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Host Response of Ornamental Palms to *Rotylenchulus reniformis*[1]

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Abstract: The responses of 20 species of ornamental palms and one cycad (*Cycas revoluta*) to two populations of the reniform nematode, *Rotylenchulus reniformis*, from southern Florida were studied in two greenhouse experiments conducted in 1989–1991 and 1991–92. Ornamental palms in pots were exposed to initial population densities of 400 and 1,500 *R. reniformis*/100 cm^3 soil for 16 and 15 months, respectively. Nematode reproduction occurred on *Acoelorrhaphe wrightii* and *Washingtonia robusta*, but not on the other palms or the cycad. In both experiments, nematode numbers on *A. wrightii* and *W. robusta* were significantly smaller than those on cowpea (*Vigna unguiculata*), a susceptible host of the nematode used as a control in these experiments. Nematodes surviving in pots containing nonhost palms for 16 months retained infectivity and were able to reproduce on susceptible cowpea in a bioassay. Sections from *Washingtonia robusta* roots infected by *R. reniformis* females showed the nematode feeding on syncytia formed by endodermal, pericyclic, and vascular parenchyma cells in a manner similar to that reported for other monocot hosts of the reniform nematode.
Key words: *Acoelorrhaphe wrightii, Archontophoenix alexandrae, Bismarckia nobilis, Carpenteria acuminata, Caryota mitis, Chamaedorea cataractarum, Chamaerops humilis, Coccothrinax* sp., *Cycas revoluta,* Florida, histopathology, infectivity, *Meloidogyne incognita,* nematode, *Neodypsis decaryi, N. lastelliana, Phoenix roebelenii, Ptychosperma elegans, Ravenea rivularis,* regulatory nematology, reniform nematode, *Rhapis excelsa,* root-knot nematode, *Rotylenchulus reniformis, Sabal palmetto,* survival, *Syagrus romanzoffiana, Thrinax morrisii, Trachycarpus fortunei, Washingtonia robusta, Wodeyetia bifurcata, Vigna unguiculata.*

The reniform nematode, *Rotylenchulus reniformis,* is common in southern Florida, where it damages vegetable and field crops (8). This pest also has regulatory significance, especially for the ornamental industry, because ornamental stocks contaminated or infested by *R. reniformis* are subject to quarantine in Arizona, California, and New Mexico, which may result in adverse economic impact for Florida growers (3–5,9). Ornamental palms are intensively grown in southern Florida (Dade County) due to the favorable growing conditions that exist for palm production. Large ornamental palms are particularly subject to contamination by the reniform nematode because they are transplanted directly into fields, many of which are infested with this pest. The major sources of infestations by *R. reniformis* in nurseries are susceptible palm species, other susceptible ornamentals (9), and weed hosts (4).

There is scarcity of information about the response of ornamental palms to *R. reniformis* because of the diversity of palm species and the frequent introduction of new palm material to the industry. Palm shipments containing *R. reniformis* are rejected for regulatory reasons, regardless of the palm's host status to the nematode. It is important however, to know the host status of ornamental palms to *R. reniformis* in order to avoid the production of palms contaminated by the nematode and consequently the adverse effects of these quarantine restrictions. The host status of 20 species of ornamental palms and one cycad to *R. reniformis* was investigated in two greenhouse experiments conducted during 1989–1991 and 1992–93.

Materials and Methods

First greenhouse experiment: A 25-cm deep Rockdale fine sand loamy soil (1) with a pH = 7.3–7.8 was collected in Dade County, Florida, in December 1989 from a recently

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plowed field infested with *R. reniformis* and *Meloidogyne incognita.* The field was plowed 5 months after the harvest of an early spring crop of snap bean (*Phaseolus vulgaris*). Initial nematode population densities (Pi) in the soil mass were estimated from an 800-cm^3 sample composed of 20 40-cm^3 cores. Vermiform reniform nematodes were extracted from the sample by centrifugal-flotation (6). Nematode population densities were expressed as nematode vermiform stages per 100 cm^3 soil.

