Bursaphelenchus cocophilus

Scientific Name
Bursaphelenchus cocophilus (Cobb, 1919) Baujard, 1989

Synonyms:
Aphelenchoides cocophilus, Aphelenchus cocophilus, Chitinoaphelenchus cocophilus, *Radinaphelenchus cocophilus,* and *Rhadinaphelenchus cocophilus.*

The red ring nematode, *Bursaphelenchus cocophilus*, was first described by Cobb (1919) as *Aphelenchus cocophilus* from specimens in Grenada. Since that time, it has undergone several name changes including the change to *Rhadinaphelenchus cocophilus* by Goodey (1960). This name is still commonly used within the scientific literature. Giblin-Davis et al. (1989b) presented morphological evidence supporting the similarities between *Rhadinaphelenchus* and *Bursaphelenchus*. Baujard (1989) synonymized the monotypic genus *Rhadinaphelenchus* with *Bursaphelenchus* creating the new combination, *B. cocophilus*. Molecular phylogenetic data presented by Ye et al. (2007) corroborates this synonymization.

Common Name
Red ring nematode, coconut palm nematode.

Type of Pest
Nematode

Taxonomic Position
Class: Secernentea, **Order:** Panagrolaimomorpha, **Family:** Aphelenchoididae

Reason for Inclusion in Manual
Palm commodity survey; national threat

Pest Description
Generalized Description: Females and males of *B. cocophilus* are 60 to 139 and 65 to 179 times longer than wide, respectively, with the greatest body width being less than 15.5 μm and total length ranging from 775 to 965 μm from little leaf symptomatic African oil palm and 812 to 1369 μm from coconut or African oil palms with typical red ring symptoms. The metacorpus and stylet in the second-stage juveniles and adults are well developed. Stylet length is between 11 to 15 μm in adults. Females have a vulval flap which appears bowed posteriorly when viewed ventrally, a long post-uterine sac (extending about 75% of the vulva-anal distance), and an elongate tail (62 to 117 μm) with a rounded terminus. Males have seven caudal papillae; one ventral preanal papilla, one pair of subventral preanal or adanal papillae, and two pairs of subventral postanal papillae. The distal ends of the spicules in the males are heavily sclerotized and the caudal alae form a spade-shaped flap. Third-stage dauer juveniles from coconut palm usually range from 700 to 920 μm and have a pointed tail with or without a

mucron. The metacorpus is usually not well developed in juveniles from the palm or the weevil vector and the stylet is not visible (Giblin-Davis et al., 2002).

Juveniles: Juveniles (Fig. 1) have high, dome-shaped heads that are not offset from the body. The tails of the second- and third-stage juveniles are conoid with or without sharply mucronate tips. Those of fourth-stage juveniles have dimorphic tips: in female juveniles they are rounded as in the female, and in male juveniles are "sharply drawn out" (Dean, 1979). The third larval stage is 0.84 mm (0.03 in) and characterized by a tapered terminal end of the body (Brammer and Crow, 2002).

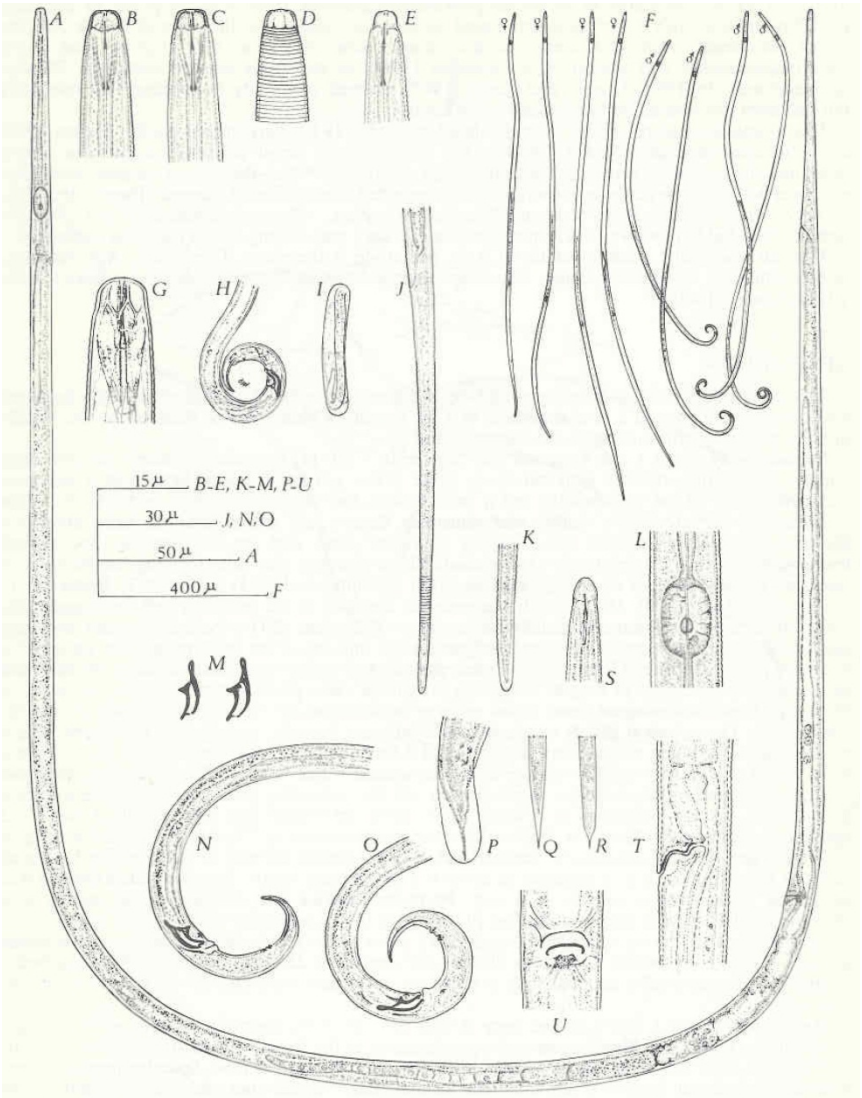
Detailed Adult Descriptions from Dean (1979):
Females (Fig. 1): Body about 1 mm [0.04 in] long and very slender, arcuate to nearly straight when relaxed; cuticle thin, marked with transverse striae, 0.6 to 1 μm apart. The lateral fields have four incisures that occupy 0.25 of the body width and a faint median line. The outer incisures are crenate. Deirids and phasmids are absent. The lip region is smooth, high, anteriorly flattened with rather straight sides, slightly narrower than and set off from the body. The head framework is prominent, sclerotized, a hexaradiate ribbed cylinder with sides forming fine bars which bifurcate basally. Spear/stylet is 11 to 13 μm long, attenuated, knobbed at the base and well developed. The stylet knobs may be obscure especially in immature specimens. The anterior part is less than half of the spear length and sharply pointed. The protractor muscles of the spear are prominent and attached to the basal plate of the labial framework. The procorpus is elongate to cylindrical. The metacorpus or median bulb is oval and usually about twice as long as wide with prominent valve plates just posterior to the center of the bulb. The dorsal oesophageal gland orifice is midway between the anterior margin of the bulb and the valve plate. Oesophageal glands overlapping the intestine dorsally and usually obscure. The nerve ring is a wide band surrounding the isthmus and about 0.5 to 1 bulb-length behind the bulb. The excretory pore a little behind the nerve ring and anterior to the hemizonid, which is about three annules long. Intestine has small granules and indistinct lumen. The vulva is slit-like and appears as an open C in ventral view, slightly overhung by a wide, thick dorsal lip. Posterior lip is also thick and heavily sclerotized. The vagina is thick-walled, slightly curved or distinctively C-shaped as it leads inwards to a distance of about 0.5 the body width. The ovary is well developed and outstretched with oocytes in a row. The postvulval uterine sac is elongate. The initial section has thickened walls probably representing part of the uterus, which extends about 0.75 the vulva-anus distance and serves as a spermatheca (often with a few large spheroid sperms). The rectum is about 1.5 anal body-widths long. The anus is distinct with an opening that is 0.25 to 0.5 that of the anal body width. The tail is elongate to subcylindrical with a rounded, unstriated terminus, 10 to 17 anal body-widths long (Dean, 1979).

