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Vertical Migration of *Meloidogyne chitwoodi* and *M. hapla* under Controlled Temperature[1]

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Abstract: Migratory ability of second-stage juveniles (J2) of two *Meloidogyne chitwoodi* races and a *M. hapla* population were compared in soil-filled columns at 12, 18, and 24 C. J2 of all populations migrated farthest at 18 C and least at 12 C. Nematode survival was significantly reduced ($P = 0.05$) at 24 C. *M. chitwoodi* J2 migrated further and in greater numbers than *M. hapla* J2 at all temperatures. A comparison with and without a host plant demonstrated no preferential migration toward the plant. Water percolation through the migration columns stimulated upward migration.

Key words: Columbia root-knot nematode, northern root-knot nematode, migration, temperature, soil moisture.

Two root-knot nematodes, *Meloidogyne chitwoodi* and *M. hapla,* are major pests of potato in the western United States; *M. chitwoodi* is the more damaging (14) with populations of 1 egg/250 cm^3 soil causing economic loss (15). Active *M. chitwoodi* second-stage juveniles (J2) have been recovered from soil collected at depths of 1.5 m (17). Deeply placed nematodes complicate management decisions. First, root-knot nematodes at lower depths are not assessed by sampling the upper 30–60 cm, the common practice in the Pacific Northwest; second, they are buffered from surface temperature and moisture extremes, and thus their survival may be enhanced; and finally, physical and chemical control procedures may not eliminate nematodes below 100 cm (4,5,21).

Deeply placed *M. chitwoodi* juveniles might be able to migrate through treated soil profile to infect growing potato roots. Migration experiments demonstrated that *Meloidogyne javanica* J2 are capable of 75-cm vertical movement (8). *M. hapla* and *M. incognita* were reported to migrate differentially over a range of soil temperatures (12). Factors affecting nematode migration have been reviewed (11).

The objective of this study was to determine and compare the migratory abilities of *M. hapla, M. chitwoodi* race 1, and *M. chitwoodi* race 2 (16) J2 over a range of temperatures common in Washington potato fields.

Materials and Methods

Migration studies were conducted in columns constructed of polyvinylchloride (PVC) rings (8.25 cm d × 5.0 cm) taped together end to end (Fig. 1). Columns were filled with fumigated soil mix (3.7% gravel, 51.6% coarse sand, 28.7% fine sand, 10% silt, 6% clay). Soil was poured in a column in 500-cm^3 increments and tamped until the column was filled. Soil bulk density in columns was 1.6 g/cm^3. Field capacity was estimated to be 10% by allowing a water-saturated column to drain freely for 48 hours (18). *M. chitwoodi* and *M. hapla* inoculum was prepared by shaking infected *Lycopersicon esculentum* cv. Columbia tomato roots in 0.05% NaOCl to extract eggs (2). Eggs were incubated on a 28-μm-pore screen immersed in water at 24 C (22). Freshly hatched J2 were collected from the water daily and stored at 12 C until adequate numbers were collected. The maximum storage period was 48 hours. The J2 suspension was adjusted to 2,000 or 5,000 nematodes/5 ml, and this volume was injected into the column through a port in one ring. The port was packed with soil and sealed after inoculation.

Two nematode assay procedures were used in these experiments. In the first, soil-

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filled rings were separated and the nematodes in each soil sample were extracted by centrifugal-flotation technique (3) (with a series of 750-, 250-, 28-μm-pore sieves) and counted. The nematodes present in the top two rings were then bioassayed by pouring the suspension around the roots of a Columbia tomato plant. The second procedure was a direct bioassay with a 4-week-old Columbia tomato planted in the soil of each ring. Plants were harvested after 20 days, the roots stained with acid fuchsin (1), and the nematodes within roots counted.

Effect of moisture and irrigation regimes on migration: Two preliminary experiments were conducted to determine the optimal moisture regime for further studies. In the first, four ring columns were packed with soil previously adjusted to 6, 8, 10, 12, and 14% moisture and the ends of the columns sealed with plastic. The bottom ring of columns was inoculated with 2,000 *M. chitwoodi* J2. In the second, four irrigation regimes were evaluated. In three regimes, columns were top irrigated to bring soil to 10% moisture and allowed to drain freely into dry soil for 24 hours before inoculation with 5,000 *M. chitwoodi* J2. One regime received only the initial water, a second received an additional 25 ml water 24 hours after inoculation, and the third received 25 ml water at 24 and 48 hours. The fourth regime had columns packed with soil premoistened to 10% and without additional irrigation to eliminate percolation. Both experiments were conducted at 18 C for 4 days. Nematodes were assayed by centrifugal-flotation technique.

