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Technical guidelines for the safe movement of cacao germplasm

Book

Published Version

End, M., Daymond, A. and Hadley, P. (2017) Technical guidelines for the safe movement of cacao germplasm. Bioversity International, pp89. ISBN 9789292550790 Available at http://centaur.reading.ac.uk/81225/

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Published version at: https://www.bioversityinternational.org/e-library/publications/detail/technical-guidelines-for-the-safe-movement-of-cacao-germplasm/

Publisher: Bioversity International

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Technical Guidelines for the Safe Movement of Cacao Germplasm

Revised from the FAO/IPGRI Technical Guidelines No. 20

(Third Update, October 2017)

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

CacaoNet
Global Network for Cacao Genetic Resources

Bioversity International

CacaoNet is an international network for cacao genetic resources coordinated by Bioversity International 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

Bioversity International is a global research-for-development organization. We have a vision – that agricultural biodiversity nourishes people and sustains the planet.

We deliver scientific evidence, management practices and policy options to use and safeguard agricultural and tree biodiversity to attain sustainable global food and nutrition security. We work with partners in low-income countries in different regions where agricultural and tree biodiversity can contribute to improved nutrition, resilience, productivity and climate change adaptation.

Bioversity International is a CGIAR Research Centre. CGIAR is a global research partnership for a food-secure future.

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Citation: End MJ, Daymond AJ, Hadley P, editors. 2017. Technical guidelines for the safe movement of cacao germplasm. Revised from the FAO/IPGRI Technical Guidelines No. 20 (Third Update, October 2017). Global Cacao Genetic Resources Network (CacaoNet), Bioversity International, Rome, Italy.

ISBN 978-92-9255-079-0
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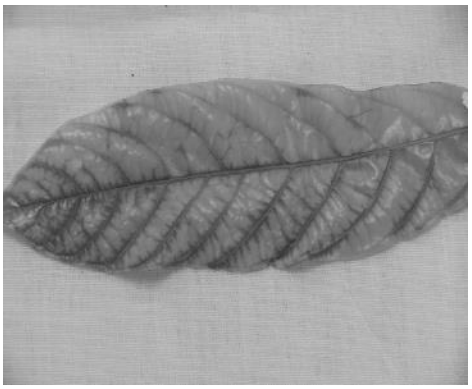

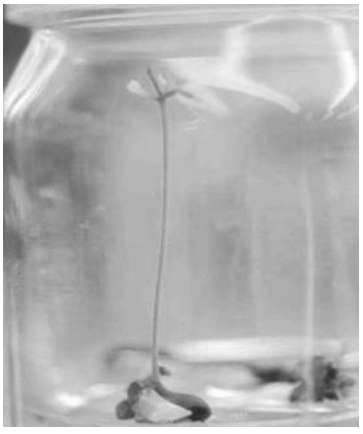

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Acknowledgements

CacaoNet would like to thank all those who have contributed to this 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 third 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 these Guidelines. We thank the many cocoa research institutes and organisations which have allowed their staff to contribute to the CacaoNet Safe Movement Working Group and, in particular, to COPAL for providing the opportunities and facilities which have enabled this Working Group to meet. The publication of these Guidelines has been supported by financial and in-kind contributions from Bioversity International, the CGIAR Research Programme on Forests, Trees and Agroforestry (FTA), the Cocoa Research Association Ltd., UK (CRA Ltd., a UK-based organization managing scientific cocoa research on behalf of Mars, Mondelēz International and the London Cocoa Trade [ICE Futures Europe]) and the University of Reading. 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

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

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 concerning export and import of germplasm or cocoa beans.

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. Bioversity International 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 regularly updated. The experts who contributed to the production of these technical guidelines are listed in this publication. Correspondence regarding this publication should be addressed to Bioversity International.

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

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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.

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

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.mynura@koko.gov.my

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. 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
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 die-back	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 a mealybugs and systemic fungi (e.g. *Ceratobasidium (formerly Oncobasidium) theobromae*).

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.

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	Special precautions
7.1 *Cacao necrosis virus* (CNV): genus *Nepovirus*	Ghana, Nigeria	Pod: Potential risk Seed: Low risk Budwood: High risk Quarantine advisable See: 5.2 Budwood SPECIAL RISK FACTOR: LATENT INFECTION UP TO TWO YEARS
7.2 *Cacao swollen shoot virus* (CSSV): genus *Badnavirus*	Benin, Côte d'Ivoire, Ghana, Liberia, Nigeria, Sierra Leone, Togo Reports also in Sri Lanka	
7.3 *Cacao yellow mosaic virus* (CYMV): genus *Badnavirus*	Sierra Leone	
7.4 Trinidad Cocoa Virus	Isolated occurences inTrinidad	Budwood: 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, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Jamaica, Mexico, Nicaragua, Panama, Peru, and western Venezuela	Pod: High risk, not recommended Seed: Moderate risk Budwood: Moderate risk Quarantine recommended SPECIAL RISK FACTOR: LONG LIVED SPORES See: 8.2.6 Quarantine measures
8.3 *Phytophthora* *Note that Phytophora species are widespread and sometimes difficult to distinguish* *P. palmivora (syn. P. arecae)* *P. megakarya*	Most cocoa-producing countries worldwide Bioko (Fernando Po), Cameroon, Côte d'Ivoire, Gabon, Ghana, Nigeria, São Tomé and Principe, Togo	Whole pods: High risk, not recommended Seed: Low risk Budwood: High risk intermediate quarantine recommended SPECIAL RISK FACTOR: PRESENCE IN SOIL

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* (cont'd)		
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	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. 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, Cuba, India, Malaysia, Philippines	
8.4 Vascular streak die-back (*Ceratobasidium theobromae)*	Most cacao-growing areas in South and South East 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, Uganda	Whole pods: Low risk Seeds: Low risk Budwood: Moderate risk See: 8.5.6 Quarantine measures
8.6 *Ceratocystis* wilt	Brazil, Cameroon, Colombia, Costa Rica, Ecuador, French Guiana, Trinidad, 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
9.2 Cocoa pod borer	Southeast Asia including Malaysia, Indonesia, the Philippines and Papua New Guinea	Pod: High risk, not recommended Seed: High risk Budwood: Moderate risk See: 9.2.6 Quarantine measures
9.3 and 9.4 Mirids and other Heteropterous plant sucking bugs	All cacao-growing regions except Carribean	Pod: Moderate risk Seed: Low risk Budwood: Moderate risk See: 9.4.6 Quarantine measures

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

Pest	Geographical spread	Special precautions
9.5 Mealybug	All cacao-growing regions	Pod: Moderate risk Seed: Low risk Budwood: Moderate risk

Table 6.2. Summary of pest risk by country (*Phytophthora palmivora* is widespread as are a number of insect 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	Witches' broom disease *Phytophthora capsici/P. tropicalis* *P. citrophthora* *P. hevea* *P. megasperma* *P. nicotianae* *Verticillium* wilt of cacao *Ceratocystis* wilt *Rosellinia* root rot
Cameroon	*Phytophthora megakarya* *Phytophthora capsici* *Ceratocystis* wilt
Colombia	Witches' broom disease *Moniliophthora* pod rot *Verticillium* wilt of cacao *Ceratocystis* wilt
Costa Rica	*Moniliophthora* pod rot *Ceratocystis* wilt *Rosellina* root rot *Phytophthora capsici*