Males (Fig. 1): The body is about 1 mm long and very slender, ventrally arcuate, more strongly curved in the tail region. The head, stylet, and oesophagus as in the female. The testis is single, anteriorly outstretched to over 0.5 of the body length with spermatogonia in a row. Spicules are small, paired, and thorn-shaped. The dorsal limb is 9 to 13 µm long with an elongated rounded apex and ends dorsally before the ventral

limb whose distal ends appears to recurve to join the dorsal limb so that the entire spicule appears notched distally. The ventral element is 7 to 8 µm long, has a distinct rostrum proximally, and appears to be connected to the dorsal limb through a transverse bar with a central hole. There is no gubernaculum, but the dorsal wall of the spicule pouch is thickened to form an apophysis. The anus has an anterior lip protruding, posterior lip protruding, or both lips protruding. The tail is strongly curved ventrally, "0.8 to 1.5 of a circle" (Dean, 1979). It is subcylindrical in the anterior half, then conoid to a pointed terminus. Bursa (caudal alae) are short, terminal, and prominent in the dorsal or ventral view with finely striated margins. The bursa envelopes the distal 30 to 50% of the tail. There are a total of seven caudal papillae; a single ventral pre-anal papilla about 3 microns before the anus and an ad-anal pair followed by two pairs of distinct ventro-submedian papillae near the base of the bursa (Giblin-Davis et al., 1989).

Biology and Ecology

The life cycle of *B. cocophilus* lasts nine to ten days (Dean, 1979). The life cycle consists of an egg stage, four juvenile stages, and an adult stage (Chinchilla, 1991). The vector, *Rhynchophorus palmarum* (the South American palm weevil), deposits the dauer third stage juvenile of the nematode as it lays its eggs on palm leaf axils or internodes (Chinchilla, 1991; Giblin-Davis et al., 2002). *B. cocophilus* propagates in the host plant. Once the weevil eggs hatch, immature nematodes will enter the larvae, where they can remain while the weevils undergo metamorphosis. Once mature, the weevils will leave the host palm carrying dauer third-stage juvenile nematodes of *B.*



A & F. Adults; B–D & G. Female head ends; E. Male head end; H, N & O. Male tail ends; I. Egg; J. Female tail; K. Female tail tip; L. Female median oesophageal bulb; M. Spicules; P. Bursa in dorsal view; Q & R. Larval tail tips; S. Larval head end; T & V. Vulva in lateral and ventral view, respectively. G, H & I. after Cobb (38); T. original Siddiqi; rest after Siddiqi (28).

Figure 1. Line drawings *of B. cocophilus* (cited as *Rhadinaphelenchus cocophilus*) from Dean (1979).

cocophilus with them to infect other host palms. *B. cocophilus* does not develop inside the weevil vectors.

