Technical guidelines for the safe movement of cacao germplasm. Revised from the FAO/IPGRI Technical guidelines No. 20 (Fourth Update 2021).

Book

Published Version

Technical Guidelines for the Safe Movement of Cacao Germplasm

Revised from the FAO/IPGRI Technical Guidelines No. 20
(Fourth Update, 2021)

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






CacaoNet
Global Network for Cacao Genetic Resources

Alliance
Bioversity International
CIAT
International Center for Tropical Agriculture
Since 1967 Science to cultivate change

The Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT) delivers research-based solutions that address the global crises of malnutrition, climate change, biodiversity loss and environmental degradation. The Alliance focuses on the nexus of agriculture, nutrition and environment. We work with local, national and multinational partners across Africa, Asia, Latin America and the Caribbean, and with the public and private sectors and civil society. With novel partnerships, the Alliance generates evidence and mainstreams innovations to transform food systems and landscapes so that they sustain the planet, drive prosperity and nourish people in a climate crisis. The Alliance is part of CGIAR, the world's largest agricultural research and innovation partnership for a food-secure future dedicated to reducing poverty, enhancing food and nutrition security, and improving natural resources. https://alliancebioversityciat.org www.cgiar.org

The Alliance of Bioversity International and CIAT coordinates the Global Cacao Genetic Resources Network, CacaoNet, with a steering committee and working groups composed of representatives from various cocoa research institutes and organizations supporting cocoa research. CacaoNet aims to optimize the conservation and use of cacao genetic resources, as the foundation of a sustainable cocoa economy (from farmers through research to consumers), by coordinating and strengthening the conservation and related research efforts of a worldwide network of public and private sector stakeholders. www.cacaonet.org

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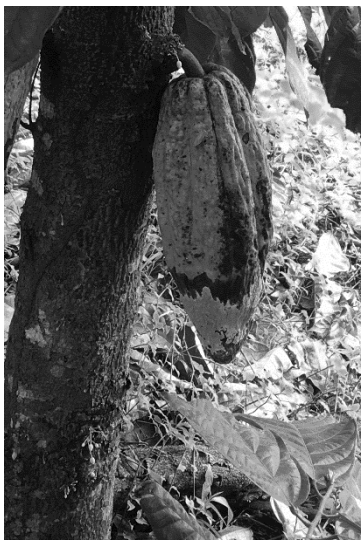

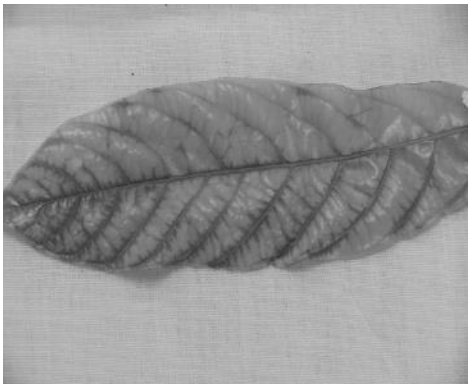

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CacaoNet would like to thank all those who have contributed to the revision of the Guidelines for the Safe Movement of Cacao Germplasm as well as those who contributed to the original FAO/IPGRI Technical Guidelines No. 20, on which this fourth update 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 from Bioversity International and the CGIAR Research Programme on Forests, Trees and Agroforestry (FTA) that allowed the publication of previous revisions. CacaoNet has received additional financial support from Mars, 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 has acted as Scientific Advisor to CacaoNet since 2010.

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

Disclaimer

Table of Contents

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 2016), 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

[1] The word 'pest' is used in this document as defined in the FAO Glossary of Phytosanitary Terms (2016): '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. 2016. Glossary of Phytosanitory Terms. ISPM No. 5 (2016) in International Standards for Phytosanitary Measures. FAO, Rome. Available from https://www.ippc.int/en/core-activities/standards-setting/ispms/

IPPC. 2016. Framework for pest risk analysis. Secretariat of the International Plant Protection Convention. Available from https://www.ippc.int/static/media/files/publication/en/2016/01/ISPM_02_2007_En_2015-12-22_PostCPM10_InkAmReformatted.pdf

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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
University of Reading
PO Box 237
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)
Subtropical Horticulture Research Station
13601 Old Cutler Road
Miami, Florida 33158
USA
Email: Osman.Gutierrez@ars.usda.gov

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),
Peti Surat 30 (PO Box 30),
Jalan Sungai Dulang,
36307 Sungai Sumun, Perak,
MALAYSIA
Contact: Nuraziawati bt. Mat Yazik
Email: nura@koko.gov.my

Cenargen Quarantine Facility
Parque Estação Biológica, PqEB, Av. W5 Norte (final)
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 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.

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 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.

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 A)	Isolated occurences inTrinidad	Budwood: potential risk
7.4 *Cacao mild mosaic virus* (CaMMV) (formerly known as Trinidad cacao virus B)	Isolated occurences in Trinidad, Puerto Rico and Brazil	Budwood: potential risk Seed: potential risk
8.1 Witches' broom disease (*Moniliophthora perniciosa*)	Brazil (Bahia, Espirito Santo, Amazonian regions), Bolivia, Colombia, Dominican Republic, 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
8.2 Moniliophthora pod rot (frosty pod rot or moniliasis disease)	Belize, Bolivia, Brazil (Acre 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

[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
8.3 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	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	
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

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

Pest	Geographical spread	Special precautions
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 Carribean	Pod: Moderate risk Seed: Low risk Budwood: Moderate risk See: 9.5 mirids
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). Users are recommended to check periodically other reports of pest/ disease outbreaks in the country in which they are working.

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) Moniliophthora pod rot (Acre State) 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
Cameroon	*Phytophthora megakarya* *Phytophthora capsici* *Ceratocystis spp.* (*C. ethacetica and C. paradoxa)* Lasiodiplodia Dieback
Colombia	Witches' broom disease Moniliophthora pod rot Verticillium wilt of cacao Ceratocystis wilt *Phytophthora nicotianae* Rosellinia root rot
Costa Rica	Moniliophthora pod rot Ceratocystis wilt Rosellina root rot *Phytophthora capsica*

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

Country	Pest risk
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
Hawaii	*Phytophthora spp*
Honduras	Moniliophthora pod rot

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

Country	Pest risk
India	*Phytophthora capsici* *Phytophthora citrophthora* *Phytophthora heveae* *Phytophthora megasperma* *Phytophthora nicotianae* Vascular streak dieback Rosellinia root rot
Indonesia	Vascular streak dieback Rosellina root rot Cocoa pod borer *Phytophthora capsica*
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 Rosellina root rot Cocoa pod borer
Mexico	Moniliophthora pod rot *Phytophthora capsici* *Phytophthora citrophthora* *Phytophthora heveae*
Nicaragua	Moniliophthora pod rot
Nigeria	*Cacao necrosis virus* (CNV) *Cacao swollen shoot virus* (CSSV) *Phytophthora megakarya*
Panama	Witches' broom disease Moniliophthora pod rot *Phytophthora capsica*

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

Country	Pest risk
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)
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] Rosellinia root rot
St Vincent	Witches' broom disease
Suriname	Witches' broom disease
Thailand	Vascular streak dieback
Togo	*Cacao swollen shoot virus* (CSSV) *Phytophthora megakarya*
Trinidad and Tobago	Witches' broom disease *Phytophthora capsici* Rosellinia root rot Ceratocystis wilt *Cacao yellow vein-banding virus* (CYVBV) and *Cacao mild mosaic virus* (CaMMV) (formerly referred to as Trinidad Cocoa Virus A and B)
Uganda	Verticillium wilt

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

Country	Pest risk
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*

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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 (O. Domfeh, unpublished)

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

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Many isolates of CSSV have been collected and are named by capital letters or the name of the locality where they were collected. Analysis of CSSV molecular variability reveals at least eight species 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, Chingandu et al. 2017, Muller et al. 2018). *Cacao mottle leaf virus* is a synonym of *Cacao swollen shoot virus* (Brunt et al. 1996).