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 hevea* *Phytophthora megasperma* *Phytophthora nicotianae*
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*
Guyana	Witches' broom disease
Honduras	*Moniliophthora* pod rot
India	*Phytophthora capsici* *Phytophthora citrophthora* *Phytophthora hevea* *Phytophthora megasperma* *Phytophthora nicotianae* Vascular streak dieback *Rosellinia* root rot
Indonesia	Vascular streak dieback *Rosellina* root rot Cocoa pod borer *Phytophthora capsici*

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

Country	Pest risk
Jamaica	*Phytophthora capsici* *Rosellinia* root rot *Moniliophthora* pod rot *Thielaviopsis* [*Ceratocystis*] *paradoxa*
Liberia	*Cacao swollen shoot virus* (CSSV)
Malaysia	*Phytophthora citrophthora* *Phytophthora hevea* *Phytophthora megasperma* *Phytophthora nicotianae* Vascular streak dieback *Rosellina* root rot Cocoa pod borer
Mexico	*Moniliophthora* pod rot *Phytophthora capsici* *Phytophthora citrophthora* *Phytophthora hevea*
Nicaragua	*Moniliophthora* pod rot
Nigeria	*Cacao necrosis virus* (CNV) *Cacao swollen shoot virus* (CSSV) *Phytophthora megakarya*
Panama	Witches' broom disease *Moniliophthora* pod rot *Phytophthora capsici*
Papua New Guinea	Vascular streak dieback Cocoa pod borer
Peru	Witches' broom disease *Moniliophthora* pod rot *Ceratocystis* wilt
Philippines	*Phytophthora citrophthora* *Phytophthora hevea* *Phytophthora megasperma* *Phytophthora nicotianae* Vascular streak dieback *Rosellinia* root rot Cocoa pod borer

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

Country	Pest risk
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 Trinidad Cocoa Virus
Uganda	*Verticillium* wilt
Venezuela	Witches' broom disease *Moniliophthora* pod rot (Western Venezuela) *Phytophthora capsici* *Phytophthora citrophthora* *Phytophthora hevea* *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

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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). *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. 2010). Therefore there is 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.

7.2.8 References

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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

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7.4 Other virus-like diseases

Trinidad virus disease was first reported in 1944 and a survey at the time suggested it was confined to Diego Martin, Santa Cruz and Maracas regions of Trinidad. Two strains (A and B) of the virus were identified on the basis of symptoms induced on the differential host, ICS 6. Strain A produces feather-like red banding in a few or all of the main veins on flush leaves, with the first leaf of the flush showing the most distinct symptoms. As the leaves mature the red vein banding disappears. In some clones a mosaic type symptom persists on mature leaves. Strain B produces a continuous vein banding extending to the fine veins, which persists even after the leaves have matured though in some varieties, this strain produces a red vein banding in young leaves which disappears as the leaves mature. Following elimination campaigns and changes of land use in the affected areas, it was thought that the virus had been eliminated. However, a reoccurrence of the virus was observed in 2009 and recent molecular sequencing studies on symptomatic leaves exhibiting the A and B phenotypes have shown that two distinct badnavirus species were present, named as cacao mild mosaic virus and cacao yellow vein banding virus, (Chingandu et al. 2017a,b). Tests have shown that the virus can be detected

using the indexing procedure described for *Cacao swollen shoot virus* above using either Amelonado or ICS 6 as the rootstock (Sreenivasan 2009, pers. comm.).

A CSSV like virus has been reported in North Sumatra (Kenten and Woods 1976), although no further published reports have been made.

7.4.1 Reference

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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 more recently by Bailey and Meinhardt (2016).

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8.1 Witches' broom disease

Update by Karina P Gramacho[1] and Nara GR Braz[2]

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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

Although *M. perniciosa* induces a variety of symptoms on vegetative shoots, flower cushions, flowers, and pods of cacao the hypertrophic growth of the infected vegetative meristems (broom) is the most characteristic symptom of the witches´ broom disease (Fig. 8.1.1, Fig.8.1.2).

8.1.3 Geographical distribution

Currently, the disease is present in Bolivia, Brazil, Colombia, Dominican Republic, Ecuador, French Guiana, Grenada, Guyana, Panama (east of Panama Canal), Peru, Trinidad and Tobago, St. Lucia, St. Vincent, Suriname, and Venezuela. In 2008, the disease was reported for the first time to occur in Union Vale, La Dauphine, and Robot estates in Saint Lucia (Kelly et al. 2009).

8.1.4 Hosts

Malvaceae Family (main host): *T. cacao, T. sylvestris, T. obovata, T. grandiflorum, T. bicolor Herrania* spp.

Solanaceae Family: *Solanum cernuum, S. gilo, S. grandiflorum* var. *setosum* (Goias, Brazil),
S. lycocarpum, S. melongena, S. paniculatum, S. stipulaceum, other *Solanum* spp., *Capsicum annuum*, *C. frutescens*.

Malpighiaceae Family: *Stigmaphyllon blanchetti*, *Heteropterys acutifolia*; *Mascagnia* cf. *sepium* (Pará, Brazil).

Others families: *Vernonia difusa, Bixa orellana*, *Arrabidaea verrucosa, Entadas gigas*, *Coussapoa eggersii*, *Barringtonia* spp., *Cecropia* spp., *Bambusa* spp., *Musa* spp.

Figure 8.1.1. Field symptoms (Source: CEPLAC/CEPEC): a) tree severely attacked in Bahia, b) terminal vegetative broom, c) diseased flower cushion, d) pod lesion

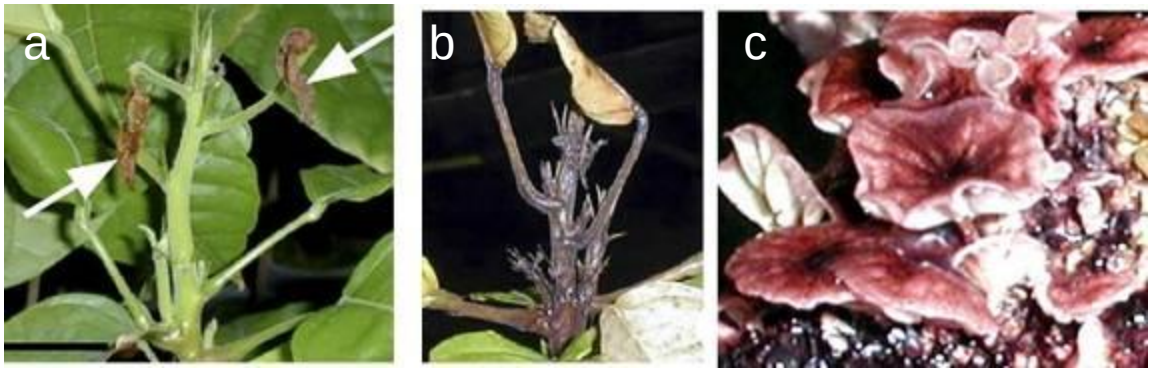

Figure 8.1.2. Greenhouse symptoms (Source: CEPLAC/CEPEC/ FITOMOL): a) terminal green broom, b) dry broom, c) "*in vitro*" basidiocarps production

8.1.5 Biology

Basidiospores, the only infective propagule of *M. perniciosa* can infect any meristematic tissues of cacao (Purdy and Schmidt 1996). Soon after infection the pathogen establishes a biotrophic relationship with its host, during which the fungus is homokaryotic, intercellular and lacks clamp connections (Calle et al. 1982, Muse et al. 1996, Orchard et al. 1994, Silva and Matsuka 1999). At this stage, it causes

hypertrophy of the tissues, loss of apical dominance, and proliferation of axillary shoots. Dissemination occurs by wind.

8.1.6 Quarantine measures

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 suitable alternate fungicde 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 one year and tested for freedom of disease before being released for general use.

