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Pathogenicity of *Pratylenchus penetrans, Heterodera glycines,* and *Meloidogyne incognita* on Soybean Genotypes[1]

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Abstract: The pathogenicity of *Heterodera glycines, Meloidogyne incognita,* and *Pratylenchus penetrans* on *H. glycines*-resistant 'Bryan,' tolerant-susceptible 'G88-20092,' and intolerant-susceptible 'Tracy M' soybean cultivars was tested using plants grown in 800 cm^3 of soil in 15-cm-diam. clay pots in three greenhouse experiments. Plants were inoculated with 0, 1,000, 3,000, or 9,000 *H. glycines* race 3 or *M. incognita* eggs, or vermiform stages of *P. penetrans*/pot. Forty days after inoculation, numbers of all three nematodes, except *H. glycines* on Bryan, generally increased with increasing inoculum levels in Experiment I. *Heterodera glycines* and *M. incognita* significantly decreased growth only of Tracy M. At 45 and 57 days after inoculation with 6,000 individuals/pot in experiments II and III, respectively, significantly more *P. penetrans* and *M. incognita* than *H. glycines* were found on Bryan. However, *H. glycines* and *M. incognita* population densities were greater than *P. penetrans* on G88-20092 and Tracy M. Growth of Tracy M infected by *H. glycines* and *M. incognita* and growth of G88-20092 infected by *M. incognita* decreased in Experiment III. *Pratylenchus penetrans* did not affect plant growth. Reduction in plant growth differed according to the particular nematode species and cultivar, indicating that nematodes other than the species for which resistance is targeted can have different effects on cultivars of the same crop species.

Key words: Glycine max, Heterodera glycines, lesion nematode, *Meloidogyne incognita,* nematode, *Pratylenchus penetrans,* resistance, root-knot nematode, soybean cyst nematode, susceptibility, tolerance.

Use of host-plant resistance is a desirable and environmentally sound nematode management tactic. However, there are few crops where nematode resistance has been identified or developed (Roberts, 1992). Where resistance has been developed, the presence of biological races (Sipes et al., 1992) or selection pressure as a result of continual use of resistant cultivars (Young, 1992, 1994) presents challenges to nematode management. Biological races may be managed through agronomically sound use of nematode-resistant, tolerant-susceptible, and intolerant-susceptible cultivars (Boerma and Hussey, 1989). Tolerant cultivars do not suffer yield loss, whereas intolerant cultivars do (Hussey and Boerma, 1992).

In addition to biological races, the presence of polyphagous communities of plant-parasitic nematodes in agricultural ecosystems may impede the effective use of resistant cultivars. For example, the cost of developing the soybean cyst nematode (*Heterodera glycines* Ichinohe) resistant cultivar, 'Forrest,' was calculated at $1.0 million, with a 403% return in 6 years (Bradley and Duffey, 1982). However, the susceptibility of Forrest to other nematodes reduced potential profits when this cultivar was planted (Bradley and Duffey, 1982). Where possible, management decisions need to account for the diversity of plant-parasitic nematodes within agroecosystems (Hargrove, 1988) and should integrate understanding of pathogenicity of various nematode species to different cultivars in specific environments.

Despite their diverse habitats and host-parasite relationships, plant-parasitic nematodes can be classified into three types of feeding behaviors: destructive (host cells killed), adaptive (host cells modified to fit nematode's sedentary nature), and neoplastic (host cells modified and undergoing new growth) (Dropkin, 1980). *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven, *H. glycines,* and *Meloidogyne incognita* (Kofoid & White) Chitwood, respectively, are examples of the three feeding behaviors. The objective of this study was to compare pathogenicity of *H. glycines, M. incognita,* and

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P. penetrans on *H. glycines*-resistant, tolerant-susceptible, and intolerant-susceptible soybean cultivars.

