# Comparison of Five Populations of Tylenchulus semipenetrans to Citrus, Poncirus, and their Hybrids

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*Abstract:* The infectivity of five populations of *Tylenchulus semipenetrans* were compared and differentiated on 10 hosts (5 *Citrus* spp., 1 *Poncirus trifoliata,* and 4 hybrids of *Citrus* spp. X *P. trifoliata*). Differences in levels of infection and development ($P = 0.01$) occurred between *Citrus* spp. and *P. trifoliata* cv. 'Pomeroy' and their three hybrids, *C. paradisi* X *P. trifoliata* cv. 'Swingle' citrumelo and *C. sinensis,* cv. 'Ruby' orange X *P. trifoliata* cv. 'Webber Fawcett 14-7', and '15-7'. *Poncirus trifoliata* cv. Pomeroy was susceptible to a California biotype 3 and highly resistant to the other citrus nematode populations. Low infection levels with California biotype 1, Arizona, and Florida populations on Swingle citrumelo, and the two Ruby orange hybrids indicated inherited resistance. Reproduction of the nematode population from Texas was greatest on the three hybrids, Swingle citrumelo, Ruby orange 14-7, and 15-7, from the California 1, Arizona, and Florida populations, but its comparable densities on *P. trifoliata* and *Citrus* spp. were not sufficiently different from these populations to consider it a separate biotype. California biotype 3 was sufficiently different from all other populations to be considered a different biotype, and it was named the "Poncirus biotype." *Key Words:* citrus-root nematode, resistance, population density, biotype.

The citrus nematode, *Tylenchulus semipenetrans* Cobb, infects citrus in Florida, Texas, Arizona, and California, and other citrus-growing areas of the world. Most citrus rootstocks are moderately to highly susceptible to *T. semipenetrans.* DuCharme (6) reported that some selections of *Poncirus trifoliata* (L.) Raf. were resistant to the citrus nematode in Argentina. Resistance or susceptibility of other *Citrus* spp., hybrids, and rutaceous plants to the citrus nematode has been reported (1, 4, 7, 8). Baines et al. (2) provided the first evidence of biotypes in *T. semipenetrans* on citrus. Six *T. semipenetrans* biotypes, four in California and two in Florida, have been differentiated by host specificity (3, 11). Each California biotype will infect citrus to some degree. In Florida, a biotype found parasitizing a grass does not infect citrus, but it is morphologically indistinguishable from biotypes infecting citrus (11). A comparison of a California and a Japanese citrus nematode population indicated a difference in infectivity between the populations on *P. trifoliata* (2). This difference and other data (5, 9) suggest that biotypes occur in other citrus-growing areas of the world.

Seed and plant material are currently being exchanged internationally. Citrus breeding for nematode resistance is determined with the native nematode populations. To develop nematode-resistant citrus rootstocks, it is necessary to consider known biotypes of *T. semipenetrans* in breeding and variety improvement programs. Knowing the reaction of several biotypes on similar hosts has provided new sources of resistance (3). Comparable infectivity data of several widely diverse populations may define differences that exist between populations.

This paper presents information on the infection potential of five populations of *T. semipenetrans* on 10 rootstocks growing under the same conditions.

## MATERIALS AND METHODS

Five populations of *T. semipenetrans* (two from California, and one each from Arizona, Texas, and Florida) were increased at Orlando, Florida. The Arizona population came from the University of Arizona Citrus Experiment Station, Yuma; two California populations, biotypes 1 and 3, were from infected citrus at the Citrus Research Center, Riverside; a Texas population was from Rio Farms near Weslaco; and a Florida population was from an infected grove near Orlando. Inoculum, obtained from infected citrus roots by root incubation,

Received for publication 10 September 1976.

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was washed in 4 μg/ml ethoxyethyl-mercury chloride and 1,000 μg/ml dihydrostreptomycin sulfate for surface disinfestation. To increase the individual populations, nematodes were pipetted around the roots of rough lemon [*Citrus limon* (L.) Burm. f.] seedlings growing in flats in a greenhouse. Six months after inoculation, rough lemon seedlings individually infected with each population were transplanted separately by nematode source into large, aboveground greenhouse growth bins (200 seedlings/bin). Each bin was placed 1.5 m from adjacent bins and contained a 1:1 mixture of Astatula fine sand and peat moss. Seedlings were grown an additional 6 months to provide a nematode density of 2,000-4,000 larvae/100 $cm^3$ soil.

