Effect of Soil Texture and the Clay Component on Migration of *Meloidogyne incognita* Second-stage Juveniles

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Abstract: The vertical migration of *M. incognita* juveniles introduced at 20 cm from the roots was studied in five natural soils, 100% silica sand, 95% silica sand with 5% clay, 90% silica sand with 10% clay, and 95% silica sand with 5% clay as a concentrated layer. In natural soils the percentage of juveniles capable of migrating 20 cm and penetrating the roots decreased when the percentage of clay and silt increased. No migration occurred in silica sand without clay particles; when 5 or 10% of clay were mixed to silica sand, 34 and 26%, respectively, of the juveniles were able to migrate 20 cm. Clay separated from silica sand in which tomatoes were grown was attractive for juveniles. It is suggested that clay particles aid in the migration of root-knot juveniles over long distances to plant roots by absorbing and holding root exudates or bacterial by-products which form a concentration gradient enabling nematodes to locate roots. *Key words:* attraction, nematode movement.

Soil texture and pore size influence the migration of plant-parasitic nematodes. Rode (9) showed that the migration of juveniles of *Globodera rostochiensis* (Wollenweber) Stone toward potato plants was greatest in sandy soil, intermediate in loamy soil, and least in clay soil. Tarjan (12) observed that *Radopholus similis* (Cobb) Thorne moved better in sandy soil than in heavy-textured soil. Baines (2) suspected that the low infection of sweet orange seedlings by *Tylenchulus semipenetrans* Cobb in coarse sand may have been caused by limited juvenile migration in this soil. Wallace (14,15) suggested that there was an optimum particle size for the movement of each nematode species in soil.

A factor responsible for stimulating or attracting nematodes left in the soil after culture of host plants has been reported for *Meloidogyne hapla* (16), *Meloidogyne* sp. (7), and *Hemicycliophora paradoxa* (6). Luc (5) suggested that this factor was absorbed on the clay fraction. Viglierchio (13) suggested that a stimulus for repulsion was absorbed on soil colloids.

To determine the influence of soil texture on the migration of second-stage juveniles of *M. incognita* (Kofoid & White) Chitwood towards a tomato plant, we studied their ability to move vertically in five different natural soils, in pure silica sand, and in three different mixtures of silica sand and clay. The movement of *M. incognita* juveniles was studied in the presence or absence of clay in which plants had been grown to determine the nature of adsorption of the stimulant.

MATERIALS AND METHODS

All the juveniles of *M. incognita* used in these experiments were derived from a culture maintained on (*Lycopersicon esculentum* L. cv. Tropic) tomato in the greenhouse. Only individuals not more than 48 h in age after hatching and extracting in a mist chamber were used for testing migration abilities.

Vertical migrations of juveniles of *M. incognita* were studied using the experimental apparatus described previously (8). A polyvinyl chloride (PVC) tube 21 cm long with an internal diameter of 2 cm was filled with soil. Three hundred juveniles were introduced into the soil 1 cm from the bottom of the tube which was then closed with a polyethylene film. The top of the tube was covered with a 85-μm screen and inserted into a hole made in the center of the bottom of a 150-cm^3 styrofoam cup. Approximately 130 cm^3 of the same soil was placed in the cup with a 4-wk-old tomato seedling. With this apparatus, vertical migration of *M. incognita* juveniles was studied in five different steam sterilized natural soils, in pure silica sand (particle size $\leq$ 250 μm), and in three different mixtures of silica sand and clay (modeling clay): (i) 95% silica sand with 5% clay added and thoroughly mixed; (ii) 90% silica sand with 10% clay; and (iii) silica sand with 5% clay as a ring at the bottom of the cup but not between the roots and the tube. The percentage of clay and silt in five natural soils is shown in Table 1.

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The experiments were conducted in a growth chamber at a constant temperature of 26 C for 7 d. Tomato plants were then washed gently from the pots and their entire root systems were stained with 0.05% cold cotton blue-lactophenol (4). Only the juveniles that had migrated up the 20-cm column and penetrated into the roots were counted and recorded.

In experiments with the five natural soils there were 10 replications for each soil type and the experiment was repeated three times. Experiments in silica sand and mixtures of silica sand and clay were repeated four times and there were five replications for each soil type.

The penetration of 4-wk-old tomato roots by *M. incognita* juveniles was studied in three different soil types: pure silica sand (particle size ≤ 250 μm) and two natural soils containing 14 or 22% clay plus silt. This study was conducted in styrofoam cups without the 20-cm column. Three hundred juveniles were added to the soil through holes just after the tomato seedling was transplanted. The plants were removed from the pots and their root systems stained after 3, 5, and 7 d. There were 10 replications for each soil and time interval.

