

Cocadviroid Coconut cadang-cadang viroid

Scientific Name

Cocadviroid coconut cadang-cadang viroid

Synonyms:

None

Common Name

Cadang-cadang viroid, coconut cadang-cadang viroid, yellow mottling disease

Type of Pest

Viroid

Taxonomic Position

Class: Not assigned, **Order:** Not assigned,

Family: Pospiviroidae

Reason for Inclusion in Manual

Palm manual, Previous Additional Pest of Concern

Pest Description

Viroids are small, circular, single-stranded molecules of infectious RNA lacking a protein coat. They are even simpler than viruses. Viroids were discovered and given this name by [Theodor Otto Diener](#), a plant pathologist at the Agricultural Research Service in Maryland, in 1971. Viroids are “the smallest known infectious agent” and fully depend on a host because they cannot produce proteins on their own to replicate (Bonfiglioli et al., 1994).

The first report of *Coconut cadang-cadang viroid* (CCCVd) was on San Miguel Island (Philippines) in 1927. It was appropriately named “cadang-cadang” disease from the word *gadan-gadan* of the local dialect meaning “dying” (Hanold and Randles, 1991a). In less than forty years, 99% of all the palms on San Miguel Island had died or had been severely affected by CCCVd. Currently about 500,000 palms die per year in the Philippines due to CCCVd (Haseloff et al., 1982). *Coconut cadang-cadang viroid* is closely related to

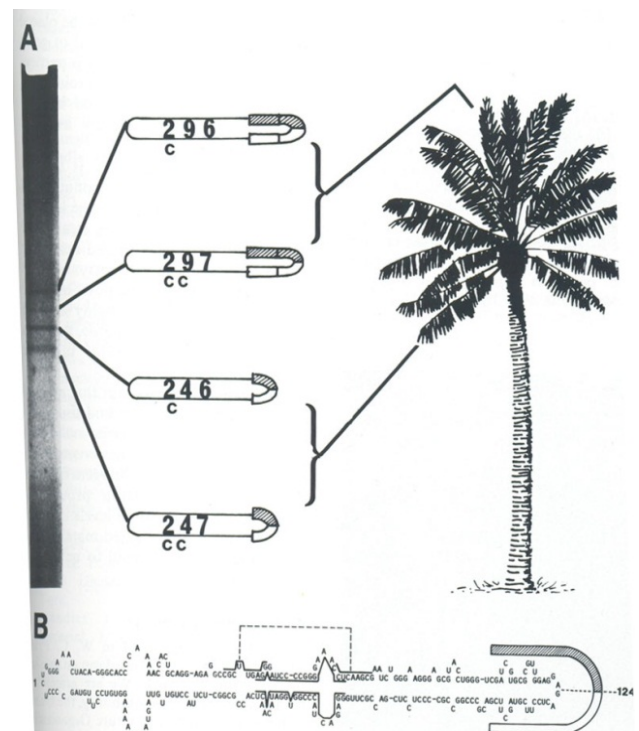


Figure 1: (A) The separation of the four molecular forms of CCCVd on a polyacrylamide gel. The small forms appear first during infection but are replaced by the larger forms (caused by duplication of the right terminus) **(B)** Sequence of the 247 nucleotide form of CCCVd (deletion of a cytosine makes the 246 form). Photo Courtesy of Dr. John Randles of University of Adelaide.

another viroid, *Coconut tinangaja viroid* (CTiVd), which is known for destroying palms in Guam. These two viroids are the only viroids found in monocotyledonous plants (monocots) (Bonfiglioli et al., 1996).

