

# Reproduction of a Florida Population of *Tylenchulus semipenetrans* on Resistant Citrus Rootstocks

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## ABSTRACT

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*Tylenchulus semipenetrans* was detected at moderately high population density on seedlings of the hybrid rootstock Swingle citrumelo growing in a Florida citrus nursery. This rootstock is considered resistant to populations of *T. semipenetrans* in Florida. Seedlings of the nematode-resistant rootstocks *Poncirus trifoliata* (PT) and Swingle citrumelo (SC), and the susceptible rootstock rough lemon (RL) were planted in the nursery and in an orchard of mature citrus trees growing on RL rootstock infected by *T. semipenetrans*. After 5 mo, the numbers of adult females per gram of root in the nursery were smaller on PT than on RL, but densities of females on SC and RL were not different. The numbers of second-stage juvenile nematodes (J2) per gram of root were not different on any rootstock seedling. In the orchard, RL supported higher numbers of both juveniles and females per gram of root than did either PT or SC. In greenhouse trials using the nursery population of *T. semipenetrans* and a population from an orchard, no differences in development of nematodes from the nursery were detected among RL, SC, or PT. All life stages of *T. semipenetrans* from the orchard were supported at higher densities on RL than on SC or PT. We conclude that a population of *T. semipenetrans* capable of circumventing resistance in PT and SC now occurs in Florida.

Additional keywords: biotype, citrus nematode, *Citrus* spp.

The first reports of biotypes of *Tylenchulus semipenetrans* Cobb were by Baines et al (1-3). They determined that a population of the species from Japan was able to infect and develop on cultivars of *Poncirus trifoliata* (L.) Raf. to a much greater degree than populations from California. *P. trifoliata* is resistant to some populations of *T. semipenetrans* and *Phytophthora parasitica* Dast., and to citrus tristeza virus. However, it is used less frequently than other rootstocks because it lacks vigor and has poor tolerance to high soil pH and salinity. The ability of the California *T. semipenetrans* populations to reproduce on *P. trifoliata* was increased, to a limited degree, by maintaining the nematodes on *P. trifoliata* rootstock for several years (2). Reports that *T. semipenetrans* populations from different parts of the world differed in their ability to infect other crop species, such as grape and olive, supported the occurrence of major differences in the parasitic abilities of populations of this nematode (1).

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O'Bannon et al (13) compared populations of *T. semipenetrans* from citrus-growing regions of the United States and suggested the name "Poncirus biotype" to describe a population from California with significant parasitic ability on *P. trifoliata*. Formal designation of a *Poncirus* biotype was in recognition of the importance of *P. trifoliata* as a source of resistance to *T. semipenetrans* (6). For example, the hybrid cultivar Swingle citrumelo (*Citrus paradisi* Macf. × *P. trifoliata*) is a widely used rootstock cultivar with a high degree of resistance to most populations of *T. semipenetrans*. An extended classification of biotypes of *T. semipenetrans* was proposed by Inserra et al (9) to include a citrus biotype that reproduces poorly on *P. trifoliata* but infects *Citrus* spp., olive, grape, and persimmon; a Mediterranean biotype with the same characteristics as the citrus biotype except the ability to infect olive; and a *Poncirus* biotype capable of infecting *Citrus* spp., *P. trifoliata* and its hybrids, and grape, but not olive. A fourth, a grass biotype of the nematode (9), was later determined to represent different species of *Tylenchulus* (10).

To date, the *Poncirus* biotype has been identified in Japan and California, where *P. trifoliata* has been used as a rootstock for decades. Widespread use of Swingle citrumelo as a rootstock in some citrus-producing regions (4) will increase the selection pressure on *T. semipenetrans*

in some areas where the *Poncirus* biotype has not yet been detected.

High numbers of *T. semipenetrans* were detected on Swingle citrumelo seedlings growing in a commercial nursery in Florida in 1992. In this paper, we report results of field and greenhouse studies to determine whether this population is different than other populations of *T. semipenetrans* in Florida with respect to its ability to infect and develop on Swingle citrumelo and *P. trifoliata*.

