POST-INFECTIONAL DEVELOPMENT OF *MELOIDOGYNE INCOGNITA* ON SUSCEPTIBLE AND RESISTANT SOYBEAN GENOTYPES†

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ABSTRACT

Moura, R. M., E. L. Davis, B. M. Luzzi, H. R. Boerma, and R. S. Hussey. 1993. Post-infectional development of *Meloidogyne incognita* on susceptible and resistant soybean genotypes. Nematropica 23:7–13.

Post-infectional development, reproduction, and fecundity of *Meloidogyne incognita* on three soybean genotypes were examined under greenhouse conditions. The genotypes Bossier, Forrest, and PI 96354 were susceptible, partially resistant, and highly resistant, respectively, to *M. incognita*. Most second-stage juveniles in roots of Forrest and PI 96354 had emigrated from roots of these genotypes by 10 days after inoculation. Nematodes parasitizing PI 96354 had the slowest developmental rate and produced 99% fewer eggs per egg mass than females on the other two genotypes. Total eggs produced on Forrest was intermediate between egg production on Bossier and PI 96354.

Key words: development, fecundity, *Glycine max, Meloidogyne incognita*, penetration, resistance, root-knot, soybean.

RESUMEN

Moura, R. M., E. L. Davis, B. M. Luzzi, H. R. Boerma y R. S. Hussey. 1993. Desarrollo posinfeccional de *Meloidogyne incognita* en genotipos susceptible y resistente de la soja. Nematrópica 23:7–13.

Se examinaron bajo condiciones de invernadero el desarrollo, la reproducción y la fecundidad de *Meloidogyne incognita* en tres genotipos de soja. Los genotipos Bossier, Forrest y PI 96354 fueron susceptible, parcialmente resistente y altamente resistente a *M. incognita*, respectivamente. La mayoría de los segundos estadíos juveniles que penetraron las raíces de Forrest y PI 96354 migraron de las raíces antes de 10 días después de la inoculación. Hembras que se desarrollaron en las raíces de PI 96354 tuvieron la tasa de desarrollo mas lente y produjeron un 99% menos huevos por masa de huevos que hembras de los dos otros genotipos. El número total de huevos producidos en Forrest fue intermedio en relación a los producidos en Bossier y PI 96354.

Palabras clave: desarrollo, fecundidad, *Glycine max, Meloidogyne incognita*, nematodo agallador, penetración, resistencia, soja.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are economically important plant parasites that cause extensive crop losses all over the world (12). Soybean [*Glycine max* (L.) Merr.] is parasitized by *M. incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, and *M. hapla* Chitwood (13). *Meloidogyne incognita*, the species most frequently found in infested soybean fields, can suppress yields of susceptible soybean cultivars by as much as 90%, and yields of resistant cultivars by 40% (8).

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The most effective tactics currently available to limit soybean losses on root-knot nematode-infested land include crop rotation, resistant cultivars, and use of nematicides. Although the use of resistant soybean cultivars is the most cost-effective tactic available to growers, these cultivars are only partially resistant, decreasing their effectiveness (9,10). Therefore, the development of root-knot nematodes on resistant cultivars is in need of further study.

Herman *et al.* (4) investigated penetration and establishment of *M. incognita* in roots of resistant and susceptible soybean genotypes and observed 54–72% emigration rates for second-stage juveniles from resistant genotypes by 5 days after inoculation. The fate of the nematodes, however, that remained in the roots of each genotype was not determined. The research reported here was conducted to assess the development and reproduction of *M. incognita* race 3 in three soybean genotypes used by Herman *et al.* (4).

MATERIALS AND METHODS

An *M. incognita* susceptible soybean genotype, Bossier [Maturity Group (MG) VII], and two *M. incognita*-resistant genotypes, Forrest (MG V) and PI 96354 (MG VI), were used. Forrest is partially resistant and PI 96354 is highly resistant to *M. incognita* (4,11).

