



FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS

INTERNATIONAL BOARD FOR
PLANT GENETIC RESOURCES

FAO/IBPGR TECHNICAL GUIDELINES
FOR THE
SAFE MOVEMENT OF
COCONUT GERMPLASM



Edited by
E.A. Frison, C.A.J. Putter and M. Diekmann

INTRODUCTION

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

Inevitably, the movement of germplasm involves a risk of accidentally introducing plant quarantine pests* along with the host plant material; in particular, pathogens that are often symptomless, such as viruses, pose a special risk. In order to minimize this risk, effective testing (indexing) procedures are required to ensure that distributed material is free of pests that are of quarantine concern.

The ever-increasing volume of germplasm exchanged internationally, coupled with recent rapid advances in biotechnology, has created a pressing need for crop-specific overviews of the existing knowledge in all disciplines relating to the phytosanitary safety of germplasm transfer. This has prompted FAO and IBPGR to launch a collaborative programme for the safe and expeditious movement of germplasm, reflecting the complementarity of their mandates with regard to the safe movement of germplasm. FAO, as the depository of the International Plant Protection Convention of 1951, has a long-standing mandate to assist its member governments to strengthen their Plant Quarantine Services, while IBPGR's mandate - *inter alia* - is to further the collecting, conservation and use of the genetic diversity of useful plants for the benefit of people throughout the world.

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

The technical guidelines are produced by meetings of panels of experts on the crop concerned, who have been selected in consultation with the relevant specialized institutions and research centres. The experts contribute to the elaboration of the guidelines in their private capacity and do not represent the organizations to which they belong. FAO, IBPGR and the contributing experts cannot be held responsible for any failures resulting from the application of the present guidelines. By their nature, they reflect the consensus of the crop specialists who attended the meeting, based on the best scientific knowledge available at the time of the meeting.

* The word 'pest' is used in this document as it is defined in the International Plant Protection Convention. It encompasses all harmful biotic agents ranging from viroids to weeds.

The technical guidelines are written in a short, direct, sometimes 'telegraphic' style, in order to keep the volume of the document to a minimum and to facilitate updating. The guidelines are divided into two parts: The first part makes recommendations on how best to move germplasm of the crop concerned and is divided into general and technical recommendations. Institutions performing indexing services and selected references are listed at the end of this first part. The second part gives descriptions of the most important pests that could be of quarantine concern.

The information given on a particular pest does not pretend to be exhaustive but concentrates on those aspects that are most relevant to quarantine. At the end of each description a few key references are given, referring mainly to geographical distribution, transmission and methods of indexing.

The procedures recommended in this booklet have been developed specifically for the movement of small quantities of germplasm exchanged for breeding, conservation or other scientific purposes. They were not developed for commercial shipments of planting material or commodities.

The present guidelines were developed at a meeting held in Ciloto, Indonesia, from 4 to 6 October 1991. The meeting was hosted by the Central Research Institute for Industrial Crops, Bogor, Indonesia.

CONTRIBUTORS

Dr R.G. Abad
Philippine Coconut Authority
Davao Research Center
Bago-Oshiro
PO Box 295
Davao City 8000
Philippines

Dr F. Engelmann
ORSTOM
Centre de Montpellier
911 Avenue Agropolis
BP 5045
34032 Montpellier Cédex
France

Dr N. Chomchalow
FAO Regional Office for Asia
and the Pacific
Maliwan Mansion
Phra Atit Road
Bangkok 2
Thailand

Ir E.A. Frison
IBPGR
Research Programme
Via delle Sette Chiese 142
00145 Rome
Italy

Dr M. Dollet
Laboratoire de Phytovirologie des
Régions Chaudes
CIRAD
BP 5035
34032 Montpellier Cédex
France

Mr H.C. Harries
International Coconut Cultivar
Registration Authority
PO Box 6226
Dar es Salaam
Tanzania

Dr S. Eden-Green
Natural Resources Institute
Central Avenue
Chatham Maritime
Kent ME4 4TB
UK

Dr F.W. Howard
University of Florida
Institute of Food & Agricultural Sciences
Fort Lauderdale
Florida 33314
USA

Dr G.V.H. Jackson
South Pacific Commission
Plant Protection Service
Private Mail Bag
Suva
Fiji

Dr D. Sitepu
Central Research Institute for
Industrial Crops
Jalan Tentara Pelajar 1
Bogor 16111
Indonesia

Ir H.T. Luntungan
Central Research Institute for
Industrial Crops
Jalan Tentara Pelajar 1
Bogor 16111
Indonesia

Dr J.J. Solomon
Central Plantation Crops Research
Institute
Kayangulam
Krishnapuram 690 533
Kerala
India

Dr C.A.J. Putter
Plant Production and
Protection Division
Plant Protection Service
FAO
Via delle Terme di Caracalla
00100 Rome
Italy

Dr J.M. Waller
International Mycological Institute
Ferry Lane
Kew
Surrey TW9 3AF
UK

Dr J.W. Randles
Waite Agricultural Research Institute
Department of Crop Protection
Glen Osmond
South Australia 5064
Australia

GENERAL RECOMMENDATIONS

- Germplasm should be collected from palms that appear healthy.
- Germplasm should not be moved from sites at which diseases of unknown etiology occur.
- Germplasm should preferably be moved as embryo cultures or pollen.
- Seednuts may be transferred under certain circumstances:
 - (i) when a thorough pest risk assessment indicates that there are no problems of quarantine concern in the area from which they were collected, or
 - (ii) from areas where diseases of quarantine concern are present **only** when embryo culture is not possible, and as long as they are germinated in quarantine.
- Seednuts should never be moved directly from areas where non-cultivable mollicutes or *Phytoplasmas* occur, to areas not affected by these pathogens.
- Embryos, seedlings and palms from which pollen is collected should be indexed for cadang-cadang and other viroids*, as well as for coconut foliar decay virus (CFDV).
- The transfer of germplasm should be carefully planned in consultation with quarantine authorities, the relevant indexing laboratory and, when appropriate, the intermediate quarantine facility. The material should be accompanied with the necessary documentation.

* Several viroid-like nucleic acid sequences related to cadang-cadang viroid are widely distributed in coconuts and understorey plants. Until such time as more is known about the significance and distribution of these viroid-like sequences, all germplasm introduced from countries where viroid-like sequences are known to occur to countries where they have not yet been reported should be indexed, and material for which tests are positive should be rejected.