8.1.7 References and further reading

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

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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. Infection can occur at very early stages of development and susceptibility decreases with increasing pod age. Initial symptoms are characterized by one or more swellings appearing on the pod (Fig. 8.2.1), or small water-soaked lesions, which enlarge into necrotic areas with irregular borders. A white fungal stroma (Fig. 8.2.2) covers the area within 3-5 days, with profuse formation of cream to light brown spores. Late infection of pods results in premature ripening showing a green and yellow mosaic pattern. In the infected pods the seeds become necrotic and compact into a mass (Fig. 8.2.3).

8.2.3 Geographical distribution

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 and Mexico (Phillips-Mora et al. 2007). It was recently reported in El Salvador (Phillips-Mora et al. 2010) and on a farm in Jamaica (IPPC 2016, Johnson et al. 2017) though efforts are now underway to contain it.

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).

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.

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

8.2.6 Quarantine measures

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)

8.3 *Phytophthora* spp.

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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. nicotinae* var *parasitica. P. katsurae, P. meadii, P. botryosa* (Surujdeo-Maharaj et al. 2016). However, only the first four species are of commercial importance.

8.3.2 Alternative hosts

Phytophthora palmivora – a very large number and wide variety of plant species, among others coconut, papaya, *Citrus* spp., *Hevea,* Mango, 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* sp. (Holmes et al., 2003) *Funtumia elastica*, *Sterculia tragacantha*, *Dracaena mannii* and *Ricinodendron heudelotii* (Opuku et al. 2002, Bailey et al., 2016). Recently Akrofi et al. (2015) recovered the pathogen from asymptomatic roots of numerous other species in cacao plantations, including Pineapple, *Athyrium nipponicum*, Papaya, Mango, Avocado, Cocoyam (*Xanthosoma sagittifolium*), 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

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.

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) (e.g. 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), 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 given 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)

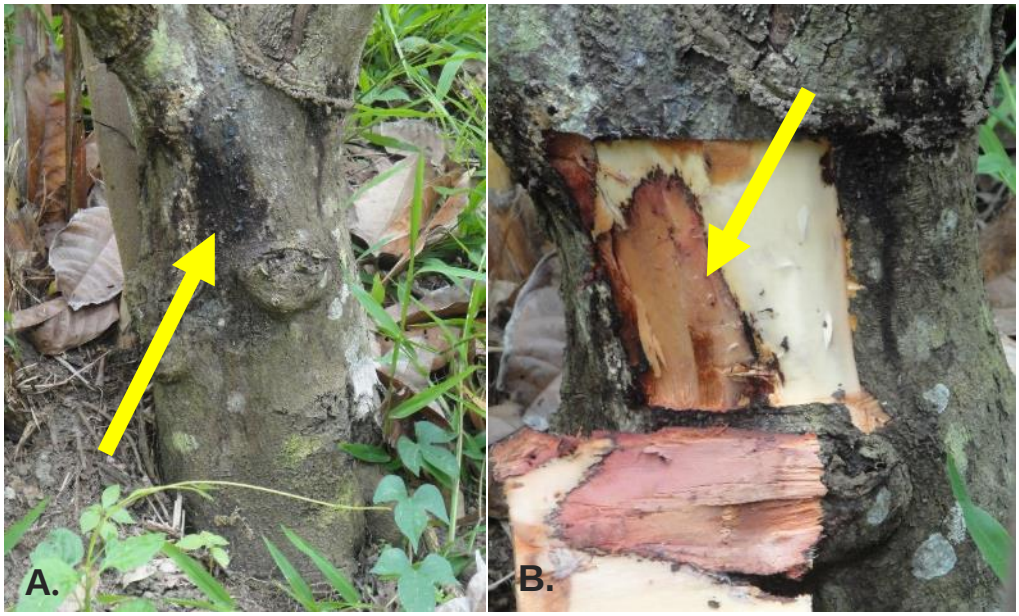

Figure 8.3.2. (A) Cacao tree trunk with canker symptoms (black discoloration) (B) discoloration of the sapwood (Dr. T Sreenivasan, CRC).

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

8.4 Vascular Streak Die-back

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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. McMahon and Purwantara (2016) further suggest that leaf necrosis and darker xylem staining observed in the VSD infected cacao in recent times, could be due to an enhanced resistance response although these authors also suggested that the necrotic symptoms could be associated with the lack of essential nutrients, such as potassium (K), reaching the canopy. Abdoellah (2009) conducted leaf nutrient analysis on infected and uninfected leaves in East Java and results indicated infected leaves had a 20% lower K concentration (on average) with Ca and Mg appearing to accumulate. Similarly, in Sulawesi, substantially lower concentrations of K were detected in infected leaves (*circa* 60% of the concentration in healthy leaves). However, similar K decreases were seen in plants exhibiting necrotic and the more usual chlorotic symptoms so further work is needed to clarify the role of K. The other possible case of the changes in field symptoms could be associated with climatic change e.g. raised temperatures or increased CO_2 levels (McMahon and Purwantara 2016) while the production of ethylene-inducing

proteins (NEPs) as demonstrated in other basidiomycete fungi that attack cacao e.g. *Moniliophthora perniciosa* (de Oliveira et al. 2012) could also be implicated in the VSD interaction. 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 South East 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 South East 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 South-East 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. Previously, introduction of the disease into New Ireland has been prevented by stringent quarantine procedures for the official movement of cacao germplasm and by a campaign of raising awareness at ports and airports of the risks involved in "unofficial" movement of cacao germplasm. Its introduction is probably via "unofficial" movement of cacao material between the island of New Britain and New Ireland. New Ireland is about 70km east of the production area in the Gazelle Peninsular in New Britain where there has been heavy infestations for many years. 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.

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. In PNG, Trinitario clones such as KA2-101 have shown durable resistance since the 1960s. Local genotypes with high resistance to VSD or with good quality attributes have been selected and tested as clones in farmers' fields with some promising results (McMahon et al. 2010). Work in India has recently suggested that VSD disease resistance is highly heritable and polygenically controlled (Minimol et al. 2016).

In addition, Rosmana et al. (2015) reported some success using *Theobroma asperellum* isolates to control VSD disease on cacao. Similarly, Vanhove et al. (2016) reported significantly lower VSD infections on plants treated with bacterial elicitors but these authors reported that *T. asperellum* did not show potential as an elicitor of systemic resistance in their work.

8.4.7 References and further reading

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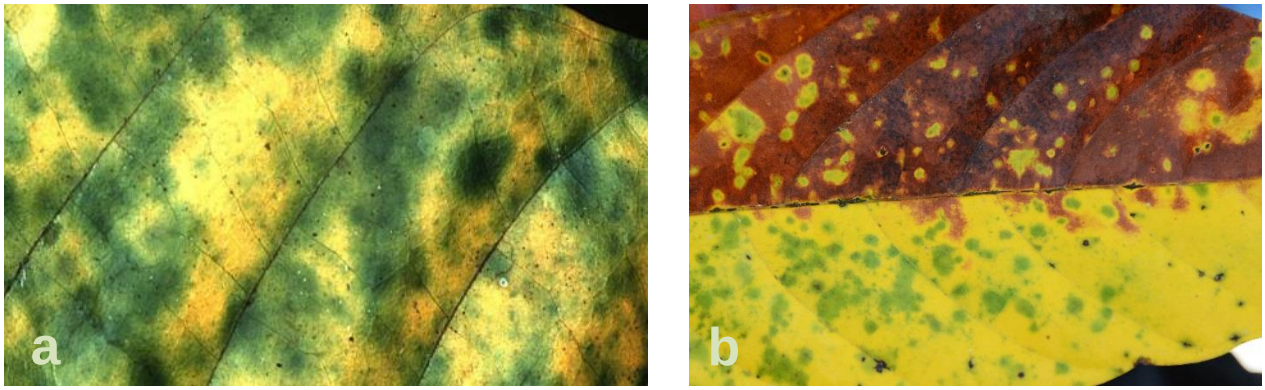

