

# Penetration Rates by Second-stage Juveniles of *Meloidogyne* spp. and *Heterodera glycines* into Soybean Roots<sup>1</sup>

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**Abstract:** The rates of soybean root penetration by freshly hatched second-stage juveniles (J2) of *Meloidogyne arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, and *Heterodera glycines* races 1 and 5 were examined over a period of 1 to 240 hours. *Heterodera glycines* entered roots more quickly than *Meloidogyne* spp. Penetration by most nematodes was accomplished within 48 hours. The increases in penetration after 48 hours were insufficient to warrant further assessments. Penetration of J2 into roots of soybean seedlings in a styrofoam container was as good or better than in a clay pot. Thus, rapid and accurate root-penetration assessments can be made at 48 hours after inoculation.

**Key words:** *Glycine max*, *Heterodera glycines*, infection, *Meloidogyne arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, nematode, penetration, root, soybean.

Several nematode taxa, including *Heterodera glycines* Ichinohe and the four most common species of *Meloidogyne*, often induce severe damage to soybean, *Glycine max* (L.) Merr. (11). Most assessments made to understand the host response to the pest or the host-parasite relationship involve characterizing root penetration by the infective second-stage juveniles (J2). The time required for J2 root penetration depends on their vigor and position in relation to the root and on soil environment, including temperature, moisture, and edaphic factors (14). Many factors, such as CO<sub>2</sub> concentration, affect nematode movement to host roots (5). Knowledge of the time required for J2 penetration of the host is critical for evaluations of nematocide efficacy, construction of nematode-life tables, and general population dynamics studies. Furthermore, it is important that this evaluation be as efficient as possible because assessment of penetration often requires large numbers of observations.

Methods of assessments for nematode infection vary with research purposes (3,4,

7,9,10). For example, potato roots were examined 4 weeks after inoculation with *Globodera* spp.; however, 4 hours were required to count all J2 in one root system (9). Although accurate evaluation of penetration is critical, efficiency also is important when large numbers of samples are involved. Subsampling may be necessary for some studies, even though the sampling error is increased (12). For example, Al-Hazmi et al. (1) used 1.0-g subsamples of corn roots and examined them at 4, 8, 16, and 32 days to assess penetration by *M. incognita* (Kofoid & White) Chitwood.

The objectives of this study were to determine i) the rate of host penetration by J2 of *M. arenaria* (Neal) Chitwood, *M. hapla* Chitwood, *M. incognita*, *M. javanica* (Treub) Chitwood, and *H. glycines* races 1 and 5; and ii) the most suitable conditions for growing soybeans for easy, accurate, and efficient evaluation.

## MATERIALS AND METHODS

**Host penetration experiment:** Experimental units consisted of a soybean seedling growing in soil artificially infested with nematodes. Soybean 'Lee 68' seeds were soaked in tap water for 5 minutes and then placed into a container of damp sterile vermiculite. The container was enclosed in a plastic bag and placed into an incubator at 27 C. Seedlings with 3-cm-long roots at 42 hours after seeding were transplanted into either a 9-cm-d styrofoam container or a 15-cm-d

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clay pot. These containers were filled with 300 (styrofoam) or 1,300 (clay pot) g of soil (92% sand, 6% silt, 2% clay; humic matter content 0.3%, bulk density 1.5 g/cm<sup>3</sup>). The clay pots were wrapped with aluminum foil; the soil surface of all containers was covered with 3 cm of perlite to retard evaporation.

Susceptible soybean roots were inoculated with freshly hatched J2 of *M. arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, and *H. glycines*. Plants were placed in a greenhouse with ambient air temperatures ranging from 25 to 31 C. The experiment was conducted twice with four replicates per treatment arranged in a randomized complete block design. Numbers of J2 in roots were determined at 1, 3, 6, 24, 48, and 120 hours in both trials; a 240-hour evaluation was added when the experiment was repeated.

*Meloidogyne* spp. were cultured on *Lycopersicon esculentum* Mill. 'Rutgers' for inoculum. The eggs of these nematodes were extracted from infected roots using a modified sodium hypochlorite extraction technique (8). *Heterodera glycines* was cultured on race-specific cultivars of soybeans. Eggs were freed by crushing egg sacs and cysts with a 40-ml glass tissue grinder (2). Eggs from each nematode group were hatched on a 25.5- $\mu$ m pore screen. Juveniles hatching during the first 24 hours were discarded; those hatching during the next 24 hours were used to infest the soil. One hundred grams of soil were removed from each container, creating a funnel-shaped depression. Roots of seedlings were placed at the bottom of the depression. Aliquants of 500 freshly hatched J2 were applied over the roots and immediately covered with the 100 g of removed soil. Water was added to each container to bring the soil to field capacity (0.03 MPa). The clay pots also were watered on days 2, 4, 6, and 8 to maintain field capacity.

To enhance visual contrast between nematodes and plant tissue, roots of seedlings were thoroughly washed and then stained using a modified sodium hypochlorite-acid fuchsin staining technique

(6). Roots were placed into a drop of glycerin in 6-cm-d inverted plastic petri dishes and flattened with slight pressure. The root system was examined with the aid of a stereomicroscope and numbers of J2 in roots determined. Nematode counts were converted to a percentage of initial inoculum.

All data were subjected to analysis of variance and Waller-Duncan Bayesian k-ratio *t*-test.

