Some Factors Affecting Penetration of Bean Roots by Larvae of Meloidogyne incognita and M. javanica

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ABSTRACT

Twenty-four-hour-old larvae of *Meloidogyne incognita* and *M. javanica,* at inoculum levels of 100 larvae per seedling, failed to penetrate roots of any of six bean *(Phaseolus vulgaris)* cultivars after exposure periods of 12, 24, or 48 hours. Under glasshouse conditions (21-24 C), freshly hatched larvae of both species, at an inoculum level of 300, penetrated roots in 48 hours. In later experiments in which the inoculum was applied to the root tips, there was penetration of the roots at inoculum levels of 100, 150, 200, and 250. There were no significant differences in penetration percentage between the cultivars and the species when the experiment was conducted under conditions of low and fluctuating temperature. In a growth chamber at higher temperature (29 and 23 C for day and night, respectively), and using larvae of variable age, significantly more *M. incognita* penetrated roots of all six cultivars than did *M. javanica.* In a similar experiment in which all larvae were of the same age, significantly more *M. incognita* penetrated roots of bean cultivars Kikara, Canadian Wonder, Masterpiece, Marathon, and Premier than did *M. javanica,* but there were no significant differences in penetration of Mexico 142 between the two species.

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Godfrey and Oliveira (6) observed that the first larvae of species of *Meloidogyne* (referred to then as *Heterodera radicicola*) penetrated the roots of pineapple (*Ananas sativus*) in less than 6 hours after inoculation. Barrons (1) demonstrated that, when plants were given an equal opportunity to become infected, just as many larvae of a species of *Meloidogyne* (reported as *H. marioni)* entered the roots of a highly resistant bean *(Phaseolus vulgaris* 'Alabama No. 1') as entered the roots of a susceptible bean (cultivar, Kentucky Wonder). Since all this work was done before Chitwood's (3) reclassification of the root-knot nematode, the actual species used in these investigations are unknown.

More recently, Riggs and Winstead (9) reported that

larvae of *M. incognita* penetrated roots of resistant and susceptible tomatoes *(Lycopersicon esculentum)* in similar numbers, but that necrotic areas appeared around the larvae within 24 hours in resistant plants. In a preliminary pot experiment, Bird and Wallace (2) found that four times as many *M. javanica* entered tomato roots as did *M. hapla,* but their growth rates were not significantly different. Reynolds et al. (8) showed that larvae of *M. incognita acrita* entered both resistant and susceptible lucerne *(Medicago sativa)* cultivars in approximately the same numbers.

No reference has been found in the literature comparing penetration of bean cultivars by *M. incognita* and *M. javanica.* The present study reports the results of studies on penetration of 6 selected bean cultivars by larvae of *M. incognita* and *M. javanica.*

MATERIALS AND METHODS.—*Phaseolus vulgaris* L. cultivars used were Kikara (a local selection), Mexico 142 and Marathon (canning cultivars), Canadian Wonder, Masterpiece and Premier (local marketing and export cultivars).

Meloidogyne incognita (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood were used. The first was obtained from egg masses produced on heavily infected Premier bean roots collected in the field at Thika, Kenya, and was maintained on the six bean cultivars in

TABLE 1. Summary of procedures used in studies on penetration of bean roots by *Meloidogyne* spp.

Experiment	Species	Inoculum level (no. of larvae/root system/cultivar)	Exposure time (hours)	Age of larvae	Experimental conditions
1	*M. incognita* *M. javanica*	100	12,24,48	24 hr	glasshouse[a]
2[b]	*M. incognita* *M. javanica*	300	48	freshly-hatched	glasshouse
3[b]	*M. incognita* *M. javanica*	200, 250	48	freshly-hatched	glasshouse
4[b]	*M. incognita* *M. javanica*	100, 150	48	freshly-hatched	glasshouse
5[b]	*M. incognita* *M. javanica*	100	24	freshly-hatched compared with 24 hr	glasshouse
6[c]	*M. incognita* *M. javanica*	100	48	freshly-hatched	room
7[c]	*M. incognita* *M. javanica*	100	48	freshly-hatched	glasshouse
8[c]	*M. incognita* *M. javanica*	100	24	freshly-hatched variable: obtained direct from pot culture	growth chamber: 29 C day 23 C night
9[c]	*M. incognita* *M. javanica*	100	48	freshly-hatched	growth chamber: 30 C day 25 C night

[a]Mean glasshouse temperatures were 21.0 C at 0830 hours; 24.0 C at 1430 hours; and 24.5 C at 1830 hours.
[b]Replicated twice.
[c]Replicated five times.

TABLE 2. Percentage penetration of *Meloidogyne incognita* and *M. javanica* larvae[a] into roots of six bean cultivars

Bean cultivar	Experiment 8		Experiment 9	
	M. incognita[b]	*M. javanica*[c]	*M. incognita*[b]	*M. javanica*[b]
Kikara	46.4[d]	11.2	42.6	21.6
Mexico 142	43.4	9.6	28.4	28.4
Canadian Wonder	36.2	4.2	41.8	14.2
Masterpiece	43.6	7.6	50.0	22.8
Marathon	43.2	2.8	54.2	31.4
Premier	41.6	3.6	56.0	28.0
LSD ($P = 0.001$) between species means	14.60		14.94	

[a]100 larvae per root system.
[b]Inoculum derived from egg masses.
[c]Inoculum derived from soil.
[d]Mean of five replicates.

the glasshouse. The second was obtained from a single egg mass isolated from a tomato plant at E.A.A.F.R.O. (Muguga) and was maintained on tomatoes and beans in the glasshouse.