Adult weevils move *B. cocophilus* around by visiting infected host palms and either ingesting the nematode or carrying them on the surface of their bodies. *B. cocophilus* can sometimes be found in the tracheal sacs of the vector, where they can move to the ovipositor of females to be injected into host plant material (reviewed in Griffith et al., 2005). Only a few (10 to 50) nematodes are needed to cause disease when inoculated into a small wound (Griffith, 1968). Around five thousand nematodes, however, are needed for disease development on natural cracks or coconut petioles (Griffith, 1968). Weevil vectors are attracted to palms with wounds or cuts in the trunks. Palms already infected and dying from red ring disease produce semiochemicals (kairomones) that are attractive to the weevil vectors (Giblin-Davis et al., 1996).

Figure 2. Cross-section of infected host palm. Characteristic red ring is visible as well as *R. palmarum* galleries (R. Giblin-Davis).

Figure 3. Host palm showing chlorosis and wilting due to *B. cocophilus* (R. Giblin-Davis).

B. cocophilus can survive long periods of time in nut husks (16 weeks), seedling tissue (90 weeks), and within the weevil. Dean (1979) states that *B. cocophilus* can survive in fresh water films for seven to eight days and in sea water for three days. *B. cocophilus* is susceptible to desiccation (Esser and Meredith, 1987).

Griffith et al. (2005) states that, “the heaviest losses due to red ring disease occur at the end of the wet season and in the first two or three months of the dry season.” Low, poorly drained areas have the highest incidence of red ring disease; while drought

conditions keep the disease in check (Esser and Meredith, 1987). The nematode survives best in wet, swampy areas and in clay rather than sandy soil. Possible root to root transmission from trees infected with *B. cocophilus* to healthy trees has also been shown (Warwick and Bezerra, 1992).

Symptoms and Signs

Internal plant damage can be observed within two to three weeks after infection by the nematode. The first external symptoms are visible about 28 days after inoculation (Giblin-Davis et al., 2002). External symptoms, however, may take up to two months to appear. The disease, caused by *B. cocophilus*, occurs most commonly in trees that are 2.5 to 10 years old, with the greatest incidence occurring in trees between four and seven years old (Griffith et al., 2005). External symptoms are generally not considered diagnostic of infection with *B. cocophilus* (Dean, 1979). Some symptoms associated with this pathogen may be caused by other pathogens or factors (like nutrient deficiency or mechanical damage) and does not necessarily mean that this species is present (Dean, 1979; Chinchilla, 1991).

Two distinct types of symptoms are caused by *B. cocophilus*: “red ring” and “little leaf disease”.

Red ring:

The nematode causes reddish lesions to form in the stem. These lesions gradually enlarge and often form the primary and most characteristic internal symptom of the disease for which the disease was named, a “red ring” when the cut stem is viewed in cross section (Fig. 2). The red ring typically occurs 14 to 21 days after inoculation with *B. cocophilus* (Giblin-Davis et al., 2002). The ring may vary in color from bright red to light pink, or cream to dark brown in *Elaeis guineensis* (African oil palm). The ring can be 3 to 5 cm wide (1.18 to 1.97 in) from the periphery, but the width may vary depending on tree size (Griffith et al., 2005). The red ring can usually be seen when the infected palm is cut crosswise from 0.3 to 2.1 meters (1 to 7 ft.) above the soil line (Brammer and Crow, 2002). The ring may not be continuous throughout the trunk length (Chinchilla, 1991). The ring may also be found in the cortex of the host roots and in the petioles (Giblin-Davis, 2001). When diseased, the soft, white cortex of the roots becomes orange to faint red in color and dry and flaky in texture (Fenwick, 1969; Griffith et al., 2005).

With red ring disease, established leaves become short, deformed, and turn yellowish-bronze before turning deep reddish-brown in color (Fig. 3). The change in color typically starts at the leaf tip spreading towards the base (Griffith et al., 2005). Older leaves will show symptoms before younger leaves. Leaves will eventually wilt and die. The oldest leaves usually break at the petiole, close to the trunk and can remain hanging down for a long period of time (Chinchilla, 1991). In coconut palm (*Cocos nucifera*), fruit typically drop prematurely (before mature) (Giblin-Davis, 2001). This usually happens around the same time that leaf symptoms develop or slightly before. Four to six weeks after symptom development, the palm crown will often topple over;

this is associated with severe internal damage caused by the larvae of the weevil vector (Griffith et al., 2005).

Little leaf:
Some African oil palms and older coconut palms will produce small, deformed leaves, which remain green with no initial necrosis. "Little leaf disease" is a chronic condition that can lead to red ring disease development. These trees usually stop producing fruit (Chinchilla, 1991; Giblin-Davis, 2001). Nematodes can be found in high numbers in young leaves, when the leaves are elongating. These leaflets eventually become partially necrosed and remain partially folded along the rachis.

Nematode Location*:*
B. cocophilus primarily invades the parenchymatous tissue of host palms (where the red ring develops) and is relatively confined to this area. At the top part of the stem, where the tissue is softer, it is possible to find the nematode both in discolored areas and in the adjacent tissues that are apparently healthy. In the tissues of the petioles, nematodes can be found in small amounts. *B. cocophilus* can also infect the roots. In samples of the roots collected at the base of the stem of diseased trees, the number of nematodes was considerably higher when compared to the number found in roots situated between 1 to 3 meters (3.28 to 9.84 ft.) away from the stem (Duarte et al., 2008). The population of the nematode is also high in the excrement of *R. palmarum* found in the larval tunnels in the apical parts of the plant (Duarte et al., 2008).

Pest Importance

Giblin-Davis et al. (2002) state that palms are important landscaping plants in subtropical areas of the United States. An introduction of *B. cocophilus*, could potentially have an impact on both the landscape and tourism industries (Giblin-Davis et al., 2002).