7.2.1 Symptoms

Symptoms of the disease are highly variable and depend on the virus strain and the stage of infection. The most characteristic symptoms on sensitive types (e.g. West African Amelonado) include a characteristic red vein banding of the young 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, Togo (Brunt et al. 1996, Kouakou et al. 2012, Oro et al. 2012, Abrokwah et al. 2016).

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 has been found in such seedlings either visually or through reverse transcription (RT) PCR screening (Ameyaw et al. 2013). While there has been the recent discovery of integrated badnaviral sequences in most of the cacao genetic groups (Muller et al. 2021), there is to date no evidence of CSSV transmission by seeds. However, plants can become infected when seeds are inoculated using viruliferous mealybugs or by sap/mechanical transmission with purified viral particles.

7.2.5 Particle morphology

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

7.2.6 Therapy

None. Once a plant is infected it cannot be cured. However, passage through somatic embryogenesis has been shown to produce virus-free clones from CSSV infected donor plants (Quainoo et al. 2008). Like most plant viral diseases, the disease can be contained or prevented if healthy plants are isolated within barriers of CSSV-immune crops.

7.2.7 Quarantine and detection measures

ELISA, ISEM and PCR techniques have been used successfully (Sagemann et al. 1985, Muller 2008, Abrokwah et al. 2016) to detect CSSV; also virobacterial

agglutination has been utilized (Hughes and Ollennu 1993). Various other successful detection methods have been reported, and these have been reviewed recently (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 PCR-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.

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Figure 7.2.1. Red vein banding on young leaf. Note the fern-like pattern of the red vein banding. (H Dzahini-Obiatey and Y Adu-Ampomah, 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, 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, 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). Recently, CaMMV was detected in Puerto Rico (Puig et al 2020) and Brazil (Ramos-Sobrinho et al. 2021), indicating it may be widespread in the Americas. Virus-like symptoms have been reported in other cocoa growing areas in the region, including Colombia, Dominican Republic, and Venezuela but have not yet been characterized.

7.4.2 Hosts

No alternative hosts are known for CaMMV or 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 (Cope 1953, Baker and Dale 1947). 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 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 in laboratory growth chambers, 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). No information is available on seed transmission of CYVBV.

Integrated badnaviral sequences were recently reported in asymptomatic cacao plants belonging to multiple genetic groups (Muller et al. 2021). These integrated sequences 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. Complete genomes of these species have not been reconstructed and they are not believed to be infective. The sequences detected so far are most similar to a region of the CYVBV genome (up to 72.5% nucleotide identity).

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. There is no evidence of CaMMV or CYVBV integrating into the genome of *T. cacao* (Chingandu et al. 2017).

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)

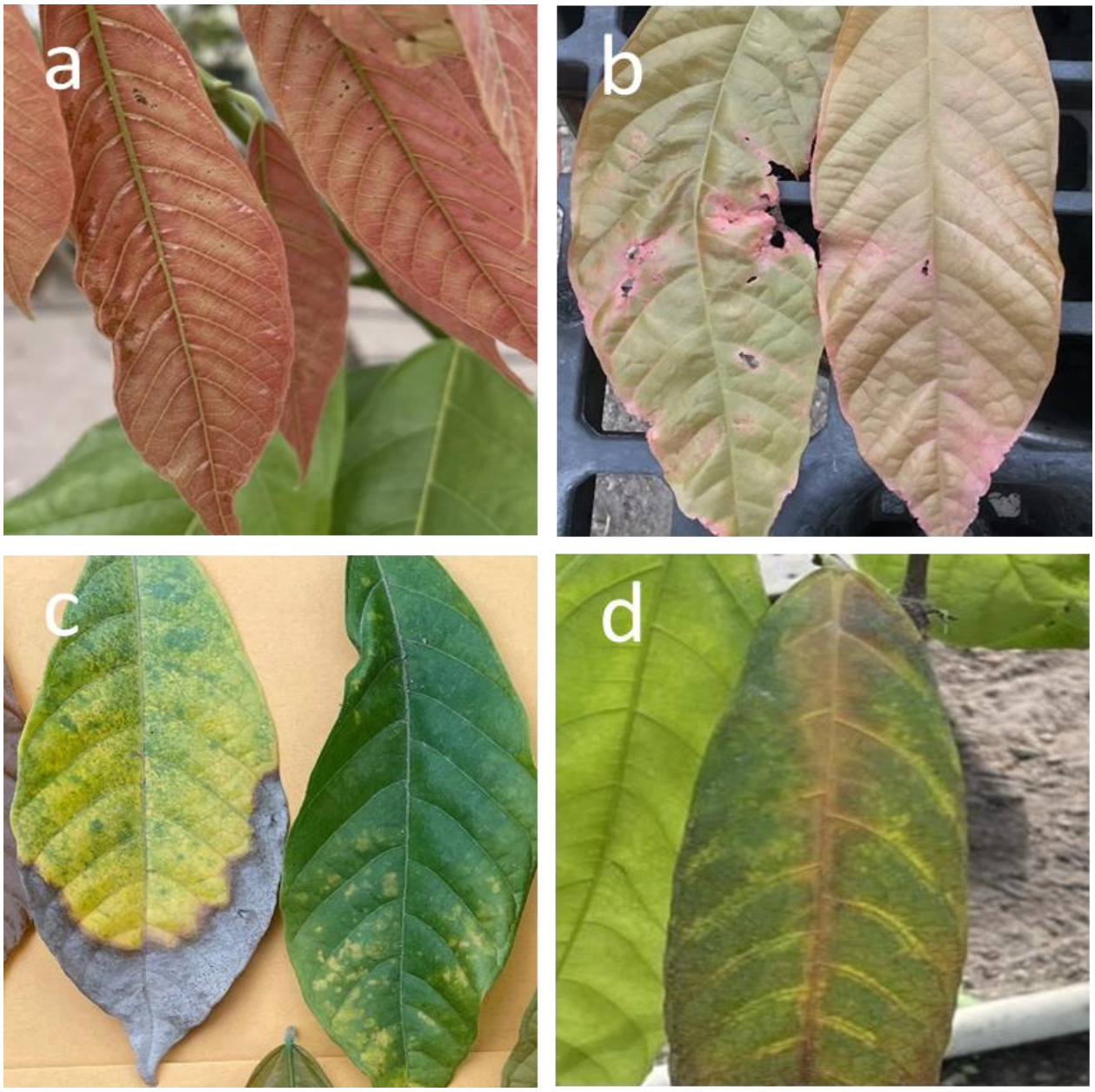

Figure 7.4.2. Foliar symptoms on trees infected with CaMMV: (a) red vein banding on young leaves, (b) pink pigmentation near veins and leaf margins, (c) mosaic on mature leaves, and (d) yellow vein banding and necrosis on midrib (AS Puig, unpublished)

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7.5 Other viruses and virus-like diseases

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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 SriLanka 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

Update by Karina P Gramacho[1] Nara G R B Patrocinio[2] and Givaldo Niella[1*]

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8.1.1 Causal agent

Moniliophthora perniciosa (Stahel) Aime & Phillips-Mora (Syn. *Crinipellis perniciosa*)

Although variability exists with the fungus there are two main biotypes, C and S biotype. Within C biotype variants seem to occur according to their country of origin (e.g. Ecuador, Peru, Brazil, Bolivia).