CCCVd size varies from 246 to 247 nucleotides early in the infection stage to 296 to 297 nucleotides later in the infection stage (Hanold and Randles, 1991b). Duplication of the right hand terminus of smaller nucleotide forms adds an additional 50 nucleotides, causing the total number of nucleotides to increase to 296 or 297 (Fig. 1) (CABI, 2006). CCCVd and CTiVd are the only viroids known to have molecular change based on the stage of the disease (CABI, 2006). CCCVd has four RNAs known as fast 1, fast 2, slow 1 or slow 2. The four RNAs that are found in CCCVd are “circular viroid-like ribonucleic acid” (Mohamed et al., 1982). The name of the four different RNAs is based on their mobility in polyacrylamide gel electrophoresis (PAGE). In the early stages of disease development, RNA fast 1 and 2 are usually observed, while in the later stages of disease development RNA slow 1 and 2 are found (Haseloff et al., 1982). Fast RNAs are known to be more infectious than the slow RNAs (Haseloff et al., 1982).

Biology and Ecology

Studies of *coconut cadang-cadang viroid* are ongoing but very little is known about the viroid including the natural mode of spread (Bigornia, 1977; Randles et al., 1980; CABI, 2006). The spread of the disease, however, can range from very slow to about 0.5 km per year (Hanold and Randles, 1991a). The disease can occur at various elevations and in a range of soil types (Bigornia, 1977). Spread may not occur by a specific route but may occur through a variety of means. The viroid can be detected in the husk and the embryo of nuts. It is seed-transmitted at a low rate of about one in 300 (Randles and Imperial, 1984). CCCVd has also been detected in pollen. It seems possible that the viroid could be transmitted unspecifically by certain coleopterous insects through feeding wounds, but a specific insect vector has not been identified.

Furthermore, younger palms are more susceptible to the viroid than older palms (Velasco, 1997). CCCVd is found in the vascular tissue and mesophyll cells of a host (Bonfiglioli et al., 1996).



Figure 2: Symptoms of CCCVd on oil palm: **Top:** Development of orange leaf spot by inoculation of CCCVd on oil palm fronds (age of fronds increases from left to right). **Bottom:** Premature loss of male florets in early stage of disease. Photo Courtesy of Dr. John Randles of University of Adelaide.



Chapter 35 Coconut cadang-cadang viroid

Cadang-cadang symptoms: (A) (right) severe non-necrotic yellow spots on a leaflet from a mid-crown frond compared with an asymptomatic leaflet (left). (B) 'brooming' symptom caused by limited or negligible development of the lamina in palms containing the mutations shown in Figure 35.3. (C) reduction in the husk, rounding, and equatorial scarring of nuts of a palm at stage E1 (left) compared with normal nuts (right).

Figure 3: Coconut cadang-cadang symptoms. Photo reproduced with permission from *Viroids* (2003). Hadidi, A., Flores, R., Randles, J., and Semancik, J. (eds.) CSIRO publishing. Photo courtesy of Dr. John W. Randles, and M.J.B. Rodriguez. Acknowledgement to CSIRO publishing,

Symptoms and Signs

Although a range of symptoms are observed with CCCVd, the symptoms alone are not diagnostic. Look for a combination of symptoms when inspecting plant tissue of coconut and oil palms reoccurring in the same tree over time. Molecular testing is necessary to identify the viroid in symptomatic tissue. Most of the time, the lower two-thirds of the leaf crown is yellow; while the upper one-third is still dark green with CCCVd infection.

Some of the general symptoms of CCCVd include: reduced nut size and production, reduced husk production in nuts, reduced tree length/stunting, "genetic" orange spotting

on fronds (especially in oil palms), and yellow spotting on leaves (Fig. 2; Fig. 3). All of these symptoms can eventually lead to plant death (Randles and Boccardo, 1982; Hanold and Randles, 2003; Hammond and Owens, 2006). If a palm patch is infected with CCCVd, death of the palms within the patch will continue until the patch is “bare” (Fig. 4) (Velasco, 1997).