*Type of experimental containers:* Three types of container systems were compared for 6 days to determine which one would allow greater J2 penetration into soybean roots. Container types tested were closed, semi-closed, and open. The closed system container consisted of a 15-cm-d pot filled with 1,300 g of soil watered to field capacity and enclosed in a 4-liter plastic bag. The semi-closed system consisted of a 9-cm-d styrofoam container filled with 300 g of soil and watered to field capacity; the soil surface was covered with 20 g of perlite. Further watering was not required in these two systems. The open system container consisted of a 15-cm-d clay pot filled with 1,300 g of soil. This system was watered twice daily. The soil type, soybean cultivar, experimental design, replicates, and statistical analyses were the same as those used in the "host penetration experiment."

## RESULTS AND DISCUSSION

*Host penetration experiment:* Penetration of soybean roots by the nematodes was rapid and usually increased little after 48 hours (Table 1). Exceptions were *M. hapla* and *M. arenaria*, which continued to increase throughout the 240 hours. Two J2 of *H. glycines* were found in the roots at 1 hour. By 3 hours, and especially by 6 hours, 17 to 40% of the inoculum was found in the roots. Root invasion by *Meloidogyne* J2 had begun within 3 hours, and 5–12% infection was evident at 24 hours and later (Table 1). In other work, *M. javanica* was able to locate and invade tobacco roots more quickly than *M. incognita* (3,4), but these two species behaved

TABLE 1. Penetration of Lee 68 soybean by second-stage juveniles of *Meloidogyne arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, and *Heterodera glycines* races 1 and 5.

Hour	Percentage of Pi penetrating roots					
	<i>M. arenaria</i>	<i>M. hapla</i>	<i>M. incognita</i>	<i>M. javanica</i>	<i>H. glycines</i> R1	<i>H. glycines</i> R5
1	0 d-e	0 d-e	0 d-e	0 c-e	<1 d-e	<1 d-e
3	<1 d-f	0 d-f	<1 d-f	<1 c-f	3 c-e	3 cd-e
6	<1 d-g	0 d-g	<1 d-g	<1 c-g	5 c-f	11 c-e
24	12 c-f	5 c-f	10 c-f	11 b-f	16 b-f	32 b-e
48	19 b-fg	9 b-g	20 ab-f	23 a-f	18 b-fg	44 a-e
120	18 b-fg	10 b-g	23 a-f	25 a-f	19 b-f	43 a-e
240	28 a-e	18 a-fg	16 b-g	20 a-efg	24 a-efg	25 b-ef

Numbers followed by same letter in columns or rows are not different ( $P = 0.05$ ) by Waller-Duncan Bayesian k-ratio  $t$ -test. Data are means of four replications and runs, except 240 hours were means of one run. a, b, c, and d represent differences ( $P = 0.05$ ) in nematodes over time (columns). e, f, g, and h represent differences ( $P = 0.05$ ) between nematodes within time (rows).

similarly on soybean in the present study. The more rapid penetration of soybean roots by *H. glycines* versus *Meloidogyne* spp. may be due to the perceptibly faster movement of the juveniles.

Inoculum efficiency varied among the six test organisms (Table 1). Inoculum efficiencies were as follows: *M. arenaria*—28%, *M. hapla*—18%, *M. incognita*—23%, *M. javanica*—25%, *Heterodera glycines* race 1—24%, and *H. glycines* race 5—44%. Another study showed that juveniles of *M. javanica* infect tobacco roots more rapidly than *M. arenaria* or *M. incognita* for 2–6 days, but levels of J2 for the three species were similar after 10 days (4). Decreased rate of penetration after 48 hours by the same age cohort inoculum is probably related to depletion of energy reserves or other vigor-related conditions of nematodes (13). Over time, root mass increases, and stained juveniles could become more difficult to locate; thus, this problem may account in part for lower counts at 240 hours.

We conclude that 48 hours is preferable for assessing penetration of roots. At this time, the root mass is easily managed and subsampling is not required. The assessment time is minimal compared to that for older roots.

*Type of experimental container:* The levels of root penetration by J2 of *H. glycines* races 1 and 5, *M. incognita*, and *M. javanica* were not different ( $P = 0.05$ ) in clay pots

and styrofoam containers (Table 2). More *M. arenaria* and *M. hapla* juveniles penetrated roots growing in styrofoam containers than in the foil-wrapped clay pots. Styrofoam containers retard water evaporation and are easy to use for short-term experiments. Their uniformity in weight, low maintenance, and ease of disposal make them ideal for this type of assessment, especially for nematicide evaluations. The time and labor necessary to assess root penetration at 48 hours is minimal. Thus, samples can readily be handled in large experiments. Because most of the host penetration by these nematodes is accomplished within 48 hours, efficient and accurate assessments can be made using these small disposable containers. Larger

TABLE 2. Comparison of 15-cm-d aluminum foil wrapped clay pots and 9-cm-d styrofoam containers on the maximum amount of penetration by *Meloidogyne arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, and *Heterodera glycines* races 1 and 5.

	Percentage J2 penetration (% of Pi)	
	Styrofoam	Clay pot
<i>M. arenaria</i>	26*	15
<i>M. hapla</i>	13*	7
<i>M. incognita</i>	6	5
<i>M. javanica</i>	3	4
<i>H. glycines</i> R1	4	5
<i>H. glycines</i> R5	4	5

\* = Penetration between clay pot and styrofoam container is different ( $P = 0.05$ ) by analysis of variance.

containers, however, must be used when a greater time period is required or when older plants are needed to meet the experimental objective(s). These short-term assessments are particularly valuable if penetration rates are the primary data desired. Longer term experiments may involve differential emigration as occurred for J2 of *M. incognita* (8) on resistant versus susceptible plants.

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