Response of Citrus Seedlings to *Radopholus similis* in Two Soils

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Abstract: *Radopholus similis* was less pathogenic to greenhouse-grown citrus seedlings in Leon loamy sand than in Lakeland fine sand. This was not affected by different watering regimes. Seedling growth reduction by the burrowing nematode in either soil, compared with noninfected controls, was significant at the 1% level. *Key Words:* Growth reduction, Soil type, Spreading decline.

The burrowing nematode, *Radopholus similis* (Cobb) Thorne, is the causal agent of spreading decline of citrus in Florida (13). Field surveys revealed that spreading decline of citrus is most generally found in the sandy ridge area of central Florida and seldom in shallow or poorly drained soils. Field observations (1) indicated that nematode population levels and growth reduction of trees vary with the natural environment (including soil types and cultural practices). *R. similis* populations also varied with tree condition and seasonal periodicity (5, 6). According to Suit and DuCharme (14), the burrowing nematode does not enter maturing roots which have formed a hardened or suberized epidermis or senescing roots in which decay of tissues has started. They mainly attack healthy, young, succulent feeder roots.

In Florida, the effects of *R. similis* on field-grown citrus vary from slight aboveground symptoms in poorly drained shallow organic soils, to typical spreading decline symptoms in the well-drained deep sands. An early report (8) indicated that trees growing in soils containing more than 3% clay are not seriously affected by the burrowing nematode. There was no evidence to show whether the nematode was present in these soils, however. The purpose of the study reported here was to investigate the influence of soils on the nematode-host interaction. This paper reports the results of two greenhouse studies of the *R. similis*-citrus interaction in two Florida citrus soils.

MATERIALS AND METHODS

The two soils used in these studies were a Leon and Lakeland type originating on the Atlantic Coastal Plain near Vero Beach and Central Florida near Clermont, respectively. The principal types of Leon soils are fine sand, the type used in these studies, and sand. The surface horizon ranges from gray to very dark gray in color. The natural soils are rather poorly drained; the water table is generally high accounting for the wetness of the soils. Water drainage is provided for citrus culture. The principal types of Lakeland soils are fine sand, used in these studies, loamy sand, and loamy fine sand. The surface horizon ranges from grayish-brown to pale brown, and is well- to excessively-drained.

The soils (Table 1) were dug from the surface 18 inches in citrus groves at the locations given above, screened to remove coarse debris, and placed in 8-inch pots. After steam pasteurization, the soils were moistened, fertilized with 400 ml of dilute 6-6-6 fertilizer solution, and maintained with occasional watering on a greenhouse bench for 5 and 3 months respectively, for each experiment.

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TABLE 1. Particle-size distribution and organic matter content of two Florida soil types from 0- to 18-inch depth used in this study.

Soil type	*p*H range	Clay (<2 μ) %	Silt (2–50 μ) %	Sand (50 μ–2 mm) %	Organic matter %	Moisture[a] %
Leon Loamy Sand	6.2–6.5	7.5	6.2	86.3	2.32	10.0
Lakeland Sand	6.1–6.6	1.7	2.0	96.3	0.20	4.5

[a] Moisture content approximates field capacity.

The Florida rough lemon (*Citrus jambhiri* Lush.) plants used in these studies were infected with *R. similis* by transplanting 5-month-old seedlings in soil bins containing Lakeland sand heavily infested with *R. similis*. Additional seedlings were lined-out in similar soil bins free of *R. similis*. After 2 months the infected and non-infected seedlings were carefully removed from the soil, shaken free of sand, selected for uniformity, and transplanted into the respective soils in 8-inch pots. Stem diameter measurements near the soil surface were taken at 2-month intervals, and dry weight measurements were obtained at harvest (18 months). Single shoots were maintained by removing new axillary buds except at the apex. To prevent excessive linear growth, tops of plants were cut back at intervals. To study nematode reproduction and population fluctuations, 2 to 3-g root samples were taken at intervals from each pot. Each sample site was marked to prevent subsequent sampling at the same spot. Nematodes were extracted from roots by incubation in jars (18) and counted at 4 and 7 days.

