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Association of the Red Ring Nematode and Other Nematode Species with the Palm Weevil, *Rhynchophorus palmarum*[1]

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Abstract: The palm weevil, *Rhynchophorus palmarum* (L.), was collected in cocoons from red ring-diseased coconut palms (*Cocos nucifera* L.) in Trinidad and Tobago. Juveniles of five species of nematodes were extracted from the genitalia and macerated bodies of newly emerged adults of the palm weevil: *Rhadinaphelenchus cocophilus* (Cobb) Goodey (the red ring nematode), *Teratorhabditis* sp., *Diplogasteritus* sp., *Mononchoides* sp., and *Bursaphelenchus* sp. Over 90% of newly emerged weevil females and males were infested internally with red ring nematode juveniles, and over 47% of the weevils contained more than 1,000 red ring nematodes each. There was no significant correlation between weevil body length and the number of red ring nematodes carried internally by each weevil. *Teratorhabditis* sp. and *Diplogasteritus* sp. were extracted from over 50% of the palm weevils, and *Monochoides* sp. and *Bursaphelenchus* sp. were found in a small proportion of the weevils. Field-collected adult weevils were also internally and externally infested with a *Rhabditis* sp., which was not observed in or on weevils allowed to emerge from field-collected cocoons.

Key words: *Bursaphelenchus* sp., *Cocos nucifera*, *Diplogasteritus* sp., entomophilic nematode, *Mononchoides* sp., palm weevil, red ring nematode, *Rhabditis* sp., *Rhadinaphelenchus cocophilus*, *Rhynchophorus palmarum*, *Teratorhabditis* sp., Trinidad.

The red ring nematode, *Rhadinaphelenchus cocophilus* (Cobb) Goodey, causes red ring disease (RRD) of the coconut palm (*Cocos nucifera* L.), oil palm (*Elaeis guineensis* Jacquin), and other palm species (3,12). The disease, named for the symptomatic red ring observed when nematode-infested palms are cut and viewed in cross-section, is reported only from the Neotropics (3,12).

In 1921 Ashby (1) suggested that the palm weevil, *Rhynchophorus palmarum* (L.), might play a role in the spread of the red ring nematode. Subsequently, other workers implicated the palm weevil as a vector for the nematode (2,13,14,19). By 1968 Griffith (8,9) had shown that red ring nematodes persisted during metamorphosis inside the palm weevil and that vertical transmission of the red ring nematode occurred during oviposition by internally parasitized females.

Weevil length was reported to be a reliable indicator of the RRD vector status of palm weevils (9–12). It was hypothesized that a genetically controlled defense mechanism caused the enzymatic digestion of invading red ring nematodes in larval weevils. The majority of weevils in Trinidad were considered homozygous dominant or heterozygous for the allele that controls the enzyme and were not vectors of RRD. In a small proportion of the weevil population, red ring nematode juveniles were supposedly capable of parasitizing weevils because of the absence of the enzyme. The large numbers of red ring nematodes were suspected to cause a significant reduction in resultant weevil size, and small weevils were considered to be primary vectors of

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RRD (12). Unfortunately, this weevil size–vector potential hypothesis has not been supported by field work in Grenada (18), El Salvador (4), and Brazil (16).

Imprecise methods have been used for determining the prevalence and intensity of nematode associations in the palm weevil. Most researchers have used field-collected adult weevils to estimate the number of associated red ring nematodes (4,16,18). Previous estimates of red ring nematode densities could have been low because the history and age of such hosts were not known and the nematodes could have been deposited into the environment prior to insect capture. In addition, we have consistently observed the palm weevil with large numbers of several species of previously unreported nematode associates (unpubl.) which may have confounded previous estimates of the numbers of red ring nematodes per weevil.

The purpose of this study was to examine newly emerged and previously emerged adults of the palm weevil from the field for internal and external nematode associates in Trinidad and Tobago. We also investigated the relationship between weevil size and the intensity of parasitism by the red ring nematode.