Egg masses were removed from tomato and bean roots with forceps, placed in watch glasses containing distilled water and incubated at 25 C for 24 hours. After hatching, larvae were collected in beakers and the volume of the resulting suspension increased to 100 ml. Three ml aliquots of the suspension were pipetted into counting dishes and the number of larvae counted under a dissecting microscope; the inoculum was then adjusted in each case to the required number of larvae per root system of each plant by the addition or removal of individual larvae.

The bean seeds were surface sterilized by soaking them in 0.1% mercuric chloride for 5 minutes, and germinated in sterile petri dishes containing moistened filter paper at 25 C for 60 hours. Seedlings of each cultivar were placed on sterilized sandy soil in paper cups 7.5 cm in diameter and 9.5 cm in height. Suspensions of *M. incognita* or *M. javanica* larvae were poured onto the roots of the seedlings which were then lightly covered with sterilized sandy soil. The seedlings were watered and then transferred to the test environments for the test period.

To demonstrate larval penetration, root systems were gently washed free of soil, stained for 1.0 minute in hot 0.01% cotton blue lactophenol, and cleared in cold lactophenol.

The procedures used in the experiments reported here are summarized in Table 1. Larval penetration percentages were not determined for Experiments 1 to 5; these were preliminary studies used to determine optimal inoculum levels and exposure times for subsequent experiments.

RESULTS.—When suspensions of 100 twenty-four-hour-old larvae of each of the two species of *Meloidogyne* were tested for penetration at exposure periods of 12, 24, and 48 hours, no larvae of either species were found in the roots of any of the bean cultivars (Experiment 1). In a subsequent experiment with 100 freshly-hatched larvae of the two species (Experiment 5), none penetrated in a 24-hour exposure.

In Experiment 2, with exposure time of 48 hours, and inoculum levels of 300 freshly-hatched larvae, both *M. incognita* and *M. javanica* entered the root tips of all cultivars except Mexico 142. In Experiment 3 and 4, in which the inoculum was applied to the root tips, larvae of both nematode species penetrated all cultivars at inoculum levels of 100, 150, 200, and 250.

Analysis of variance of the penetration percentage of *M. incognita* and *M. javanica* after 48 hours of exposure at constant inoculum level (100 freshly-hatched larvae) revealed no significant differences between bean cultivars or species of *Meloidogyne,* either at room or glasshouse temperatures (Experiments 6 and 7). When experiments were conducted under growth chamber conditions (Experiments 8 and 9), results (Table 2) indicated that while there were no significant differences between cultivars, there were highly significant differences ($P<0.001$) between nematode species. At growth chamber temperatures of 29 and 23 C (day and night, respectively; Experiment 8) with larvae of varying age, a striking difference was noted between *M. incognita* and *M. javanica.* In a similar experiment (Experiment 9) in which larvae were all of the same age, differences between species were again highly significant; but, in addition, differences in penetration of different bean cultivars were also observed (Table 2).

DISCUSSION.—The larvae used in Experiment 1 had been kept in a water suspension at room temp for more than 24 hours and may have been weak. This could explain their failure to penetrate. Dropkin (5) reported that larval infectivity of *H. rostochiensis* declined rapidly when larvae were stored in tap water at room temp for extended periods. Similar observations were reported by Thomason et al. (10) who found that infectivity of *M. javanica* was reduced more rapidly than motility after 4 days' storage in tap water at 3 or 27 C. They suggested that larvae of *M. javanica* were injured by low temp while at high temp the decline in infectivity was due to high respiratory rate and depletion of energy needed for penetration of the host root.

When the exposure period was increased to 48 hours, penetration by freshly-hatched, active larvae of *M. incognita* and *M. javanica* occurred at an inoculum level of 300 on all cultivars except Mexico 142. Mexico 142 root systems were of greater length at the time of inoculation and it was assumed that, for this reason, larvae did not reach the root tips, the point of entry for most nematode larvae (4, 6). When the inoculum was applied to the root tips, there was penetration of all cultivars at inoculum levels of 100, 150, 200 and 250.

Penetration is apparently influenced both by age of larvae and prevailing temp: high percentages of *M. incognita* and *M. javanica* invaded the roots when young, freshly-hatched larvae were used, provided the temp was high. Bird and Wallace (2) reported that at 15-20 C *M. hapla* invaded tomato roots in significantly greater numbers than *M. javanica,* at 20-25 C approximately equal numbers of both species penetrated, and at 25-30 C *M. javanica* showed a significantly higher invasion rate than *M. hapla.* However, *M. javanica* invaded tomato roots in near equal numbers at all three temp regimes. Hu (7) reported that optimum temp range for *M. incognita* and *M. javanica* for penetration of sugar cane was 20-30 C, and Wallace (11) reported a similar temp range for *M. javanica* for penetration of tomato roots.

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