Four Biotypes of Tylenchulus semipenetrans in California Identified, and Their Importance in the Development of Resistant Citrus Rootstocks

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Abstract: Four biotypes (pathotypes) of the citrus nematode, *Tylenchulus semipenetrans*, occurring in California, U.S.A. were differentiated on the basis of differences of infectivity on 'Homosassa' sweet orange, 'Troyer' citrange, 'Pomeroy' and 'Rubidoux' *Poncirus trifoliata*, 'Thompson Seedless' grape, and 'Manzanillo' olive. A method for differentiating biotypes of *T. semipenetrans* is described. Field observations indicate that biotypes of this nematode are very stable. The importance of using highly infective biotypes in the development and selection of satisfactory citrus-nematode-resistant rootstocks is emphasized. *Key Words*: *Tylenchulus semipenetrans* biotypes.

Biotypes of the citrus nematode, *Tylenchulus semipenetrans* Cobb, that differ in ability to infect (and reproduce on) some hosts, have been described (3). They occur in the U.S.A. and in other countries where citrus and other susceptible crops are grown. In southern California, the citrus nematode attacks many *Citrus* spp., grape (*Vitis vinifera* L.), olive (*Olea europaea* L.), persimmon (*Diospyros lotus* L.), and *Poncirus trifoliata* Raf. A population of *T. semipenetrans* that infected grass (*Andropogon rhizomatus* Swallen) in Florida, but not rough lemon [*C. limon* (L.) Burm.], sour orange (*C. aurantium* L.), or *P. trifoliata* was described by Stokes (9). Baines et al. (3) described two biotypes of citrus nematode in California that produced different levels of infection (no. adult females/cm of feeder root) on 'Homosassa' sweet orange (*C. sinensis* L.), and 'Troyer' citrange (F_1 hybrid of *C. sinensis* × *P. trifoliata*) in pots. Recently, populations of citrus nematode that produce high levels of infection on 'Davis B' and 'Rubidoux' *P. trifoliata*, and Troyer citrange were found in southern California. Information on the occurrence and distribution of highly infective biotypes of the citrus nematode in California is needed to develop satisfactory citrus nematode-resistant rootstocks. This paper presents information on some hosts used to distinguish biotypes of *T. semipenetrans*, and a method for testing infectivity of nematode populations.

MATERIALS AND METHODS

Four citrus nematode populations were collected from citrus or other hosts in the field and increased for testing. Biotype 1 (3) was collected on sweet orange in field 18A, Citrus Research Center (C.R.C.), Riverside, May 1967, and was maintained on Troyer citrange seedlings in pots. Biotype 2(3) was collected on sweet orange at Highgrove, California, April 1967 and was maintained on Homosassa sweet orange. Biotype 3 (Table 2) was collected on Davis B *P. trifoliata* in C.R.C. field 3A, November 1968, and was maintained on Troyer citrange seedlings. Biotype 4 (Table 2) was collected on large olive trees near Strathmore, California, November 1968, and was maintained on Homosassa sweet orange. Larvae obtained from roots in a mist chamber were treated (disinfected) in 40 ppm of copper sulfate for 1 h. The copper sulfate solution was removed by pouring the suspension onto filter paper supported on a plastic grid. Larvae were washed from the filter paper and placed around roots of Homosassa sweet orange or Troyer citrange seedlings in pots containing steam-pasteurized sandy loam for increase. Larvae from each culture were used to inoculate other Homosassa sweet orange or Troyer citrange seedlings in a steam-pasteurized sandy loam in four pots 25-30 cm in diam. After 5-6 mo, larvae obtained by the above method were used in our tests.