TECHNICAL RECOMMENDATIONS

A. Movement of pollen

- At the time of collecting the pollen, leaf samples should be taken, following the procedures described below, and indexed for viroids and CFDV where appropriate:
 - Cut about 50 g of leaflets from the middle of a frond between positions 5 and 10; wipe the leaflets free of moisture and debris; remove mid-ribs, cut into 20 cm lengths, place in a plastic bag and seal it.
 - Keep samples cool (but do not freeze) and immediately consign by courier or air freight to the indexing laboratory, enclosing an import permit issued by the receiving country.
 - Notify the testing laboratory by telex or fax when the sample is despatched.
- Established methods for pollen collecting (Baliñgasa & Santos, 1978) which are used to prevent pollen contamination from neighbouring palms will also prevent contamination by air-borne pests, if carefully applied. They include the following steps:
 - Surface sterilise the spathe just before it opens (e.g., with 3.5% sodium hypochlorite* or 70% ethanol), and cover with an isolation bag. Remove the outer sheath, wrap insecticide-impregnated cotton waste around the base of the stalk of the inflorescence and tie the bag in place.
 - Collect male flowers once or twice between 7 to 14 days after bagging. Surface sterilise the sleeve of the isolation bag, open it and insert a plastic collecting bag. Cut off entire stalks with anthesing male flowers and put them in the collecting bag, remove the collecting bag and close and sterilise the sleeve.
 - Open the collecting bag in an isolation box (or room) and strip the male flowers from the stalks. Transfer to a paper bag. Close the bag, lay it flat and leave overnight (up to 16 h) at 40°C. In the morning use a roller to crack the flowers inside the unopened paper bag. Allow the flowers to

* Equivalent to undiluted 'household' bleach or a 2:5 v:v dilution of commercial (8%) bleach in water, giving about a 0.8% to 1.0% level of available chlorine in the solution.

dry for a further 8-24 h. In the isolation box (or room), open the bag and sieve (60 and 100 mesh) to remove unwanted flower parts. Transfer the pollen to clean plastic bags or glass vials and temporarily store at 4°C.

- At this stage pollen should be tested for viability (by germination or fluorescence), and inspected for the presence of mites, nematodes, fungi or bacteria.
- Viable pollen free of visible pests should be dried to 6-8% moisture content under vacuum or in a desiccator.
 - For short-term storage (up to a few months) vials should be kept in a closed container at -18°C.
 - For medium or long-term storage (months to years) vials should be sealed under vacuum. Vacuum-sealed vials can be stored under ambient conditions and despatched without refrigeration.
 - Cryopreservation in liquid nitrogen is also possible.
- Viable pollen, free of visible pests, should be stored pending the results of viroid (and where appropriate virus) indexing; if these are negative, the pollen may be released for use.
- Pollen from palms growing in areas infected with *Phytophthora* and non-cultivable mollicutes should be stored and used only if the source palms are still healthy after a time exceeding the incubation period of the particular disease.
- Once pollen has received health clearance, it should be rehydrated and the viability tested before use.

B. Movement of embryo cultures

- Embryos should be extracted using the method described below and whenever possible, they should be grown *in vitro* in the country of origin.
- If tissue culture facilities are not available, either in the country of origin or destination, embryos should be sent on culture media to a third country where they can be grown.
- If embryos cannot be extracted in the country of origin, seednuts should be sent to a third country, using the procedure recommended in Section C below, for embryo culture.

- Mature seednuts should be taken from the palm when at least one nut in the bunch turns from the fresh to the dry colour.
- Seednuts should be dehusked and rinsed in common bleach (3.5% sodium hypochlorite - see footnote on page 7).
- Embryos should be extracted, preferably under sterile conditions using a laminar flow cabinet, or in the field using the equipment described by Assy Bah *et al.* (1987) in the following manner:
 - Crack nuts equatorially and use a 20 mm corkborer to remove a cylinder of endosperm containing the embryo.
 - Disinfect the cylinders by placing batches of 25 in calcium hypochlorite (45 g/l for 20 mm).
 - Place individual cylinders in sterile Petri dishes, and extract the embryos.
 - Rinse each embryo in sterile distilled water (15 ml) and place them on sterilised semi-solid growth medium in the culture vessels (see details on page 14).

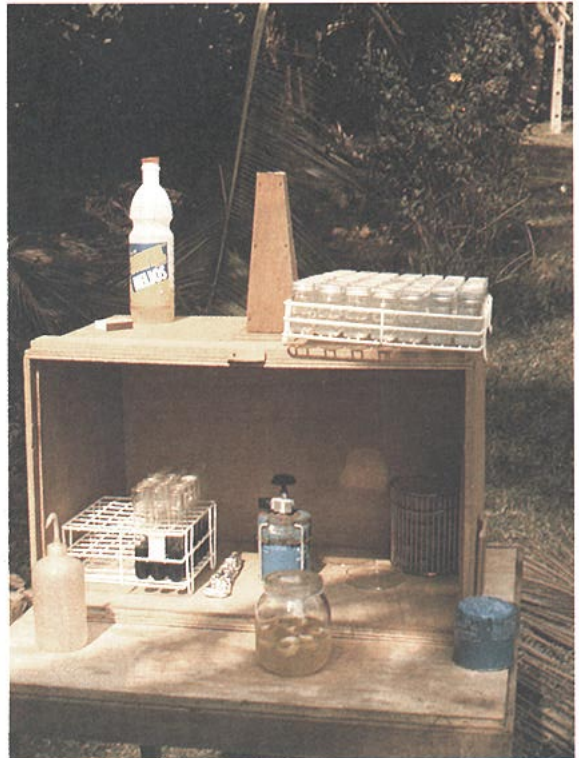


Fig. 1. Field equipment for the extraction of coconut embryos. (Dr. Florent Engelmann, ORSTOM, Montpellier)

- Cultures should be incubated at 27°C in the dark until gemmules emerge, then exposed to light (12 h/24 h photoperiod, 3000 lux).
- Embryos should be subcultured monthly on the growth medium and the haustorium should be removed when the gemmule is 2-4 cm long.
- When plantlets are well developed (at least one fully expanded leaf and one or more principal roots >3 cm), a sample should be taken of each for indexing, using the following procedure:
 - Cut approximately 0.5 g (equivalent to c. 10 cm) from the distal part of the youngest expanded leaf, wipe free of moisture or culture medium and seal in a plastic bag.
 - Keep samples cool (but do not freeze) and immediately consign by courier or air freight to the indexing laboratory, enclosing an import permit issued by the receiving country.
 - Notify the testing laboratory by telex or fax when the samples are despatched.
- Material should be released only when the indexing procedures confirm freedom from viroids, and CFDV where appropriate.

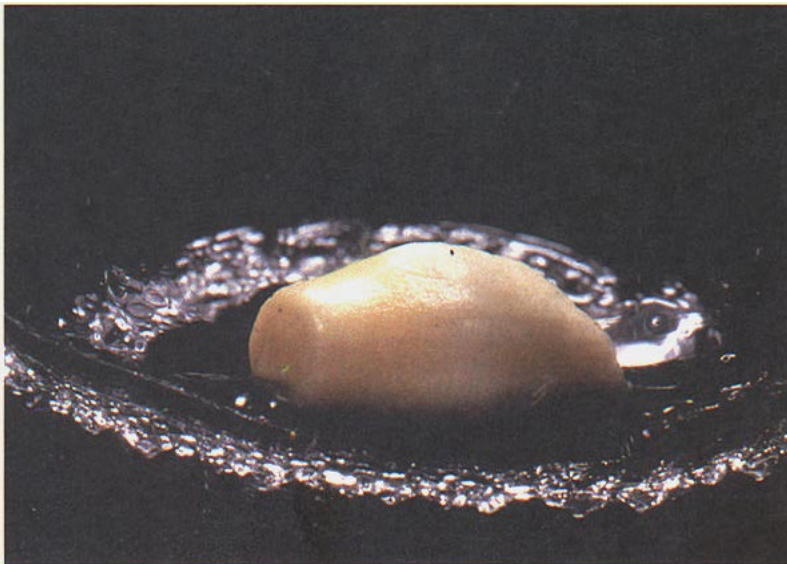


Fig. 2. Excised coconut embryo on culture medium. (Dr. Florent Engelmann, ORSTOM, Montpellier)