Materials and Methods

Soybean hosts and growth conditions: Three greenhouse experiments were conducted to determine the effect of *H. glycines* race 3, *M. incognita,* and *P. penetrans* on growth of three maturity group VI soybean cultivars. Each experiment included, in relation to *H. glycines,* resistant Bryan (Boerma et al., 1991), tolerant-susceptible G88-20092 (Boerma et al., 1993), and intolerant-susceptible Tracy M soybean cultivars (Boerma and Hussey, 1989). Bryan soybean also has a moderate level of resistance to *M. incognita* (Boerma et al., 1991). Seeds were germinated on plastic trays, and single seedlings were transplanted into 15-cm-diam. clay pots containing 800 cm^3 of steam-sterilized sandy loam soil (87% sand, 8% silt, 5% clay, pH 7.0). Plants were placed in a completely randomized design on a greenhouse bench at 25 ± 2 °C and with a 16-hour photoperiod at 400 to 450 $\mu E \cdot s^{-1} \cdot m^{-2}$. All treatments were watered to saturation daily with tap water and twice weekly with full-strength Hoagland solution (Hoagland and Arnon, 1939).

Nematode inoculum and experiments: Heterodera glycines and *M. incognita* were obtained from soybean and tomato greenhouse cultures, respectively. Eggs were collected by crushing cysts or egg masses extracted from soil and roots (Jenkins, 1964; Niblack and Hussey, 1985). Excised root cultures of *P. penetrans* were obtained from the Ohio State University Tissue Culture Laboratory, Columbus, Ohio. Roots and agar were macerated with a blender, and the number of nematodes was determined. Nematode inocula were suspended in tap water, and aliquants containing the required number of nematodes were pipetted into three or four 1-cm-diam. × 2- to 3-cm-deep holes around each plant at 1 week after germination. Controls received the same volume of tap water but without nematodes.

Experiment I dealt with establishing the relationships among the inoculum levels of 0 (control), 1,000, 3,000, and 9,000 individuals/species, population development, and effects on shoot dry weight 40 days after inoculation. Each treatment was replicated three times. *Heterodera glycines* and *M. incognita* were inoculated as eggs; *P. penetrans* inoculum consisted of all life stages. At the end of the experiment, shoots were cut and dried at 80 °C for 48 hours. Nematode population densities were estimated from 1.0 g of fresh roots collected at random (Hussey, 1985) and 100 cm^3 soil (Jenkins, 1964), and developmental stages were determined microscopically. The relationships between the level of nematode infection and nematode inoculum level and between nematode infection levels and shoot dry weight were analyzed with regression analysis, and slopes were compared (Steele and Torrie, 1980) with general linear models procedures (SAS Institute, Cary, NC).

In Experiments II and III, the effect of 6,000 individuals/species on shoot dry weight was tested at 45 and 57 days after inoculation, respectively. Each treatment was replicated four times. Nematode inoculum and determinations of shoot dry weight and nematode developmental stage were as in Experiment I. Nematode numbers were determined after staining whole root systems (Hussey, 1985). Relationships between nematode infection levels and shoot dry weights within a cultivar and infection levels among cultivars were analyzed with analysis of variance (SAS Institute, Cary, NC), and means were separated with Tukey's multiple-range test (Steele and Torrie, 1980).

Results

In Experiment I, only adult stages ranging from pre-egg-laying to fully developed females of *H. glycines* or *M. incognita* were found in root systems of all three cultivars. Few eggs or vermiform stages of either nematode were extracted from soil. All vermiform stages of *P. penetrans* were found.

The numbers of all three nematodes per gram fresh root weight, except *H. glycines* on Bryan, increased with increasing inoculum level in all three cultivars (Fig. 1). Higher

