets. The insect uses these stylets to penetrate the plant’s cortical tissues to tap into the phloem from which they may also imbibe virus particles. The stylets often exceed half of the insect’s body length, but are capable of being withdrawn undamaged in seconds should the insect be disturbed. Reproduction may be sexual and/or parthenogenetic. Males lack mouthparts in those species that do retain sexual reproduction, so only adult females and female nymphs are vectors of viruses. Most species lay eggs, often adjacent to the mother and in masses of several hundred eggs protected by white fluffy ovisacs. However, some species including *Formicoccus (Planococcoides) njalensis* (Laing) (Fig. 9.8.1.) a widespread vector of CSSV in West Africa, either give birth to live young or the eggs hatch within a few minutes of being laid. Newborn and newly hatched nymphs, barely visible to the unaided human eye, are the principle dispersive stage of the insect. They mostly walk giving rise to radial spread of virus diseases, but they can also be carried often long distances by wind currents giving rise to jump spread of viruses. Young nymphs often settle within apical buds so may inadvertently be transported with budwood unless the safeguards outlined in the general precautions are followed. They also squeeze between cracks in the bark and in fissures on the surface of developing pods. Nymphs can also feed on the cotyledons of any cacao seeds damaged during pod-splitting, so it is also a wise precaution to dip pods in an insecticide before live seeds are extracted and exported.

Figure 9.8.1. Adults and nymphs of *Formicoccus njalensis* (WP N'Guessan, CNRA)

9.8.4 References

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9.9 Ambrosia beetles

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9.9.1 Causal agents

Some 135 species of Ambrosia beetles (Coleoptera: Scolytinae) have been recorded from cacao (Bigger, 2012) almost all of which are capable of inflicting serious damage from invasion by phytopathogenic fungi into their feeding tunnels. Of greatest concern are *Xylosandrus compactus* (Eichhoff) (senior synonym of *Xyleborus morstatti* Hagedorn) because of its ubiquity and small size (female *ca*. 1.7 x 0.8 mm), and *Xyleborus ferrugineus* (Fabricius) (female *ca*. 2.7 x 0.9 mm) because of its symbiotic association with the fungus *Ceratocystis cacaofunesta* which causes wilting and dieback of branches, or even death of the whole tree, in South America and the Caribbean. Both species are known to attack healthy cacao. Eighteen fungal species have been identified associated with *X. compactus*; some are saprophytic while others such as *Lasiodiplodia* (*Botryodiplodia*) *theobromae* (Pat.) Griffon & Mauble.) and *Fusarium decemcellulare* Brick (are phytopathogenic. The status of both beetle species on cacao is summarised in detail by Entwistle (1972), although the scale markers he presents for *Xylo. compactus* are twice their actual size.

9.9.2 Symptoms

Many species in the genera *Xylosandrus* and *Xyleborus* bore into trunks or small branches causing dieback so are particularly dangerous as pests of nursery plants. The adult beetle excavates multi-branching galleries often subepidermally but sometimes penetrating on older branches into the wood to a depth of 5cm or more. Often the first signs of infestation are wilting of young stems which eventually die back. Peeling back the bark to expose any surface tunnels in the cambium is not always definitive for *Xylo. compactus* as females often bore radial holes straight to the pith in thinner stems (see Fig 23.1D in Entwistle, 1972) whereas *Xyle. ferrugineus* does produce such multibranched galleries.

9.9.3 Geographical distribution

Xylo. compactus is widely distributed in Africa, Asia and South America. It has been introduced in some Pacific Islands and also occurs in Italy and France. It has been recorded infesting cacao in Cameroon, Ghana, Indonesia, Ivory Coast, Malaysia W. Malaysia, Nigeria, Papua New Guinea, Sierra Leone and Uganda. *Xyle. ferrugineus* is similarly widely distributed and has been recorded from cacao in Brazil, Costa Rica, Ecuador, Mexico, Trinidad & Tobago, Venezuela and Zaire.

9.9.4 Host plants other than *T. cacao*

Both species are highly polyphagous. *Xylo. compactus* attacks over 220 plant species belonging to 60 families (EPPO, 2020), including several important crop plants, but probably the host most frequently documented is coffee *Coffea arabica* and *C. canephora*. *Xyle. ferrugineus* has an even wider host range including many mostly tropical tree crops.

