Relative Tolerance of Selected Soybean Cultivars to *Hoplolaimus columbus* and Possible Effects of Soil Temperature[1]

A. P. NYCZEPIR and S. A. LEWIS[2]

Abstract: Eleven soybean [*Glycine max* (L.) Merr.] cultivars resistant to one or more plant-parasitic nematodes, and one resistant to the Mexican bean beetle (*Epilachna varivestis* Muls.), were tested for susceptibility to *Hoplolaimus columbus*. All cultivars were parasitized, but nematode reproduction varied. 'Pickett-71' was the most susceptible host among the cultivars tested. 'Dyer' and three 'P.I. cultivars' were most tolerant when yield/plant and total yield were compared for fumigated and unfumigated plots, even though their yield potential was low. 'Hardee,' 'Coker 4504,' 'W-4,' 'D71-9257,' and 'ED-371' appeared tolerant throughout the growing season and yielded well in unfumigated soil. Infection and reproduction of *H. columbus* in 'Forrest' soybean roots were greater at 30 ± 1 C than at 20 or 25 ± 1 C. Plant height and root weight varied with the soil treatments. *Key Words:* tolerant, reproduction, infectivity, ecology.

The lance nematode, *Hoplolaimus columbus* Sher, is widespread in the middle and upper coastal plain of South Carolina, where about 31,000 ha of agricultural land are infested (6). Soybean [*Glycine max* (L.) Merr.], an important cash crop in South Carolina, is a primary agronomic host for *H. columbus*. The nematode damages this host, thus contributing to low yields (2, 10). On the basis of a 10% loss in soybean yield over the lance-nematode-infested area, the average dollar loss during 1972–77 was about $834,000 annually. Early-season control has been with nematicides applied with subsoiling. Use of resistant varieties would minimize the costs and hazards associated with chemical application, but no genes for resistance have been discovered.

Temperature, which affects host response and the rate at which nematodes infect and reproduce within roots, must be considered in pathogenicity tests in the greenhouse and growth chambers. Although *H. columbus* can survive high soil temperatures (4), its infectivity and reproduction have not been studied at field temperatures.

This investigation tested 12 soybean cultivars for susceptibility to *H. columbus* and determined the optimum soil temperature for infectivity and reproduction of *H. columbus* on soybean roots.

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[2] Respectively Graduate Research Assistant and Associate Professor, Department of Plant Pathology and Physiology, Clemson University, Clemson, South Carolina 29631.

MATERIALS AND METHODS

Field studies: The cultivars tested were 'P.I. 88788,' 'P.I. 89772,' 'P.I. 90763,' 'Dyer,' 'Forrest,' 'Pickett-71,' 'Bragg,' 'D71-9257,' 'ED-371,' 'Coker 4504,' 'Hardee,' and 'W-4,' representing maturity classes III–VIII. Hardee and W-4 were selected because of their good performance in field plots infested with *H. columbus*. The other cultivars were used because they were known to have genes for resistance to one or more plant-parasitic nematodes, and one cultivar was resistant to the Mexican bean beetle. Recommended soybean cultivars for South Carolina belong to maturity classes VII and VIII. The plot was on a Varina sandy loam soil infested with *H. columbus* near Blackville, South Carolina. Other plant-parasitic nematodes in the soil included *Scutellonema brachyurum* Steiner, *Helicotylenchus exallus* Sher, *Macroposthonia curvatum* Raski, *Paratrichodorus porosus* Allen, and *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven. All populations were fewer than 15/250 cm^3 of soil. DBCP (1,2-dibromo-3-chloropropane) was injected 45 cm deep with a single chisel under the row at 10 kg (ai)/ha 7 days before planting. Fifty seeds were planted per 3.04 m of row.

Treatments were replicated six times in a randomized complete block experiment with a split-block design. Fumigation made up the whole plots and soybean genotypes the subplots. Each subplot consisted of two consecutive rows bordered by guard rows of soybean. The soybean cultivar 'Bragg' was included since it is very susceptible to injury by *H. columbus*.

The number of nematodes in the soil was determined 17, 56, and 152 days after planting. Average initial population (Pi) estimated from 144 samples was 237 ± 43 nematodes/250 cm^3 of soil. Soil was sampled on days corresponding to physiological plant ages: primary-leaf stage, 14 days before bloom, and when pods turned brown. Soil samples were collected from each whole plot, for a total of 144 soil samples. Nematodes were extracted from each sample (250 cm^3) by centrifugal flotation (9).

Numbers of *H. columbus* in the roots were determined 94, 130, 140, and 154 days after planting, respectively when the majority of the plants were at early pod development, pod fill, pod yellowing, and pod browning. Bird's modification (1) of Young's technique (11) was used to extract nematodes from randomly chosen roots collected from individual subplots. Fresh and dry root weights were recorded.