8.1.2 Symptoms

M. perniciosa can infect all actively growing tissues (shoots, flower cushions, pods), 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 that develop following infection of terminal and axillary buds. Stem swellings are formed following infection of the main axis at an internode or node involved. 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 on Fig. 8.1.1 and Fig. 8.1.2.

Infection of flower cushion may form hypertrophied flowers, vegetative brooms, and parthenocarpic carrot-shaped or strawberry-shaped pods. (Fig. 8.1.2 A-C).

Pods can be infected at any stage, being most susceptible when they are young (0- to 2-months old). Infected pods suffer hypertrophy, distortion, early ripening, and external necrotic lesions of the tissues that cause the pod to mummify. Levels of internal damage depend on when the infection occurs and can vary from watery rot to a dry compacted bean mass (Fig. 8.1.2D). Although, in most cases, the seeds become partly/completely cemented to each other and the pod wall, infections of maturing pods can result in localized necrotic areas on the pod 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 Surinam 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, Dominican Republic, 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.

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 different 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 *Moniliophthora* spp. 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 (Source: CEPLAC/CEPEC/SEFIT) of Witches' broom disease: a) tree severely attacked in Bahia, b) terminal vegetative broom partially dry, c) diseased flower cushion, d) pod lesion with necrotic lesion and watery seed/beans

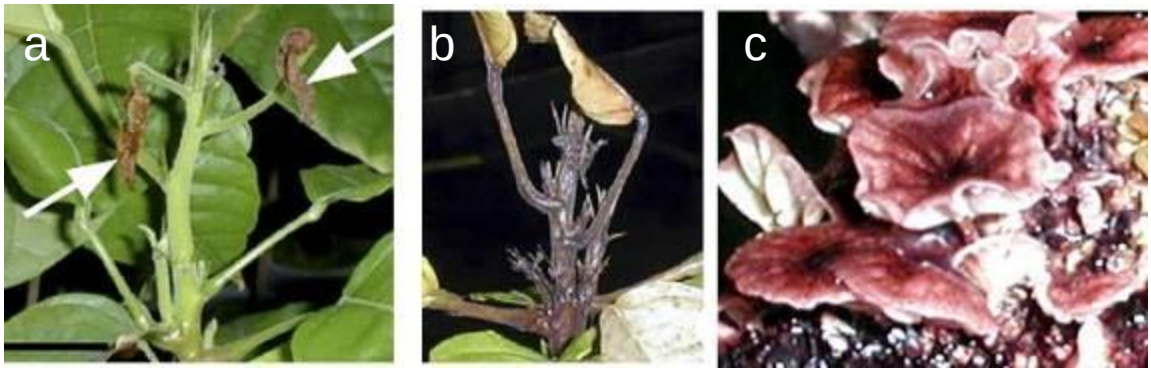

Figure 8.1.2. Plantlets with Witches' broom symptoms (Source: CEPLAC/CEPEC/ SEFIT): a) terminal green broom followed by necrosis of stems of the brooms from their tips (arrows), b) terminal dry broom, c) "*in vitro*" basidiocarps production

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 about 4–8 weeks after the onset of rain. The basidiocarps can form on any necrotic tissues, such as stem, seeds/beans, leaf vein or pod tissue that has undergone necrosis (Fig. 8.1.3 A-C).

Wind is the main mode of spore dissemination, although dispersal can also occur by water, and human beings. 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, but the infection may become latent, and symptoms will develop when the plant restarts growth (Purdy and Schmidt 1996, Silva et al. 2002). The length of the biotrophic phase will vary according to factors such as the WBD strain, genotype of the host, 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).

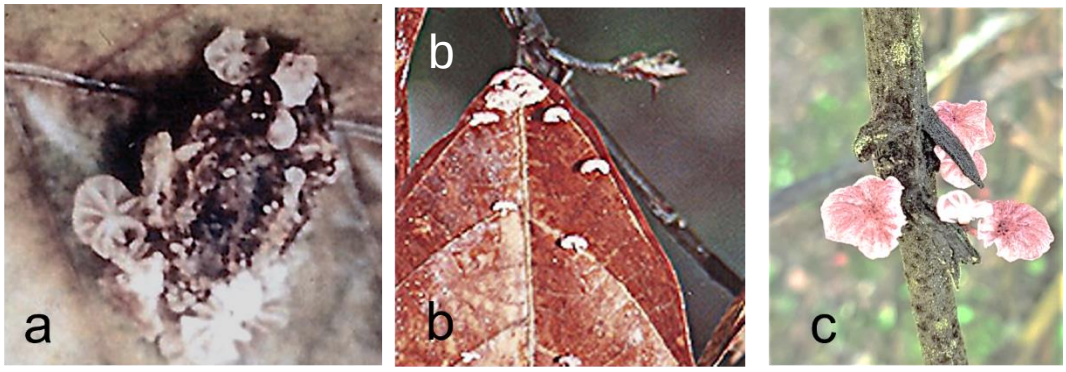

Figure 8.1.3. Basidiocarps production on (a) necrotic seeds/beans, (b) leaf vein and (c) stems (dry brooms) of cacao.

8.1.6 Quarantine measures

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

- Fruits (inc. Pods): Fruiting bodies, hyphae; borne internally; borne externally
- Leaves: Hyphae; borne internally
- Stems (above ground)/shoots/trunks/branches: Fruiting bodies, hyphae; borne internally; borne externally; usually invisible to naked eye.
- Seeds: hyphae, invisible to naked eye

Anthropogenic activity is responsible for long-distance dissemination, as spores are short-lived, and the fungus can survive as hidden infections in plant parts. 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.

The fungus can be transported on entire plants or their parts (seeds, pods, leaves, and stems (shoots/branches/ budwood, etc.). Consequently, 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.

Although *M. perniciosa* may be seed transmitted, movement as seed is the safest method of moving germplasm. Seeds should be collected from apparently healthy pods, 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 in insect-proof glasshouses under strict supervision in a quarantine station for at least a year to assure that plants are free of disease 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 signaled 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) and has recently been reported in an urban area of Acre State (CEPLAC 2021).

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 & 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 some days, 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.

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

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

8.3 *Phytophthora* spp.