The ornamental palms used in this experiment were obtained from stock material from a commercial nursery in southern Florida. One-year-old plants of *Chamaerops humilis, Coccothrinax* sp., *Ptycosperma elegans, Syagrus romanzoffiana,* and *Washingtonia robusta,* and two-year-old *Bismarckia nobilis, Neodypsis decaryi, Phoenix roebelenii, Ravenea rivularis,* and a cycad, *Cycas revoluta,* were planted, respectively, in 15-cm-d and 25-cm-d plastic pots containing the infested Rockdale soil. Cowpea, *Vigna unguiculata* cv. California No. 5, a good host of *R. reniformis,* were grown in additional 15-cm-d pots containing nematode-infested soil to serve as a susceptible check. All plants were distributed arbitrarily among six replicates on three greenhouse benches in December 1989 and maintained for 16 months at 15–32 C. Irrigation, fertilization, and soap sprays to control insect and mite pests were provided as needed.

A 120-cm^3 sample of soil and roots was removed from each pot 4, 9, and 13 months after planting. A subsample of roots (2.5 g) was separated from the soil, washed gently, and examined with a stereomicroscope for sedentary nematode females and egg masses. Nematode densities on roots were expressed as swollen females and eggs per g fresh root. Vermiform stages of *R. reniformis* from soil were extracted as described above. Plants were harvested 16 months after planting and final population densities (Pf) were assessed in 250 g soil and 3 g roots with the same procedures. Pots containing cowpea were reseeded every 4 months. To test the infectivity of residual *R. reniformis* populations in the pots at the end of the host test, 1,400 cm^3 soil were removed from each pot and transferred to clean 15-cm-d pots. Cowpeas were planted and maintained for 4 months in a greenhouse. Reproduction of *R. reniformis* on cowpea roots was assessed with procedures described above.

Data were subjected to analysis of variance and separation of means by Tukey's studentized range test at $P = 0.05$. Analyses were conducted with SAS (SAS Circle, Cary, NC) programs.

Second greenhouse experiment: A Rockdale soil (pH = 7.5) infested with another population of *R. reniformis* and *M. incognita* was collected in Dade County in the fall of 1991. The field in this new site had been plowed recently after a corn (*Zea mays*) crop. The infested soil was placed in 25-cm-d pots and sown with cowpea seeds to increase the nematode population density. Cowpea was grown for 9 months and reseeded every 90 days. In August 1992, individuals of each of 20 palm and one cycad species were planted singly in the infested soil in 15-cm-d pots. Species tested included the nine palms and one cycad used in the first experiment, with 11 additional palm species: *Acoelorrhaphe wrightii, Archontophoenix alexandrae, Carpenteria acuminata, Caryota mitis, Chamaedorea cataractarum, Neodypsis lastelliana, Rhapis excelsa, Sabal palmetto, Thrinax morrisii, Trachycarpus fortunei,* and *Wodyetia bifurcata.* The plant species tested in the first experiment were included in a second experiment to confirm results and to check for possible differences in host preferences of the two populations of reniform nematode. Plants were arranged arbitrarily on four greenhouse benches in six replicates and maintained at 15–32 C. Plants were grown in the nematode-infested soil for 15 months, at which time plants were harvested and their root systems examined for nematode infection. Procedures used to assess *R. reniformis* Pi and Pf in the soil, to assess nematode infection in the roots, and to perform statistical analyses were the same as those used in the first experiment.

Histopathology: Washingtonia robusta roots infected by the nematode were washed free of soil and cut into 4–5 mm lengths, fixed in FAA, dehydrated in a tertiary butyl alcohol series and embedded in paraffin (7). Embedded roots were sectioned 10–15 μm thick, stained with safranin and fast-green, mounted in Dammar xylene, and examined with the aid of a compound microscope (7).