Figure 8.4.1. a) Vascular streak dieback: chlorotic leaf (M. Holderness, CABI) and b) Leaf showing necrosis and scattered islets of green tissue (AJ Daymond, University of Reading)

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

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

8.5 *Verticillium* wilt of cacao

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8.5.1 Causal agent

Verticillium dahliae Klebahn

8.5.2 Symptoms

Over 200 mainly dicotyledonous species including herbaceous annuals, perennials and woody species are host to *Verticillium* diseases (Agrios 2005). 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, on cotton, Schnathorst and Mathre (1966) described *V. dahliae* pathotypes as defoliating or non-defoliating, but other authors (Bell 1973, Ashworth Jr 1983) have suggested a continuum of symptoms related to the relative aggressiveness amongst strains of *V. dahliae*, rather than the occurrence of distinct pathotypes. 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 of *Verticillium*, plant variety and stage of plant development, temperature, soil moisture, and nutrition, particularly potassium content (Resende 1994). 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).

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 (Subbarao et al. 1995). *Verticillium dahliae* and *V. albo-atrum* cause disease in temperate and subtropical regions but are less destructive in the tropics. *Verticillium dahliae* appears to be favoured by higher temperatures than *V. albo-atrum*, as can be deduced from its geographical distribution (Fradin and Thomma 2006, Resende 1994). *Verticillium dahliae* is more destructive in warmer climates, whereas *V. albo-atrum* is more apt to cause problems in crops in northern latitudes with humid climates. 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 (Resende 1994).

In Brazil, *Verticillium* wilt is a serious problem in the States of Bahia and Espírito Santo (Resende et al. 1995, Agrianual 2009). This disease is more common in regions subject to rainfall shortages, causing annual plant mortality of up to 10% on unshaded cacao areas (Almeida et al. 1989).

Verticillium wilt disease is the most serious disease of cacao in Uganda inducing losses of up 30% (Emechebe et al. 1971). It has been recognized in Uganda for many years and may be a reason why cacao has not become a significant crop there (Leakey 1965, Resende et al. 1995, Sekamate and Okwakol 2007). *Verticillium* wilt has recently been reported in the Province of North Kivu in the Democratic Republic of Congo, most likely as a result of spread from Uganda. *Verticillium dahliae* has also been found on cacao in Colombia (Granada 1989, Resende et al. 1995).

8.5.4 Alternative hosts

Cotton and many other dicotyledonous species.

8.5.5 Biology

Verticillium dahliae Kleb. is a root inhabiting fungus with a necrotrophic life cycle. This anamorphic form of an ascomycete, belonging to family Plectospharellaceae, Class Sordariomycetes, is a common causal agent of wilt diseases in many crop plants (Domsch et al. 2007).

The vegetative mycelium of *V. dahliae* is hyaline, usually branched, septate and multinucleate (Fig. 8.5.2 A). Conidiophores are erect, bearing whorls of slender awl-shaped divergent phialides. Conidia are ellipsoidal to ovoid (Fig. 8.5.2 A), 15-50 (-100) μm in diameter, hyaline, mainly 1-celled, 3-8 μm long and are produced on long phialides positioned in a whorl or spiral-like shape around the verticillate conidiophores (Resende 1994, Gómez-Alpízar 2001, Fradin and Thomma 2006). Microsclerotia, considered resting structures, are commonly observed.

Two species of *Verticillium, V. dahliae* Klebahn and *V. albo-atrum* Reinke & Berthold, are very similar. Taxonomically, *V. dahliae* is separated from *V. albo-atrum* mainly by

the presence of microsclerotia (Fig. 8.5.2 C) as resting structure and these withstand adverse environmental conditions up to 13 years (Schnathorst 1981, Resende 1994). *Verticillium dahliae* appears to be favoured by temperatures of 25 – 28°C while *V. albo-atrum* of 20 – 25°C (Resende 1994). *Verticillium dahliae* causes monocyclic disease, meaning that only one cycle of disease and inoculum production occurs during a growing season. In contrast, *V. albo-atrum* may produce conidia on infected plant tissues that become airbone and contribute to spread of the disease. Therefore, the diseases caused by *V. albo-atrum* can sometimes be polycyclic (Fradin and Thomma 2006).

As *Verticillium* wilt is a monocyclic disease, inoculum levels of *V. dahliae* (microsclerotia per g of soil) in the soil at planting time, play a critical role in wilt development on many crops (Xiao and Subbarao 1998, 2000). A wide range of genera and plant species are colonized by *V. dahliae*, including members of the families Malvaceae such as cacao and cotton, Solanaceae, Compositae, Convolvulaceae, Papilionaceae, Labiatae and Chenopodiaceae (Resende et al. 1994).

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 senescense 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 B and 8.5.2 C).

8.5.6 Quarantine measures

Efforts should be made to prevent the entry of the pathogen in the main cacao-producing regions. 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. Vascular discolouration symptoms are often observed. The absence of the pathogen must be confirmed

through direct isolation in an alcohol agar medium before being dispatched (Freitas and Mendes 2005). *Verticillium dahliae* can be isolated from the xylem of roots, stems, branches, twigs and even leaves and seeds. Recent efforts to detect both specie4s of *Verticillium* are mainly concentrated on the use of DNA hybridization probes. An ELISA test for *V. albo-atrum* is in use in France for testing certified pelargoniums (CABI/EPPO).

According to Pereira et al. (2008), disease control can be achieved through the use of genetic resistance associated with cultural measures, such as the use of healthy seedlings, removal of infected crop residues, balanced fertilization, irrigation and proper application of systemic fungicides, although the use of these products can be impracticable, since the fungus survives in plant debris or soil, as microsclerotia for prolonged periods. Even though genetic resistance is desirable, genetic material with satisfactory level of resistance is not yet available, although cv. POUND 7 has been highlighted in tests of "screening" to be partially resistant to the disease.

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. Such measures are as relevant in a national certification scheme as for international phytosanitary certification (CABI/EPPO).

