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11 INFLUENCE OF MOVEMENT OF JUVENILES ON DETECTION OF FIELDS INFESTED WITH *MELOIDOGYNE*

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Introduction

Numerous observations indicate that although there have been considerable improvements in nematode extraction from soil, discrepancies still exist between the number of juveniles of *Meloidogyne* extracted from field soil and the degree of infection observed on host plants grown in these fields. Thus while classical extraction methods frequently do not reveal the presence of *Meloidogyne*, susceptible crops grown in fields from which the soil samples have been taken are often heavily parasitized by the nematodes (de Guiran, 1966a).

In extraction methods currently used (Seinhorst, 1956, 1962; de Guiran, 1966a; Gooris and d'Herde, 1972; Demeure and Netscher, 1973) relatively small soil samples (100 or 250 cm^3) are analyzed. Sample size together with the uneven distribution of root-knot nematodes in the soil, related to the presence of egg-masses, could partly explain the discrepancies referred to. The only way to improve the situation is to increase the size of the samples or if this is not possible, to increase the number of samples.

Root-knot nematodes appear to be capable of moving over relatively large distances in short periods of time. Johnson and McKeen (1973) demonstrated that a population

of *M. incognita* localized at a depth of 120-125 cm induced galling of roots of tomatoes situated in the horizon between 0 and 15 cm at the time of the first harvest. Attacks of crops grown in seemingly uninfested land could be explained by assuming that juveniles present in large volumes of soil are capable of reaching and infecting plants.

To test this assumption a series of experiments was conducted to determine the degree of migration of 2nd-stage juveniles of *Meloidogyne* and the relation to the detection of these nematodes in the field.

Experimental results

Field experiments

Five experimental plots previously infested respectively with different field populations of: *M. javanica*; a mixture of *M. javanica*, *M. incognita* and a form intermediate between *M. arenaria* and *M. incognita*; *M. incognita* (two populations) were kept fallow during the dry season (mid-August until November) to decrease nematode numbers. In November, in each plot 20 soil samples (0-20 cm horizon) were taken at 50 cm intervals in a transect and 250 cm^3 of soil from each site were processed to recover *Meloidogyne* (Demeure and Netscher, 1973). At alternate sampling sites in each plot a 4-week-old *Meloidogyne*-susceptible tomato (*Lycopersicon esculentum* cv. Roma) was planted. From each of the other 50 sampling sites, two dm^3 of soil were taken and placed in plastic pots, into each of which a 4-week-old Roma tomato plant was transplanted, and the pots placed in the soil at each planting site. Eight days after transplanting, all the tomato plants were removed from the field, the root systems stained with cold cotton blue-lactophenol (de Guiran, 1966b) and the numbers of juveniles in the roots were counted (Table 1). No differences were detected between populations of *Meloidogyne*.

TABLE 1

Assessment of numbers of *Meloidogyne* juveniles and samples infested, using different techniques (from Prot and Netscher, 1978).

Technique	Mean no. *Meloidogyne*	% samples with *Meloidogyne*
Soil extraction	19	47
Plants in pots	33	64
Plants *in situ*	109	96

If the soil samples had been analyzed by Demeure and Netscher's method only, it would have been concluded that the field was slightly infested with large areas free of *Meloidogyne* (Table 1). Examination of the roots of the plants grown in pots showed an increase in the efficiency of detection. The two techniques are analogous since they represent different methods of analyzing the number of juveniles present in a given volume of soil.

Examination of roots of tomato plants transplanted directly in the field revealed a much higher infection and the field appeared to be almost completely infested, only 4% of the sampling sites being free of *Meloidogyne*. These results can be explained by assuming movement of juveniles of *Meloidogyne* to the indicator plants followed by penetration. The results could also be explained by assuming that the indicator plant had induced eggs close to the roots to hatch, but this hypothesis is difficult to reconcile with the results when tomatoes were grown in pots of soil.