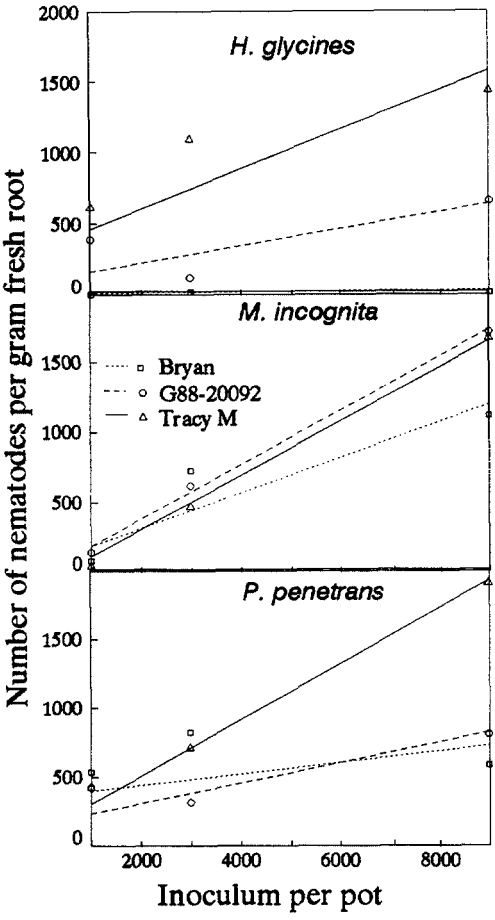

FIG. 1. Relationships between inoculum levels of 0, 1,000, 3,000, and 9,000 individuals of each of *Heterodera glycines, Meloidogyne incognita,* and *Pratylenchus penetrans,* and the mean numbers of nematodes found per gram of fresh root weight of Bryan, G88-20092, and Tracy M soybean cultivars at 40 days after inoculation (Experiment I). Regression equations are as follows: i) For *H. glycines, Y*(Bryan) = 7.61 + (0.0203*X*), r^2 = 0.28; G88-20092 = 103.5 + (0.0596*X*), r^2 = 0.43*; Tracy M = 349.1 + (0.1358*X*), r^2 = 0.59*; ii) For *M. incognita, Y*(Bryan) = 79.2 + (0.1241*X*), r^2 = 0.78*; G88-20092 = −3.9 + (0.1922*X*), r^2 = 0.86*; Tracy M = −78.6 + (0.1914*X*), r^2 = 0.88*; iii) For *P. penetrans, Y*(Bryan) = 356.7 + (0.0404*X*), r^2 = 0.12; G88-20092 = 148 + (0.0741*X*), r^2 = 0.63*; Tracy M = 106.5 + (0.2015*X*), r^2 = 0.91*. *Meloidogyne incognita* and *H. glycines* inocula consisted of eggs, and *P. penetrans* inoculum consisted of eggs and vermiform stages. Regression equations with starred r^2 values are significant according to Tukey's multiple-range test ($P \leq 0.05$).

numbers of *H. glycines* were found on Tracy M than on G88-20092 ($P \leq 0.05$). Similar numbers of *M. incognita* were found on G88-20092 and Tracy M but more than from Bryan ($P \leq 0.05$). Similar numbers of *P. penetrans* infected Bryan and G88-20092 but were lower than the numbers that infected Tracy M (Fig. 1).

Heterodera glycines had no effect on growth of either Bryan or G88-20092, but shoot dry weight of Tracy M decreased with increasing levels of inoculum ($P \leq 0.05$) (Fig. 2). Although shoot weight tended to decrease with increasing inoculum levels of *M. incognita* in all three cultivars, reduction in shoot weight was significant only for Tracy M ($P \leq 0.05$). *Pratylenchus penetrans* had no effect on shoot weight on any cultivar (Fig. 2).

In Experiments II and III, all developmental stages of *H. glycines* and *M. incognita* were present (Fig. 3). However, the proportion of adults generally was greater in Experiment II than in Experimental III.

In Experiment II, more *M. incognita* and *P. penetrans* than *H. glycines* were found in roots of Bryan ($P \leq 0.05$); however, more *H. glycines* and *M. incognita* than *P. penetrans* were generally found in G88-20092 and Tracy M (Table 1). In Experiment III, all nematodes infected Tracy M similarly, but more *H. glycines* and *M. incognita* than *P. penetrans* infected G88-20092, and more *M. incognita* and *P. penetrans* were found in Bryan (Table 1). However, more nematodes infected all three cultivars in Experiment III than in Experiment II (Table 1).

