
Coconut Cadang-cadang Viroid Infection of African Oil Palm*

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Cadang-cadang is an uncontrollable quarantinable lethal disease reported to have killed over 40 million coconut palms in the central Philippines since first being described in 1914. It spreads naturally by unknown means, attempts at eradication have never been successful, and in the absence of genetic resistance, it is managed only by removal of affected palms and replanting. Extraction, fractionation, physical characterisation, expression of symptoms in inoculated coconut seedlings, recovery of viroid, and sequencing, have confirmed that Coconut cadang-cadang viroid (CCCVd; Cocadviroid, Pospiviroidae) is the causal agent of cadang-cadang disease.

CCCVd has been detected in oil palm plantations derived from DxP hybrids in Oceania and SE Asia. There is a close association between the detection of the viroid and the presence of a specific type of orange spotting (OS) of oil palm leaflets. Oil palms containing CCCVd and showing OS are markedly stunted, and there is evidence for horizontal and vertical spread of the viroid and OS in commercial plantations.

Viroid molecules isolated from a Malaysian oil palm have been shown to be closely related in sequence and structure to type CCCVd isolates. Scaling up of the detection and sequencing of the oil palm viroid molecules is needed to study their incidence, transmission, spread, pathogenicity, replication and host response to infection. Viroids are molecular plant pathogens which cause economic losses in many species. The oil palm industry needs to determine the effect of viroid infection on yield and quality, and to evaluate the benefits of excluding infected germplasm from future oil palm plantations.

Keywords: *Oil palm, Elaeis guineensis, coconut palm, cadang-cadang disease, CCCVd, cocadviroid, sequence variants, molecular diagnostic methods.*

Coconut cadang-cadang viroid (CCCVd) occurs in Malaysian coconut and oil palms. Two questions arise. Firstly, is the viroid in Malaysia the same as the viroid which causes the lethal disease of coconut palm in the central Philippines? Secondly, what is the economic effect of the viroid on commercial oil palm production? This article briefly describes

viroids, cadang-cadang disease and CCCVd, outlines the evidence that CCCVd infects commercial oil palm, indicates its potential economic impact, and suggests that viroid-free planting material should be evaluated for improving production. In this article the term CCCVd refers not only to the viroid sequenced from coconut palm (Haseloff *et al.*, 1982;

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GenBank accession no. J 02050.1) but also to the closely similar molecules identified in oil palm (Vadamalai *et al.*, 2006; GenBank accession nos. DQ097183–DQ097185. *Figure 1*).

WHAT ARE VIROIDS?

Viroids (Hadidi *et al.*, 2003; Flores *et al.*, 2005) are extremely small, circular, naked, replicating, plant pathogenic RNA molecules (246 - 400 nucleotides in size). They are the smallest known pathogens, containing less than 5 per cent of the information held by the smallest viruses. They do not contain genes but appear to cause disease by an effect on the basic processes of information transfer in plant cells. There are about 30 described viroid species, which cause stunting, poor fruit quality, reduced yield, and sometimes death of their plant host. They are spread by vegetative propagation practices, mechanical damage, and some are also transmitted by pollen and seed. Viroid infection is diagnosed either biologically, using symptoms and host range, or by biochemical methods, using molecular properties of the viroid.

CADANG-CADANG DISEASE

Cadang-cadang (dying-dying) has killed over 40 million coconut palms in the central Philippines since the first reported outbreak around 1914 (Hanold & Randles, 1991a; Randles & Rodriguez, 2003). It is similar to Tinangaja disease of coconuts which destroyed the coconut industry of Guam. Both diseases are irreversible and lethal over a term of 5-15 years. Their most serious economic impact arises early in disease development because palms become unproductive about two years after symptoms first appear. Cadang-cadang spreads at about 500 metres

per year, and new outbreak areas are still reported, but the mode of field spread and the disease cycle remain unknown. Attempts at eradication have not been successful and resistant cultivars are not known. Cadang-cadang is managed in the central Philippines by the removal of palms showing the early stages of disease, and subsidised replanting with yield improved hybrid seedlings (Hadidi *et al.*, 2003). National quarantine is also in force, adding to the economic impact of the disease.

