Pathogenicity of the Lesion Nematode, *Pratylenchus brachyurus*, on Six Soybean Cultivars[1]

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Abstract: Pathogenicity tests of *Pratylenchus brachyurus* on selected greenhouse-grown soybean cultivars indicated the nematode reduced seed yield of 'Hood' but not that of 'Custer', 'Bragg', 'Dyer', or 'Pickett'. Root weights of all cultivars were reduced. Damage and numbers of nematodes within soybean roots growing at 13, 21, and 29 C were greater at higher temperatures. At 29 C, root pruning was prominent in 'Hood' and 'Pickett' but limited in 'Custer' and 'Hill'. Root pruning was not observed at 13 C and only 'Pickett' showed pruning at 21 C. Plant height and foliage weight were not affected. *P. brachyurus* had no effect on the emergence of 'Pickett' or 'Bragg' soybeans. Nematode counts from roots of 'Pickett' at intervals after inoculation indicated that hatching of second generation second-stage larvae occurred about 15 days after egg laying. An average of 68% of the initial inoculum penetrated the roots within five days of inoculation, the highest observed was 81% in five days. Details of structural damage in penetrated tissues were studied in sectioned roots. In soybean roots infected by *P. brachyurus* and/or *Rhizoctonia solani*, greater damage occurred with nematode and fungus combined than with either acting alone. *Key Words*: Interaction, Resistance.

Little is known concerning the pathogenicity and economic importance of nematodes (other than root-knot and cyst nematodes) on soybeans, *Glycine max* (L.) Merr., in the southeastern United States. *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven, has been found associated with soybeans in several states (9, 12, 19). Ferris and Bernard (7) reported 25% soybean root weight reduction with *P. alleni* at typical field population levels. In a two-year study, Ross et al. (22) found no significant yield reduction the first year in 'Lee' soybean plots infested with spiral (*Helicotylenchus dihystera*), stunt (*Tylenchorhychus claytoni*), and lesion (*P. brachyurus*) nematodes; yields the following year were considerably reduced by all three nematodes, however. *P. brachyurus* and *P. zeae* along with *Belonolaimus longicaudatus* and *T. claytoni* have been associated with substantial yield reductions in 'Lee' soybeans in North Carolina (17). Endo (5) found that 'Lee' and 'Peking' soybeans were susceptible to *P. brachyurus* and *P. zeae*.

Soil temperature is an important environmental factor for plant parasitic nematodes. Graham (10) found *Pratylenchus* populations increased more rapidly on corn and tobacco in soil at 26.7–32.2 C than at 15.6–26.7 C.

A number of nematode-fungus disease complexes have been recognized and many were discussed in recent reviews (8, 18). In a root rot of wheat (16) involving *P. minyus* and *Rhizoctonia solani*, the two pathogens together were necessary for full disease expression. Taylor and Wyllie (23) reported that either *Meloidogyne javanica* or *M. hapla* combined with *R. solani* reduced soybean emergence more than either pathogen alone.

The major objectives of the study reported below were to determine: (i) the effect of *P. brachyurus* on seedling emergence, plant growth and yield of selected soybean cultivars, (ii) the effect of soybean cultivar and soil temperature on root populations of *P. brachyurus*, and (iii) the relationship of *P.*

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brachyurus and *R. solani* to soybean emergence.

MATERIALS AND METHODS

Stock cultures of *P. brachyurus*, originally collected from a soybean field in Escambia County, Alabama, were maintained in the greenhouse on soybean cultivars 'Pickett' and 'Hood'.

Nematode inoculum was obtained by mist chamber extraction. Aliquot samples, containing adults and larvae, were used for inoculation. Control inoculum was the filtrate of a nematode suspension passed through a 325-mesh sieve, and thus contained microorganisms or other substances associated with the nematodes. Nematodes were placed around soybean roots or seeds with a 10-ml pipette.

No soybean cultivar has been reported resistant to *Pratylenchus*. Those used in this study were selected for known susceptibility or relative resistance to other nematodes. 'Custer' is resistant to *Heterodera glycines* (soybean cyst nematode), but susceptible to *Meloidogyne sp.* (root-knot nematode) (11). 'Bragg' is resistant to root-knot, but susceptible to *Rotylenchulus reniformis*, (reniform nematode) (20). 'Hood' is susceptible to each of the three nematodes above (personal communication, R. V. Rebois, U. S. Department of Agriculture, Plant Industry Station, Beltsville, Md.). 'Pickett' is resistant to the reniform (20) and soybean cyst nematodes (1, 11), but susceptible to root-knot nematodes (11). Seeds of each cultivar were carefully selected for uniformity of size and seed coat, surface-sterilized 10 min in 20% Clorox, then rinsed several times in distilled water.