Migration rate study: Twelve-ring columns were constructed with the inoculation port in the bottom ring. Columns were set in clay pots of air-dried soil in both this study and the following temperature study (Fig. 1). Columns were adjusted to 10% moisture 24 hours before 2,000 *M. chitwoodi* race 1 J2 were inoculated. Columns were top irrigated daily with 25 ml distilled water that had percolated through soil containing a 2-month-old Columbia tomato plant. Columns were maintained at 19–20 C. Three replicate columns were harvested after 3, 6, and 9 days. Nematodes were assayed by centrifugal-flotation technique and bioassay of extracted nematodes.

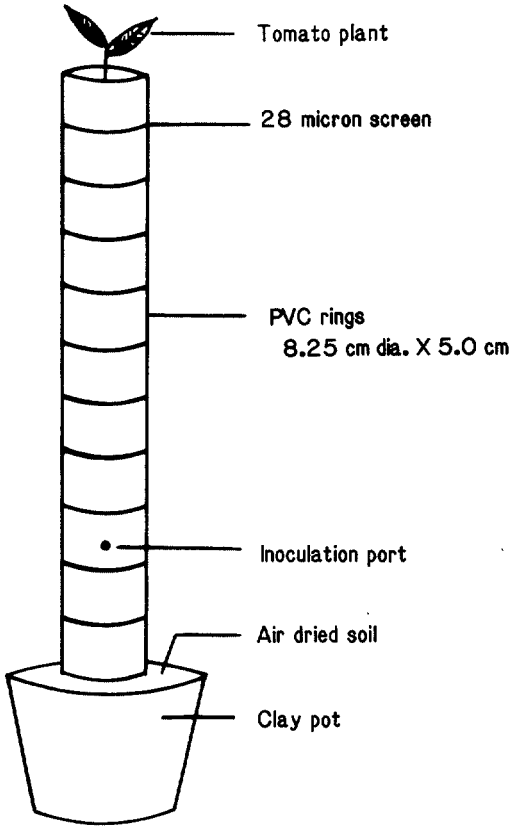


FIG. 1. Column used in migration studies.

To ascertain whether nematodes were passively moved in this system, one series of 12-ring columns with two rings below the inoculation port was inoculated with 2,000 J2 previously killed in hot acid fuchsin. Columns were dismantled after 6 days. Nematodes were assayed by centrifugal-flotation technique.

Effect of temperature on migration: Columns were constructed of 11 rings with the inoculation port in ring 3 (Fig. 1). The bottom of the top ring was covered with 28-μm-pore nylon screen. This ring contained a 4-week-old Columbia tomato plant or soil alone. Three *Meloidogyne* populations from the Washington State University collection (6) were used in the study: *M. hapla* (WAMh1 population), *M. chitwoodi* race 1 (WAMc1 population), or *M. chitwoodi* race

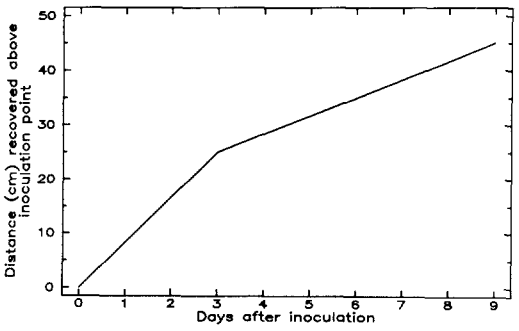


FIG. 2. *Meloidogyne chitwoodi* race 1 (WAMc1 isolate) migration rate at 19–20 C in presence of tomato root leachates.

2 (ORMc8 population). Columns were adjusted to 10% moisture 24 hours before inoculation with 5,000 J2 of either population. A plastic bag was loosely placed over the top of each column to reduce transpirative and evaporative water loss. Columns were placed in temperature cabinets at 12, 18, and 24 C with a 16-hour photoperiod. The experiment was terminated after 9 days and soil was directly bioassayed. Soil samples (30–40 g) were collected from each ring in three columns at each temperature for soil moisture determination.