## MATERIALS AND METHODS

**Field trials.** Moderately high population densities of *T. semipenetrans* (500 second-stage juveniles [J2] per 100 cm<sup>3</sup> of soil; 200 adult females per gram of root) were detected on Swingle citrumelo seedlings in a citrus nursery in Pasco County, Florida, in early July 1992. To confirm that the nematode population represented a resistance-breaking biotype, the ability of this population to reproduce on resistant rootstocks was compared with an orchard population. Eight-month-old seedlings of three citrus rootstock cultivars were planted in randomly chosen sites in the nursery on 21 July and in a citrus nematode-infested orchard in Polk County on 22 July 1992. One seedling each of *P. trifoliata* (PT), Swingle citrumelo (SC), and the susceptible rootstock rough lemon (RL) (*Citrus limon* (L.) Burm. f.) were planted in groups within the rows of the SC seedlings in the nursery and beneath the canopies of individual, mature Valencia orange trees on RL rootstocks in the orchard. Sixteen replicate groups of seedlings were planted in the nursery, and eight replicate groups were planted in the orchard. Three replicate groups of plants in the nursery and two in the orchard were lost due to seedling death. Eight additional groups of seedlings, each consisting of a single seedling of PT, SC, and the susceptible species sour orange (SO) (*Citrus aurantium* L.) were also planted in the orchard at the same time. One replicate group was lost due to seedling death. All seedlings of each rootstock used at both sites were from the same seed lots, produced and grown by the Division of Plant Industry of the Florida Department of Agriculture and Consumer Services. The groups of seedlings containing RL, SC, and PT were grown for 4 mo in both the nursery and

the orchard. The groups containing SO, SC, and PT were grown in the orchard for 11 mo.

At the end of the growth periods, seedlings were dug from the soil, and 2 g of fibrous roots from each plant were maceration-processed to extract female and J2 *T. semipenetrans* (7). Nematode

counts were expressed per gram of fresh root tissue; and for each population, numbers on PT and SC were compared to those on RL or SO by means of a paired *t* test.

**Greenhouse trials.** Two greenhouse studies were conducted under controlled conditions to compare the nursery

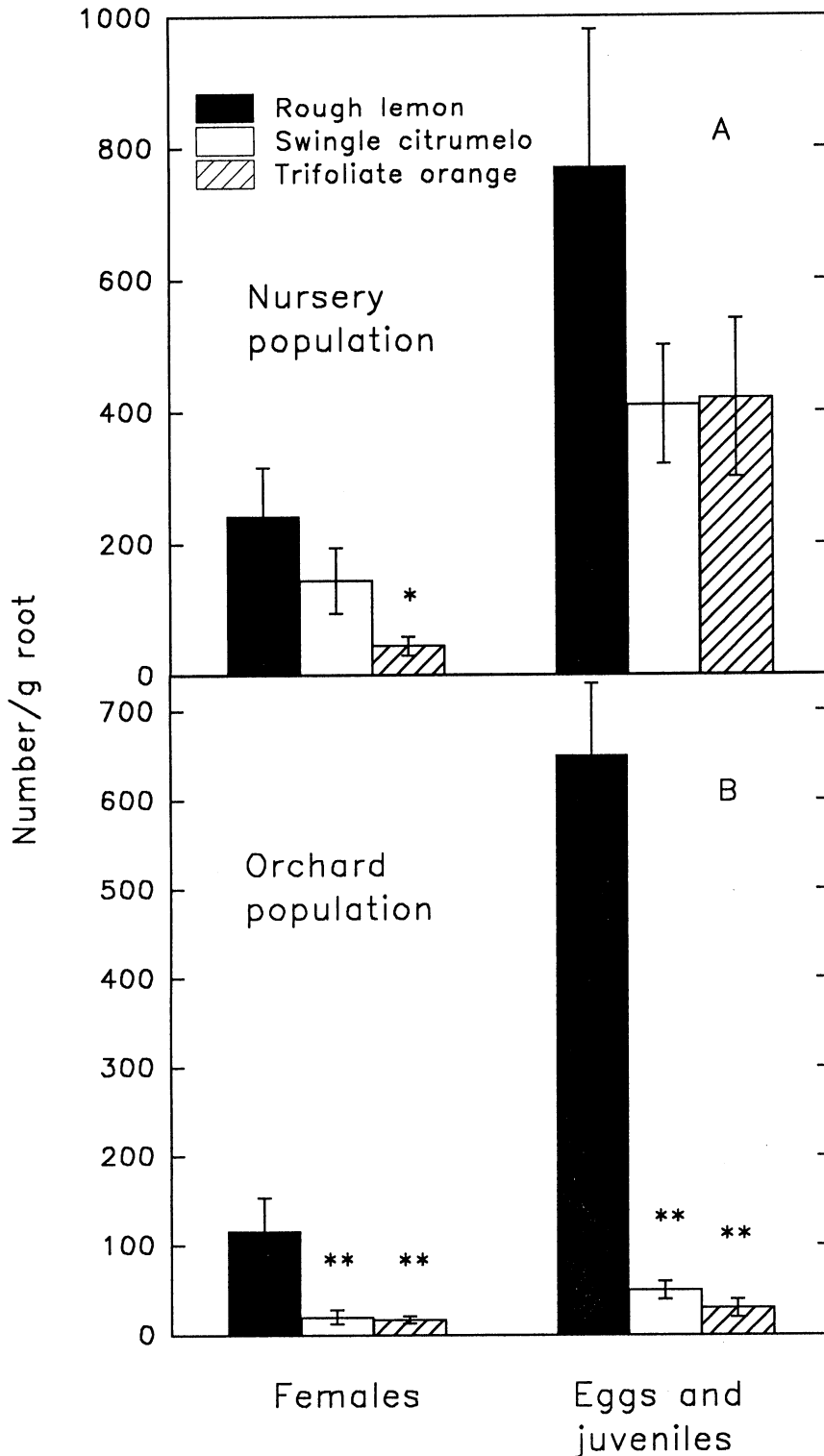
population of *T. semipenetrans* with a population from a second orchard in Polk County. In the first experiment, 8-mo-old seedlings of SC, PT, and RL were planted in 1-L clay pots containing steam-sterilized sandy soil (97% sand, 2% silt, 1% clay) mixed 1:1 (v/v) with an organic potting mixture. Ten replicates of each rootstock were randomly arranged and grown at average temperatures of 21–33 C. Plants were fertilized weekly with 250 ml per pot of a commercial formulation of micronutrients and 20:10:20 (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O) at 10 ml/L.