Fig. 3. Coconut plantlet grown from an excised embryo in a culture tube (Dr. Florent Engelmann, ORSTOM, Montpellier)

- Plantlets should be transferred to sterilised damp sand. They should be maintained at 100% humidity for the first 2 weeks by enclosing plants in a clear plastic-covered frame and watered as needed. After the first month, a nutrient solution (see page 14) should be applied every 2 days. After 2 months, the plants should be transferred to a suitable potting mix (Assy Bah *et al.*, 1989).
- Embryos excised from seednuts originating from areas where non-cultivable mollicutes occur should be cryopreserved (Assy Bah & Engelmann, 1992) or maintained under slow growth conditions for 1 and 2 years respectively. Parent palms should be observed for that period and embryos should only be released if the source palm has not shown disease symptoms.

C. Movement of seednuts

- Seednuts should only be transferred where circumstances prevent the extraction of embryos in the country of export or when a thorough pest risk assessment fails to show problems of quarantine concern.

- Mature seednuts should be taken from the palm when at least one nut in the bunch turns from the fresh to the dry colour. After removing the stalks and calyces, they should be partially dehusked, leaving a layer of fibre up to 3 cm thick. Seednuts should be harvested and dispatched without delay to minimize the risk of germination before they reach the importing country.
- In the country of export, the seednuts should be fumigated with methyl bromide at normal atmospheric pressure at a rate of 32 g/m³ for 3 h at 21°C or above, or with aluminium phosphide at the recommended dosage, and following fumigation, treated with a suitable fungicide. It should be noted that methyl bromide may affect germination.
- After arrival in the country of destination, the seednuts should be inspected for the presence of insect pests and re-fumigated or destroyed if any are found.
- Unless a thorough pest risk assessment has failed to show problems of quarantine concern in the country of origin, the seednuts should be sown under containment and leaf samples from each seedling should be indexed for viroids, and CFDV where appropriate, following the procedure described below:
 - Take 2 g of leaf tissue (c. 20 cm) at the earliest opportunity from the youngest expanded leaf. Wipe the leaflets free of moisture and debris, remove mid-ribs, and place in a sealed plastic bag.
 - Keep samples cool (but do not freeze) and immediately consign by courier or air freight to the indexing laboratory, enclosing an import permit issued by the receiving country.
 - Notify the testing laboratory by telex or fax when the samples are despatched.
- Seedlings should be released from quarantine if the results of the indexing are negative.
- In exceptional circumstances, such as where the country of import lacks adequate post-entry quarantine facilities, seednuts should be germinated under containment in intermediate quarantine. Seedlings should be indexed for viroids and CFDV as mentioned above, and, if the results are negative, forwarded as seedlings to the importing country.

EMBRYO CULTURE MEDIA AND CULTURE VESSELS

The media used for the growth or storage of the embryos differ only in the concentration of sucrose. Compositions are as follows:

Basal medium for storage and transport

Murashige and Skoog's (1962) mineral solution (macro- and micro-nutrients):

Macro-elements:

NH_4NO_3	1650	mg/l
KNO_3	1900	mg/l
$\text{CaCl}_2 \times 2\text{H}_2\text{O}$	440	mg/l
KH_2PO_4	170	mg/l
$\text{MgSO}_4 \times 7\text{H}_2\text{O}$	370	mg/l

Micro-elements:

H_3BO_3	6.2	mg/l
$\text{MnSO}_4 \times 4\text{H}_2\text{O}$	22.3	mg/l
$\text{ZnSO}_4 \times 7\text{H}_2\text{O}$	8.6	mg/l
KI	0.83	mg/l
$\text{Na}_2\text{MoO}_4 \times 2\text{H}_2\text{O}$	0.25	mg/l
$\text{CuSO}_4 \times 5\text{H}_2\text{O}$	0.025	mg/l
$\text{CoCl}_2 \times 6\text{H}_2\text{O}$	0.025	mg/l

Morel and Wetmore's (1951) vitamins:

Thiamine	1	mg/l
Pyridoxin	1	mg/l
Calcium pantothenate	1	mg/l
Nicotinic acid	1	mg/l
Inositol	100	mg/l
Biotin	0.01	mg/l

Fe EDTA	41	mg/l
Sodium ascorbate	100	mg/l
Agar	8	g/l
Activated charcoal	2	g/l
Adjust to pH 5.5		

Growth medium

Basal medium with the addition of 60 g/l sucrose.

Culture vessels

- For embryo culture, use glass or autoclavable plastic test tubes (24 x 150 mm) containing 20 ml of growth culture medium, with plastic or glass caps. Seal tubes with plastic film.
- For transport, use disposable sterile plastic test tubes (13.5 x 95 mm) containing 5 ml of transport culture medium. Seal tubes with plastic film.

NUTRIENT SOLUTION FOR COCONUT PLANTLETS GROWING IN SAND

For 1 l of watering solution, mix:

- 10 ml of Solution A
- 10 ml of Solution B
- 1 ml of Solution C
- 1 ml of Jacobson's iron solution
- 978 ml of water

Solution A:

Potassium nitrate (KNO ₃)	27.4 g/l
Calcium nitrate (Ca(NO ₃) ₂ x 4H ₂ O)	109.5 g/l

Solution B:

Potassium phosphate (KH ₂ PO ₄)	13.7 g/l
Magnesium sulphate (MgSO ₄ x 7H ₂ O)	27.4 g/l
Ammonium sulphate ((NH ₄) ₂ SO ₄)	13.7 g/l

Solution C:

Potassium chloride (KCL)	274.0 mg/100 ml
Boric acid (H ₃ BO ₃)	300.0 mg/ 100 ml
Manganese sulphate (MnSO ₄ x H ₂ O)	170.0 mg/100 ml
Zinc sulphate (ZnSO ₄ x 7H ₂ O)	274.0 mg/100 ml
Ammonium molybdate ((NH ₄) ₆ Mo ₇ O ₂₄ x 4H ₂ O)	274.0 mg/100ml
Copper sulphate (CuSO ₄ x 5H ₂ O)	137.0 mg/100 ml
Sulphuric acid (H ₂ SO ₄ , density 1.83)	13.7 µl/100 ml

Jacobson's iron solution:

EDTA	2.61 g/100 ml
FeSO ₄ , x 7H ₂ O	2.49 g/100 ml

Selected references

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INDEXING LABORATORIES

Waite Agricultural Research Institute
Department of Crop Protection
Glen Osmond
South Australia 5064
Australia

Laboratoire de Phytovirologie des Régions Chaudes
CIRAD
BP 5035
34032 Montpellier Cédex
France

DESCRIPTIONS OF PESTS

Viral disease

Foliar decay

Cause

Coconut foliar decay virus (CFDV). An icosahedral virus, 20 nm in diameter, with an associated circular single-stranded DNA of 1291 nucleotides.