The following plant selections were used in the host resistance study: five *Citrus:* rough lemon *(C. limon),* 'Rangpur' lime (*C. reticulata* var. *austera* Sw.), *C. macrophylla* Wester, 'Homosassa' sweet orange (*C. sinensis* (L.) Osb.), *C. volkameriana* Ten. & Pasq.; one *Poncirus:* 'Pomeroy' CRC 1717 *(P. trifoliata);* four hybrids: 'Swingle' citrumelo (*C. paradisi* Macf. X *P. trifoliata*), 'Carrizo' citrange (*C. sinensis* X *P. trifoliata*); and two citrange selections: '14-7' and '15-7' [Ruby orange X Webber-Fawcett trifoliate orange (*C. sinensis* X *P. trifoliata*)], hereafter called Ruby orange hybrids 14-7 and 15-7. Twenty 5-month-old seedlings of each selection were transplanted into the growth bins containing individual populations of *T. semipenetrans.* The seedlings were grown in the bins for 6 months with regular greenhouse maintenance. To satisfy replication requirements, 10 uniform seedlings of each selection were transplanted into 20-cm clay pots containing a 1:1 mixture of sand and peat and randomly distributed on greenhouse benches. Seedlings were grown at greenhouse ambient temperature (22-32 C).

Six months after being transplanted into pots, the seedlings were harvested by carefully removing roots from pots and gently washing the roots in running tap water. A 3- to 4-gm portion of lateral feeder roots was cut from each seedling and placed in a moist jar for root incubation in the dark at 23-24 C. After 3 and 5 days, 50 ml of water was poured over the roots, roiled vigorously, and decanted into beakers. Five-ml samples were withdrawn and placed in counting dishes, and citrus nematode larvae were counted. After the second reading (5 days), roots were removed from jars and weighed moist. Nematode numbers were calculated as citrus nematode larvae per gm root.

## RESULTS AND DISCUSSION

Numbers of *Tylenchulus semipenetrans* larvae on *Citrus* spp. were significantly higher ($P = 0.01$) than those on *Poncirus trifoliata* cv. Pomeroy or the three hybrids, Swingle citrumelo, Ruby orange 14-7, and 15-7. Numbers of California biotype 3 (CA3) were generally lower than the other four populations on the *Citrus* spp.; however, numbers of CA3 were significantly higher ($P = 0.01$) on Pomeroy and three hybrids than were the California 1, Florida, and Arizona populations (Fig. 1). The densities of the populations other than CA3 were very low and not significantly different among each other on Pomeroy, an indication reflecting the high level of resistance of certain *Poncirus* selections. Swingle citrumelo and the two Ruby orange hybrids supported significantly lower ($P = 0.01$) numbers of Arizona, Florida, and California 1 populations than either Texas or California 3. The *Poncirus*-resistant factor seemed greater in these hybrids when compared to that of the Carrizo citrange, which was similar to the *Citrus* spp. The Texas population was lower but not significantly different from California 3 on three of the hybrids, but it was significantly higher than California 3 ($P = 0.01$) on Carrizo citrange.

Differences in numbers of larvae/gm root among the five populations were most evident among Pomeroy, Swingle citrumelo, and the two Ruby orange hybrids (Table 1). Because of the relatively high numbers of nematodes found on all *Citrus* spp. and Carrizo citrange, we classified these selections as susceptible to all populations tested.

Levels of significance based on analyses of variance (LSD) comparing logarithmic nematode densities showed that California biotype 3 was different from Arizona, California 1, Florida, and Texas on 6, 7, 6, and 5 hosts, respectively. Its specificity, especially on *Poncirus,* suggests the name *T. semi-*

