ASSOCIATION OF THE RED RING NEMATODE, *RHADINAPHELENCHUS COCOPHILUS*, WITH WEEVILS FROM ECUADOR AND TRINIDAD[†]

Karin Gerber,[1,‡] Robin M. Giblin-Davis,[1] and Jenny Escobar-Goyes[2]

Fort Lauderdale Research and Education Center, University of Florida, IFAS, 3205 College Avenue, Fort Lauderdale, FL 33314, U.S.A.;[1] and Department of Crop Protection, University of Guayaquil, P. O. Box 3293, Guayaquil, Ecuador.

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ABSTRACT

Gerber, K., R. M. Giblin-Davis, and J. Escobar-Goyes. 1990. Association of the red ring nematode, *Rhadinaphelenchus cocophilus*, with weevils from Eccuador and Trinidad. Nematrópica 20:39–49.

Cocoons with larvae, prepupae, or pupae of the palm weevil, *Rhynchophorus palmarum* (Coleoptera: Curculionidae), were collected from red ring diseased (RRD) coconut palms, *Cocos nucifera* in Ecuador and Trinidad. Each stage of the weevil was removed from its cocoon, surface-disinfested, and allowed to emerge as an adult. Only 31% of the newly emerged weevils from RRD coconut palms from Ecuador (n = 16) were infested internally with the red ring nematode, *Rhadinaphelenchus cocophilus*, compared with 83% of the weevils (n = 24) from Trinidad. Infestation levels of the newly emerged palm weevils with *Diplogasteritus* sp. and *Teratorhabditis* sp. were low. In Ecuador, the weevil *Dynamis borassi* was observed to do significant damage to the inflorescences of 15–20-year-old coconut palms. It can carry a high number of red ring nematodes internally through metamorphosis suggesting that it may be a vector of RRD in Ecuador.

Key words: coconut palm, *Cocos nucifera, Diplogasteritus, Dynamis borassi*, Ecuador, palm weevil, red ring disease, red ring nematode, *Rhadinaphelenchus cocophilus, Rhynchophorus palmarum, Teratorhabditis*, Trinidad, *Xyleborus*.

RESUMEN

Gerber, K., R. M. Giblin-Davis, y J. Escobar-Goyes. 1990. La asociación del nematodo del anillo rojo, *Rhadinaphelenchus cocophilus*, con gorgojos del Ecuador y Trinidad. Nematrópica 20:39–49.

Se recolectaron cuncunas con larvas, prepupas o pupas del gorgojo de la palma, *Rhynchophous palmarum* (Coleoptera: Curculionidae) de cocoteros afectados de la enfermedad del anillo rojo en Trinidad y Ecuador. Cada estadío del gorgojo fue removido de su cuncuna, desinfectado en superficie y dejado para emerger como adulto. Solamente el 31% de los gorgojos recién emerjidos provenientes de cocoteros del Ecuador (n = 16) fueron infestados internamente con el nematodo del anillo rojo, *Rhadinaphelenchus cocophilus*, en contraste con el 83% de los gorgojos (n = 24) de Trinidad. Niveles de

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[‡]Present address: Federal Institute of Agrobiology, Wieningerstrasse 8, A-4025 Linz, Austria.

infestación de con *Diplogasteritus* sp. y *Teratorhabditis* sp. en gorgojos recién emerjidos fue bajo. En Ecuador, se observó que el gorgojo *Dynamis borassi* puede causar daño significativo a la inflorescencia de cocoteros de 15 a 20 años. Este gorgojo además puede mantener un alto número de nematodos del anillo rojo internamente a través de la metamorfósis, sugiriendo que puede ser un vector de la enfermedad del anillo rojo en Ecuador.

Palabras claves: cocotero, *Cocos nucifera*, *Diplogasteritus*, *Dynamis borassi*, Ecuador, enfermedad del anillo rojo, gorgojo de la palmera, nematodo del anillo rojo, *Rhadinaphelenchus cocophilus*, *Rhyncophorus palmarum*, *Teratorhabditis*, Trinidad, *Xyleborus*.

