

To the Graduate Council::

I am submitting herewith a thesis written by Franklin S. Wartman entitled "Pathogenicity and Reproduction of Pratylenchus alleni and P. brachyurus on Selected Soybean Cultivars." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

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PATHOGENICITY AND REPRODUCTION OF PRATYLENCHUS ALLENI AND

P. BRACHYURUS ON SELECTED SOYBEAN CULTIVARS

A Thesis

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ABSTRACT

The lesion nematode, Pratylenchus allenii, a potential pest of soybean, was found in soil samples from four of nine west Tennessee counties. Twelve soybean cultivars ('Bedford', 'Bragg', 'Centennial', 'Coker 136', 'Dare', 'Essex', 'FFR 666', 'Forrest', 'Lee 74', 'McNair 500', 'Pickett 71', and 'York'), recommended for cultivation in Tennessee were tested for reproductive suitability to P. allenii and P. brachyurus in the greenhouse. For these suitability studies, 1000 larvae and adults were pipetted onto or around each seed or seedling in clay pots. Ten weeks after inoculation, roots were harvested, blotted dry, and weighed. Nematodes were extracted from the roots in a mist chamber for two weeks. Numbers of P. allenii per gram of fresh root of 'Pickett 71' were significantly greater than most other cultivars, and 'Lee 74' was more suitable than 'Coker 136', 'Dare', 'Essex', or 'McNair 500', but this difference was not obtained in subsequent experiments. All soybean cultivars tested were suitable for reproduction of P. allenii. The ratio of males and of adults to the total recovered population were not significantly different among the cultivars. No significant differences in reproduction of P. brachyurus among the cultivars were noted. In a host range test, numbers of P. allenii increased significantly on snapbean, tomato, wheat, soybean, cowpea, corn, and potato, but declined on tobacco, lima bean, alfalfa, cabbage, cotton, watermelon, and peanut. The potential of lesion nematodes for reproduction on certain crops in rotation with soybeans should be taken into account when designing pest management strategies for soybean.

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CHAPTER I

INTRODUCTION

Soybeans are one of the major agricultural crops in Tennessee (American Soybean Association, 1979). The soybean cyst nematode, Heterodera glycines Ichinohe, is the major nematode pest on soybeans (Riggs, 1977). However, lesion nematodes, Pratylenchus spp., may be significant pests on soybeans and have been shown to cause as much as 25% reduction in root weight (Ferris and Bernard, 1962). Various species of Pratylenchus occur in Tennessee, primarily in the soybean-producing (Klobe, 1976; Bernard, 1980) and tobacco-growing (Ponchillia, 1975) regions of the state.

The objectives of these studies were to: (1) determine the frequency and identity of species of Pratylenchus in western Tennessee soybean fields; (2) study pathogenicity and reproduction of P. alleni Ferris, 1961, and P. brachyurus (Godfrey, 1929) Filipjev and Schuurmans-Stekhoven, 1941, on selected soybean cultivars; and (3) ascertain the suitability of selected crop species as hosts for P. alleni.

CHAPTER II

LITERATURE REVIEW

Distribution

Pratylenchus brachyurus was first described in Hawaii on pineapple (Godfrey, 1929). The nematode is now known to occur throughout the southern United States. P. brachyurus is found in Georgia, Alabama, Louisiana, North and South Carolina, Texas, Florida, and Virginia (Anonymous, 1960); Maryland (Jenkins et al., 1957); and South Dakota (Norton et al., 1968). It has been reported on soybeans in Louisiana (Fielding and Hollis, 1956); Mississippi (Rebois and Golden, 1978); and Alabama, Florida, and Georgia (Rebois and Cairns, 1968). P. brachyurus was also shown to occur on soybeans in Maryland (Jenkins et al., 1957), but a later study (Golden and Rebois, 1978) did not find it associated with that crop.

