MORPHOMETRIC COMPARISONS OF GEOGRAPHIC AND HOST ISOLATES OF THE RED RING NEMATODE, *RHADINAPHELENCHUS COCOPHILUS*[1]

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ABSTRACT

Gerber, K., R. M. Giblin-Davis, R. Griffith, J. Escobar-Goyes, and A. D'Ascoli Cartaya. 1989. Morphometric comparisons of geographic and host isolates of the red ring nematode, *Rhadinaphelenchus cocophilus*. Nematrópica 19:151–159.

Morphometrics of adult females and males of the red ring nematode, *Rhadinaphelenchus cocophilus*, were compared from red ring diseased (RRD) stem tissue from the coconut palm, *Cocos nucifera* L., from several plantations in Trinidad; from a RRD coconut palm in Esmeraldas, Ecuador, and from oil palms, *Elaeis guineensis*, with RRD and little leaf symptomatology in San Felipe, Venezuela. Also, juvenile *R. cocophilus* (J2, JIII) from coconut palm tissue and JIII's from the palm weevil vector of RRD, *Rhynchophorus palmarum*, were measured. Morphometric differences observed between the different populations of adult *R. cocophilus* in this study probably represent infraspecific variability.

Key words: African oil palm, coconut palm, *Cocos nucifera, Elaeis guineensis*, Ecuador, little leaf, morphometrics, palm weevil, red ring disease, red ring nematode, *Rhadinaphelenchus cocophilus, Rhynchophorus palmarum*, Trinidad, Venezuela.

RESUMEN

Gerber, K., R. M. Giblin-Davis, R. Griffith, J. Escobar-Goyes y A. D'Ascoli Cartaya. 1989. Comparaciones morfométricas entre aislamientos geográficos y de hospedantes del nematodo del anillo rojo, *Rhadinaphelenchus cocophilus*. Nematrópica 19:151–159

Se compararon las morfometrías de machos y hembras adultos del nematodo del anillo rojo *Rhadinaphelenchus cocophilus* procedentes de tejidos de cocoteros (*Cocos nucifera*) afectados, de varias plantaciones en Trinidad, de un cocotero de Esmeraldas, Ecuador, así

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como de palmas aceiteras (*Elaeis guineensis*) de San Felipe, Venezuela que mostraban sintomatología de "hoja chica" y de anillo rojo. También se tomaron medidas de juveniles (J2, JIII) de tejidos de cocotero y JIII del gorgojo de la palma (*Rhynchophorus palmarum*), vector del anillo rojo. Las diferencias morfométricas observadas entre las diferentes poblaciones adultas de *R. cocophilus* en este estudio se deben probablemente a variaciones conespecificas.

Palabras claves: anillo rojo, cocotero, *Cocos nucifera*, Ecuador, *Elaeis guineensis*, gorgojo de la palma, hoja chica, morfometría, nematodo del anillo rojo, palma aceitera africana, *Rhadinaphelenchus cocophilus, Rhynchophorus palmarum*, Trinidad, Venezuela.

INTRODUCTION

The red ring nematode, *Rhadinaphelenchus cocophilus* (Cobb) Goodey, was first described from roots of the coconut palm, *Cocos nucifera* L., from Grenada, West Indies (2). The nematode is known as the causal agent of the destructive red ring disease (RRD) which affects coconut palms, African oil palms (*Elaeis guineensis* Jacquin) and several other species of palms in the Neotropics (1,4,10,19). Presently, RRD of the coconut palm is reported to occur in all countries of Central America, in many countries of South America (Brazil, Colombia, Ecuador, Guyana, Peru, and Venezuela) and the southern Caribbean (Grenada, St. Vincent, Tobago, and Trinidad) (4). Natural occurrences of RRD of the oil palm have been reported from Brazil (5,17,27), Colombia (13,20,24,25,30), Costa Rica (23), Ecuador (Oriente; pers. obs. Escobar-Goyes), Guyana (4), Surinam (15), and Venezuela (18,21). Symptoms of RRD vary from different geographical locations and are affected by host palm species, cultivar, age, and growing conditions (4,12,16). *Rhadinaphelenchus cocophilus* also is associated with little leaf symptoms in coconut and oil palms in Surinam (11,15). Coconut palms in Guyana also showed this disorder and the red ring nematode was recovered from young leaves (11). The palm weevil, *Rhynchophorus palmarum* (L.), acts as a vector for this serious disease (4,10).