Inocula were collected (12) from roots of SC from the nursery and from RL roots from the orchard. Eggs and J2 were separated by repeated passes through a 38- $\mu$ m-pore sieve. Following surface disinfestation with 0.10% CuSO<sub>4</sub> for 15 min, nematodes and eggs were rinsed three times and added to the soil by syringe in four places 3 cm from the base of the seedlings. Seedlings were inoculated on three occasions, 4 days apart, beginning 7 days after transplanting. Approximately 160,000 J2 and 96,000 eggs were added to each pot. After 5 mo, plants were removed from pots, 100 cm<sup>3</sup> soil was processed for 48 hr on Baermann funnels, and all life stages of the nematode were extracted from 2 g of fibrous roots (7). Nematodes were expressed per 100 cm<sup>3</sup> of soil or gram of fresh root; and for each population, infection levels on *P. trifoliata* and SC were compared to those on RL by analysis of variance followed by Dunnett's test comparing all means against a control (15).

The second greenhouse experiment was conducted in the same manner as the first, except that six replicate SO (susceptible) and SC (resistant) seedlings were used as rootstock cultivars. Inoculum (2,300 J2 per plant) was obtained from roots of plants used in the first experiment. Treatment means of SO and SC rootstocks in this experiment were compared using *t* tests of transformed ( $\log X + 1$ ) data.

## RESULTS

**Field trials.** The differences in nematode numbers on SC and PT compared to RL were less for seedlings planted in the nursery than for those planted in the orchard (Fig. 1). Numbers of female nematodes per gram of fibrous roots on SC and PT in the nursery were 59 and 18%, respectively, of those on RL. Numbers of J2 nematodes per gram of fibrous root were 53 and 55% as numerous on SC and PT, respectively, as on RL (Fig. 1A). In the orchard, adult female nematodes per gram of fibrous roots were 20 and 17% as numerous on SC and PT, respectively, as on RL; and comparable percentages for numbers of juveniles were 7 and 5% (Fig. 1B). All differences in the numbers of nematodes on the susceptible compared to the resistant rootstocks grown in the orchard



**Fig. 1.** Development of two populations of *Tylenchulus semipenetrans* on the rootstock seedlings rough lemon, *Poncirus trifoliata*, and Swingle citrumelo at two different field locations: (A) a nursery in Pasco County and (B) an orchard in Polk County, Florida. Bars = mean standard error. Asterisks denote significant differences (\*  $P = 0.05$ , \*\*  $P = 0.01$ ) between population development on rough lemon and other rootstock cultivars as determined by paired *t* tests of data from each planted group of seedlings.

were highly significant ( $P = 0.01$ ). In the nursery, only PT was different than RL ( $P = 0.05$ ) with respect to adult female nematodes.