Pratylenchus alleni was first described by Ferris (1961) from the roots of several soybean cultivars in a field in Illinois. It has since been reported in several other states in the Midwest, including Iowa (Norton and Burns, 1971), Missouri (Norton et al., 1968), Ohio (Brown and Riedel, 1978), and Kansas (Dickerson, 1979).

The genus Pratylenchus was first observed in Tennessee in 1889 by Scribner. He noticed a nematode affliction of potatoes distinct from that caused by root knot nematodes, Meloidogyne spp. Sherbakoff and Stanley (1943) mentioned this same problem again and gave a brief description of Pratylenchus scribneri Steiner.

Ponchillia (1975), in a survey of Tennessee burley tobacco fields, found P. neglectus in 9% of the fields sampled. Klobe (1976), who surveyed seven counties in the soybean-producing region of west Tennessee for plant-parasitic nematodes, found Pratylenchus spp. in all seven counties. Bernard (1980) conducted a survey of Tennessee for various plant-parasitic nematodes and found nine different species of Pratylenchus in the state, including P. allenii.

Environment

Populations of Pratylenchus spp. are influenced significantly by environment. Norton and Burns (1971) found that P. allenii colonized soybean roots better in a dry soil region than in a wet region. They also noted that the soil moisture regime affected the sex ratio of the reproducing nematodes.

Dickerson (1979) reported that P. allenii reproduced well on soybeans at 32 and 37.5 C and less favorably at 27.5 C, but did well at 27.5 C on 'Rutgers' tomato. Acosta and Malek (1978) in a more extensive test showed that 30 C was the optimum temperature for population development of P. allenii and P. brachyurus but that populations of these nematodes would also increase as much as five-fold at 20 C. Lindsey and Cairns (1970) had previously shown that the optimum temperature for population increase, at least for P. brachyurus, varied with host cultivar. The work of Acosta and Malek seems to lend considerable support to that concept.

Host Range

Pratylenchus brachyurus has a fairly wide host range. It was first found on pineapple (Godfrey, 1929) and at that time was shown

to reproduce well on cowpea. Graham (1951) and Good et al. (1958) showed that P. brachyurus could be a serious pest of tobacco and peanuts, respectively. Fielding and Hollis (1956) found P. leiocephalus Steiner (= P. brachyurus) in fields of cotton, soybean, strawberry, sugarcane, and sweet potatoes in Louisiana.

Endo (1959) conducted a more extensive host range study and found that after two months, P. brachyurus could increase significantly on cultivars of corn, clover, cotton, and sudan grass as well as tobacco. He also found that after five months, high population levels were produced on soybean, peanut, watermelon, and milo.

Norton et al. (1968) reported finding P. brachyurus commonly associated with wheat and unspecified range grasses. Johnson and Nusbaum (1968) did more extensive work on soybean and corn, and noted that Pratylenchus spp. population levels were affected not only by cultivar but also by the presence of other species of plant-parasitic nematodes. Tarjan and O'Bannon (1969) found that P. brachyurus could be a serious pest of citrus. Koen (1967) reported its effects on potatoes in South Africa and showed that corn, sunflower, soybean, common bean, wheat, oats, and several weeds would allow penetration and reproduction of the nematode.

The host range of P. alleni has not been extensively investigated. The nematode was originally found on soybean (Ferris, 1961), who also found that populations would increase on wheat and oats. Hackney and Dickerson (1975) found that P. alleni did well on tomato. Dickerson (1974) found that dry-edible beans were suitable hosts. Brown and Riedel (1978) reported P. alleni from potato roots in Ohio.

Soybean Cultivar Suitability

Since the first reports by Endo (1959) that P. brachyurus reproduces well on soybean, several studies have been conducted to determine resistance or tolerance among various soybean cultivars. Ross et al. (1967) showed that P. brachyurus not only increases on soybean but that it does cause yield reduction.