Meloidogyne incognita race 3 was propagated on greenhouse-grown tomatoes (*Lycopersicon esculentum* Mill.) cv. Rutgers. Galled roots were harvested 55 days after inoculation and eggs were collected by the technique described by Hussey and Barker (6). Infective second-stage juveniles (J2) were hatched from eggs on sieves and collected every 24 hr. The J2 obtained in the first 24 hr were discarded and the subsequent 48-hr-aged J2 cohort was used as inoculum. Preliminary experiments were conducted to determine the appropriate inoculum levels, soil type, and harvesting intervals. All experiments were conducted under greenhouse conditions (temperature range 21–35°C) with supplemental light from 400-watt multi-vapor phosphor coated lamps to provide a 16-hr photoperiod. Four seeds of each soybean genotype were planted in 474-cm^3 styrofoam cups filled with 400 cm^3 of a methyl bromide-fumigated sandy loam soil amended with sand to a texture of 80% sand, 12% silt, and 8% clay. After 4 days, the seedlings were thinned to one plant per cup and 6 days later were inoculated with an 8-ml suspension of J2. Bossier seedlings were inoculated with 2 000 J2 each, while Forrest and PI 96354 received 8 000 J2 per cup. The lower inoculum used for Bossier was to reduce infection sites with multiple J2 and competition that could affect J2 development. The inoculum was dispensed into 8-cm-deep holes made in the soil around each seedling. Two days after the seedlings were inoculated, they were removed from the cups and their root systems were washed with tap water prior to transplanting into clay pots containing the same type of soil. Washing roots and transplanting seedlings synchronized infection by limiting J2 penetration of roots to a 48-hr period. The experimental design was a randomized complete block with three treatments and five replicates.

Plants of each genotype were harvested at 4, 10, 20, 30, and 45 days after inoculation. At 4, 10, and 20 days four replicates were harvested, but at 30 and 45 days five replicates were harvested. Root systems were carefully removed from the soil, washed, and stained with acid fuchsin (1). Ten nematodes were randomly dissected from root systems of each replicate and the developmental stage of each nematode was noted using the following categories, as described by

Triantaphyllou and Hirschmann (14): vermiform second-stage juvenile; swollen, sexually undifferentiated second-stage juvenile; late second-stage female juvenile; late second-stage male juvenile; third or fourth stage female juvenile; third or fourth stage male juvenile; adult female with no eggs; and adult female with eggs observed inside or outside the body. Adult males were not considered in the enumeration of life stages.

To evaluate reproduction, the total number of eggs per root system and the number of eggs per egg mass were assessed. Before staining root systems from the 30 and 45 day harvest, five egg masses were picked from each of five replicates and eggs remaining on the root systems were collected with 1% NaOCl (6). Eggs per egg mass were quantified by dissolving the gelatinous matrix of individual egg masses in 1% NaOCl contained in a 1.5-ml microcentrifuge tube. When evaluating nematode development within roots and nematode fecundity, infection sites with a single nematode were selected and root tips were not observed after the 4-day harvest. Chi-square analysis was used to compare rates of nematode development; reproduction rates and fecundity were analyzed by analysis of variance.

RESULTS

Post-infectional development: Development of *M. incognita* was affected by soybean genotype, with development being slowest in PI 96354 (Table 1). Four days after inoculation most nematodes in roots of each genotype were vermiform J2 and

Table 1. Post-infectional development of *Meloidogyne incognita* race 3 on susceptible (Bossier), partially resistant (Forrest), and highly resistant (PI 96354) soybean genotypes.

		Second-stage juvenile (J2)				Developed beyond second stage			
			Swollen						
				Sexually differentiated		Third or fourth stage juvenile		Adult female	
Genotype	Days after inoculation	Vermiform	Sexually undifferentiated	Female	Male	Female	Male	Without eggs	With eggs
Bossier	4	40	—	—	—	—	—	—	—
	10	—[z]	7	30	3	—	—	—	—
	20	—	6	—	—	—	11	20	3
	30	—	1	2	1	—	6	2	28
	45	—	—	—	—	—	—	5	35
Forrest	4	40	—	—	—	—	—	—	—
	10	2	23	10	5	—	—	—	—
	20	—	18	9	1	—	4	8	—
	30	—	4	—	1	1	—	15	19
	45	—	5	—	1	—	2	9	23
PI 96354	4	40	—	—	—	—	—	—	—
	10	18	22	—	—	—	—	—	—
	20	2	25	8	—	—	1	4	—
	30	—	9	1	—	—	11	10	9
	45	—	3	5	—	1	6	20	5

Nematode development at 10, 20, 30, and 45 days after inoculation differs on all three genotypes ($P < 0.05$) by chi-square analysis. On each date, 10 nematodes from each of four plants were examined.

[z]Dash indicates that no nematodes of the developmental stage indicated were observed for that genotype on that date.

present in comparable numbers among genotypes. Ten days after inoculation swollen J2 were observed in roots of all genotypes, with sexually differentiated J2 observed only in roots of Bossier and Forrest. Also at this harvest interval, considerably fewer nematodes were present in roots of Forrest and PI 96354 than at 4 days (data not presented).

