

## Host Status of Citrus and Citrus Relatives to *Tylenchulus graminis*<sup>1</sup>

R. N. INSERRA, J. H. O'BANNON, AND W. M. KEEN<sup>2</sup>

**Abstract:** Studies were conducted on the host suitability of four citrus rootstocks—rough lemon (*Citrus limon*), sour orange (*C. aurantium*), trifoliolate orange (*Poncirus trifoliata* cv. Argentina), and Swingle citrumelo (*C. paradisi* × *P. trifoliata*)—to *Tylenchulus graminis* which was previously considered a “grass” race of *T. semipenetrans*. In an uncultivated field, sour orange seedlings grown with *T. graminis*-infected broomsedge (*Andropogon virginicus*) were not infected with this nematode after 18-month's exposure to *T. graminis* population densities ranging from < 0.01 to 0.4 second-stage juveniles (J2)/cm<sup>3</sup> soil. In a greenhouse test, two *T. graminis* populations from two Florida locations did not infect sour orange seedlings grown for 2 years in soil naturally infested with 0.3 and 1.3 J2/cm<sup>3</sup>. Rough lemon, trifoliolate orange, and Swingle citrumelo seedlings suppressed *T. graminis* initial population densities of 7 to final values of < 0.1 J2/cm<sup>3</sup> soil. Final values of > 70.0 J2/cm<sup>3</sup> occurred in soil with broomsedge. These findings provide conclusive evidence that *T. graminis* is a specific parasite of grasses and does not infect citrus.

**Key words:** *Andropogon virginicus*, broomsedge, *Citrus aurantium*, *Citrus limon*, citrus nematode, *Citrus paradisi* × *Poncirus trifoliata*, host preference, *Poncirus trifoliata*, rough lemon, sour orange, survival, Swingle citrumelo, trifoliolate orange, *Tylenchulus graminis*, *Tylenchulus semipenetrans*.

*Tylenchulus graminis* Inserra et al. is a widespread parasite of native grasses in Florida (1,2). Before its description, *T. graminis* was considered the “grass” race of the citrus nematode *T. semipenetrans* Cobb (5,6). Previous greenhouse tests provided evidence that this nematode did not attack several *Citrus* spp., including rough lemon (*Citrus limon* (L.) Burm. f.), sour orange (*C. aurantium* L.), and trifoliolate orange (*Poncirus trifoliata* (L.) Raf.) seedlings, which were not infected when they were grown in association with *T. graminis*-infected blue stem grass (*Schizachyrium rhizomatum* (Swallen) Gould) (4,5). In these studies, neither the initial nematode population densities nor the survival densities were reported.

A complete knowledge of the host status of citrus and citrus relatives to *T. graminis* is of great importance in Florida because, before its description, it was subject to the same regulatory restrictions as *T. semipenetrans*. Field and greenhouse experiments

were conducted in 1986–88 with selected rootstocks commonly used by citrus growers in Florida to determine their possible suitability as hosts for *T. graminis*.

### MATERIALS AND METHODS

**Field experiment:** This study was conducted on a 200-m<sup>2</sup> site in an uncultivated field with characteristic native flora in Highlands County in central Florida where *T. graminis*-infected broomsedge (*Andropogon virginicus* L.) was abundant. This grass provided a nematode inoculum source year round (1). Other *T. graminis* nonhost plant species were also present in the site. The area was fenced to keep out game animals, such as deer.

*Tylenchulus graminis* initial population densities in soil were assessed by collecting 40 soil plugs, 50 g each, around broomsedge plants in the site. Soil plugs from adjacent plants were mixed together and processed by the sugar flotation method (3). In June 1986, 125 5-month-old sour orange seedlings were transplanted adjacent to *T. graminis*-infected broomsedge plants. Seedlings were watered as necessary; however, there was a high seedling mortality rate due to broomsedge root competition and grazing effect of rabbits that came through the fence. Only 14 seed-

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<sup>1</sup> Contribution No. 377, Bureau of Nematology, Florida Department of Agriculture and Consumer Services, Gainesville.

<sup>2</sup> Nematologists and Agricultural Products Supervisor, Bureau of Nematology and Bureau of Plant Inspection, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL 32602.

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FIG. 1. Left to right, trifoliolate orange cv. Argentina, Swingle citrumelo, and rough lemon grown in association with broomsedge in soil infested with 7 *Tylenchulus graminis* J2/cm<sup>3</sup> for 10 months. *Tylenchulus graminis* final population density was 10 times higher than the initial density, but no infection was detected on citrus roots. Scale bar = 10 cm.

lings survived and became established. Three months after transplanting, soil samples around the combined citrus and broomsedge plants were collected again to determine *T. graminis* density changes after the rainy summer months (June–August 1986).

Half of the citrus seedlings were removed randomly from the site in June 1987 and the remainder in December 1987. *Tylenchulus graminis* population densities in the root and rhizosphere were determined at each sampling. *Tylenchulus graminis* second-stage juveniles (J2) and males were extracted from roots by incubation (8). To obtain adult females, root segments were macerated, sieved, and centrifuged in sugar solution (3). Nematode life stages were recorded as either nematodes per cubic centimeter of soil or nematodes per gram of fresh root weight.