Greenhouse studies: Ten (Table 3) of the 12 soybean cultivars used in the field were tested for host suitability in the greenhouse. Varina sandy loam soil infested with *H. columbus* was fumigated with methyl bromide (0.1 kg/0.2 m^3 soil). Three weeks after fumigation, 3,000 ± 100 *H. columbus* were added per 15-cm-diam pot of soil (F + L), while other pots were left nematode-free (F). The infested field soil (ca. 5400 *H. columbus*/pot) was used as a check (NF). Nematode inoculum for this experiment was extracted from infested soil by Cobb's method (3). The soil and water that passed through the screen series was poured over a 325-mesh (45-μm) screen and checked for nematodes. If no nematodes were present, 5-ml portions were then added into each of the control pots (F).

Before planting, seeds were treated with Thiram fungicide and inoculated with *Rhizobium japonicum*. Two seeds were planted in each pot. After emergence, the plants were thinned to one/pot. Treatments were replicated six times in a randomized complete block with the soybean genotypes representing the whole plots and the presence of *H. columbus* as subplots. The experiment was terminated at 100 days. Data collection and nematode extraction procedures were as described for the field study.

Temperature study: The influence of temperature on the infection and reproduction of *H. columbus* on Forrest soybeans was studied in temperature tanks maintained at 20, 25, and 30 ± 1 C. Varina sandy loam soil infested with *H. columbus* was used in 15-cm-diam plastic pots. Treatments were: 1) field soil, with a Pi of 5,800 *H. columbus*/pot (NF); 2) methyl-bromide-fumigated soil (0.1 kg/0.2 m^3 soil) to which were added 2,300 ± 100 *H. columbus* per pot (F + L); and 3) methyl-bromide-fumigated soil without nematodes (F). Treatments were replicated six times per temperature tank in a randomized complete block, with temperatures representing whole plots and nematodes as subplots.

Forrest soybean seedlings growing in vermiculite were removed before the primary-leaf stage, the roots were dusted with *Rhizobium japonicum*, and the seedlings were transplanted into the planting hole containing nematodes. Control pots (F) were inoculated with a soil-water suspension from which nematodes had been removed by sieving. Soil moisture was maintained near field moisture capacity (11.8% oven dry wt) as measured with tensionmeters.

After 60 days, the nematode population in soil and roots was determined. The dry weight of roots and shoots and plant height were measured. The data were subjected to analysis of variance, and least-significant difference (LSD) values were calculated.

RESULTS

Field studies: No soybean cultivar was highly resistant. All root systems were penetrated by juvenile and adult *H. columbus*. Soil populations of *H. columbus*, for both fumigated and unfumigated plots, increased as the cultivars matured. However, P.I. 90763 had the fewest ($P = 0.05$) nematodes in the rhizosphere at the last sampling date (at pod browning). The number of *H. columbus*/g dry weight decreased from the first sampling (at early pod development) to the last sampling (at pod browning) for all cultivars. At early pod development in fumigated soil, there were more nematodes in roots of P.I. 89772 than in roots of Dyer, ED-371, Pickett-71, P.I. 90763, W-4, Bragg, or Forrest (Table 1). Numbers of nematodes in roots of the different cultivars grown in fumigated soil did not differ at

TABLE 1. Numbers of *Hoplolaimus columbus*/gram dry root weight for 12 soybean cultivars in fumigated soil at Blackville, South Carolina.

Genotype	Total *H. columbus* 8/29	Total *H. columbus* 10/4	*H. columbus* juveniles 8/29	*H. columbus* juveniles 10/5
P.I. 89772[y]	532 a[x]	—	499 a	—
Coker 4504	409 ab	283 a	357 ab	256 a
P.I. 88788	389 ab	—	336 ab	—
D71-9257	247 ab	175 a	230 ab	152 a
Hardee	236 ab	334 a	207 ab	283 a
Dyer	144 b	432 a	140 b	399 a
ED-371	127 b	286 a	112 b	268 a
Pickett-71	122 b	475 a	103 b	446 a
P.I. 90763	29 b	—	75 b	—
W-4	74 b	263 a	68 b	263 a
Bragg	63 b	143 a	56 b	120 a
Forrest	46 b	390 a	45 b	372 a

[x]Numbers within a column followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple-range test.
[y]Obtained only one sample from plant introduction (P.I.) genotypes because these matured and senesced early.

TABLE 2. Yield of 12 soybean genotypes in the field at Blackville, South Carolina, as affected by preplant soil fumigation with DBCP.

