The Effect of Soil Type on Movement and Infection Rate of Larvae of Tylenchulus semipenetrans

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Abstract: Most of the *Tylenchulus semipenetrans* larvae applied on the surface of four soils in pots 14.5-cm deep moved no further downward than 6.5 cm, and remained in the upper half of the pot. The percentage of second-stage larvae that developed into adult females on 'Homosassa' sweet orange in the soils were: sandy loam, 6.8%; in the same soil with inoculation holes, 8.6%; loamy sand, 5.4%; coarse sand, 0.2%; and in sand-peat (2:1, v/v) mixture 0.04%. Low percentage infection in coarse sand and sand-peat mixture may have been caused by restriction of larval migration in the coarse sand, and by low pH or toxic organic acids in the sand-peat mixture. Good root development occurred throughout all soils. *Key Words:* citrus nematode, soil type, infection, sweet orange seedlings.

A soil medium that is suitable for determining the susceptibility of citrus seedlings to the citrus nematode (*Tylenchulus semipenetrans* Cobb) should be favorable for penetration, movement, and development of second-stage larvae and for the growth of the host. Citrus nematode larvae are attracted to micro- and macrowounds that involve one to several epidermal cells or considerable root tissue (5). Thus, mechanical wounding of roots should be avoided when determining the natural resistance of roots to the citrus nematode. Differences in the development of the citrus nematode on citrus roots in different soils have been observed, and peat (organic matter) mixed into a mineral soil interfered with the counting of mature female nematodes. O'Bannon (3) reported that the citrus nematode reproduced more rapidly on rough lemon [*Citrus limon* (L.) Burm.] and sour orange [*C. aurantium* L.] in a loamy sand that contained 3.2% or 9.2% organic matter (peat) than in a loamy sand without additional organic matter. Tarjan (6) reported that the citrus nematode moved a much shorter distance in a sandy loam that contained 85.6% sand, 5.4% silt, 9.0% clay, and 3.5% organic matter than in a loamy sand of 97.5% sand, 2.5% clay, and 0.5% organic matter. The effects of some chemical and physical properties of four soils on the movement of second-stage citrus nematode larvae and on their development on sweet orange roots are reported herein.

MATERIALS AND METHODS

Square plastic pots 13.5 cm wide and 14.5 cm high that tapered toward the bottom (average volume, 2 liters) were used. The pots had one drainage hole on each of the four sides. The holes were covered with a piece of paper towel to prevent soil loss. Pore space and bulk density of the soils was determined by covering the holes with tape, filling with oven-dried soil (110 C), shaking, and then slowly adding water until saturation was reached. Percent of water retained after draining and before irrigating was determined.

The soils, except peat, were pasteurized by heating with steam at 80 C for 1 h. The loamy sand and sandy loam received N, P, K, Cu, and Zn salts before planting. Sweet orange seedlings [*C. sinensis* (L.) Osbeck 'Homosassa'] approximately 8 cm high were planted one per pot. The seedlings were inoculated 7 weeks after planting by pouring 40,000 recently hatched second-stage larvae of *T. semipenetrans* in 250 ml of water onto the soil surface. In one treatment, the 40,000 larvae in 24 ml of water were distributed equally in six 0.8 diam × 5 cm deep holes made by withdrawing glass rods, which had been placed in the soil at planting. After inoculation, the holes were filled with soil. Treatments were replicated 10 times and pots were randomized on a bench in a glass house at 20-27 C. When the soil surface became dry, deionized water was added to bring the soil to the drained weight. The seedlings in coarse sand or in sand-peat received 250 ml of complete nutrient solution weekly, in addition to water. Two months after inoculation, the soil was removed carefully from the pot without disturbing the roots and then was cut in half horizontally. All roots were removed separately from the top and bottom portions of the soil, carefully washed free of soil, blotted dry, and weighed. They were stored in 10% formalin and numbers of mature females were determined by a staining (acid fuchsin in

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lactophenol), blender maceration, and sieving method (1).

RESULTS

Physical properties and pH of the four soils are given in Table 1. In the sand-peat mixture, the hydrophilic organic matter absorbed large amounts of water and the spaces between the sand particles apparently were filled with water and organic material after complete wetting. When the other soils were saturated the pore spaces were filled with water and a little air. Air entered the soils when water was lost by evaporation, transpiration, and drainage. Good root development and plant growth occurred in all four soils (Table 2), which suggests that soil aeration was favorable for both sweet orange roots and citrus numatode.

In the sandy loam inoculated through six holes, and in the same soil inoculated on the surface, 8.6 and 6.8%, respectively, of the larvae developed into mature females. In the loamy sand, coarse sand, and in the sand-peat mixture, 5.4, 0.2, and 0.04% of the larvae developed into adult females. Between 74.1 and 91.5% of the adult females occurred on roots in the top half (6.5 cm) of the pots (Table 2).