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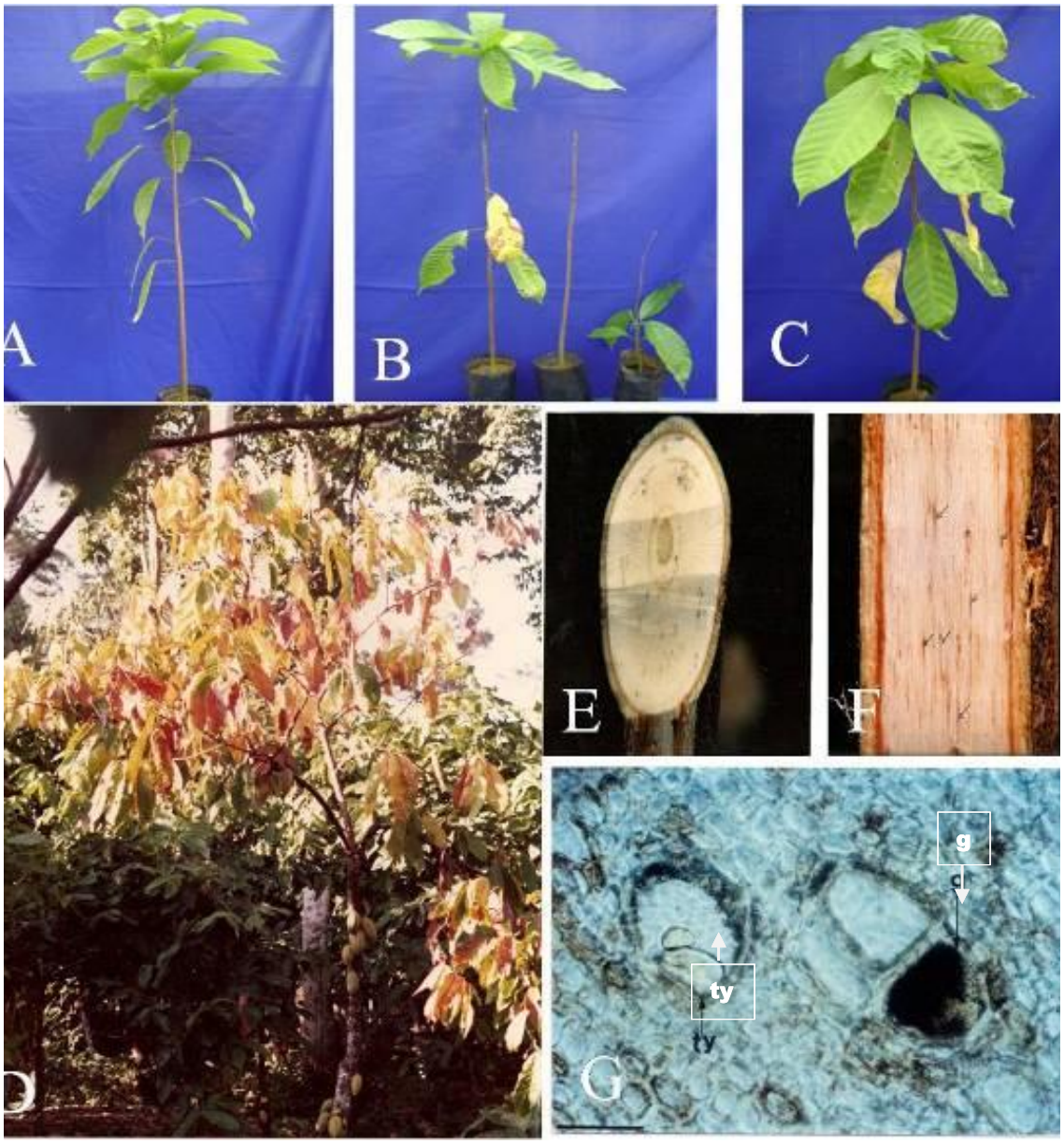

Figure 8.5.1. External (A-D) and internal (E-G) symptoms of *Verticillium dahliae* – cocoa interactions (MLV Resende, Univ. Federal de Lavras, Brazil):

- **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 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).

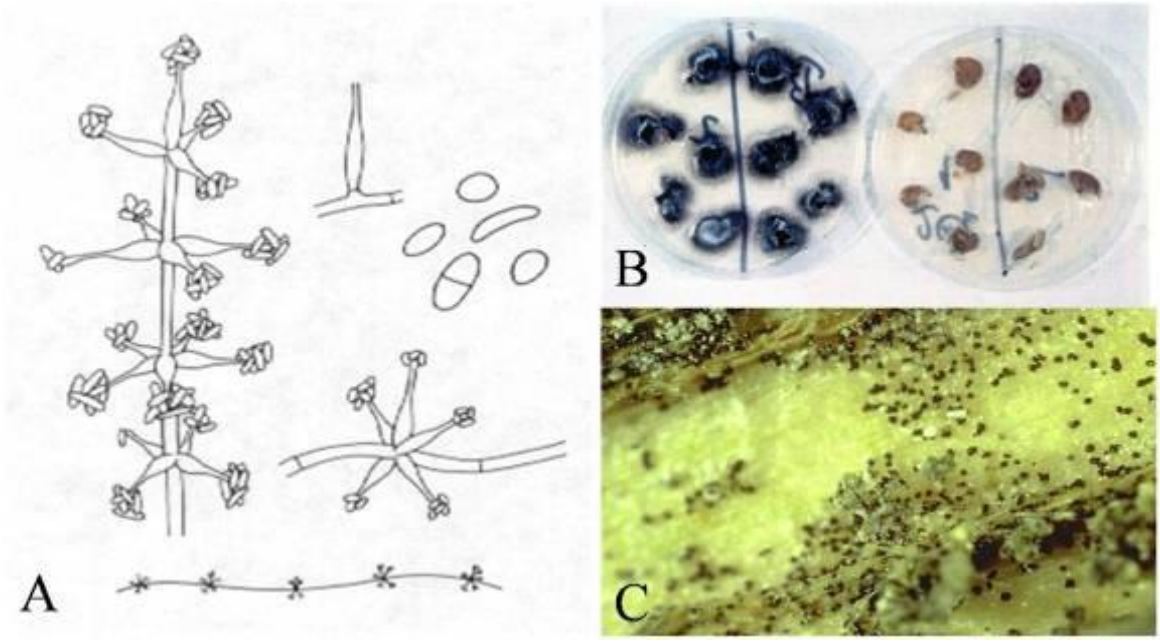

Figure 8.5.2. Biological cycle of *Verticillium dahliae*:

A Line drawing of hyphae, conidiophores and conidia of *Verticillium* spp. (Gómez-Alpízar 2001)

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).

8.6 *Ceratocystis* wilt of cacao or mal de machete

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8.6.1 Causal agent

Ceratocystis cacaofunesta Engelbr. & T.C. Harr.

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).

Ceratocystis cacaofunesta is a serious pathogen of cacao *(Theobroma cacao)* and related *Herrania* spp., causing wilt and death of infected trees. The cacao pathogen is a member of the Latin American clade of the *C. fimbriata* species complex, which has substantial genetic variation and a wide range of hosts. For an extensive review of the genus refer to CABI Crop Protection Compendium, CABI Publishing Updated 2001 by CJ Baker and TC Harrington (CAB International 2001).

8.6.2 Symptoms

Infected trees show limp, brown foliage on a single branch or across the whole tree, depending if only a branch or the main stem is infected; the first symptom is a general yellowing and slow wilt of the infected part of the branch/tree, which progressively turn brown. Typically *Ceratocystis* wilt is recognised through limp brown foliage that hang from the tree without falling even when shaking the branch or tree (Fig. 8.6.1). 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 ambrosia beetles is pushed to the outside of the stem or branch, and is seen on the base of the tree as light, powdery masses (Fig. 8.6.2). This is recognised as the first positive sign of *Ceratocystis* wilt; frequently the frass is seen even before the yellowing of the tree is visible.

8.6.3 Alternative hosts

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

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 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. This differentiation certainly explains the variation in aggressiveness observed when dealing with artificial inoculations (C. Suárez-Capello, personal observation).

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) (Iton and Conway 1961) and may be spread by wind or rainsplash to infect wounds on other trees (Iton 1960). Machete blades are another efficient means of spreading the fungus (Malaguti 1952).

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 of 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.

Infected trees show heavy infection at the base, perhaps due to infection of wounds near ground level. Spores in the wind-dispersed frass or spores carried by fungal-feeding insects may infect fresh wounds. The name *'mal de machete'* comes from the association of such infections with machete wounds.

8.6.6 Quarantine

The mycelium of the fungus is as infective as the spores (both conidia and ascospores), they readily germinate on water without any dormancy; after penetration an extensive growth of mycelium is produced within the cacao tissue well before any symptom is visible.