To determine whether attractive or repellent substances were absorbed on the clay, ten 4-wk-old tomato seedlings were grown in a pot containing 600 g of silica sand and 90 g of clay as a layer at the bottom of the pot. After 4 or 6 d of tomato growth this clay was removed and used in an experiment employing PVC tubes 12.5 cm long with an internal diameter of 2 cm and closed at both ends by a polyethylene film. At the middle of the tube a 0.3-cm-d opening was made for introducing the nematodes. The tubes were cut halfway every 2.5 cm, allowing division of the column of sand in the tube into five fractions of 2.5 cm. During the experiments the cuts were closed with adhesive tape. Sections 1, 2, and 3 of the tube were filled with 95% silica sand plus 5% clay which had not supported the growth of tomato plants. Sections 4 and 5 were filled with 95% silica sand plus 5% clay which had grown tomato seedlings previously. Fifteen percent water by weight was added to the mixture of sand and clay. Immediately after preparing the sand tube, 0.1 ml of water containing 300 juveniles was introduced with a syringe into the center of section 3 of the column. The tubes were maintained horizontally at 26 C and divided into five parts 48 h after introduction of the juveniles. Nematodes were extracted by placing the sand from each section in a Baermann funnel (1) for 48 h.

The experiments using a clay which had supported tomato growth for 4 d had six replications and were repeated six times. The experiments using clay which had supported the tomato growth for 6 d were repeated three times with 12 replications the first time and 10 replications the second and third times. The experiments were also repeated six times with four replications with each tube filled with silica sand mixed with 5% of clay which had not supported tomato growth.

A similar set of experiments was conducted using bentonite instead of modeling clay. Ten 4-wk-old tomato plants were grown for 7 d in a pot containing 150 g of silica sand and 10 g of bentonite as a layer at the bottom of the pot. After 7 d of tomato growth the bentonite was mixed with silica sand. Four hundred grams of silica sand was added to 10 g of bentonite with 30 g of water adsorbed on the bentonite layer and another 55 g of water was added. This mixture of sand and bentonite was used in an experiment in PVC tubes 6 cm long and divided in three sections of 2 cm each. The bentonite used in sections 1 and 2 had not supported plant growth, but that used in section 3 had grown tomato for 7 d. The control tubes used bentonite which had not been used for tomato growth in all three sections. Immediately after preparing the sand tube we introduced 150 juveniles in 0.1 ml of water into the center of section 2. The tubes were maintained horizontally at 26 C. Forty-eight hours after introduction of the juveniles the sand was divided into three parts. The nematodes were extracted from the sand as described previously. The experiment was repeated three times with ten replications.

RESULTS

The percentage of juveniles able to migrate 20 cm and penetrate tomato roots

Table 1. Migration of *Meloidogyne incognita* juveniles toward tomato roots in five different soil types.

Soil type	% clay	% silt	% clay & silt	% migration & penetration
1	8.8	5.2	14.0	31.5 a
2	10.2	11.9	22.1	12.6 b
3	9.2	16.0	25.2	9.5 b
4	13.2	19.5	32.7	0 c
5	12.5	23.5	42.0	0 c

Numbers followed by same letter are not significantly different, according to an analysis of variance ($P = 0.05$).

decreased as the percentage of clay and silt increased (Table 1). There was a significant difference in the rate of migration in soil which contained only 14% clay plus silt and in soils which contained 22.1 and 25.2% clay plus silt. No migration was observed when the soils contained more than 30% clay plus silt. In pure silica sand less than 1% of the juveniles migrated 20 cm and penetrated (Table 2). When 5% clay as added to the silica sand the percentage of migration was more than 30%, and with 10% clay the percentage of migration was 26.4%. When 5% clay was added as a layer at the bottom of the pot, but not between

Table 2. Migration of *Meloidogyne incognita* juveniles toward tomato roots in four different soil types.

Soil type	% clay	% silt	% clay & silt	% migration & penetration
sand 250 μ	0	0	0	0.5 a
sand 250 μ	5	0	5	34.4 b
sand 250 μ	10	0	10	26.4 b
sand as a layer 250 μ	5	0	5	0.6 a

Numbers followed by same letter are not significantly different, according to an analysis of variance ($P = 0.05$).

the roots and the tube, the percentage of migration was similar to the all-sand system.

No differences in penetration (Table 3) were observed in two natural soils and in pure silica sand when the juveniles were introduced directly into the pot around the tomato roots for 3, 5, and 7 d.

In a tube of sand mixed with 5% clay which had not been used to grow a tomato plant, the juveniles were equally distributed on both sides of the tube 48 h after their introduction into the center of the tube (Fig. 1A). When the reference clay in sections 4 and 5 was replaced by clay used to grow tomato plants, the juveniles were mainly aggregated in sections 4 and 5 containing the clay used to grow tomato plants for 4 d (Fig. 1B) or 6 d (Fig. 1C). The numbers of juveniles found in the two sides of these tubes were significantly different. The same results were observed when bentonite was used as the clay fraction (Fig. 2).

DISCUSSION

Soil texture appears to be important for vertical migration of juveniles of *M. incognita*. These differences in migration in soils of different structure could occur because nematode species generally have an optimum particle size for movement (15). This could explain in part the greater pathogenicity of the genus *Meloidogyne* in sandy soils (10,11) and the quick reinfestation that is sometimes observed in a sandy soil as nematodes move upward after a poor soil fumigation (Van Gundy and Lembright, personal communication).