Symptoms

A frond between positions 5 and 11 below the spearleaf first shows chlorosis in leaflets, over about one quarter of the frond. The whole frond becomes chlorotic, as do the immediately adjacent fronds, so that a central whorl in the crown appears yellow. These fronds collapse when marginal necrosis is observed. They hang down through the normal lower whorls of the crown. The severity of symptoms depends on the cultivar and in some cases symptoms disappear in a 'remission' phase. In susceptible coconut palms, the crown dies within 6 months to 2 years after symptoms first appear.



Fig. 4. Symptoms of coconut foliar decay disease in Vanuatu, showing yellowing and collapse of fronds from a point on the lower petiole. Collapsed fronds die rapidly, e.g. the one at left of trunk. (Dr. John Randles, Waite Agricultural Research Institute, Glen Osmond)

Natural host range

CFDV has been detected only in *Cocos nucifera* L. In Vanuatu where the disease is endemic, 'Vanuatu Tall' and 'Vanuatu Dwarf' are hosts but are essentially symptom-free, whereas material introduced to Vanuatu, particularly 'Malayan Red Dwarf', shows severe disease symptoms.

Geographical distribution

Vanuatu, and suspected in other areas.

Transmission

By the planthopper, *Myndus taffini* Bonfils (Cixiidae). No seed or mechanical transmission has been demonstrated.

Therapy

None.

Indexing

Dot-blot hybridization test using nucleic acids extracted from virus-enriched fractions of coconut leaf bound to a nylon membrane and a complementary radioactively labelled DNA probe.

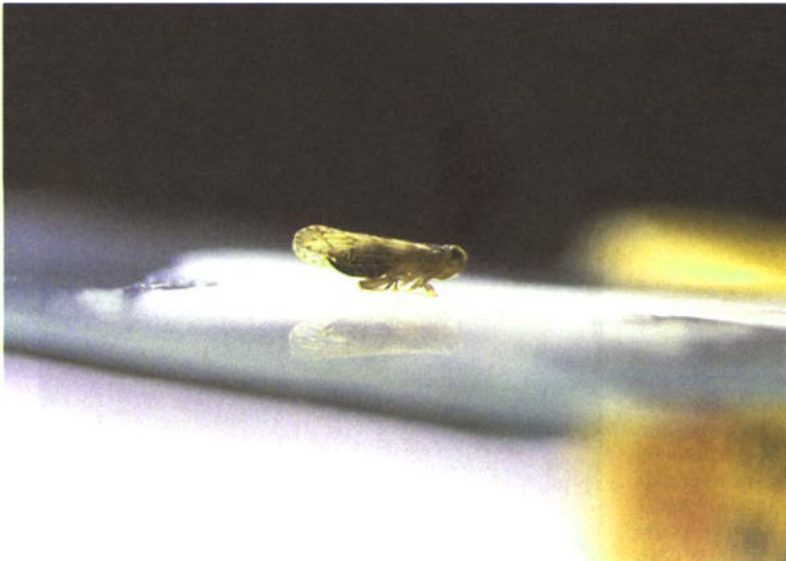


Fig. 5. *Myndus taffini*, vector of coconut foliar decay virus. (Dr. John Randles, Waite Agricultural Research Institute, Glen Osmond)

References

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- Hanold, D., Langridge, P. & Randles, J.W. 1988. The use of cloned sequences for the identification of coconut foliar decay disease-associated DNA. *J. gen. Virol.* **69**:1323-1329.

Viroid diseases

1. Coconut cadang-cadang

Cause

Coconut cadang-cadang viroid (CCCVd). Circular, single-stranded RNA, highly base-paired, forming a stable rod-like structure. The size of the viroid *in vivo* is related to the developmental stage of the disease. In coconut palm, the minimal 246 or 247 nucleotide form is the first form detected after infection, and this is progressively replaced by larger (287 to 302 nucleotides) forms as the symptoms of disease appear and develop. Each form is also accompanied by a dimer, and a linear molecule accompanies its corresponding circular monomer and dimer. CCCVd belongs to the potato spindle tuber viroid subgroup, the members of which share a characteristic conserved sequence.

Fig. 6. An area of high incidence of cadang-cadang disease in the Philippines, showing trees at early, mid and late stages of disease development. (Dr. John Randles, Waite Agricultural Research Institute, Glen Osmond)



Symptoms

The earliest symptoms in naturally infected coconut palm are rounding of nut shape; the development of equatorial nut scarifications; and the appearance of fine, translucent, bright yellow leaf spots. Inflorescences then become necrotic, nut production declines and then ceases, frond production slows, and general chlorosis appears, followed by death of the crown. Eight to 16 years elapse between first symptoms and death of the palm. Artificially inoculated seedlings show varying severities of leaf spotting and stunting. Some palms die soon, those that continue to develop never flower. Symptoms are unreliable for diagnosis at a single observation. No resistance has been found.

Natural host range

Cocos nucifera L., *Elaeis guineensis* Jacquin and *Corypha elata* Roxburgh.

Geographical distribution

CCCVd occurs in certain parts of the Philippines (Hanold & Randles, 1991).

Transmission

In the field, natural transmission is observed but the mechanism remains as yet unknown. A low rate of seed transmission has been observed, but these results need to be confirmed. Artificial inoculation has been achieved by high pressure injection of nucleic acid extracts into the shoots of germinating nuts. Pollen transmission is suspected.

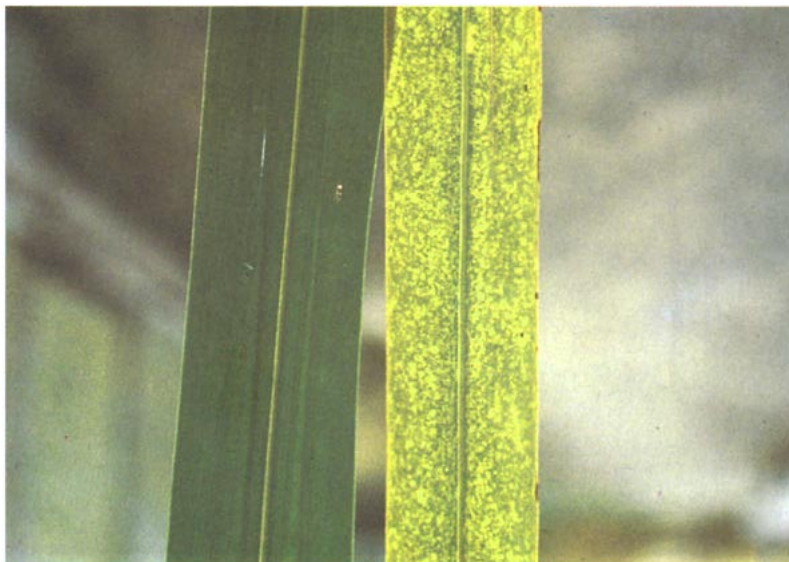


Fig. 7. Leaflets from healthy palm (left) and a palm with late stage disease showing non-necrotic chlorotic spotting (right). (Dr. John Randles, Waite Agricultural Research Institute, Glen Osmond)

Fig. 8. Nuts from healthy palm (left) and diseased palm showing rounding, equatorial scarifications and reduced husks (right). (Dr. John Randles, Waite Agricultural Research Institute, Glen Osmond)



Therapy
None.

