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Penetration and Development of *Meloidogyne incognita* on Roots of Resistant Soybean Genotypes[1]

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Abstract: *Meloidogyne incognita* penetration and development were studied in roots of highly resistant (PI 96354, PI 417444), resistant (Forrest), and susceptible (Bossier) soybean genotypes. Although more second-stage juveniles (J2) had penetrated roots of PI 96354 and PI 417444 than roots of Forrest and Bossier by 2 days after inoculation, fewer J2 were present in roots of PI 96354 at 4 days after inoculation. Juvenile development in all genotypes was evident by 6 days after inoculation, with the highest number of swollen J2 present in roots of Bossier. At 16 days after inoculation, roots of PI 96354 had 87%, 74%, and 53% fewer J2 than were present in roots of Bossier, Forrest, and PI 417444, respectively. Differential emigration of J2, not fewer invasion sites, was responsible for the low number of nematodes in roots of the highly resistant PI 96354. Some 72% of the J2 penetrating the roots of this genotype emerged within 5 days after inoculation, whereas 4%, 54%, and 63% emerged from roots of Bossier, Forrest, and PI 417444, respectively. Penetration of roots of PI 96354 decreased the ability of J2 emerging from these roots to infect other soybean roots.

Key words: emigration, *Glycine max*, infectivity, *Meloidogyne incognita*, plant introduction, root penetration, soybean.

Among the *Meloidogyne* species causing significant soybean (*Glycine max* (L.) Merr.) losses in the southeastern United States (5), *M. incognita* (Kofoid & White) Chitwood is the species most frequently found infesting soybean fields in Georgia. This nematode can damage and suppress yields of susceptible soybean cultivars by as much as 90% and yields of resistant cultivars by 40% (14).

The most effective management tactics to maximize soybean yields on nematode-infested fields include use of resistant cultivars, crop rotation, and nematicides. The present lack of effective, inexpensive nematicides, the potential hazards of pesticides in the environment, and the wide host ranges of some nematode species make using resistant cultivars a critical management tactic. Currently the use of resistant cultivars is the most economical and environmentally tolerable nematode management tactic available to soybean growers. Although many root-knot resistant soybean cultivars are available, they are only partially resistant (15,21). This partial resistance can allow high residual soil population densities of eggs and second-stage juveniles (J2) of *Meloidogyne* species to build up by harvest time which could severely damage subsequent susceptible crops.

In a greenhouse screen of genotypes from the Southern Soybean Germplasm Collection, two plant introductions (PI), PI 96354 and PI 417444, were identified as highly resistant to *M. incognita* (16). In field microplot experiments with increasing initial population densities of *M. incognita*, soil population densities of J2 at harvest time were lower on both PI than on Forrest, a standard resistant cultivar (8). In addition, at the highest initial population density, 62% fewer J2 were present in roots of PI 96354 than in roots of the other two resistant genotypes at 14 days after planting.

The present experiments were conducted to further evaluate the effects of the highly resistant soybean PI on root penetration and subsequent development of *M. incognita*.

Materials and Methods

A *M. incognita*-susceptible soybean genotype, Bossier (Maturity Group [MG] VII), and three *M. incognita*-resistant genotypes,

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Forrest (MG V), PI 417444 (MG VI), and PI 96534 (MG VI), were used in these experiments. A mixture of three collections of *M. incognita*, host race 3, selected for their aggressiveness to soybean (11) was propagated on tomato, *Lycopersicon esculentum* Mill. cv. Rutgers, in a greenhouse. Nematode inoculum was obtained by collecting infective J2 from galled roots in a modified Seinhorst mist chamber for 3 days after discarding J2 emerging during the first 24 hours (1).

Penetration experiments: Four seeds of Bossier, Forrest, PI 417444, and PI 96354 were planted in 474-cm^3 styrofoam cups filled with 400 cm^3 soil mix (loamy sand soil, sand, and attapulgite clay, 3:1:1) previously fumigated with methyl bromide at 1.36 kg/800 liters soil. After 5 days the seedlings were thinned to one plant per cup and inoculated with 2,000 J2 per cup. Plants were grown on greenhouse benches under supplemental light from 400-watt Multi-Vapor phosphor-coated lamps to provide a 16-hour photoperiod. Nematode penetration was determined by staining the entire root system with acid fuchsin (2) and counting vermiform and swollen J2 with a stereomicroscope. The experimental design was a randomized complete block with six replications. In the first experiment, roots were harvested and stained at 2-day intervals for 16 days to assess J2 penetration and development. In the other greenhouse experiments the roots were stained on 2, 4, 8, 12, and 16 days after inoculation.

