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Characterization of a New Burrowing Nematode Population, *Radopholus citrophilus*, from Hawaii[1]

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Abstract: Karyotype, host preference, isozyzme patterns, morphometrics, and mating behavior of two burrowing nematode populations from Hawaii, one infecting *Anthurium* sp. and the second infecting *Musa* sp., were compared with *Radopholus similis* and *R. citrophilus* populations from Florida. The population from *Anthurium* sp. had five chromosomes (n = 5), and that from *Musa* sp. had four (n = 4). Neither of the Hawaiian nematode populations persisted in roots of *Citrus limon* or *C. aurantium. Anthurium clarinerivum* and *A. hookeri* were hosts of the burrowing nematode population from anthurium in Hawaii and of *R. citrophilus* from Florida, whereas the two anthurium species were poor hosts of the population from *Musa* sp. in Hawaii and *R. similis* from Florida. The isozyme pattern of the population isolated from anthurium was identical to that of *R. citrophilus,* whereas the pattern of the population from banana in Hawaii was identical to that of *R. similis.* Mating behavior between the burrowing nematode population isolated from *Anthurium* sp. and a Florida population of *R. citrophilus* supports their close taxonomic relationship. Mating was observed between the population from *Anthurium* sp. and the Florida population of *R. citrophilus* but not between the Hawaiian burrowing nematode population isolated from *Musa* sp. and a Florida population of *R. citrophilus.* These findings indicate that a previously unidentified population of *R. citrophilus* which does not parasitize citrus occurs in Hawaii.
Key words: anthurium, banana, biotype, citrus, ornamental, race, *Radopholus similis, Radopholus citrophilus.*

The burrowing nematode, *Radopholus similis* (Cobb) Thorne, infects more than 250 plant species in most tropical and subtropical areas of the world except Israel and Taiwan (15). Two physiological races of the burrowing nematode (5) were recently designated as sibling species based on differences in chromosome number (8,10), isozyme patterns (12), mating behavior (11), and host preference (7). The banana race was named *R. similis* and the citrus race *R. citrophilus* Huettel, Dickson, and Kaplan (9). *Radopholus citrophilus* has been reported only from Florida (1–3,6).

Two burrowing nematode populations collected from roots of anthurium and banana were sent to us by W. J. Apt, University of Hawaii, for identification. The populations differed in their ability to reproduce in roots of anthurium. Studies were conducted to characterize karyotype (chromosome number), mating behavior, isozyme patterns, morphometrics, and host preference of these populations.

Materials and Methods

Burrowing nematode-infested roots were obtained from a commercial planting of *Anthurium andraeanum* L. on Hawaii and from banana *Musa* sp. corms growing on Oahu. Anthurium roots and banana corms were chopped, rinsed, and maintained in aerated water. Nematodes that emerged were handpicked, surface sterilized, and cultured on carrot discs (7).

The number of chromosomes (karyotype) (8), mating behavior (11), electrophoretic isozyme patterns (12), and morphometrics of the two nematode populations were compared with those of previously characterized *Radopholus similis* and *R. citrophilus* populations from Florida (8,11,12). Female nematodes were fixed in FAA for at least 48 hours before measuring stylet and body length, distance from vulva to tail tip and anus to tail tip, and greatest body width.

The ability of the two nematode populations from Hawaii to persist in root systems of citrus was compared with that of Florida *R. similis* and *R. citrophilus* populations maintained in carrot disc culture but originally isolated from bananas and

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from a citrus grove with spreading decline symptoms, respectively. Astatula fine sand (typic, uncoated quartz ippsamments) in which 6-month-old rough lemon, *Citrus limon* L. (Burm. f.), or sour orange, *C. aurantium* L., seedlings were growing was infested, 1 month after transplanting, with 100 nematodes from each of the four populations. Treatments were replicated 10 times and randomized on a greenhouse bench. Two studies were conducted for 3 and 5 months in a greenhouse with soil temperature at 24 ± 2 C. At the end of each experiment, roots were rinsed free of sand and all fibrous roots from each root system were collected. Nematodes were extracted from roots by jar incubation (21) and recovered on a 38-μm-pore sieve. Root weights were determined after drying at 76 C for 48 hours. Data were expressed as nematodes per gram root dry weight.

Reproduction of *R. citrophilus* and *R. similis* was compared on *A. clarinerivum* Lind. and *A. hookeri* Lind. grown in either astatula fine sand or in a soil mix (91% sand, 4% silt, 5% clay, 1% organic matter). Five plants of each anthurium species, approximately 10 cm high, were transplanted individually into 15-cm-d clay pots and maintained in a greenhouse. The soil around each plant was infested with 50 nematodes of one of the four populations obtained from carrot disc cultures. Roots were collected 7 weeks later from each pot, rinsed, blotted dry, weighed, chopped, and incubated for 48 hours in aerated water. Data were expressed as nematodes per gram of fresh root.