There are questions about whether different host isolates of *Rhadinaphelenchus* are the same species (23). An analysis of intra- and interdemic morphometric variability in *R. cocophilus* populations is needed as a first step towards resolving such questions. This study was done to compare the morphometrics of adults of *R. cocophilus* from different geographical regions, palm hosts, and palms with different symptomatology. In addition, measurements from juveniles of different developmental stages of *R. cocophilus* were compared.

MATERIALS AND METHODS

Red ring nematode isolates from coconut palms from Trinidad and Ecuador: Red ring nematode-infected stem tissue was collected from 3-6-year-old coconut palms in Trinidad (Manzanilla, Cedros, Wallerfield; 1987 and

1988) and from a 3-year-old coconut palm in Ecuador (La Tola, Esmeraldas, near power station of Escamarlas; January 1988). Persistent stages (3rd stage juveniles; JIII) of the red ring nematode usually were numerous in the basal portion of the coconut stem where a solid ring of reddish discoloration was observed. Adult nematodes, juvenile stages, and eggs were recovered from uncoalesced reddish lesions in the upper stem portions and heart of trees. Red ring nematodes from coconut palms were extracted from discolored tissues as described by Giblin-Davis et al. (6).

Red ring nematode isolates from the palm weevil, Rhynchophorus palmarum: A male palm weevil that was still in its cocoon was collected from a coconut palm with RRD in Manzanilla, Trinidad. The weevil was allowed to swim for 2 hours to remove externally associated nematodes. Subsequently, it was killed, dissected, and placed on a Baermann funnel overnight. Red ring nematodes obtained from the genital capsule of the weevil were measured in temporary water mounts.

Red ring nematode isolates from oil palms in Venezuela: Red ring nematodes were extracted from upper stem tissue of a 29-year-old oil palm (tenera hybrid) with RRD at the plantation C. A. Bananera Venezolana, San Felipe (April 1988). An indistinct ring 7 cm from the stem periphery was observed in this 5-m-tall palm. The ring consisted of two portions: an outer, light-brown portion (2 cm wide) and an inner irregular yellow portion (3 cm wide). Adult red ring nematodes were extracted from the 3-m stem height, where the ring was distinct with a brown portion (1 cm wide) and highlighted by a dark brown line.

Additionally, discolored tissue from unfolded bud leaves and the growing zone of an 18-year-old oil palm with little leaf symptoms only, was collected and red ring nematodes extracted. The discoloration was orange-brown and occurred only at the edge of the soft, cream-white tissue of unfolded, young leaves. Pinkish spots also could be found in the soft heart region of this palm. Adults, juveniles, and eggs of *R. cocophilus* were recovered from this discolored tissue, however numbers were low. Discolored oil palm tissues were soaked in tap water for several hours, suspensions were concentrated, fixed with 4% formalin, and processed slowly to glycerin (28).

All juveniles of *R. cocophilus* were hand-picked, heat-relaxed, mounted in water, and measured (Table 1). Also, 20 female and 20 male red ring nematodes (coconut palm, Trinidad) were observed and measured in temporary water mounts, while all other populations were fixed in 4% formalin, processed to glycerin (28), and measured from permanent mounts (Tables 2 and 3).

Statistical analyses were made using the general linear models procedure and means were separated using the Waller Duncan *k*-ratio *t*-test ($P \leq 0.05$, $k = 100$) (26).

Table 1. Morphometrics of juveniles of *Rhadinaphelenchus cocophilus* from coconut tissue and from the palm weevil, *Rhynchophorus palmarum*, from Trinidad[w].

	J2-Coconut palm[w]	JIII-Coconut palm[w]	JIII-Palm weevil[w]
n[x]	15	30	30
L (μm)	$340^c \pm 39^y$ (265–383)	$827^a \pm 52$ (707–914)	$786^b \pm 40$ (674–849)
a	$40.9^c \pm 4.8$ (32.1–51.1)	$89.3^a \pm 6.1$ (77.6–107.0)	$70.2^b \pm 5.6$ (59.5–80.0)
c	$9.3^c \pm 0.8$ (7.5–10.2)	$10.3^b \pm 0.6$ (9.2–11.8)	$10.6^a \pm 0.6$ (9.6–11.9)
c′	$6.3^c \pm 0.6$ (5.1–7.2)	$11.7^a \pm 1.2$ (9.6–14.8)	$9.6^b \pm 0.9$ (8.3–11.8)
MBV[z] (μm)	$49^c \pm 4$ (40–56)	$71^a \pm 3$ (64–75)	$67^b \pm 3$ (62–74)
EP[z] (μm)	—	$103^a \pm 4$ (96–109)	$94^b \pm 4$ (84–98)
GBW[z] (μm)	$8^c \pm 1$ (7–9)	$9^b \pm 1$ (8–11)	$11^a \pm 1$ (10–13)
Tail (μm)	$37^c \pm 4$ (30–44)	$81^a \pm 8$ (70–98)	$74^b \pm 5$ (64–86)
ABW[z] (μm)	$6^c \pm 1$ (5–7)	$7^b \pm 1$ (6–9)	$8^a \pm 1$ (6–9)
Stylet (μm)	12 ± 1 (11–13)	—	—