In the first experiment regular and supplemented water schedules were maintained to study the effects of two moisture levels on the nematode population. In the regular watering, the soil was watered, as determined by weight, to maintain the soil moisture between 50 and 100% field capacity. In the supplemented water treatment series, seedlings were given one or more extra waterings per week. This amounted to 1 to 1½ times more water than given plants receiving a regular water schedule. There were nine replicates in each treatment randomized on greenhouse benches. Greenhouse temperatures ranged from 16–35 C. Seedlings were harvested after 18 months.

In a second experiment, to study in detail the effects of *R. similis* on citrus seedlings grown in the two soils, only the regular watering schedule was maintained. Nine replicate pots with a single seedling per pot were used. Seedlings were harvested after 12 months. Analyses of variance were calculated for all data.

RESULTS

In the first experiment, root samples taken in October when seedlings were transplanted from infection bins into their respective soils averaged 160 *R.* similis per g of root. Six months later *R. similis* populations had increased fivefold in the Lakeland soil but less than twofold in the Leon soil (Fig. 1B). Subsequent sampling showed a rapid population decrease in both soils and water schedules. After 18 months, the termination of the experiment, the difference between nematode populations on seedlings in the Leon and Lakeland soils was significant at the 5% level. The different water schedules had no significant effect on population levels in either of the soil types in this experiment.

R. similis reduced stem diameters of infected rough lemon seedlings in both soils 14% after 6 months (Fig. 1A). Growth of non-infected seedlings was similar in both

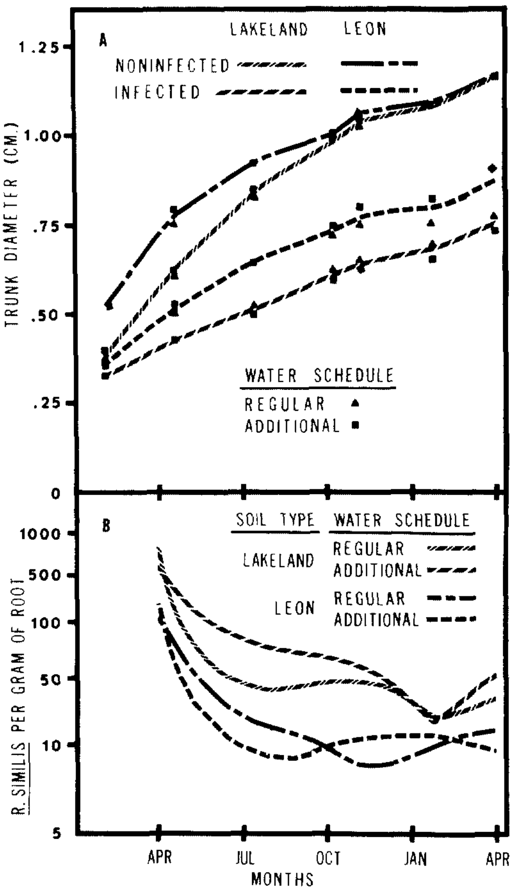

FIG. 1. Soil type and watering effects on: **A.** Citrus seedling stem diameters; and **B.** *Radopholus similis* populations on rough lemon seedling.