Indexing

The various CCCVd forms are detectable by a combination of polyacrylamide gel electrophoresis (PAGE) using the range of forms of CCCVd as size markers to identify the bands, and hybridization analysis with specific radioactive RNA probes (Northern blotting) (Hanold & Randles, 1991).

References

- Hanold, D. & Randles, J.W. 1991. Coconut cadang-cadang disease and its viroid agent. *Plant Dis.* **75**(4):330-335.
- Imperial, J.S., Bautista, R.M. & Randles, J.W. 1985. Transmission of the coconut cadang-cadang viroid to six species of palm by inoculation with nucleic acid extracts. *Plant Path.* **34**(3):391-401.
- Randles, J.W. & Imperial, J.S. 1984. Coconut cadang-cadang viroid. *CMI/AAB Descriptions of Plant Viruses*, No. **287**.

2. Coconut tinangaja

Cause

Coconut tinangaja viroid (CTiVd). Single-stranded circular RNA with 254 nucleotides and about 64% nucleotide sequence homology to CCCVd. It is highly base-paired and stable.

Symptoms

Fine, yellow, leaf spots; nuts may be scarified and round or, more frequently, small and elongated and lacking a kernel. Diseased palms decline and die in a similar manner to cadang-cadang.

Natural host range

Only known from *Cocos nucifera* L.

Geographical distribution

Guam.

Transmission

Means of natural transmission not known.

Therapy

None available.



Fig. 9. Nuts from tinangaja affected palms showing severe and mild spindling, compared with a normal nut (right). (Dr. John Randles, Waite Agricultural Research Institute, Glen Osmond)

Indexing

Nucleic acids are extracted from coconut leaves and fractionated by PAGE using CCCVd as a size marker. CTiVd is detectable by hybridization analysis with a radioactive probe specific for CCCVd (Northern blotting) (Hanold & Randles, 1991). CTiVd is sufficiently similar to CCCVd for the probe to bind under conditions of high stringency. No method of biological testing is available.

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- Hanold, D. & Randles, J.W. 1991. Detection of coconut cadang-cadang viroid-like sequences in oil and coconut palm and other monocotyledons in the south-west Pacific. *Ann. appl. Biol.* **118**(1):139-151.
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3. Other viroid-like sequences

Assays of coconut and other monocotyledons from countries in the Pacific region have identified viroid-like sequences similar, but not identical, to CCCVd. They are detected by the Northern blotting technique with a complementary RNA probe specific for CCCVd. They are not associated with typical cadang-cadang symptoms and they cannot be consistently associated with specific disease symptoms. Surveys from South Asia to French Polynesia have shown that a high proportion of palms contain such viroid-like sequences. Since they have been found in each area where tests have been conducted, it is likely that they are present in other coconut growing areas. Until such time as more is known about the significance and distribution of these viroid-like sequences, all germplasm introduced from countries where viroid-like sequences are known to occur to countries where they have not yet been reported should be indexed, and material for which tests are positive should be rejected.

Reference

- Hanold, D. & Randles, J.W. 1991. Detection of coconut cadang-cadang viroid-like sequences in oil and coconut palm and other monocotyledons in the south-west Pacific. *Ann. appl. Biol.* **118**(1):139-151.

Mollicute diseases

1. Blast

Cause

Probably a non-cultivable mollicute, formerly referred to as mycoplasma-like organism (MLO).

Symptoms

Blast is a nursery disease. Tissues at the base of the spearleaf become necrotic, eventually affecting the meristem and turning into a strong-smelling soft rot. When the seednut is opened, a very strong putrid odour is detectable.

Natural host range

Reported from *Cocos nucifera* L. and *Elaeis guineensis* Jacquin.

Geographical distribution

Africa. Similar symptoms have been reported in oil palm or coconut in South America and in Indonesia.

Transmission

Recilia mica Kramer (Jassidae) is a vector of blast

Quarantine measures

Blast is a nursery disease and does not occur on adult trees. There is therefore no known risk associated with the movement of embryos or nuts.

Reference

Julia, J.F. 1979. Mise en évidence et identification des insectes responsables des maladies juvéniles du cocotier et du palmier à huile en Côte d'Ivoire [Isolation and identification of insects carrying juvenile diseases of the coconut and the oil palm in the Ivory Coast]. *Oléagineux* **34**(8/9):385-393.

2. Lethal yellowing (LY) and similar diseases

Cause

A non-cultivable mollicute, formerly referred to as mycoplasma-like organism (MLO).

Symptoms

The earliest symptom is an abnormal shedding of fruits of all ages, usually accompanied by the appearance of one or more blackened, newly-opened inflorescences. This is followed by a progressive discoloration and shedding of foliage, upwards from the oldest fronds, but some yellowing may be observed in younger leaves. An isolated yellow leaf in mid-crown is an inconsistent symptom, but when present is highly indicative of LY. A dry necrosis develops in the young, newly-expanding spear leaf and progresses downwards to the soft internal tissues above the growing point, where a wet, foul-smelling internal rot develops. The growing point itself may remain intact until most of the foliage is affected, but the whole of the crown eventually rots and falls off within 3-6 months of the appearance of the first symptoms. Bright yellow fronds are characteristic of the disease in 'Jamaica Tall' and 'West Africa Tall'; in some other varieties, fronds may turn bronze or brown. Symptoms in pre-bearing palms follow a similar pattern, but seedlings, up to about 18 months old, are not affected in the field. In older seedlings the incubation period is 6 to 12 months. Symptoms in other palm species are generally similar to those in coconut, but the sequence of spear, necrosis and discoloration of leaves may differ.



Fig. 10. Plantation in Jamaica destroyed by lethal yellowing. (Dr. F.W. Howard, University of Florida, Fort Lauderdale)



Fig. 11. Inflorescence of coconut palm with lethal yellowing. (Dr. F.W. Howard, University of Florida, Fort Lauderdale)

Natural host range

Reported from at least 30 other palm species in Florida, including date palm (*Phoenix dactylifera* L.), and in *Pandanus utilis* Bory. Certain coconut varieties show a high degree of resistance to LY in the Americas but do not show the same degree of resistance to the disease in trials in Tanzania. Observations in Ghana support the results in Tanzania.

Geographical distribution

Bahamas (New Providence and possibly other islands), Cayman Islands, Cuba, Dominican Republic, Haiti, Jamaica, Mexico (Yucatan peninsula and Gulf coast) and USA (southern Florida, southern Texas). Diseases in Africa associated with non-cultivable mollicutes and similar to lethal yellowing are: **Cape St. Paul Wilt** (Ghana), **Kaincobe** (Togo), **Kribi** (Cameroon) and **lethal disease** (Tanzania). Diseases in Nigeria (**Awka wilt**), Kenya and Mozambique are symptomatologically similar to lethal yellowing but have not yet been associated with a non-cultivable mollicute.

