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Data Sheets on Quarantine Pests

Coconut cadang-cadang viroid

IDENTITY

Name: Coconut cadang-cadang viroid
Taxonomic position: Viroids
Common names: CCCVd (acronym)
Cadang-cadang disease (English)
EPPO computer code: CKCCXX
EPPO A1 list: No. 192
EU Annex designation: II/A1

HOSTS

Coconuts, *Corypha elata* and oilpalms (*Elaeis guineensis*). In addition, arecanuts, *Chrysalidocarpus lutescens*, dates, *Ptychosperma macarthurii*, *Roystonea regia* and *Veitchia merrillii* are susceptible to inoculation with CCCVd (Anon., 1985; Imperial *et al.*, 1985). CCCVd-like sequences have also been identified in some symptomless herbaceous monocotyledonous species growing near CCCVd-infected coconuts (Hanold & Randles, 1991a).

GEOGRAPHICAL DISTRIBUTION

EPPO region: Absent.
Asia: Philippines (detailed map in Hanold & Randles, 1991a).
Oceania: Solomon Islands.
EU: Absent.

In Guam, a similar disease 'tinangaja disease' is caused by a different, but related viroid, coconut tinangaja viroid (CTiVd), which has 64% sequence homology with CCCVd. Coconuts and oilpalms from other countries in Asia and the South Pacific have been found to contain nucleic acid sequences similar to CCCVd. These are probably not the same as CCCVd, but appear closely related and their pathogenicity is uncertain. Viroids in herbaceous plants appear to be different from those in coconuts, but with a high degree of homology. Different strains could be species-related. Similar viroid-like sequences have also been detected in palm material from South America and Africa (Hanold & Randles, 1991b).

BIOLOGY

The disease is rarely seen in palms before they commence flowering but, after this, incidence increases more or less linearly with age, with a rate of increase in the number of diseased palms ranging from 0.1 to 1% per year. Areas of high incidence may become low incidence over a 20-30 year cycle (Randles *et al*., 1988).

Mode of spread is still unclear, but several routes may be involved. Diseased palms are not clustered. Above-ground or aerial movement of the viroid could account for new

infections arising up to 500 m ahead of the disease front, but no insect or other animal vectors have been found as yet. The viroid can be detected in purified pollen and pollen transmission studies are being carried out (Hanold & Randles, 1991a). The viroid has been detected in the husk and embryo of nuts and can be seed transmitted, but only at a low frequency. Mechanical transmission through wounds caused by cultural practices or insect feeding wounds might also occur (Randles & Imperial, 1984; Hanold & Randles, 1991a).

DETECTION AND IDENTIFICATION

Symptoms

Early stage (lasting 2-4 years): Yellow leaf spots appearing water-soaked in reflected light, translucent yellow in transmitted light. Nuts become small and rounded, with characteristic equatorial scarifications.

Medium stage (lasting around 2 years): Leaf spots become numerous, giving the lower two-thirds of the crown a yellowish appearance. Inflorescences become necrotic, infertile and nut production ceases. Frond production and size decline.

Late stage (lasting around 5 years): Leaf spots almost confluent. Whole crown yellow/bronze-coloured and much reduced in size and number of fronds. Leaflets become brittle and palm dies. Time from appearance of first symptoms to tree death ranges from around 8 to 16 years and is generally greater in older palms (Zelazny *et al*., 1982; Hanold & Randles, 1991a).

Morphology

Infectivity is associated with naked, low molecular weight, single-stranded, predominantly circular, viroid ribonucleic acid. CCCVd contains a region of around 44 nucleotides which is common to most viroids ('the central conserved sequence'). The viroid is presumed to have a natural rod-like structure including a partially double-stranded region comprising 13 base pairs, with small single-stranded loops (Haseloff *et al*., 1982).

CCCVd has a number of molecular forms; the minimum infectious form is 246 nucleotides in size, but an additional cytosine may be inserted at position 197. A variable region exists at the right-hand end of the molecule, at which reiteration of either 41, 50 or 55 nucleotides can occur, producing large forms of 287 to 301 nucleotides. These are unique features of CCCVd, as all other known viroids have only been detected as a single molecular form. Dimeric forms with the same sequence variations as their respective monomers are also associated with the disease, but are always isolated in smaller amounts than their monomers (Randles *et al*., 1988). A detailed description of the sequences and structures of CCCVd is given by Haseloff *et al*. (1982).

Only the lower molecular weight forms and their dimers are present in fronds of infected palms at the early stage of the disease. Fronds produced subsequently contain progressively larger amounts of the large 287-301 nucleotide forms and smaller amounts of the 246/247 forms. A systematic progression has been observed in those forms containing either one or two cytosine residues at nucleotide position 197. If the 246 nucleotide form appears first, the transition is 246, 247, 296, 297. If the 247 form is the first detected, only the 297 form appears later in the infection. The nature of viroid replication and pathogenesis is not yet understood, but the increase in size of CCCVd with disease progress may be related to the development of more severe symptoms. Alternatively, these molecular changes may be the product of faulty viroid replications, induced by cell metabolic changes (Randles *et al*., 1988).

Detection and inspection methods

Symptomatology is not reliable for disease diagnosis.

CCCVd can be detected by size and structure using one- or two-dimensional polyacrylamide gel electrophoresis (with a silver stain). This technique is preferred for field surveys and disease monitoring, based around a mobile laboratory (Imperial *et al*., 1985; Hanold & Randles, 1991a).

Dot-blot molecular hybridization is a more sensitive method. CCCVd is cloned or amplified by the polymerase chain reaction (PCR) and the clones or PCR product are used as templates for synthesis of radioactively-labelled complementary nucleic acid probes (Randles & Palukaitis, 1979; Imperial *et al*., 1985). These are used in hybridization assays to detect nucleotide sequences similar to those of CCCVd (Barker *et al*., 1985).

The most reliable and sensitive method currently in use comprises a combination of these techniques, 'gel electroblot hybridization', in which nucleic acids are separated by one- or two-dimensional gel electrophoresis, transferred to a membrane filter and then hybridized with radioactive probe. These techniques are reviewed by Hanold & Randles (1991a).

MEANS OF MOVEMENT AND DISPERSAL

The CCCVd-like viroids can be transmitted by seed or pollen and occur in almost all plant parts.

PEST SIGNIFICANCE

Economic impact

The disease results in the premature decline and death of coconut palms. Total losses of about 30 million palms and annual yield losses of about 22 000 t of copra have been attributed to cadang-cadang disease in the Philippines (Zelazny *et al*., 1982).

Control

No known control measures exist for CCCVd in the field. Specific control recommendations cannot be developed until the epidemiology of CCCVd is more clearly understood. Potential control strategies include elimination of reservoir species, vector control, mild strain protection and breeding for host resistance. Eradication of diseased plants is usually performed to minimize spread but is of dubious efficacy due to the difficulties of early diagnosis.

Phytosanitary risk

CCCVd was recently added to the EPPO A1 list of quarantine pests, and is also considered as a quarantine pest by CPPC, NAPPO and APPPC. In the EPPO region, CCCVd certainly has potential pest significance on many species of palms, grown as outdoor or indoor ornamentals. The addition to the EPPO list harmonizes it with EU Directive Annex II/A1.

PHYTOSANITARY MEASURES

Ideally, no imported palm seed or propagating material (including embryo cultures) should be moved into the EPPO region from infested countries unless it is shown to be free from viroids by molecular diagnostic methods.

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