By 20 days after inoculation nematodes had matured to adult females on all genotypes. Very few nematodes remained in roots of the resistant genotypes, especially PI 96354. A higher percentage of adult females had developed on Bossier (57%) than on Forrest (20%) and PI 96354 (10%). Females produced eggs only on Bossier. Third and fourth stage males were observed in roots of all three genotypes, with the lowest percentage on PI 96354. Adult males were observed in roots of Bossier and Forrest, but were not counted (Fig. 1). Necrotic tissue in infection sites was rare but was observed on PI 96354 and Forrest by day 20.

At the last two harvest dates (30 and 45 days after inoculation), progressively more adult females with eggs were observed in roots of all genotypes with the lowest percentage occurring on PI 96354. At 30 days, more swollen second, third, and fourth stage males were observed on PI 96354 than on the other genotypes. By 45 days, almost all of the nematodes observed in roots of Bossier had matured to fecund adult females. However, for the resistant genotypes, Forrest and PI 96354, respectively, only 57% and 12% of the nematodes in the roots had eggs inside or outside the body.

Nematode reproduction and fecundity: The different rates of nematode development on the three genotypes were reflected in nematode reproduction. Thirty days after inoculation, Forrest had 75% and PI 96354 had 97% fewer eggs per root system than the susceptible Bossier, and at 45 days these values were 56% and 99% lower than that of Bossier (Table 2). Total eggs per root system on Bossier and Forrest at 45 days did not differ significantly even though Forrest had received four times the initial inoculum level applied to Bossier.

Egg production per adult female also was affected by soybean genotype. At 30 days after inoculation, adult females on Forrest had an average of 40 and PI 96354 had an average of 68 fewer eggs per egg mass than Bossier. By 45 days, the adult females examined on Forrest had numerically more eggs per egg mass than the females on Bossier (not statistically significant at $P = 0.05$). Adult females on PI 96354 produced 99% fewer eggs per egg mass than females on Bossier and Forrest at 45 days after inoculation (Table 2).

DISCUSSION

The data presented here confirm the previously reported susceptibility of Bossier, partial resistance of Forrest, and high level of resistance of PI 96354 to *M. incognita* (4,5,11). The equally high numbers of J2 observed in root systems of all three genotypes at 4 days after inoculation also are similar to initial rates of penetration previously observed for these soybean genotypes (4). Nematodes usually enter roots of resistant and susceptible cultivars equally (7).

The differences in root-knot nematode development in the three soybean genotypes indicate that distinct types of interactions occur between this nematode and these genotypes. By 10 days after inoculation, many J2 had egressed the roots of PI 96354. Some of the nematodes remaining inside the roots never matured while others had initiated development and died.