Two experiments were conducted to determine the number of J2 that emerged from the roots. In the first, seedlings were removed from the cups 2 days after inoculation, root systems were washed, and seedlings were transplanted into sterilized soil mix in new cups. At time of transplanting, one group of seedlings was harvested and their roots were stained for enumerating nematodes. The remaining plants were harvested at 4, 8, 12, and 16 days after inoculation. In the second, at 2 and 4 days after inoculation, each root system was washed carefully to remove the soil and J2 adhering to the root surface. The root systems of the intact plants were placed individually in 150-ml glass beakers filled with 50 ml sterilized distilled water and incubated in a growth chamber at 25 C. Juveniles emerging from roots were collected by changing the water daily for 5 days and counted with a stereomicroscope. After collecting J2 for 5 days, roots were excised, blotted dry, and weighed, and the number of root tips was determined. Roots were stained to enumerate the number of J2 remaining in the root systems.

Another experiment was conducted to determine how rapidly J2 penetrated roots. Bossier, Forrest, and PI 96354 seeds were germinated in ragdolls (3), and after 3 days the seedlings were placed on the surface of sterilized sand in a tray (4). Root tips were lightly covered with sand and inoculated with 250 freshly hatched J2 from eggs collected with 0.5% NaOCl (10) and placed on a Baermann funnel at room temperature. Seedlings were stained as described at 6, 12, and 24 hours after inoculation, and J2 in the roots were counted.

Infectivity experiment: This experiment was conducted to assess the infectivity of J2 of *M. incognita* that had emerged from the roots of Bossier and PI 96354. Surface-sterilized soybean seeds were germinated in ragdolls wrapped with aluminum foil to prevent desiccation. The ragdolls were placed in glass beakers filled with sterilized distilled water and incubated at 25 C. After 6 days the seedlings were transferred to another ragdoll and root tips were placed between two strips of Miracloth (2 cm wide). After ragdolls were incubated for 3 days at 25 C, the seedlings were inoculated by adding 2,000 J2 to the Miracloth (3). Four days later the plants were removed from the ragdolls, roots were washed, and individual plants were transferred to 30 ml sterilized distilled water in test tubes and incubated in a growth chamber at 25 C for 5 days. Second-stage juveniles emerging from the roots were collected for inoculum.

Seeds of Bossier, Forrest, and PI 96354 were germinated in ragdolls. After 3 days

TABLE 1. Numbers of *Meloidogyne incognita* vermiform and swollen juveniles in root systems of susceptible (Bossier) and resistant (Forrest, PI 417444, and PI 96354) soybean genotypes at 2–16 days after inoculation.

Genotype	2	4	6	8	10	12	14	16
				Vermiform				
Bossier	95	206	148	152	40	15	7	4
Forrest	96	174	146	50	34	78	51	7
PI 417444	150	225	34	11	6	9	42	37
PI 96354	182	103	37	22	22	14	62	26
LSD ($P = 0.05$)	24	38	23	23	11	10	19	6
				Swollen				
Bossier	0	0	217	361	675	850	1,028	1,317
Forrest	0	0	90	139	168	264	484	521
PI 417444	0	0	97	109	131	145	164	193
PI 96354	0	0	49	59	84	107	116	143
LSD ($P = 0.05$)	ns	ns	26	29	56	55	66	64

Data are average of six replications.

the seedlings were transferred to the surface of sterilized sand. The roots were lightly covered with sand, and each seedling was inoculated with 250 J2 that had emerged from soybean roots. Second-stage juveniles freshly hatched from eggs as previously described were used as inoculum controls. The roots were stained 2 days after inoculation, and J2 inside were enumerated. The experimental design was a randomized complete block with four replications.

Statistical analysis: Each experiment was analyzed separately with analysis of variance. In experiments with multiple sampling dates, each date was analyzed separately. The effects of genotypes on nematode and plant traits were compared by Fisher's protected least significant difference ($P = 0.05$).