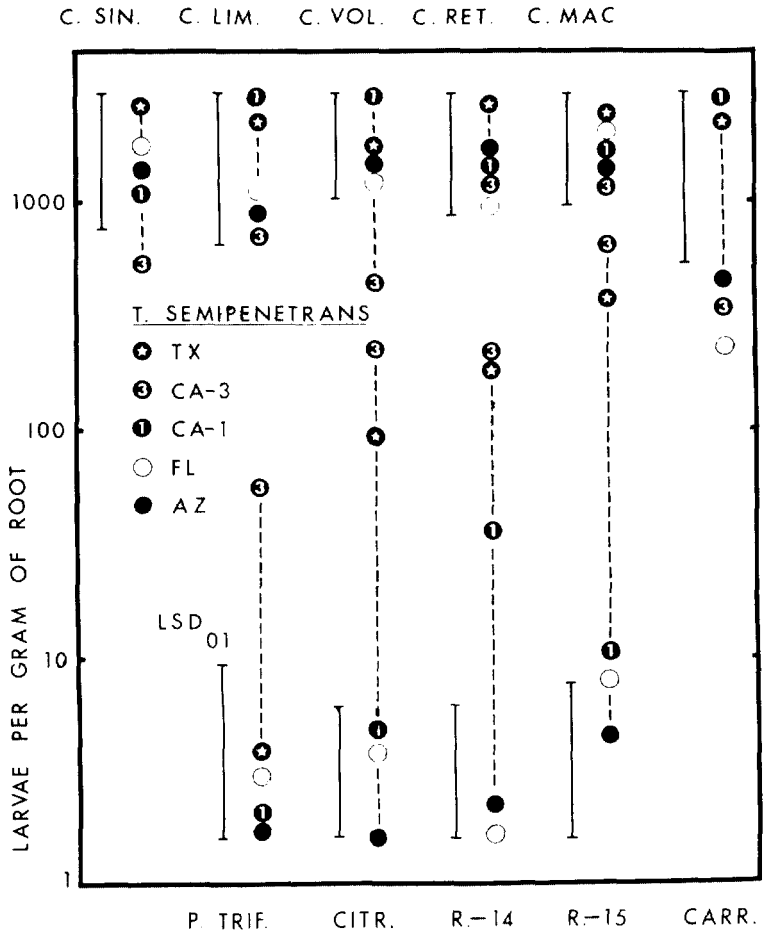


FIG. 1. Log numbers of five citrus nematode populations, Texas (TX), California 3 (CA3), California 1 (CA1), Florida (FL), and Arizona (AZ), on 5 *Citrus* spp., [*C. sinenis* (C. SIN.), *C. limon* (C. LIM.), *C. volkameriana* (C. VOL.), *C. reticulata* (C. RET.), and *C. macrophylla* (C. MAC.)]; 1 *Poncirus trifoliata* (P. TRIF.); and 4 *Citrus* spp. X *P. trifoliata* hybrids, [Swingle citrumelo (CITR.), Ruby orange 14-7 (R-14), Ruby orange 15-7 (R-15), and Carrizo citrange (CARR.)].

TABLE 1. Comparison of five pathotypes (Arizona, Florida, California 1, Texas, and California 3) of the citrus nematode to *Citrus, Poncirus,* and their hybrids.

| Citrus nematode population[a] | *Poncirus* sp. | Mean number of larvae/gm root/host[y] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Hybrids | | | | *Citrus* spp. | | | | |
| | Pomeroy | Swingle citrumelo | Ruby orange 14-7 | Ruby orange 15-7 | Carrizo citrange | Homosassa sweet orange | Rough lemon | *C. volkameriana* | Rangpur lime | *C. macrophylla* |
| AZ | 1 a | <1 a | 4 a | 5 a | 534 a | 1,743 ab | 1,787 a | 1,460 b | 1,469 a | 1,615 a |
| FL | 3 a | 3 ab | 1 a | 10 a | 442 a | 1,934 ab | 1,740 a | 1,437 b | 1,010 a | 2,691 a |
| C1 | 1 a | 6 b | 39 b | 26 a | 2,874 b | 943 ab | 3,032 a | 2,355 b | 1,760 a | 1,759 a |
| TX | 10 a | 131 c | 157 c | 550 b | 2,249 b | 2,496 b | 1,744 a | 1,978 b | 2,451 a | 2,200 a |
| C3 | 209 b | 284 c | 367 c | 714 b | 563 a | 752 a | 890 a | 475 a | 1,269 a | 1,617 a |

[y]Nematode means with different letters are significantly different ($P<0.05$) by Duncan's multiple range test.
[a]AZ (Arizona), FL (Florida), C1 (California 1), TX (Texas), C3 (California 3).

*penetrans* Poncirus biotype. Numbers of the Texas citrus nematode population were higher on Swingle citrumelo and the Ruby orange hybrids than Arizona, California 1, and Florida populations. The comparable high and low population levels on the *Citrus* spp. and Pomeroy, respectively, indicate the Texas population is not distinct from the Arizona, California 1, and Florida populations, even though the reproduction potential is greater.

The three hybrids, Swingle citrumelo, Ruby orange 14-7, and 15-7, appear to be unsuitable as hosts to the Arizona, Florida, and California 1 populations. Previous studies (4) showed the two Ruby orange hybrids to be free or only slightly infected with the California 1 biotype. Carrizo citrange is of the same parentage as Troyer citrange, which was previously (2, 10) shown to be susceptible to both California 1 and 3. In this study, Carrizo supported moderate to high densities of all five populations.

As observed in these studies, after approximately a 1-year exposure to the 10 hosts, the five populations remained stable. The ability to differentiate nematode biotypes by host is very important in any long-term, citrus rootstock-breeding program. These comparisons provide a better perspective of infection types of *T. semipenetrans*.

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