

Response of Resistant Soybean Plant Introductions to *Meloidogyne incognita* in Field Microplots¹

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Abstract: The response of two soybean plant introductions, PI 96354 and PI 417444, highly resistant to *Meloidogyne incognita*, to increasing initial soil population densities (Pi) (0, 31, 125, and 500 eggs/100 cm³ soil) of *M. incognita* was studied in field microplots for 2 years. The plant introductions were compared to the cultivars Forrest, moderately resistant, and Bossier, susceptible to *M. incognita*. Averaged across years, the yield suppressions of Bossier, Forrest, PI 417444, and PI 96354 were 97, 12, 18, and < 1%, respectively, at the highest Pi when compared with uninfested control plots. Penetration of roots by second-stage juveniles (J2) increased linearly with increasing Pi at 14 days after planting. At the highest Pi, 62% fewer J2 were present in roots of PI 96354 than in roots of the other resistant genotypes. Soil population densities of *M. incognita* were lower on both plant introductions than on Forrest. At 75 and 140 days after planting, PI 96354 had the lowest number of J2 in the soil, with 49% and 56% fewer than Forrest at the highest Pi. The resistance genes in PI 96354 should be useful in a breeding program to improve the level of resistance to *M. incognita* in soybean cultivars.

Key words: *Glycine max*, *Meloidogyne incognita*, population density, resistance, root-knot nematode, root penetration, soybean, yield response.

The use of resistant cultivars is the most economical means of limiting damage to soybean (*Glycine max* (L.) Merr.) by root-knot nematodes (9). Resistance to *Meloidogyne incognita* (Kofoid & White) Chitwood, the *Meloidogyne* sp. most frequently found in soybean fields (4), is common in soybean cultivars adapted to the southern United States (13). This resistance is incomplete, however, and moderately high residual soil population densities of *M. incognita* usually occur following resistant cultivars (9,11). Although levels of nematode damage to resistant soybeans may be low, the high residual *M. incognita* densities may damage subsequent root-knot susceptible crops. New soybean cultivars with higher levels of resistance to *M. incognita* than currently available are needed to manage this nematode more effectively.

Several soybean genotypes in the Southern Germplasm Collection were found to have high levels of resistance (few eggs produced per root system) to *M. incognita* (10).

Two plant introductions, PI 96354 and PI 417444, that had the highest levels of resistance (10) could provide genes to improve the level of *M. incognita* resistance in soybean cultivars.

These experiments were conducted to determine the response of plant introductions highly resistant to *M. incognita* to various initial population densities of *M. incognita* in field microplots.

MATERIALS AND METHODS

In 1987 and 1988, microplot experiments were conducted at the University of Georgia Plant Sciences Farm near Athens, Georgia. Four soybean genotypes, Bossier (Maturity Group [MG] VII), Forrest (MG V), PI 417444 (MG VI), and PI 96354 (MG VI) were used in the experiments. Bossier is susceptible and the other three genotypes have varying levels of resistance to *M. incognita* (10).

Meloidogyne incognita (race 3), established from a mixture of three collections selected for their aggressiveness to soybean (6), was cultured on greenhouse-grown tomato, *Lycopersicon esculentum* Mill. cv. Rutgers. Nematode inoculum was obtained by collecting eggs with 0.5% NaOCl as described by Hussey and Barker (5).

Microplots (75 cm d [1] with fiberglass walls) were established in Appling coarse

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sandy loam soil which was limed and fertilized according to the soil test recommendations of the University of Georgia Extension Service. Four weeks before planting, microplots were fumigated with methyl bromide at 0.12–0.19 kg/m². At planting, four initial population densities (Pi) of *M. incognita* (0, 31, 125, and 500 eggs/100 cm³ soil) in 1,600 ml of water were mixed into the top 23 cm of soil of each microplot. The four genotypes and four Pi were arranged as factorial combinations with four replications in a randomized complete block design.