CCCVd

CCCVd was isolated from diseased coconut palms and reinoculated to produce symptoms in seedling coconuts, confirming that it is the causal agent of cadang-cadang (Randles *et al.*, 1977). Diagnosis of cadang-cadang disease is based on detection of the viroid by molecular tests. Its molecular structure, infectivity, and sequence showed that the viroid was distinct from other known viroid species, including Tinangaja viroid. Sequencing (Haseloff *et al.*, 1982) showed that there were various forms of the viroid, that changes in its molecular structure followed a pattern related to the stage of the disease, and that a particularly severe form of cadang-cadang disease was associated with only two or three point mutations in the viroid sequence (Rodriguez & Randles, 1993; *Figure 1*).

DIAGNOSIS OF VIROID INFECTION

Biochemical diagnosis of viroids uses methods which identify their specific nucleotide sequence. The simplest and fastest method, which is useful for testing large numbers of samples, is based on a hybridisation assay in which a labelled probe with a sequence 'complementary' to the target viroid sequence finds and binds (hybridises) to only those

molecules in a mixture which are either identical to, or very similar to, the viroid under assay. This is a laboratory based method as the conditions for hybridisation need to be strictly controlled for optimal sensitivity and specificity. Surveys of the incidence, distribution and epidemiology of viroids require high skill levels, advanced laboratory equipment and specialised biochemicals. Additional diagnostic methods such as the reverse-transcription polymerase chain reaction (RT-PCR; Hodgson *et al.*, 1998) and the isolation, cloning and sequencing of viroid molecules are more complex and are used principally for research and the development of improved diagnostic methods.

SURVEY OF COCONUT PALMS IN MALAYSIA

Following the detection of CCCVd by northern blot hybridisation assay in a commercial oil palm growing in the Solomon Islands (Hanold & Randles, 1989) the Australian Centre for International Agricultural Research (ACIAR) supported a survey for the viroid in tropical monocotyledonous species in South-East Asia and Oceania (Hanold & Randles, 1998). Included were 1024 coconut palms from 48 sites in 27 countries. On average, RNA molecules related to CCCVd were detected in about half of these samples. The incidence varied from country to country, and between sites within countries. There was a higher than average incidence in Thailand, Malaysia and western Indonesia. For example, in Selangor, Malaysia, 9 out of 10 dwarf-type coconut palms, which had been selected because they showed various phenotypes such as a severely tapered trunk, failure to produce nuts, or production of abnormal rounded nuts, contained the CCCVd sequence.

SURVEY OF OIL PALMS IN MALAYSIA

Elaeis guineensis (oil palm) is susceptible to CCCVd, and oil palms in the cadang-cadang affected area of the Philippines became naturally infected with CCCVd (Randles *et al.*, 1980). Both naturally and artificially (Imperial *et al.*, 1985) infected palms are stunted, often chlorotic, and show yellow or orange leaf spotting similar to that seen in CCCVd-infected coconut palms. The orange leaf spotting (OS) frequently seen in oil palm plantations (Figure 2), and commonly referred to as genetic orange spotting, was shown to be associated with CCCVd infection in a series of experiments in the Solomon Islands (Hanold & Randles, 1989, 1991b). Oil palms sampled in Malaysia during the ACIAR survey were grouped according to their expression of OS. Of the palms in which CCCVd was detected, the majority (9/14) also showed OS. This suggests that there is an association between infection and the OS phenotype (that is, if OS is seen on oil palm leaves it is probable that the palm will contain CCCVd), but that infection can also occur in asymptomatic palms.