Endo (1967) reported that there was no difference in susceptibility between soybean cultivars 'Lee' and 'Peking'. Lindsey (1969) evaluated 'Custer', 'Hill', 'Hood', and 'Pickett' at three different temperatures and found that reproduction and pathogenicity, as measured by root weight losses, vary according to the cultivar as well as to the temperature.

Lindsey and Cairns (1971) found that 'Bragg' especially, but also 'Custer', 'Hill', 'Dyer', and 'Pickett' were tolerant to P. brachyurus. All cultivars had slightly reduced root weights and a sixth cultivar, 'Hood', was susceptible and had reduced seed yield.

Schmitt (1976) evaluated twenty-five cultivars for P. brachyurus and among his results, found that 'Dare' and 'York' did not support very high numbers and could be called resistant. 'Bragg' was susceptible and tolerant and 'Essex', 'Forrest', 'Pickett 71', 'Lee 74', and 'Coker 136' were susceptible and not tolerant.

Acosta et al. (1978), in an evaluation of fifty-one cultivars for susceptibility to P. scribneri, found that nematode numbers decreased on 'York', 'Forrest', 'Essex', 'Dare', and 'Bragg' but increased on 'Lee 74' and 'Pickett 71'.

Less extensive cultivar testing has been done with P. alleni. Ferris and Bernard (1962) found that P. alleni reduced the weight of soybean root systems by up to 25%, although there was no noticeable difference in top growth. They found 500 to 1000 P. alleni per gram of root emerging from 'Kent', 'Clark', 'Harosoy', 'Shelby', and 'Lindarin' and 1900 per gram of root emerging from 'Lincoln' in field tests. In the greenhouse, they found means of 1350 for 'Lincoln' and 3650 for 'Adams'.

Dickerson (1974) reported that 'Columbus', 'Williams', 'Cutler', 'Calland', 'Wayne', and 'Clark 63' were resistant to P. alleni. In a temperature effects test, Dickerson (1979) found that P. alleni would reproduce more abundantly on 'Clark 63' than on 'Cutler 71'.

CHAPTER III

MATERIALS AND METHODS

General

Pratylenchus alleni was originally collected from a snap bean field in Cumberland County, Tennessee, and increased on soybean and navy bean. A culture of P. brachyurus was obtained from R. S. Hussey, University of Georgia, and maintained on soybean. All experiments were performed in the greenhouse. Root systems were harvested 70 days after inoculation and weighed before extraction. Nematodes were extracted from chopped roots on funnels placed in a mist chamber, 10 sec every 10 min for 14 days.

Soybean Cultivar Suitability

Two tests were conducted to determine the suitability of soybean cultivars as hosts for P. alleni. In the first, 1000 P. alleni were pipetted onto two seeds planted in each 12 cm pot containing a steam-sterilized field soil-peat mix. An identical series of uninoculated control plants was also prepared. After seedling emergence, plants were thinned to one per pot. Twelve cultivars were used ('Bedford', 'Bragg', 'Centennial', 'Coker 136', 'Dare', 'Essex', 'FFR 666', 'Forrest', 'Lee 74', 'McNair 500', 'Pickett 71', and 'York'). The experiment consisted of five plant replications arranged in a randomized block design in the greenhouse. Pots without germinating seedlings were replanted.

In the second P. alleni experiment, two seeds were planted in 6 cm clay thumb pots containing a field soil-sand mix and thinned to one per pot after emergence. Eight days after planting, seedlings were transplanted to 12 cm clay pots and inoculated by pipetting 1000 P. alleni onto the root ball. This experiment consisted of eight cultivars ('Bedford', 'Bragg', 'Centennial', 'Essex', 'Forrest', 'Lee 74', 'Pickett 71', and 'York') and eight plant replications arranged in a Latin square design in the greenhouse.

A root weight loss experiment was also performed with eight plant replications and three cultivars ('Pickett 71', 'Bragg', and 'Essex').