9.9.5 Biology

Ambrosia beetles cultivate fungal symbionts within tunnel systems excavated by females. The fungi multiply on the tunnel walls and provide the sole food for adults and larvae. *Xylo. compactus* predominantly attacks current year shoots, whereas *Xyle. ferrugineus* normally attacks branches larger than 10 cm diameter including recently felled logs. *Xylo compactus* may also bore into tap roots of seedlings. In both species, females produce males from unfertilized eggs while fertilized eggs produce female progeny. Males remain in the brood galleries which are blocked by females post-oviposition, thereby protecting the brood from natural enemies. Mating is primarily between siblings within the galleries. Pupation and mating of brood adults occurs in the infested plant material. Eggs laid in a loose cluster inside the gallery hatch in 3–5 days. The complete lifecycle occurs in *ca*. 30 days.

9.9.6 Quarantine measures

Because they reproduce by arrhenotokous parthenogenesis, the transfer of even an individual female has the potential to initiate an infestation. The most likely source of international transfer is via infested budwood as the female entrance holes are typically <1 mm in diameter so are easily overlooked. Within the twigs, the females and brood are not susceptible to contact pesticides either by spraying or by dipping. All budwood pieces should be inspected microscopically for the presence of entry holes prior to despatch.

9.9.7 References and further reading

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9.10 Phytophagous mites

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9.10.1. Causal agents:

Other than in the Americas, phytophagous mites have received little attention on cacao. Entwistle (1972) cites just two examples, a *Tetranychus* sp. in Nigeria and a *Metatetranychus* sp. in Ghana. Phytophagous mites on cacao are represented by, Eriophyidae (gall mites), three genera with one species each (Rodrigues et al. 2017); Tarsonemidae (white mites), three genera and twelve species probably mainly feeding on algae, fungi and lichens (Ochoa et al. 1995, Rezende et al. 2015, Sousa et al. 2018, 2020); Tenuipalpidae (flat mites), two genera and six species (Castro et al. 2021); Tetranychidae (red spider mites), six genera and thirteen species (Anon 2021 Migeon and Dorkeld, 2021) with the inclusion of *Tetranychus urticae* Koch; Tuckerellidae (ornate or peacock mites), three *Tuckerella* spp. (Escobar-Garcia et al. 2021a). Several species among these families damage a range of crops in the Neotropics, including cacao. Probably owing to a scarcity of taxonomic specialists, their importance elsewhere is unknown. Only species listed as economically important are named below.

9.10.2 Symptoms:

The cacao bud mite *Aceria reyesi* (Nuzzaci) Eriophyidae attacks the terminal buds of branches, causing atrophy, premature leaf fall and shortening of the internodes and in severe infestations death of the tree (de Carvalho et al. 2018). *Brevipalpus yothersi* Baker (Tenuipalpidae) feed mostly on the surface of pods causing scarring and superficial surface lesions concentrated in the pod grooves (Escobar-Garcia et al. 2021b). An accumulation of feeding punctures by *Tetranychus mexicanus*, (McGregor), *T. urticae*, and Tetranychidae in general, leads to whitening, yellowing or bronzing of leaves, followed by desiccation, and eventually defoliation and sometimes death of the shoot. *Tetranychus* spp. mainly colonise leaf lower surfaces while other family members prefer upper leaf surfaces e.g. *Oligonychus yothersi* (McGregor). *Tuckerella* spp. feed in fissures in branches and on pod epicarps where they induce corky extrusions which cause severe malformation as pods develop.