Update by G Martijn ten Hoopen1, S Nyassé2 and R Umaharan3

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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.) 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 others 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 sagittifoilium*), Cocoyam or 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 originating from Africa. It is present in Gabon, São Tomé and Principe, Bioko (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. *P. tropicalis*/*P. capsici* is found in the Americas, Caribbean, Asia and Africa (e.g. Brazil, Dominican Republic, El Salvador, Guatemala, India, Jamaica, Mexico, Trinidad, Venezuela, Cameroon), 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 Cameroon 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 Mefenoxam 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) 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 (Dr GM ten Hoopen, CIRAD)

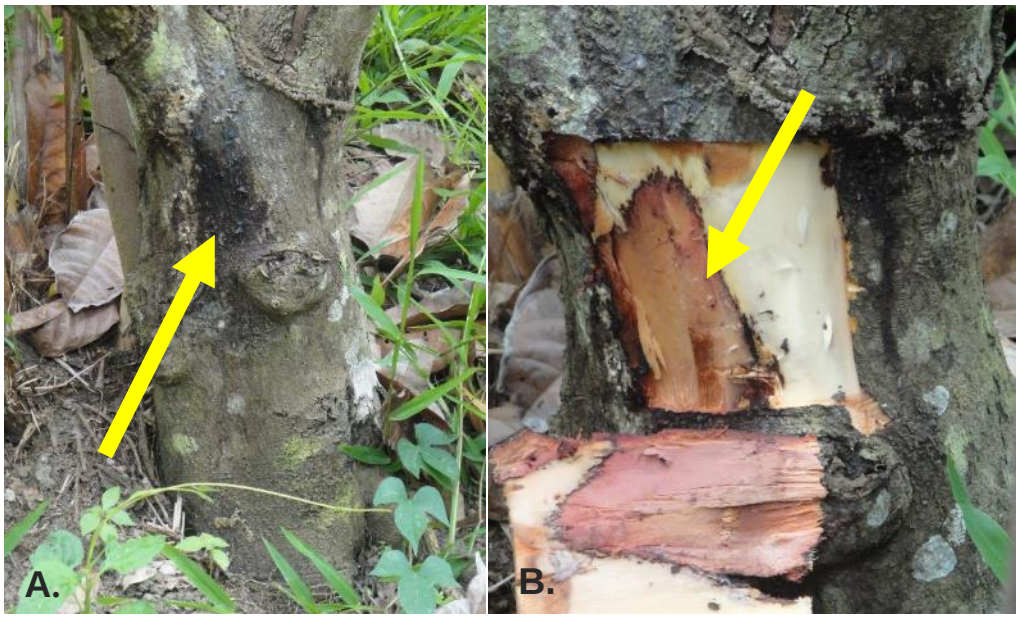

Figure 8.3.2. (A) Cacao tree trunk with canker symptoms (black discoloration) (B) discoloration of the sapwood (Dr T Sreenivasan, CRC).

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

8.4 Vascular Streak Dieback (VSD)

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8.4.1 Causal agent

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

Synonym: *Oncobasidium theobromae* P.H.B. Talbot & Keane

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 of the pathogen, evident as a white, flat, velvety coating over the leaf scar and adjacent bark. Presence of these basidiocarps 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 and West Java, in Sumatra, in Kalimantan, the Moluccas and in the large areas of new

cacao plantings in 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 is only known from cacao so the geographical distribution generally reflects the occurrence of cacao in South and Southeast Asia and Melanesia. Its most easterly natural limit 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 or the New World.

8.4.4 Alternative hosts

Avocado.

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 recently (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 (Asman et al. 2018, Rosmana et al. 2015, 2019, Vanhove et al. 2016).

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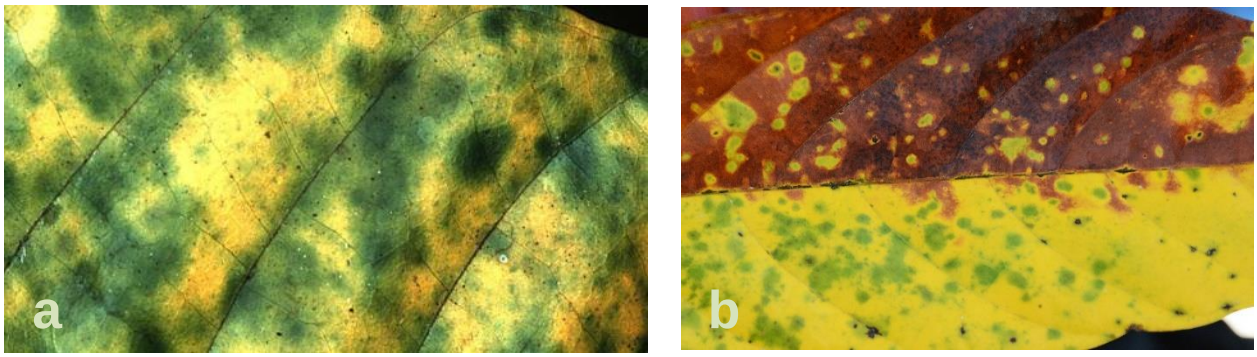

Figure 8.4.1. a) Vascular streak dieback: chlorotic leaf (M. Holderness, CABI) and b) Leaf showing necrosis and scattered islets of green tissue (AJ Daymond, University of Reading)

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).

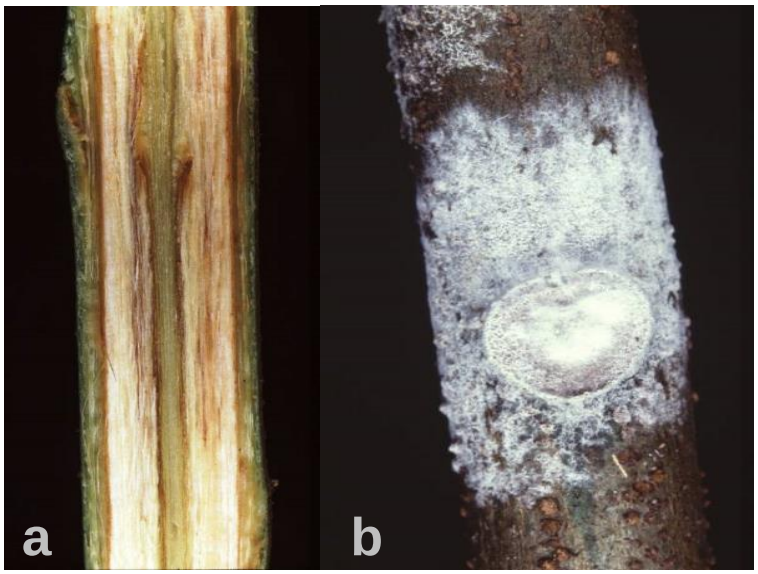

Figure 8.4.3. a) VSD infected stem section showing brown streaking (CABI) and b) VSD fruiting body (CABI).