In the second orchard experiment (Fig. 2), differences in nematode population densities on susceptible and resistant rootstocks were less than in the first experiment. At the end of 11 mo in the orchard, adult female nematode numbers on SC and PT were 49 and 61% of those on SO (Fig. 2A). Levels of eggs and juveniles per gram of root on SC and PT were 25% ( $P = 0.05$ ) and 44% (ns) the levels on SO (Fig. 2B). Numbers of eggs and juveniles per adult female on the susceptible SO tended to be about double the numbers on the two resistant rootstocks (Fig. 2C).

**Greenhouse trials.** The two populations of *T. semipenetrans* differed in their ability to infect SC and PT in the greenhouse (Fig. 3). In the first experiment, numbers of adult females per gram of roots for the nursery population were 85 and 77% as numerous on SC and PT as on RL (Fig. 3A). Only 2% as many females of the orchard population developed on SC as on RL, and no females were recovered from PT. Numbers of eggs and J2 per gram of roots showed similar differences with 56 and 72% as many on SC and PT as on RL for the nursery populations and only 1% as many developing on both resistant rootstocks as on the susceptible for the orchard population (Fig. 3B). More J2 and males on average were detected in soil from resistant rootstocks than from RL for the nursery population, while only 7 and 5% as many nematodes of the orchard population were detected in soil from SC and PT as from RL (Fig. 3C). All measures of population development on resistant rootstocks were different ( $P = 0.01$ ) than measures on the susceptible rootstock for the orchard population. No rootstock effects were detected in any measure of nematode population development for the nursery population.

In the second greenhouse experiment, no differences were detected in numbers of females, eggs, or juveniles per gram of root or of nematodes in the soil between SO or SC rootstocks inoculated with the nursery population (Fig. 4). However, nematode reproduction for the nursery population was generally lower on SC than on SO. Mean numbers of females per gram of root on SC were only 33% of those on SO rootstock. Similarly, only 61% as many juveniles and eggs per gram of roots and 39% as many juveniles and males per unit of soil were recovered from SC as from SO rootstocks. Nematodes in soil for SC were 10% of those for SO ( $P = 0.01$ ), using the orchard population. Eggs and juveniles per gram of root and females per gram of root were 4% ( $P = 0.01$ ) and 5% ( $P = 0.01$ ) as numerous, respec-

