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Occurrence of Biotypes in *Radopholus citrophilus*

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Abstract: Two morphologically and karyotypically identical populations of the citrus burrowing nematode, *Radopholus citrophilus,* differed in their ability to damage and reproduce in roots of citrus rootstocks previously identified as either resistant or tolerant. These populations are considered to be biotypes, and their occurrence may explain the appearance of spreading decline symptoms in plantings of rootstocks previously considered resistant.

Key words: burrowing nematode, citrus, control, resistance, taxonomy.

The causal agent of spreading decline (12), the citrus burrowing nematode *Radopholus citrophilus,* Huettel, Dickson, and Kaplan (7), has been managed for more than 25 years by the "push and treat" program (13), barriers (11), a "nematode-free nursery stock" certification program, and resistant or tolerant rootstocks (2). Although these approaches have been successful in limiting the spread of this disease in Florida citrus, the recent ban on ethylene dibromide (EDB) and the current lack of a registered, economically feasible alternative nematicide has terminated the "push and treat" and barrier programs. The nursery stock certification program will continue to limit introduction of *R. citrophilus* into noninfested groves. Use of nematode-resistant or nematode-tolerant rootstocks remains a primary means of burrowing nematode management.

Rootstocks used for control of the citrus burrowing nematode are not completely incompatible with this pest. Low populations of *R. citrophilus* have been associated with these rootstocks, and there is concern that resistance-breaking biotypes of this nematode may develop (10).

Trees affected with spreading decline show poor growth, dieback in the upper canopy, reduction in tree size and fruit and leaf numbers, and poor response to fertilization (12). We have observed spreading decline symptoms in isolated plantings of sweet orange (*Citrus sinensis* [L.] Osbeck) on Milam lemon (*C. limon* [L.] Burm. f. cv. Milam) and Carrizo citrange (*C. sinensis* × *Poncirus trifoliata* [L.] Raf.) rootstocks reported to be resistant or tolerant to *R. citrophilus* (2,8). The purpose of this study was to determine if the appearance of spreading decline symptoms in a burrowing nematode-resistant rootstock planting resulted from the development of new burrowing nematode biotypes capable of reproducing in, and causing damage to, roots of burrowing nematode-resistant rootstocks.

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TABLE 1. Population densities of two *Radopholus citrophilus* populations on five citrus rootstocks, greenhouse Experiment 1 (average of 10 replications).

	Rootstock									
	Ridge Pineapple		Algerian navel		Milam lemon		Carrizo citrange		Rough lemon	
Month	1†	2	1	2	1	2	1	2	1	2
6	13	69*‡	12	163**	0	60*	28	118*	288	81
9	12	101**	8	115**	0	131**	0	329**	481	78
12	14	495**	18	369**	5	157**	71	657**	424	185

† *R. citrophilus* population 1 obtained from roots of rough lemon and population 2 from roots of Milam lemon from trees in groves showing symptoms of spreading decline.

‡ *R. citrophilus* per gram moist root weight. Data were transformed (log x + 1) and population 1 vs. 2 evaluated at 6, 9, or 12 months postinoculation by Tukey's Honestly Significant Difference Test, $*P = 0.05$, $**P = 0.01$.

MATERIALS AND METHODS

Greenhouse cultures of two *Radopholus citrophilus* populations were maintained in bins containing rough lemon (*Citrus limon* [L.] Burm. f.) trees growing in Astatula fine sand (hyperthermic, uncoated typic quartzipsamments). One *R. citrophilus* population (P1) was isolated from roots of rough lemon, a *R. citrophilus*-susceptible rootstock. A second *R. citrophilus* population (P2) was isolated from roots of Milam lemon, a *R. citrophilus*-resistant rootstock. Both populations were isolated from groves exhibiting spreading decline symptoms.

These populations were used as inoculum sources in Experiment 1 and in other experiments conducted between 1975 and 1978. Subsequently, monoxenic cultures were developed using 6-mm carrot disks surface sterilized by flaming in 95% ETOH. Nematodes were surface sterilized by incubation in 1.0% 8-hydroxyquinoline hemisulfate salt (20 minutes) and rinsing twice in 0.1% streptomycin sulfate. Carrot disks were inoculated with nematodes in suspension in the streptomycin sulfate solution at 23 C. Such cultures were subsequently used to obtain nematode inoculum for Experiment 2 and other experiments conducted between 1979 and 1983.