Results and Discussion

The burrowing nematode population collected from anthurium in Hawaii had five chromosomes, whereas that from banana on Oahu had four. These karyotypes were consistent with those of *R. citrophilus* (n = 5) and *R. similis* (n = 4) (10).

Behavior of nematodes isolated from anthurium in attraction and mating tests toward *R. citrophilus* was identical to that observed in previous intraspecific attraction studies involving other populations (11). Males from the anthurium population were attracted to *R. citrophilus* females, and copulation was observed. Males of the banana population from Hawaii copulated with females of *R. similis* from Coto, Costa Rica; Machala, Ecuador; and Florida. *Radopholus similis* males coiled as they approached females isolated from anthurium, but copulation was not observed. This behavior was similar to that of interspecific burrowing nematodes observed previously (11).

Table 1. Measurements in μm of 30 female *Radopholus citrophilus* from anthurium on Hawaii.

Character	Range	Mean	Standard deviation
Stylet length	17.6–22.4	18.9	1.0
Vulva–tail tip	242.4–318.0	282.0	22.8
Anus–tail tip	58.4–74.4	69.5	4.5
Body length	584.0–734.0	672.0	54.0
Greatest body width	20.0–34.0	28.8	6.6

Morphometrics of the two Hawaiian populations were similar to each other and to those reported for *R. similis* and *R. citrophilus* (19,20). Measurements of the population from anthurium are listed in Table 1. No unusual morphological characteristics were observed. Lateral lines in the terminus of the lateral field as previously reported by Sher (18) were observed.

An initial study indicated that the Hawaiian burrowing nematode population isolated from anthurium reproduced on citrus (13); however, we were unable to repeat this. Neither population, from anthurium or banana, from Hawaii reproduced in, or caused damage to, roots of citrus (Fig. 1). However, nematode population densities of a Florida isolate of *R. citrophilus* averaged 142 and 712 per gram root dry weight in roots of rough lemon and sour orange, respectively. Neither Hawaiian population of the burrowing nematode reproduced in citrus in the greenhouse in Hawaii (W. J. Apt, pers. comm.). Both populations reproduced on okra, with the anthurium population increasing to greater numbers than the banana population (Table 2).

Anthurium had been identified previously as a host of a burrowing nematode (17,18). In our study, anthurium appeared to be a poor host for *R. similis* but a good host for *R. citrophilus* (Table 3). Although previous studies indicated that soil type (texture) influenced burrowing nematode population densities (4,16), we found no