The CCCVd-infected leaves in the palm crown are typically smaller than those observed in healthy palms. Leaf scarring is often found on the trunk of the infected palm. Palms that are infected with CCCVd before flowering have leaf scars that are spread farther apart than healthy plants. In contrast, plants infected with CCCVd after flowering have leaf scars that are closer together than healthy plants. Water-soaked leaf spots may appear, but this symptom is not unique to CCCVd (Velasco, 1997). Another possible symptom (although rarely found in the field and in only about 3% of experimental inoculations) is lamina reduction. This symptom can lead to a “brooming symptom” where parts of the frond contain only the midrib (Fig. 4) (Hanold and Randles, 1991a).

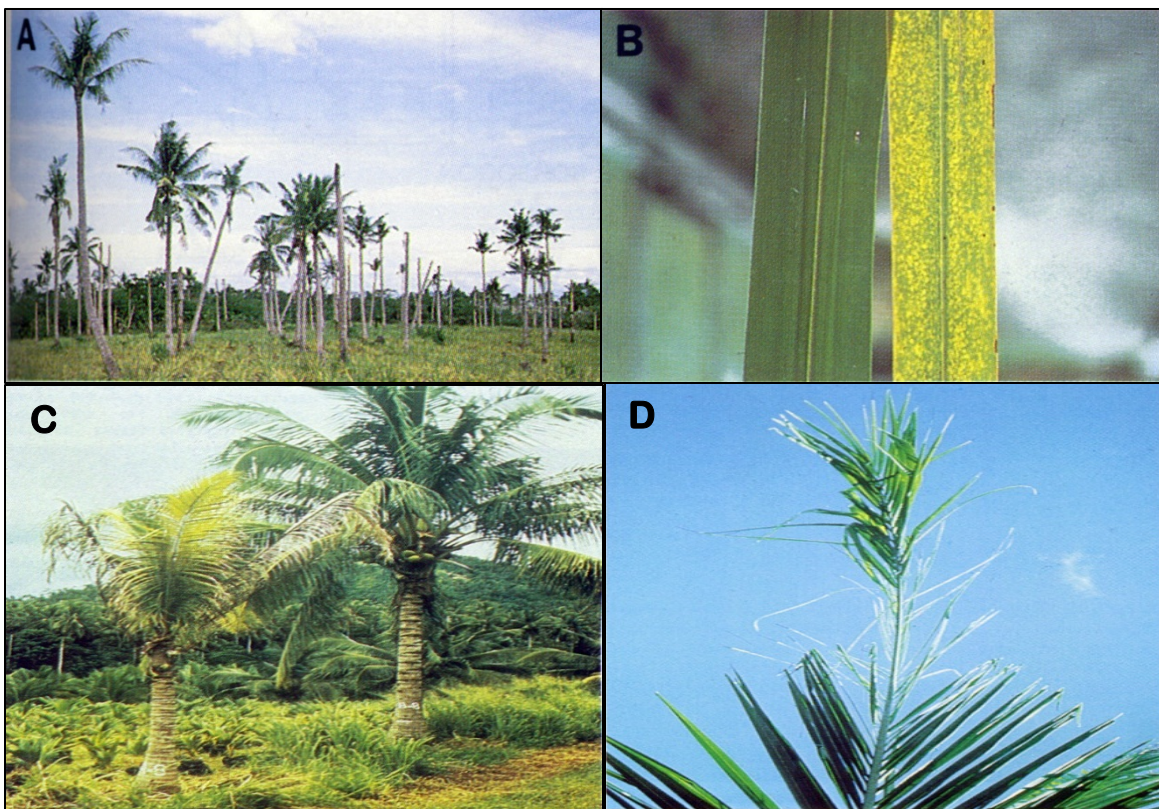


Figure 4: (A) A field where CCCVd has infected palms. This field shows different stages of the infection from early to late stage. (B) Comparison of a healthy palm leaf (left) to a palm leaf that is showing the symptoms of non-necrotic chlorotic spotting (right). (C) On the left is a palm affected by CCCVd that is exhibiting yellowing, stunting, no nuts, and reduced crown symptoms. On the right is a healthy palm. (D) Reduction of lamina in an inoculated palm, causing the “brooming” syndrome. Photo courtesy of Dr. John Randles of University of Adelaide.