*Greenhouse experiment 1:* Two different *T. graminis* populations collected from another site in Highlands County and from Sarasota County on the central west coast of Florida were used in this experiment. The inoculum source was naturally infested field soil containing 0.3 and 1.3 *T. gra-*

*minis* J2/cm<sup>3</sup> from Highlands and Sarasota counties, respectively. Two-month-old sour orange seedlings were transplanted into 16 10-cm-d pots, each containing 800 cm<sup>3</sup> infested soil from Highlands County (eight pots) or Sarasota County (eight pots). These two *T. graminis* populations were also maintained in the greenhouse on broomsedge plants grown concurrently with the inoculated citrus seedlings. Sour orange seedlings transplanted into eight pots, each containing 800 cm<sup>3</sup> soil infested with 3 *T. semipenetrans* J2/cm<sup>3</sup>, collected from a citrus grove near Frostproof, in central Florida, served as controls.

Seedlings were grown at 20–25 C and were watered and fertilized as necessary. Two years after transplanting, all plants were removed from the pots and nematodes were extracted from soil and roots as previously described.

*Greenhouse experiment 2:* To study the effect of a high population density of *T. graminis* on rough lemon, trifoliolate orange, and Swingle citrumelo (*C. paradisi* Macf. × *P. trifoliata*), test seedlings were grown alone or together with broomsedge (Fig. 1) in *T. graminis*-infested soil.

To obtain a pure source of *T. graminis* inoculum, nematode-free broomsedge seedlings grown in 25-cm-d pots containing 5 dm<sup>3</sup> steam pasteurized soil were inoculated with hand-picked specimens of *T. graminis* from Sarasota County and maintained in a greenhouse at 20–25 C. After 1 year the roots from all plants were shaken free of soil and cut into small pieces and remixed with soil from all pots. Thirty-five 15-cm-d pots were filled with 2 dm<sup>3</sup> of the root–soil mix containing ca. 7 *T. graminis* J2/cm<sup>3</sup>. Seven-month-old seedlings of the three *Citrus* spp. and 4-month-old broomsedge plants were transplanted alone or in combination, in five replicates. The pots were randomly arranged on a greenhouse bench and grown at 20–25 C and watered and fertilized as necessary. After 10 months, all plants were removed from the pots and nematode densities in soil and roots were determined. Nematode density means from pots with broomsedge were separated using Duncan's multiple-range test.

## RESULTS

*Field experiment:* The initial population density of *T. graminis* in the soil around broomsedge plants was ca. 0.15 J2/cm<sup>3</sup>, with ca. 1.5 females/g broomsedge root. Other ectoparasitic nematodes detected in soil samples from the plot were not considered relevant to this study. The nematode population densities in soil with the combined broomsedge and sour orange seedlings, 3 months and 1 year after transplanting, were 0.12 and < 0.1 J2/cm<sup>3</sup>, respectively. No *T. graminis* were detected in roots of sour orange seedlings. Eighteen months after transplanting, there was an increase in *T. graminis* population density to 0.4 J2/cm<sup>3</sup> soil and 7 females/g broomsedge roots. Again, no nematodes were detected in the roots of any sour orange seedling.

*Greenhouse experiment 1:* After 2 years no *T. graminis* infection was detected on the sour orange seedlings grown in the two naturally infested soils from Highlands and Sarasota counties. The infection level on

the broomsedge plants grown in these two soils persisted at the original population densities detected at the beginning of this study. These results suggest that the decline of *T. graminis* population densities to undetectable levels in the pots with sour orange seedlings was mainly because this citrus species was not a suitable host for the nematode. Population densities of *T. semipenetrans* on citrus seedlings grown in nematode-infested soil increased from 3 to 27 J2/cm<sup>3</sup> soil with 159 J2 and 236 females/g sour orange roots.

*Greenhouse experiment 2:* In pots with citrus seedlings grown alone in *T. graminis*-infested soil, no nematode infection was detected on the roots of any rough lemon, trifoliolate orange, or Swingle citrumelo, even though J2 and males were detected in the soil. The population densities in soil, however, decreased from initial levels of 7 to < 0.1 J2/cm<sup>3</sup> after 10 months (Table 1). In pots with citrus seedlings and broomsedge combined, there was no evidence of infection of any of the three citrus rootstocks tested; however, *T. graminis* J2 densities in the soil increased on broomsedge to levels 5 and 12 times greater than the initial population density (Table 1). Density of *T. graminis* J2, males, and females in soil with broomsedge only was lower ( $P = 0.05$ ) than in soil with mixed roots; however, root nematode density was greater ( $P = 0.05$ ) (Table 1).

## DISCUSSION

Under field conditions *T. graminis* densities on broomsedge are much lower than *T. semipenetrans* densities on citrus (0.1–2.0 vs. 30–150 J2/cm<sup>3</sup> soil) (1,7). Factors such as plant species diversity, competition with other nematodes, unstable environmental conditions (extreme variations in moisture and temperature values, plant stress), and seasonal root growth may play an important role in density suppression under uncultivated conditions. Interestingly, in our survey we have often observed *T. graminis* specimens infected with *Pasteuria* sp. which, with other nematode antagonist organisms, must influence soil population levels.