Nematode treatments caused no significant effect on plant growth in Experiment II. In Experiment III, shoot weights of Tracy M infected by *H. glycines* and *M. incognita* were lower compared to the control and *P. penetrans* ($P \leq 0.05$). Shoot weight of G88-20092 infected by *M. incognita,* compared to other treatments, was less ($P \leq 0.05$) (Table 1).

DISCUSSION

The lower levels of *H. glycines* and *M. incognita* infection on Bryan compared with G88-20092 and Tracy M confirm the reported resistance of the cultivar (Boerma et al., 1991, 1993). The numbers of *M. incognita* found on Bryan, however, were higher than expected. It is likely some sampling er-

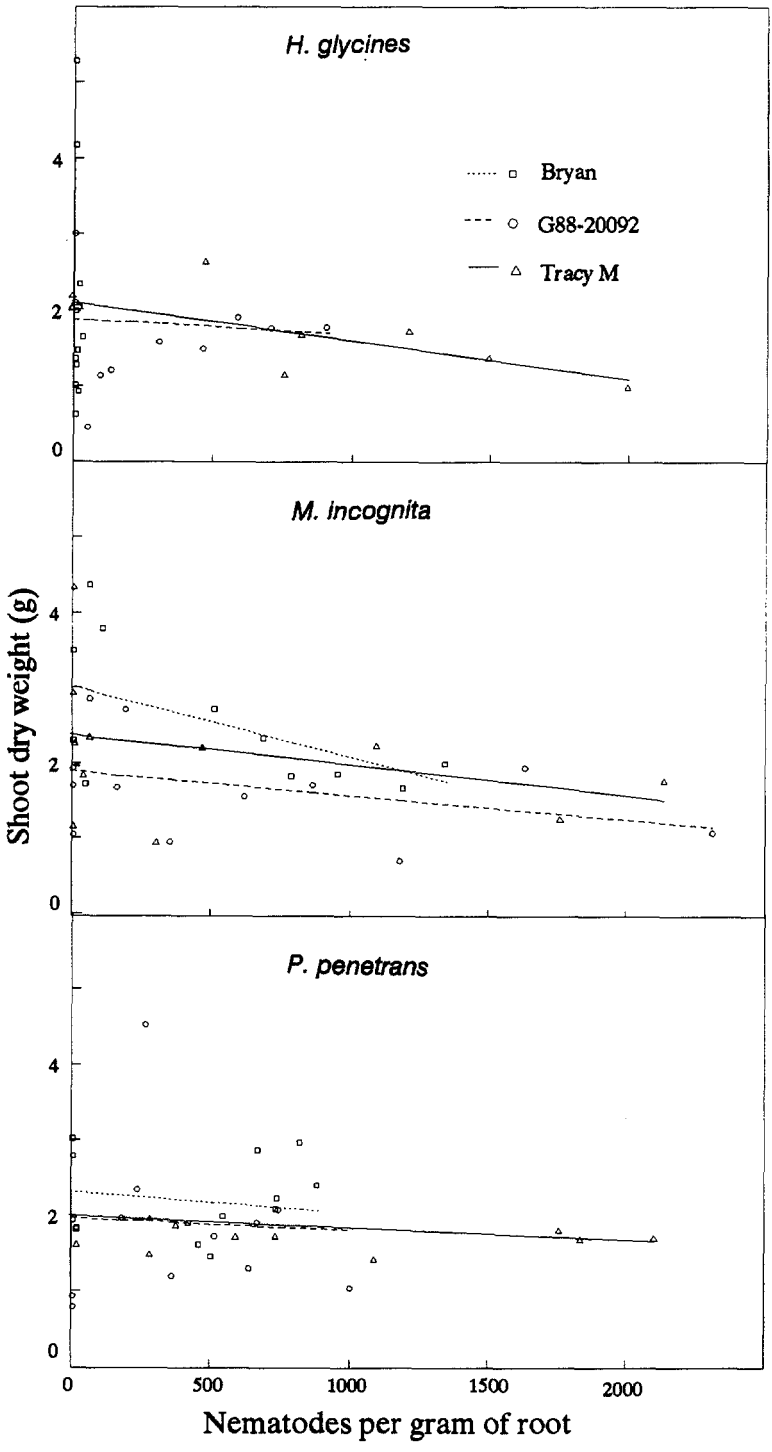

FIG. 2. Relationships between the numbers of each of *Heterodera glycines, Meloidogyne incognita,* and *Pratylenchus penetrans* found per gram of fresh root weight of Bryan, G88-20092, and Tracy M soybean cultivars and changes in shoot dry weight at 40 days after inoculation (Experiment I). Regression equations are as follows: i) For *H. glycines,* Y(Bryan) = 2.093 + (0.0044X), r^2 = 0.004; G88-20092 = 1.84 – (0.0002X), r^2 = 0.01; Tracy M = 2.04 – (0.001X), r^2 = 0.46*; ii) For *M. incognita,* Y(Bryan) = 2.98 – (0.0.0009X), r^2 = 0.27; G88-20092 = 1.97 – (0.0003X), r^2 = 0.13; Tracy M = 2.35 – (0.0.004X), r^2 = 0.38*; iii) For *P. penetrans,* Y(Bryan) = 2.29 – (0.00001), r^2 = 0.001; G88-20092 = 1.91 – (0.0001X), r^2 = 0.003; Tracy M = 1.95 – (0.0002X), r^2 = 0.10. *Meloidogyne incognita* and *H. glycines* inocula consisted of eggs, and *P. penetrans* inoculum consisted of eggs and vermiform stages. Regression equations with starred r^2 values are significant according to Tukey's multiple-range test ($P \leq 0.05$).

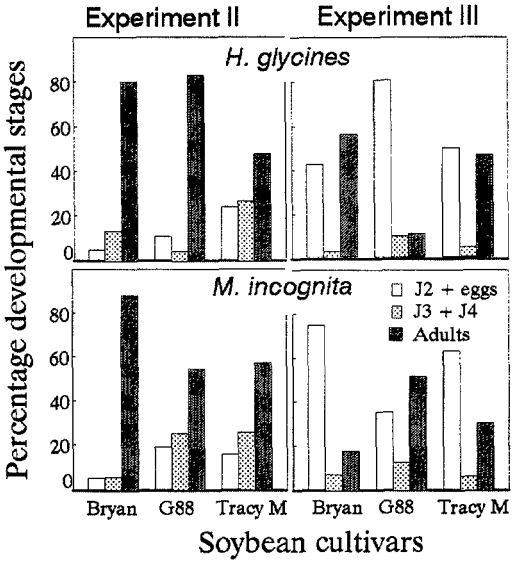