Inocula of mycorrhizal fungi (*Gigaspora margarita*, *Glomus etunicatum*, *G. macrocarpum*, and *G. mosseae*) and *Bradyrhizobium japonicum* were added within the row in each microplot. Forty-five seeds were planted in a center row inside each microplot. Twenty seeds were planted in a row adjacent to the center row for determination of root penetration by *M. incognita* second-stage juveniles (J2). Seeds of the same genotype planted inside microplots were also planted in border rows (96.5 cm apart) on both sides of the microplot and between microplots within rows. Seedlings in the center row of each microplot were thinned to 20 plants 3 weeks after planting. Microplots were irrigated as needed to provide suitable moisture for plant growth.

All 20 seedlings in the row adjacent to the center row were removed to determine nematode penetration 14 days after planting. Five root systems were selected randomly and stained (3) and the number of J2 per root system was determined. Soil samples were taken 65, 90, and 120 days after planting in 1987 and 75 and 140 days after planting in 1988 for extraction of J2. Six 2.5-cm-d soil cores taken to 20–30 cm deep were composited and J2 were extracted from 250 cm³ soil by a combination of elutriation (2) and centrifugal flotation (7). Plant mortality was observed and seed yield per microplot was measured.

For statistical analysis nematode data were transformed to log₁₀ (X + 1) values to remove the correlation between treatment means and variances and are report-

ed as antilogs of the data analyzed. Analysis of variance for nematode soil population densities was conducted separately for each year. Means of nematode soil population densities for each genotype were compared by Fisher's protected least significant difference ($P = 0.05$) when genotype \times Pi interaction was insignificant ($P > 0.05$). Relative seed yield was calculated on a per replication basis as the yield of each nematode-infested microplot divided by the yield of the noninfested microplot. Relative yields and J2 penetration data were combined over years after a separate analysis showed similar trends. Regression models (linear and quadratic) were used to determine yield response to Pi. Separate response curves for relative yield were fitted for each genotype. The effects of genotypes on absolute yield and J2 penetration were compared by protected least significant difference at each Pi level.

RESULTS

Seed yields were slightly higher in 1988 than in 1987, but in both years there were no differences in yields among genotypes in the absence of *M. incognita* (Table 1). Both yield suppression and plant mortality of Bossier increased with Pi, both exceeding 90% at the highest Pi (Table 1). Yield response of Bossier to Pi was best described as quadratic, $R^2 = 0.83^{**}$ (Fig. 1). Except for PI 417444 which performed poorly in 1987, seed yields of the resistant genotypes were not affected by *M. incognita* Pi (Table 1). Of the resistant genotypes only Forrest exhibited any plant mortality, and it occurred only at the higher Pi (Table 1).

Numbers of *M. incognita* J2 penetrating root systems of each genotype increased linearly as Pi increased (Table 2). All resistant genotypes had fewer J2 present in roots at 14 days after planting than were present in roots of Bossier at low (31 eggs/100 cm³) and intermediate Pi, except for PI 417444 at the intermediate Pi (125 eggs/100 cm³). At the highest Pi (500 eggs/100 cm³), fewer (63–69%) J2 were present in PI 96354 roots than in roots of the other three genotypes (Table 2).

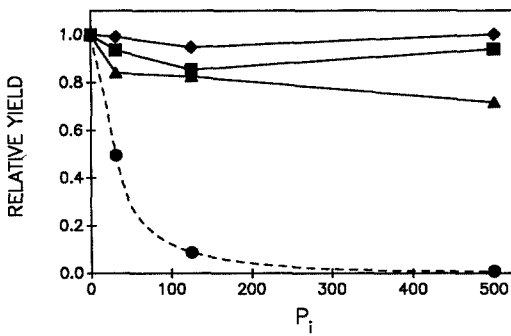


FIG. 1. Relative yield responses of four soybean genotypes to increasing initial population densities (Pi) of *Meloidogyne incognita* in field microplots. Data are averages of 1987 and 1988 experiments. Solid lines connect means for genotypes which showed no significant linear or quadratic regression of relative yields (Y) on Pi (X): PI 96354 (◆), PI 417444 (▲), and Forrest (■). Dashed line connects means for Bossier where the response was quadratic and $Y = 0.895 - 0.852X + 0.001X^2$, $R^2 = 0.83$.