Glasshouse experiments

Movement of juveniles of *M. javanica* in the presence of absence of a susceptible tomato plant (cv. Roma) was studied using the experimental set up illustrated in Fig. 1. A glass tube 25, 50, 75 or 200 cm long and 1.2 cm internal diameter was attached either at the side (Fig. 1A) or

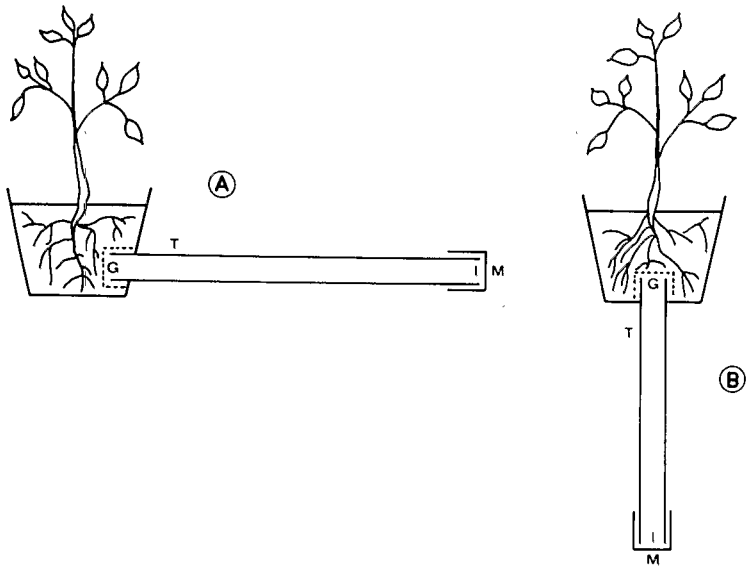

Fig. 1. Experimental set up for studying horizontal (A) and vertical (B) movement of juveniles of *Meloidogyne javanica*. G: stainless steel screen; I: inoculation site; M: polyethylene film; T: glass tube.

in the centre of the base (Fig. 1B) of 6 cm diameter pots with a capacity of 100 cm^3. Pots and tubes were filled with sterile sandy soil and 300 juveniles were placed at a vertical distance of 0, 25, 50, 75 and 100 cm from the roots. Pots were watered daily. Nine days later the roots were stained with cold cotton blue lactophenol (de Guiran, 1966b) and juveniles inside the roots counted. Soil samples from pots planted with tomatoes and from unplanted controls were extracted by elutriation and the number of juveniles counted. Fig. 2 shows the relation between distance of inoculation site and number of juveniles recovered in roots and pots.

An analogous experiment was made, placing 300 juveniles either horizontally or vertically at a distance of 25 and 50 cm from the roots. Counts were made at 1, 3, 5, 7, or 9 days after inoculation (Fig. 3).

The two experiments allow the following conclusions to

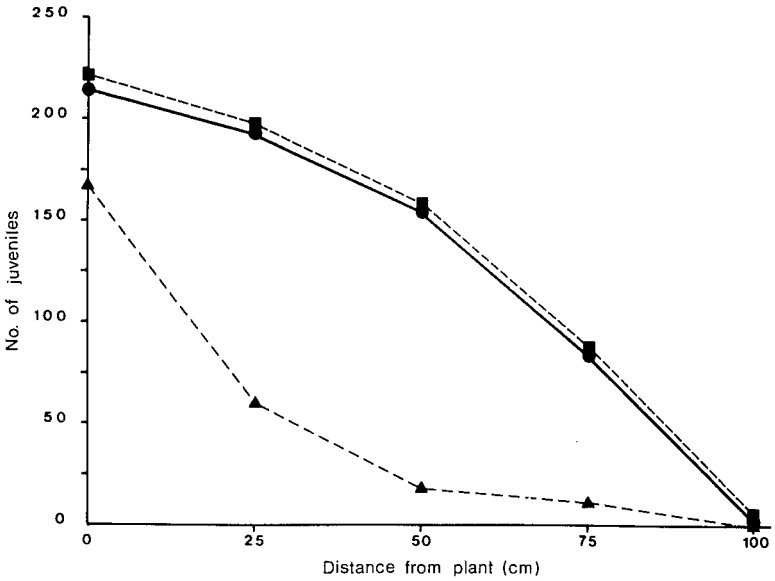

Fig. 2. Vertical movement of juveniles placed at different distances from root systems of tomato plants. •——•: juveniles counted in roots; ■---■: total number of juveniles in roots and soil; ▲—▲: Number of juveniles recovered from soil of controls.

be made:

- Juveniles of *M. javanica* are capable of moving as much as 75 cm and penetrating roots within a period of 9 days.