TABLE 1. Density variations of three nematode life-stages on three citrus rootstocks and broomsedge grown alone or in combination for 10 months in soil infested with 7 *Tylenchulus graminis* second-stage juveniles (J2)/cm<sup>3</sup>.

	Nematodes/cm <sup>3</sup> soil			Nematodes/g fresh root wt.		
	J2	♂	♀	J2	♂	♀
Broomsedge	36.0 a	18.0 a	9.0 a	176.0 a	34.0 a	176.0 a
Rough lemon	0.1†	0.1	0.0	0.0	0.0	0.0
Trifoliolate orange	0.1	0.0	0.0	0.0	0.0	0.0
Swingle citrumelo	0.1	0.0	0.0	0.0	0.0	0.0
Rough lemon + broomsedge	88.0 b	25.0 b	23.0 b	121.0 b	27.0 b	118.0 b
Trifoliolate orange + broomsedge	94.0 b	33.0 b	31.0 b	70.0 b	13.0 b	129.0 b
Swingle citrumelo + broomsedge	93.0 b	20.0 b	31.0 b	100.0 b	19.0 b	131.0 b

Values are means of five replicates. Means with the same letter in vertical columns are not significantly different according to Duncan's multiple-range test ( $P = 0.05$ ).

† Treatments without a suitable host (broomsedge) were not included in the statistical analysis.

We have found that under optimal soil moisture, temperature, and other plant growth conditions, *T. graminis* increased to relatively high numbers (Table 1) on a suitable host, such as broomsedge, as does *T. semipenetrans* on citrus.

Many *T. graminis* females were detected in the soil samples from our experiments. High numbers of mature females in the soil resulted from the disproportion between the body length of swollen females (222–355  $\mu\text{m}$ ) and the diameter of broomsedge roots (140  $\mu\text{m}$ ) (Fig. 2A, B). The thin layer of cortical cells (60  $\mu\text{m}$  thick) does not provide a good anchorage for the swollen females (Fig. 2B); thus they were easily dislodged from roots. Nematodes displaced from the broomsedge roots into the soil occurred most frequently in treatments

with combined broomsedge and citrus during the separation of their root systems. Broomsedge roots alone were less disturbed, thus a higher percentage of nematodes remained attached to the roots and lower numbers were recovered from soil.

It is worth mentioning that, for regulatory purposes, citrus roots cannot be visually separated from those of broomsedge when these plants are grown together. In our experiments, the presence of citrus roots contaminated with *T. graminis*-infected broomsedge roots occasionally resulted in the detection of *T. graminis* life stages after citrus root incubation. Contaminated citrus roots can be detected only after microscopic examination of the citrus root mass.

In Florida, all *T. graminis* life stages were

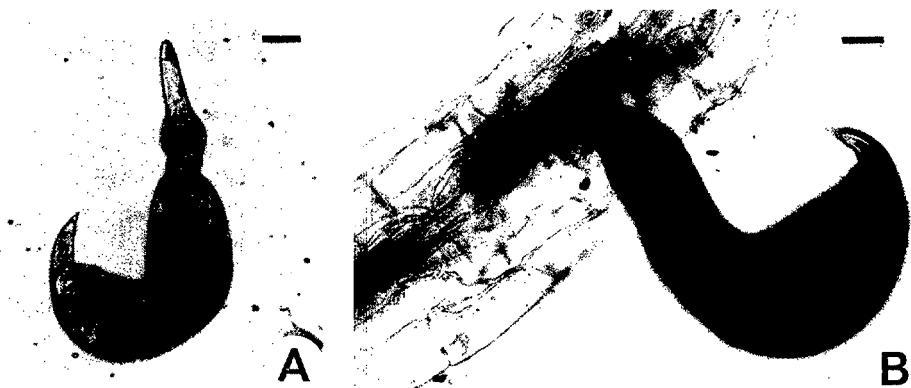


FIG. 2. *Tylenchulus graminis*. A) Mature female entire body. Note the short anterior body portion that is not swollen and is easily dislodged from root tissue. B) Swollen posterior body portion of a mature female protruding from the surface of a broomsedge feeder root. Note the disproportion between nematode body length and root diameter. Scale bars = 25  $\mu\text{m}$ .

found on native grass hosts year round (1), similar to *T. semipenetrans* on citrus. Fluctuations of *T. graminis* populations (from < 0.1 to 0.4 J2/cm<sup>3</sup> soil) observed in the field experiment are related to several factors previously mentioned and also to the biology of the host. *Tylenchulus graminis* levels reached a peak during broomsedge blooming (November–December) and decreased after seed production, when plants became senescent and languished before undergoing the regenerative process in February–March. Broomsedge and other perennial grasses that persist and produce new stolons and root flushes throughout the year provide an excellent continuous source of nutrients for this nematode, as does citrus for *T. semipenetrans*.

These studies and those previously reported (4,5) show conclusively that *T. graminis* does not pose a threat to citrus in Florida.

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