Results and Discussion

First greenhouse experiment: Rotylenchulus reniformis Pi was 400 nematodes/100 cm^3 soil. Population density of *M. incognita* was low (1 second-stage juvenile/kg soil). Four months after planting, *R. reniformis* population densities in soil in all pots with ornamentals declined, whereas those with cowpea increased (Table 1). Cowpea pots contained significantly more vermiform *R. reniformis* per 100 cm^3 soil than any of the ornamentals at all sampling dates (Table 1). Nematode numbers in soil did not differ among ornamentals except that, after 13 months, significantly more nematodes were associated with *W. robusta* than with any of the other ornamentals (Table 1). Numbers of sedentary females per g fresh root generally were related to soil population level; cowpea had significantly more females than any of the ornamentals (Table 2). Among the ornamentals, only *W. robusta* consistently supported infection by reniform nematode females (Table 2). The number of eggs per g root showed *W. robusta* to be a modest host, supporting less nematode infection and reproduction than cowpea (Table 2). One swollen female without eggs was found attached to a *R. rivularis* root at the 4-month sampling date, but there was no further evidence of *R. rivularis* as a host of the nematode (Table 2). No eggs were recovered from any of the other ornamentals (Table 2).

When the soil remaining from the host test was bioassayed with cowpea, final soil population densities tended to reflect the residual nematode densities that followed harvest of ornamentals, with greater values in soil for *W. robusta* and cowpea than any other of the ornamentals (Table 1). Also, females and eggs per g root were significantly more numerous on cowpea plants growing in soil from cowpea than in that from any of the ornamentals except *W. robusta* (Table 2). *Meloidogyne incognita* galls and egg masses were observed on roots of *P. roebelenii, R. rivularis, S. romanzoffiana,* and *W. robusta.* Cowpea was a poor host for this population of *M. incognita.* Slight infection by root mealybugs, *Rhizoecus* sp., was also detected in the roots of all ornamentals tested.

Second greenhouse experiment: At planting, soil Pi was 1,500 *R. raniformis*/100 cm^3 soil, three times more than in the first experiment. Population densities of *M. incognita*

TABLE 1. *Rotylenchulus reniformis* per 100 cm^3 soil from nine ornamental palms, a cycad, and cowpea after 4, 9, 13, and 16-month exposure to initial population densities (Pi) of 400 nematodes/100 cm^3 soil, and following a 4-month cowpea bioassay.

Plant species	Months after transplanting				After 4-month cowpea bioassay
	4	9	13	16	
Bismarckia nobilis	70 b	80 b	50 c	40 b	1,380 bc
Chamaerops humilis	60 b	40 b	50 c	30 b	970 bc
Coccothrinax sp.	50 b	30 b	40 c	10 b	1,870 abc
Neodypsis decaryi	100 b	50 b	50 c	20 b	800 bc
Phoenix roebellenii	120 b	70 b	50 c	30 b	1,830 abc
Ptychosperma elegans	70 b	90 b	60 c	40 b	850 bc
Ravenea rivularis	70 b	60 b	80 c	40 b	1,540 abc
Syagrus romanzoffiana	60 b	40 b	30 c	10 b	670 c
Washingtonia robusta	80 b	880 b	950 b	250 b	3,130 a
Cycas revoluta	70 b	30 b	30 c	20 b	1,320 bc
Vigna unguiculata	1,710 a	7,610 a	3,040 a	2,170 a	2,510 ab

Data are means of six replications. Means followed by the same letters are not different ($P = 0.05$) by Tukey's studentized range test.

TABLE 2. Swollen females and eggs of *Rotylenchulus reniformis* per gram fresh root on nine ornamental palms, a cycad, and cowpea after 4, 9, 13, and 16-month exposure to initial population densities (Pi) of 400 nematodes/100 cm^3 soil and on cowpea roots following a 4-month bioassay.