8.5 *Verticillium* wilt of cacao

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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 & 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 & 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 sulfate-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). Cultural measures including 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 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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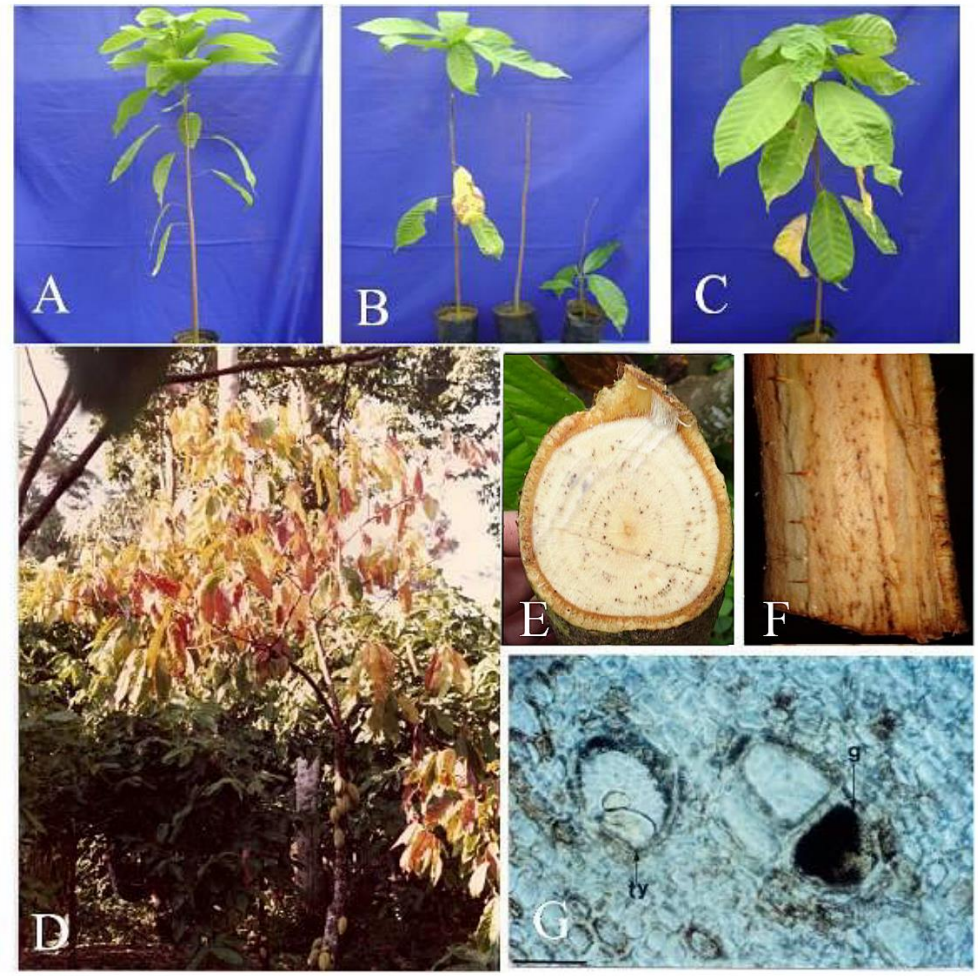

Figure 8.5.1. External (A-D) and internal (E-G) symptoms of *Verticillium dahliae* – cocoa interactions (MLV Resende, Univ. Federal de Lavras, Brazil):

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).

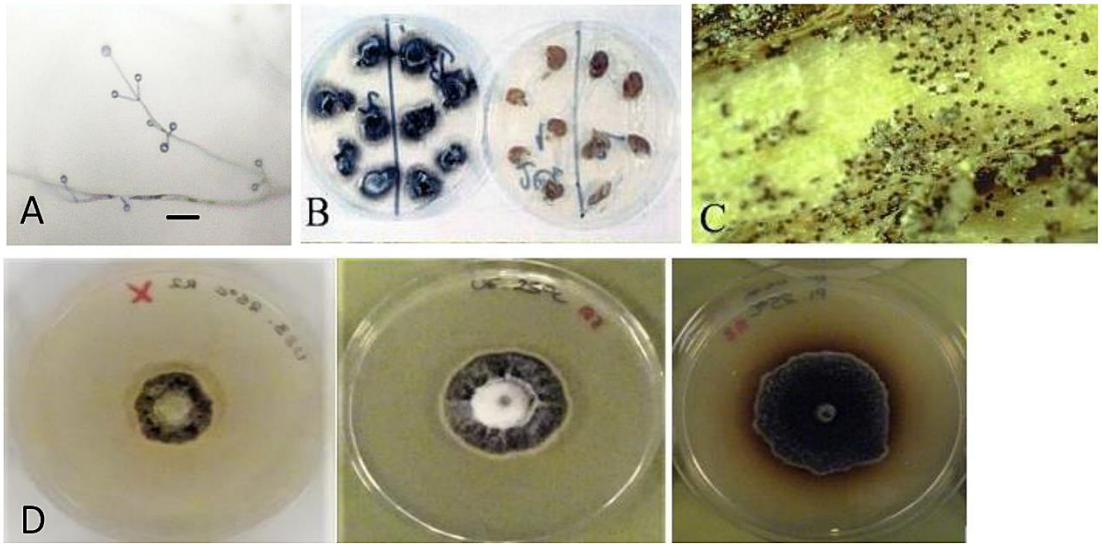

Figure 8.5.2. Biological cycle of *Verticillium dahliae*:

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

Update by Carmen Suárez-Capello[1]

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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 host-specialized form of *Ceratocystis fimbriata*, now known as *C. cacaofunesta* (Engelbrecht and Harrington, 2005). Earlier studies suggested the *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 over a period of 2 to 4 weeks, though the leaves remain attached to the plant (Delgado and Suárez 2003). Typically, Ceratocystis wilt is recognized through limp brown foliage that hangs from the tree without falling, even when the branch is shaken. Ambrosia beetles of the genus *Xyleborus* are attracted to the diseased trees and bore into the branches or main stem (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. 8.6.2). This is recognized as the first positive sign of Ceratocystis wilt; frequently the frass is seen even before the yellowing of the tree is visible.

Once inside the plant, the fungus causes a necrosis of the ray parenchyma cells, compromising the xylem; such lesions advance in the direction of the plant apex, although the cross-section is also thoroughly colonized (Harrington 2004) leading to the expression of the chlorosis and stem darkening symptoms.

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) was first reported on cacao in western Ecuador in 1918 (Rorer 1918). It was reported to be causing extensive damage in Colombia after 1940, Venezuela in 1958 (Thorold 1975), Costa Rica in 1958 (Thorold 1975) and Trinidad in 1958 (Spence and Moll 1958). Reports of the disease stretch from Guatemala (Schieber and Sosa 1960) and Central America to northern South America, including the Peruvian Amazon (Soberanis et al. 1999),

Ecuador, Colombia and Venezuela (Thorold 1975). In Brazil, the disease was reported in the south-western Amazon (Rondônia) in 1978 (Bastos and Evans 1978) and more recently in Bahia (Bezerra 1997), which is out of the native range of *T. cacao*. The disease is also found in French Guiana (M Ducamp, pers. comm.).