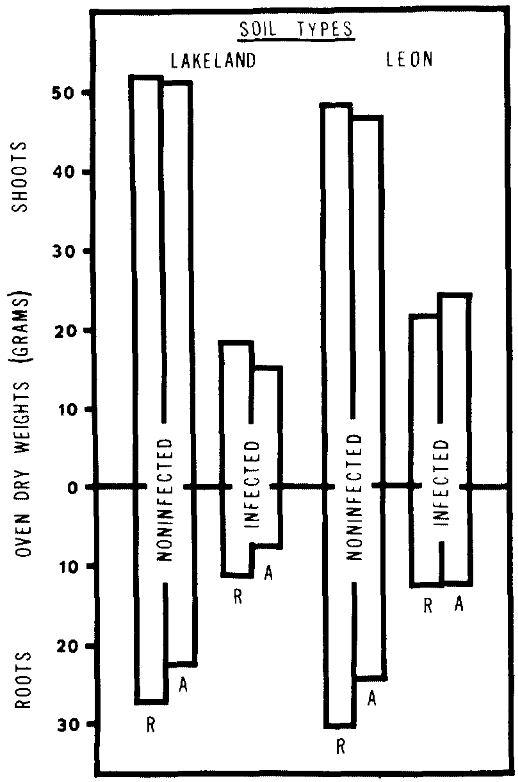

FIG. 2. Interaction of soil type, watering regime, and *Radopholus similis* infection on root and shoot dry weights of 18-month-old greenhouse-grown rough lemon seedlings.

soils after 1 year (Fig. 1A). Growth retardation of infected seedlings by the nematode was significantly ($P = 0.01$) greater in each soil compared to the non-infected seedlings. There was a 25 and 35% respective reduction in seedling size in the Leon and Lakeland soils at the termination of this experiment which was significant at the 5% level. Different water schedules were not a significant factor in growth of seedlings as influenced by nematodes under these conditions.

Oven-dry weights of non-infected rough lemon seedlings in either soil were approximately the same. As with the trunk diameter measurements, the differences between dry weight of infected and non-infected seedlings in both soils were highly significant ($P = 0.01$) (Fig. 2). Dry weight differences between infected seedlings growing in Leon soil were significantly ($P = 0.05$) greater than infected seedlings in Lakeland soil. Even though the root system of seedlings growing in the Lakeland soil was significantly less than seedlings in the Leon type, it supported a significantly greater population.

In the second experiment, all seedlings were maintained on a regular water schedule, since supplemental watering under our greenhouse conditions did not affect the severity

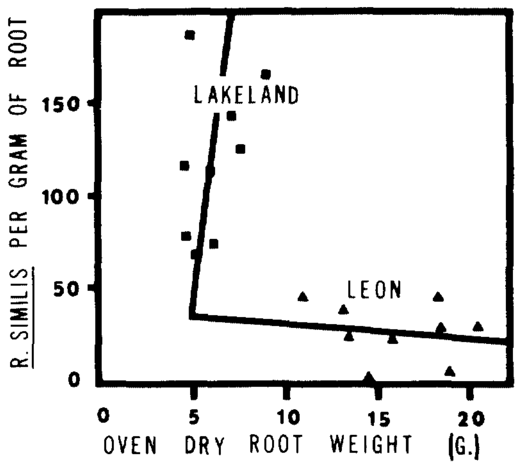

FIG. 3. Correlation of oven dry root weights and numbers of *Radopholus similis* on greenhouse-grown rough lemon seedlings in Lakeland fine sand (■) and Leon fine sand (▲).

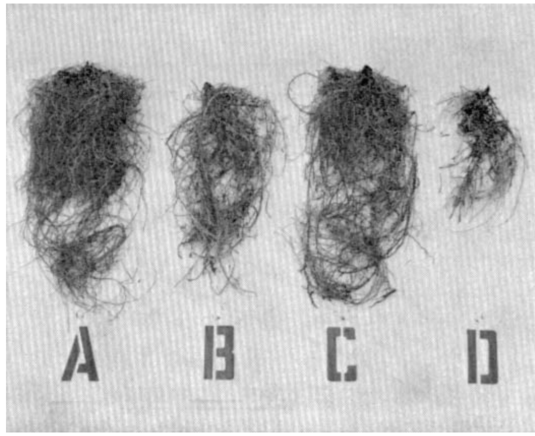

FIG. 4. Pathogenicity of *Radopholus similis* to roots of 12-month-old greenhouse-grown rough lemon seedlings grown in: Leon fine sand, **A.** non-infected, **B.** infected; and Lakeland fine sand, **C.** non-infected, **D.** infected.

of seedling damage or reproduction of *R. similis* in the first test.