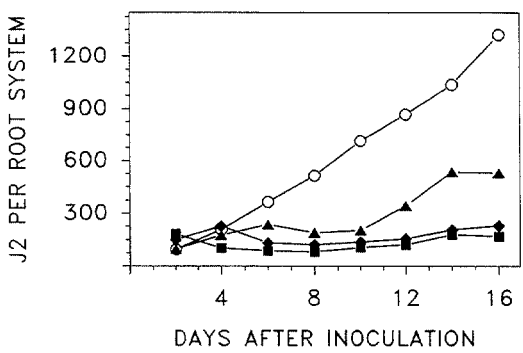

FIG. 1. Total *Meloidogyne incognita* juveniles (J2) in root systems of susceptible (Bossier ○) and resistant (Forrest ▲, PI 417444 ◆, and PI 96354 ■) soybean genotypes at different days after inoculation. Data are means of six replications. LSD ($P = 0.05$) = 24, 38, 27, 36, 53, 56, 60, and 65 for each sampling date starting at 2 days.

RESULTS

Penetration experiments: Penetration and subsequent development of J2 of *M. incognita* in roots were affected by soybean genotype. By 2 days after inoculation, the number of J2 in Bossier and Forrest roots did not differ, but a greater number of J2 penetrated roots of PI 41744 and PI 96354 (Table 1). Fewer vermiform J2 were present in roots of PI 96354 than in roots of the other genotypes at 4 days. At 6 days after inoculation, the number of vermiform juveniles in roots of both resistant PI decreased precipitously. In contrast, vermiform J2 numbers in roots of Forrest and Bossier did not decline until 8 and 10 days after inoculation, respectively.

Juvenile development was evident in all genotypes by 6 days after inoculation, with the greatest number of swollen juveniles being present in Bossier roots (Table 1). At 16 days after inoculation, the total number of J2 (vermiform and swollen) present in the root systems was greatest for Bossier (1,321) and lowest for PI 96354 (169) (Fig. 1). In this experiment, 88%, 68%, and 29% fewer J2 were present in roots of PI 96354 at 16 days after inoculation than in roots of Bossier, Forrest, and PI 417444, re-

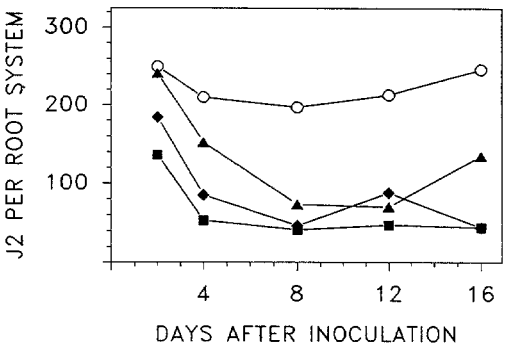

FIG. 2. Number of *Meloidogyne incognita* juveniles (J2) in root systems of susceptible (Bossier ○) and resistant (Forrest ▲, PI 417444 ◆, and PI 96354 ■) soybean genotypes at different days after inoculation. Roots of seedlings were exposed to inoculum for only 2 days. Data are average of six replications. LSD ($P = 0.05$) = 39, 39, 18, 19, and 20 for each sampling date starting at 2 days.

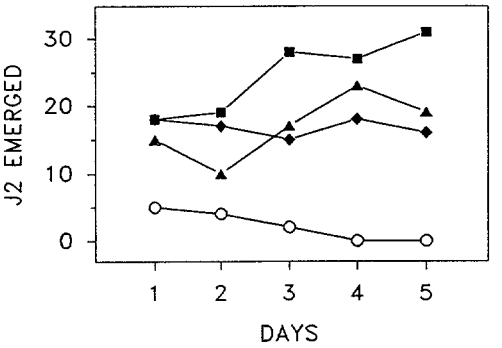

FIG. 3. Daily emigration of second-stage juveniles of *Meloidogyne incognita* from root systems of resistant (Forrest ▲, PI 417444 ◆, and PI 96354 ■) and susceptible (Bossier ○) soybean genotypes. Data are average of six replications. LSD ($P = 0.05$) = NS, 10, 19, 22, and 25 for each sampling date starting at 1 day.

spectively (Fig. 1). Similar results were obtained when this experiment was repeated with five harvest dates.