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

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

- Seeds

Therefore, infested cuttings of *T. cacao* are the most likely, and may be only, means by which *C. cacaofunesta* can be spread to new areas. In consequence, transport of whole plants, graftwood or cuttings from areas where *C. cacaofunesta* is present should be avoided and vegetative planting materials collected only from areas free from the fungus if possible. Budwood from plants grown where the disease is present should be sent and maintained in an intermediate quarantine station in a disease-free area and budded onto rootstocks of resistant material preferably grown in a disease free area. As with other diseases of the xylem, the scion should be maintained for several successive growth flushes to confirm that it is free from *C. cacaofunesta*. Treatment of the cuttings with insecticide-fungicide is recommended.

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

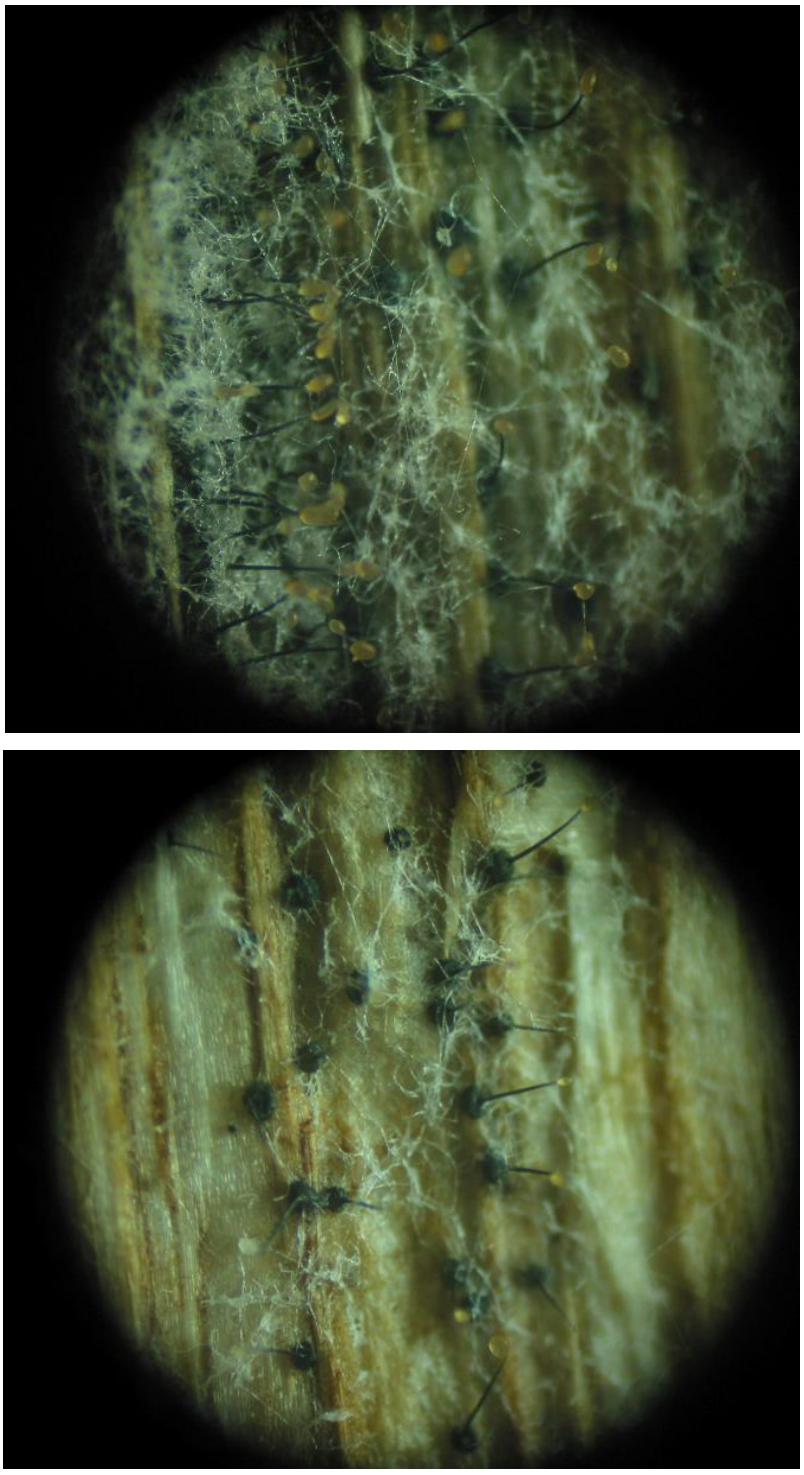

Figure 8.6.3. Perithecia of *Ceratocystis cacaofunesta* growing over the xylem of cocoa branches inoculated with the pathogen (C Suárez-Capello, UTEQ, Ecuador)

8.7 *Rosellinia* root rot

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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. and *Erythrina* spp. among others (Waterston 1941, Saccas 1956, Booth and Holliday 1972, Sivanesan and Holliday 1972, Aranzazu et al. 1999, Ten Hoopen and Krauss 2006).

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 neighboring 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 of them 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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9. Insect pests

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A rich diversity of insects 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 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 9.3 and 9.4) and Mealybugs (see Section 9.5). 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 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

Immature infested pods show pre-ripened yellow patches (Fig. 9.2.1). Larval entry holes on the pod surface are barely visible to the naked eye, but they can be detected by shaving the husk. Larvae leave characteristic 1-2 mm diameter exit holes in pod walls (Fig. 9.2.2). Beans from infested pods often clump together and are difficult, if not impossible, to extract (Fig. 9.2.3). Beans may begin to germinate within pods that are infested when nearly ripe (Azhar 1986).

9.2.3 Geographical distribution

The pest is widely distributed throughout Southeast Asia including Malaysia, Indonesia, the Philippines and Papua New Guinea.

Figure 9.2.1. Uneven yellowing of immature pods due to pod borer infestation (A Alias, Malaysian Cocoa Board)

Figure 9.2.2. Pod borer larval exit hole in a pod wall (A Alias, Malaysian Cocoa Board)

Figure 9.1.3. Beans clumped into a solid mass from pod borer feeding (A Alias, Malaysian Cocoa Board)

9.2.4 Host plants

Other known hosts include fruits of *Nephelium lappaceum, N. mutabile, Euphoria malaiense* and *Pometia* spp. (Family: Sapindaceae), *Cynometra cauliflora* (Family: Leguminosae*)* and *Cola nitida* (Family: Malvaceae). The Sapindaceae and Leguminosae species may be the original host of pod borer as cacao is not indigenous to Southeast Asia.

9.2.5 Biology

The life cycle of CPB is illustrated in Fig. 9.2.4. Female moths may each lay 40-100 (maximum 300) eggs. The 0.6 mm long oval and strongly flattened eggs are usually laid singly near furrows on the pod surface. The eggs hatch after *circa* three days, changing during maturation from an orange colour to nearly colourless. Newly hatched larvae bore immediately through the pod walls (Fig. 9.2.5). Inside the pod, the larvae feed for 14-21 days on the mucilage, pulp, placenta and sometimes the testas of the cotyledons. Once mature, larvae bore out through the pod wall (Fig. 9.2.6) and pupate within silken cocoons on leaves, pods or dry leaf litter on the ground (Fig. 9.2.7). Pupae change colour from an initial light green to dark grey as they mature. The adults, which are *circa* 5 mm long with a 13 mm wingspan, emerge after a 6-8 day pupation period. 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 is normally about one week and, exceptionally, up to 30 days. A generation is usually completed within 27-33 days.