FIELD STUDIES IN OTHER OIL PALM PLANTATIONS

The relationship between OS and CCCVd is yet to be studied in detail, but a pilot trial in the Solomon Islands indicated that oil palms with both OS and CCCVd were more stunted (~30%) than those with CCCVd alone (~7%) (Hanold & Randles, 1998). Evidence was also obtained for vertical spread of OS by seed transmission in greenhouse grown oil palm seedlings, as well as for the natural horizontal spread of CCCVd at one plantation site with a possible primary focus of four palms with

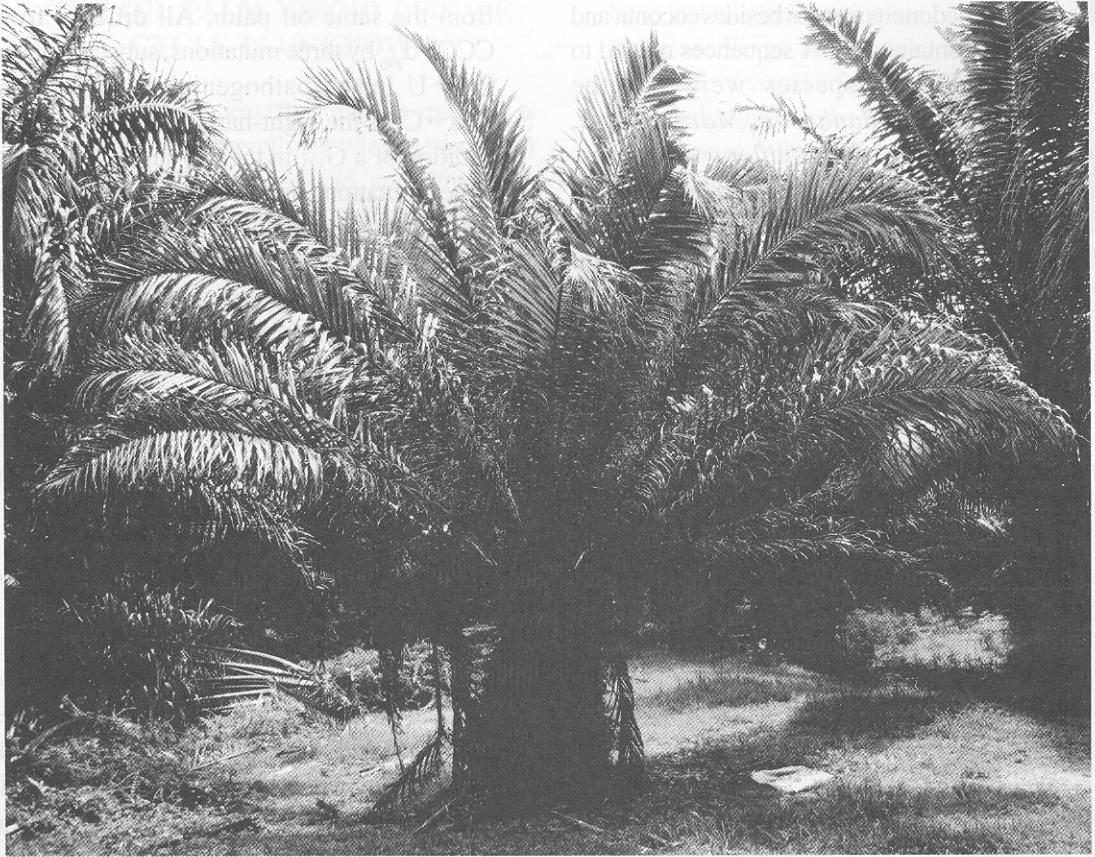


Figure 2 Orange leaf spotting (OS) symptom observed in an oil palm. The fronds are bright orange in colour and the palm is stunted.

OS symptoms (Hanold & Randles 1991b, 1998). In 1988, 9/14 palms were positive for CCCVd, in 1991 14/14 were positive. In Indonesia, a survey of a three-year-old planting of oil palm near Medan, North Sumatra, on newly cleared land, showed an incidence of OS in the range of 1-2 per cent. CCCVd was detected both in OS and asymptomatic palms. The *E. guineensis* growing in Bogor, Indonesia, which was used as the 'dura' parent of the current commercial oil palm hybrid had OS, as did 'pisifera' parents growing near Medan (Randles, unpublished). There is evidence for a decline in yield in Sumatran oil palm of about 25 per cent from 1985 to 1989. In one pilot trial

in Papua New Guinea, evidence was obtained for the mechanical transmission of viroid from oil palm to oil palm (Hanold, unpublished results). Such data raise various hypotheses. One hypothesis that should be tested urgently is that the viroid is present in planting material including both seed and tissue culture derived plantlets, and that this provides primary foci for significant secondary spread during the life of oil palm plantations.