Transmission

In Florida, transmission of the disease by the planthopper, *Myndus crudus* van Duzee (Cixiidae), has been demonstrated. This species occurs in parts of the Caribbean and adjacent areas. Vectors in Africa are unknown, but another species, *Myndus adiopodoumeensis* Synave, is under investigation in Ghana. There is no evidence for seed transmission, but infective vectors, and possibly symptomless infections, could be carried on seedlings.

Therapy

No efficient method is available. Tetracycline antibiotics will give remission of symptoms but will not eliminate the non-cultivable mollicute from palms.

Indexing

There is no reliable indexing method for non-cultivable mollicutes. It may be possible to detect non-cultivable mollicutes in suspect material by electron microscopy or fluorescent staining (DALI), but the concentration of non-cultivable mollicutes is too low for confident indexing by these techniques. The highest concentrations of non-cultivable mollicutes are observed in the phloem of actively growing regions, such as root tips, expanding leaf bases or immature inflorescence stalks; non-cultivable mollicutes have rarely been observed in fully-expanded leaves. The concentration of non-cultivable mollicutes in coconut is generally lower than in many other palm species. Nucleic acid probes are being developed.

Quarantine measures

There is no evidence for transmission of non-cultivable mollicutes through seed, embryo cultures or pollen. However, as a precaution, if material must be taken from an affected area to an area not affected by the same disease, only embryo cultures or pollen should be transferred directly to the country of destination. These should be preserved by an appropriate technique (see Technical Recommendations) and used only if the parent palm remains free of disease for a period that exceeds the incubation period of the disease.

References

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3. Root wilt or Kerala wilt

Cause

A non-cultivable mollicute, formerly referred to as mycoplasma-like organism (MLO).

Symptoms

Symptoms are only obvious in palms that are more than 30 months old. The most consistent and diagnostic symptom is the characteristic bending of the leaflets called 'flaccidity'. In older palms, yellowing and marginal necrosis of the older leaves also develops. Roots of diseased palms show degenerated phloem, disorganized tracheal elements and tylosis in the metaxylem. They eventually rot. Inflorescence necrosis develops in some cases. The disease is not lethal, but significantly reduces production.

Natural host range

Only known from *Cocos nucifera* L.

Geographical distribution

India (parts of Kerala and Tamil Nadu States).

Transmission

The lace bug *Stephanitis typica* (Distant) is a vector (Mathen *et al.*, 1990). *Proutista moesta* (Westwood) is a putative vector (Rajan & Mathen 1985; Anonymous, 1989). There is no evidence of seed transmission. Symptoms develop 9 to 24 months after inoculation.



Fig. 12. Palm tree affected by root wilt. (Dr. J.J. Solomon, Central Plantation Crops Research Institute, Krishnapuram, Kerala)

Therapy

No efficient method is available. Tetracycline antibiotics give temporary remission of symptoms, but do not eliminate the non-cultivable mollicute from the plants.

Indexing

No reliable indexing method is available. It may be possible to detect non-cultivable mollicutes in suspect material by electron microscopy, fluorescence staining (DAPI), Dienes' staining or serological tests, but these techniques are not reliable enough for indexing.

Quarantine measures

As for lethal yellowing.

References

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4. Tatipaka disease

Cause

Probably a non-cultivable mollicute, formerly referred to as mycoplasma-like organism (MLO).

Symptoms

Reduction in number and size of leaves, fasciation, leaves become light green and develop chlorotic spots. Abnormal bending of fronds, tapering of stem and production of small-sized inflorescences bearing atrophied empty nuts can also occur.



Fig. 13. Palm tree affected by Tatipaka disease (Dr. J. J. Solomon, Central Plantation Crops Research Institute, Krishnapuram, Kerala)

Natural host range

Only known from *Cocos nucifera* L.

Geographical distribution

India (East and West Godavari, Srikakulam and Nellore districts in Andhra Pradesh).

Transmission

Unknown.

Therapy

No efficient method is available. Tetracycline antibiotics give temporary remission of symptoms, but do not eliminate the non-cultivable mollicute from the plants.

Quarantine measures

As for lethal yellowing.

References

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Fungal diseases**1. Bole rot, shoot rot and other *Marasmiellus* diseases****Cause**

Marasmiellus cocophilus Pegler and *M. inoderma* (Berk.) Singer (syn. *Marasmius palmivorus*). The role of *M. cocophilus* as a primary pathogen of coconut has been questioned and predisposing factors, including other diseases, may be involved in the disease's etiology.

M. albofuscus, *M. crinisequi* and *Rigidoporus zonalis* have also been associated with coconut.

Symptoms

M. cocophilus is associated with a lethal bole rot of seedlings and young palms in eastern Africa. Leaves wilt, become yellow and bronze, and, on mature palms, remain attached as a 'skirt' around the trunk. The spear leaf dies and a foul-smelling soft rot develops at the base of the leaves and spathes. A reddish dry

rot with a narrow yellow margin and cavities lined with fungal mycelium occurs at the base of the bole. Roots are destroyed. In the Solomon Islands, outer leaves of seedlings die prematurely as brown rots, often associated with white mycelium, attack leaf bases. Younger leaves are successively colonized and plants may snap at the junction of the stem and nut. Roots decay as they penetrate the leaf bases, and the rot extends to the bole, developing a reddish-brown margin. Where root damage is extensive, seedlings develop a little-leaf symptom when field planted, but recover and grow normally.

M. inoderma colonizes the shoot as seednuts germinate. Early infection destroys the embryo, leading to invasion of the nut cavity and colonization of the endosperm. Later infections are seen as areas of white or pinkish mycelium on the shoot, which may cause rots and slow development or, occasionally, lead to post-emergence death.

M. albofuscus is associated with a superficial, brown, basal trunk rot of mature palms.

M. crinisequi, a pathogen of cocoa, and *Rigidoporus zonalis*, a tree root pathogen, have also been detected growing from seednuts.

Alternative hosts

M. cocophilus has been reported from several grasses, including *Eleusine indica* (L.) Gaertner, *Echinochloa colona* (L.) Link and *Cynodon dactylon* (L.) Pers. *M. inoderma* is a pathogen of roots of maize and banana, and causes a sheath rot.



Fig. 14.
Sporophores of
Marasmiellus
cocophilus on a
seedling coconut.
(Dr. Graham
Jackson, South
Pacific Comision,
Suva)

Geographical distribution

M. cocophilus occurs in Kenya, Tanzania and Solomon Islands.

M. inoderma is cosmopolitan.

Biology and transmission

M. cocophilus causes death in palms up to 8 years old, seedlings being highly susceptible on transplanting to the field. Spread occurs through soil, root contact between palms, infected coconut debris and probably by air-borne basidiospores. Infection occurs via wounds. Sporocarps occur on exposed roots, on leaf bases of seedlings, exposed tops of seed nuts and on the soil surface (growing from coconut debris). Infection by *M. inoderma* occurs through the calyx end of the nut; the fungus then colonizes the fibrous husk tissues and grows beneath the operculum as it is raised by the emerging embryo. Infection may occur whilst nuts are on the palm. Sporocarps are often seen growing from seednuts in the germination nursery. Both species can occur as saprophytes and be transmitted on coconut debris.

Quarantine measures

- Embryo and pollen transfer should be carried out using the techniques described in the Technical Recommendations
- Healthy nuts should be partially de-husked and treated with an appropriate fungicide.