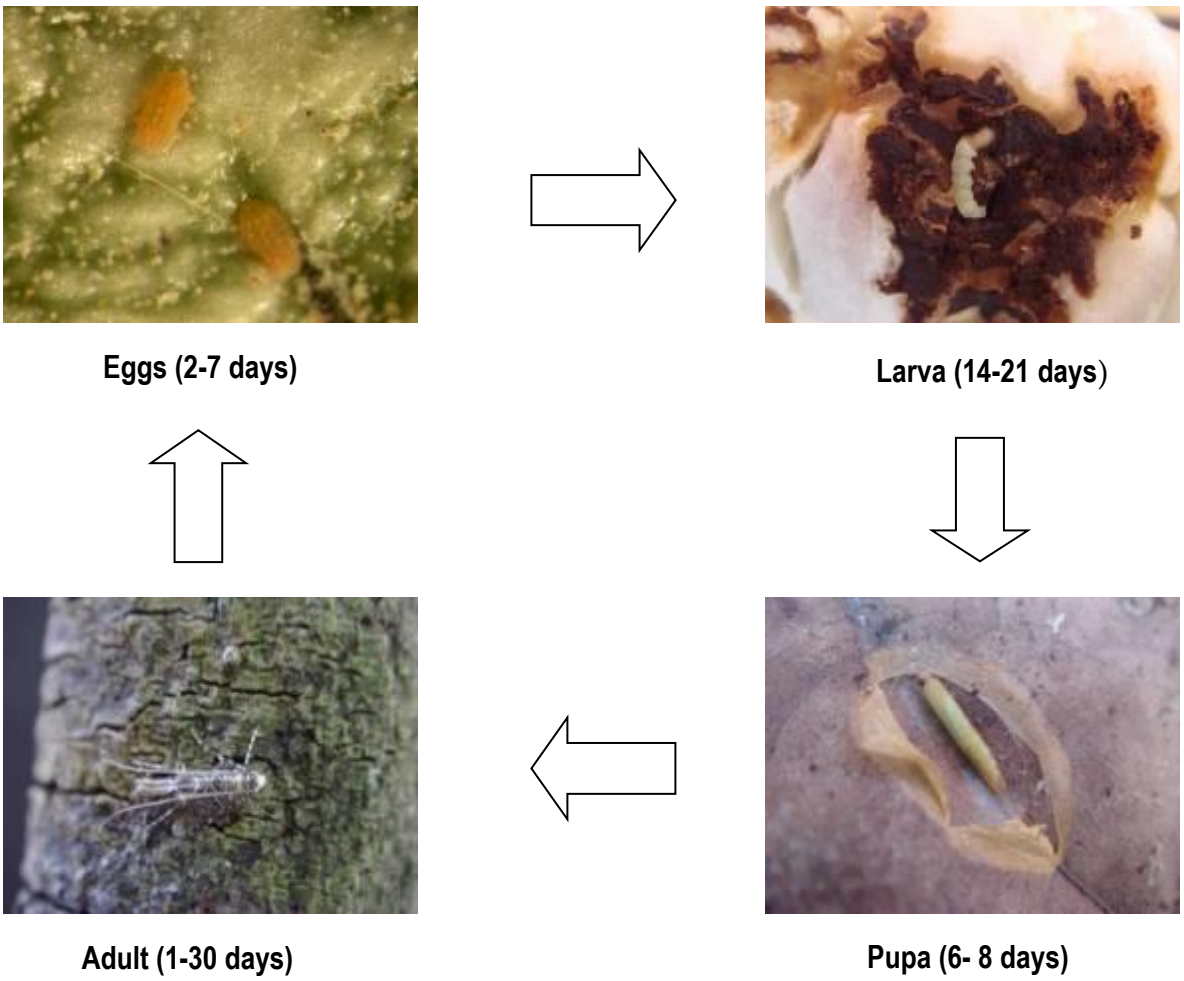

Figure 9.2.4. Life cycle and duration of the life stages of cocoa pod borer (A Alias, Malaysian Cocoa Board)

9.2.6 Quarantine recommendations

When transferring seed:

1. Whole unopened pods should NOT be sent from infected areas.
2. The source of the seeds should be clean pods with no signs of insect boring or fungus inside the pod.
3. 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 9.2.5. Newly hatched pod borer larva tunnelling into the pod wall (A Alias, Malaysian Cocoa Board)

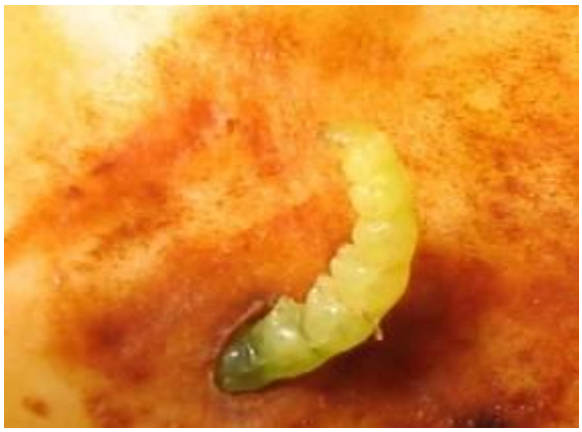

Figure 9.2.6. Pod borer larva emerging from its exit tunnel in the pod wall (A Alias, Malaysian Cocoa Board)

Figure 9.2.7. Pod borer pupa under its silk cocoon on leaf litter (A Alias, Malaysian Cocoa Board)

9.2.7 References

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9.3 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 (9.4) is included to cover the Mosquito bug (*Helopeltis theobromae*) which is of particular concern in Southeast Asia.

9.3.1 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. 9.3.1) and *Distantiella theobroma* (Fig. 9.3.2) 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. 9.3.3 and Fig. 9.3.4) 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.

9.3.2 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.

9.3.3 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.

9.3.4 References

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Figure 9.3.1. Adults of Sahlbergella singularis (KF N'Guessan, CNRA)

Figure 9.3.2. Adults of *Distantiella theobromae*

Figure 9.3.3. Mirids lesions (dark colour) on cacao pods (KF N'Guessan, CNRA)

Figure 9.3.4. Larvae of Mirids on cocoa twig and Mirids lesions (dark colour) on cocoa pod (KF N'Guessan, CNRA)

9.4 Mosquito bug

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9.4.1 Causal agent

Helopeltis theobromae (Miller) (Hemiptera: Miridae)

Common synonym *Helopeltis theivora (Waterhouse) (*Hemiptera: Miridae).

9.4.2 Symptoms

Both nymph and adult of *Helopeltis* infest young shoots (Fig. 9.4.1) cacao pods and peduncles on which a single pest can produce approximately 25-35 lesions per day. Fresh lesions are water-soaked and dark green in colour. The lesions will turn dark and slightly concave. Old lesions are also dark in colour but are usually convex (Fig. 9.4.2). Infestation on the shoots often occurs when only a few pods are available or as an alternative food source (Alias 1983). The infestation on shoots can be recognized by oval shaped black colour lesions, which are about 4-7mm in length. *Helopeltis* feed on the parenchymatous husk tissue of the cacao pod, and this usually induces cherelle wilt. Young pods, especially those less than three months old (Fig. 9.2.3), have little chance of surviving (Wan Ibrahim 1983). Mirid damage may lead to invasion by secondary pests (Fig. 9.2.4) or disease organisms and severe infestations on the cacao pod will lead the pod to crack. Pods usually die either due to *Helopeltis* infestation itself or fungal infestations through the lesions (Gerard 1968). In very serious infestations, the entire tree looks burnt.