	Months after transplanting								After 4-month cowpea bioassay	
	4		9		13		16			
Plant species	Females	Eggs	Females	Eggs	Females	Eggs	Females	Eggs	Females	Eggs
Bismarckia nobilis	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	32 b	448 bc
Chamaerops humilis	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	27 b	400 bc
Coccothrinax sp.	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	29 b	415 bc
Neodypsis decaryi	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	23 b	421 bc
Phoenix roebelenii	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	34 b	463 bc
Ptychosperma elegans	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	34 b	402 bc
Ravenea rivularis	<1 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	38 b	446 bc
Syagrus romanzoffiana	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	20 b	258 c
Washingtonia robusta	0 b	0 b	5 b	78 b	19 b	309 b	12 b	253 b	115 a	1074 ab
Cycas revoluta	0 b	0 b	0 b	0 b	0 b	0 b	0 b	0 b	17 b	294 c
Vigna unguiculata	13 a	192 a	110 a	948 a	153 a	1,196 a	122 a	1,193 a	105 a	1,751 a

Data are means of six replications. Means followed by the same letter are not different (P = 0.05) by Tukey's studentized range test.

were low as were those of the first experiment. At palm harvest 15 months after planting, counts of vermiform stages in soil and females and eggs from roots were consistent with results from the first experiment. Nematode populations declined with most palms and the cycad significantly more than with cowpea (Table 3). Counts of sedentary females and eggs per g root gave evidence of reniform nematode reproduction only on cowpea, *W. robusta,* and one new host, *A. wrightii,* which supported 2 females and 34 eggs per g fresh root (Table 3). Although neither females nor eggs per g roots differed significantly among ornamentals, average numbers of both recovered from *W. robusta* were substantially greater than those from *A. wrightii* (Table 3). There was no evidence of nematode reproduction on *T. fortunei* and *W. bifurcata,* but one swollen female was observed on the roots of a plant of each species. *Meloidogyne incognita* galls and egg masses were detected on the roots of *A. alexandrae* and also on those of the palms found infected by this nematode in the first experiment, but not on those of cowpea. Root mealybugs were also found on palm and cycad roots in this experiment.

TABLE 3. Numbers of *Rotylenchulus reniformis* on 20 ornamental palms and one cycad grown for 15 months in soil infested with 1,500 nematodes/100 cm^3 soil.

Plant species	Nematodes per 100 cm^3 soil	Females and eggs per g fresh root	
Acoelorrhaphe wrightii	580 bc	2 b	34 b
Archontophoenix alexandrae	380 c	0 b	0 b
Bismarckia nobilis	370 c	0 b	0 b
Carpenteria acuminata	400 bc	0 b	0 b
Caryota mitis	400 bc	0 b	0 b
Chamaedorea cataractarum	350 c	0 b	0 b
Chamaerops humilis	400 bc	0 b	0 b
Coccothrinax sp.	400 bc	0 b	0 b
Cycas revoluta	260 c	0 b	0 b
Neodypsis decaryi	360 c	0 b	0 b
N lastelliana	480 bc	0 b	0 b
Phoenix roebelenii	330 c	0 b	0 b
Ptychosperma elegans	400 bc	0 b	0 b
Ravenea rivularis	410 bc	0 b	0 b
Rhapis excelsa	270 c	0 b	0 b
Sabal palmetto	450 bc	0 b	0 b
Syagrus romanzoffiana	250 c	0 b	0 b
Thrinax morrisii	280 c	0 b	0 b
Trachycarpus fortunei	320 c	<1 b	0 b
Washingtonia robusta	860 b	16 b	351 b
Wodyetia bifurcata	270 c	<1 b	0 b
Vigna unguiculata	1,380 a	167 a	2,568 a

Data are means of six replications. Means followed by the same letter are not different ($P = 0.05$) by Tukey's studentized range test.

Histopathology: Histological examination of sections of *W. robusta* roots infected by *R. reniformis* sedentary females showed anatomical alterations similar to those induced by *R. reniformis* in the roots of other monocots such as banana (10). Semiendoparasitic females fed in the root stele and established a syncytium with dense cytoplasm and hypertrophied nuclei in the endodermis (Fig. 1A,B). The syncytium involved endodermal, pericyclic, and vascular parenchyma cells (Fig. 1B).