In Trinidad, red ring disease can kill 35% of young coconut trees. In Venezuela, 35% of oil palms (*Elaeis* spp.) were killed by red ring disease over a 10-year period (Brammer and Crow, 2002). Chinchilla (1991) states that losses of 5 to 15% in oil and coconut palm plantations as a result of *B. cocophilus* is common in several countries in Central and South America.

B. cocophilus most often attacks *Cocos nucifera* trees that are four-to-seven years old. These trees usually die six to eight weeks after symptoms appear. Older trees may last up to 20 weeks after symptom expression (Esser and Meredith, 1987). Some trees may live several years after infection. When compared to healthy trees, trees that have been infected with red ring disease for more than three years are noticeably stunted (Chinchilla, 1991). In host plants, *B. cocophilus* blocks water pathways in the leaves, stem, and roots. This reduces the host palm's water absorption.

Known Hosts

Host palms two years and younger are generally not susceptible to *B. cocophilus* (Giblin-Davis, 2001). This species is known to infect over 17 species in the Palmae

family. The disease severity and symptoms will vary depending on the host species and environmental conditions (Giblin-Davis et al., 2002).

Major hosts
Cocos nucifera (coconut) and *Elaeis guineensis* (African oil palm) (EPPO, 2012).

Other hosts
Acrocomia intumescens, *Attalea cohune* (Cohune nut), *Bactris gasipaes*, *Euterpe pacifica*, *Jessenia polycarpa*, *Mauritia mexicana*, *Oenocarpus distichus*, *Phoenix canariensis* (Canary Island date), *P. dactylifera* (date palm), and *Roystonea regia* (Cuban royal palm) (Schuilling and van Dinther, 1981; Esser and Meredith, 1987; Griffith et al., 2005).

Experimental hosts
Acrocomia aculeata (gru-gru palm), *Mauritia caribea* (cocorite palm), *M. flexuosa* (Ita palm), *Maximiliana maripa* (cucurite palm), *Roystonea oleracea* (cabbage palm), and *Sabal palmetto* (Sabal palm) (Esser and Meredith, 1987; Griffith et al., 2005; Giblin-Davis et al., 2002).

Known Vectors (or associated organisms)

Bursaphelenchus cocophilus parasitizes the South American palm weevil *Rhynchophorus palmarum* (Fig. 4), which serves as a vector. This weevil was found in the area of San Diego, California in May of 2011 and Alamo, Texas in May 2012. Delimitation surveys were initiated and other finds have been made in the same general geographic areas within 2.5 miles and 5 miles of the United States/Mexico Border, respectively (Bech, 2011; 2012).

Gerber et al. (1990) and Giblin-Davis (2001) also list *Dynamis borassi* as a vector of this pathogen. Martyn (1953) and Dean (1979) also list the weevil *Rhinostomus* (*Rhina*) *barbirostris* as a vector of *B. cocophilus*.

Figure 4. Lateral view of the vector *Rhynchophorus palmarum* (top) and dorsal view (bottom) (Jennifer C. Giron Duque, University of Puerto Rico, Bugwood.org).

Other weevils could serve as potential vectors, including *Metamasius hemipterus* and *Rhynchophorus cruentatus*, which are both present in the United States (Mora et al., 1994). Bulgarelli et al. (1998), however, found no evidence to support *M. hemipterus* as a vector of *B. cocophilus* in Costa Rica.

Dean (1979) lists several species of ant, termite, and spider that have been reported as vectors, although he does state that many of these may not be as important in *B. cocophilus* movement. It is highly unlikely, however, that these species are competent vectors of *B. cocophilus*.

Known Distribution

Caribbean: Grenada, St. Vincent and the Grenadines, and Trinidad & Tobago. **Central America:** Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama (including the San Blas Islands). **North America:** Mexico. **South America:** Brazil, Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, and Venezuela (Ashby, 1924; Govindankutty and Koshy, 1979; Noriega et al., 1992; Mora et al., 1994; CABI, 2012; EPPO, 2012).

Griffith et al. (2005) and CABI (2012) state that records from the Bahamas, Barbados, Dominica, Dominican Republic, Haiti, Jamaica, and Puerto Rico are questionable and are not included in the distribution information above.

Pathway

B. cocophilus would be able to move with any of its vectors. Its main vector, *Rhynchophorus palmarum*, can move through infected plants, like nursery stock. Natural spread can occur through adult flight of *R. palmarum* (EPPO, 2005). Both sexes of *R. palmarum* are considered strong fliers and can fly over half a mile in one flight (Hagley, 1965). Esser and Meredith (1987) state that *B. cocophilus* can also move through seeds, seedlings, tools, vehicles, and animals. Furthermore, natural movement may occur through movement from infected to non-infected roots, although survival in soil is short (Esser and Meredith, 1987; Warwick and Bezerra, 1992).

B. cocophilus has not been intercepted at U.S. ports of entry; however, its main vector, *R. palmarum*, has been intercepted (AQAS, 2012). *R. palmarum* has been intercepted ten times with seven additional interceptions being identified at the genus level only. These interceptions occurred between January 1986 to October 2009 in airports (8), at land borders (2), and maritime ports (7). Most interceptions occurred on fruit and other plant parts. One interception occurred on *Cocos nucifera* (coconut), the main host of *B. cocophilus*. Most intercepted host material originated from Ecuador (6), Mexico (3), Guatemala (2), Peru (3), El Salvador (1), and Africa (2) (AQAS, 2012).