[w]Measurements of heat-relaxed specimens in temporary water mounts.
[x]n = number of nematodes measured.
[y]Mean ± standard deviation and (range); means in a row that are followed by the same letter are not significantly different according to a Waller Duncan *k*-ratio *t*-test ($P \leq 0.05$, $k = 100$).
[z]MBV = distance from anterior end to midpoint of metacorpus valve; EP = distance from anterior end to excretory pore; GBW = greatest body width; and ABW = anal body width.

RESULTS AND DISCUSSION

Juveniles: The J2 stage was significantly smaller than the persistent JIII from the coconut palm or the JIII from the weevil (Table 1). A well developed stylet was observed consistently in the J2 stage, whereas a stylet was not seen in the JIII stage from the coconut palm or the weevil host. The metacorpus was pronounced in the J2 stage, whereas in the JIII it was weakly developed. The excretory pore was distinct in the JIII but could not be observed in the J2 stage. The tail shape was blunt in the J2 stage and pointed with or without a mucron in the JIII stages. No differences between the tail shape of JIII stages from the coconut palm tissue and the weevil were noticed. JIII inside the palm weevil underwent some significant shortening and widening which suggested

Table 2. Morphometrics of females of *Rhadinaphelenchus cocophilus* from different locations, hostplants, and association with either red ring disease (RRD) or little leaf.

	Trinidad[w] Coconut palm RRD	Trinidad Coconut palm RRD	Ecuador Coconut palm RRD	Venezuela Oil palm RRD	Venezuela Oil palm Little leaf
n[x]	20	15	10	10	10
L (μm)	1 030^{c} ± 95[y] (812–1 235)	1 088^{bc} ± 105 (922–1 274)	1 198^{a} ± 86 (1 085–1 362)	1 140^{ab} ± 123 (1 000–1 369)	838^{d} ± 35 (774–912)
a	81.0^{c} ± 9.9 (62.5–95.0)	94.9^{b} ± 9.4 (74.4–107.4)	120.6^{a} ± 13.7 (98.6–138.2)	96.7^{b} ± 8.9 (84.8–114.1)	77.4^{c} ± 7.3 (69.8–95.0)
b	11.2^{c} ± 1.1 (8.7–12.7)	12.5^{b} ± 0.9 (11.2–13.9)	13.7^{a} ± 1.2 (12.2–15.7)	12.9^{b} ± 0.9 (11.5–14.7)	10.4^{d} ± 0.4 (9.7–11.0)
c	11.3^{c} ± 0.7 (10.0–12.3)	11.9^{b} ± 0.6 (10.6–12.7)	10.9^{c} ± 0.8 (9.6–12.4)	12.8^{a} ± 0.9 (11.4–14.2)	12.0^{b} ± 1.0 (10.2–13.8)
c′	13.8^{bc} ± 1.8 (11.4–17.8)	14.6^{b} ± 2.1 (10.4–16.8)	22.9^{a} ± 3.8 (17.0–28.8)	14.0^{bc} ± 1.2 (12.1–16.5)	12.9^{c} ± 1.7 (10.8–16.4)
V	65.2^{a} ± 1.7 (60.5–68.2)	66.6^{a} ± 1.1 (64.8–68.6)	65.9^{a} ± 2.7 (59.8–68.8)	65.0^{a} ± 3.8 (56.3–68.0)	67.2^{a} ± 1.6 (64.7–69.4)
Stylet (μm)	14^{a} ± 1 (13–15)	12^{b} ± 1 (12–13)	12^{c} ± 1 (11–12)	12^{b} ± 1 (12–13)	12^{c} ± 1 (11–12)
GBW (μm)	13^{a} ± 2 (11–18)	12^{b} ± 1 (9–14)	10^{c} ± 1 (9–11)	12^{b} ± 1 (10–13)	11^{bc} ± 1 (9–12)
Gonad length (μm)	203^{c} ± 58[z] (103–283)	376^{a} ± 79 (238–535)	350^{a} ± 64 (272–476)	265^{b} ± 118 (144–502)	265^{b} ± 69 (135–328)
Postvulval sac length (μm)	195^{ab} ± 28 (118–255)	210^{a} ± 16 (176–245)	182^{bc} ± 38 (130–240)	200^{ab} ± 32 (160–255)	166^{c} ± 28 (121–198)
ABW (μm)	7^{a} ± 1 (6–7)	6^{a} ± 1 (6–7)	5^{c} ± 1 (4–6)	6^{a} ± 1 (6–7)	6^{b} ± 1 (5–6)
Tail (μm)	92^{b} ± 8 (79–107)	92^{b} ± 9 (73–104)	110^{a} ± 6 (102–117)	89^{b} ± 8 (81–101)	71^{c} ± 7 (62–82)