INTRODUCTION

Red ring disease (RRD) of the coconut palm, *Cocos nucifera* L., is caused by the red ring nematode, *Rhadinaphelenchus cocophilus* (Cobb) Goodey. RRD is usually lethal to its host and can cause serious losses to coconut and oil palm plantations in the Neotropics (3,8). RRD has been observed in Trinidad since 1905 (8) and has been studied extensively there. The first report of RRD in Ecuador was in 1967 (2). The palm weevil, *Rhynchophorus palmarum* L., (Fig. 1 A–B) is generally regarded as the vector of the red ring nematode and has been known as 'Gualpa' for more than 60 years in the coconut growing areas of Ecuador (12).

The association between *R. palmarum* and *R. cocophilus* varies depending upon location (8). Recent studies in Trinidad (4) showed that over 90% of newly emerged *R. palmarum* were infested internally with red ring nematode juveniles and > 47% contained > 1 000 red ring nematodes each. No information is available on the interaction between the red ring nematode and *R. palmarum* in Ecuador. The incidence of red ring nematode-infested coconut palms was reported as being low in Ecuador and the palm weevil is considered a major pest of healthy palms without being associated with *R. cocophilus* (8).

This study was done to confirm observations of the occurrence of RRD in Ecuador and to compare the association between *R. cocophilus* and palm weevils from Trinidad and Ecuador. During this study, observations concerning *Dynamis borassi* Fabricius (Coleoptera: Curculionidae) and *Xyleborus* sp. (Coleoptera: Scolytidae) were made. Details of the association of these two species with RRD and the decline of coconut palms in Ecuador are presented.

MATERIALS AND METHODS

Cocoons with larvae, prepupae, or pupae of the palm weevil, *R. palmarum*, were collected from RRD coconut palms from the Cocal plantation of Huggins Trust in Manzanilla, Trinidad during February – May 1988. Collections in Ecuador were made at the Haciendas Acapulco, Costa Rica, Lagartillo, Las Penas, Molina, (Esmeraldas Province) and Hacienda Camilita (Manabi Province) during January – February 1988. The age of the red ring nematode and weevil-infested