Soil population densities of *M. incognita* J2 differed among the genotypes with the lowest densities associated with PI 417444 and PI 96354. Genotype × Pi interactions were not detected. In 1987, differences in J2 population densities for the genotypes were first observed 90 days after planting. Among the resistant genotypes, PI 96354 had the lowest densities (Table 3). Final population densities of J2 at the high Pi were 20–28% lower on PI 417444 and PI 96354 than on Forrest, the resistant standard, and the mean number of J2 did not differ across Pi levels for PI 417444 and PI 96354 (Table 3). At all Pi, regressions of J2 population densities on sampling dates were quadratic and homogeneous for all cultivars.

The population increase of *M. incognita* in 1988 was similar to that observed in 1987 on all genotypes, but observed differences among the resistant genotypes were greater (Table 4). Compared with Forrest at the high Pi, PI 96354 had 49% and 56% fewer J2 per 100 cm³ soil at 75 and 140 days after planting, respectively. Reproductive factors were inversely related to Pi on all genotypes and were lowest for PI 96354.

TABLE 1. Seed yields and mortalities of susceptible (Bossier) and resistant (Forrest, PI 417444, and PI 96354) soybean genotypes with increasing initial population densities of *Meloidogyne incognita* in field microplots in 1987 and 1988.

Pi†	1987		1988	
	Seed yield (g/plot)	Mortality (%)	Seed yield (g/plot)	Mortality (%)
Bossier				
0	71.2 a	0	75.5 a	0
31	26.0 b	44	47.6 b	29
125	0.2 c	99	13.0 b	55
500	0.0 c	100	4.4 b	90
Mean	24.3	61	35.1	43
Forrest				
0	82.0 a	0	78.3 a	0
31	65.8 a	0	78.2 a	0
125	57.9 a	1	74.9 a	0
500	70.1 a	4	73.2 a	2
Mean	69.0	1	76.1	< 1
PI 417444				
0	51.2 a	0	75.4 a	0
31	34.4 b	0	69.0 a	0
125	30.6 b	0	72.8 a	0
500	27.2 b	0	64.4 a	0
Mean	35.9	0	70.4	0
PI 96354				
0	83.7 a	0	82.5 a	0
31	81.4 a	0	81.9 a	0
125	79.4 a	0	76.8 a	0
500	87.5 a	0	78.6 a	0
Mean	83.0	0	79.9	0

Data are averages of four replications. Means within columns followed by different letters indicate significant difference based on Fisher's (protected) LSD ($P = 0.05$) for comparison between genotype at the same Pi levels only.

† Pi = initial population density of *M. incognita* eggs/100 cm³ soil.

DISCUSSION

Meloidogyne incognita reproduces on resistant soybean cultivars and may suppress yields of resistant cultivars by as much as 40% (8). In field microplot studies (11), *M. incognita* reproduced on resistant soybean cultivars although at a lower level than on a susceptible cultivar. *Meloidogyne incognita* soil population densities were still increasing under a resistant cultivar at 120 days after planting, whereas the densities plateaued after 90 days under susceptible cultivars presumably because of lack of food resulting from heavily damaged roots.

TABLE 2. Numbers of *Meloidogyne incognita* juveniles in root systems of four soybean genotypes 14 days after planting in field microplots.

Pi†			PI	PI
	Bossier	Forrest	417444	96354
31	14 a	8 b	6 bc	4 c
125	55 a	36 b	59 a	27 b
500	177 a	162 a	150 a	56 b
Linear model	**	**	**	**
R ²	0.57	0.68	0.54	0.50

Data are averages of 1987 and 1988 experiments, each with four replications. Means within rows followed by different letters indicate significant difference based on Fisher's (protected) LSD test ($P = 0.05$).

** = significant at $P = 0.01$ based on F -test.

† Pi = initial population density of *M. incognita* eggs/100 cm³ soil.

Nematode reproduction on resistant cultivars often results in a high residual soil population density of eggs and J2 by harvest that could cause excessive damage to root-knot susceptible crops the following year. In contrast, the two highly resistant plant introductions in our studies, PI 417444 and PI 96354, were very effective in suppressing J2 population densities in the soil when compared with a standard resistant cultivar, Forrest. These results establish the effectiveness of *M. incognita* resistance in the plant introductions under field conditions. They had lower gall indices, fewer eggs per root system, and fewer eggs per gram of root than Forrest in greenhouse experiments (10).