Genotype	Yield (g/3 m of row) Fumigated (g)	Yield (g/3 m of row) Unfumigated (g)	Mean Difference[b] (g)
Pickett-71	883	437	433 a[a]
Hardee	1053	752	245 b
ED-371	763	607	193 bc
Bragg	797	551	161 bc
Coker 4504	977	898	106 cd
W-4	897	829	71 cde
Forrest	508	551	16 def
D71-9257	617	641	7 def
Dyer	163	112	− 18 def
P.I. 90763	56	121	− 43 ef
P.I. 88788	75	201	− 58 ef
P.I. 89772	108	102	− 94 f

[a]Numbers followed by the same letter are not significantly different ($P = 0.05$) by Duncan's multiple-range test.
[b]The mean difference for yield was adjusted for plant number.

pod fill (10/4), pod yellowing, or pod browning. In unfumigated soil the number of *H. columbus* (total and juveniles)/g dry root weight did not differ within sampling dates. The dry root weights of cultivars were greater in fumigated soil at early pod development, except for W-4, Dyer, Hardee, and Coker 4504. Plant height of Bragg was especially influenced by soil fumigation, whereas D71-9257 and Dyer did not respond.

Yield response to fumigation was greatest in Pickett-71, and absent in Dyer, P.I. 89772, P.I. 90763, and P.I. 88788 (Table 2). The latter four cultivars had low yields, regardless of treatment. Yields were better for ED-371, Hardee, Coker 4504, W-4, Bragg, D71-9257, and Forrest than for the other cultivars. Bragg and Forrest grew more vigorously in fumigated plots, however.

Greenhouse studies: In unfumigated soil (NF), populations of nematodes in soil were higher with W-4 than with Bragg and D71-9257 (Table 3). No differences occurred among soybean cultivars in F + L. Roots of Bragg harbored more nematodes than P.I. 88788, P.I. 90763, or P.I. 89772 in treatment F + L, but no differences occurred between cultivars in the NF treatment. There were greater numbers of juveniles than of adults in both treatments (NF and F + L). There were no significant differences between cultivars in total numbers of *H. columbus* or numbers of juveniles/g dry root weight. Dry weights of roots were always greatest in fumigated soil without nematodes.

The root weight of Hardee was greater than those of Forrest, P.I. 89772, P.I. 90763, or P.I. 88788; and dry root weights of Pickett-71 and the three P.I. cultivars also differed when expressed as the mean difference between F and F + L. Shoot weights among cultivars did not differ, but shoots of plants without nematodes (F) weighed more. All cultivars parasitized by *H. columbus* (F + L) were shorter than plants in fumigated soil (F). The size of D71-9257 was least influenced by the nematode.

Temperature study: Root populations of adults and juveniles were greater at 30 C than at 25 and 20 C (Fig. 1). More ($P = 0.01$) juveniles were recovered from soil at 30 than at 25 or 20 C, whereas adult soil populations did not differ with temperature. In all treatment-temperature combinations there were more adults than juveniles in the soil, but more juveniles than adults in the roots. Effects of soil temperature and nematode populations on plant growth were independent.

TABLE 3. Population of *Hoplolaimus columbus* (all stages) in the soil and roots of 10 greenhouse-grown soybean genotypes in unfumigated and fumigated soil to which were added about 3,000 *H. columbus.*

Genotype	Fumigated soil + *H. columbus*		Uufumigated soil	
	H. columbus in soil	*H. columbus* in roots	*H. columbus* in soil	*H. columbus* in roots
Bragg	1047 a[a]	4980 a	247 b	1551 a
Hardee	983 a	3249 abc	353 ab	1297 a
P.I. 88788	935 a	386 c	510 ab	1116 a
D71-9257	882 a	3827 abc	261 b	1966 a
Pickett-71	855 a	2322 abc	426 ab	1889 a
P.I. 89772	776 a	1266 bc	368 ab	1407 a
Coker 4504	732 a	2841 abc	330 ab	1858 a
Forrest	702 a	4085 ab	428 ab	1499 a
P.I. 90763	533 a	163 c	451 ab	974 a
W-4	387 a	1995 abc	740 a	1254 a

[a]Numbers within a column followed by the same letter are not significantly different (P = 0.05) by Duncan's multiple-range test.

Dry root weight decreased as soil temperature increased in F + L, whereas in NF dry root weights increased from 20 to 25 C and decreased from 25 to 30 C (Fig. 1). Root weights in uninfested soil (F) were greatest at the highest temperature. Root weights were greater in NF and F treatments than in F + L when all temperatures were combined, with no significant differences between NF and F.