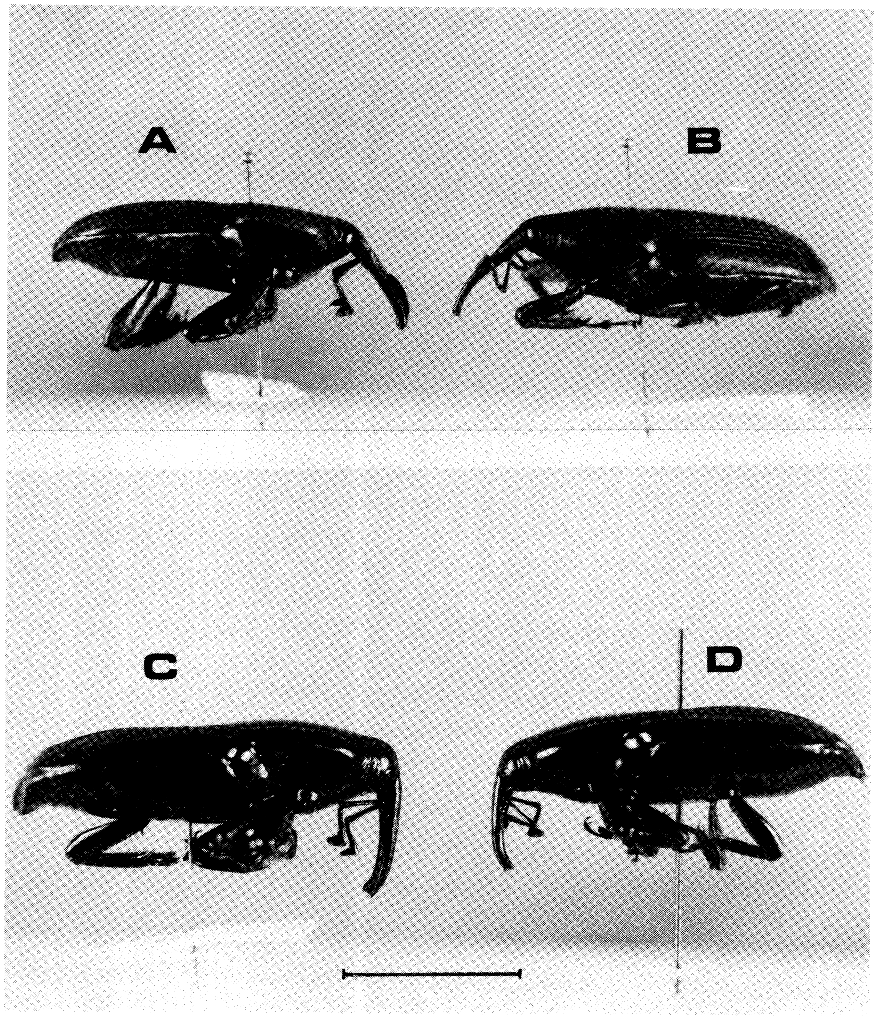

Fig. 1. Weevil species associated with red ring nematodes in coconut palms collected from La Tola, Esmeraldas Province, Ecuador in January 1988. A) Male *Rhynchophorus palmarum*. B) Female *Rhynchophorus palmarum*. C) Male *Dynamis borassi*. D) Female *Dynamis borassi*. (Bar = 2 cm).

coconut palms usually ranged from 3–8 years in Trinidad and from 4–10 years in Ecuador. In both countries, the coconut palms were mostly tall cultivars with unknown parentage.

Each weevil was removed carefully from its cocoon, immersed in Lugol's solution (1 g iodine, 2 g potassium iodide, and 100 ml water) for 3 minutes, rinsed in distilled water, and patted dry. Nematodes which occurred externally and numerous mites were removed and killed

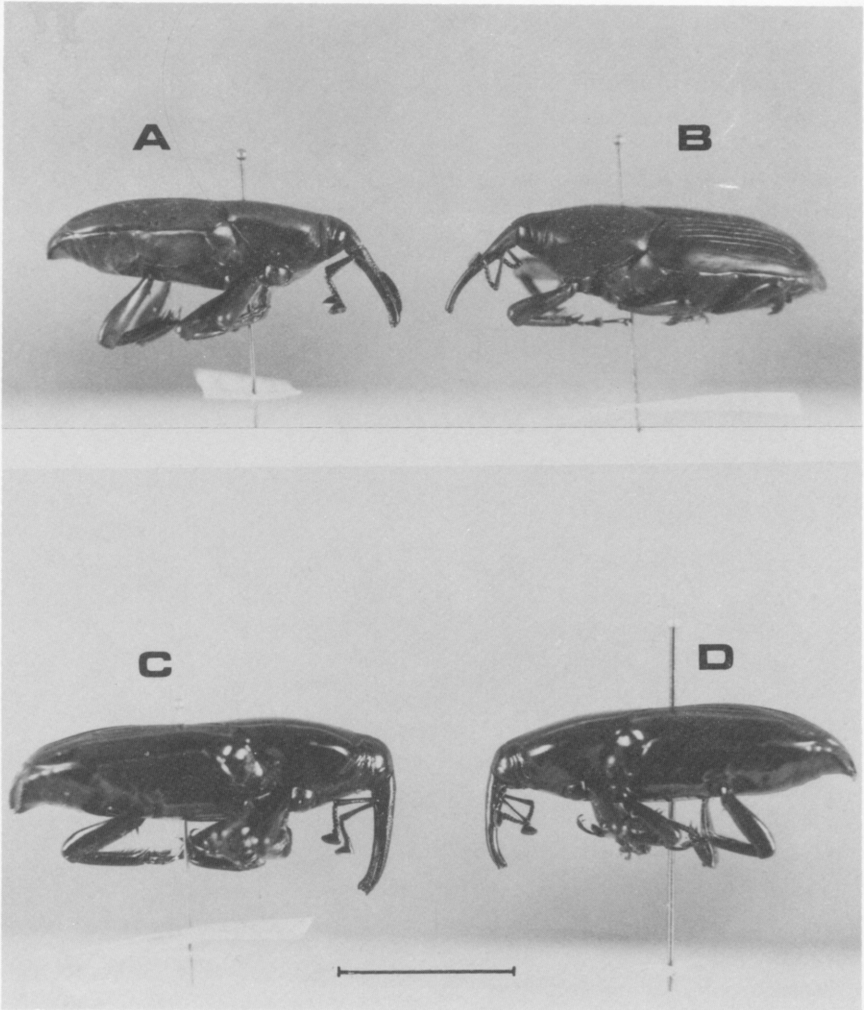

Fig. 1. Weevil species associated with red ring nematodes in coconut palms collected from La Tola, Esmeraldas Province, Ecuador in January 1988. A) Male *Rhynchophorus palmarum*. B) Female *Rhynchophorus palmarum*. C) Male *Dynamis borassi*. D) Female *Dynamis borassi*. (Bar = 2 cm).