Seeds and cuttings of differential hosts were planted in a propagation bed or in flats in a steam-pasteurized sandy loam soil. Seedlings 10-15 cm high, and newly rooted soft-wood cuttings of 'Manzanillo' olive or of 'Thompson Seedless' grape were transplanted, one/2-liter plastic pot (Fig. 1-A). After 4-7 wk the seedlings and cuttings were inoculated by pouring 20,000 second-stage larvae onto the soil surface, which was kept moist and shaded for 24 h to protect larvae from desiccation. The pots had no drain holes and were maintained at 26 C in control temperature

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tanks (Fig. 1-B). When the soil surface became dry, 200 ml of demineralized water was applied. This decreased the soil-water suction in most of the soil to 5-7 centibars. Soil moisture conditions were favorable for development of roots throughout the soil. The experimental design consisted of six randomly distributed pots of each treatment. Two months after inoculation, the soil mass was removed intact from the pot and cut in half horizontally. Roots in the top halves were freed of soil by soaking and gently washing in water, and stored in 10% Formalin (2). Later, the roots from each pot were rinsed with water, blotted, the fine feeder roots removed and weighed, and the number of adult females/cm of feeder root determined by a staining, blending, and sieving method (3). The average length of feeder roots in two 0.5-g lots was determined and used to calculate the density of adult females on the sample of roots.

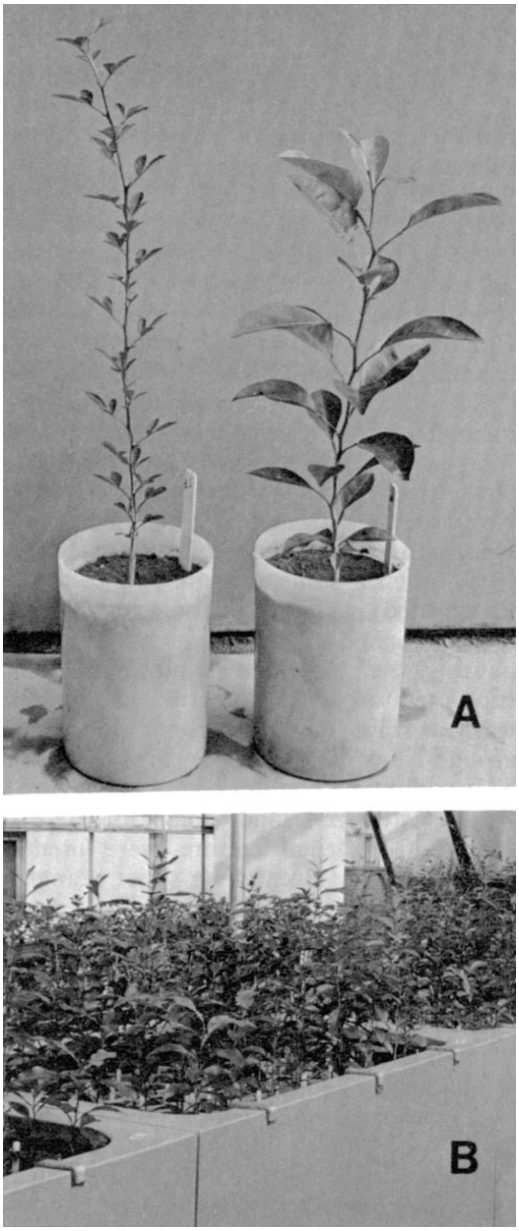

FIG. 1-(A, B). Testing the infectivity of biotypes of the citrus nematode on differential hosts. **A.** Young seedling of 'Troyer' citrange (left) and of 'Homosassa' sweet orange (right) 6 wk after inoculation. **B.** View of temp control tanks used for maintaining soil at 27 C.

The susceptibility of Troyer citrange and of five selections of *P. trifoliata* to biotypes 1 and 3 under field conditions, and to biotype 3 in pots in a greenhouse were determined. Young trees of 'Frost nucellar Washington' navel orange on six different rootstocks were planted in a Ramona sandy loam infested with *T. semipenetrans* biotype 3 (C.R.C. field 3A). The experimental design consisted of six blocks, with two trees of each rootstock per block. Seedling trees of each rootstock also were planted in a similar soil in C.R.C. field 13C that was infested with *T. semipenetrans* typical of biotype 1. One seedling of each type was planted per block with six replications. Both fields were infested (337-400 second-stage larvae/50 cc of soil from 0-60 cm depth) from old orange trees that had been removed 8-12 mo before the test trees were planted in 1963. Nematode infection was determined after 4 yr in field 13C and after 7 yr in field 3A. Roots were collected from 10- to 30-cm depth at the drip line of the trees in the field, and from those in the greenhouse test and were processed as described above.