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 they 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 locates 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

C. cacaofunesta typically enters cacao plants through fresh wounds, such as pruning or pod harvesting wounds (Malaguti 1952) and moves through the host in the secondary xylem. Ambrosia beetles of the genus *Xyleborus* often attack the wood of infected trees (Saunders 1965), first attracted by the strong banana odour that the fungus produces. The frass which is pushed to the outside of the stem or branch as the beetles excavate their galleries, contains viable inoculum of the fungus (asexual spores, either conidia or thickwalled aleurioconidia) that may be spread by wind or rainsplash (Iton and Conway 1961) Although it is possible that this frass transmits the infection to other plants (Iton, 1960), the most efficient means of spreading the fungus are "machete" blades and pruning tools (Malaguti, 1952). Frequently, infected trees show heavy infection at the base, perhaps through infection of wounds near groundline. The name 'mal de machete' comes from the association of such infections with machete wounds.

The fungus moves through the xylem, often concentrating in the vascular rays, causing a deep stain wherever it grows. It moves systemically and slowly through the plant like a vascular wilt fungus, but it more readily kills the parenchyma tissue. The fungus will also kill the cambium and bark tissue, creating a canker on the stem or branch, usually associated with a weakening of 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.

The fungus sporulates heavily on the cut surfaces of diseased branches. These sporulating mats produce perithecia (fruit bodies) (Fig. 8.6.3) that exude sticky spore masses for insect dispersal. The mats produce a characteristic banana-like

odour that attracts fungal-feeding beetles, which can serve as vectors after helping to disseminate the fungus within the cacao tissue through their galleries.

8.6.6 Quarantine

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 naked eye
- Growing medium accompanying plants

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 which 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 movement of plants or plant parts, since unrestricted movement of cuttings or other propagative material is potentially dangerous. In consequence, transport of 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*.

Molecular or serological diagnostic techniques for *C. cacaofunesta* have not yet been reported though there are DNA sequences of ITS-rDNA and other genes unique to *Ceratocystis* species which could be developed for diagnosis (CABI 2021). 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.

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Figure 8.6.1. A young, infected tree with limp brown foliage (C. Suárez-Capello, UTEQ, Ecuador)

Figure 8.6.2. Abundant frass from Ambrosia beetles at the base of an infected tree (C Suárez-Capello, UTEQ, Ecuador)

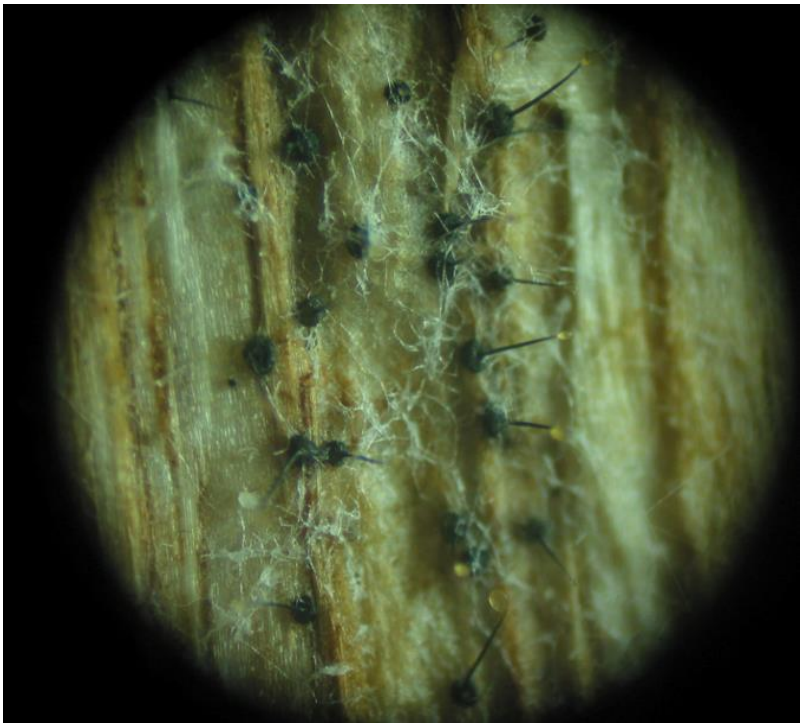

Figure 8.6.3. Perithecia of *Ceratocystis cacaofunesta* growing over the xylem of cocoa branches inoculated with the pathogen (C Suárez-Capello, UTEQ, Ecuador)

8.7 *Rosellinia* root rot

Update by Fabio Aránzazu Hernández[1], Darwin H. Martínez Botello[1] and G Martijn ten Hoopen[2]

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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*. 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. 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

Update by Andrews Y. Akrofi[1]; Eric Kumi-Asare[2] and Ishmael Amoako-Atta[2].

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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).

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8.8.1. Pink Disease

Erythricium salmonicolor (Berk. & Broome) Burdsall (Syn. *Corticium salmonicolor* Berk. & Broome) (syn. *Phanerochaete salmonicolor* Berk. & Broome, Julich). Known as "malaidie 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, Colombia, Ghana, 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 basidioma 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).

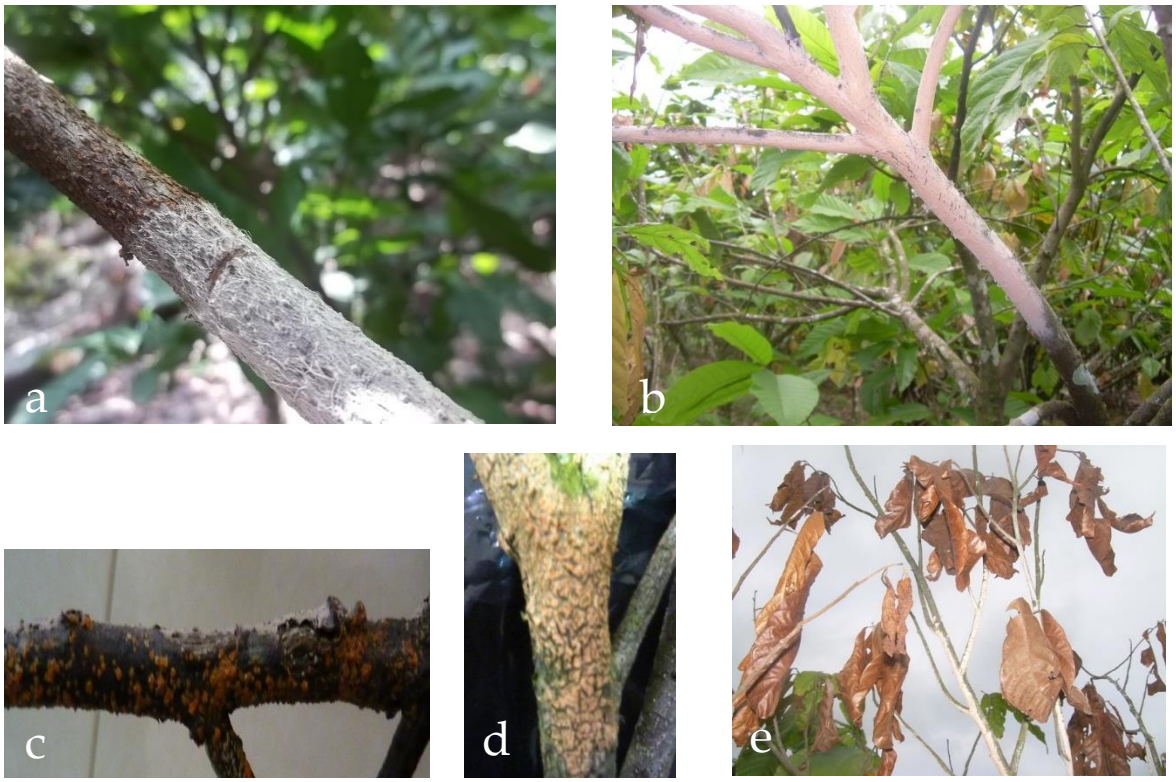

Fig. 8.8.1 Symptoms of pink disease on cacao: (a) white/light pink vegetative mycelia which can be easily overlooked when the bark is wet; (b) pink to salmon encrustation/pustules on the branch; (c) creamy pustules which are more conspicuous on the underside of infected branches; (d) orange fruiting bodies which develop from the creamy pustules on dying infected stems and (e) dieback in infected branch with dead leaves hanging (Source: Andrews 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: Eric 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, rain water 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 and Priya 2010) and in the Phillipines (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: Eric 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.