To reduce the risk of importation and dissemination of *Rhynchophorus ferrugineus*, *R. palmarum*, and *B. cocophilus*, a Federal Order (dated January 25, 2010) prohibits the importation of all plants for planting of *Acrocomia, Astrocaryum, Attalea, Bactris, Brahea, Butia, Calamus, Chamaerops, Desmoncus, Euterpe, Manicaria, Mauritia, Metroxylon, Oncosperma, Roystonea, Sabal*, and *Washingtonia* from all foreign

countries, with the exception of seed, until a pest risk analysis has been completed and determined whether effective mitigation measures exist (APHIS, 2010). All propagules of *Cocos nucifera* are prohibited except seed from Costa Rica and Jamaica with a written permit and special certification via 7CFR 319.37-5(g), which deals specifically with lethal yellowing disease of palm. *B. cocophilus* is known to occur in Costa Rica.

Giblin-Davis (1990) stated that one pathway of introduction of *B. cocophilus* is through the arrival of infested *R. palmarum* that have traveled undetected in a shipment of coconut seed nuts. Giblin-Davis et al. (2010) also stated that de-husked coconut fruit would not serve as a pathway for either *B. cocophilus* or its vector *R. palmarum*. There may be a risk of introducing *B. cocophilus* on imported improperly composted and refined coir from areas where the nematode is present. This material is used by the ornamental plant industry as either a soil conditioner or as a component of soil mixes used in containerized plant production (Giblin-Davis et al., 2010).

Potential Distribution within the United States

This species is unlikely to establish in most of the United States due to the low density of this host plant species. Florida is the only state that has a moderate density of these host plants of *B. cocophilus* (USDA-NCRS, 2012). The main host plant, *Cocos nucifera* (coconut) is also found in other states including Hawaii and North Carolina and is planted as an ornamental in the southern United States from California to Florida. It is also found in Puerto Rico and the U.S. Virgin Islands. *Elaeis guineensis* (African oil palm) is only listed as occurring in Florida. Many of the other host species are not listed as occurring in the United States, although *Phoenix* spp. are found in Arizona, California, Florida, Hawaii, Massachusetts, and Maryland. *Sabal* spp. occur in Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, and Texas, as well as Puerto Rico and the U.S. Virgin Islands (USDA-NRCS, 2012).

In addition, the known weevil vector*, R. palmarum*, is known to be present at a low level in California and Texas near the United States/Mexico border (Bech 2011, 2012). *B. cocophilus* is known to be present in southern Mexico. Special attention should be focused in these areas.

Survey

CAPS-Approved Method*: Visual survey for symptoms of nematode infestation with host and/or vector (*R. palmarum*) collection (where the vector is known to occur).

Host samples or extracted nematodes should be sent to Dr. Lynn Carta:
Dr. Lynn Carta, Ph.D.
Research Plant Pathologist
Nematology Laboratory
USDA-ARS BARC-W, Bldg. 010A, Rm. 110
10300 Baltimore Avenue, Beltsville, MD 20705

Office phone: 301-504-8787 Lab phone: 301-504-7039
Email: lynn.carta@ars.usda.gov

1. Sample collection:
   A. Host collection: The well-established methods for obtaining samples of nematodes from living trees are used.
      a. A stainless steel tube, sharpened at one end, is driven at an angle of 45° at the point selected for sampling. The tube needs to penetrate a minimum of 5 cm into the tree trunk. Multiple samples should be taken from an individual palm and samples should be taken from 0.3 to 2.1 meters (1 to 7 ft.) above the soil line.
      b. An alternative method: Two holes, up to six inches (15 cm) deep, can be drilled per tree at six inches (15-cm) from both ends using a 2.125-inch (5.4-cm), self-feeding wood bit using an electrical or 18-Volt-battery-powered drill. The wood shavings from the two trees are mixed together, and a minimum 200 g of wood shavings are collected as one lab sample (Carta, personal communication).

   Bore holes made with the tube should be sealed with silicone (type used to seal bathroom fixtures) to prevent the entry of weevils, because it is easy to control, inert, and creates a good seal. Holes can also be filled with wooden dowels.

   B. Vector collection: Please follow the **Protocol for Preparing and Forwarding Suspect South American Palm Weevil from Survey Traps for Confirmation and to Maximize Red Ring Nematode Detection**.

2. Nematode extraction: Submit samples to a nematology diagnostic lab for identification.
   A. From host material and soil: Nematodes can be extracted by several techniques: sedimentation/sieving and Seinhorst two-flask technique.
      a. Sedimentation/Sieving: The extracted core is placed in a blender with 50 ml of water and processed for 2 min. The contents of the blender are then poured into a dish and left for 20 min for the nematodes to emerge. The nematodes are then recovered by sieving. The red ring nematodes are often highly mobile in water (swimming and coiling), leading to knots of clumped nematodes or resuspension of nematodes after centrifugation. In coconut and the palmiste palms, the nematodes are most active in the stem tissue (except in the very necrotic regions). The core tissue generally shows a

Figure 6. Seinhorst two-flask technique. Photo from Hoooper (1986).

red cylinder of necrotic red ring tissue (Griffith et al., 2005).

b. Two-Flask Technique (Seinhorst, 1955): From Hopper (1986): "This is a simple but efficient technique for extracting small to average-sized nematodes. It gives a cleaner extract than that usually obtained by direct sieving and is useful for recovering nematodes, which do not readily move through a filter. The apparatus is shown below in Fig. 6. Thoroughly mix a soil sample of about 200 g with 750 ml of water in a 1 liter beaker…..Wash the soil/water mixture through a hemispherical domestic sieve, with mesh of about 2 mm aperture, into a large wide-stem funnel fitted with a plug. When all the soil has passed through the sieve, pull out the plug allowing the slurry to run into a wide-neck, 2 L Erlenmeyer (conical) flask. Wash the funnel clean with a little water and top up the flask with water, removing any froth that accumulates. If a flask with a standard ground glass joint, 35 mm diameter, is available the appropriate funnel is used; otherwise a short plastic funnel may be attached with a rubber sleeve, or more easily, using Hooper's method (1961). The funnel aperture should be about 12 mm in diameter to obtain a suitable rate of sedimentation/elutriation.