9.10.3 Geographical distribution:

Aceria reyesi has been found on cacao in Brazil, Costa Rica, Cuba, Ecuador and Venezuela and, because of its microscopic size and cryptic behaviour, may have been spread undetected more widely in the Neotropics and beyond. In view of Beard et al.'s (2015) revision of the *Brevipalpus phoenicis* species complex, it seems likely that early records of feeding damage to cacao attributed to *B. phoenicis* (Geijskes) in Cuba, Honduras, Malaysia and India, (Castro et al. 2021) and in Malaysia (Lim 1998) probably refer to *B. yothersi*. Similarly, the almost worldwide distribution of *B. phoenicis* (Castro et al. 2021) on other hosts may also refer mainly to *B. yothersi*, as Beard et al. (2015) confirmed the latter's presence in 32 countries globally whereas they list the distribution of *B. phoenicis* as Costa Rica and, on plant imports, the Netherlands and USA. *B. yothersi* also damages cacao in Peru (Escobar-Garcia et al. 2021b). *Tetranychus mexicanus* occurs in Mexico and most Neotropical countries (Migeon and Dorkeld, 2021) while *T. urticae* is ubiquitous. *Tuckerella ornata* Tucker, originally described from South Africa, also occurs in Brazil, Costa Rica, Cuba, Guadeloupe, Namibia, Philippines and Zambia. *Tu knorri* Baker & Tuttle, originally described from Thailand, also occurs in Brazil, China, , Costa Rica, Cuba, Dominican Republic, Iran and the Philippines. *Tu pavoniformis* (Ewing), originally described from Hawaii, also occurs in Cuba, Dominican Republic, Costa Rica, Trinidad & Tobago and Venezuela.

9.10.4. Host plants other than *T. cacao*:

Aceria reyesi has been recorded from cacao only. The cacao-infesting tenuipalpids, tetranychids and tuckerellids are polyphagous. For example, *B. yothersi* is recorded from 42 plant families and is a serious pest of citrus, while *T. mexicanus* is reported from 44 plant families. The *Tuckerella* spp. on cacao are serious pests of citrus and also infest a wide range of other plants, including crops.

9.10.5. Biology:

Aceria reyesi adults are carrot-shaped (ca. 0.2x0.04mm), tapering from head to rear, and translucent white. Unlike most mites, they have four legs only, located near the head. Reproduction is sexual and several overlapping generations of mites may inhabit a single bud. *Brevipalpus yothersi* adults are similarly small (ca. 0.2x0.18 mm), shield shaped, dorso-ventrally flattened and orange-red in colour. Males are rare, so they probably reproduce mainly by parthenogenesis. Dense colonies may develop on infested pods and they are known to exploit surface fissures created by *Tuckerella* spp. (Escobar-Garcia et al. 2021b). Two-spotted spider mites, *Tetranychus urticae*, reproduce by parthenogenetic arrhenotoky, in which unfertilized eggs develop into males and fertilized eggs become females. Virgin females initially produce male offspring; later, when sexually mature, the sons mate with their mothers, a reproductive strategy common among Tetranychidae. Adult *T. urticae* females are elliptic about 0.4 mm long and are greenish-yellow or almost translucent with two dark abdominal spots. Males are similar but smaller. Nymphs lack the dark spots which are accumulations of body wastes visible through the translucent body wall. Colonies are often clothed in silk webbing which aids wind dispersal. Female *Tetranychus mexicanus* are similar in size and shape to *T. urticae*, but are a uniform blood red colour. Motile stages of *Tuckerella* spp. are small (ca 0.3 x 0.2 mm) oval in outline and dorso-ventrally flattened. Ochoa (1989) presents a taxonomic key to four species of *Tuckerella* in Costa Rica which includes the three species found on cacao (*Tu ornata* (Tucker), *Tu. knorri* Baker & Tuttle, and *Tu pavoniformis* (Ewing). They are carmine red in colour with white fan like setae around the periphery, and in transverse rows dorsally, plus, depending on species, either five or six pairs of flagellate caudal setae equal in length to the body. Setae also aid wind dispersal. Males are common, so reproduction is probably sexual. On cacao, they colonise flower cushions, fissures in bark, and pods. Their feeding galls the surface of developing pods creating severe deformities (Escobar et al. 2021a).

9.10.6. Quarantine measures:

Phytophagous mites are internationally important quarantine pests**.** While established colonies may be visible to an unaided eye, new infestations started by dispersing individuals may only be detected by careful microscopic examination of plant material prior to export. Even then, eriophyids such as *A. reyesi* hidden as they are between terminal bud scales may be easily overlooked. Furthermore, immersing shoots in a contact acaricide may not be wholly effective against motiles and eggs hidden deep within buds or bark fissures. Survival of a single individual of a parthenogenetically reproducing species can start a new infestation. All cacao acquisitions from Neotropical countries should be inspected using a microscope on arrival, and plants derived from buddings should be kept isolated until freedom from infestation is confirmed.