Second-stage juveniles of *Meloidogyne* spp. readily penetrate roots of susceptible and resistant soybean cultivars (14). In previous studies (12) there were no differences among resistant and susceptible cultivars in penetration by *M. incognita* 7 days after inoculation; however, there were 27% fewer nematodes in roots of resistant cultivars than in roots of susceptible cultivars 14 days after inoculation, indicating J2 emerged from roots of the resistant soybean. In our studies, roots of PI 96354 contained 65% fewer nematodes than resistant Forrest 14 days after inoculation, a trend which was reflected in soil J2 population densities midway to late in the season.

TABLE 3. Soil population densities of *Meloidogyne incognita* juveniles (J2) on four soybean genotypes at different initial population densities in field microplots 65, 90, and 120 days after planting in 1987.

Pi†	J2/100 cm ³ soil			RF‡
	65	90	120	
Bossier				
31	4	269	897	29.6
125	14	790	2,053	19.6
500	50	2,293	1,832	4.0
Mean	23 a	1,117 a	1,594 a	
Forrest				
31	4	180	410	13.6
125	10	532	1,225	9.9
500	51	1,038	1,400	2.8
Mean	22 a	583 b	1,012 b	
PI 417444				
31	5	153	448	14.7
125	15	531	915	7.3
500	36	916	1,128	2.3
Mean	19 a	533 b	830 c	
PI 96354				
31	4	103	513	17.2
125	12	421	885	7.2
500	43	878	1,019	2.0
Mean	20 a	467 c	839 c	

Data are averages of four replications. Means within columns followed by different letters indicate significant difference based on Fisher's (protected) LSD test ($P = 0.05$) for comparison of genotype means only.

† Pi = initial population density of *M. incognita* eggs/100 cm³ soil.

‡ RF = reproductive factor; i.e., number of J2 at 120 days/number of eggs Pi.

The large differences in seed yield for PI 417444 between 1987 and 1988 might be attributed to the methyl bromide treatment because poor growth of PI 417444 occurred in one block. Although yields of the two plant introductions were comparable to yields of the cultivars in our experiments, the plant introductions exhibited undesirable agronomic traits, such as excessive plant height and seed shattering, which would reduce their yield under normal production conditions.

The extreme aggressiveness of the *M. incognita* population used in our studies was reflected in the high mortality of the susceptible cultivar, Bossier, even at a low Pi. This population is a mixture of collections from three southern states (6,15) selected

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TABLE 4. Soil population densities of *Meloidogyne incognita* juveniles (J2) on four soybean genotypes at different initial population densities in field microplots 75 and 140 days after planting in 1988.

Pi†	J2/100 cm ³ soil		RF‡
	75	140	
Bossier			
31	13	1,899	63.9
125	71	3,663	29.4
500	554	1,000	2.1
Mean	213 a	2,187 a	
Forrest			
31	8	832	27.2
125	37	1,333	10.7
500	170	1,853	2.7
Mean	72 b	1,339 b	
PI 417444			
31	9	263	8.5
125	66	421	3.4
500	200	991	2.0
Mean	92 b	558 c	
PI 96354			
31	6	186	6.0
125	31	363	2.9
500	87	827	1.6
Mean	41 c	459 c	

Data are averages of four replications. Means within columns followed by different letters indicate significant difference based on Fisher's (protected) LSD test ($P = 0.05$) for comparison of genotype means only.

† Pi = initial population density of *M. incognita* eggs/100 cm³ soil.

‡ RF = reproductive factor; i.e., number of J2 at 140 days/number of eggs Pi.

for screening germplasm to detect genotypes possessing high levels of *M. incognita* resistance. Soybean genotypes resistant to this population should be at least as effective in suppressing development and reproduction of less aggressive *M. incognita* populations.

Our results indicate that the high level of resistance present in PI 96354 suppressed reproduction of an aggressive *M. incognita* population under field conditions. The resistance genes in this plant introduction should be useful in breeding programs to improve the level of resistance in soybean cultivars to *M. incognita*.

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