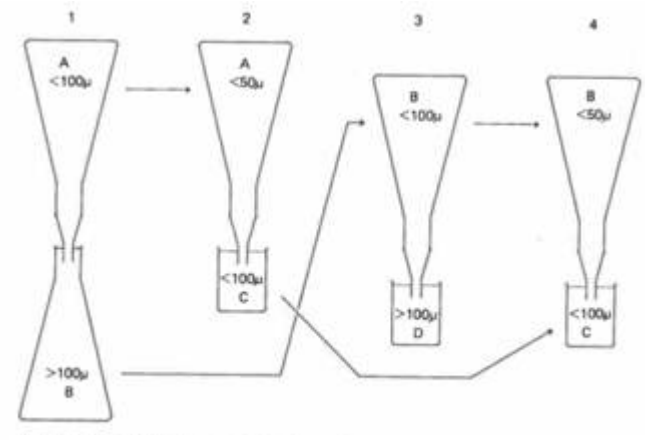


Figure 7: Procedure in Seinhorst's two-flas, extraction process. Photo from Hoooper (1986).

With a finger-tip closing the funnel orifice, shake the flask to mix the contents thoroughly and invert it over a similar flask filled with water. The funnel orifice should be just immersed and the finger-tip quickly removed; the soil particles and nematodes then sediment out differentially. This and the subsequent stages, where each flask is inverted over a beaker of water, are shown diagrammatically in Fig. 7. At each change shake the flask before setting it into its new position. Each stage runs for 10 min. The figures on the containers A, B, C, D (Fig. 7) show the size of the soil particles found in each at the end of the prescribed time. After the three 10 min periods, pour the contents of A and B through the 53 µm aperture (300 mesh/inch). The sieving are collected in a beaker and concentrated by settling. If necessary, the sieving may be cleaned and concentrated by one of the filtering methods described."

B. From vectors: Gerber and Giblin-Davis (1990) outline a procedure to extract nematodes from *R. palmarum*. Each weevil was placed in a beaker with 50

ml of tap water for two hours to remove external nematodes. Nematodes in the suspension were identified and counted. Each weevil was decapitated and dissected separately. The genital capsule (ovipositor or aedeagus) was removed and placed in a Baermann funnel with a small piece of cotton at the funnel outlet. The rest of the weevil body was macerated and extracted separately on a Baermann funnel. After 24 hours, the nematode suspensions were collected, and nematodes were counted, identified, and cultured on glycerol supplemented potato dextrose agar and nutrient agar. Cocoons were also individually extracted and examined for nematodes after weevil emergence on a Baermann funnel.

3. Survey Site Selection: The main hosts of this pathogen are *Cocos nucifera* (coconut) and *Elaeis guineensis* (African oil palm). These species are used as ornamentals in certain parts of the southern United States. Low, poorly drained areas have the highest incidence of red ring disease, while drought conditions keep the disease in check (Esser and Meredith, 1987). Additionally sites known to have the weevil vector *R. palmarum* should be surveyed as well for the red ring nematode.

*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:

Host collection: Fenwick and Maharaj (1963) used an 8 inch length of ½ in. diameter stainless steel tubing of approximately 1/16 in. wall thickness; one end was sharpened to form a circular cutting edge and the other end was cross-drilled to make a tommy bar. The tube was driven into the tree using a mallet and withdrawn with the aid of the tommy bar. The tissue core was then driven out of the tube using a 5/16 in. bar. The extent and depth of the red ring area was visible at this point. Cores were stored in individual polyethylene bags for laboratory examination. Cores left by the borer were plugged and sealed to prevent the entry of weevils.

Nematode extraction: Fenwick (1963) and Schuilling and van Dinther (1981) used 15 g of chopped tissue suspended in 250 ml of water and blended the suspension in an electric mixer for 30 seconds. The resulting suspension was made up to 1 or 2 liters in a bottle or Erlenmyer flask and allowed to stand for 30 minutes. The contents of the bottle were then sedimented over another container filled with water. After 30 minutes, the contents of the lower bottle were discarded. The contents of the top bottle were sieved four times through a 60 µm sieve.

Key Diagnostics/Identification

CAPS-Approved Method*:

The CAPS-Approved identification method is morphological. *B. cocophilus* is a relatively long, vermiform nematode with long tapering tails, about 1 mm for both females and males, but are also very thick. The vulva is positioned one-third body length from tail tip. Stylets are small, 11 to 13 *µ*m long and are often obscure (Bridge and Starr, 2007).

Detailed descriptions of the adult male and female as well as larva can be found in Dean (1979). Giblin-Davis et al (1989b) also provides information on the morphological features of *B. cocophilus*.

*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:
Culture: Giblin-Davis et al. (1989a) showed that *B. cocophilus* can survive longer than 70 days in autoclaved red ring stem tissue infusion water that was unsupplemented or supplemented with *D*-glucose, lactose, or Bacto-lactose broth and *D*-glucose.

Easily Confused Pests

The only other known nematode known to cause severe damage in *Cocos nucifera* (coconut) is *Radopholus similis* (burrowing nematode) (Griffith et al., 2005). In coconut palms, *R. similis* causes non-specific general decline symptoms including: stunting, yellowing, reduction in number and size of leaves and leaflets, delay in flowering, button shedding, and reduced yield. Infestation by *R. similis* produces small, elongate, orange-colored lesions on tender creamy-white roots (Griffith et al., 2005).