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10.Parasitic nematodes

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Parasitic nematodes play an important role in cacao production though their impact is difficult to assess since the symptoms they cause can often be mis-attributed to abiotic stresses. The presence of root knot nematodes on cacao roots has been known since 1900 (Sosamma et al. 1979), and most of the early works on the diagnosis and control of nematodes in cacao were carried out in cacao growing countries of West Africa and in Jamaica (Meredith 1974). A large number of plant parasitic nematodes species are known to be associated with healthy and diseased cacao plants (Orisajo 2009). Cacao is seriously affected by nematodes of *Meloidogyne* spp. and estimated losses from these nematodes, based on pathogenicity studies, range from 15–30% but can be as high as 40-60% (Fademi et al. 2006). Damage by this nematode is most serious on seedlings, where the losses can be as high as 100%. However, knowledge of the actual yield losses in cacao caused by nematodes, especially those from other genera, is very limited. Based on the published findings, other nematodes are as detrimental to cacao as *Meloidogyne* spp. when their population densities are high (Fademi et al. 2006).

10.1 Causal agents

Over 25 genera of endoparasitic and ectoparasitic nematodes are known to be associated with cacao (Sosamma et al. 1979, Campos and Villain 2005). *Meloidogyne* spp. have been reported as the most damaging due to their pathogencity and wide distribution throughout cacao growing regions (Villain et al. 2018). Campos and Villain (2005) and Villain et al. (2018) list several species of *Meloidogyne* and the countries where they have been found to affect cacao production, including *M. arenaria* and *M. thamesi* (Brazil), *M. incognita* (Nigeria, India, Malaysia, Venezuela, Brazil), *M. exigua* (Bolivia), *M. javanica* (Malawi, Central Africa).

10.2 Symptoms

Infected plants show reduced plant height, stem diameter, and dry weight often associated with the formation of small leaves. Stem dieback, wilting, yellowing and browning of leaves, are common symptoms of nematode infestation (Fig. 10.1). However, it can be difficult to distinguish symptoms caused by nematode infestation from similar symptoms caused by other biotic and abiotic stresses (Orisajo et al. 2021, Okeniyi et al. 2009). Roots of infected plants show swelling of hypocotyls and roots. Formation of gall knots on roots, the rupture of cortex, total disorganization of the stele, destruction of the xylem, phloem, pericycle, and endodermis and abrupt end of tap root with scanty feeder roots are other symptoms observed on infected roots (Fig. 10.2) (Asare-Nyako and Owusu 1979, Afolami 1982, Afolami and Ojo 1984, Campos and Villain 2005).

10.3 Geographical distribution

Root-knot nematode on cacao was first reported in 1900 (Sosamma et al. 1979). Nematode infestation on cacao is recorded in most of the cacao growing regions of the world (Table 10.1). Nematode infestation has been reported throughout the Congo (1921), Zaire (1921), Côte d'Ivoire (1930), São Tomé (1930), Java (1941), Ghana (1955), Malawi (1960), Nigeria (1967), Brazil (1968), India (1980), Costa Rica (1980), Bolivia (1982), Peru (2007), Malaysia, Java, Philippines, Papua New Guinea, Jamaica, Venezuela and Ecuador (Sosamma et al. 1979, Sosamma et al. 1980, López -Chaves et al. 1980, Sharma 1982, Crozzoli et al. 2001, Wood and Lass 2001, Campos and Villain 2005, Arévalo-Gardini et al. 2007, Orisajo 2009, Villain et al. 2018).

Table 10.1. Geographical distribution of endoparasitic and ectoparasitic nematodes associated with cacao

Genera	Geographic Distribution
Anguillulina	Nigeria
Aphelenchoides	Brazil, Nigeria, Peru, Venezuela
Aphelenchus	Brazil ,Peru
Atylenchus	Costa Rica, Peru,
Basiria	Brazil
Belonolaimus	Brazil
Boleodorus	Brazil
Criconema	Venezuela
Criconemella	Côte d'Ivoire
Criconemoides	Brazil, Costa Rica, Côte d'Ivoire, Ecuador, Ghana, Malaysia, Nigeria, Peru, Venezuela
Crossonema	Peru
Diphtherophora	Brazil
Discocriconemella	Côte d'Ivoire
Ditylenchus	Peru
Dolichodorus	Belize, Brazil, Costa Rica
Dorylaimidos	Ecuador, Peru,
Dorylaimus	Peru