FIG. 3. Percentage of second-stage juveniles and eggs (J2 + eggs), third- and fourth-stage juveniles (J3 + J4), and all adult stages of *Heterodera glycines* and *Meloidogyne incognita* on Bryan, G88-20092 (G88); Tracy M per gram fresh root weight 45 and 57 days after inoculation in Experiments II and III. *Meloidogyne incognita* and *H. glycines* inocula consisted of eggs.

ror may be involved because subsequent studies have shown fewer *M. incognita*-infected Bryan than in this study. Nonetheless, the development of *H. glycines* and *M. incognita* generally was slower on Bryan than on either G88-20092 or Tracy M (Fig. 3). This is in agreement with the mechanism of resistance, which allows infection but limits development of females in resistant soybeans (Melton et al., 1986).

The general increase in the numbers of nematodes with increasing inoculum level shows that the inoculum range used here may have been below the maximum carrying capacity. The relationship between the numbers of nematodes in roots and plant growth, however, differed by cultivar and nematode. The data confirm the tolerance of G88-20092 to *H. glycines* (Boerma et al., 1993). *Heterodera glycines* caused no significant weight loss on G88-20092 despite high levels of infection, whereas Tracy M suffered loss in shoot weight with fewer nematodes than did G88-20092. Similar numbers of *H. glycines* and *M. incognita* caused similar weight loss on Tracy M, suggesting that the cultivar may be equally susceptible to both nematodes. More *M. incognita* than *H. glycines* were required to affect growth of G88-20092, suggesting that G88-20092 may be less susceptible than Tracy M to *M. incognita.* By definition, however, G88-20092 may not be considered tolerant to *M. incognita.*

