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Root Penetration by *Meloidogyne incognita* Juveniles Infected with *Bacillus penetrans*[1]

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Abstract: *Bacillus penetrans* inhibited penetration by *Meloidogyne incognita* second-stage juveniles (J2) into tomato roots in the laboratory and greenhouse. Spores from this Florida population of *B. penetrans* attached to J2 of *M. javanica, M. incognita,* and *M. arenaria.* A greater proportion of J2 of *M. javanica* were infected than were J2 of either *M. incognita* or *M. arenaria,* and a greater number of spores attached to *M. incognita* than to *M. arenaria.*

Key words: bacterial spore parasite, biological control, root-knot nematode.

Prasad (4) reported several experiments that suggested infection of *Meloidogyne* spp. second-stage juveniles (J2) by *Bacillus penetrans* (Thorne) Mankau reduced the number of nematodes entering roots; our field evaluations (unpubl.) indicated the same. Stirling (7) reported also that in field plots treated with *B. penetrans,* reduced numbers of juveniles of *M. javanica* (Treub) Chitwood invaded tomato roots. Our objectives were to determine if *B. penetrans* infection decreased the ability of *M. incognita* to penetrate tomato roots and to determine if spores of this population of *B. penetrans* attached to J2 of the other two *Meloidogyne* spp. important in Florida, *M. javanica* and *M. arenaria* (Neal) Chitwood.

Materials and Methods

Root penetration by M. incognita

Laboratory: The effect of *B. penetrans* infection of *M. incognita* J2 on their penetration into tomato roots (*Lycopersicon esculentum* Miller cv. Rutgers) was investigated using a modified penetration inhibition test (1). Ten cubic centimeters quartz sand (99.3% sand, 0.3% silt, 0.4% clay with a sand particle size distribution of 22% 1–0.5 mm, 41.4% 0.5–0.25 mm, 35.4% 0.25–0.1 mm, 0.5% 0.1–0.05 mm) and 4 ml water were added to 25-ml glass vials into which were injected 50 *M. incognita* J2 in water. Each of two treatments—J2 infected with *B. penetrans* collected from MacClenny, Florida, and noninfected (control) J2—was replicated 25 times, and the vials were arranged in a completely randomized design.

Infected J2 were obtained by adding healthy J2 to 100 cm^3 *B. penetrans*-infested potting soil (90.6% sand, 3.9% silt, 5.5% clay) that had been moistened and incubated for 3 days at 28 C before addition of nematodes. After 3 days at 28 C, the J2 were extracted from the soil by modifications of a sieving and centrifugation method (3). The control J2 were treated identically except they were added to soil not infested with *B. penetrans.*

A random sample of 30 J2 from each treatment was examined (at 200×) to determine the number of attached spores before adding J2 to the bioassay vials. Twenty-five J2 from the infested soil each had more than 20 spores attached to their cuticles. Five J2 each had 13 spores attached,

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TABLE 1. Numbers of spores of *Bacillus penetrans* attached to samples of juveniles (J2) of *Meloidogyne incognita* used in greenhouse experiments on penetration of roots by the nematode.

Experiment	Duration (days)	Number of J2/pot	Number of spores/J2*	Frequency
1	30	325	0	10
			1	8
			2	3
			5	1
			10	1
			12	1
			15	1
			16	1
			>20	4
2	14	325	0	20
			1	4
			2	2
			3	1
			4	1
			5	1
			8	1
3	14	35	0	12
			1	10
			2	1
			3	1
			4	1
			9	1
			12	1
			16	1
			>20	2

* No spores were observed on juveniles from the control.

one had 10 spores, and three each had a single spore. None of the 30 J2 from noninfested soil had attached spores.

Twenty-four hours after infesting the vials with *M. incognita* J2, one tomato seedling was planted in each vial. The vials were placed randomly in an incubator at 28 C with a cycle of 13 hours light (260 lux) and 11 hours dark. After 7 days, galls on roots were counted and the effect of parasitism by *B. penetrans* on the formation of galls by nematodes was tested with the Wilcoxon rank sum test (2).

Immediately after the galls were counted, the seedlings were transplanted into 10-cm-d clay pots containing potting soil and grown in a greenhouse. After 14 days in the greenhouse, the seedlings were removed from pots and the root systems were stained with acid fuchsin in lactophenol to aid in counting the nematodes within the roots (6). This procedure was undertaken to corroborate that the root galls present were formed by nematodes at established feeding sites and to account for J2 that might have entered the roots but had not initiated galls within 7 days.

TABLE 2. Number of galls on tomato roots 7 days after inoculation with 50 juveniles (J2) of *Meloidogyne incognita* not infected (control) and infected with *Bacillus penetrans*.

Control*		Infected*	
Number of galls/plant	Frequency	Number of galls/plant	Frequency
0	6	0	17
1	4	1	7
2	4	3	1
3	5		
4	1		
5	2		
6	1		
7	2		

* The Wilcoxon rank sum test indicated fewer galls formed by infected J2 than than by healthy J2 ($P < 0.001$).