8.8.3.5 References and further reading

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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: Ishmael Amoako-Atta & Eric 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 *Phythophthora* 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.

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, 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 quarantine recommendations for 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 Cocoa Pod Borer (see section 9.2).

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.

9.1.1 References

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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. In contrast, green patches are visible on mature pods (Fig. 9.2.1). These symptoms are due to larvae tunneling inside the pod (Bakar et al. 2021). 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). Cocoa beans in infected pods are hardened and clumped together (Fig. 9.2.5), making extraction from the pod husk and mucilage difficult (Lee et al. 2013). Beans may also begin to germinate within pods that are infested when nearly ripe (Azhar 1986).

9.2.3 Geographical distribution

CPB was first detected in a cocoa plantation in Sulawesi, Indonesia, in the 1860s. The pest was recorded in the Philippines in 1936, in Malaysia in 1980 and in Papua New Guinea in 2006 (Saripah & Alias 2016, Yen et al. 2010). 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, 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, Iamba and Masu 2020, Niogret et al. 2019, Saripah et al. 2021, Shapiro et al. 2008, Sulistyowati 2015).

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

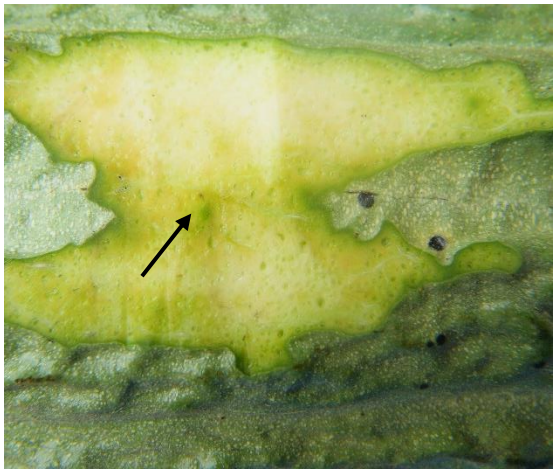

Figure 9.2.2. The entry hole is visible after the pod husk was shaved (Saripah B, Malaysian Cocoa Board)

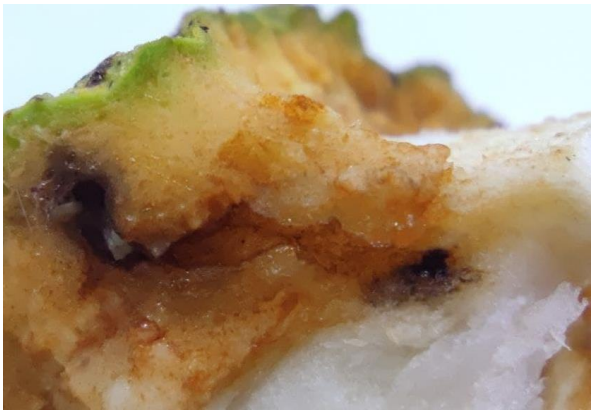

Figure 9.2.3. Galleries of larval infestation on the mucilage and pod husk (Saripah B, Malaysian Cocoa Board)

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

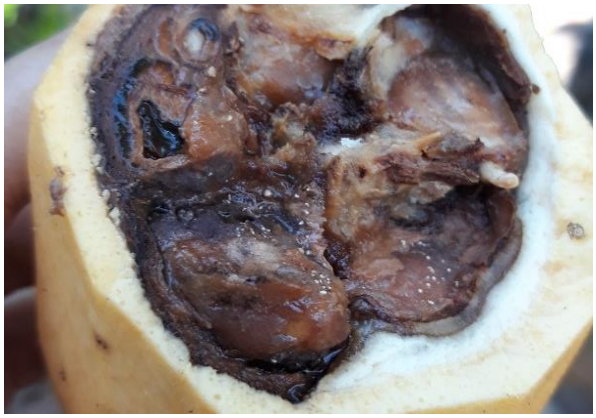

Figure 9.2.5. Beans clumped into a solid mass resulting from cocoa pod borer feeding at a heavy level of infestation (Saripah B, Malaysian Cocoa Board)

9.2.4 Host plants

CPB is known to attack fruits from the Sapindaceae family including *Nephelium lappaceum* (rambutan), *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). *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).

9.2.5 Biology

The life cycle of CPB is relatively short, approximately 27 to 33 days as illustrated in Fig. 9.2.6. 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 behavior 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 and bore immediately through the pod walls (Fig. 9.2.7). Inside the pod, the larvae feed for 14-21 days on the mucilage, pulp, placenta, and sometimes the testas of the cotyledons. 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. 9.2.8) 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. 9.2.9).

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 recommendations

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.