Unless otherwise indicated, roots were stained in hot acid fuchsin-lactophenol and cleared with lactophenol (14).

For yield studies, seeds of 'Custer', 'Bragg', 'Hood', 'Dyer', and 'Pickett' soybeans were planted in sterile sandy soil in ten 8-inch clay pots. After emergence, soil in five pots of each cultivar was inoculated at the rate of 1000 nematodes per plant; remaining pots were treated as controls. All plants were fertilized monthly with Hoagland's solution. At maturity, seed pods were harvested and counted; seeds were counted, dried, and weighed; and roots dried and weighed. Nematodes were extracted from the soil by Seinhorst elutriator and from roots by mist chamber extraction and nematode counts determined.

Effect of soybean cultivar and soil temperature on reproduction and pathogenicity of the lesion nematode was determined for 'Custer', 'Hood', 'Hill', and 'Pickett' soybeans. Sandy loam soil was passed through a 10-mesh screen, autoclaved for 15 min, aged two weeks to eliminate toxicity, and portioned into 5-oz. size plastic cups, 200 g per cup. Seeds (one per cup) were germinated in the soil at ambient greenhouse temperatures. Two days after emergence, 18 cups of each cultivar were selected for healthy appearance and uniform height. Six cups of each cultivar were randomly distributed in each of three constant temperature tanks (water bath type) at 13, 21, and 29 C. Soil and water bath temperatures were recorded continuously with a thermograph. Following a 3-day adjustment period, three cups of each cultivar at each temperature were inoculated with 100 nematodes per plant; the three remaining cups received control inoculum. Thirty days after inoculation, height and top weight of each plant was measured. Root system of each plant was washed free of soil, stained, and nematodes and eggs were counted. A portion of each root system was preserved in Navashin's fluid (as modified by Randolph) (13) and embedded in paraffin after dehydration in tertiary butyl alcohol. Transverse and longi-

tudinal sections were cut at 25–30μ and stained with safranin and fast green.

For emergence tests, 'Pickett' and 'Bragg' soybeans were planted in steam treated soil in flats (50 × 35 × 8 cm) and inoculated with: (i) 100 nematodes per seed, (ii) 1000 nematodes per seed, (iii) sterile water, and (iv) nematode wash water. Two flats with 10 seeds each were used for each treatment. Final count of seedling emergence was made 10 days after inoculation. Roots of all seedlings in each treatment were stained.

The time-rate of *P. brachyurus* entry into root systems was determined by inoculating 3-day-old 'Pickett' seedlings growing individually in sterile soil in 5-oz. size plastic cups at 29 C with 100 nematodes. At intervals after inoculation, the root system was removed from each of three cups, washed free of soil and stained; the total number of nematodes in each root system counted.

Effect of *P. brachyurus* and *Rhizoctonia solani* on seedling emergence was determined with 'Pickett' soybeans because that cultivar is susceptible to infection by both pathogens. Nematode inoculum was prepared as previously described except nematodes were surface-sterilized by successive single rinses in distilled water, 0.02% mercuric chloride, streptomycin sulfate solution, and sterile water. The fungus was maintained in pure culture on potato-dextrose-agar (PDA); "standard dosage" *Rhizoctonia* inoculum was prepared in the manner described by McKeen and Mountain (15) and incorporated into the soil prior to planting. The soil was contained in small plastic flats with sealed drainage holes to maintain soil moisture and prevent contamination. Treatments were: (i) 100 nematodes per seed; (ii) 20 ml *Rhizoctonia* per flat; (iii) 100 nematodes per seed plus 20 ml *Rhizoctonia* per flat; (iv) nematode wash-water plus sterile PDA. Each treatment had two flats with ten seeds per flat. The test was terminated 3 weeks after emergence and the number of surviving plants counted. Roots were washed free of soil, stained and cleared and examined for nematodes and fungi. One small section of each root system was removed prior to staining, rinsed in sterile water, and placed on sterile PDA to check for presence of *R. solani*.

