

## SHORT COMMUNICATIONS

J.-C. PROT and C. NETSCHER <sup>1)</sup>: *Improved detection of low population densities of Meloidogyne.*

Observations in tropical Africa have repeatedly shown that although extraction of nematodes from soil fails to recover juveniles of *Meloidogyne* spp., subsequent culture of susceptible crops in such fields have often produced plants severely parasitized by *Meloidogyne* spp. This is often due to the rapid increase of small populations of these nematodes under conditions favourable to the parasites. The detection of very low densities therefore becomes important because the presence of root-knot nematodes may influence the choice of cropping sequence and cultural methods.

It was thought that the elutriation technique used to extract nematodes from the soil failed to recover all nematodes contained in egg-masses, but even improved techniques developed to extract juveniles present in egg-masses (de Guiran, 1966a; Gooris & d'Herde, 1972; Demeure & Netscher, 1973) failed to eliminate the discrepancy between absence of nematodes in soil samples and presence of root-knot in the field. Because soil examination entailed extraction of only 250 ml, a technique was sought enabling examination of larger soil samples while avoiding the manipulation of large quantities of soil.

Recently Prot (1977) has shown that juveniles of *Meloidogyne* can migrate over distances up to 50 cm in a short time and infect susceptible plants.

To evaluate the effect this phenomenon could have on the detection of small numbers of *Meloidogyne* in the field and to improve detection techniques, two experiments were carried out.

### *Experiment 1*

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*; and another population of *M. incognita* were kept fallow during the rainy season (mid-August until November 1976) to allow nematode populations to decrease. In November 1976, 20 soil samples (0-20 cm horizon) were taken from each of the five plots in a straight line at 50 cm intervals and 250 ml of soil from each site were processed to recover *Meloidogyne* (Demeure & Netscher, 1973). From these 100 samples an average of 19 juveniles per l were recovered; *Meloidogyne* was not

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recovered from 53 samples. At every other sampling site in the plots a 4-week-old *Meloidogyne* susceptible plant (*Lycopersicon esculentum* var. Roma) was planted. From each of the other 50 sampling sites, 2 l 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 sampling site. Eight days after transplanting, the 100 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. A mean number of 33 juveniles was found in tomatoes grown in pots containing infested soil; 36% of the plants were not infested. In

TABLE I

Mean number of juveniles per sample and percentage of samples with *Meloidogyne*, detected using different techniques

	Soil extraction	Indicator plants grown in pots	Indicator plants <i>in situ</i>
Mean number	19	33	109
Percentage of samples with <i>Meloidogyne</i>	47	64	96

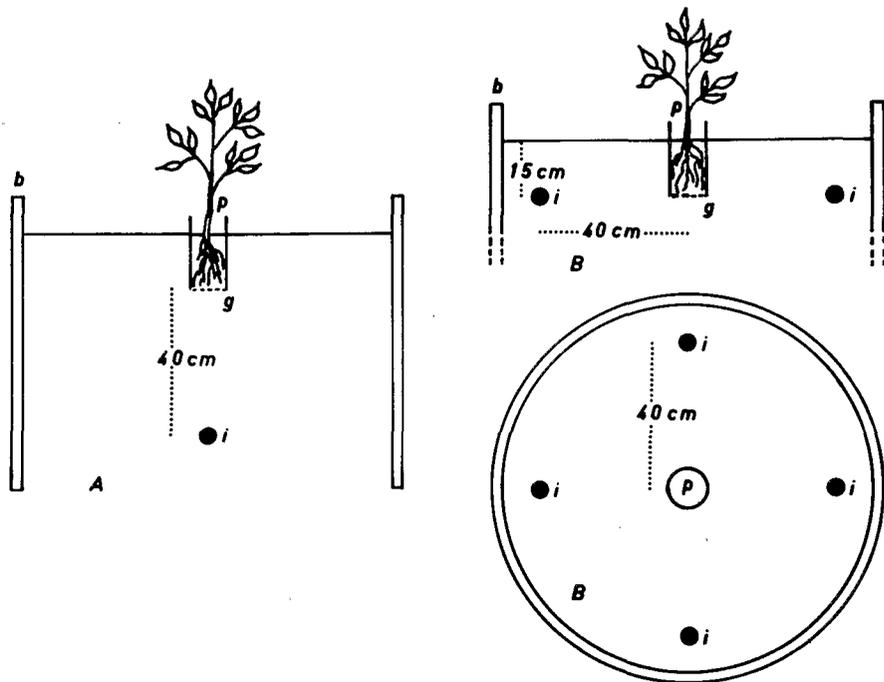


Fig. 1. Arrangement of plants and inoculation sites in microplots (Experiment 2). A = inoculation 40 cm below plant; B = inoculation 40 cm from plant horizontally, b = wall of microplot, g = stainless steel screen, p = 4-week-old Roma tomato in polyvinyl chloride cylinder, and i = point of inoculation.

the tomatoes grown *in situ*, a mean of 109 juveniles per plant was found and only 4% of the plants were not infected. No differences were detected between species of *Meloidogyne*. Results are summarized in Table I.