CCCVd-RELATED RNA IN OTHER MONOCOTYLEDONS

The ACIAR survey showed that many tropical

monocotyledonous species besides coconut and oil palm contained RNA sequences related to CCCVd. These species were in the *Arecaceae*, *Pandanaceae*, *Marantaceae*, *Zingiberaceae*, *Commeliniflorae* and others (Hanold & Randles, 1998; 2003). Thus, a reservoir of CCCVd exists which is a potential source of infection for commercial oil palm plantings. None of these RNAs have been sequenced.

HOW SIMILAR ARE OIL PALM AND COCONUT CCCVd MOLECULES?

Viroids with minor differences in nucleotide sequence can vary greatly in their pathogenicity (Flores *et al.*, 2005). Examples from a range of viroids, including CCCVd (Rodriguez & Randles, 1993; *Figure 1a*), show that point mutations at only two or three sites distinguish severe from mild viroid isolates. A study of pathogenicity therefore must include sequencing of the viroid. Sequences give detailed information which can define a particular isolate of a viroid. However, if an overview of the range of mutants and variants in a survey is required, indirect assays such as the ribonuclease protection assay (RPA) allow many samples to be compared in a short time frame (Vadamalai *et al.*, 2009).

Direct sequence comparisons

Comparison of the sequence of a 297 nucleotide (nt) CCCVd molecule from an oil palm (Vadamalai *et al.*, 2006) with the published 296 nt form of CCCVd from coconut with cadang-cadang (Haseloff *et al.*, 1982) is depicted in *Figure 1*. The CCCVd₂₄₆ form with mutation sites associated with a severe phenotype in coconut (Rodriguez & Randles, 1993) is included for comparison. Overall, three size classes, 297, 293, and 270 nt were described

from the same oil palm. All differed from CCCVd₂₉₆ by three mutations, substitutions of C₃₁→U in the pathogenicity domain and A₁₇₅→C in the right-hand terminus, and an addition of a G₁₂₅ in the 297 and 293 classes. Other mutations and deletions accounted for the different sizes. This is the first report that a range of sequence variants of CCCVd can coexist in one oil palm. A number of other variant molecules smaller than the minimal viroid were also sequenced (Vadamalai, 2005) but are of unknown function. These were sub-viroidal molecules, consisting of multiple repeats of a 22 nt sequence, together with other partial viroid sequences.

Detection of the range of sequence variation by RPA

The RPA identifies molecules which are identical to a given sequence (using a 'probe' prepared from a specific clone), together with those which are mutants of that sequence. It uses the principles of hybridisation and the property of particular ribonuclease enzymes to digest mismatched regions of a double-stranded hybrid RNA molecule. The value of RPA is that it detects identical and variant molecules in the same sample, and therefore provides an overview of the range of variation within one palm as well as between a number of different palms. Results from a survey of oil palms (Vadamalai *et al.*, 2009) confirmed the direct sequencing results that multiple variants can co-exist in a single palm, and showed that both asymptomatic and OS palms contain CCCVd variants.

A further advantage of RPA over other diagnostic techniques such as RT-PCR (Hodgson *et al.*, 1998) is that it is robust, independent of inhibitors in extracts, and is potentially more sensitive.

THE OIL PALM VIROID DISEASE CYCLE IS UNKNOWN

A disease cycle describes the life cycle of a plant pathogen, generally showing the source(s) of infection, means of spread, and alternative hosts. Plant pathologists develop disease management procedures from knowledge of pathogen disease cycles. The disease cycle of CCCVd in both coconut and oil palm is unknown. Although there is evidence for a low rate of seed transmission of cadang-cadang disease in coconut palm, the disease cycle is not described and so it is not available as a model for oil palm.