Materials and Methods

Cocoons with larvae, prepupae, pupae, or adults of the palm weevil were collected from May 1987 to May 1988 from 3–8-year-old RRD-coconut palms in King's Bay, Tobago, and in Manzanilla, Toco, and Cedros, Trinidad. The palm weevil cocoons were mostly obtained from RRD-trees which broke off at the crown ("popped-neck") because of excessive larval feeding. Cocoons, found at the basal to mid portion of the petioles, and sometimes in the decaying stem base, were stored individually in closed containers with perforated lids in the laboratory (27 ± 3 C) until the adult weevil emerged. Upon emergence each weevil was weighed and sexed and the body length was measured with calipers. Weevil measurements were taken from the rostrum (measured from the tip of the epistoma to the anterior ocular suture) and along the dorsal midline from the anterior ocular suture (tip of head) to the tip of the pygidium. Each weevil was placed separately in a beaker with 50 ml tap water for 2 hours to remove external nematodes. Nematodes in the suspension were identified and counted. This procedure was used because in our exploratory dissections and observations, 2 hours in water was sufficient to remove all associated nematodes from the exterior of the weevil. We did not observe nematodes associated under the elytra or on the wings of weevils. 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. Cocoons were also individually extracted and examined for nematodes after weevil emergence on a Baermann funnel.

Adult weevils that had previously emerged in the field were collected arbitrarily from cut petioles, crown regions, and the stems of RRD-coconut palms from Manzanilla and Centeno, Trinidad, from May 1987 to May 1988. These adults, which had already undergone different activities (i.e., feeding, mating, flying, ovipositing), were dissected and extracted as previously described within 3 days of collection.

Glycerol-supplemented potato dextrose agar (GPDA) (6) and nutrient agar (NA) were used as culture media for xenic culture attempts of dauer juveniles of free-living and predaceous nematodes extracted from palm weevils or from empty cocoons.

Total length of each weevil, length from tip of head to tip of pygidium, weight at emergence, and total number of internal nematodes per individual weevil were compared for sex and location-related differences ($P \leq 0.05$) using the general linear models procedure (17). Separation of

TABLE 1. Internal association of dispersal stage juveniles of *Rhadinaphelenchus cocophilus*, *Teratorhabditis* sp., and *Diplogasteritus* sp. with newly emerged adults of *Rhynchophorus palmarum* from Trinidad and Tobago, May 1987–May 1988.

Location†	Nematodes from female weevils			Nematodes for male weevils		
	Mean ± SD‡	Range	Infested (%)	Mean ± SD‡	Range	Infested (%)
	Rhadinaphelenchus cocophilus (45 ♀, 44 ♂)					
Total internal	1,838 ± 2,107	4–7,808	100	2,126 ± 2,515	1–11,003	91
Genital capsule	1,240 ± 1,805	2–5,880	47	899 ± 1,444	1–4,360	52
Macerated body	1,415 ± 1,975	4–7,080	89	1,694 ± 2,504	1–10,980	86
	Teratorhabditis sp. (30 ♀, 31 ♂)					
Total internal	330 ± 977	17–3,972	53	564 ± 1,676	2–7,960	71
Genital capsule	59 ± 85	7–350	50	551 ± 1,745	2–7,890	65
Macerated body	489 ± 1,290	9–3,920	30	171 ± 310	1–900	26
	Diplogasteritus sp. (30 ♀, 31 ♂)					
Total internal	924 ± 1,663	9–6,590	67	780 ± 1,174	3–4,100	77
Genital capsule	868 ± 1,653	2–6,480	50	332 ± 497	4–2,140	65
Macerated body	321 ± 615	3–2,420	57	514 ± 951	2–4,030	65

† Total internal = nematodes recovered from the genital capsule and (or) macerated body of the weevil; genital capsule = nematodes recovered from the genital capsule only; macerated body = nematodes recovered from the macerated body.
‡ Only weevils infested with a given species of nematode were used in the mean and SD calculations (zeros were omitted).

means was done with a Waller Duncan *k*-ratio *t*-test (*k*-ratio = 100, $P \leq 0.05$), and correlations were measured using the correlation procedure (17).