The fine particles of clay and slit appear to be obstacles to *Meloidogyne* migration, but the clay particles also appear to have a function in attracting nematodes over large

Table 3. Penetration of tomato roots by *Meloidogyne incognita* juveniles in three different soil types.

Soil types	% clay & silt	% penetration after 3 d	5 d	7 d
1	14	48.6	81.4	77
2	22.1	35.2	51.5	67.9
sand 250 μ	0	40	53.4	68

Each number represents the mean of 10 replications.

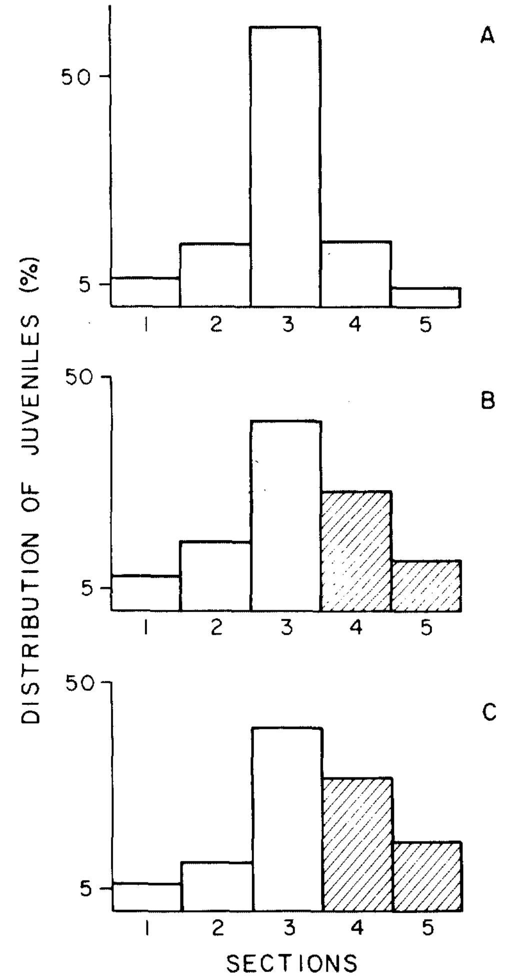

Fig. 1. Distribution of juveniles of *M. incognita*, 48 h after their introduction at the center of section 3, in columns of sand mixed with A) 5% clay which had not been used to grow tomato plants; and B, C) 5% clay which had not been used to grow tomato plants in sections 1, 2, and 3 and 5% clay used to grow 10 tomato plants for 4 d (B) or 6 d (C) in sections 4 and 5.

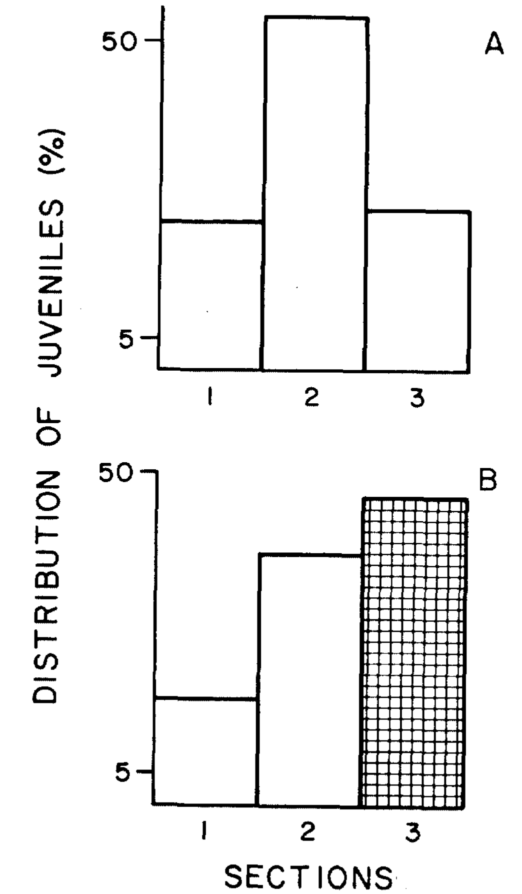

Fig. 2. Distribution of juveniles of *M. incognita* 48 h after their introduction in the center of section 2: A) in a column of sand mixed with 2.5% bentonite which was not used to grow tomato plants; B) in a column of sand mixed with 2.5% bentonite not used to grow tomato plants in sections 1 and 2 and with 2.5% bentonite used to grow 10 tomato plants for 7 d in section 3.

distances in soil. The addition of only 5% clay increased migration markedly over that in pure sand. It is difficult to conceive that the addition of 5% of clay could change the soil texture sufficiently to explain this effect. It is possible that the clay particles added to the silica sand cause migration of root-knot juveniles over large distances to plant roots by holding some root exudate either on the clay or in a water film and establishing a gradient which helps nematodes to locate roots.

It is also possible that some root exudates, carried by soil water, are metabolized by the bacteria living on the clay particles and that gradients of these byproducts could attract the nematodes to the roots. The clay particles may also transform or select some of the root exudates carried by the percolating water. Bird (3) observed that *Meloidogyne* juveniles were not attracted by tomato root exudates, but when these exudates were absorbed on anionic exchange resin they become attractive.

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