DISCUSSION

The low percentage (0.2%) of the larvae that developed on sweet orange roots in coarse sand and in the sand-peat mixture (0.04%) indicates that some factors were unfavorable for infection and/or development of the nematodes. Since sweet orange made good growth in these soils, it is assumed that these factors were not unfavorable for the growth of the host. As the coarse sand with large pore spaces became dry, the water occurred mainly at the points of contact between the sand particles and apparently restricted the movement of and trapped the larvae (9). The large interconnecting pore spaces in the coarse sand likely made it possible for the larvae to move through this soil more easily than through the two finer-textured soils when they were wet. This may account for the slightly higher percent of adult females that occurred on roots in the bottom half of the pots that contained coarse sand than on similar roots in the other soils.

TABLE 1. Chemical and physical properties of four soils.

Type	pH	Particle sizes: Sand (%)	Silt (%)	Clay (%)	Pore space (%)	Water when drained (%)	Average water before irrigation (%)
Coarse sand	6.9	98.7	1.0	0.3	34.8	21.7	7.7
Loamy sand	7.2	75.0	24.0	1.0	37.5	25.2	7.0
Sandy loam	7.0	74.1	11.4	14.5	32.2	20.5	7.6
Sand-peat[a]	4.0				51.0	50.7	12.3

[a]Mixture (2:1, v/v).

TABLE 2. Effect of soil type on the movement of *Tylenchulus semipenetrans* larvae and on percentage of larvae infecting sweet orange seedlings.

Soil type	Dry wt tops (g)	Roots in top half of pot: Weight (g)	Adult female nemas/g root	Adult nemas (%)	Roots in bottom half of pot: Weight (g)	Adult female nemas/g root	Adult nemas (%)	Larvae that matured (%)
Sandy loam (control)	1.81	1.73	0[z]		2.08	0		
Sandy loam	1.84	1.75	1233 a	79.1	1.94	293 a	20.9	6.8 ab
Sandy loam (holes 5 cm deep)	1.64	1.83	1503 a	79.5	2.00	356 a	20.5	8.6 a
Loamy sand	.99	1.45	1354 a	91.5	1.03	183 b	8.5	5.4 b
Coarse sand	1.61	1.70	39 b	74.1	1.23	19 c	25.9	0.2 c
Coarse sand - peat (2:1, v/v)	2.87	2.36	6 b	89.6	1.80	1 c	10.4	0.04 c

[z]Numbers followed by different letters are significantly different $P = 0.05$.

The sandy loam soil was favorable for the development of sweet orange seedlings and the second-stage larvae moved readily into and through this soil. An additional 1.8% of the larvae developed to maturity when they were placed in holes than when they were poured onto the surface of the sandy loam. In both methods of inoculation, 79.1% and 79.5% of the adult females occurred on roots in the top half of the pots. Migration in this soil as in the other soils was limited, or the larvae were attracted to roots. The increased infection does not appear to justify the additional operations involved in making the holes. The development of the citrus nematode in these soils in similar to that reported by Van Gundy et al. (8). Migration data in this study represent one nematode generation, and may be used to determine the susceptibility of citrus seedlings to *T. semipenetrans.*

Elmiligy and Norton (2) reported that seven organic acids (including fulvic and humic acids) were inhibitory to the survival or reproduction of *Aphelenchus avenae* Bastian, *Helicotylenchus pseudorobustus* (Steiner) Golden, *Meloidogyne hapla* Chitwood, and *Xiphinema americanum* Cobb. However, reproduction of *H. pseudorobustus* and *M. hapla* increased in proportion to increasing amounts of muck added to sand. O'Bannon (3) reported that adding peat moss to Eustis fine sand accelerated the increase of the citrus nematode. He adjusted the pH to 6.4-6.9 by adding dolomite lime. Sayre et al. (4) mentioned that degree of ionization of some organic acids is important in their toxicity. Van Gundy and Martin (7) reported that pH levels 4.3 and lower greatly decreased rates of reproduction of *T. semipenetrans,* and also the growth of Homosassa sweet orange seedlings. Thus, it is not certain whether the fewer nematodes obtained from soil was due to the effects of pH 4.3 on the growth of sweet orange seedlings or on the rate of reproduction of *T. semipenetrans.* Since sweet orange seedlings grew well in the sand-peat mixture at pH 4.0 and also at pH 3.8-4.1 in hydroponic culture (H. D. Chapman, *unpublished*),this author believes that the low infection in the sand-peat mixture was due to toxicity of pH 4.0 or of organic acids (humic or fulvic), or other factors on the infectivity of *T. semipenetrans* larvae.

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