RESULTS

The level of infection produced by four populations (biotypes) of *T. semipenetrans* on six hosts are presented in Table 1. Biotypes 1 and 2 obtained from sweet orange, biotype 3 from Davis B *P. trifoliata* and biotype 4 from olive were highly infective on Homosassa sweet orange. Homosassa sweet orange is selected for a differential host, since a population of *T. semipenetrans* on *Andropogon rhizomatus* in Florida did not infect sweet orange (9). Biotype 1 produced a higher level of infection on sweet orange and on Troyer citrange than the other biotypes,

TABLE 1. Average number of adult female *Tylenchulus semipenetrans* per centimeter of root (greenhouse test)

Nematode Biotype[u]	Differential host					
	Sweet Orange[v]	Troyer Citrange	Pomeroy *P. trif.*[w]	Rubidoux *P. trif*[w]	Grape[x]	Olive[y]
1	2.93 z	2.78 c	0.00 a	0.00 a	0.15 b	0.01 a
2	1.93 ab	0.23 a	0.01 a	0.00 a	0.03 a	0.15 b
3	1.41 a	2.25 bc	0.49 b	1.48 b	0.05 a	0.00 a
4	2.06 ab	1.79 b	0.00 a	0.02 a	...	0.43 c

[u] Biotypes 1 and 2 were obtained from *Citrus* sp., 3 from *Poncirus trifoliata*, and 4 from olive.
[v] 'Homosassa' sweet orange, *Citrus sinensis.*
[w] *Poncirus trifoliata*, 'Pomeroy'; *P. trifoliata*, 'Rubidoux.'
[x] Thompson Seedless' grape, *Vitis vinifera.*
[y] 'Manzanillo' olive, *Olea europaea.*
[z] Host-infection means followed by different letters are statistically different ($P = 0.05$) by Duncan's multiple range test.

slight on grape and olive, and none on 'Pomeroy' and Rubidoux *P. trifoliata*. Biotype 2 produced high infection on sweet orange, slight on Troyer citrange and olive, a trace on grape and Pomeroy, and none on Rubidoux *P. trifoliata*. Biotype 3 produced high infection on sweet orange, Troyer citrange, and Rubidoux *P. trifoliata*, slight on Pomeroy *P. trifoliata*, a trace on grape, and none on olive. Biotype 4 produced high infection on sweet orange and Troyer citrange, slight on olive, a trace on Rubidoux, and none on Pomeroy *P. trifoliata*. Biotype 3 produced a higher level of infection on *P. trifoliata* than biotypes 1, 2, and 4. The six differential hosts are considered adequate for differentiating the four biotypes of *T. semipenetrans* that occur in California.

In field 13C, biotype 1 produced none or only trace infection on 'Benecke,' 'Christiansen,' 'Kryder 28-3,' Pomeroy, or Rubidoux *P. trifoliata*, and moderate infection on Troyer citrange (Table 2). In field 3A, biotype 3 produced much higher infection on all of these hosts than occurred in field 13C. Levels of infection produced by biotype 3 on six hosts in field 3A are comparable to those obtained in the greenhouse test (Table 2). Infection differences observed may in part be related to the inoculum levels. In the greenhouse, numbers of adult females/cm of root that developed from one inoculation was measured, whereas in the field roots were exposed repeatedly to different levels of inocula. Benecke and Pomeroy *P. trifoliata* both showed a high level of resistance to the four biotypes tested.

DISCUSSION

Homosassa sweet orange, Troyer citrange, and Pomeroy and Rubidoux *P. trifoliata* were adequate for distinguishing important differences in degree of infectivity of populations of *T. semipenetrans* on *Citrus* spp. and relatives. Thompson Seedless grape and Manzanillo olive are satisfactory hosts for differentiating pathotypes of this nematode on these horticultural crops.