- Migratory movements are relatively fast as a considerable proportion of the juveniles were able to move vertically over a distance of 50 cm in three days.

- Movement over large distances is not exceptional; in 9 days 60% of the juveniles moved 25 cm, 50% 50 cm and 25% 75 cm.

With the same technique, vertical migration of four field populations was studied: one population of *M. javanica*, one of *M. incognita*, one with a perineal pattern intermediate between *M. arenaria* and *M. incognita* and one population containing a mixture of these species.

Migration was studied as a function of distance by

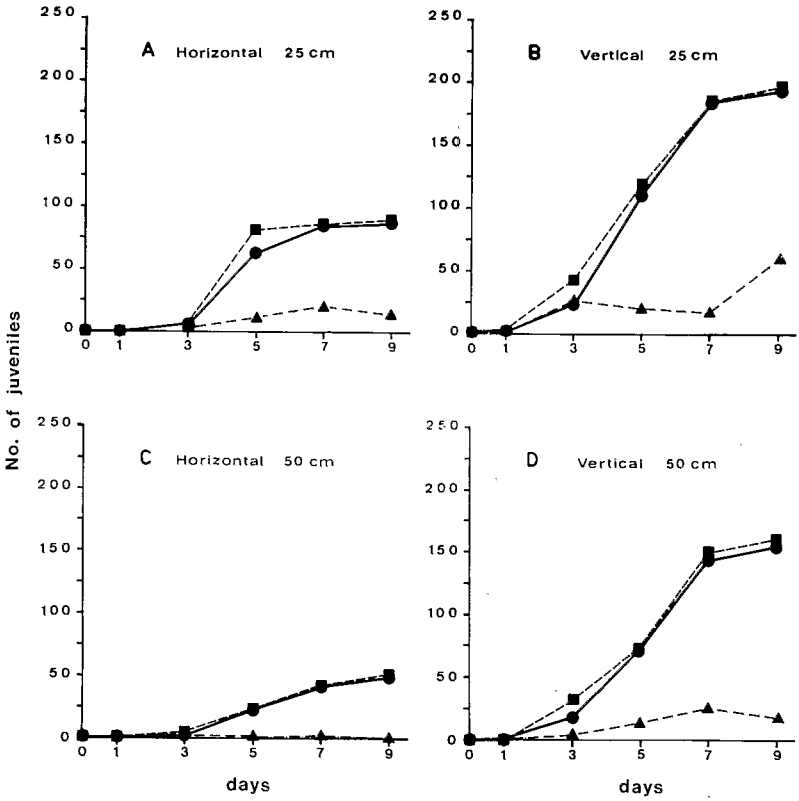

Fig. 3. Vertical and horizontal movement of *M. javanica* as a function of time. A: horizontal movement, inoculation site 25 cm from roots; B: vertical movement, inoculation site 25 cm from roots; C: horizontal movement, inoculation site 50 cm from roots; D: vertical movement, inoculation site 50 cm from roots; ●—●: juveniles counted in roots; ■--■: juveniles counted in roots and soil; ▲—▲: juveniles counted in soil of controls.

introducing the juveniles at 0, 5, 10, 25 and 50 cm from the root systems and leaving the plant in position for nine days. Migration was also studied as a function of time; juveniles were placed 25 cm from the roots and the experiment terminated 1, 3, 5, 7 and 9 days after introduction. Each treatment was replicated five times.