[w]Heat-relaxed specimens observed in temporary water mounts; all others permanent glycerin mounts.
[x]n = number of nematodes measured.
[y]Mean ± standard deviation (range); means in a row that are followed by the same letter are not significantly different according to a Waller Duncan k-ratio t-test ($P \leq 0.05$, $k = 100$).
[z]Six specimens with reflexed gonads (material from Cedros, Trinidad).

that they were parasitic and capable of sequestering nutrients from the weevil (Table 1).

Females: Significant morphometric differences were observed among the different female populations of *R. cocophilus* (Table 2). The greatest differences in females appeared to be between the little leaf oil palm population from Venezuela and the RRD coconut palm isolate from Ecuador (Table 2). Females of the little leaf oil palm population were significantly shorter than those of the other measured populations. The

Table 3. Morphometrics of males of *Rhadinaphelenchus cocophilus* from different locations, hostplants, and association with either red ring disease (RRD) or little leaf.

	Trinidad[x] Coconut palm RRD	Trinidad Coconut palm RRD	Ecuador Coconut palm RRD	Venezuela Oil palm RRD	Venezuela Oil palm Little leaf
n[y]	20	15	10	10	10
L (μm)	1 155[a] ± 87[z] (924–1 235)	999[a] ± 102 (813–1 150)	1 039[a] ± 79 (858–1 109)	1 017[a] ± 77 (841–1 111)	866[b] ± 71 (789–965)
a	121.9[bc] ± 11.1 (93.4–139.5)	116.6[c] ± 15.8 (92.6–143.8)	150.9[a] ± 9.5 (136.0–165.5)	129.7[b] ± 13.3 (113.8–150.0)	99.3[d] ± 11.8 (78.9–116.1)
b	11.2[a] ± 0.6 (10.2–12.3)	11.2[a] ± 1.0 (8.9–13.2)	11.7[a] ± 1.1 (9.8–13.6)	11.3[a] ± 0.9 (10.1–12.8)	10.3[b] ± 0.6 (9.6–11.2)
c	27.1[ab] ± 2.5 (22.5–33.8)	25.7[b] ± 2.6 (20.3–30.4)	28.1[a] ± 2.6 (24.1–32.1)	26.1[b] ± 2.2 (22.5–29.2)	22.4[c] ± 2.8 (18.8–26.5)
c′	5.4[a] ± 0.4 (4.6–6.1)	5.3[a] ± 0.4 (4.8–6.1)	5.8[a] ± 0.8 (5.1–7.7)	5.6[a] ± 0.5 (4.8–6.4)	5.4[a] ± 0.8 (4.7–6.8)
Stylet (μm)	12[a] ± 1 (12–13)	13[a] ± 1 (12–13)	11[c] ± 1 (11–12)	11[b] ± 1 (11–12)	12[bc] ± 1 (11–12)
GBW (μm)	9[a] ± 1 (7–10)	9[a] ± 1 (7–10)	7[c] ± 1 (6–8)	8[b] ± 1 (7–9)	9[a] ± 1 (7–10)
Spicule dorsal	8[d] ± 1 (7–10)	13[a] ± 1 (12–14)	9[c] ± 1 (8–10)	12[b] ± 1 (11–13)	11[b] ± 1 (10–13)
ABW (μm)	7[a] ± 1 (6–8)	7[a] ± 1 (7–8)	6[b] ± 1 (6–7)	7[a] ± 1 (5–8)	7[a] ± 1 (6–9)
Tail (μm)	39[a] ± 4 (32–49)	39[a] ± 2 (34–43)	37[a] ± 4 (31–46)	39[a] ± 4 (32–46)	39[a] ± 4 (33–46)

[x]Heat-relaxed specimens observed in temporary water mounts; all others permanent glycerin mounts.
[y]n = number of nematodes measured.
[z]Mean ± standard deviation (range); means in a row that are followed by the same letter are not significantly different according to a Waller Duncan *k*-ratio *t*-test ($P \leq 0.05$, $k = 100$).