Results and Discussion

Of the five cultivars tested, only 'Hood' showed a significant yield reduction (Table 1); however, marked reduction in root number and size in all cultivars except 'Bragg' occurred in the presence of *P. brachyurus*. Apparently, the amount of root damage sustained was not a limiting factor in yield of 'Custer', 'Dyer', and 'Pickett'. Ferris and Bernard (7) found *P. alleni* reduced root weight of soybeans 25% with no measurable effect on top growth. They suggested that under field conditions where either water or nutrients were limited, root growth reduction would be reflected in reduced growth and yield. 'Bragg' soybeans appeared to be tolerant to damage by *P. brachyurus*; tests at higher inoculum levels would be useful in further evaluating that cultivar. Nematode populations in this study were lower than those reported by Ross et al. (22), but were comparable to other studies of *Pratylenchus* on soybeans (5, 6, 7).

P. brachyurus caused no significant reduction in top growth of 'Custer', 'Hood', 'Hill', or 'Pickett' growing at 13, 21, or 29 C. Relative root weights of soybean cultivars are shown in Figure 1. Destruction of secondary roots occurred with all cultivars at 29 C, giving the roots a "pruned" appearance. Roots of 'Hood' were the most severely pruned, followed by 'Pickett', 'Custer', and 'Hill'; those of 'Pickett' showed a limited amount of pruning at 21 C. Nematode numbers in roots were highest at the

TABLE 1. Growth and yield of five soybean cultivars inoculated with *P. brachyurus*

Cultivar	Treatment	Seed pods/ plant	Seed/ plant	Seed dry wt./ plant	Root wet wt./ plant
Custer	check	10.0	13.8	1.50	7.39
	inoculated	11.0	16.0	1.79	3.39
Bragg	check	8.7	13.2	1.11	4.33
	inoculated	12.0	18.6	2.25	6.24
Dyer	check	12.0	15.6	1.78	8.37
	inoculated	11.0	17.0	1.90	3.94
Hood	check	15.6	20.8	2.42	9.21
	inoculated	7.2**	9.4**	0.78**	2.17**
Pickett	check	18.9	32.6	4.74	8.77
	inoculated	17.2	32.3	4.42	4.24

** Student-t value significant at 1% level, other values not significant

two higher temperatures (Fig. 2). Roots of 'Custer', 'Hood', and 'Pickett' plants contained most nematodes at 29 C and fewest at 13 C. At 29 C, roots of 'Pickett' contained highest populations, followed by 'Custer', 'Hill', and 'Hood'. Populations in 'Custer' and 'Hill' roots dropped sharply between 21 and 13 C. Egg-laying was more correlated with cultivar than temperature at 21 or 29 C (Fig. 3), suggesting temperature may alter the physiology of the soybean rather than of the nematode at 21 and 29 C. Physiology of both the soybean and the nematode is probably altered at 13 C. This indicates that optimum temperature for increase of

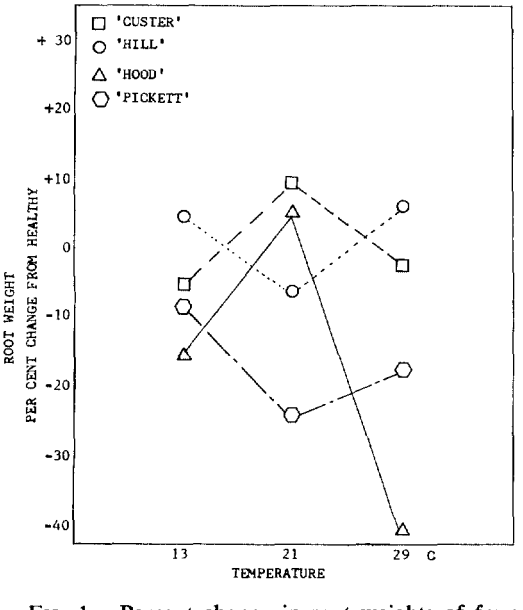

FIG. 1. Percent change in root weights of four soybean cultivars 30 days after inoculation.

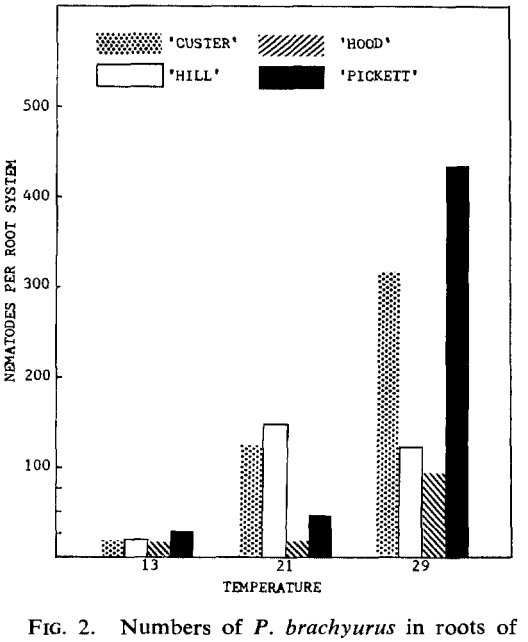

FIG. 2. Numbers of *P. brachyurus* in roots of four soybean cultivars 30 days after inoculation.