The results of these greenhouse tests indicated that the ornamental palms tested were poor or nonhosts of *R. reniformis.* Reniform nematode infection and reproduction occurred only on *A. wrightii* and *W. robusta.* Both nematode populations used in these two experiments were able to infect *W. robusta,* confirming the susceptibility of this palm to reniform nematode infection. The nematode reproduction potential on these two palms, however, was significantly lower than that on cowpea, a good host.

The ability of *R. reniformis* to reproduce on *A. wrightii* and *W. robusta* favors contamination problems in nursery operations with a history of *R. reniformis* infestations. In these nurseries, *A. wrightii* and *W. robusta* play the same role as weed hosts in maintaining sources of nematode inoculum and contamination to nonhost palms through root contact (3). The infection without reproduction on *R. rivularis, T. fortunei,* and *W. bifurcata* suggests that continuous long exposure of these palms to *R. reniformis* may induce selection of nematode populations able to reproduce on these palms.

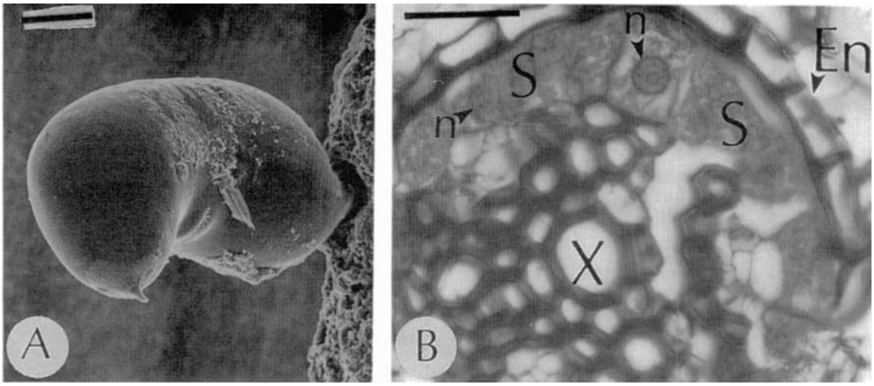

FIG. 1. *Washingtonia robusta* roots infected by *Rotylenchulus reniformis*. Scale bars = 45 μm in A and 20 μm in B. A) SEM micrograph of a female nematode with swollen posterior body protruding from the root surface. B) Root cross section showing a syncytium (S) with dense cytoplasm and hypertrophied nuclei (n) induced by the nematode in the stele. En = endodermis; X = xylem.

In our studies, *R. reniformis* survived and remained infective for 16 months in Rockdale soil planted with nonhost palms, as reported for survival in other soils (2). This long nematode survival and the lack of effective nematicides registered for use in ornamental nurseries make nematode control difficult for regulatory purposes.

In southern Florida, the most effective practice to exclude *R. reniformis* and other regulated nematodes from nursery palms is to grow palms in clean soil in containers that are not in direct contact with the ground. This practice can be applied successfully for small palms. Large palms must generally be grown directly in the field; even if grown in fumigated soil, they are subject to contamination by *R. reniformis* from weed and palm hosts, such as *A. wrightii* and *W. robusta*. Efforts to eliminate food sources for the nematode through effective weed control in infested fields with large nonhost palms are hindered mainly by the long nematode survival in absence of the hosts.

Meloidogyne incognita commonly occurs in association with *R. reniformis* in Rockdale soils of Dade County (8). This root-knot nematode, which in our study infected 5 out of 20 ornamental palms, further complicates the nematological problems on palms for the ornamental industry in southern Florida. Simultaneous infections by *M. incognita, R. reniformis,* and root mealybug were observed on two plants of *W. robusta* in the second experiment. The parasitic relationship among these three pests on palm hosts was not determined, but deserves further study.

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