"a" ratio of the population from Ecuador was significantly smaller than all other populations examined. The stylet was easier to observe in heat-relaxed specimens mounted in water. The excretory pore was very difficult to see in fixed specimens; it was observed 95.4 ± 8.1 μm (80–112 μm; n = 16) from the anterior end in the population from Trinidad (water mounts). All females showed a distinct vulval flap which also has been observed with the scanning electron microscope (SEM) (6). The ovary usually was outstretched but six specimens from coconut palm tissue collected in Cedros, Trinidad, showed reflexed ovaries.

Males: Males were similar morphometrically to the female populations (Table 3). Significant differences were observed among the different populations. The little leaf population from oil palm in Venezuela was significantly shorter. Males from coconut palm tissue from Ecuador were extremely slender ("a" ratio, Table 3).

Observations of the morphology of *R. cocophilus* with light microscopy (LM) did not reveal any distinguishing morphological features among the populations studied. This concurs with the observation that morphological differences could not be detected between populations of *R. cocophilus* from RRD coconut palms from Manzanilla, Trinidad or RRD oil palms from San Felipe, Venezuela with the SEM or LM (6). Van Hoof and Seinhorst (11) compared the nematodes associated with little leaf of coconut and oil palm in Surinam with *R. cocophilus* isolated from RRD coconut from Trinidad and concluded that they were infraspecific. Measurements of populations of *R. cocophilus* have been reported from coconut palms from Grenada (n = ?) (2), from Brazil (5 females and 5 males) (14), and from Trinidad (10 females and 10 males) (8,9) and from coconut and oil palms from Costa Rica (23). In addition, Thorne (29) published measurements of this nematode without reference to the host, location, or number of specimens measured. Lordello and Zamith (14) reported that variation between populations of red ring nematodes from coconut from Grenada (2), Trinidad (9), and Brazil (14) were similar enough to preclude the possibility of geographical races. Conversely, Salazar and Chinchilla (23) have suggested that "L" and "a" ratio differences between two populations from coconut and two populations from oil palm in Costa Rica indicate the presence of different *Rhadinaphelenchus* species.

Differences in disease symptomatology caused by infestations of *R. cocophilus* may be more affected by host variability and growing conditions of the host than by pathological differences in nematode populations. For example, *R. cocophilus* isolated from RRD coconut palm tissue from Tucacas and from RRD oil palm stem tissue from San Felipe, both in Venezuela, were equally effective in inducing RRD in 10-year-old oil palms in Maracay, Venezuela (3). Stem inoculations of coconut and oil palms in Surinam with *R. cocophilus* from RRD coconut produced typical RRD within 5 months in 40% of 4–7-year-old coconut and oil palms, and little leaf symptomatology in 40% of the oil palms and 20% of *Mauritia flexuosa* L. 1 year after inoculation (16). Stem inoculation with pieces of leaves from oil palm with little leaf produced red rings with *R. cocophilus* in >25% of 5–8-year-old coconut palms in Surinam (12). Maas (16) suggested that growing conditions rather than host age were the most important determinant for *R. cocophilus*-induced disease symptomatology; lethal RRD was expressed in succulent and vigorously growing palms, whereas less vigorously growing palms developed little leaf. Little leaf symptomatology also could be an expression of host-palm resistance, because oil palms in San Felipe, Venezuela, do not necessarily die from little leaf but are affected in terms of their productivity. Adult palm weevils are abundant in the plantation and are caught in traps regularly. In contrast to most situations in coconut palm plantations, the palm weevil does not develop in oil palms at this plantation and uses coconut palms or other palms growing in the surrounding area to complete its life cycle. This means that the red ring nematodes are probably transported to oil palms from other palm species.

Variability in morphometrics of plant-parasitic and mycophagous nematodes, such as *Ditylenchus* and many of the aphelenchids, can be induced by different hosts (7,22). Considering the variability reported previously for morphometrics of *R. cocophilus* (2,8,9,14,23), and the lack of data concerning the effects of host and host condition on the morphometrics of inoculated *R. cocophilus* we conclude that the morphometrics of populations measured in this study probably represent normal infraspecific variability.

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