The life span for a CCCVd-infected palm is about eight years for younger palms (22-year old palms) and 16 years for older palms (44-year old palms). The early stage of disease development, which usually lasts two to four years, is divided into three substages:

- In E1, the first early substage, the nuts usually “round out” with strong “equatorial scarification.”
- In E2, the second early substage, yellowing occurs, “water-soaked leaf spots appear,” round nuts with equatorial scarification are more frequent, necrosis appears at the tips of inflorescences, and male florets may be lost.
- In E3, the final early substage, nuts are usually not being produced, larger leaf spots appear, “new inflorescences [are] stunted and sterile” and fibrous tissue remains attached to petioles as “winging” (Hanold and Randles, 1991a).

During the middle stage, which usually lasts two years, nut production slows down or completely stops, leaf spots grow and are abundant, and “inflorescences become necrotic (brown/dead).” During the final stage of disease development, usually lasting about five years, the crown is reduced, necrotic leaf spots are still present, and the palm is nearing death or dead (Hanold and Randles, 1991a).

Pest Importance

Coconut cadang-cadang viroid (CCCVd) and *Coconut tinangaja viroid* (CTiVd) are of concern not only because of their lethality but because diseased palms cease production of nuts many years before they die. The diseases are frequently unrecognized by growers. Non-producing palms may be kept for years in hope that they will become productive again or simply because there is reluctance to cut down a living palm whether it is bearing or not.

CCCVd is the main threat to coconut production in the Philippines. By 1990, CCCVd had damaged over 30 million palms in central Philippines (Hanold and Randles, 1991b). This suggests serious implications for the numerous countries where coconut is considered an important crop. Annual loss of palm in the Philippines, where CCCVd is known to occur is in the range of 200,000 to 500,000 palms with a total loss of 40×10^6 trees since the disease was first recognized (Randles and Rodriguez, 2003). Because palms may cease fruit production five years before death and because the replacement palms do not reach full bearing age for five to eight years, each infected palm may interrupt production from that site for 10 to 13 years.

Known Hosts

Major hosts

Cocos nucifera (coconut palm), *Corypha elata* (buri palm), and *Elaeis guineensis* (African oil palm) (Imperial et al., 1985; Hanold and Randles, 1991b; Bonfiglioli et al., 1994).

Experimental hosts

Adonidia merrillii (manila palm), *Areca catechu* (betel nut palm), *Caryota cumingii* (fishtail palm), *Chrysalidocarpus lutescens* (yellow butterfly palm), *Livistona rotundifolia* (round-leaf fountain palm), *Phoenix dactylifera* (date plum), *Ptychosperma macarthurii* (macarthur palm), and *Roystonea regia* (royal palm) (Imperial et al., 1985; Hanold and Randles, 1991a; Maramorosch, 1999; PCA, n.d).

Known Vectors or Associated Organisms

The viroid is thought to cause infection through feeding wounds of coleopteran insects, but this has not been confirmed (Handles and Randles, 1991a).

Known Distribution

Asia: Philippines (Randles and Boccardo, 1982).

Distribution of CCCVd-related or -like RNAs*:

Asia: Malaysia, Sri Lanka, Thailand, and West Indonesia (Hammond and Owens, 2006; Vadamalai et al., 2006; Vadamalai et al., 2008)

*CCCVd-related or like RNAs are structurally and genetically similar to CCCVd. These other viroids usually contain RNA that leads to the “genetic” orange spotting symptoms that occurs in CCCVd infected plants, especially in oil palm.

Pathway

CCCVd can spread through mechanical inoculation primarily through the use of human machetes and other tools from palm to palm when used without proper sanitation (Hanold and Randles, 1991b; Maramorosch, 1999).

Although pollen and seed have very low transmission rates, they can also be responsible for pathogen movement (Hanold and Randles, 1991a; Pacumbaba et al., 1994; Vadamalai et al., 2008).