Bursaphelenchus gerberae was also found recently to be in association with the vector of red ring nematode *Rhynchophorus pamarum* in Trinidad and could potentially be confused with *B. cocophilus* (Giblin-Davis et al., 2006).

References

APHIS. 2010. Federal Import Quarantine Order: Palm Pests *Rhynchophorus ferrugineus* Olivier (Red Palm Weevil), *R. palmarum* Linnaeus (Giant Palm Weevil), and *Bursaphelenchus cocophilus* Cobb (Red Ring Nematode). January 25, 2010. http://www.aphis.usda.gov/import_export/plants/plant_imports/federal_order/downloads/2010/Palm%20Pests_1-25-10.pdf.

AQAS. 2012. Interception data on *Bursaphelenchus cocophilus.* All interceptions. Queried October 1, 2012 from: https://mokcs14.aphis.usda.gov/aqas/HomePageInit.do.

Ashby, S.F. 1924. Red ring disease of coconut. Proceedings 9th West Indies Conference, Jamaica. Pgs. 167-172.

Baujard, P. 1989. Remarques sur les genres des sous-familles Bursahphelenchidae Paramanov, 1964 et Radinaphelenchinae Pramanov, 1964 (Nematoda: Aphelenchoididae). Revue de Nematologie 12: 323-324.

Bech, R.A. 2011. Detection of South American Palm Weevil (*Rhynchophorus palmarum*) in California. USDA-APHIS-PPQ. DA-2011-45. August 5, 2011.

Bech, R.A. 2012. Detection of South American Palm Weevil (*Rhynchophorus palmarum*) in Texas. USDA-APHIS-PPQ. DA-2012-22. June 15, 2012.

Brammer, A. S. and Crow, W.T.. 2002. Featured Creatures, Red Ring Nematode. University of Florida Institute of Food and Agricultural Sciences, Department of Entomology and Nematology and Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

Bridge, J., and Starr, J.L. 2007. Plant Nematodes of Agricultural Importance: A Color Handbook. Academic Press, Boston. Pgs. 111-113.

Bulgarelli, J., Chinchilla, C., and Oehlschlager, C. 1998. The red ring/little leaf syndrome and *Metamasius hemipterus* captures in oil palm in Cost Rica. ASD Oil Palm Papers 17-24.

CABI. 2012. Crop protection compendium: global module. Commonwealth Agricultural Bureau International, Wallingford, UK. http://www.cabi.org/compendia/cpc/.

Chinchilla, C.M. 1991. The red ring little leaf syndrome in oil palm and coconut palm. ASD Oil Palm Papers No. 1, 1-17. Accessed February 1, 2012 from: http://www.asd-cr.com/ASD-Pub/Bol01/b01c1.htm

Cobb, N.A. 1919. A newly discovered nematode, *Aphlenchus cocophilus* n. sp., connected with a serious disease of the coconut palm. W. Indian Bull. 17: 203-210.

Dean, C.G. 1979. Red ring disease of *Cocos nucifera* L. caused by *Rhadinaphelenchus cocophilus* (Cobb, 1919) Goodey, 1960. An annotated bibliography and review. Technical Communication No. 47 of Commonwealth Institute of Helminthology, St. Albans. Commonwealth Agricultural Bureaux. 70 pp.

Duarte, A.G., De Lima, I.S., Da Araujo,J.V., Duarte, A.G., De Albuquerque, A.L.S., and De Menezes Cruz, M. 2008. Disposition of the nematode *Bursaphelenchus cocophilus (*Cobb) Baujard, in coconut palm trees with the red ring disease. Rev. Bras. Frutic. Online. 30(3): 622-627. In Portuguese. English Abstract. http://www.scielo.br/pdf/rbf/v30n3/10.pdf.

EPPO. 2005. Data sheets on quarantine pests, *Rhynchophorus palmarum*. EPPO Bulletin 35: 468-471.

EPPO. 2012. EPPO PQR Database. Version 5.0.5540. www.eppo.org.

Esser, R.P. and Meredith, J.A. 1987. Red ring nematode. Nematology Circular No. 141. Florida Department of Agriculture and Consumer Services, Division of Plant Industry. 4 pp.

Fenwick, D.W. 1963. Recovery of *Rhadinapehlenchus cocophilus* (Cobb, 1919) Goodey, 1960 from coconut tissues. Journal of Helminthology 37: 11-14.

Fenwick, D.W. 1969. Red ring disease of coconut palm. In: J.E. Peachy (ed.) Nematodes of Tropical Crops Technical Communication No. 40.C.A.B. St. Albans Herts, UK. Pg. 89-98.

Fenwick, D.W., and Maharaj, S. 1963. Observations on the course of red ring disease of coconuts caused by the nematode *Rhadinaphelenchus cocophilus* (Cobb, 1919) Goodey, 1960 in naturally infected trees. Journal of Helminthology 37 (1/2): 21-26.

Gerber, K., Giblin-Davis, R.M., and Escobar-Goyes, J. 1990. Association of the red ring nematode, *Rhadinaphelenchus cocophilus*, with weevils from Ecuador and Trinidad. Nematropica 20(1): 39-49.

Giblin-Davis, R.M., Gerber, K., and Griffith, R. 1989a. *In vivo* and *in vitro* culture of red ring nematode, *Rhadinaphelenchus cocophilus*. Nematropica 19(2): 135-142.

Giblin-Davis, R.M., Mundo-Ocampo, M., Baldwin, J.G., Gerber, K., and Griffith, R. 1989b. Observation on the morphology of the red ring nematode, *Rhadinaphelenchus cocophilus* (Nemata: Aphlenchoididae).Revue Nematol. 12(3): 285-292.