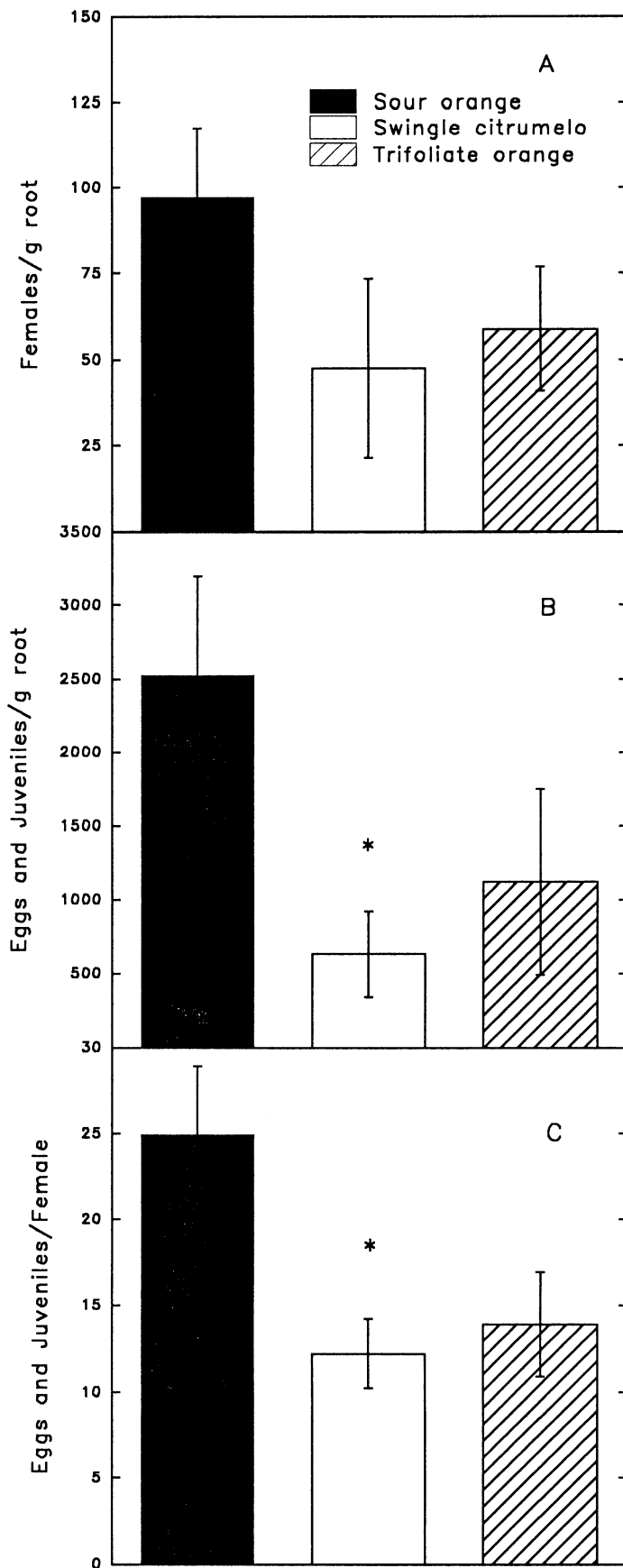


Fig. 2. Population development of *Tylenchulus semipenetrans* on the rootstock seedlings sour orange, *Poncirus trifoliata*, and Swingle citrumelo planted beneath mature citrus trees in the field. Asterisks denote significant differences ( $P = 0.05$ ) between population development on sour orange and other rootstock cultivars as determined by paired *t* tests of data from each planted group of seedlings. Bars = mean standard error.