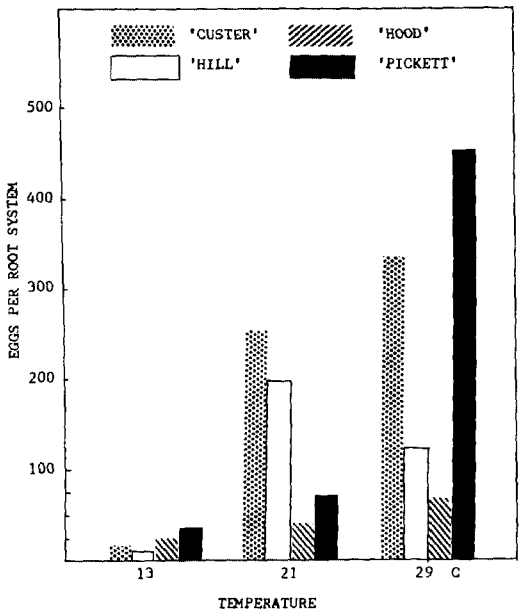

FIG. 3. Numbers of *P. brachyurus* eggs in roots of four soybean cultivars 30 days after inoculation.

P. brachyurus varies with the cultivar of a specific host. Dropkin (4) observed similar results with root-knot nematodes.

P. brachyurus caused damage to the cortical parenchyma of the root. Cavities and tunnels were formed as a result of nematode feeding and movement. Some infected cells were void of cytoplasm. Exposed to safranin fast-green, walls of cells affected by the nematode stained red, while walls of normal cells stained green. The altered staining reaction of the walls extended two or three cell layers deep on each side of a cavity and may have been caused by toxic enzyme secretion as suggested by Brooks and Perry (3). Nematodes moved intracellularly, as evidenced by the presence of nematodes and eggs within cells and by the mechanical rupture of cell walls. By tracing the altered staining of the cells in serial root sections, it was found that the nematode moved parallel to the longitudinal axis of the root as well as in a circular path around the stele. Nematodes were occasionally found in the vascular cylinder, but usually did not penetrate the endodermis. No nematode damage was observed in the vascular system, nor were hypertrophy or hyperplasia seen in any root tissues. The epidermis was ruptured in some sections, but appeared intact in others.

Nematodes were found in tap roots and secondary roots at 21 and 29 C but rarely in secondary roots at 13 C. The restriction of nematodes to the tap root at 13 C may be attributed to the low temperature. Wallace (24) noted that extremely low or high temperature restricted the movement of plant-parasitic nematodes. Thus, the nematodes may have penetrated the tap root and were unable to migrate to the secondary roots when these were formed later. Or, the entrance of the nematode into the secondary roots may have been limited by restricted movement at low soil temperature.

Numbers of nematodes in the root system of 'Pickett' soybeans at intervals after inoculation are shown in Figure 4. An average of 68% of the initial inoculum penetrated the roots within five days of inoculation. A marked increase, due to the hatching of second-stage larvae, occurred after 15 days. Congregation of nematodes within the cortex was observed after 10 days and appeared due to hatching of eggs deposited at a location rather than nematodes in the soil being attracted to this injured area of root. In one 12-mm length of root, 90 nematodes and 76 eggs were counted. The ability of the nematode to multiply in the roots up to 400% in 35 days indicates that high populations could build up during the growing season of a soybean plant. High percentage of infection (up to 81%) by the original inoculum contributed to this rate; however, field experimentation is needed to supplement this information.