Table 10.1. Geographical distribution of endoparasitic and ectoparasitic nematodes associated with cacao (cont'd)

Genera	Geographic Distribution
Eutylenchus	Nigeria
Haplolaimus	Brazil, Costa Rica
Helicotylenchus	Brazil, Costa Rica, Côte d'Ivoire, Ecuador, Ghana, Malaysia, Nigeria, Peru, Philippines, Venezuela,
Hemicriconemoides	Brazil, Venezuela, Nigeria
Heterodera	Belize, Brazil, Nigeria
Longidorus	Belize, Brazil, Costa Rica, Côte d'Ivoire, Ghana, Nigeria
Neodiplogaster	Guatemala
Meloidogyne	Brazil, Costa Rica, Côte d'Ivoire, Ecuador, Ghana, India, Java, Malawi, Malaysia, Nigeria, Papua New Guinea, Peru, São Tomé, Venezuela, Zanzibar
Mesocriconema	Nigeria, Venezuela
Monotrichodorus	Venezuela
Mononchus	Ecuador, Peru,
Ogma	Venezuela
Paralongidorus	Nigeria
Parachichodorus	Brazil
Paratylenchus	Belize, Côte d'Ivoire, Ghana, Peru, Venezuela
Peltamigrattus	Brazil, Venezuela
Pratylenchus	Brazil, Costa Rica, Côte d'Ivoire, Ecuador, Ghana, India, Indonesia, Jamaica. Malaysia, Nigeria, Peru, Venezuela
Psilenchus	Nigeria, Peru, Venezuela,
Rhabditidos	Ecuador, Peru
Rhadinaphelenchus	Peru
Radopholus	Côte d'Ivoire, Jamaica, Nigeria
Rotylenchulus	Belize, Brazil, India, Indonesia, Jamaica, Nigeria, Peru, Venezuela,
Rotylenchus	Brazil, Ecuador, Nigeria, Peru, Venezuela,
Scutellonema	Brazil, Jamaica, Nigeria, Peru,
Tetylenchus	Nigeria
Trichodorus	Belize, Brazil, Costa Rica, Côte d'Ivoire, Ghana, India, Mexico, Nigeria, Peru, Venezuela
Trophurus	Brazil, Côte d'Ivoire, Venezuela
Tylenchorhynchus	Brazil, Costa Rica, India, Mexico, Nigeria, Peru, Venezuela
Tylenchulus	Brazil, Peru
Tylenchus	Brazil, Costa Rica, Nigeria, Peru, Venezuela,
Xiphidorus	Venezuela
Xiphinema	Brazil, Ecuador,Ghana, Malaysia, Mexico, Nigeria,Perú, Philippines Venezuela

Source: Tarjan and Jiménez (1973), Sosamma et al. (1979), López -Chaves et al. (1980), Afolami and Caveness (1983), Sharma (1977), Sharma (1982), Crozzoli (2002), Crozzoli et al. (2001), Wood and Lass (2001), Campos and Villain (2005), Arévalo-Gardini et al. (2007), Arévalo-Gardini (2008), Arévalo-Gardini (2014), Okeniyi et al. (2016), Orisajo (2009), Popoola (2018), Bustamante (2019), Daramola et al. (2020) McQueen et al. (2020), Adewale and Dada (2020), Maosa et al. (2024), Vera-Velez et al. (2024).

Figure 10.1. Dieback of cocoa caused by root-knot nematodes (left) compared with a healthy plant
(Orisajo, 2018)