RESULTS AND DISCUSSION

Five species of nematodes were extracted internally from newly emerged palm weevils. Juveniles of three species of nematodes (*R. cocophilus*, *Teratorhabditis* sp., and *Diplogasteritus* sp.) were recovered in relatively large numbers from 53–100% of newly emerged palm weevils of both sexes. Ninety-one percent of males and 100% of females were infested internally with red ring nematode juveniles (Table 1). Two percent of 45 females and 5% of 44 males of the palm weevil, newly emerged from the cocoon, were externally contaminated with red ring nematodes (fewer than 90 each). Only one of the 76 cocoons inspected for nematodes after weevil emergence contained red ring nematodes (n = 456). This is not surprising, since Griffith (9) reported that nematode persistence was very poor after the host palm had died. Our results suggest that the red ring nematodes present inside the adult weevils were carried through metamorphosis, as previously reported (8).

Over 47% of the palm weevils dissected were internally infested with more than 1,000 red ring nematodes and over 73% were infested with more than 100 red ring nematodes (Fig. 1). Transmission curves have not been reported for palm weevils infested with red ring nematodes, and the effects of nematode density per vector on host longevity or fecundity have not been quantified. This kind of study should now be possible since the palm weevil can be successfully cultured free of red ring nematodes in the laboratory on pineapples and sugarcane (7). An approximate 10% nematode transfer efficiency has been reported in the analagous nematode–vector association between *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle and cerambycid beetles which together cause the pine wilt disease (20). Assuming a 10% nematode transfer efficiency during oviposition for the palm weevil and a required dose of 100 red ring nematodes into a wound for successful RRD initiation (9), 47% of the newly emerged females from this survey (Fig. 1) would have been capable of vectoring the RRD. This is a considerably higher estimate for potential weevil vectors in Trinidad than the previously reported level of 16% (9,12).

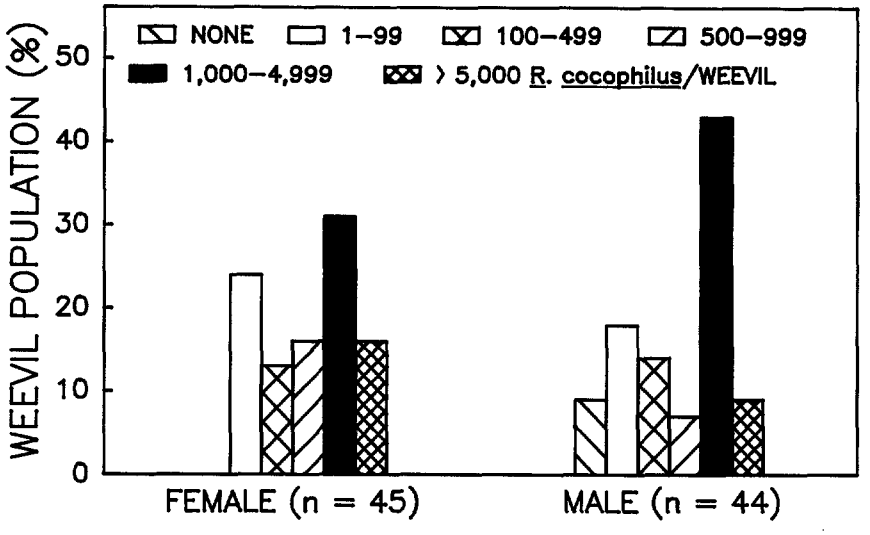

FIG. 1. Proportions of newly emerged *Rhynchophorus palmarum* internally infested with different density classes of *Rhadinaphelenchus cocophilus* juveniles.

Male palm weevils may have been capable of horizontally transferring red ring juveniles to females during copulation because they had large numbers of red ring juveniles in their genital capsules (Table 1, Fig. 1). However, their potential role in the transmission of RRD remains uncertain.

All stages of *Teratorhabditis* sp. were extracted from 82% of the 76 cocoons extracted after adult palm weevil emergence. Fifty-three percent of 30 females and 71% of 31 males examined were infested internally with dauer juveniles of *Teratorhabditis* sp. (Table 1). Most of the palm weevils had burdens of fewer than 499 dauer juveniles of *Teratorhabditis* sp. (Fig. 2).

All stages of *Diplogasteritus* sp. were extracted from 20% of the 76 extracted cocoons. Dauer juveniles of *Diplogasteritus* sp. were observed in 67% of 30 newly emerged

FIG. 2. Proportions of newly emerged *Rhynchophorus palmarum* internally infested with different density classes of *Teratorhabditis* sp. dauer juveniles.