by this method. Surface-disinfested weevils were placed individually in a closed container with a perforated lid that contained a single tissue paper moistened with 4 ml of distilled water. The containers were held at 27 ± 3 C until adult emergence. At emergence, each weevil was weighed, sexed, and the body length measured with calipers. Weevil measurements were taken as described by Gerber and Giblin-Davis (4) along the dorsal midline of the weevil. Newly emerged weevils were forced to swim for 2 hours in 50 ml of tapwater to check for external nematodes. Any nematodes recovered in the water were identified and counted. Each rinsed weevil was decapitated and dissected; the genital capsule and macerated body were extracted separately for 24 hours in Baermann funnels, each with a small piece of cotton at the outlet (4). Nematode suspensions were collected and nematodes were counted.

In Ecuador, cocoons with pupae (Esmeraldas, n = 3; Manabi, n = 2) and adults (Esmeraldas, n = 4; Manabi, n = 2) of the weevil, *D. borassi* (Fig. 1 C–D) were recovered from 10–20-year-old coconut palms that were declining, or showed crown, inflorescence, and/or leaf stalk larval feeding damage, but showed no red ring symptomatology. The five pupae were surface-disinfested of nematodes and allowed to emerge separately, as described for *R. palmarum*. The newly emerged *D. borassi* adults were not sexed but were measured, weighed, and cleaned prior to dissection and nematode harvest as described above.

Total length of each weevil, weight at emergence, and numbers of internally associated red ring nematodes ($\log_{10}$ transformed) were compared for sex, location, and stage-related differences ($P \leq 0.05$) with an ANOVA using the general linear models procedure for unbalanced data (11). Separation of means was done with a Waller Duncan *k*-ratio *t*-test ($k = 100$) and correlations were determined (11).

RESULTS AND DISCUSSION

Rhynchophorus palmarum, collected as pupae from red ring nematode-infested coconut palms, disinfested externally, and allowed to emerge separately, were free of nematodes externally but were infested internally (Table 1). There was a greater proportion of females and males of *R. palmarum* parasitized (total internal and macerated body) with red ring nematodes from Trinidad than from Ecuador (Table 1). The mean number of red ring nematodes per infested *R. palmarum* was not significantly different between locations for total internal and genital capsule infestations. However, males from Ecuador emerged with significantly fewer red ring nematodes than females or males and females from Trinidad. This may be due to the relatively low numbers of male weevils that emerged and were examined from Ecuador. There was no significant difference ($P = 0.30$) between the number of red ring nematodes

Table 1. Internal association of dispersal stage juveniles of *Rhadinaphelenchus cocophilus* with adults of *Rhynchophorus palmarum* from red ring diseased coconut palms from Trinidad and Ecuador that were disinfested externally of nematodes as pupae and allowed to emerge (January 1988–May 1988).

	Trinidad[x]				Ecuador[x]			
	Females (n = 13)		Males (n = 11)		Females (n = 9)		Males (n = 7)	
	No. nematodes[y]	Infested (%)	No. nematodes	Infested (%)	No. nematodes	Infested (%)	No. nematodes	Infested (%)
Total internal[z]	2 243 ± 2 768 (15–8 780)	85	1 169 ± 1 497 (41–4 722)	82	648 ± 640 (195–1 100)	22	4 362 ± 7 838 (16–13 613)	43
Genital capsule	1 346 ± 1 706 (2–3 580)	31	1 587 ± 2 089 (6–4 510)	36	33 (33)	11	6 808 ± 9 607 (15–13 600)	29
Macerated body	1 753[a] ± 2 098 (15–5 770)	85	464[a] ± 493 (41–1 430)	82	631[a] ± 663 (162–1 100)	22	24[b] ± 29 (1–57)	43

[x]Weevil pupae were collected from Manzanilla, Trinidad and Esmeraldas Province, Ecuador from red ring nematode-infested coconut palms.
[y]Mean ± standard deviation; only weevils that were infested with red ring nematode were used in the mean and S. D. calculations and statistical comparisons (zeros were omitted). General linear models procedure was performed with $\log_{10}$ transformed values and means separation was performed only on rows with a significant F value using the Waller-Duncan k-ratio t-test. Means with different letters are significantly different at $P \leq 0.05$.
[z]Total internal = red ring nematodes recovered from the genital capsule and (or) macerated body of the weevil; Genital capsule = red ring nematodes recovered from the genital capsule only; Macerated body = red ring nematodes recovered from the macerated body.

recovered from adults reared from the different stages (larvae, prepupae, or pupae) from Trinidad, suggesting that red ring nematode juveniles parasitize *R. palmarum* before or at the time of cocoon preparation and are carried internally through metamorphosis.