Differences of infectivity of biotypes were adequately expressed by one generation in a

TABLE 2. Susceptibility of five *Poncirus trifoliata* selections and of 'Troyer' citrange to *Tylenchulus semipenetrans* in two fields and in a greenhouse.

Host cultivars	Number of Adult Female Nematode/cm of Root[w]		
	Biotype 3 G.H.[x]	Biotype 3 Field 3A[y]	Biotype 1 Field 13C[z]
Poncirus selections			
'Benecke'	1.0 a	1.1 a	0.0 a
'Christiansen'	0.5 a	3.8 b	0.01 a
'Kryder 28-3'	0.6 a	1.6 a	0.0 a
'Pomeroy'	0.6 a	1.0 a	0.0 a
'Rubidoux'	1.7 b	6.0 c	0.01 a
'Troyer' citrange	2.0 b	8.2 c	4.39 b

[w] Column means followed by uncommon letters are significantly different ($P = 0.05$) by Duncan's multiple range test.
[x] G.H. – Small seedlings in a greenhouse. Data were taken seven weeks after inoculation.
[y] The trees were 7 hr old, growing in a sandy loam.
[z] The trees were 4 yr old, growing in a sandy loam.

greenhouse. Eggs were produced by the adult females that developed on the resistant hosts; reproduction has been verified in other tests in pots and on trees in orchard plantings. In the field, infection on selections of *P. trifoliata* and Troyer citrange 3-30 years of age reached typical levels. The level of infection likely was limited by the ability of the larvae to invade and set-up feeding sites, and also by the ability of females to reproduce on resistant hosts (3, 12).

Observations at the C.R.C. indicate that some populations of the citrus nematode remain stable in infectivity and apparently do not mutate frequently. Thus, 36-yr-old Troyer citrange trees had slight infection of their roots, Pomeroy, Rubidoux, 'Rusk,' and 'Webber-Fawcett' *P. trifoliata* of the same age had none or only light infection after long periods of exposure to biotype 1. Similarly, navel orange on Troyer citrange rootstock exposed to high inoculum levels of biotype 2 for 3-5 yr in three commercial orchards had trace infection. On the other hand, navel orange trees on Troyer citrange, or on five selections of *P. trifoliata* rootstocks developed infection typical of biotype 3 on six pairs of trees of each rootstock that were randomly distributed in a 2-ha field, 4-5 yr after planting. This field had been in navel orange on susceptible sour orange rootstock for approximately 30 yr prior to the present planting. The soil contained 337 second-stage larvae in 50 cc of soil in the 0-30 cm depth and 1,090 in the 30-60 cm soil depth, which are assumed to be biotypes 1 and 3 and combinations between them. Biotype 3 appeared to come to the fore by host selection pressure. Other supporting evidence for the stability of biotypes is that *T. semipenetrans* has not been observed on olive in France (6), Israel and Italy (conversation with Eli Cohn and F. Lamberti), but it frequently occurs on this host in California, USA (5). Mixtures of biotypes occurring in the field usually are difficult to recognize in tests of short duration, and may require long periods of host selection on a resistant host.

Naturally occurring citrus nematode populations were used in the present tests, since it would be difficult to obtain homozygous populations by simply developing colonies from single individuals. This nematode reproduces by amphimixis or mitotic parthenogenesis (11). Macaron and Ritter (7) observed that males are also formed from second-stage females. Populations of other nematodes that developed from single individuals have been shown to vary in infectivity. Allen (1) and Riggs and Winstead (8) showed that progeny from a single egg mass of *Meloidogyne incognita acrita* or from larvae of *M. incognita* differ greatly in pathogenicity on certain hosts, which supports the contention that such differences are largely of a genetic nature (10).

Benecke and Pomeroy *P. trifoliata* are good sources of resistance in a program to develop new nematode resistant rootstocks by breeding (4) and also for rootstocks for oranges.

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