Greenhouse: Tomato plants were inoculated with noninfected or *B. penetrans*-infected J2 which were obtained as described previously. In three experiments carried out for 14 or 30 days, 35 or 325 J2 were added to each of six pots per treatment (Table 1). In each experiment, random samples of 30 J2 were examined to determine the number of attached spores before they were added to the pots (Table 1).

At the end of the experiments, plants were removed from the pots. When 35 juveniles were used, the entire root system was stained with acid fuchsin in lactophenol; when 325 J2 were used, a 4-g random sample of roots was stained. The numbers of nematodes within the roots in the two treatments were compared with the Wilcoxon rank sum test.

Attachment of spores to Meloidogyne spp. J2

Random subsamples of 30 cm^3 dried potting soil infested with *B. penetrans* were placed in 30-cm^3 plastic cups. The soil was moistened with 10 ml distilled water, and the cups were incubated for 3 days at 28 C; noninfested potting soil was the control. Treatments were *M. javanica, M. incognita,* and *M. arenaria.* Each treatment was replicated four times with cups arranged in a randomized design on an incubator shelf. Then 1,000 J2 of *M. javanica, M. incognita,* or *M. arenaria* in water were injected into the cups, and the cups were incubated for 3 days at 28 C. The J2 were extracted from the soil by the method described previ-

TABLE 3. Numbers of *Meloidogyne incognita* in tomato roots 14 or 30 days after inoculation with juveniles (J2) not infected (control) and infected with *Bacillus penetrans* in the greenhouse.

Experiment	Control	Infected
1*	28	8
	38	24
	38	32
	41	32
	61	38
	62	53
2*	30	1
	34	25
	35	36
	59	41
	63	45
	67	58
3†	3	0
	6	0
	9	1
	9	1
	13	2
	18	7

In Experiments 1 and 3 fewer infected than noninfected J2 developed in the roots ($P = 0.066$ and $P = 0.004$). In Experiment 2 no difference could be claimed ($P = 0.197$).

* Nematodes per 4 g roots.

† Nematodes per root system.

ously, and a random sample of 50 J2 per cup was examined (at 200×) for parasitism by *B. penetrans*. The independence of the percentage of infected J2 and the species of *Meloidogyne* was tested with the log likelihood ratio G statistic (5).

RESULTS

Root penetration by M. incognita

Laboratory: Fewer galls occurred on roots of tomato plants inoculated with *M. incognita* J2 infected with *B. penetrans* than on roots inoculated with noninfected J2 (Table 2). The total number of galls formed on the roots after 7 days was 10 for the infected nematodes and 61 for the control nematodes; after 21 days, the number of infected nematodes in roots was 9 compared with 101 noninfected nematodes.

Greenhouse: In Experiments 1 and 3, but not in Experiment 2, fewer infected than noninfected nematodes developed in tomato roots (Table 3).

Attachment of spores to Meloidogyne spp. J2

Spores of *B. penetrans* attached to the cuticles of nematodes of all three species. The numbers of 200 J2 of the three species of *Meloidogyne* with spores attached were *M. javanica* 157, *M. incognita* 95, and *M. arenaria* 105. A greater proportion of *M. javanica* J2 were infected than were those of either *M. incognita* or *M. arenaria*, and the corresponding proportions for *M. incognita* and *M. arenaria* were not found to be different ($\alpha = 0.04$). Nearly all infected *M. javanica* J2 and the majority of infected *M. incognita* J2 had more than 15 attached spores; the infected *M. arenaria* J2 had fewer than seven attached spores, except for one heavily infected specimen.

DISCUSSION

Our results demonstrate that *B. penetrans* infection of *M. incognita* J2 decreases root penetration by the J2. Prasad (4) reported greenhouse tomatoes inoculated with *M. incognita* had fewer galls on roots when grown in soil containing *B. penetrans* than in bacterium-free soil and *M. javanica* J2 heavily infected with *B. penetrans* formed fewer galls on tomato roots than did noninfected J2. In other treatments of Prasad's study, J2 with moderate infection produced intermediate numbers of galls on tomato roots, suggesting that inhibition of root penetration may be related to the number of spores attached to a J2. This relationship was confirmed experimentally (7). The influence of the number of infected J2, and their degree of infection, and the lowered incidence of root penetration, as well as the mechanism by which penetration is inhibited, need investigation.

The population of *B. penetrans* used in the present study attaches to *M. javanica* and *M. arenaria* as well as *M. incognita*. Assuming that attachment of spores indicates a host–pathogen relationship between the organisms, the Florida population of *B. penetrans* may be promising for use against the three most important *Meloidogyne* spp. (*M. javanica, M. incognita, M. arenaria*) in Florida (R. A. Dunn, pers. comm.). Moreover, since this population of *B. penetrans* attached in greater numbers to J2 of *M. javanica* than *M. incognita*, it may more effectively inhibit *M. javanica*.

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