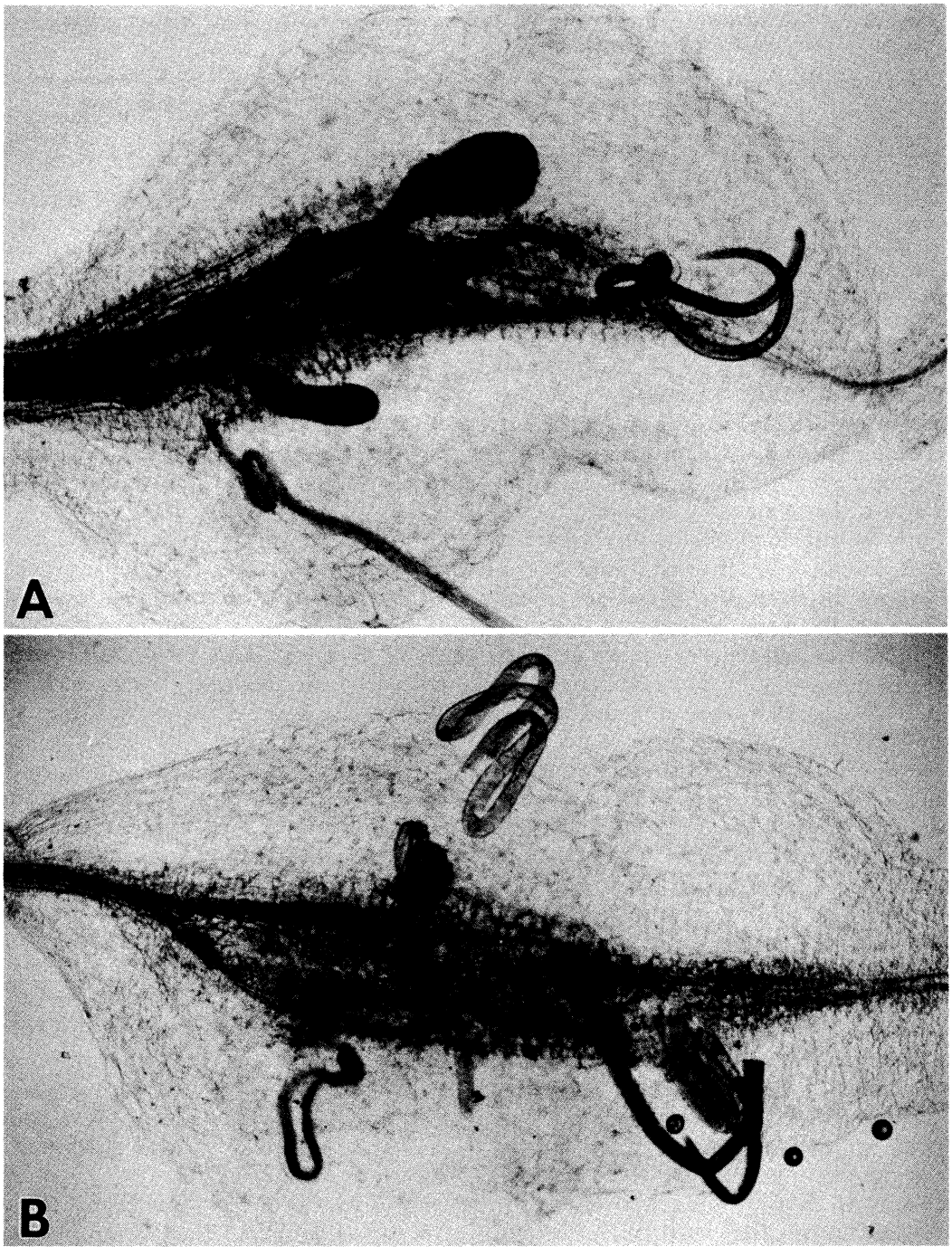

Fig. 1. Galls on soybean 20 days after inoculation with *Meloidogyne incognita* race 3. A) Adult males and females in root gall on root-knot susceptible soybean cultivar Bossier. B) Adult males in root gall on root-knot resistant soybean cultivar Forrest.