A first principle in disease control is that only pathogen-free germplasm should be planted. A priority for CCCVd research should

be to test whether production of viroid-free oil palm seedlings and ramets will affect the incidence of viroid infection in plantations. The knowledge gaps in the oil palm viroid cycle is diagrammatically shown in *Figure 3*. Thus, while it is certain that ramets produced from infected parent palms will also be infected, there is a lack of information on whether the viroid spreads *via* pollen, seed, mechanical harvesting or vectors. There is also a lack of information on whether the source of viroid is oil palm or other infected plant species.

A second research priority is to test whether exclusion of CCCVd from oil palm plantations will improve palm oil yield and quality. Viroid-free schemes applied to other crops have generally given dramatic improvements in plant health and product yield

KNOWLEDGE GAPS IN THE OIL PALM VIROID CYCLE

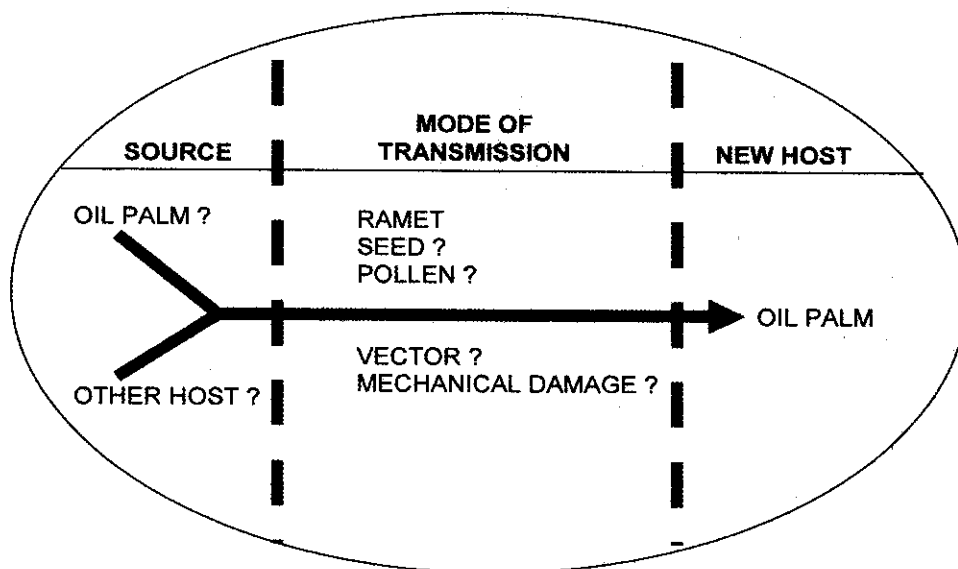


Figure 3 Diagram representing the spread of oil palm viroid from source to new host, where gaps in knowledge are indicated with a ?. It is not known whether oil palm or alternative plant species are the source of infection. Note that the viroid will be transferred by vegetative propagation via ramets if their parent palm is infected, but that other potential modes of spread are yet to be studied.

and quality (Hadidi *et al.*, 2003).

CONCLUSIONS

The work described here indicates that the extent of infection of commercial oil palm with CCCVd is likely to be high in all countries where it is grown. Such a scenario is feasible because commercial oil palm has a very narrow genetic base and breeders have not tried to exclude viroids from germplasm. Moreover, viroids have been known only since 1970, and the required modern diagnostic tools are not generally available to breeders. There is evidence that one or more of the commercial oil palm parents showed OS, and our survey results indicate that OS is closely associated with CCCVd infection. Asymptomatic infection occurs as well and these palms may still be a source of viroid spread. CCCVd is a plant pathogen and it is likely that it affects oil palm health and yield. Research is therefore needed to determine the incidence, economic effects, disease cycle and management procedures for CCCVd. This should be given equal priority with other attempts to improve oil palm productivity globally. Excluding CCCVd from germplasm may raise the attainable yield (Agrios, 2005) of oil palm.