The mean length and weight of newly emerged *R. palmarum* from disinfested larvae were significantly less than those from prepupae and pupae (Table 2). The reason for these morphometric differences is not known but may be due to sampling error. Size of weevil adults is positively correlated to nutrition, and oviposition is not synchronized in *Rhynchophorus*. Developmental asynchrony and competition for limited resources would imply that adults reared from pupae and prepupae received better nutrition than those reared from larvae. In addition, female *R. palmarum* from Ecuador were usually heavier than males or females from Trinidad (Table 2). In adult weevils that emerged from surface-disinfested pupae, there was no significant correlation between the total weevil length in females and the number of red ring nematode parasites (Trinidad, $r = 0.458$, $P = 0.12$, n = 13; Ecuador, $r = 0.317$, $P = 0.41$, n = 9). In male *R. palmarum*, there was a significant positive correlation between weevil length and red ring nematode parasitism in Trinidad ($r = 0.640$, $P = 0.03$, n = 11), but not in Ecuador ($r = 0.297$,

Table 2. Measurements of newly emerged *Rhynchophorus palmarum* adults from Trinidad and Ecuador disinfested externally of nematodes at various stages and reared to adults.

Location	Sex	Stage disinfested	n	Total length (mm)[z]	Weight at emergence (g)
Trinidad	F	larva	16	$44^{c} \pm 5$ (35–51)	$1.55^{d} \pm 0.39$ (0.85–2.23)
Trinidad	M	larva	2	$46^{c} \pm 1$ (45–47)	$1.91^{d} \pm 0.35$ (1.66–2.16)
Trinidad	F	prepupa	6	$54^{ab} \pm 3$ (49–56)	$3.09^{abc} \pm 0.46$ (2.56–3.75)
Trinidad	M	prepupa	3	$52^{ab} \pm 3$ (49–54)	$2.81^{c} \pm 0.50$ (2.76–2.86)
Trinidad	F	pupa	13	$52^{ab} \pm 4$ (46–58)	$2.63^{c} \pm 0.56$ (1.56–3.68)
Trinidad	M	pupa	11	$55^{a} \pm 3$ (51–62)	$2.92^{bc} \pm 0.55$ (2.17–3.66)
Ecuador	F	pupa	9	$54^{ab} \pm 3$ (51–59)	$3.56^{a} \pm 0.45$ (3.05–4.39)
Ecuador	M	pupa	7	$51^{b} \pm 5$ (44–57)	$3.21^{ab} \pm 0.32$ (2.76–3.75)

[z]Mean ± standard deviation (range); means followed by different letters in a column are significantly different according to the Waller Duncan *k*-ratio *t*-test at $P \leq 0.05$.

$P = 0.52$, n = 7). These results do not support the contention that total length may be an indicator of the number of red ring nematodes carried by *R. palmarum* (7).

The proportion of red ring nematode-infested adult *R. palmarum* with > 1 000 juveniles of *R. cocophilus* internally was similar in Trinidad and Ecuador (between 40–50%) (Table 3). The major difference between the association of red ring disease with *R. palmarum* from Trinidad and from Ecuador was the low proportion of infested weevils found in RRD coconut palms in Ecuador (Table 3).