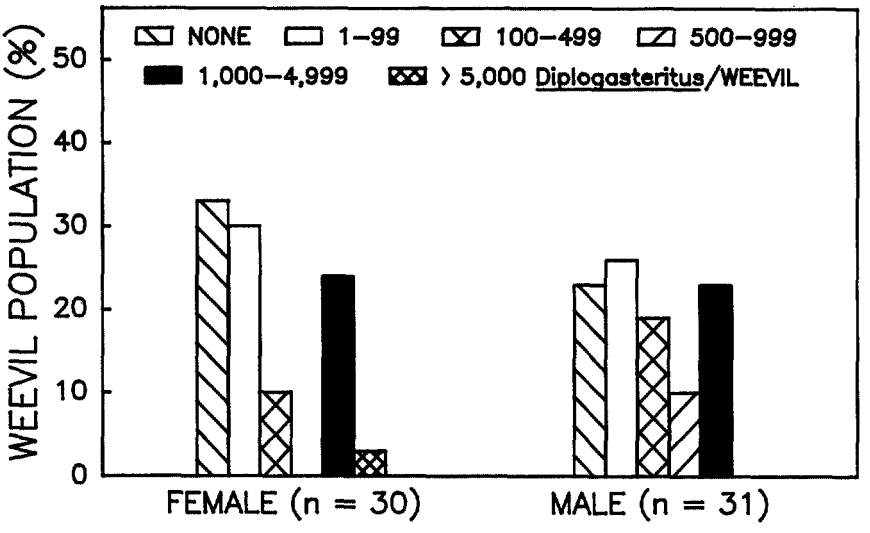

FIG. 3. Proportions of newly emerged *Rhynchophorus palmarum* internally infested with different density classes of *Diplogasteritus* sp. dauer juveniles.

females and 77% of 31 males of the palm weevil (Table 1, Fig. 3). Over 20% of the palm weevils of both sexes were internally infested with more than 1,000 *Diplogasteritus* dauer juveniles per weevil (Fig. 3).

All stages of *Mononchoides* sp. were extracted from 91% of the 76 cocoons harvested after adult palm weevil emergence. Yet, only 7% (two) of the 30 females were infested internally with 130 and 1,480 dauer juveniles of *Mononchoides* sp. and 16% of 31 males were infested internally with 80 ± 34 (30–110). The high prevalence of *Monochoides* sp. in the cocoons, but low prevalence in adult palm weevils, suggests a situation which prevented the formation of resistant dispersal stages and the natural association with the weevil. The palm weevil may not be the preferred host for this nematode. Many insects are attracted to dying palms and are commonly associated with the palm weevil. Insects occupying the same habitat can function as surrogate commensal hosts for nematodes (5). *Mononchoides adjunctus* Massey has been reported to be associated with bark beetles in ponderosa pine (15).

Bursaphelenchus sp. (close to *B. corneolus* Massey) was extracted from 5% of 76 cocoons at two sites (Manzanilla and Cedros, Trinidad); 160 dauer juveniles were extracted internally from a single newly emerged male palm weevil from Manzanilla.

Dauer juveniles of *Teratorhabditis* sp. from palm weevils were cultured xenically on NA and GPDA and needed to be subcultured about once every 3 weeks. *Diplogasteritus* sp. were cultured xenically on NA and GPDA, but needed to be subcultured on NA at least once every 2 weeks. *Mononchoides* sp., a predaceous genus in the order Diplogasterida, was cultured on prey from cultures of *Teratorhabditis* sp. and (or) *Diplogasteritus* sp. on NA or GPDA. In addition, we observed that this *Mononchoides* sp. was capable of consuming juveniles of the red ring nematode in water suspensions. *Bursaphelenchus* sp. was cultured xenically on GPDA and needed to be subcultured monthly.

Adult palm weevils from the field, of unknown life history and age, were parasitized with red ring nematodes to a lesser degree that newly emerged weevils from cocoons. Only 53% of 15 female weevils were parasitized with 1,561 ± 1,446 (12–3,560) red ring nematodes and 53% of 15 male weevils carried 2,678 ± 2,212 (23–6,680) red ring nematodes internally. Thirty-three percent of 15 female weevils and 13% of 15 male weevils were associated

TABLE 2. Morphometrics of newly emerged palm weevils from cocoons from red ring diseased-coconut palms from Trinidad and Tobago.

