Journal of Nematology 16(4):368–371. 1984.

Variability in Reproduction of Four Races of *Meloidogyne incognita* on Two Cultivars of Soybean[1]

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Abstract: Variability in the reproduction of the four races of *Meloidogyne incognita* on the soybean cultivars Pickett 71 and Centennial was studied in growth chamber experiments. Analysis of variance in the number of eggs produced by the races 6 weeks after the plants had been inoculated with 5,000 eggs of each race revealed that the nematode race by soybean cultivar interaction was highly significant ($P = 0.001$). Races 1, 3, and 4 produced from about 5,000 to 15,000 eggs per root system on Pickett 71 and only from about 300 to 600 eggs per root system on Centennial. In contrast, race 2 produced about 8,000 eggs per root system on Centennial and about 1,200 eggs per root system on Pickett 71. In a second experiment, in which the plants were inoculated with 2,000 second-stage juveniles, race 1 and race 2 produced about 13,000 and 3,000 eggs per root system, respectively, on Pickett 71 and about 600 and 10,000 eggs per root system, respectively, on Centennial. The results suggest that *M. incognita* resistance in soybean is race-specific.

Key words: Meloidogyne incognita, root-knot nematodes, resistance, *Glycine max.*

Variability in pathogenicity of nematodes has historically been a problem in studies of plant resistance to root-knot nematodes. Some of this variability in the genus *Meloidogyne* was explained in 1949 when Chitwood separated root-knot nematodes into four species (1). Now, more than 50 species of *Meloidogyne* are recognized, but only four are common in cultivated soils. Taylor and Sasser (14) reported in 1978 that of 250 populations of root-knot nematodes collected worldwide, 60% were *M. incognita,* 24% were *M. javanica,* 9% were *M. hapla,* and 6% were *M. arenaria.* When the *M. incognita* populations were subjected to the host differential test (14), 60% failed to reproduce on tobacco (*Nicotiana tabacum* L. cv. NC95) and on cotton (*Gossypium hirsutum* L. cv. Deltapine 16). These populations were designated race 1. Of the remaining populations, 22% reproduced on tobacco but not cotton, 9% reproduced on cotton but not tobacco, and 5% reproduced on both cotton and tobacco. These populations were designated races 2, 3, and 4, respectively.

Variability in the pathogenicity of root-knot nematodes to soybean (*Glycine max* [L.] Merr.) was first reported in 1959 (2). In 1968, a population that was similar to *M. incognita* in morphology, but which could reproduce on the moderately root-knot resistant soybean cultivar Bragg, was found in Louisiana. This population was subsequently named a subspecies of *M. incognita* (4).

The soybean cultivar Centennial has high resistance to *M. incognita* (9,10). This resistance is associated with a hypersensitive reaction and the phytoalexin glyceollin (10). Since resistance of such a high degree sometimes defines pathogen races (15), we measured the reproduction of the four races of *M. incognita* on Centennial and the related soybean cultivar Pickett 71, and subjected the data to ANOVA to elucidate any race by cultivar interaction (15).

Materials and Methods

One population of each of the four races of *M. incognita* was supplied by the International Meloidogyne Project at N.C. State University. The nematodes were maintained on tomato (*Lycopersicon esculentum* L. cv. Tropic) or pepper (*Capsicum frutescens* L. cvs. Yolo Wonder or California Wonder) plants in the greenhouse.

In the first experiment, 2-week-old plants of Pickett 71 and Centennial soybeans were inoculated with suspensions of eggs of each race. The egg suspensions were prepared by blending the roots of culture plants in a 0.5% NaOCl solution for 60 seconds (7). The macerate was quickly poured through nested 500-μm-pore and 20-μm-pore sieves and rinsed with tap water. The residue on the 20-μm-pore sieve was diluted to make

Received for publication 4 November 1983.

[1] The authors thank Kelly Moore and Marc Monson for technical assistance and Carol Adams for advice on the statistical analyses.

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a suspension containing 1,000 eggs/ml. Each soybean plant was inoculated with about 5,000 eggs. The plants were grown in sand in 7.5-cm lengths of 3.5-cm-interior-diameter polyvinylchloride tubing standing upright on clay bricks (11). The experiment was a split-plot design with one level of subsampling. The races of *M. incognita* were assigned to the main plots, and the two cultivars were assigned to the subplots. Each cultivar appeared three times in a subplot in a completely random arrangement. The main plots were replicated three times in a randomized complete block design. The first experiment was conducted in a growth chamber at 27 C with a 15-hour photoperiod and a light intensity of 25,000 lux. Plant nutrients were supplied weekly in Hoagland's solution (6). The plants were harvested 6 weeks after inoculation, and the roots were carefully washed of sand, blotted dry, weighed, and stored in vials in 4% formaldehyde solution. Eggs were later separated from roots by maceration in NaOCl solution as described earlier, except that the 20-μm-pore sieve residue was suspended in 100 ml of tap water and stained with one drop of acid fuchsin–lactophenol. Eggs from each root system were counted in duplicate samples. The numbers of eggs per root system were transformed to $\log_{10}$ before statistical analysis.