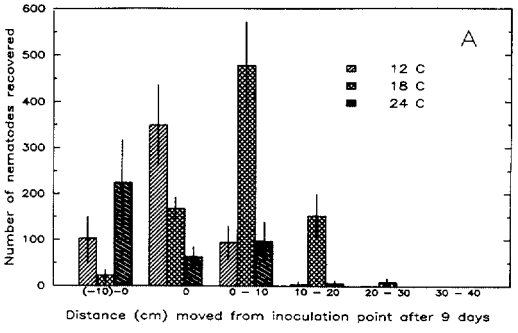
RESULTS

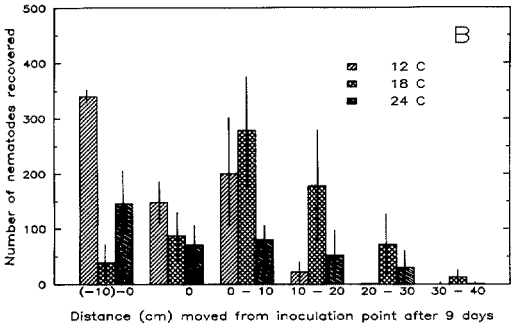
Effect of moisture and irrigation regimes on migration: In preliminary studies, J2 moved poorly in soil below field capacity (6 and 8%), but moved well above it. Therefore, all further experiments were conducted with soil initially adjusted to 10% moisture. Soil moisture levels at termination of temperature studies were also consistently between 9 and 10%. Upward juvenile migration was enhanced by water percolation downward. In columns containing soil premoistened to 10% and without additional irrigation, 98% of recovered nematodes were in the inoculation ring. In columns where moisture was raised to 10% by top irrigation, 43% of recovered nematodes were above the inoculation ring, but only 1% above 10 cm. Top irrigation with 25 ml water at 24 hours or 24 and 48 hours increased the percentage of nematodes recovered above 10 cm to 3.8% and 34%, respectively. Therefore, in all further experiments, columns were top irrigated daily with 25 ml water.

Migration rate: Five to eight percent of J2 were recovered in the inoculation rings on all three sampling dates. Less than 4% of the nematodes were recovered above 25, 35, and 45 cm on days 3, 6, and 9, respectively. Migration was most rapid during the first 3 days, > 8 cm/day, and slowed to > 3 cm/day during the last 6 days (Fig. 2). No nematodes were recovered above 50 cm after 9 days. Bioassays demonstrated that juveniles recovered in the upper rings were infective. In columns inoculated with heat-killed juveniles, 94% of the recovered nematodes were in the inoculation ring and 6% were in the ring immediately below. Thus, there was no passive nematode movement upward and only slight movement downward. No nematodes were recovered more than 5 cm below the inoculation ring.

Effect of temperature on migration: Juveniles of all isolates migrated farthest at 18 C (Fig. 3A–C). Migration was the least at 12 C for all isolates, and the majority of nematodes were recovered at or below the inoculation point. The majority of WAMh1 and WAMC1 individuals were also recovered at or below the inoculation point at 24 C. Significantly fewer ($P = 0.05$) nematodes of all isolates were recovered at 24 C than at 12 or 18 C. *M. hapla* migrated less than either *M. chitwoodi* race and moved above 20 cm only at 18 C. Juveniles of both *M. chitwoodi* races migrated above 30 cm and penetrated the tomato plants above 35 cm. *M. chitwoodi* J2 (ORMc8 population) displayed similar migration patterns at 18 C in columns with or without a tomato plant (Fig. 4).

The temperature study was repeated three times, and migration trends were similar in each study. There was some variability among columns in all treatments, as evidenced by the standard errors shown in Figures 3 and 4. The nematode recovery rates were 6–17% of initial inoculum, whether assayed by centrifugal-flotation





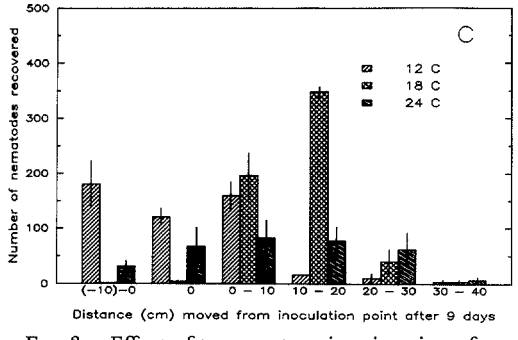


FIG. 3. Effect of temperature in migration of second-stage juveniles. A) *Meloidogyne hapla* (WAMh1 isolate). B) *M. chitwoodi* race 1 (WAMc1 isolate). C) *M. chitwoodi* race 2 (ORMc8 isolate). Vertical lines represent standard error.

technique or direct bioassay. We were not able to greatly reduce the variability nor improve nematode recovery with modifications that were made in different experimental runs.