References

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2. *Phomopsis* leaf spot

Cause

Phomopsis cocoina (Cooke) Punith.

Synonyms: *Phomopsis cocoes* Petch

Phoma cocoina Cooke

Phyllosticta cocos Cooke

Symptoms

Causes a leaf spot and husk rot. On leaflets, the visible symptoms are circular to slightly irregular, dark brown lesions with black stromatic bodies containing pycnidia. Symptoms on husks are similar but lesions are a lighter brown.

Alternative hosts

Corypha umbraculifera.

Geographical distribution

Australia, Guam, India, Jamaica, Kenya, Malaysia (Sabah, Sarawak), Mauritius, Nepal, Papua New Guinea, Puerto Rico, Seychelles, Solomon Islands, Sri Lanka, and Trinidad and Tobago.

Biology and transmission

The pathogen is dispersed by water-borne conidia exuded during wet weather from lesions on the tree and on coconut debris beneath. It can grow saprophytically on dead coconut tissue and as a secondary invader of damaged tissue, and can be dispersed on husks.

Quarantine measures

- Embryo and pollen transfer should be carried out using the techniques described in the Technical Recommendations.
- Healthy nuts should be partially de-husked and treated with an appropriate fungicide.

References

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- Punithalingam, E. 1985. *Phomopsis cocoina*. *CMI Descriptions of Pathogenic Fungi and Bacteria*, No. **828**.

3. *Bipolaris* leaf blight

Cause

Bipolaris incurvata (Ch. Bernard) Alcorn.

Synonyms: *Drechslera incurvata*

Helminthosporium incurvatum

Symptoms

Coconut leaf blight. Generally minor, but can be severe in the nursery. Commences as a brown leaf spot, enlarging to produce lesions with pale centres and wide, dark brown margins. Similar symptoms can be caused by other fungi of no quarantine significance, as described under 'Other fungal diseases' below.

Alternative hosts

None reported.

Geographical distribution

Australasia, Central and South America, Pacific and Seychelles.

Biology and transmission

Wind dispersed conidia can remain viable for at least 3 months. Disease develops most rapidly when the K/N balance is disturbed. Heavy dew favours infection. Can presumably be dispersed on leaf debris and nuts.

Quarantine measures

- Embryo and pollen transfer should be carried out using the techniques described in the Technical Recommendations.
- Healthy nuts should be partially de-husked and treated with an appropriate fungicide.

References

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4. Bud rot and fruit rot

Cause

Phytophthora palmivora (Butler) Butler *sensu lato*. *P. arecae* is now regarded as part of this complex.

Phytophthora katsurae Ko and Chang. This species is close to *P. heveae*, with which it has been confused.

Symptoms

Bud rot. First symptoms observed are chlorosis and the collapse of the youngest leaves. The bud rots and the spear leaf withers; successive leaves turn yellow and die so that all central fronds are killed with a remaining outer fringe of green fronds. Eventually the whole palm dies.



Fig. 15. Palm tree affected by bud rot, caused by *Phytophthora palmivora*, resulting in wilting of the youngest leaves. (Ms. E.C. Concibido, Philippine Coconut Authority, Davao City)

Fruit rot. Nuts, 2 to 5 months old, can be attacked. Symptoms begin as water-soaked lesions usually appearing near the perianth. These turn brown and become irregular in shape. They spread into the husk and may reach the endosperm (Concibido 1990; Quillec and Renard 1984). Premature nut fall occurs at any time. The pathogen can also affect the inflorescence.



Fig. 16.
Coconuts with
fruit rot
symptoms,
caused by
Phytophthora
palmivora. (Ms.
E.C. Concibido,
Philippine
Coconut
Authority,
Davao City)

Alternative hosts

P. palmivora sensu lato has a wide host range and there is a tendency for some pathotypic specialization, although this is not strong (Joseph & Radha, 1975). Isolates from Southeast Asia are particularly variable.

P. katsurae occurs on *Castanea* spp. and *Cocos nucifera* L.

Geographical distribution

P. palmivora is cosmopolitan, but is a principal pest of coconut in the Caribbean, the Pacific and Southeast Asia.

P. katsurae occurs on coconuts in the Caribbean area, Hawaii and Vanuatu and seems to be the main coconut *Phytophthora* species in West Africa.

Biology and transmission

The disease is most active during wet weather. Spores are dispersed by rain splash and in air currents. Palms may be predisposed by damage and adverse growing conditions. Resistant chlamydospores can survive in soil and coconut debris, including nut tissue. Nuts may be infected internally, but then do not germinate.

Quarantine measures

- Embryo and pollen transfer should be carried out using the techniques described in the Technical Recommendations.
- Healthy nuts should be partially de-husked and treated with an appropriate fungicide to reduce the probability of seed transmission.

References

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5. Leaf blight ('lixá pequena', 'lixá grande')

Cause

Catacauma torrendiella, *Coccostroma palmicola* and *Botryosphaeria* sp.

C. torrendiella is the most important and the most widespread primary parasite. *Botryosphaeria* sp. establishes itself with help from *C. torrendiella* and plays a major role in the etiology of leaf diseases. *C. palmicola* is a minor parasite but also facilitates access for *Botryosphaeria*.

Symptoms

C. torrendiella is associated with the drying out of coconut leaves, especially older leaves. The initial symptom is small black stromata beneath the epidermis, concentrated on the upper side of the lamina. These stromata then turn into a multitude of small black spheres (perithecia) either in a line along the veins or

distributed at random, giving the leaf a rough, warty appearance, hence the name 'lixa pequena' in Portuguese. All these stromata lead to localized drying out, which then becomes generalized. In cases of serious infection, the leaf petiole and rachis also have a large number of black stromata, as, more rarely, do the nuts. The first symptoms can be detected along the leaflet veins of leaves 8 or 9 (about 5 to 6 months old). The leaves, which are green but drooping, leave the bunches with no support, which often causes the bunch peduncle to break, leading to premature nut-fall. A hyperparasite, *Septofusidium elegantulum*, may invade the stromata, giving leaflets a white to pinkish, powdery appearance. *Coccostroma palmicola* is a leaf parasite forming large, greenish and cracked stromata (perithecia), concentrated along the edge of leaflets, hence the name 'lixa grande' (in contrast to 'lixa pequena') in Portuguese. *Botryosphaeria* sp. penetrates the lamina, aided by *C. torrendiella* and *C. palmicola*. It forms large necrotic areas, exacerbating drying out of the lamina. These necrotic areas have numerous *Botryosphaeria* pycnidia and, more rarely, *Botryosphaeria* perithecia. This symptom is called 'queima das folhas' in Portuguese. When the parasite reaches the rachis, the tissues turn brown and a gummy exudate flows from the rachis and sometimes forms large masses of resin or brown stalactites. On nuts, this parasite leads to large brown patches, but does not cause nutfall. The *C. torrendiella/Botryosphaeria* sp. complex causes a 30% to 50% reduction in assimilating area, causing a substantial drop in production.

Alternative hosts

Catacauma spp. have been observed on native oil palms in South America, though there is no certainty that they belong to the same species that causes disease in coconuts.