In experiment two, juveniles of *M. incognita* race 1 and race 2 were collected from continuously aerated tap water in which roots of culture plants had been incubated in 1-liter Erlenmeyer flasks in a water bath at 28 C. After the first 24 hours, the flasks were drained to remove free-living nematodes and any previously hatched root-knot juveniles and refilled with fresh tap water. Every 24 hours thereafter, the incubation water was passed through a 20-μm-pore sieve and the residue was placed in Baermann funnels to remove root debris and inactive juveniles. Juveniles collected from the funnels were stored at 12 C until a sufficient number for the experiment had been collected. Pickett 71 or Centennial plants growing in 160 ml of sand in styrofoam cups on a bench in the growth chamber were inoculated with about 2,000 juveniles of either race 1 or 2. Six weeks later the eggs were separated from the roots and counted as described earlier. The egg numbers per root system were transformed to $\log_{10}$ and analyzed as a 2 × 2 factorial with 11 replications arranged in a completely randomized design.

Results and Discussion

Host–pathogen compatibility is often described qualitatively. The host is classified as either resistant or susceptible, and the pathogen is described as either virulent or avirulent (13). Compatibility of a host and pathogen can also be determined quantitatively by measuring infection frequency, length of latent period, or pathogen reproduction (15). Quantitative data are desirable in studies of the differences in compatibility due to host cultivar and pathogen isolate, because the data can be subjected to a two-factor ANOVA and the cultivar-by-isolate interaction term tested for significance (15). A significant interaction term is evidence of physiological specialization. If the interaction is nonsignificant, the changes in compatibility due to cultivar and to isolate vary independently. Significant main effects of either cultivar or of isolate reveal differences in horizontal resistance among the cultivars and in aggression among the isolates. Usually, one or both of the main effects is significant when the interaction is significant (15).

The race-by-cultivar interaction was highly significant ($P = 0.001$) in our experiments, indicating that there is physiological specialization in the soybean–*M. incognita* host–pathogen relationship. The interaction was significant in our experiments because there was a reversal in the rankings of the compatibility of the races with the two soybean cultivars. Race 2 produced more eggs on Centennial than did races 1, 3, or 4. The order was reversed on Pickett 71 (Table 1). A reversal in the order of ranking due to pathogen isolate and host cultivar is considered to be evidence of a gene-for-gene relationship (13). According to the gene-for-gene model, a single resistance gene in the host defines two pathogen races, and conversely, a single virulence gene in the pathogen defines two host cultivars. To obtain reversed ranking, at least two genes for resistance, each matched by a different gene for virulence, is required (13). If this model explains our results, then the genotypes at

TABLE 1. Numbers of eggs produced by *Meloidogyne incognita* races 1–4 on the roots of two soybean cultivars 6 weeks after the plants were inoculated with 5,000 eggs or 2,000 second-stage juveniles.

		Eggs per root system		Eggs per gram roots	
	Race	Pickett 71	Centennial	Pickett 71	Centennial
Eggs	1	5,128 ab*	302 b	8,650†	842
	2	1,230 b	8,318 a	942	3,824
	3	14,125 a	631 b	7,499	361
	4	10,965 ab	442 b	7,357	395
Juveniles	1	13,183 a	575	3,337	179
	2	3,162	10,233	1,081	2,477

* Means followed by the same number are not significantly different ($P = 0.05$) according to Duncan's new multiple-range test performed on data transformed to $\log_{10}$.
† These means were calculated before the egg counts were transformed to $\log_{10}$.

the resistance gene loci in the two soybean cultivars and at the virulence gene loci in the four nematode races are as shown in Table 2. Study of the segregation for resistance to the races in a cross of Pickett 71 and Centennial is needed to confirm this interpretation. Unfortunately, a corresponding study of the segregation for virulence in the nematode is not possible, as it reproduces parthenogenetically (14).

The numbers of eggs per root system that we recovered are lower than the numbers Hussey and Boerma reported (8); however, they grew the plants in larger containers and for a longer time after inoculation than we did in our study. After adjustment for root fresh weight, the number of eggs the nematodes produced in our experiments was similar to the numbers reported by Hussey and Boerma. It was evident that Centennial was considerably more resistant to *M. incognita* than any of the 18 cultivars they tested.

Hussey and Boerma (8) found no evidence of differential compatibility between six soybean cultivars and three populations of *M. incognita* collected from the southern United States. Dropkin (2) obtained results similar to ours, but with the cultivars Harosoy and Roanoke and populations of *M. incognita acrita* from Maryland and California.

We used one population of each of the four races of *M. incognita* in our experiments. Other populations might not give the same reactions that we have found, however, because the host differential test defines races with tobacco and cotton differentials, not soybean. Unless compatibility of *M. incognita* with cultivars of other host species, such as soybean, and with the tobacco and cotton differentials, involves the same virulence genes in the nematode, more races of the nematode could exist than can be defined by the host differential test. This may explain why the cowpea cultivar Colosus was resistant to one population of *M. incognita* (3) but susceptible to another (5), even though both populations were race 3 by the host differential test.

The host differential test reaction of the subspecies of *M. incognita* from Louisiana that was able to overcome the resistance of the soybean cultivar Bragg has not been

TABLE 2. Genotypes of two soybean cultivars and the races of *Meloidogyne incognita* predicted from host suitabilities.

Cultivar	Cultivar genotype	*M. incognita** race	Race genotype	Compatibility†
Pickett 71	V1r1R2R2	1, 3, 4	V1V1v2v2	+
		2	v1v1V2V2	−
Centennial	R1R1r2r2	1, 3, 4	V1V1v2v2	−
		2	v1v1V2V2	+

* *M. incognita* race as determined from the host differential series test.
† + = compatible; − = incompatible.

reported. It will be interesting to see if it also types as race 2, and to evaluate its compatibility with Pickett 71 and Centennial soybeans.

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