### Experiment 2

Soil in a cement cylinder 1 m in diameter and 0.8 deep buried in the soil was treated with Vapam (60 ml of Vapam diluted with 10 l of water) and allowed to lay fallow for 6 months. Four-week-old Roma tomato plants, were transplanted into polyvinyl chloride tubes 6 cm in diameter and 20 cm long, closed at the bottom by a screen with mesh size of 35  $\mu\text{m}$  to allow recovery of all roots. The tubes were inserted 15 cm deep in the soil at the centre of each microplot and were left in place for 10 days. Roots were stained as previously described, and examined for the presence of *Meloidogyne* juveniles. One of the indicator plants became infected, so the corresponding microplot was excluded from the experiment.

In the middle of eleven cement microplots, 2000 juveniles of *M. javanica* (from eggs hatched for four days) were injected 55 cm deep in the soil. In the remaining ten microplots 2000 juveniles in lots of 500 were placed 15 cm deep at four equidistant points 40 cm from the centre, as shown in Fig. 1. A 4-week-old Roma tomato, growing in a polyvinyl chloride cylinder as previously described, was inserted in the soil in the middle of each microplot so that the bottom of the cylinder was 15 cm below the soil surface. The cylinders were removed after 10 days, the soil washed from the tomato roots and the roots stained with cold cotton blue-lactophenol for microscopic examination. In the microplots in which juveniles were placed 40 cm below the indicator plant all eleven plants became infected. A mean number of 677 juveniles was recorded per plant. In those plots in which the juveniles were placed 40 cm from the plant horizontally, a mean of 209 juveniles per plant was observed. Nine of the ten plants tested became infected (Table II).

TABLE II

Mean number of infected and healthy root apices and number of juveniles observed in roots of tomato (*var. Roma*) 10 days after soil inoculation with 2000 juveniles of *Meloidogyne javanica* 40 cm from the roots

A. Juveniles introduced into the soil 40 cm horizontally from roots (Mean of ten replicates).

Root apices		Juveniles		Total
infected	noninfected	Total in apices	In other root parts	
48.6	274.6	193.7	15.4	209.1

B. Juveniles introduced into the soil 40 cm vertically from roots (Mean of eleven replicates).

125.9	202.1	623.72	57.7	677.5
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### Discussion

Results of Experiment 1 have shown that indicator plants, grown *in situ* provide the best means to detect *Meloidogyne*. Prot (1977) has shown that juveniles of

*Meloidogyne* can migrate horizontally and vertically 50 cm in 9 days to a susceptible plant under laboratory conditions. Our microplot experiment has demonstrated the same ability of *Meloidogyne* juveniles in simulated field conditions. This helps to explain the observation in Experiment 1, where results of soil sampling were compared with indicator plants. When a susceptible plant is transplanted in the field and if 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  $V = \frac{4/3 r^3}{2} l = 261.8 \text{ l}$  of soil. According to Poisson's law,

the probability of finding juveniles in a l of soil at population densities of one juvenile per 2 l of soil and 1 juvenile per l, assuming random distribution of the nematodes, is 0.61 and 0.37 respectively; yet at these densities there may be 131 and 262 juveniles in the hemisphere capable of reaching and infecting a susceptible plant. Egg-masses will give a more clumped 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.

The data presented here confirm the reliability of indicator plants as a bio-assay system for *Meloidogyne*, as suggested by Godfrey (1934), particularly because of its ability to migrate comparatively long distances to infect susceptible plants.

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S. O. ADESIYAN & R. A. ODIHIRIN<sup>1</sup>): *Root knot nematodes as pests of yams (Dioscorea spp.) in Southern Nigeria.*

The occurrence and the pathogenicity of some root-knot nematodes on yam tubers in Southern Nigeria is more important than was thought earlier. Unny & Jerath (1965) reported root-knot nematodes on yam in Eastern Nigeria, but no mention was made of the yam species attacked and possible amount of damage.

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