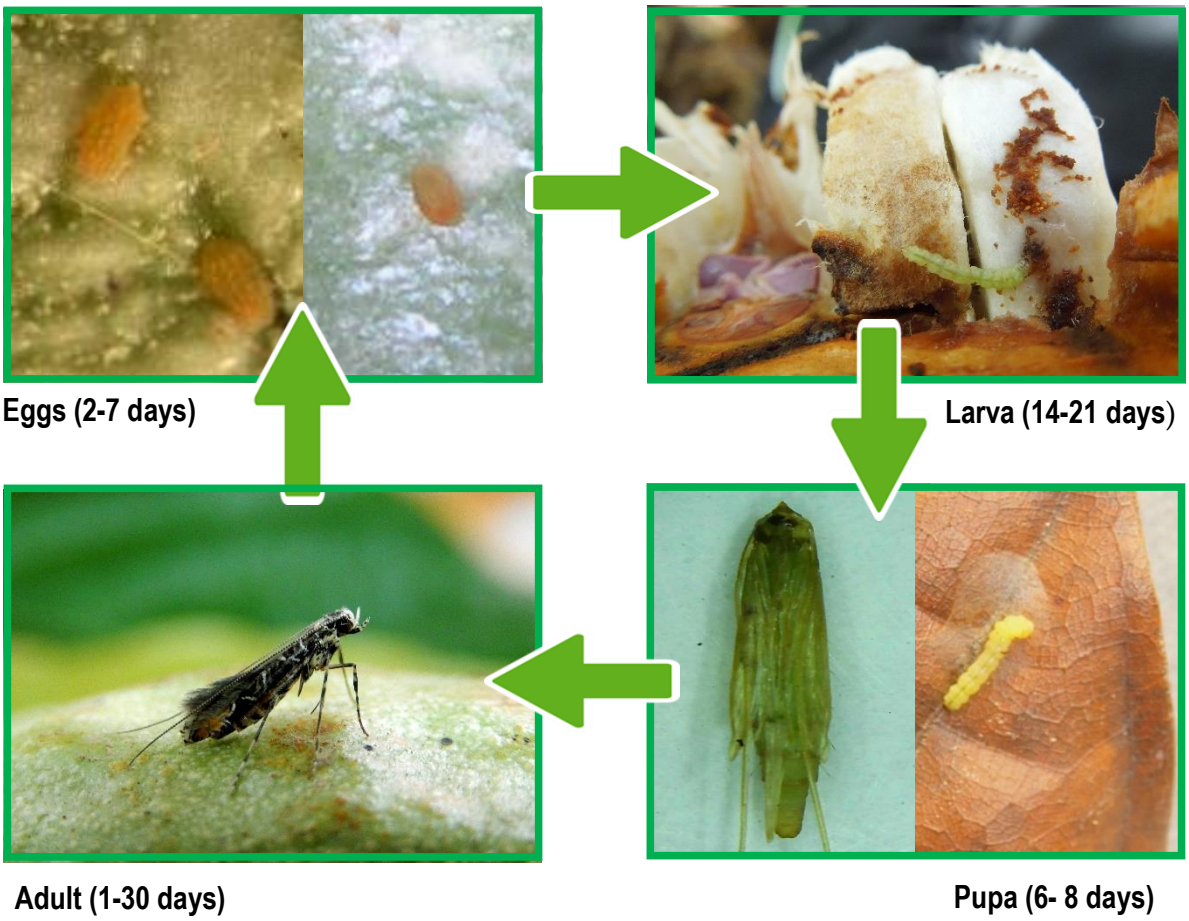

Figure 9.2.6. Life cycle and duration of the life stages of cocoa pod borer (Saripah B, Malaysian Cocoa Board)

Figure 9.2.7. Newly hatched cocoa pod borer larva tunneling into the pod wall (A Alias, Malaysian Cocoa Board)

Figure 9.2.8. Cocoa pod borer larva emerging from its exit tunnel in the pod wall (Saripah B, Malaysian Cocoa Board)

Figure 9.2.9. Cocoa pod borer pupa under its silk cocoon on a pod surface and leaf litter (Saripah B, Malaysian Cocoa Board)

9.2.7 References and further reading

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9.3 Cocoa Fruit 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 is similar to 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* (Lepidoptera: Cossidae)

The larvae of this moth bore into woody stems, branches and roots of cocoa in West and Central Africa, 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 *Cryptophlebia encarpa* from Malaysia and Papua New Guinea and *Ecdytolopha aurantianum* from Venezuela and *E. punctidescanum* from Trinidad, the Gracillariids *Marmara* spp. from Brazil, Trinidad and Tobago, *Spulerina* spp. from West Africa and the Noctuid *Characoma stictigrapta* 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. 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, 4 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* (Fig. 9.5.1) and *Distantiella theobroma* (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* 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* 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.

9.5.4 References

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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

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. The estimated yield loss in Indonesia has been estimated as 50-60% if the infestation is at a high level (Siswanto et al. 2020). 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. Infestation by *Helopeltis* usually increases particularly in the rainy season (MCB, 2013).

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

Figure 9.6.2. Old lesions on cocoa pod are dark in color (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 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.

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).

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 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 important.

9.7.3 Symptoms

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. Wounds created on fruits attacked by the bug are subsequently invaded by opportunistic fungi (e.g. *Fusarium decemcellulare* (anamorph of *Calonectria rigidiuscula)*) and other rot causing fungi (e.g. *Phytophthora spp)* to cause diebacks and fruit rots respectively (Akrofi et al. 2016).

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*, 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 21 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.

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*, *Planococcus citri* and *Pseudococcus longispinus*), 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* (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 theobromae* (syn. *Botryodiplodia theobromae)* and *Fusarium decemcellulare* (anamorph of *Albonectria rigidiuscula)* 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 multibranching 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 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.

9.10.7. References:

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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. Campos and Villain (2005) list several species of *Meloidogyne* and the countries where they have been found to affect cacao production, including *M. arenaria* (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). Roots of infected plants show swelling of hypocotyls and roots. Formation of gall knots on roots, 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), Côte d'Ivoire (1930), São Tomé (1930), Ghana (1955), Malawi (1961), Nigeria (1967), Brazil (1968), India (1980), Costa Rica (1980), Bolivia (1982), Peru (2007), Malaysia, Java, Philippines, Papua New Guinea, Jamaica, Venezuela andEcuador (Sosamma et al. 1979, 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).

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

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

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

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

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).

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

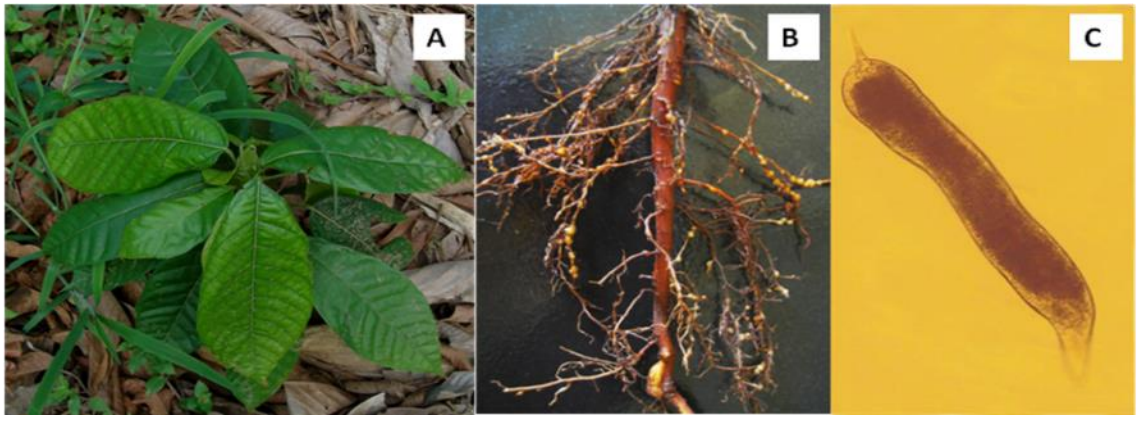

Figure 10.2. Symptoms of damage of *Meloidogyne* spp. on cacao plants

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 (Arévalo-Gardini, 2007)

10.4 Alternative hosts

Each species of *Meloidogyne* has a range of plant species and cultivars that it will infect though 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 theshade plants commonly used in cacao plantations, such as banana and *Inga* spp. are often sources of inoculum (Sosamma et al. 1980).

In South America and Central America *M. exigua* is a very serious pest of *Coffea arabica* but is polyphagous on many crops including cacao 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).

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 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). Although nematicides and steam sterilization have been used to control nematodes in the nursery (Afolami, 1993), there are few chemical control methods that 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, Lezaun 2016).

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