At the end of an experiment the tomato plant was removed from the pot and the root system stained with cold

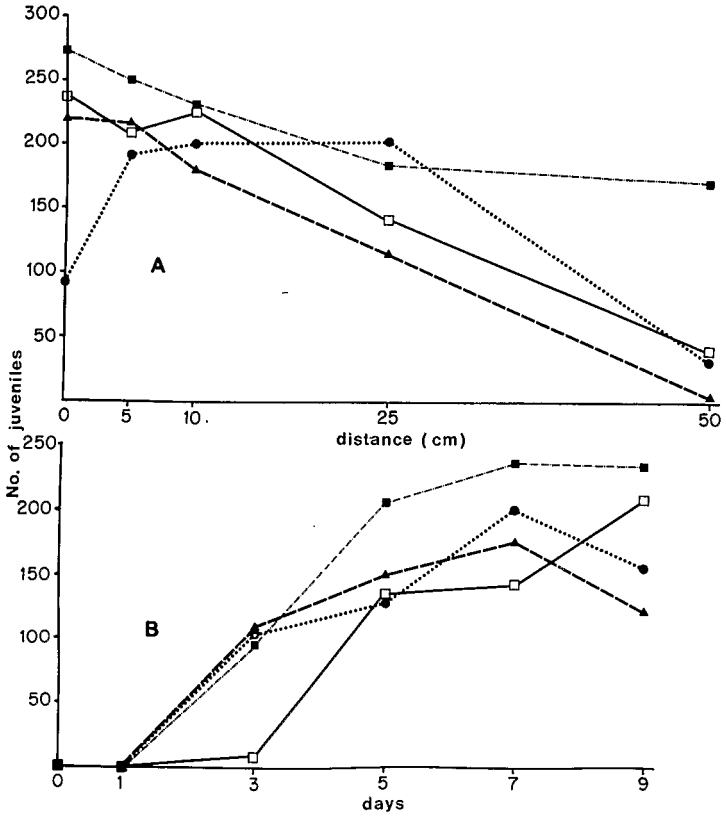

Fig. 4. Vertical migration of juveniles of four natural populations of *Meloidogyne*. ■---■ *M. incognita*; □—□ *M. javanica*; ▲—▲ population intermediate between *M. arenaria* and *M. incognita*; ●····● population including the three previous species. A: vertical migration as a function of the distance between root systems and inoculum (abscissa); ordinate, number of juveniles in the roots after 9 days. B: vertical migration of juveniles placed 25 cm from root systems as a function of time; abscissa: time of experiment in days; ordinate: number of juveniles found in the roots, (from Prot, 1977, *Rev.Nematol.*1, 109-111; with permission).

cotton blue lactophenol (de Guiran, 1966b). Only juveniles found in the roots were counted. Nematodes that entered the pots but did not infect were ignored.

The mean numbers of juveniles in the roots 9 days after inoculation and placed 0, 5, 10, 25 and 50 cm from

the root systems are shown in Fig. 4A. Fig. 4B represents the mean penetration of juveniles when placed 25 cm from the roots 1, 3, 5, 7 and 9 days before examination.

In the experiments in which distance between the point of introduction of nematodes and roots was varied, the results were approximately the same for the four populations at distances less than 25 cm. The sole exception occurred with the heterogeneous population at a distance of 0 cm (in direct contact with the roots). In this case in three of the five replicates many of the roots died resulting in an unrealistically low penetration figure. When nematodes were introduced 5 or 10 cm from the roots, penetration was similar to that obtained when the same populations were introduced at a distance of 0 cm.

At a distance of 25 cm, mean percentages of the four populations varied between 50 and 66%. At a distance of 50 cm, approximately 50% of the *M. incognita* population penetrated the tomato roots within 9 days (Fig. 4A). This figure is similar to that obtained with a clone of *M. javanica* (Prot, 1977b). Less than 15% penetration was observed at this distance with the other three populations.

In the experiment in which the distance was constant (25 cm) the rapid rate of movement is evident, i.e. in three of four populations approximately 33% of the juveniles reached and penetrated the roots within three days. Within seven or nine days a penetration of more than 50% was achieved with each population.