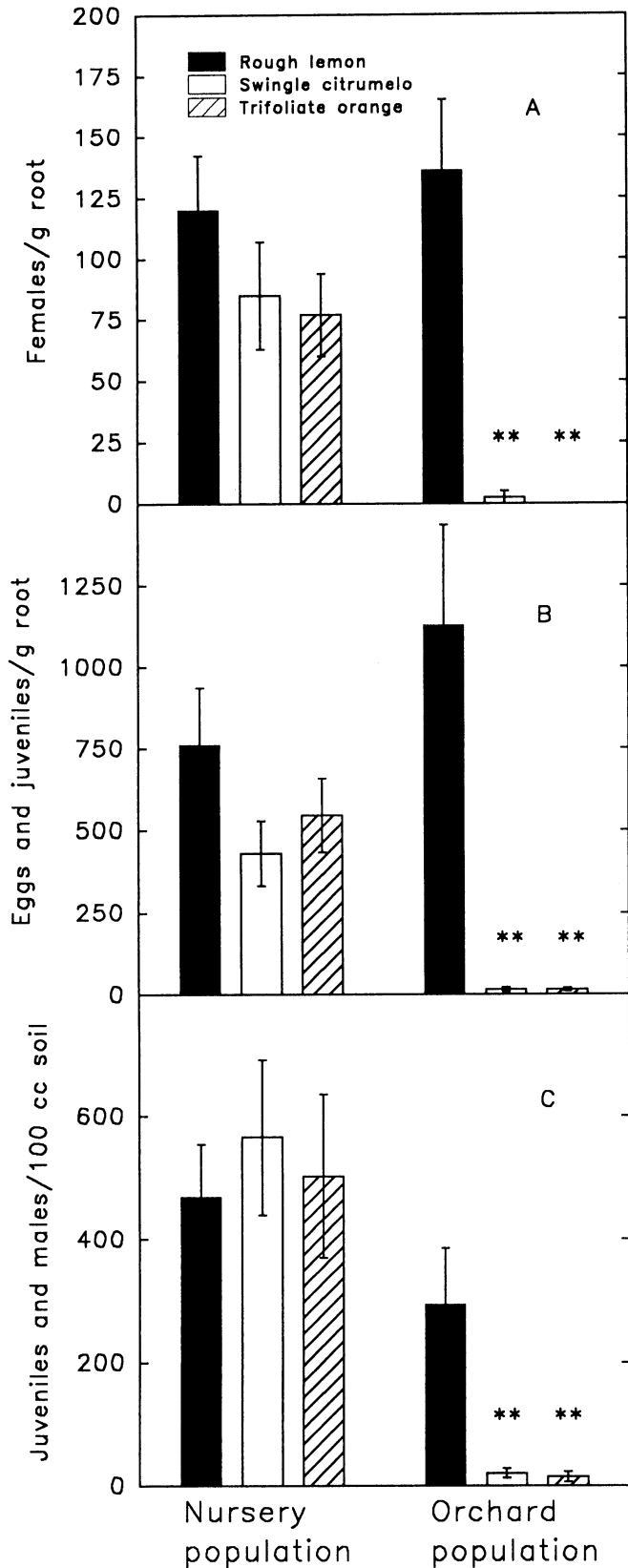


Fig. 3. Development of two populations of *Tylenchulus semipenetrans* on the rootstock seedlings rough lemon, *Poncirus trifoliata*, and Swingle citrumelo in the greenhouse. Bars = mean standard error. Asterisks denote significant differences ( $P = 0.01$ ) between population development on rough lemon and other rootstock cultivars as determined by Dunnett's test to compare all treatments with a control.