Potential Distribution within the United States

CCCVd is known to be exotic to all palm producing states and territories in the United States including the continental United States, the Pacific region (Hawaii, Guam, and American Samoa), and the Caribbean region (Puerto Rico and the U.S. Virgin Islands). In 2013 or 2014, a commodity-based survey for palm is planned by the CAPS Program (via Farm Bill funding), which will include CCCVd and CTiVd. CTiVd, known only to occur in Guam, is related (65% homology) to CCCVd.

Florida is the most vulnerable state in the continental United States based on the presence of hosts.

Survey

CAPS-Approved Method*: Visual survey for symptomatic plants is the approved method for *Coconut cadang-cadang viroid*. Normally leaf (leaflet) tissue is used for diagnosis/identification, but the viroid is present in most host tissues.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at <http://caps.ceris.purdue.edu/>.

Literature-Based Methods:

Visual survey/soil sampling:

Protacio and Sill (1970) published a symbolic key based on information for survey and sampling of coconut trees in the Philippines.

Important visual symptoms include:

- Scattered small or large yellow or yellow-green circular spots on a healthy dark green leaf (the spots may be translucent);
- White or whitish-greenish irregular spots can occur on the pinnae, apex or anywhere on the blade of the leaf;
- Water soaked spots. Eventually the spots will lead to a brown-yellow mottle and/or streaks that cover the leaf;
- Green, pale-green and/or yellow green stripes;
- Leaf veins can become brown, light-green, or yellow;
- “Longitudinal fluting or wrinkling of leaflet”;
- Leaf color is whitish green (Protacio and Sill, 1970).

These symptoms are seen best in reflected, alternating with transmitted light.

Key Diagnostics/Identification

CAPS-Approved Method*:

Reverse transcriptase polymerase chain reaction (RT-PCR) is the approved method for *Coconut cadang-cadang viroid*.

All palm viroid samples should be sent to Dr. Rosemarie Hammond for sample processing. Palm samples should be received as freshly harvested leaflets exhibiting symptoms of CCCVd. Samples from plants with atypical or no symptoms can also be included as the disease is difficult to recognize based on symptoms alone, because symptoms develop in stages depending upon the age of the palm.

Dr. Rosemarie Hammond, Ph.D.
Molecular Plant Pathology Laboratory

USDA-Agricultural Research Service
Building 004, Room 204, BARC-West
Beltsville, MD, USA 20705
Voice: (301) 504-5203
Fax: (301) 504-5449
Email: rose.hammond@ars.usda.gov

For shipment, leaves must be packaged according to APHIS regulations and under permit. Each sample, in a separate plastic bag, will be labeled according to cultivar (when known) and plant species, date sample was collected, location of plant sampled, and name and institution of sample collector; each bag should contain leaves collected from only a single plant. Samples must be shipped to the testing laboratory by overnight express service.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at <http://caps.ceris.purdue.edu/>.

Literature-Based Methods:

Electrophoresis: When cellular nucleic acids purified from approximately 1g of coconut tissue are analyzed on polyacrylamide gels, the molecular forms of CCCVd can be identified by their relative electrophoretic mobility (Fig. 1).

Dot Blot Hybridization: CCCVd has been cloned and can also be amplified by polymerase chain reaction. The clones and PCR product can be used as templates for synthesis of radioactively labeled complementary RNA or DNA probes. These are used in hybridization assays to detect nucleotide sequences similar to CCCVd. Extracts to be tested are applied to a supporting membrane and the presence of the viroid is detected by its specific hybridization with the probe. The radioactive label causes darkening of exposed x-ray film, while samples without the viroid show no signal. This diagnostic method is called “dot blot hybridization”. This method is more sensitive than electrophoresis and the viroid can be detected many months before symptoms appear.