In an experiment conducted to determine if the differences observed in J2 penetration of roots of the four genotypes were due to variation in number of root tips (invasion sites) or size of the root systems, fresh root weight (1.06 g) and number of root tips (116) for Bossier did not differ from PI 417444 (1.02 g and 112) or PI 96354 (1.06 g and 126) 4 days after inoculation. In this experiment, the total number of J2 in roots of PI 96354 at 16 days after inoculation was 89%, 79%, and 63% fewer than in roots of Bossier, Forrest, and PI 417444, respectively (data not presented).

Exposing roots of each genotype to nematode inoculum for only 2 days limited the numbers of J2 penetrating the roots (Fig. 2), relative to the penetration experiment. At 16 days after inoculation, 45% fewer J2 were present in roots of Forrest than in Bossier, whereas PI 417444 and PI 96354 each had 82% fewer J2 in their roots than did Bossier.

Emigration of J2 from root systems of each genotype after exposure to inoculum for 2 and 4 days varied with genotype. Juveniles emerging from roots of PI 96354 increased daily over the 5-day test period, whereas those leaving Bossier roots decreased over time (Fig. 3). Juvenile emigration from roots of Forrest and PI 417444 was intermediate and changed little over the five collection dates. Greater numbers of J2 emigrated from root systems of the resistant genotypes than from roots of Bossier, with the highest number leaving PI 96354 roots (Table 2). Emigration rates (number of emerging J2 ÷ number of J2 penetrating roots) for 5 days were 4%, 54%, 63%, and 72% for Bossier, Forrest, PI 417444, and PI 96354, respectively. The mean number of J2 remaining in roots did not differ among the resistant genotypes (Table 2). In this experiment, Forrest had the greatest number of root tips at 4 days (239), Bossier had the fewest (126), and the PI root tip numbers were intermediate.

Data from our experiments indicated that J2 penetrated roots of PI 96354 more rapidly than roots of Bossier and Forrest. Therefore, J2 penetration was assessed at 6, 12, and 24 hours after inoculation. In this experiment, the J2 penetration rate (J2 in roots ÷ J2 in inoculum) was highest for PI 96354. At 6, 12, and 24 hours after inoculation, 9%, 17%, and 34% of the J2 penetrated roots of PI 96354, respectively, whereas only 5%, 9%, and 16% of the J2 penetrated roots of Bossier by these times (Table 3). Juvenile penetration rates for

TABLE 2. *Meloidogyne incognita* juveniles emerged from or remaining in root systems of four soybean genotypes during 5 days beginning 2 and 4 days after inoculation.

Genotype†	Emerged‡			Remaining		
	2	4	Means	2	4	Means
Bossier	14	9	11 c	254	307	280 a
Forrest	93	77	85 b	84	63	73 b
PI 417444	82	98	90 ab	59	49	54 b
PI 96354	118	129	123 a	58	36	47 b

Data are average of four replications. Means within columns followed by different letters indicate significant difference based on Fisher's (protected) LSD test ($P = 0.01$) for comparison of genotype means only.

† Seedlings were inoculated with 2,000 second-stage juveniles.

‡ Juveniles emerged were collected for 5 days.

Forrest were intermediate to those for PI 96354 and Bossier.

Infectivity experiment: Juveniles emerging from roots of PI 96354 were less infective than those from Bossier. In this experiment, only 7–10% of the J2 that emigrated from roots of PI 96354 were infective, compared to 19–25% of the J2 leaving roots of Bossier. In contrast, 34–41% of freshly hatched J2 infected roots of Bossier, Forrest, and PI 96354 (Table 4).

DISCUSSION

Second-stage juveniles of *Meloidogyne* species generally penetrate roots of resistant cultivars as readily as roots of susceptible cultivars of most crop species, precluding a barrier to penetration as a common form of resistance (9). In fact, by 2 days after inoculation, greater numbers of *M. incognita*-infective J2 penetrated roots of highly resistant *Gossypium barbadense* than roots of a susceptible cotton cultivar (18). In our study, more J2 usually initially penetrated roots of the highly resistant soybean PI than roots of susceptible Bossier.