DISCUSSION

Less than 0.1% of inoculated *M. chitwoodi* J2 were able to migrate 45 cm and penetrate bioassay plants. We have found that

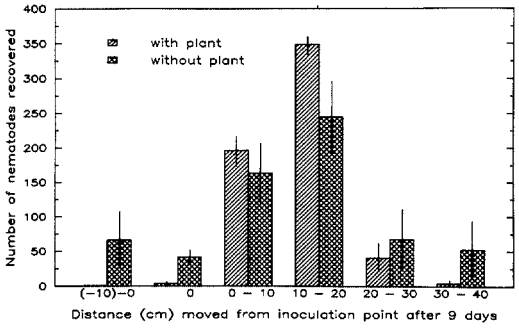


FIG. 4. Effect of Columbia tomato plant on the migration of *Meloidogyne chitwoodi* (ORMc8 isolate) second-stage juveniles. Vertical lines represent standard error.

the inoculation of tomato plants with the same freshly hatched J2 populations resulted in only 10–12% infection (unpubl.). In contrast, Prot (8) reported a 50% recovery rate of *M. javanica* juveniles in tomato roots placed 50 cm above the inoculum. The size of the columns and soil texture may account for this discrepancy. Prot (8–13) in his studies used 1.5-cm-d columns, whereas we used 8.24-cm columns. Wallace (20) hypothesized that nematode movement in soil is dependent on the soil pore size in relation to the nematode body diameter and the soil matrix potential. Prot (13) reported that migration of *M. incognita* J2 was inhibited in pure 250-μm silica sand and in sand amended with over 32% silt and clay. Soil used in our experiments had a much larger coarse sand fraction, 51.6%, than soil used by Prot (8). Migration may have been enhanced in our experiments if a finer textured sandy loam soil had been used.

Short-distance attraction of nematodes to roots is well documented (11). Long-distance attraction to roots and differential migration of *M. javanica* toward resistant and susceptible tomato roots has been reported (8). Our data showed no differential migration of *M. chitwoodi* in the presence or absence of a suitable host; however, the percolation of water through columns enhanced vertical migration. Moisture gradient studies demonstrated that *M. javanica* (10) and *Globodera rostochiensis* (19) J2 aggregate at the moist end of the gradient.

Prot (10) reported that *M. javanica* J2 were repulsed by salt concentrations close to those found in soils. He hypothesized that movement toward wetter soil was actually movement toward a lower salt concentration. These studies suggest that the flushing action of water added during our studies may stimulate the juveniles to move vertically.

Differential migration has been reported for four natural *Meloidogyne* populations (9). In our study, populations of the two *M. chitwoodi* races displayed similar migration patterns; however, *M. hapla* was less motile than *M. chitwoodi.* The greater activity of *M. chitwoodi* J2 may be a component in its greater pathogenicity on potato under field conditions.

Second-stage juveniles of all isolates migrated at the experimental temperatures, which represent the extremes that we recorded at 20 cm in potato plots during the 1984 and 1985 growing seasons (unpubl.). Survival in columns of these cool-temperature adapted *Meloidogyne* species was reduced at 24 C, a temperature rarely reached in the potato root zone. Juvenile migration was retarded but not halted at 12 C, a typical early season soil temperature. Migration and recovery was greatest at 18 C, a soil temperature recorded shortly after plant emergence in both years.

M. chitwoodi has the potential to migrate up into the treated soil profile after soil fumigation. Since active *M. chitwoodi* have been recovered below 1 m, sampling methods may need to be modified to assay this soil strata. Nonfumigant nematicides may be necessary to restrict nematode migration into the root zone following fumigation. Such combination treatments have proven effective in experimental plots (7) and are presently used in Pacific Northwest commercial potato production.

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