Although the cultivars performed as expected with respect to *H. glycines,* their suitability to other nematodes shows that these nematodes could be a problem where these cultivars are grown. Despite similar numbers of *P. penetrans* in all three cultivars to those of *M. incognita* and *H. glycines* that decreased growth in G88-20092 and Tracy M, *P. penetrans* did not affect plant growth. This difference suggests that either damage thresholds may not have been reached or that the cultivars may have some level of tolerance to *P. penetrans.* It is not known how the observed levels of infection may affect plant growth over a longer duration than that used in this study.

Comparison of nematode damage thresholds is difficult because results vary by nematode species, host cultivar, soil texture, and other edaphic factors (Hussey and Boerma, 1992). The overall differences between nematode numbers and effect on plant growth probably reflect the differences in food requirements and pathogenicity of the nematode species in the root systems (Melakeberhan and Ferris, 1988). Differences in the carrying capacity of the different cultivars is probably one reason why the large numbers of nematodes, especially *P. penetrans,* did not result in more severe reduction of plant growth than observed in this study. Another reason could be a combination of initial level of infection and the short duration of the study. In this study, *M. incognita* and *H. glycines* infections decreased shoot weight at 57 days at the highest initial infestation level compared with 45 days at lower infestation levels. Nonetheless, the general increase in infection with increasing inoculum level suggests that the inoculum levels were within the pathogenicity threshold ranges for *M. incognita* and *H. glycines* in one or more cultivars.

The correlations in this study between

TABLE 1. Relationships between numbers of *Heterodera glycines, Meloidogyne incognita,* and *Pratylenchus penetrans* and shoot dry weight of *H. glycines*-resistant (Bryan), tolerant-susceptible (G88-20092), and intolerant-susceptible (Tracy M) soybean cultivars at 45 days (Experiment II) and 57 days (Experiment III) after inoculation.

	Experiment II[a]		Experiment III[a]	
Nematode treatment	Nematodes per gram fresh root	Shoot dry weight (g)	Nematodes per gram fresh root	Shoot dry weight (g)
		Bryan[b]		
Check	0[c]	1.47 a	0	1.63 a
H. glycines	31 b	1.03 a	62 b	1.90 a
M. incognita	138 a	1.60 a	287 ab	1.92 a
P. penetrans	131 a	1.11 a	438 a	1.13 a
		G88-20092[b]		
Check	0	1.18 a	0	1.41 a
H. glycines	254 b	1.27 a	1,394 b	1.20 a
M. incognita	549 a	0.69 a	2,416 a	0.58 b
P. penetrans	80 c	1.02 a	170 c	1.47 a
		Tracy M[b]		
Check	0	1.24 a	0	1.60 a
H. glycines	210 ab	0.88 a	758 a	0.48 b
M. incognita	446 a	1.87 a	501 a	0.61 b
P. penetrans	128 b	1.49 a	1,250 a	1.29 a

Data are means of four replications from Experiments II and III. Means within each column and cultivar followed by the same letters are not different according to Tukey's multiple-range test ($P \leq 0.05$).

[a] Inoculum consisted of 6,000 *M. incognita* and *H. glycines* eggs and mixed stages of *P. penetrans*.

[b] Plants were 1 week old at the time of inoculation.

[c] Zero values not included in data analyses.

growth reduction and yield (growth) loss do not necessarily apply to field conditions. However, it is clear that the presence of polyphagous communities of plant-parasitic nematodes under field conditions may be a problem (Lawn and Noel, 1986) and that infections by *M. incognita* and *P. penetrans* (nontarget nematodes) can affect use of cultivar resistance to manage *H. glycines*. One way to maximize use of nematode-resistant cultivars in polyphagous communities may be to suppress nontarget nematodes, which requires an understanding of the agroecosystem complex, host-parasite interactions, and the environment (Lawn and Noel, 1986; Melakeberhan and Webster, 1993; Wallace, 1987).

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