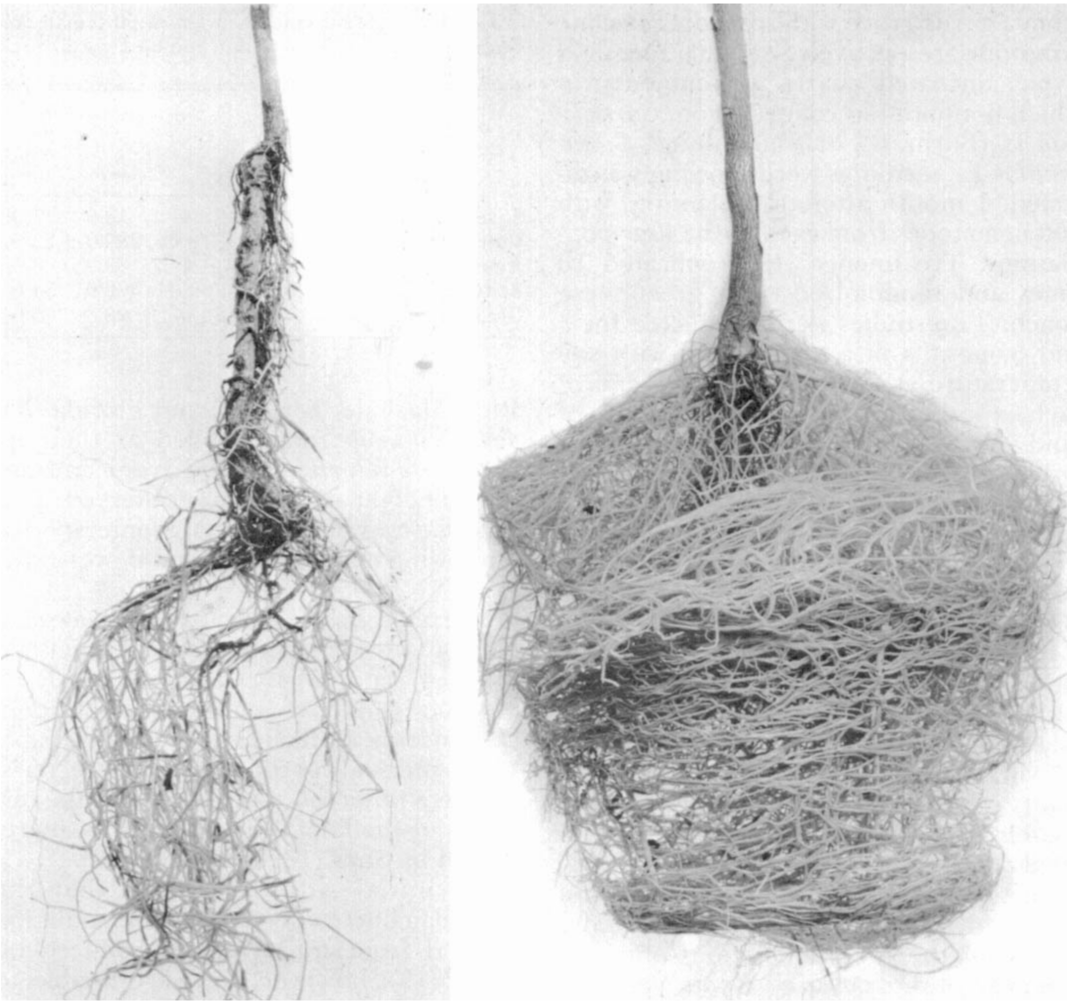

FIG. 1. Root systems of *Citrus aurantium* grown for 5 months in astatula sand infested with *Radopholus citrophilus.* Left) *Radopholus citrophilus* from Florida. Right) *Radopholus citrophilus* from anthurium on Oahu.

significant interaction between soil type and (or) plant species.

Analyses of homogenates of the nematode population from anthurium had isozyme bands which were identical to those previously determined for *R. citrophilus* (9,12). The population from banana also had 16 isozyme bands with two bands for phosphoglucose isomerase as in populations of *R. similis* from Central and South America (12).

The chromosome number, mating behavior, isozyme pattern, and morphometrics indicate that the burrowing nematode population from banana in Oahu is *R. similis* and that from anthurium in Hawaii is *R. citrophilus.* Hawaii's *R. citrophilus* is unique in that it did not damage or reproduce in rough lemon or sour orange roots. Differences in host preference have recently been identified among *R. citrophilus* populations from Florida (14). *Radopholus citrophilus* biotype 1 does not reproduce well or dam-

TABLE 2. Population densities of *Radopholus* from Hawaii in roots of okra.

Population source	Time (months)		
	2	4	6
Anthurium	205 a	194 a	151 a
Banana	37 b	42 b	87 b

Data are nematodes per gram of root fresh weight. Means followed by the same letter within columns are not significantly different according to Duncan's multiple-range test ($P = 0.05$).

TABLE 3. Population densities of four *Radopholus* populations in roots of *Anthurium clarinerivum* and *A. hookeri* growing in two soil types (I and II)* 7 weeks after inoculation.

Radopholus population	*A. clarinerivum*		*A. hookeri*	
	I	II	I	II
Anthurium	52.0 a	117.0 a	67.0 a	66.0 a
R. citrophilus	46.0 a	98.0 a	64.0 a	145.0 a
Banana	0.7 b	1.0 b	2.0 b	0.0 b
R. similis	0.5 b	0.1 b	3.0 b	1.0 b

Data are nematodes per gram of root fresh weight. Means followed by the same letter within columns are not significantly different according to Duncan's multiple-range test ($P = 0.05$).

* Soil type I = 90.6% sand, 3.9% silt, 5.5% clay, 1.9% organic matter. Soil type II = 97% sand, 0% silt, 3% clay, 0.13% organic matter.

age the roots of several citrus rootstocks, but it does damage and persist in great densities in roots of rough lemon and sour orange rootstocks. In contrast, the population designated as *R. citrophilus* biotype 2 occurs in significantly greater densities and causes greater damage than biotype 1 to the root systems of the citrus rootstocks Milam lemon (*C. limon* (L.) Burm. f. cv. Milam), Algerian navel and Albritton sweet oranges (*C. sinensis* (L.) Osbeck), and Carrizo citrange (*C. sinensis* × *Poncirus trifoliata* (L.) Raf.) which were previously considered to be resistant or tolerant to *R. citrophilus* (14).

Our findings confirm that karyotype, isozyme patterns, morphometrics, host preferences, and mating behavior may aid in differentiating the burrowing nematode sibling species *R. citrophilus* and *R. similis.* Use of such techniques to differentiate these two species may not be feasible for regulatory agencies because time, funding, facilities, expertise, or adequate numbers of specimens to conduct such procedures may not be available. However, our findings support the sibling species concept as a meaningful and reproducible system for classifying burrowing nematodes. It provides researchers with better means for characterizing populations. Such approaches have stimulated research efforts to develop rapid, economic, and precise methods for burrowing nematode identification by concerned regulatory agencies.

Kaplan and O'Bannon (14) identified two biotypes of *R. citrophilus* that differed in their ability to reproduce in, and damage roots of, citrus rootstocks. We have identified an *R. citrophilus* population that does not persist in roots of citrus; this population could also be considered a biotype or a race. Since relatively few populations of *R. citrophilus* have been evaluated, we prefer to consider the three populations of *R. citrophilus* characterized to date as distinct populations rather than biotypes or races. As additional populations of *R. citrophilus* are studied, perhaps a natural system for their classification will evolve.

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