In addition to *R. cocophilus,* two genera of nematodes were recovered from disinfested weevils. *Teratorhabditis* sp. was rarely recovered from disinfested pupae removed from their cocoon and allowed to emerge. This contrasts with the high degree of association of *Teratorhabditis* in newly emerged *R. palmarum* from cocoons reported by Gerber and Giblin-Davis (4) and suggests that *Teratorhabditis* sp. has a more peripheral association with its host than *R. cocophilus* and may infest the insect at the time of adult emergence. *Diplogasteritus* sp. was recovered from 31% (n = 16; 371 ± 566 [range = 7–1 325 nematodes/host]) and 50% (n = 2; [479 nematodes/host]) of the disinfested weevil larvae from Trinidad that emerged as adult females and males, respectively. *Diplogasteritus* sp. infested < 15% of the treated pupae from either Trinidad or Ecuador that were allowed to emerge. Infestations were < 67 nematodes per insect. These infestation levels are much lower than reported for weevils reared from cocoons in Trinidad (4). None of the other species of nematodes reported from *R. palmarum* (4) were found in adults that emerged from disinfested stages of the weevil in either country.

Three of the five *D. borassi* from Ecuador, which were reared from disinfested pupae to adults, were parasitized with 1 995 and 4 (Esmeral-

Table 3. Percentage of adults of *Rhynchophorus palmarum* that were infested internally with different levels of *Rhadinaphelenchus cocophilus* juveniles.

	Trinidad		Ecuador	
No. of nematodes	% of all weevils (n = 24)[z]	% of infested weevils (n = 20)	% of all weevils (n = 16)	% of infested weevils (n = 5)
0	17	—	69	—
1–99	21	25	13	40
100–499	8	10	6	20
500–999	13	15	0	0
1 000–4 999	33	40	6	20
>5 000	8	10	6	20

[z]Newly emerged adult males and females of *R. palmarum* from surface-disinfested pupae that had been collected from red ring diseased coconut palms from Trinidad and Ecuador.

das Province), and 181 (Manabi Province) juveniles of *R. cocophilus*. None of the six adult weevils in cocoons were parasitized.

A large number of insects in a variety of orders have been implicated as potential vectors for RRD (3,9). However, the role of insects other than *R. palmarum* as vectors of RRD has not been established because of the very low numbers of red ring nematodes carried (< 12 nematodes per insect) (9). Dean (3) reported that *D. borassi* (= *Dynamis politus* Gyllenhal) serves as a vector of RRD in Brazil but gave no details.

At the plantation Acapulco (Esmeraldas Province, Ecuador) the crown regions of 15 mature and bearing coconut palms (10–15 years old, > 7 m tall, green and yellow cultivars) were checked from a ladder. Only trees that had observable inflorescence damage from ground level were examined in the crown region. Unopened inflorescences infested with weevil larvae or cocoons were harvested. In four palms the identity of the weevils was confirmed as *D. borassi* by rearing to the adult stage. The identity of the large weevils in the remaining 11 trees was not confirmed due to the death of the collected pupae, but it is assumed that they also were *D. borassi*. Up to five larvae and four cocoons were observed in one inflorescence. The weevils seemed to complete their life cycle inside the inflorescence, although this was not confirmed. Damaged inflorescences usually remained closed, broke off easily at the base, and often were dried out. Weevil damage to the inflorescences on coconut palms 15–20 years old was very common and widespread at the Haciendas Acapulco, Costa Rica, Molina, and Lagartillo, Esmeraldas Province, and Hacienda Juananu, Manabi Province, Ecuador. In many of the coconut estates, weevil control involved insecticide applications every 3 months into the crown region of the palm combined with removal of attacked inflorescences. More detailed research concerning the biology and control of *D. borassi* is needed in Ecuador.

Dynamis borassi reportedly lays its eggs in the terminal tissue of *C. nucifera, Syagrus schizophylla* Martius, and *Syagrus vagans* Bondar. The larvae tunnel down from the tip of these palm hosts to the base of the trunk and the life cycle is completed in about 6 months. The biology of *D. borassi* and *R. palmarum* is similar except for the preference by *D. borassi* for young, live terminal tissue and for its longer life cycle (12). It is possible that coconut palms attacked and stressed by infestations of *D. borassi* could be attacked subsequently by red ring nematode-infected *R. palmarum*. This secondary infestation could foster an association between *D. borassi* and *R. cocophilus* which could explain our observations.

That *D. borassi* carried internally a large number of red ring nematode juveniles through metamorphosis in Ecuador, suggests that it may be a vector for the disease under certain circumstances. In addition, it suggests that other members of the genus *Rhynchophorus*, such as the palmetto weevil, *R. cruentatus* Fabricius, from North America, may be able to transmit the red ring nematode if allowed to complete its life cycle in an appropriate red ring diseased host (6).