Another test was conducted to determine the suitability of soybean cultivars as hosts for P. brachyurus. One thousand P. brachyurus adults and larvae were pipetted onto the roots of one six-day-old soybean seedling per 12 cm clay pot and the pots were then filled with a steam-sterilized field soil-sand mix. An identical series of non-inoculated controls was also prepared. The same twelve cultivars as the first P. alleni experiment were used and the experiment was replicated five times.

After extraction, nematodes were counted. Pratylenchus alleni were counted as males, females, and juveniles; P. brachyurus were counted as adults and juveniles.

Host Range

The host range test for P. alleni consisted of fourteen different hosts with five replications arranged in a randomized block design.

Soybean (Glycine max (L.) Merr. 'Pickett 71'), snapbean (Phaseolus vulgaris L. 'Blue lake'), lima bean (Phaseolus lunatus L. 'Henderson's Baby Bush'), peanut (Arachis hypogaea L. 'Florunner'), alfalfa (Medicago sativa L. 'Tempo'), cotton (Gossypium hirsutum L. 'Hancock'), Cowpea (Vigna unguiculata (L.) Walp. subsp. unguiculata 'California Blackeye'), tobacco (Nicotiana tabacum L. 'NC 95'), tomato (Lycopersicon esculentum Mill. 'Rutgers'), corn (Zea mays L. 'Burpee Golden Cross Bantam'), wheat (Triticum aestivum L. 'Monon'), watermelon (Citrullus lanatus (Thunb.) Matsum. and Nakai 'Charleston Gray'), cabbage (Brassica oleracea capitata L. 'Burpee Copenhagen Market'), and potato (Solanum tuberosum L. 'Kennebec') were started in thumb pots. All were eleven-day-old seedlings except for alfalfa (six day); cabbage, potato, and tomato (28 days); and tobacco (8 cm high). All plants were transplanted to 12 cm clay pots and inoculated by pipette with 1000 P. alleni adults and larvae. To determine numbers of P. alleni in the soil, 100 ml soil samples from each pot, except wheat, were also analyzed using a sugar flotation-centrifugation method (Jenkins, 1964).

Distribution of Pratylenchus spp. in Tennessee Soybean Fields

Five random samples from each of nine west Tennessee counties were solicited by letters written to Extension personnel in those counties. Nematodes were extracted with a sugar flotation-centrifugation method (Jenkins, 1964). Pratylenchus spp. were selected, killed, and fixed with hot 4% formalin, then processed to glycerin by a rapid method (Seinhorst, 1959) and mounted on permanent slides for identification. The nematodes were identified to species with the key of Loof (1978) and published descriptions.

CHAPTER IV

RESULTS AND DISCUSSION

Distribution

P. scribneri was the most frequent Pratylenchus species found in soil from soybean fields in the present survey. P. alleni was also common but Pratylenchus neglectus (Rensch) Filipjev and Schuurmans-Stekhoven was found infrequently (Table 1). Klobe (1976) found Pratylenchus spp. to be frequent in west Tennessee soybean fields, but did not identify taxa to species. Bernard (1980) found P. alleni in Dyer county and P. scribneri in Madison and Lauderdale counties. P. scribneri is the most widespread member of the genus in Tennessee but P. alleni also occurs frequently. P. alleni and especially P. scribneri are potentially serious pests of soybean (Ferris and Bernard, 1962; Acosta et al., 1978) and should be studied more closely.

Soybean Cultivar Suitability

All soybean cultivars were suitable hosts for reproduction of P. brachyurus (Table 2). While reproduction varied widely among the cultivars, no significant differences occurred, either in total nematodes or in number of P. brachyurus per gram of fresh root. Root weights were not significantly different. These data, while not significant, appear to agree in some ways to those of Schmitt (1976), who found that the cultivar 'York' was not very susceptible but that 'Bragg' and 'Forrest' were susceptible. The remainder of the data, however, did not parallel Schmitt's findings. Schmitt (1980) also showed 'Forrest' to be more susceptible than 'Essex'.