Greenhouse studies

Preliminary studies and six experiments were conducted to compare the reproductive potential of the two *R. citrophilus* populations on a series of citrus rootstocks. The two experiments described here in detail were representative of all trials conducted.

Experiment 1: Equal numbers of 8-month-old seedlings of Ridge Pineapple and Algerian navel sweet oranges, Carrizo citrange, and Milam and rough lemons were planted in two soil tanks containing Astatula fine sand. Each tank was previously infested with one of the two nematode populations. Eight months later, seedlings were selected for uniformity and transplanted to 20-cm-d clay pots containing steam-pasteurized Astatula sand. The 10 replicate pots in each treatment were randomized on greenhouse benches. Nematode populations were determined in 2–3-g samples of fibrous roots removed from each pot 6, 9, and 12 months after transplanting. Each sample site within a pot was marked to avoid subsequent sampling at the same site. Root samples were washed, and nematodes were recovered by jar incubation after 4 and 7 days at 26 ± 1 C (14). Roots were weighed moist, and data were expressed as nematodes per gram moist root weight.

Experiment 2: Six-month-old seedlings of Carrizo citrange, Milam and rough lemons, and Albritton sweet orange were planted in 20-cm-d clay pots containing steam-pasteurized Astatula sand. Ninety juvenile and adult nematodes extracted from carrot cultures were added to the soil around each seedling. Eight pots of each nematode population–citrus variety combination were randomized on a greenhouse bench. Plants were harvested at 6, 9, and 12 months postinoculation. At harvest, root systems were washed and all fibrous roots collected; nematodes recovered from fibrous roots were incubated in jars for 7 days at 26 ± 1 C (14). Subsequently, these roots were dried for 24 hours at 76 C, dry root weights were determined, and data were expressed as nematodes per gram dry root weight.

TABLE 2. Population densities of two *Radopholus citrophilus* populations on four citrus rootstocks, greenhouse Experiment 2 (average of eight replications).

	Rootstock							
	Albritton sweet orange		Milam lemon		Carrizo citrange		Rough lemon	
Month	1†	2	1	2	1	2	1	2
6	34	3,928**‡	19	762**	109	784**	1,053	1,059
9	3	653**	20	481**	67	1,123**	747	409*
12	4	514**	59	360**	160	829**	473	963**

† *R. citrophilus* population 1 obtained from roots of rough lemon and population 2 from roots of Milam lemon from trees in groves showing symptoms of spreading decline.

‡ *R. citrophilus* per gram dry root weight. Comparison of population 1 vs. 2 as per Table 1 evaluated by Tukey's Honestly Significant Difference Test, $*P = 0.05$, $**P = 0.01$.

Stem diameters, 7.6 cm above the soil line, were recorded before the final harvest (12 months postinoculation).

Morphometric and karyotypic analysis of nematode populations

Twenty specimens of each of the two populations were obtained from carrot cultures, killed in hot triethanolamine formalin (TAF) (1), and observed in TAF. Female body, neck, stylet and tail length, width, V, a, b, and c ratios; and male body, stylet, spicule, excretory pore to head end, and tail length, as well as height and width of head were measured using a light microscope. Karyotypic analyses were conducted as described by Huettel and Dickson (4).

RESULTS

The two *Radopholus citrophilus* populations differed consistently in their ability to 1) reproduce on roots of Carrizo citrange, Milam lemon, and Albritton, Algerian navel, and Ridge Pineapple oranges and 2) reduce the growth of these rootstocks previously considered tolerant to the burrowing nematode. Significant populations of *R. citrophilus,* originally isolated from roots of Milam lemon (P2), were present in roots of four rootstocks previously reported as resistant or tolerant to *R. citrophilus* (Table 1). Similarly, in Experiment 2, P2 reproduced on three citrus species previously considered incompatible with the burrowing nematode (Table 2). Fibrous root weight and stem diameters (Table 3) were significantly reduced by P2, compared with P1.

Nematodes of the two *R. citrophilus* populations were similar in morphology (Table 4) and karyotype (five chromosomes).