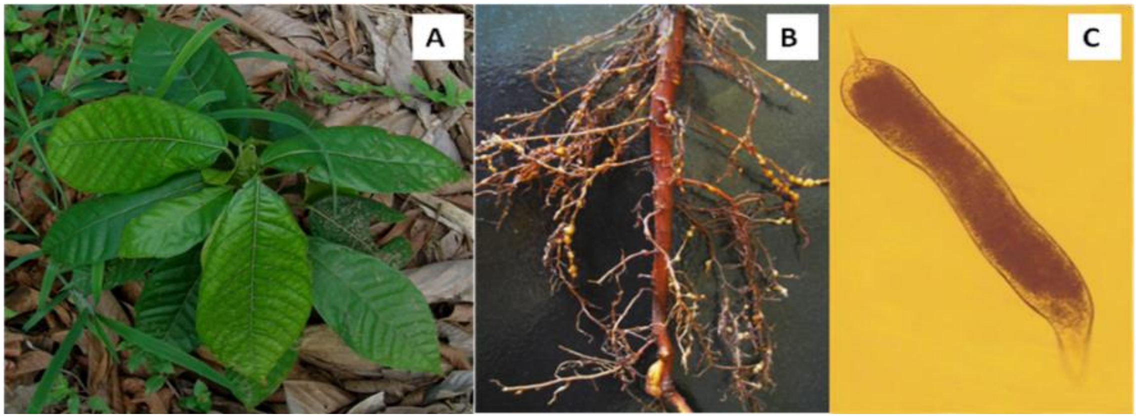

Figure 10.2. Symptoms of damage of *Meloidogyne* spp. on cacao plants

A. Plant showing reduced growth one month after transplant into nematode infested soil
B. Roots with galls
C. Second larval stage of a female
Source: Instituto de Cultivos Tropicales (E Arévalo-Gardini, 2007)

10.4 Alternative hosts

Each species of *Meloidogyne* has a range of plant species and cultivars that it will infect. However, the severity of symptoms expressed will depend on the susceptibility of the plant host. Approximately 165 species of host plants to *Meloidogyne* spp. are reported. *M. arenaria*, *M. incognita* and *M. javanica* have a wide host range (Taylor and Sasser 1983) and some of the shade plants commonly used in cacao plantations, such as banana and *Inga* spp. are often sources of inoculum (Sosamma et al. 1980, Villain et al. 2018). In South America and Central America *M. exigua* is a very serious pest of *Coffea arabica* but is polyphagous on many crops including cacao (Botello et al. 2019, Oliveira et al. 2005, Taylor and Sasser 1983, Sasser and Carter 1985).

10.5 Biology

A large number of plant parasitic nematodes are known to be associated with diseased cacao seedlings. Banana, used as a shade plant, is the primary source of inoculum. Infested nursery soil leads to infested seedlings, which will disseminate nematodes into plantations and runoff water may also spread the nematodes (Campos and Villain 2005, Villain et al. 2018).

10.6 Quarantine measures

The following plant parts are likely to carry the pathogen in trade and transport:

- Roots (eggs and galls often invisible to the naked eye but usually visible using a light microscope
- Growth media accompanying plants, especially soil, can carry eggs and galls.

It is important to carry out an efficient inspection of plant material for indications of nematode infestation as part of any quarantine procedure (Oostenbrink 1972). Eggs and galls can be present in the soil as well as the roots, so movement of any whole plants with associated soil will risk spread of the pest.

Seedlings obtained in the nursery must be carefully examined for the presence of *Meloidogyne* before being transplanted. If infestation is suspected, the plant material should not be transplanted without root treatment with hot water. Where possible, materials with resistance or immunity to nematode infestation should be used for propagation (Taylor and Sasser 1983, Okeniyi et al. 2009). Organic amendments such as poultry litter and cattle manure, and plant leaf extracts from *Ocimum gratissimum*, *Carica papaya*, *Azadirachta indica*, *Vernonia amygdalina*, *Bixa orellana*, *Acalypha ciliate*, *Jatropha gossypifolia* and *Allium ascalonicum*, have been shown to have a suppressive effect on plant-parasitic nematodes, or to reduce populations in the soil (Orisajo et al. 2008, Orisajo, 2009, Orisajo et al. 2012, Orisajo et al. 2021). Other studies report the reduction of density of parasitic nematodes with the use of cocoa pod husk and/or neem leaves (Okeniyi et al. 2016). Although nematicides and steam sterilization have been used to control nematodes in the nursery (Afolami, 1993), few chemical control methods are environmentally safe and economically viable for use in a perennial tree crop such as cacao in the field. Integrated management systems incorporating good hygiene, organic soil amendments and development of biological control are advocated (Orisajo 2018, Orisajo et al. 2021, Lezaun 2016, Villain et al. 2018).

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