Table 1. Distribution of Pratylenchus species in soybean fields in selected west Tennessee counties.

County	Collected species	Positive samples/ total samples
Chester	<u>P. scribneri</u>	1/5
Crockett	<u>P. alleni</u> , <u>P. scribneri</u>	2/5
Dyer	<u>P. alleni</u> , <u>P. neglectus</u> , <u>P. scribneri</u>	6/21
Fayette	<u>P. scribneri</u>	2/5
Lauderdale	<u>P. neglectus</u> , <u>P. scribneri</u>	3/5
Madison	<u>P. alleni</u>	2/5
McNairy	<u>P. scribneri</u>	1/5
Obion*	<u>P. alleni</u> , <u>P. neglectus</u> , <u>P. scribneri</u>	12/17
Tipton	<u>P. scribneri</u>	1/5

Table 2. Reproduction of Pratylenchus brachyurus on selected soybean cultivars.

Cultivar	Number of nematodes extracted*	
	Per plant	Per g fresh root
FFR 666	3454 a**	1553 a**
Bedford	3959 a	1444 a
Forrest	3646 a	1374 a
Bragg	2858 a	1232 a
Dare	4739 a	1156 a
Centennial	2800 a	842 a
Essex	3899 a	841 a
Lee 74	2957 a	591 a
McNair 500	1804 a	523 a
Pickett 71	1656 a	459 a
York	1830 a	372 a
Coker 136	1770 a	348 a

*Numbers recovered after 14 days incubation in a mist chamber; initial inoculum 1000 adults and larvae.

**Means of five replications. Column means followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P=0.05$).

All soybean cultivars tested were suitable hosts for P. alleni when reproduction was measured on a plant basis. However, results varied considerably on the basis of root weight (Table 3). In the first experiment, P. alleni reproduced best on 'Pickett 71', and better on 'Lee 74' than on 'McNair 500', 'Dare', 'Coker 136', or 'Essex', but these results were not confirmed in later tests. From the data, it is evident that there were no definite signs of resistance. The use of resistant cultivars is important in nematode control, and cultivar resistance under field conditions needs to be determined.

Higher numbers of males were extracted from soybean cultivars in the first experiment (42-48%) than in the second (35-42%; Fig. 1). The percentage adults was also higher in the first experiment (74-79%) than in the second (51-54%; Fig. 2). This variation may be due to two factors. The first experiment was conducted in the fall and the second in the spring. The temperature was lower in the greenhouse in the fall than in the spring. There was also a difference in the soil mix of each experiment. More adults and slightly more males were produced during the cooler period and in the more organic soil. Norton and Burns (1971) found 26% in wet soil and 53% adults in dry soil. With the exception of the adults in the first test, the data of this test seemed to be similar to those of Norton and Burns.

There are several possible explanations for the inconclusive results of these experiments. It has been shown by several researchers including Lindsey and Cairns (1970) that environmental factors such

Table 3. Reproduction of Pratylenchus alleni on selected soybean cultivars.

Cultivar	Number of nematodes extracted					
	Experiment 1*		Experiment 2**		Experiment 3**	
	Per plant	Per g root	Per plant	Per g root	Per plant	Per g root
Pickett 71	3810 a ⁺	571 a	4312 a	334 a	4959 a	531 a
Lee 74	2553 a	390 ab	4335 a	331 a		
Centennial	2659 a	317 bc	3415 a	349 a		
Bragg	2104 a	276 bc	4564 a	380 a	4049 a	260 a
Bedford	2145 a	254 bc	2903 a	366 a		
FFR 666	1754 a	231 bc				
York	2064 a	165 bc	3039 a	188 a		
Forrest	1478 a	159 bc	3924 a	380 a		
McNair 500	1230 a	127 c				
Dare	1325 a	121 c				
Coker 136	1111 a	118 c				
Essex	1206 a	108 c	5910 a	419 a	5519 a	454 a

* Means of five plant replications.