Root samples for *R. similis* determination were taken at 3-month intervals for 12 months from seedlings in each soil. There were no discernible differences in populations in the two soils after 3 months; after 6, 9 and 12 months, however, seedlings in the Lakeland soil had respectively 152, 112 and 357% more nematodes per g of root than seedlings in the other soil. Final populations averaged 119 and 26 *R. similis* per g of root from seedlings growing in Lakeland and Leon soils. Mean dry-root weights of *R. similis*-infected seedlings growing in Lakeland and Leon soils were 6.0 and 16.1 g, respectively compared to 20 and 22 g for the non-infected. *R. similis*-infected seedlings growing in either soil showed a highly significant reduction in total dry weights, compared to non-infected seedlings.

Fig. 3 shows *R. similis* numbers and dry root weights from each seedling growing in the two soils. The consistently higher numbers of *R. similis* extracted from roots growing in the Lakeland soil resulted in severe reduction in root growth. Even though the roots were severely damaged they continued to sustain greater numbers of *R. similis* than seedlings in the Leon soil. The relation of nematode numbers and root condition in Lakeland soil compared with Leon soil represents a tenfold difference. Root systems of *R. similis*-infected seedlings and non-infected seedlings in the two soils are illustrated in Fig. 4.

DISCUSSION

Plant-parasitic nematodes occur in all soil types, but generally only exceed a certain population limit under rather specific soil conditions (10, 17). Some plant-parasitic nematodes are known to cause greater economic damage in sandy soils (7, 10, 16). Others cause greater damage in soils with finer texture or higher organic matter (11, 12, 17). Many factors in soils such as moisture, aeration, *p*H, salinity and other organisms can influence nematode activity; however, it seems apparent that soil texture and organic matter content, as typified by these two soil types, also exert considerable influence on the host-parasite relation.

Our greenhouse studies and field surveys of several groves show that *R. similis* will survive and increase best in deep, well-drained sands, but less well in shallow, poorly drained soils. Growth reduction and root deterioration of infected greenhouse-grown seedlings was much more severe in the well-drained, low organic Lakeland soil. Even though seedlings in each soil were initially inoculated under the same conditions to achieve uniformity of infection, the nematode population rapidly declined in the Leon soil where conditions were not favorable for nematode infection and reproduction. DuCharme and Price (4) suggested that secondary organisms may influence the numbers of nematodes in roots by rendering tissues unsuitable for nutrition of the nematode, thus forcing the nematodes to leave. In these studies no attempt was made to maintain the soils free of other organisms. Soil samples analyzed at the termination of the experiments by dilution plate analysis (9) for fungal flora indicated fungi were equally prevalent in both soils. This may account in part for the general reduction in nematode population as shown in Fig. 1. In spite of the severely reduced root system of the infected seedlings growing in the Lakeland soils (Fig. 4), they still maintained a higher population than the seedlings in the Leon soil. An important consideration is that, even though populations on seedlings in the Leon soils were low in both experiments, they still caused moderate stunting and debilitation of seedlings.

The possibility of cultural control of the burrowing nematode has been advanced (2, 15). Bryan reported (3) that groves infected by the burrowing nematode can be kept productive by maintaining adequate soil moisture in the grove to overcome drought effects on the weakened root systems. The limited study on soil moisture reported here was not sufficient to draw any conclusions, except that burrowing nematode populations will persist even under high moisture conditions.

This study has attempted to explain in part why the symptoms of spreading decline are more pronounced in some areas than in others. This is particularly so in the deep, well-drained sandy soils in the "Ridge" section of Florida, as compared to the shallower, high organic soils. This will result in some groves remaining productive longer than others.

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