In Trinidad, the association between *R. palmarum*, the red ring nematode, and coconut palms involved mostly trees that were 3–10 years old. *Rhynchophorus palmarum* infestations without incidence of RRD were rare. The latter probably explains the high infestation level of the palm weevil population with red ring nematodes in Trinidad (4). We did not observe *D. borassi* in Trinidad. In 1930, one male of *D. borassi* was reported from Trinidad (12). It is apparently rare.

Our observations in Ecuador suggest a more complicated situation for RRD when compared with what has been observed for Trinidad. For example, 38% of the 29 declining or dead palms that we cut in Ecuador had symptoms of RRD. These 11 palms were < 10 years old, in various stages of decline, and had a typical red ring in cross section. Seventy-three percent of these 11 RRD coconut palms were infested with *R. palmarum* of various stages, 18% were infested with both *R. palmarum* and *Xyleborus* sp., and 9% were infested with *Xyleborus* sp. only. Cocoons of *R. palmarum* in these RRD palms were usually found in the leaf stalks or in the stem base. Twenty-four percent of the 29 cut palms were infested with *R. palmarum* without red ring symptoms. These palms ranged in age from 5–20 years and *R. palmarum* cocoons were found mostly in the stem base, leaf stalks, or unopened inflorescences. Seven percent of the 29 cut palms contained various stages of *R. palmarum* and *D. borassi* without red ring symptomatology. These two palms were > 10 years old and weevil cocoons were recovered from unopened inflorescences. Although these palms were asymptomatic for red ring disease, the disinfested pupae of both weevil species from one of the palms contained red ring nematodes internally. Because the weevils carrying red ring nematodes were found in the inflorescence, the red ring nematodes probably were restricted to that tissue. Blair (1) reported that the red ring nematode is not capable of passing through the fruitstalk into the stem tissue. The numerous small bundles separated by thin bands of lignified parenchymal cells seem to prevent movement of the red ring nematode (2). RRD was not observed in mature palm trees that were only attacked in the inflorescence by palm weevils in Ecuador. One of the 29 cut palms was infested with *D. borassi* without symptoms of RRD. This palm was 20 years old and the cocoons of *D. borassi* were recovered from an unopened inflorescence. Twenty-eight percent of the 29 cut palms did not have a red ring, and weevils that were collected from these palms were not reared successfully out for confirmation of identity. In more than half of these eight palms, *Xyleborus* sp. was recovered.

In Ecuador, the internal diagnosis of RRD in coconut palms can be confused with a reddish discoloration which is observed in *Xyleborus* sp.

infested stem and leaf tissue. The scolytid *Xyleborus* sp. (locally called 'Cernidera') or a possible bacterial or fungal pathogen associated with the insect causes a discoloration of the stem tissue which is dark pink to reddish-brown in color. Sometimes stem tissue was observed with a diffuse ring in cross section. This discolored stem tissue of the few *Xyleborus* infested trees that we examined did not contain red ring nematodes. It is not clear whether *Xyleborus* sp. is a secondary or primary attacker of palms in Ecuador. In Manabi, however, only *Xyleborus* sp. infestations and damge were observed in three dying coconut trees (20 years old). *Xyleborus perforans* Wollaston is reported to be a pest of unhealthy trees in Jamaica and the Seychelles but is a primary pest in Fiji (10). Generally, very little information is available on this potentially important coconut pest in Ecuador. *Xyleborus* sp. also can occur together with RRD and palm weevils in Ecuador and its potential role in the disease complex is unclear.

There are differences between the RRD complex that we observed in Trinidad and Ecuador. For example, the percentage of *R. palmarum* that were associated with *R. cocophilus* from RRD coconut palms was much lower in Ecuador. Gerber et al. (5) have reported morphometric differences between *R. cocophilus* isolated from coconut palms in Ecuador and Trinidad. It is possible that geographic differences in the coconut hosts, nematode populations, and weevil vectors exist which acount for the variability between the RRD associations in Ecuador and Trinidad. It also is possible that the association between *R. palmarum* and *R. cocophilus* may be relatively recent in Ecuador and that the dynamics between the nematode and its vector(s) have not stabilized. *Dynamis borassi* may be a vector of *R. cocophilus* in Ecuador and also may serve to predispose palms to attack by *R. palmarum* with or without the red ring nematode.

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