** Means of eight plant replications.

⁺ All plants inoculated with 1000 P. alleni each. Column means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (P=0.05).

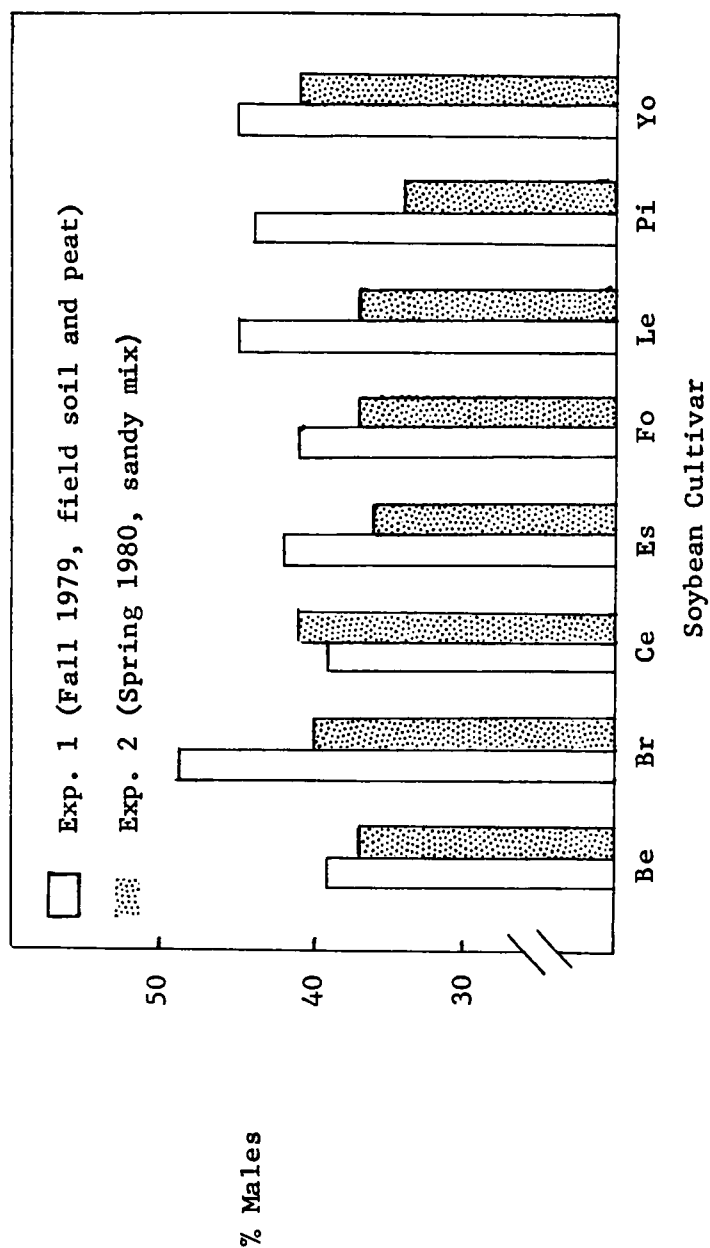


Figure 1. Sex ratios of *Pratylenchus alleni* extracted from selected soybean cultivars ('Bedford', 'Bragg', 'Centennial', 'Essex', 'Forrest', 'Lee 74', 'Pickett 71', and 'York') as a percentage of total extracted adult nematodes.