	Female (n = 45)		Male (n = 44)	
	Mean ± SD	Range	Mean ± SD	Range
Total length (mm)†	53 ± 5	38–60	53 ± 4	44–58
Body length (mm)	40 ± 4	28–46	40 ± 3	33–44
Weight (g)	2.68 ± 0.67	0.90–3.87	2.65 ± 0.50	1.47–3.49

† Length of the rostrum (measured from the tip of the epistoma to the anterior ocular suture) plus body length (measured from the anterior ocular suture to the tip of the pygidium along the dorsal midline of the body).

with red ring nematodes externally. In addition to *Teratorhabditis* sp., *Diplogasteritus* sp., and *Mononchoides* sp., a bisexual rhabditid nematode, *Rhabditis* sp., was extracted from the genital capsule and macerated whole body of weevils. Seventy-three percent of 15 female palm weevils were internally infested with 1,118 ± 2,120 (7–7,420) *Rhabditis* sp., and 67% of 15 male palm weevils carried 675 ± 487 (19–1,332) *Rhabditis* sp. The majority of *Rhabditis* sp. occurred as dauer juveniles inside the adult weevils, but a few adult nematodes were observed. Surprisingly, the *Rhabditis* sp. was never recovered from newly emerged adult weevils. The data suggest some artifact in our laboratory rearing procedure that selected against this nematode or that the *Rhabditis* sp. is acquired post-emergence. This nematode goes through its life cycle quickly in laboratory cultures, and it is prone to die off if not subcultured weekly on NA. Thus, under the artificial conditions of this study, the nematodes may have died in the cocoons before adult weevil emergence when they might have normally associated with the weevil host. This *Rhabditis* sp. is very similar to the *Rhabditis* sp. previously illustrated from the palm weevil (9).

Only two species of nematodes, *Rhadinaphelenchus cocophilus* and *Rhabditis* sp., have been previously reported to be associated with the palm weevil (9). The *Teratorhabditis* sp., *Diplogasteritus* sp., *Mononchoides* sp., *Bursaphelenchus* sp., and *Rhabditis* sp. observed in this study all appear to be new species that are commensally associated with the palm weevil. The red ring nematode appears to be parasitically associated with the weevil, as previously reported (12).

There was no significant sex or location-related differences ($P > 0.05$) between weevils for the parameters of total length, length from tip of head to tip of pygidium, weight at emergence, total internal red ring nematodes, total internal *Teratorhabditis* sp., total internal *Diplogasteritus* sp., or total internal *Mononchoides* sp. The mean pooled total length, length from the anterior ocular suture (tip of head) to tip of pygidium, and weight at emergence are in Table 2. Our measurements of the palm weevil were similar to those reported by Wattanapongsiri (21).

There was no significant correlation between total length in female weevils ($r = 0.139$, $P = 0.36$, n = 45) or males ($r = 0.016$, $P = 0.92$, n = 44) and the total number of red ring nematodes extracted internally. In addition, there was no correlation between weevil length (tip of head to tip of pygidium) and the total number of red ring nematodes extracted internally in females ($r = -0.163$, $P = 0.28$, n = 45) or in males ($r = 0.044$, $P = 0.78$, n = 44). Weevil weight at emergence was correlated with total length and length from tip of head to tip of pygidium (females: $r = 0.827$, $P = 0.0001$, n = 45; males: $r = 0.770$, $P = 0.0001$, n = 44) but not with total number of red ring nematodes extracted internally (females: $r = -0.189$; $P = 0.21$, n = 45; males: $r = -0.053$; $P = 0.73$, n = 44). In addition, there were no significant correlations between any weevil size parameter and total number of red ring nematodes extracted internally in previously emerged field-collected weevils of either sex.

We conclude that neither length nor emergence weight can be used as reliable indicators of the red ring nematode vector potential of the palm weevil populations in Trinidad and Tobago. In addition, future work on the vector potential of field populations of the palm weevil should focus on newly emerged weevils and care should be taken in distinguishing the red ring nematode juveniles from the equally numerous co-occurring nematode associates.

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