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REFERENCES

- AGRIOS, G N. 2005. Plant disease epidemiology. *Plant Pathology* (5th Edition). New York: Academic Press. 273 pp.
- FLORES R, RANGLES J W, OWENS R A, BAR- JOSEPH M and DIENER, T O. 2005. *Viroids*. In: *Virus Taxonomy. VIII Report of the International Committee on Taxonomy of Viruses* (C M Fauquet, M A Mayo, J Maniloff, U Desselberger and A L Ball, eds.). 1145-1159. Elsevier/Academic Press, London.
- HADIDI A, FLORES R, RANGLES J W and SEMANCIK J S. 2003. *Viroids*. CSIRO Publishing, Australia. 370 pp.
- HANOLD D and RANGLES J W. 1989. Cadang-cadang-like viroid in oil palm in the Solomon Islands. *Plant Disease*, **73**: 183.
- HANOLD D and RANGLES J W. 1991a. Coconut cadang-cadang disease and its viroid agent. *Plant Disease*, **75**: 330-335.
- HANOLD D and RANGLES J W. 1991b. Detection of coconut cadang-cadang viroid-like sequences in oil and coconut palm and other monocotyledons in the south-west Pacific. *Annals of Applied Biology*, **118**: 139-151.
- HANOLD D and RANGLES J W. (EDITORS). 1998. Report on ACIAR-funded research on viroids and viruses of coconut palm and other tropical monocotyledons 1985-1993. Monograph MN45 1993 <http://www.aciar.gov.au/web.nsf/doc/JFRN-5J473D> ACIAR, Canberra.
- HANOLD D and RANGLES J W. 2003. CCCVd-related molecules in oil palms, coconut palms and other monocotyledons outside the Philippines. In: *Viroids* (Hadidi, A, Flores, R, Randles, J W and Semancik, J S, eds.). CSIRO Publishing, Australia. 336-340.
- HASELOFF J, MOHAMED N A and SYMONS R H. 1982. Viroid RNAs of cadang-cadang disease of coconuts. *Nature*, **299**: 316-321.
- HODGSON R A J, WALL G C and RANGLES J W.

1998. Specific identification of coconut tinangaja viroid (CTiVd) for differential field diagnosis of viroids in coconut palm. *Phytopathology*, **88**: 774-781.
- IMPERIAL J S, BAUTISTA R M and RANGLES J W. 1985. Transmission of the coconut cadang-cadang viroid to six species of the palm by inoculation with nucleic acid extracts. *Plant Pathology*, **34**: 391-401.
- RANGLES J W, BOCCARDO G, RETUERMA M L and RILLO E P. 1977. Transmission of the RNA species associated with cadang-cadang of coconut palm and insensitivity of the disease to antibiotics. *Phytopathology*, **67**: 1211-1216.
- RANGLES J W, BOCCARDO G and IMPERIAL J S. 1980. Detection of the cadang-cadang associated RNA in African oil palm and buri palm. *Phytopathology*, **70**: 185-189.
- RANGLES J W and RODRIGUEZ M J B. 2003. Coconut cadang-cadang viroid. In: *Viroids* (Hadidi, A, Flores, R, Randles J W and Semancik, J S, eds.) CSIRO Publishing, Australia. 233-241.
- RODRIGUEZ M J B and RANGLES J W. 1993. Coconut cadang-cadang viroid (CCCVd) mutants associated with severe disease vary in both the pathogenicity domain and the central conserved region. *Nucleic Acids Research*, **21**: 2771.
- VADAMALAI G. 2005. An investigation of oil palm orange spotting disorder. PhD Thesis. The University of Adelaide, Adelaide. URL <http://thesis.library.adelaide.edu.au/public/adt-SUA20060321.085810/index.html>
- VADAMALAI G, HANOLD D, REZAIAN A M and RANGLES J W. 2006. Variants of the Coconut cadang-cadang viroid isolated from an African oil palm (*Elaeis guineensis* Jacq.) in Malaysia. *Archives of Virology*, **151**: 1447-1456.
- VADAMALAI G, PERERA A A F L K, HANOLD D, REZAIAN M A and RANGLES J W. 2009. Detection of coconut cadang-cadang viroid sequences in oil and coconut palm by ribonuclease protection assay. *Annals of Applied Biology*, **154**: 117-125.



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