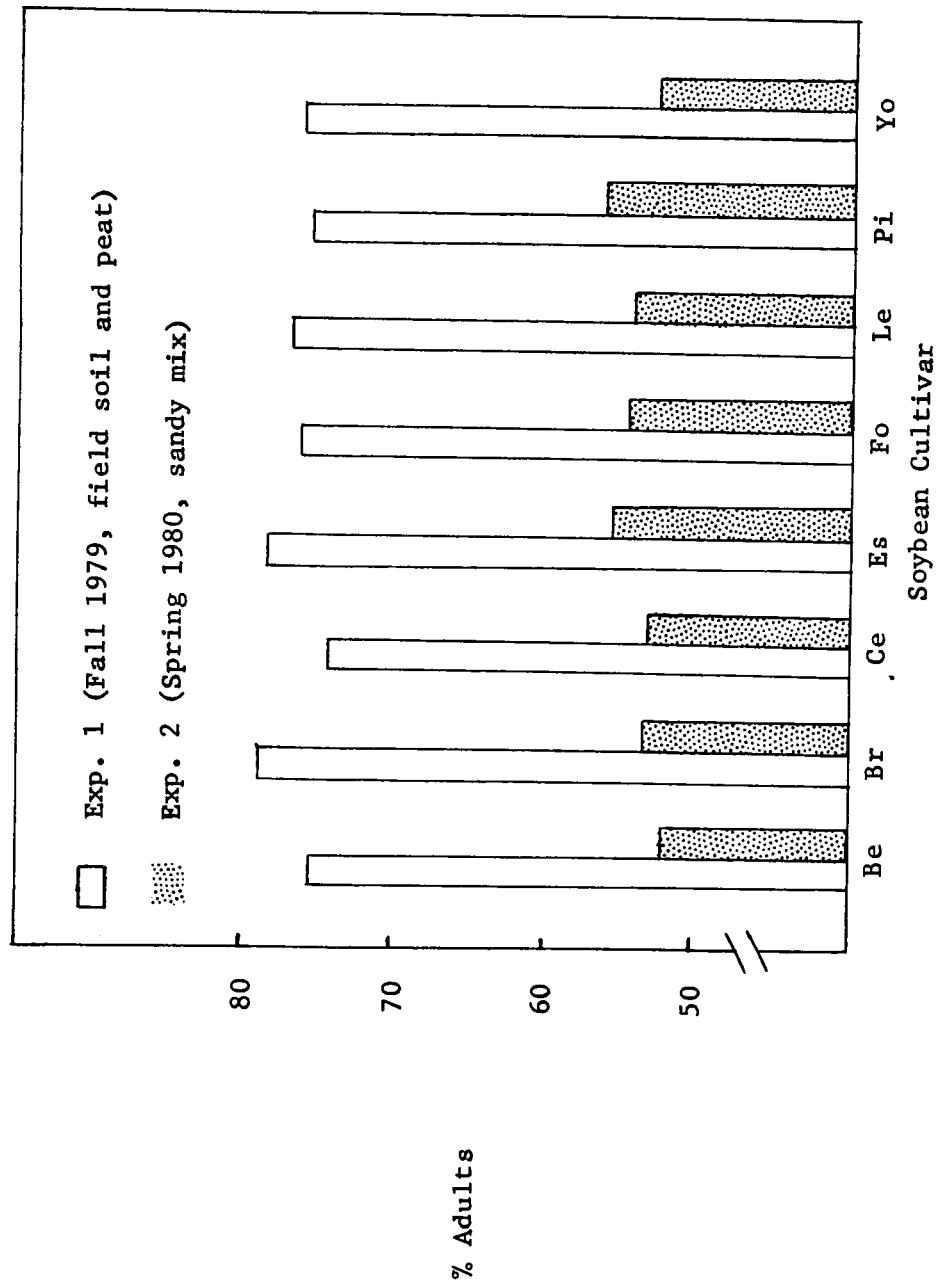


Figure 2. Proportion of adult *Pratylenchus alleni* extracted from selected soybean cultivars ('Bedford', 'Bragg', 'Centennial', 'Essex', 'Forrest', 'Lee 74', 'Pickett 71', and 'York') as a percentage of total extracted nematodes.

as soil moisture, soil type, and temperature have considerable impact on the effects of different soybean cultivars on Pratylenchus populations. These effects should be a consideration in testing soybean cultivars for Pratylenchus resistance, since it would be important to know how different cultivars perform over a range of conditions.