On the basis of these results it is concluded that the capability to migrate over relatively large distances is common within populations from West Africa. It seems logical to assume that the same ability exists within *Meloidogyne* populations from other geographical areas as well. It should be noted that populations do vary in this character, e.g. higher penetration figures were obtained in both experiments with the *M. incognita* population than

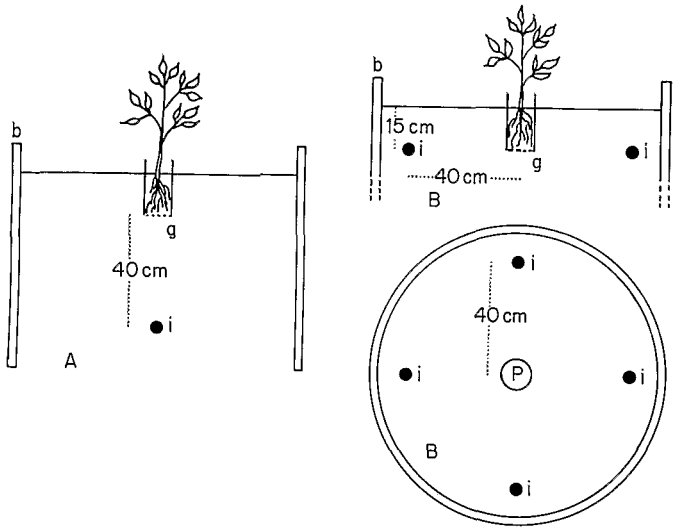

Fig. 5. Arrangement of plants and inoculation sites in microplots. A = inoculation 40 cm below plant; B = inoculation 40 cm from plant horizontally, b = wall of micro plot, g = stainless steel screen, p = 4 week-old Roma tomato in polyvinyl chloride cylinder; i = point of inoculation, (from Prot and Netscher, 1978, with permission).

with the others tested.

Experiments in microplots

Vertical and horizontal movement of juveniles of *M. javanica* was studied in cylindrical micro plots of 1m diameter. Before beginning the experiment, absence of *Meloidogyne* was verified by growing a susceptible tomato plant in the centre of each plot for 10 days, after which the plants were uprooted and examined for the presence of *Meloidogyne*. A 4-week-old tomato seedling (cv. Roma) was placed in sterile soil contained in a tube 6 cm in diameter and 20 cm tall, closed at the bottom by a stainless steel screen with 35 µm mesh. One tube was placed in the centre of each of 21 micro plots. Two thousand juveniles of *Meloidogyne* were inoculated 40 cm under the screen (Fig. 5A) or four aliquots of 500 juveniles were

TABLE 2

Infection of roots of tomato cv. Roma 10 days after inoculation with 2000 juvenile *M. javanica* 40 cm from roots (from Prot and Netscher, 1978)

Root apices		No. juveniles	
Infected	Non-infected	In apices	other root parts
A. 40 cm horizontal[1]			
49	275	194	15
B. 40 cm vertical[2]			
126	202	624	58

[1]10 replicates [2]11 replicates

inoculated at a depth of 15 cm and 40 cm from the tubes (Fig. 5B). Tubes were removed 10 days after inoculation and the tomato roots were stained and the number of juveniles that had penetrated were counted (Table 2). The results confirm those obtained in glasshouse studies and demonstrate that under conditions approximating to those occurring in the field, juveniles of *M. javanica* are capable of moving vertically or horizontally and subsequently infesting tomato plants.

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Discussion

Viglierchio (1961) reported that the horizontal movement of *Meloidogyne hapla* and *M. incognita acrita* was only a few centimetres but our experiments show that juveniles of *M. javanica* are capable of moving over distances up to 75 cm in 9 days. These migrations over relatively long distances are not exceptional as 50% of the juveniles moved 50 cm vertically in 9 days; Rode (1962) observed movement of *Globodera rostochiensis* juveniles over distances of 45 cm occasionally.

The great capability by juveniles of *Meloidogyne* may explain partially why it is so difficult to determine the level of infestation of slightly infested soils. If the juveniles can migrate 50 cm in 10 days and infect roots, the potential inoculum represents all juveniles located in a hemisphere of soil with a radius of 50 cm or 261.8 dm^3 of soil. According to Poisson's law, the probability of finding juveniles in 1 dm^3 of soil at population densities of 1 juvenile per 1 or 2 dm^3 of soil, assuming random distribution of the nematodes, is 0.37 to 0.61 respectively; yet at these densities there may be 131 or 262 juveniles in the hemisphere capable of reaching and infecting a susceptible plant. Egg masses will give a more contagious distribution, thus increasing the frequency of negative samples. It is therefore not surprising that *Meloidogyne* infections occur in fields thought from routine sampling to be free of these nematodes.