DISCUSSION

The two *R. citrophilus* populations examined differ with respect to their ability to overcome citrus rootstock defense mechanisms and to cause disease. In this

TABLE 3. Influence of two *Radopholus citrophilus* populations on dry fibrous root weight (g) and stem diameter (mm) of four citrus rootstocks (greenhouse Experiment 2) (average of eight replications).

	Rootstock							
	Albritton sweet orange		Milam lemon		Carrizo citrange		Rough lemon	
Month	1†	2	1	2	1	2	1	2
Fibrous roots								
6	3.0	0.6**‡	4.2	2.7**	2.4	2.0	2.3	1.5
9	4.7	0.9**	5.0	1.7**	3.0	1.9**	2.0	2.0
12	8.1	2.2**	6.2	2.2**	3.1	2.1*	4.0	3.0
Stems								
12	9.0**	6.0	9.9**	6.9	8.2**	6.1	5.9	6.3

† *R. citrophilus* population 1 from roots of rough lemon and population 2 from roots of Milam lemon from trees in groves showing symptoms of spreading decline.

‡ Comparisons evaluated by Tukey's Honestly Significant Difference Test, $*P = 0.05$, $**P = 0.01$.

and previous studies (identified as *R. similis* Orlando I and Orlando II) (4–6), they appear to be morphologically and karyotypically identical. Both populations have similar electrophoretic patterns for total protein and isozymes (5,6). Based on the above facts, they should be considered separate biotypes. Biotype 1 is designated as that population which reproduces poorly in Milam lemon and does not cause significant damage. Biotype 2 reproduces well in (and significantly damages) Milam lemon, Carrizo citrange, Albritton sweet, Algerian navel, and Ridge Pineapple orange rootstocks.

Rootstocks resistant or tolerant to *R. citrophilus* have often been planted in groves where spreading decline had been observed. In many instances, subsequent tree growth was deemed adequate following implementation of "push and treat" control strategies and good citricultural practices. O'Bannon and Ford (10) speculated that the persistence of low nematode population levels in roots of these rootstocks might lead to resistance-breaking races. Their prediction appears to have been correct because, in some locations, spreading decline symptoms have recurred in plantings on Milam lemon or Carrizo citrange rootstocks. Biotype 2 was isolated from such a location. Our findings indicate that poor rootstock performance may result from the presence of resistance-breaking biotypes. Poor rootstock performance may also be attributed to variability in the inheritance of genes conferring resistance to *R. citrophilus* (3, Kaplan, unpubl.).

Use of nematode-resistant citrus rootstocks alone may not be adequate to manage spreading decline, and this control method needs to be reevaluated. Ideally, these rootstocks should be planted in soil treated with a fumigant or contact nematicide that has eliminated a large portion of the *R. citrophilus* population. However, with increasing awareness of possible environmental pollution and cost of treatment, adequate reduction of soil nematodes with nematicides has proven difficult to achieve. Control of spreading decline should focus on use of rootstocks in conjunction with other management practices (9) which will enhance tree tolerance to nematode damage and reduce soil nematode populations with minimal adverse effects on the environment.

TABLE 4. Morphometrics (in μm) of two populations of *Radopholus citrophilus*.

	Population 1†	Population 2	+/−
Females (n = 20)			
Stylet length	18.7‡	19.4	0.9
Tail length	70.4	65.3	4.3
Neck length	165.2	157.5	6.8
Width	23.4	23.9	2.8
Body length	744.0	697.2	51.0
V (%)	60.0	59.0	
Ratios			
a	31.8	29.2	
b	4.5	4.4	
c	10.6	10.6	
Males (n = 20)			
Stylet length	15.1	15.4	0.7
Head height	6.6	6.8	0.2
Head width	8.3	8.5	0.3
Spicule length	19.9	19.6	1.0
Tail length	76.5	70.2	3.6
Body length	646.8	642.0	29.1
Excretory pore to head end	95.9	93.2	3.4

† *R. citrophilus* population 1 obtained from roots of rough lemon and population 2 from roots of Milam lemon from trees in groves showing symptoms of spreading decline.

‡ No differences in any parameter used to compare populations 1 and 2 by Tukey's Honestly Significant Difference Test ($P = 0.05$).

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