Kumar and Viswanathan (1972) have shown that physiological races of Pratylenchus spp. may occur. The importance and control of such variable species would be more difficult to assess in different areas.

Southards and Nusbaum (1967) found that tobacco plants of the same cultivar varied in their response to infection by P. brachyurus and that this variation was genetic in nature. This finding fits in quite well with what was found in these experiments. There was sometimes considerable variation in nematode numbers among replicates of the same cultivar. This phenomenon would be of considerable importance in resistance breeding. Soybean cultivars, tolerant to high Pratylenchus population levels, could be bred through selection of the most tolerant individuals from large numbers of plants.

Finally, P. alleni may be relatively insensitive to differences among the cultivars, so that reproductive variation within cultivars may have been due to experimental error of inoculation and plant handling.

Host Range

Snapbean, tomato, wheat, soybean, cowpea, corn, and potato were suitable hosts for P. alleni (Table 4). Populations were sharply

Table 4. Reproduction of Pratylenchus alleni on selected plant species.

Host	Total nematodes extracted						
	Per plant		Per g root		S. D.	% Juveniles:females:males	
	Mean*	Range	Mean*	Range			
Snapbean	15423	6292-25616	7663	1118	419-1534	419	56:29:15
Tomato	8917	5824-15194	3835	836	529-1288	299	54:29:17
Wheat	7190	4752-10272	2817	110	55-186	48	42:34:24
Soybean	6550	3072-9064	2221	355	111-544	355	49:30:21
Cowpea	5943	3642-11314	3108	433	203-1179	419	43:33:24
Corn	5855	3936-8928	1924	192	137-249	40	51:31:18
Potato	3817	1088-7032	2493	153	32-274	110	39:37:24
Tobacco	335	192-426	94	28	13-39	11	45:37:18
Lima Bean	326	124-440	129	21	13-24	5	33:31:36
Alfalfa	143	56-276	140	9	4-19	7	44:26:30
Cabbage	51	8-124	45	3	1-10	4	48:32:20
Cotton	46	16-74	25	5	2-8	3	39:51:10
Watermelon	14	6-18	5	2	1-4	1	24:66:10
Peanut	10	2-26	10	1	1-2	1	40:33:27

*Each value is the mean of five plant replications; each plant inoculated with 1000 nematodes.

lower than the initial inoculum level on tobacco, lima bean, alfalfa, cabbage, cotton, watermelon, and peanut. Snapbean was the best host of the crops tested. There were no discernible trends in sex ratios between the hosts. Snapbean, tomato, soybean, wheat, and potato were previously reported as good hosts for P. alleni (Dickerson, 1974; Hackney and Dickerson, 1975; Ferris and Bernard, 1962; Brown and Riedel, 1978). Tobacco and cotton which are reported as good hosts for some other Pratylenchus spp. (Graham, 1951; Hollis, 1956), were not suitable hosts in this experiment.

A potential problem is suggested for growers since several of these good hosts are used as rotation crops with soybeans to control the soybean cyst nematode. Should P. alleni become the principal nematode through cultural management of other nematodes, a serious pest problem might result from the use of several good hosts.

CHAPTER V

CONCLUSIONS

Pratylenchus alleni is widespread in Tennessee soybean fields along with other species of Pratylenchus. All soybean cultivars recommended for Tennessee are suitable hosts for this potential pest. Although P. brachyurus is not known in Tennessee soybean fields, it also reproduces on the recommended soybean cultivars. Cultivars in this study had no discernible effect on proportions of males or adults of P. alleni. Soybean breeders should consider adding tolerance to Pratylenchus spp. into new cultivars. Because of the wide distribution of P. alleni, rotation recommendations should be based on the use of crops which are poor hosts for P. alleni. This could minimize population increases that might affect subsequent soybean plantings.

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LITERATURE CITED

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