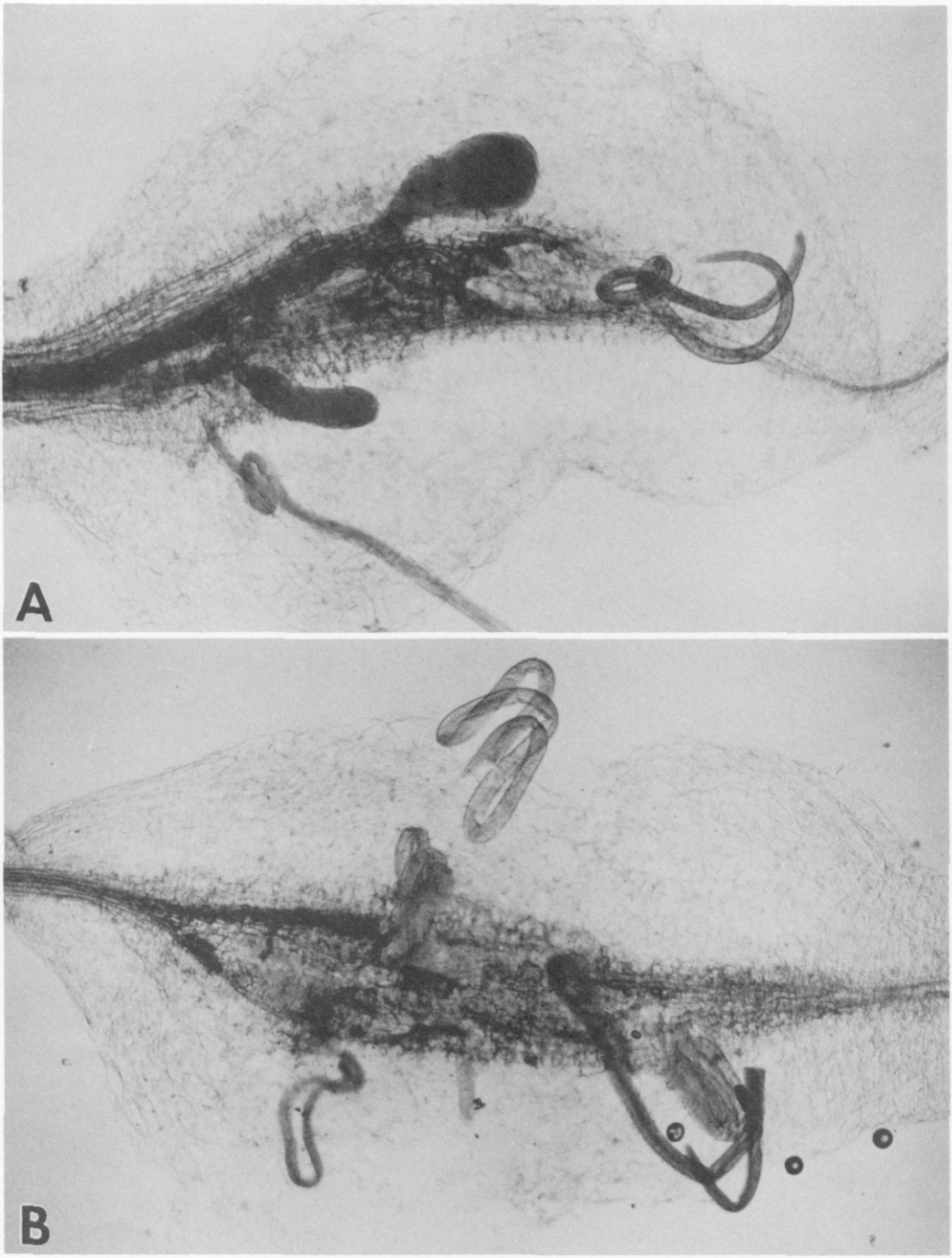

Fig. 1. Galls on soybean 20 days after inoculation with *Meloidogyne incognita* race 3. A) Adult males and females in root gall on root-knot susceptible soybean cultivar Bossier. B) Adult males in root gall on root-knot resistant soybean cultivar Forrest.

Table 2. Reproduction and fecundity of *Meloidogyne incognita* race 3 on three soybean genotypes in greenhouse pots 30 and 45 days after inoculation.[y]

Genotype	Eggs/root system		Eggs/egg mass	
	30 days	45 days	30 days	45 days
Bossier	6 400 a[z]	30 400 a	75 a	99 a
Forrest	1 630 b	13 300 ab	35 b	128 a
PI 96354	176 b	352 b	7 c	1 b

The data are means of five replicates; for eggs/egg mass each replicate is a mean of five egg masses.
[y]Bossier was inoculated with 2 000 eggs/plant; Forrest and PI 96354 were inoculated with 8 000 eggs/plant.
[z]In each column means followed by the same letter are not significantly different ($P < 0.05$) according to Fisher's least significant difference test.

The egression of *M. incognita* juveniles that is so striking on soybean is not uncommon for nematode resistant plants (4). Although many J2 also emigrated from Forrest, the nematodes remaining in the roots developed normally, very similar to that observed for Bossier. Resistance in soybean to *Heterodera glycines* has been shown to be stage related and does not affect males and females equally in all resistant genotypes (3). The number of eggs produced by *M. incognita* females in roots of Forrest and Bossier were comparable but significantly fewer females developed in roots of Forrest. High levels of juvenile emigration and poor nematode development confer to PI 96354 a high level of resistance to *M. incognita*.

Soybean genotype had a striking effect on root-knot nematode adult female fecundity. Although a few adult females developed on the highly resistant PI 96354, eggs were rarely found in egg masses produced by these nematodes. Hatching of eggs on Bossier probably contributed to the greater number of eggs per egg mass observed on Forrest compared to the susceptible Bossier cultivar at 45 days. This is supported by the data at 30 days after inoculation showing egg production by females on Forrest is delayed compared to that by females that developed on Bossier.

Males developed on all genotypes. Environmental and nutritional stresses unfavorable for the development of female root-knot nematodes induce male development (15). Development of a large number of males on Bossier, as well as a relatively low number of eggs per female on this genotype, indicates that soybean, in general, is not a highly suitable host for *M. incognita*. This conclusion is supported by the fact that development of *M. incognita* is slower on soybean than on good hosts (13).

Frequently in this investigation, only males were observed inside galls on resistant genotypes. This observation supports the view that the presence of galls may not be a completely reliable parameter to screen plant species for resistance to root-knot disease, as previously pointed out by Fassuliotis (2).

Since some development and reproduction of *M. incognita* does occur on Forrest, continuous cropping of this resistant cultivar could select for *M. incognita* biotypes that would adversely affect crop performance. The resistance genes in PI 96354, by comparison, confer a superior level of root-knot resistance that is effective throughout the nematode's life cycle, and the few females that do develop reproduce only at very low rates. These factors should be taken into consideration,

when using resistant cultivars for the management of this pathogen and when breeding soybean for *M. incognita* resistance.

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