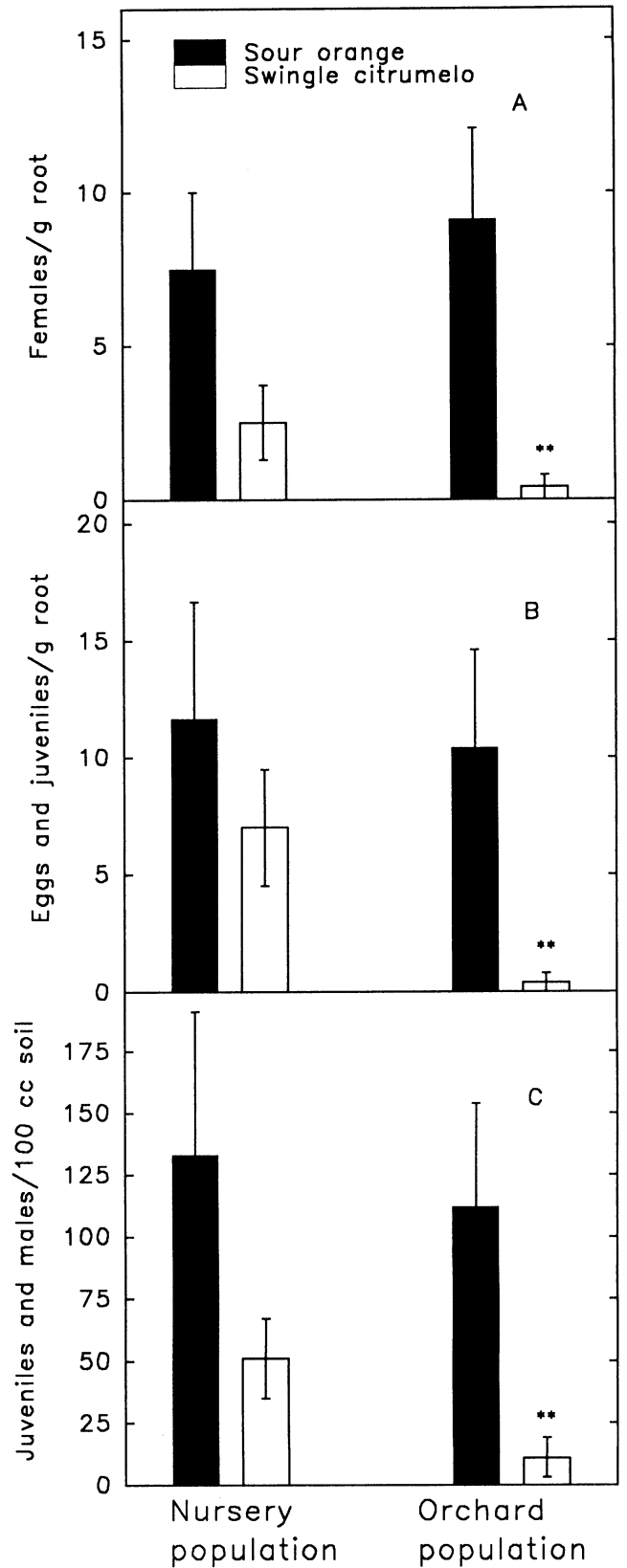


Fig. 4. Development of two populations of *Tylenchulus semipenetrans* on the rootstock seedlings sour orange and Swingle citrumelo in the greenhouse. Bars = mean standard error. Asterisks denote significant differences ( $P = 0.01$ ) between populations within a population as determined by  $t$  tests of transformed ( $\log X + 1$ ) data.

tively, on SC as on SO, with the orchard population.

## DISCUSSION

Based on the results of these studies, we conclude that the nursery population is a different biotype of *T. semipenetrans* with respect to its ability to infect PT and SC than has been detected previously in Florida. Numbers of J2 nematodes recovered from SC and PT in the field and greenhouse were comparable to those reported for the *Poncirus* biotype defined by O'Bannon et al (13). However, further host-range studies are necessary to determine whether the nursery population behaves exactly as the *Poncirus* biotype defined by Inserra et al (9).

Although there were few significant differences in population densities of the nursery population on any rootstock in either the field or the greenhouse, average densities were generally highest on the susceptible rootstocks RL and SO. Rough lemon rootstock is known to be a better host of *T. semipenetrans* than are many other rootstocks susceptible to this nematode (5,14). Other *Poncirus* biotype populations attain higher population densities on other rootstock cultivars of the genus *Citrus* than on PT (1,13).

It is not surprising that resistance-breaking populations of *T. semipenetrans* will infect and reproduce at lower levels on SC and PT than on other susceptible rootstocks. Van Gundy and Kirkpatrick (16), Kaplan (11), and Gottlieb et al (8) all concluded that resistance to *T. semipenetrans* derived from PT is based on several mechanisms including a hypersensitive response, periderm formation at the feeding site, toxic substances, and unknown factors that result in low population densities of the nematode at the rhizoplane. Thus, populations of *T. semipenetrans* unaffected by one of the mechanisms (e.g., a hypersensitive response) may be able to reproduce at moderate levels on PT and SC but may be affected somewhat by other mechanisms. Conversely, most hybrids of PT are likely to be less susceptible to infection by *T. semipenetrans* than the susceptible citrus parent cultivar if PT can provide any of several different resis-

tance genes during meiosis. However, only those hybrids receiving a sufficient number of genes for highly effective resistant mechanisms (i.e., Swingle citrumelo) will appear to be highly resistant (F. Gmitter, P. Ling, and L. Duncan, unpublished).

The origin of the nursery population in this study is unknown. It may have developed in situ in response to selection pressure. Other known populations of the *Poncirus* biotype have developed only in areas with a high incidence of use of PT and its resistant hybrids (2,3). The citrus nursery certification program enacted by the Florida Department of Agriculture and Consumer Services in cooperation with citrus growers prevents the movement of new biotypes from citrus nurseries to new citrus orchards. However, surveys of older plantings on SC rootstock are needed to determine the extent to which resistance-breaking biotypes may be present in the state.

When seedlings were grown under trees in an orchard, the average population density that developed on SC and PT compared to RL was proportionately higher than when seedlings were grown in the greenhouse. This is most likely because the nematodes were only introduced into pots during an 8-day period in the greenhouse; whereas in the orchard, nematodes could continuously move from the roots of trees to those of the seedlings. The susceptible rootstock, SO, used in the second orchard trial supports somewhat lower population densities of *T. semipenetrans* than does RL (14). Nevertheless, the differences between nematode infection of the susceptible and resistant rootstocks were very small. However, differences in infection of SO and SC were greater when they were tested in pots with a more restricted inoculation period. These data suggest that interplanting young trees on resistant rootstocks among existing trees infected by citrus nematodes may result in considerably higher infection of the young trees than would occur if resistant trees were planted as a block in nematode-infested soil free of existing infected trees. The former situation would significantly increase the selection pressure on the resistant rootstock and would prob-

ably increase the rate at which resistance-breaking biotypes develop (2).

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