Molecular hybridization of dot-blotted extracts has been used by collecting nuclei acid from chopped leaflets. The dot blots extracts are probed on nitrocellulose sheets with ³²P labeled DNA probes complementary to CCCVd. An autoradiogram will reveal the resulting banding pattern (Imperial et al., 1985; Mohamed and Imperial, 1984).

A ³H-cDNA probe has been used for hybridization in solution (Mohamed and Imperial, 1984). The PAGE procedure uses nucleic acids from a leaflet to elute RNA from the gel (Imperial et al., 1985; Mohamed and Imperial, 1984; Mohamed et al., 1985).

PCR: RT-PCR and PCR for CCCVd used primer G2-1 for RT-PCR and primers G2-1 and D9-1 for conventional PCR. RT-PCR needs to be used to be able to separate CCCVd from CTiVd (Hodgson et al., 1998).

A commercial diagnostic kit is available: “Coconut *cadang-cadang* viroid (CCCVd) RT-PCR detection kit; Norgen Biotek Corp., Thorold, ON Canada, Cat # 47900.” This kit is being tested for its sensitivity and specificity for CCCVd detection via a Farm Bill agreement with Dr. Hammond and USDA APHIS.

Easily Confused Pests

CCCVd is commonly confused with CTiVd, which is found on Guam. CTiVd is 254 nucleotides in length and has 64 to 65% nucleotide sequence homology to CCCVd

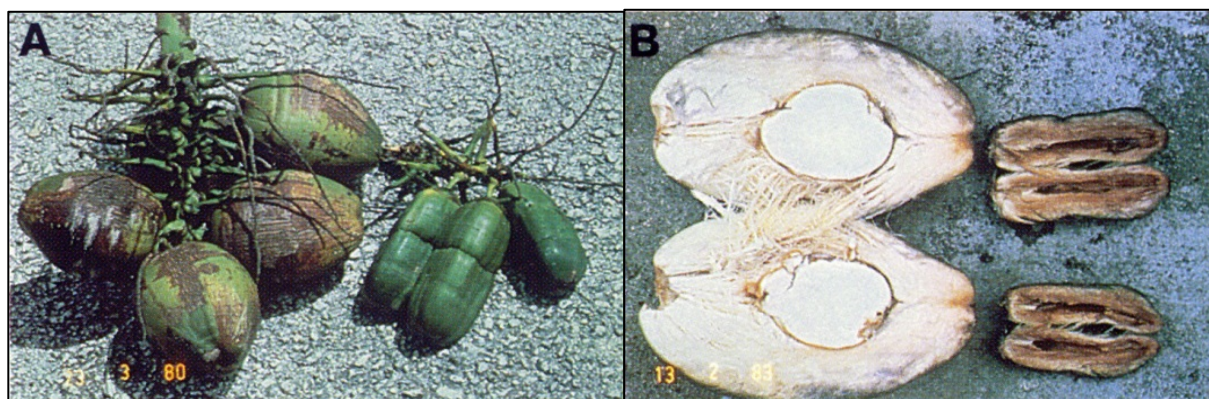


Figure 6: Symptoms of CTiVd are shown: **(A)** The nuts on the right have the small, elongated shape characteristic of CTiVd. **(B)** The left nut is healthy and right nut is diseased and shows lack of kernel. Photo Courtesy of Dr. John Randles of University of Adelaide.

(Hanold and Randles, 1991b). CTiVd has similar visual disease symptoms as well. Both viroids are known to have three stages of infection (early, middle, late) (Hodgson et al., 1998). Water-logging and nutrient deficiency due to CTiVd cause yellowing of the palm, showing similar symptoms to CCCVd (Bigornia, 1977). Symptoms of both viroids include: spotting on the leaves, reduced crown, and death. The main difference in symptomatology between the two viroids is the effect on the fruit. Plants infected with CCCVd have nuts that are greatly reduced and round. Plants infected with CTiVd have nuts that are “small, elongated mummified husks with no kernel” (Fig. 6) (Keese et al., 1988).

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Draft Log

July, 2016: Updated 'Potential Distribution Within the United States' section