Giblin-Davis, R.M. 1990. The red ring nematode and its vectors. Nematology Circular No. 181. Florid a Department of Agriculture and Consumer Services Division of Plant Industry. September 1990.

Giblin-Davis, R. M., Oehlschlager, A.C., Perez, A., Gries, G., Gries, R., Weissling, T.J., Chinchilla, C. M., Peña, J.E., Hallett, R.H., Pierce, Jr, H.D., and Gonzalez, L.M. 1996. Chemical and behavioral ecology of palm weevils (Curculionidae: Rhynchophorinae). Florida Entomologist 79(2): 153-167.

Giblin-Davis, R. M. 2001. Box 5.2, Red-ring disease. In F. W. Howard, D. Moore, R. M. Giblin-Davis, and R. G. Abad (eds.) Insects on Palms. CAB International, Oxon, UK. pp. 276-277.

Giblin-Davis, R. M., Lehman, P.S., and Inserra, R. N. 2002. *Bursaphelenchus cocophilus* Red Ring Disease of Coconut. Exotic Nematode Plant Pests of Agricultural and Environmental Significance to the United States. The Society of Nematologists. Accessed February 2, 2012 from: http://nematode.unl.edu/pest1.htm.

Giblin-Davis, R.M., Kanzaki, N., Ye, W., Center, B.J., and Kelley, T.W. 2006. Morphology and systematics of *Bursaphelenchus gerberae* n. sp. (Nematoda: Parasitaphelchidae), a rare associate of the palm weevil, *Rhynochophorus palmarum* in Trinidad. Zootaxa 1189: 39-53.

Giblin-Davis, R.M., Inserra, R.N., Stanley, J. D., and Hodges, G.S. 2010. Pest Alert: The Red Ring Nematode, *Bursaphelenchus cocophilus* (Cobb, 1919) Baujard, 1989 (Nematoda: Tylenchida). DACS-P-01748. Florida Department of Agriculture and Consumer Services, Division of Plant Industry. 6 pp.

Goodey, J.B. 1960. *Rhadinaphelenchus cocophilus* (Cobb, 1919) n. comb, the nematode associated with 'red ring' disease of coconuts. Nematologica 5: 98-102.

Govindankutty, M.D., and Koshy, P.K. 1979. Nematodes associated with the coconut palm (*Cocos nucifera* L.). Technical Bulletin 2 – Nematodes, Fungi, Insects, and Mites Associated with the Coconut Palm. Central Plantation Crops Institute Kasaragod, India. Pgs. 1-20.

Griffith, R. 1968. The mechanism of transmission of the red ring nematode. Journal of the Agricultural Society of Trinidad and Tobago 67: 436-457.

Griffith, R., R. M. Giblin-Davis, P. K. Koshy, and V. K. Sosamma. 2005. Nematode parasites of coconut and other palms. M. Luc, R. A. Sikora, and J. Bridge (eds.) In Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. C.A.B. International, Oxon, UK. Pp. 493-527.

Hagley, E. A. C. 1965. On the life history and habits of the palm weevil, *Rhynchophorus palmarum* [sic]. Annals of the Entomological Society of America 58(1): 22-28.

Hooper, D.J. 1961. A method for attaching the funnel to the flask for use in the Seinhorst two-Erlenmeyer flask soil extraction technique. Nematologica 6: 336. – modified plate in Hooper, 1986 plate 4 III.

Hooper, D.J. 1986. Extraction of free-living stages from soil. In: Laboratory methods for work with plant and soil nematodes (Ed. J. F. Southey), 6th Edition. Her Majesty's Stationery office, London. 202pp.

Martyn, E.E. 1953. Red ring disease of coconuts in Trinidad and Tobago. Tropical Agriculture 32: 45-53.

Mora, T.L.S., Calvache, G.H., and Avila, P.M. 1994. The spread of *Rhadinaphelenchus cocophilus* (Cobb) Goodey, causal agent of red ring – little leaf in oil palm. San Carlos De Guaroa (Meta.) Palmas 15: 15-27.

Noriega, C.D.H., Becerra, L.N., and Hernandez, R.F. 1991. Coconut diseases in the coast of Guerrero, Mexico. Revista Mexicana de Fitopatologia 9: 84-93.

Schuiling, M., and van Dither, J.B.M. 1981. 'Red ring disease' in the Paricatuba oilpalm estate, Para, Brazil. A case study. Zeitschrift Fur Angewandte Entomologie 91: 154-169.

Seinhorst, J.W. 1955. Een eenvoudige method voor het afschieden van aaltjes uit grond. Tijdschr. PlZiekt. 61: 188-190.

Seinhorst, J.W. 1956. The quantitative extraction of nematodes from soil. Nematologica 1:249-267.

USDA-NRCS. 2012**.** The PLANTS Database. National Plant Data Team, Greensboro, NC. Accessed February 2, 2012 from: http://plants.usda.gov.

Warwick, D.R.N., and Bezerra, A.P.T. 1992. Possible root transmission of red ring nematode (*Rhadinaphelenchus cocophilus*) to coconut palms. Plant. Disease 76: 809-811.

Ye, W., Giblin-Davis, R.M., Braasch, H., Morris, K., and Thomas, W.K. 2007. Phylogenetic relationships among *Bursaphelenchus* species (Nematoda: Parasitaphelenchidae) inferred from nuclear ribosomal and mitochondrial DNA sequence data. Molecular Phylogenetics and Evolution. 43:1185-1197.

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Draft Log

July, 2016: Updated mapping information.