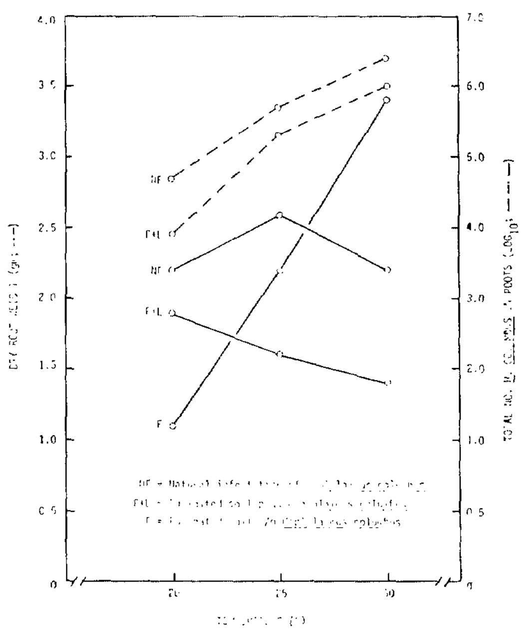

FIG. 1. Influence of *Hoplolaimus columbus* on dry root weight of Forrest soybean, and numbers of *Hoplolaimus columbus* in roots at different soil temperatures at 60 days.

Temperature did not influence the dry weight of shoots, whereas soil fumigation did. Shoots weighed more in NF than in F and F + L. There were no differences in shoot weights between F and F + L. Soil fumigation appears to have influenced other soil factors in addition to nematodes (i.e., mycorrhizal development, nitrogen-fixing actinomycetes). Soil temperatures and treatments acted independently of each other. Both variables had significant influences on plant height. Plants were taller at 25 C in NF than at 30 C in F + L and F.

DISCUSSION

Hoplolaimus columbus is predominantly an endoparasitic nematode on soybean roots (10). Oocyte development does not occur until after feeding has begun on a suitable host, with egg-deposition occurring primarily within the epidermis and cortical areas of the root (5, 10). The number of juveniles in the roots aways exceeded the number of adults, indicating that all varieties were suitable hosts. The population in the rhizosphere was predominantly adult. Most of the internal root damage was therefore attributed to the developing preadult stages.

H. columbus penetrated and reproduced in the roots of all soybean cultivars tested, indicating that none of the cultivars was highly resistant. In unfumigated field soil there were no significant differences between cultivars as regards numbers of nematodes in roots. In the fumigated soil, a separation of means was possible, but the array of nematode counts formed a succession which does not suggest distinct differences in cultivar resistance. The greenhouse study substantiated the field study in that the cultivars were all good hosts for *H. columbus*. Fumigation improved the yields of most cultivars. Most of the cultivars which were not influenced by DBCP yielded poorly in both fumigated and unfumigated soil. Something other than nematodes limited their yield. These cultivars (P.I.'s 89772, 90763, and 88788) were of an early-maturity class (III-IV) planted in areas with shorter growing seasons. Soybeans grown in South Carolina are of the maturity class VII-VIII. Thus, the difference in climate and growing season may have affected the physiological development of the plant.

W-4, Hardee, Coker-4504, ED-371, and D71-9257 in the field had more vigorous growth than the other lines throughout the growing season. Hussey (8) hypothesized that soybean lacked the root-damage-compensating feature of some other agronomic crops. It would follow, therefore, that a large root system would alleviate the stress of nematode feeding and injury. In this study the root systems of the tolerant lines W-4, Hardee, Coker 4504, and ED-371 were more profuse and weighed more than those of the other cultivars. Furthermore, all had desirable agronomic traits (yield, lodge resistance, uniform maturity).

Research in developing nematode resistance in soybean has been directed toward the sedentary endoparasitic nematodes. The probability of discovering a high level of resistance (major gene) to a migratory endoparasite is remote, since the physiology of the host is not directed toward support of the parasite. Generally, factors for resistance are attributed to the production of toxic metabolites, lack of substances necessary for development, lack of attraction, or plant-tissue hypersensitivity (7). In these experiments we have seen evidence of differences in cultivar tolerance to *H. columbus*, not a high level of resistance, and therefore would expect other plant factors to be responsible for these differences.

Soil temperature influenced the population dynamics of *H. columbus* on Forrest soybean. In fumigated soil, soybean growth increased with temperature over the range tested, whereas growth of infected soybean decreased. This decrease in soybean growth was attributed to increased feeding, penetration, and reproduction by *H. columbus*.

The increase of *H. columbus* at 30 C corresponded with similar increases in field populations in the summer. Soil temperatures averaged more than 27 C at the 10-cm depth in four months of the field experiment.

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