9.4.3 Geographical distribution

The pest is widely distributed throughout South East Asia including Malaysia, Indonesia and Papua New Guinea.

9.4.4 Host plants

Other known host plants for *Helopeltis* are mango, cashew, guava, *Acalypha* spp. and Japanese Cherry (Khoo et al. 1991). *Helopeltis theivora* has also infested tea plantations in North East India as reported by Sarmah and Bandyopadhyay (2009).

Figure 9.4.1. *Helopeltis* infestation on young shoots (B Saripah, Malaysian Cocoa Board)

Figure 9.4.2. Old lesions on cocoa pod are dark in colour (B Saripah, Malaysian Cocoa Board)

Figure 9.4.3. *Helopeltis* infestation on a cherelle (B Saripah, Malaysian Cocoa Board)

Figure 9.4.4. Secondary pest infestation (B Saripah, Malaysian Cocoa Board)

9.4.5 Biology

The life cycle of *Helopeltis* is between 21-35 days. 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. 9.4.5) 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. 9.4.6).

Figure 9.4.5. *Helopeltis* nymph which is a light green colour (B Saripah, Malaysian Cocoa Board)

Figure 9.4.6. *Helopeltis* adult, usually up to 5.5 mm in length (B Saripah, Malaysian Cocoa Board)

9.4.6 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.

9.4.7 References

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9.5 Mealybugs and other insects

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9.5.1 Mealybugs

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.

9.5.1.1 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.

9.5.1.2 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. 9.5.1.) 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 9.5.1. Adults and nymphs of *Formicoccus njalensis* (WP N'Guessan, CNRA)

9.5.2 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.

9.5.3 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.

9.5.4 References

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10. Parasitic nematodes

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Parasitic nematodes play a very important role in cacao production. 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 Jamaica (Meredith 1974). A large number of plant parasitic nematodes 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 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, actual yield losses in cacao caused by other nematode genera are very limited. Based on the published findings, other nematodes are as detrimental to cacao as *Meloidogyne* spp. when their populations 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 countries where this has created problem for cacao, 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. Stem dieback, wilting, yellowing and browning of leaves and formation of small leaves and dried leaves, which fall before the plant dies, 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 Côte d'Ivoire, Ghana, Nigeria, São Tomé, India, Malaysia, Java, Philippines, Papua New Guinea, Jamaica, Venezuela, Costa Rica, Brazil, Ecuador, Peru, Bolivia (Sosamma et al. 1979, Lopez-Chaves et al. 1980, Sharma 1982, Crozzoli et al. 2001, Wood and Lass 2001, Campos and Villain 2005, Arévalo 2008).

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
Criconemella	Ivory Coast
Criconemoides	Brazil, Costa Rica, Peru, Venezuela, Ivory Coast, Ghana, Nigeria, Malaysia
Crossonema	Peru
Diphtherophora	Brazil
Discocriconemella	Ivory Coast
Ditylenchus	Peru
Dolichodorus	Brazil, Costa Rica
Dorylaimidos	Peru
Dorylaimus	Peru
Eutylenchus	Nigeria
Haplolaimus	Brazil, Costa Rica
Helicotylenchus	Brazil, Venezuela, Peru, Costa Rica, Ivory Coast, 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, Peru, Nigeria, Ivory Coast, Suriname
Hemicriconemoides	Brazil, Venezuela, Nigeria
Heterodera	Brazil, Nigeria
Longidorus	Brazil, Costa Rica, Ivory Coast, Ghana, Nigeria
Neodiplogaster	Guatemala
Meloidogyne	Venezuela, Brazil, Costa Rica, Peru, Ghana, Nigeria, Ivory Coast, Zanzibar, Malawi, India, Papua New Guinea, Sao Tomé, Java, Malaysia
Mesocriconema	Venezuela
Mononchus	Peru
Ogma	Venezuela
Paralongidorus	Nigeria
Parachichodorus	Brazil
Paratylenchus	Peru, Venezuela, Ivory Coast
Peltamigrattus	Brazil, Venezuela
Pratylenchus	Brazil, Costa Rica, Peru, Venezuela, Ivory Coast, Nigeria, Ghana, Indonesia, India, Jamaica. Malaysia
Psilenchus	Peru, Venezuela, Nigeria
Rhabditidos	Peru
Rhadinaphelenchus	Peru
Radopholus	Ivory Coast, Jamaica, Nigeria
Rotylenchulus	Brazil, Peru, Venezuela, Indonesia, India, Jamaica
Rotylenchus	Brazil, Peru, Venezuela, Nigeria
Scutellonema	Brazil, Peru, Jamaica, Nigeria
Trichodorus	Brazil, Costa Rica, Venezuela, Peru, Mexico, India, Ivory Coast, Ghana, Nigeria
Trophurus	Brazil, Venezuela, Ivory Coast
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, Venezuela, Ghana, Mexico, Philippines

Source: Tarjan and Jiménez (1973), Sosamma et al. (1979), Lopez-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).

Figure 10.1. Stunted growth, chlorosis, reduction in leaf size, and wilting of *M. incognita*–infested cacao seedlings (left) compared to a similar age healthy plant (right) in soil amended with poultry litter (Orisajo et al. 2008)

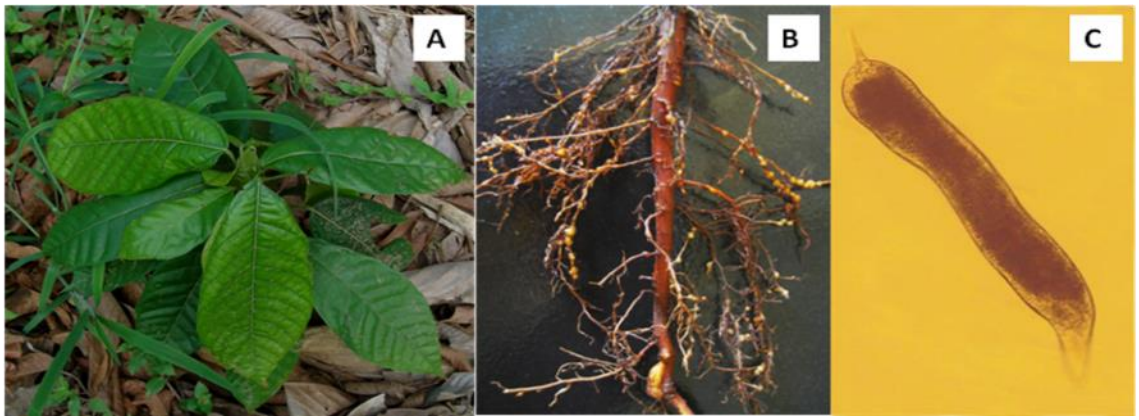

Figure 10.2. Symptoms of damage of *Meloidogyne* spp. on cacao plants

A. Plant after a month of transplant
B. Roots with galls
C. Second larval stage of a female
Source: Instituto de Cultivos Tropicales

10.4 Alternative hosts

Each species of *Meloidogyne* has plant species and cultivars that are very susceptible, moderately susceptible, susceptible and immune. 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), in many cases shade plants

commonly used for tropical plants, such as banana and *Inga* sp. can become a source of inoculum in the cacao plantations (Sosamma et al. 1980). In South America and Central America *M. exigua* is a very serious pest of *Coffea arabica*. There have been few additional hosts registered 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

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). 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). Chemical control with nemastatic products of *Meloidogyne* in roots of perennial crops that are already established is not effective. In Nigeria, Alofami (1993) controlled the nematodes in nursery soil treated with the nematicide Basamid and steam sterilization of nursery soil.

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ISBN: 978-92-9255-079-0

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