TABLE 3. *Meloidogyne incognita* juveniles in roots of Bossier, Forrest, and PI 96354 soybean at 6, 12, and 24 hours after inoculation (HAI).

HAI†	Bossier	Forrest	PI 96354
6	12 b	14 b	22 a
12	23 b	28 b	43 a
24	40 c	61 b	86 a

Data are average of four replications. Means within rows followed by different letters indicate significant difference based on Fisher's (protected) LSD test ($P = 0.01$).

† Seedlings were inoculated with 250 freshly hatched second-stage juveniles.

Even though endoparasitic nematodes initially penetrate roots of resistant cultivars, in some plant species fewer nematodes are present in roots of the resistant cultivar than in roots of a susceptible cultivar a few days after inoculation. For example in soybean, similar numbers of *M. incognita* J2 were present in roots of resistant and susceptible cultivars at 7 days after inoculation, but there were 27% fewer J2 in roots of resistant cultivars than in roots of susceptible cultivars at 14 days after inoculation (20). Similar observations have been reported for cotton (18), alfalfa (6,22), and tomato (7). Postinfectional reduction in number of J2 in roots of resistant cultivars has been correlated frequently (22), but not always (17), with emigration of nematodes from roots. In resistant alfalfa, the reduction in *M. incognita* J2 numbers

TABLE 4. Infection of soybean genotypes by second-stage juveniles (J2) of *Meloidogyne incognita* that emerged from roots of Bossier and PI 96354 soybean compared with freshly hatched J2 (control).

Genotype	Source of J2	J2/root system
Bossier	PI 96354	17 c
	Bossier	62 b
	Control	85 a
Forrest	PI 96354	23 c
	Bossier	56 b
	Control	87 a
PI 96354	PI 96354	24 c
	Bossier	48 b
	Control	102 a

Data are average of four replications. Means within columns followed by different letters indicate significant difference based on Fisher's (protected) LSD test ($P = 0.01$) for comparison within each genotype.

in roots at 4 days after inoculation correlated with J2 emigrating from the roots (22). In another study (19), a higher number of *Globodera rostochiensis* J2 emigrated from roots of a resistant potato cultivar than from roots of a susceptible cultivar, but only during the first 4 days after inoculation.

Our results indicate that the low number of J2 previously observed in roots of highly resistant PI 96354 and PI 417444 at 14 days after inoculation in field microplots (8) was due to emigration of J2 from roots and not to a different number of invasion sites (root tips). In fact, only 28% of the J2 that penetrated roots of PI 96354 over 4 days remained in the roots during the next 5 days. In contrast, 96% of the infective J2 remained in roots of the susceptible cultivar (Bossier) during the same period.

The stimulus responsible for emigration of J2 from roots of resistant plants remains unknown. Huang (9) suggested that emigration of J2 from roots shortly after penetration might be due to the absence of specific nutrients. Since some J2 initiated development in roots of the highly resistant PI in our study, egress of J2 is probably not nutritionally related. More than likely, emigration reflects an active response in roots of the resistant genotypes that creates conditions adverse to the majority of J2 which penetrate the roots. Postinfectional resistance mechanisms previously reported in soybean resistant to *M. incognita* include a hypersensitive necrotic response (12,23) and glyceollin accumulation (13).

Infectivity of *M. incognita* J2 that emerged from roots of PI 96354 was lower than for J2 that emerged from roots of the susceptible cultivar (Bossier) and freshly hatched J2. The basis for J2 emerging from roots of the highly resistant PI being physiologically less fit remains unknown. Similar results were obtained with *G. rostochiensis* J2 that emerged from roots of a resistant potato cultivar (19).

In conclusion, differential emigration of *M. incognita* J2 resulted in lower numbers of nematodes remaining in roots of highly resistant PI than in roots of a susceptible soybean cultivar or a standard resistant cultivar. Furthermore, emigration of most of the J2 that penetrate roots of the highly resistant PI 96354 contributes to its effectiveness in suppressing J2 soil population densities under field conditions (8). The factor(s) responsible for making root tissue of the highly resistant PI 96354 an unfavorable habitat for infective *M. incognita* J2 warrants further study.

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