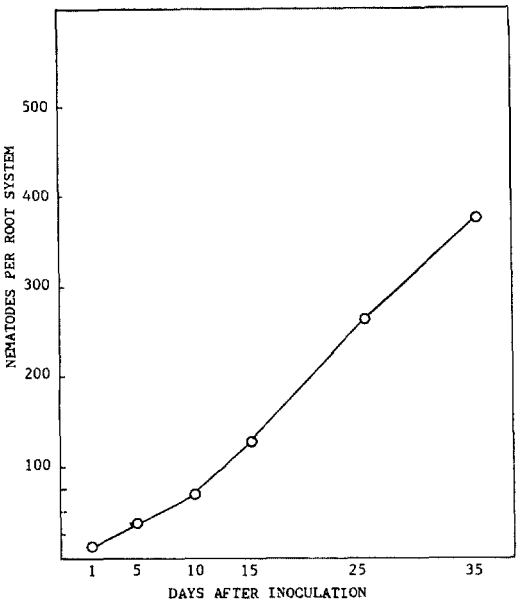

FIG. 4. Numbers of *P. brachyurus* per root of Pickett soybean at intervals after inoculation.

Neither 100 nor 1000 nematodes per seed caused reduction in the emergence of soybeans; however, low nematode counts (10–20% of inoculum) in the roots after 10 days indicated low infectivity in this test (data not shown).

The experiment involving mixed infection with *P. brachyurus* and *R. solani* was terminated 21 days after planting and the numbers of plants surviving expressed as a percentage of the check were, by treatment: *Rhizoctonia* alone, 63%; *Pratylenchus* alone, 100%; and *Rhizoctonia* plus *Pratylenchus*, 21%. Damage to soybeans at 10 days after planting is shown in Figure 5. Lesions were prominent on roots and hypocotyls of plants growing in soil inoculated with *Rhizoctonia* plus *Pratylenchus* or *Rhizoctonia* alone. Lesions occurred on roots, but not on hypocotyls of plants growing in *Pratylenchus*-infested soil. Acid fuchsin-lactophenol stain revealed nematodes in the roots from soil infested with *Rhizoctonia* plus *Pratylenchus* and *Pratylenchus* alone. *Rhizoctonia* was reisolated only from roots that had been originally inoculated with the fungus. It is well-known that *R. solani* can cause severe post-emergence damping-off of soybeans. This test indicated that the combination of *P. brachyurus* and *R. solani* caused greater damage than either pathogen alone. The exact role of the nematode is unknown. Reynolds and Hanson (21) considered damage of cotton seedlings by root-knot

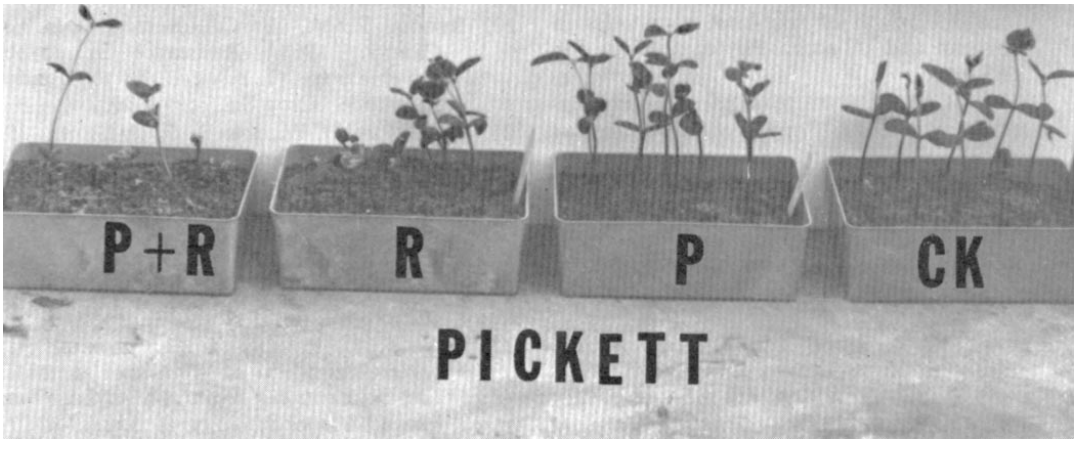

FIG. 5. Effect of *Praytlenchus brachyurus* (P) and *Rhizoctonia solani* (R) on Pickett soybean 10 days after planting.

nematodes to be responsible for increased incidence of damping-off caused by *R. solani*. Brodie and Cooper (2) found root-knot nematodes capable of prolonging the susceptible period for cotton seedlings exposed to *R. solani*. They assumed that the nematodes delayed the peridermal development, extending the period of susceptibility to *Rhizoctonia*.

These greenhouse studies indicated that *P. brachyurus* can cause severe damage to soybeans which results in significant yield reductions. However, soybean cultivars tested which currently show resistance to other major soybean nematode pathogens appear to be tolerant to *P. brachyurus*. Only after field experimentation can these cultivars be fully evaluated for resistance to *P. brachyurus*.

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