Geographical distribution

C. torrendiella, *C. palmicola* and *Botryosphaeria* sp. are known from Brazil. *C. torrendiella* and *Botryosphaeria* sp. have been observed in French Guiana.

Biology and transmission

There is still very little known about the biology of parasites causing leaves to dry out. *C. torrendiella* penetrates the stomata located on the underside of leaves or on the upper side of the central vein of the leaflet. The spermagonial stage (sub-cuticular stromata), enclosing numerous filiform spermatia, is followed by a perithecial stage, which is the infectious phase. *Botryosphaeria* sp. infection takes place when conidia or ascospores enter the wounds caused by *C. torrendiella*. Rain and wind are probably the major factors involved in parasite dispersal over both short and long distances. However, contaminated plant debris, nuts and pollen should not be ruled out as a source of primary inoculum.

Quarantine measures

The lack of knowledge about *C. torrendiella* ascospore viability currently rules out any nut and pollen exports from infected zones. Germplasm transfer should only be considered through embryo culture.

References

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6. Other fungal diseases

Stem bleeding

Caused by *Ceratocystis paradoxa*. Infects via wounds which then exude a reddish liquid. A cosmopolitan but usually mild pathogen with a wide host range. It poses no quarantine threat.

Leaf spots and blights

Associated with several cosmopolitan but mildly pathogenic fungi such as *Pestalotiopsis palmarum*, *Exserohilum turcicum*, and *Botryodiplodia theobromae*. These have wide host ranges and usually only cause damage to palms predisposed to infection by other factors. They pose no quarantine threat.

Ganoderma butt and root rots

Caused by *Ganoderma boninense* and other *Ganoderma* spp. Soil-borne fungi which attack palms and other tropical tree crops. Initial symptoms are chlorosis and wilt of the foliage with an internal, yellow-bordered, brown rot developing at the stem base. Bracket shaped sporocarps are produced around the base as palms die. Not seed transmitted.

Protozoan disease

Hartrot, Fatal wilt, Cedros wilt or Marchitez

Cause

Phytomonas spp. (plant trypanosomatids). There is a specific association between the syndrome and the pathogen, but Koch's postulates have not yet been fulfilled.

Symptoms

The earliest symptom is a yellowing or browning of the oldest leaves, starting from the tips and spreading to the base of the leaf. Immature nuts can fall but mature nuts generally remain longer on the palm. Yellowing progresses to younger leaves while older leaves become necrotic. Inflorescences also become necrosed and collapse. Finally, when most of the leaves are brown and almost all the nuts have dropped, a rot develops beneath the spear leaf, extending into the meristematic zone, and the palm dies. Generally, death occurs within one month of the first appearance of symptoms.



Fig. 17. Hartrot on Malayan Yellow Dwarf in Surinam; first stage: yellowing of lower leaves. (Dr. M. Dollet, Institut de Recherches pour les Huiles et Oléagineux, Montpellier)

Alternative hosts

Phytomonas spp. occur also on oil palm, and it has recently been shown that *Phytomonas* from oil palm can infect coconut (M. Dollet, pers. comm.).



Fig. 18. Hartrot on hybrid coconut (tall x dwarf) in French Guiana. (Dr. M. Dollet, Institut de Recherches pour les Huiles et Oléagineux, Montpellier)

Geographical distribution

Brazil, Colombia, Costa Rica, Ecuador, Grenada, Guyana, French Guiana, Nicaragua, Peru, Surinam, Trinidad and Tobago, Venezuela.

Transmission

Lincus croupius, *L. tumidifrons* and *L. lethifer* (Heteroptera, Pentatomidae) are reported as vectors in South America. Other *Lincus* spp. are suspected vectors. In Brazil, another pentatomid, *Ochlerus* sp., is also suspected to be a vector.

Quarantine measures

Embryo and pollen transfer should be carried out using the techniques described in the Technical Recommendations.

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Nematodes

Red ring disease

Cause

Bursaphelenchus cocophilus (Cobb) Goodey

Synonym: *Rhadinaphelenchus cocophilus*

Symptoms

Young palms between 30 months and 10 years old are most commonly attacked. Older leaves yellow, dry, and turn brown. Nuts are shed prematurely. Internally, and diagnostically, a red, or reddish brown band forms, 2-4 cm wide and 2-5 cm in from the periphery. This extends throughout the stem but is clearest about 1 m above ground level. Red streaks may appear in the petioles, and the roots

become orange to faint red, dry and flaky. The key sign is the presence of the nematode in the reddened tissues. Infected trees invariably die.



Fig. 19. Cross-section of coconut stem, showing red ring caused by *Bursaphelenchus cocophilus* (Dr. Robin Giblin-Davis, University of Florida, Fort Lauderdale)

Alternative hosts

Roystonea, *Elaeis* and *Phoenix* palms, amongst others.

Geographical distribution

Mexico, Central and northern South America, and southern Caribbean (Trinidad and Tobago, St. Vincent, Grenada).

Biology and transmission

The nematodes colonize parenchymatous tissues of roots, stems and leaves, entering palms at the leaf axils. They do not enter the vessels, although these become blocked by tyloses and this affects water uptake. Cavities appear as the parenchyma cells break down and become filled with nematodes. The life-cycle of the nematode is 9-10 days. The nematode is transmitted by the palm weevil, *Rhynchophorus palmarum*. Juvenile nematodes are transmitted during oviposition and other activities. Palm weevil larvae that develop in infested palms become parasitized by the juvenile nematodes, which persist through to the adult stage, thus completing the infection cycle. Seed transmission is most unlikely. It has been shown that artificially inoculated seednuts can produce infected seedlings, but the nematodes did not survive.

Quarantine measures

Embryo and pollen transfer should be carried out using the techniques described in the Technical Recommendations.

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Diseases of uncertain etiology

Disease	Cause	Geographical distribution
• Bristle top	Unknown	Guam, Jamaica, Tanzania
• Bronze leaf wilt	Unknown	Formerly used for unknown diseases in Guyana, but possibly confused with <i>Phytophthora</i> . Has also been used to refer to unknown disease in Nigeria, possibly Awka MLO. The name should be avoided if possible to prevent confusion.
• Dry bud rot	Possibly a virus	Côte d'Ivoire
• Finschhafen disease	Unknown	Papua New Guinea
• Frond rot	Physiological disorder	Jamaica, Philippines
• Leaf scorch	Unknown	Sri Lanka
• Malaysia wilt	Unknown	Malaysia
• Natuna wilt	Unknown	Indonesia (Natuna Islands and Kalimantan)
• Socorro wilt	Unknown	Philippines (elevated areas of Socorro, Oriental Mindoro)
• Stem necrosis	Possibly a MLO	Malaysia, Sumatra

Arthropod pests

Movement of coconut germplasm using the procedures recommended here will exclude the risk of introducing scale insects, mealy bugs, aphids, mites and other arthropods which could be carried on pollen or fruits.

FAO/IBPGR Technical Guidelines for the Safe Movement of Germplasm are published under the joint auspices of the Plant Production and Protection Division of the Food and Agriculture Organization of the United Nations (FAO) and the International Board for Plant Genetic Resources (IBPGR).

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