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Pratylenchus, Paratylenchus, Helicotylenchus, and Other Nematodes on Soybean in Missouri[1]

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Abstract: Eighteen species in eight genera of plant-parasitic nematodes were identified from soil samples collected from soybean fields throughout Missouri. The genera represented were *Helicotylenchus, Heterodera, Hoplolaimus, Meloidogyne, Paratylenchus, Pratylenchus, Tylenchorhynchus,* and *Xiphinema.* Three fields, each with high densities of *Helicotylenchus pseudorobustus, Pratylenchus hexincisus,* or *Paratylenchus projectus,* were planted in 1989 with six soybean cultivars, with plots of each cultivar either not treated or treated with 5.43 kg/ha aldicarb, to determine whether economically important relationships existed. In none of the sites were nematode densities affected by either aldicarb treatment or cultivar, nor were seed yields related to nematode densities; however, mean seed yield was significantly lower in the *P. projectus* site. In 1990, seed yield was negatively correlated ($r = -0.34$, $P < 0.05$) with *P. projectus* density at planting. Based on the present and previous studies, *H. pseudorobustus* and *P. hexincisus* do not appear to be of economic interest on soybean, but *P. projectus* probably deserves more study.
Key words: Glycine max, Helicotylenchus, Heterodera glycines, lesion nematodes, nematode, *Paratylenchus,* pin nematode, plant-parasitic nematode, *Pratylenchus,* soybean, spiral nematode.

Soybean, *Glycine max* (L.) Merr., is the most economically important row crop in Missouri, particularly in the northern and central; part of the state, where about 80% of the soybeans are produced (22). The principal soybean pathogen in north Missouri is the soybean cyst nematode, *Heterodera glycines* Ichinohe, which was first identified from the region in 1976 (E. Palm, pers. comm.). Although the occurrence of other soybean pathogenic nematodes in north Missouri is probable and would be of interest, no systematic surveys have been conducted to determine their presence or distribution. A list of species associated with soybean in north Missouri was compiled from records kept since 1988. These species were identified from samples taken in soybean research sites and from samples collected from soybean fields and submitted through Agricultural Extension offices to the University of Missouri-Columbia nematology laboratory. This is the first report of some of these species in Missouri.

A number of plant-parasitic nematodes have been reported associated with soybean in north central states other than Missouri, but very few have been studied further (16). In a survey of plant-parasitic nematodes at a research farm in Missouri, three soybean fields were identified with relatively high densities of lesion (*Pratylenchus*), pin (*Paratylenchus*), or spiral (*Helicotylenchus*) nematodes. Species in each genus are common in soybean fields, and occasionally are observed in high densities when *H. glycines* is not present. Research plots were established in these fields to determine whether the host–parasite relationships warranted further investigation to evaluate their economic importance.

MATERIALS AND METHODS

The nematology laboratory at the University of Missouri-Columbia provides "complete nematode profiles" to growers requesting such analysis of soil samples submitted either directly to the laboratory or through University Extension Specialists (referred to hereafter as "extension samples"). The profiles comprise the generic identities and densities of all plant-parasitic nematodes extracted from a 100-cm^3 subsample, along with an indication of whether the occurrence or density of an individual genus is cause for concern. Similar profiles are prepared for all plant-

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parasitic nematode research sites. From 1988–91, profiles were completed on 312 extension samples and 38 research sites. For all vermiform plant parasites, samples were standardized by water displacement to 100 cm^3 and processed by semiautomatic elutriation (4) and centrifugal flotation (21). Root segments were removed by hand from the samples, placed in a 5% streptomycin sulfate solution and shaken for 48 to 72 hours for extraction of endoparasites (3). Root segments were then dried and weighted. Plant-parasitic nematodes were identified to genus at ×40 magnification. Adult females were hand picked and processed to glycerin (21) for species identification. For identification of *Meloidogyne* spp., seedlings of *Lycopersicon esculentum* Mill. cv. Rutgers were transplanted into infested soil and allowed to grow for 45 days, at which time species were identified based on the perineal patterns exhibited by 10 females (5). Identification of *Heterodera* spp. was based on observations and measurements of cysts and second-stage juveniles extracted from original soil samples (11).

Soybean fields at the University of Missouri Agronomy Research Farm near Columbia were surveyed for the identities and densities of plant-parasitic nematodes. The genera *Pratylenchus, Paratylenchus,* and *Helicotylenchus* were frequently identified in low densities in mixed populations and in association with *H. glycines;* however, in some fields in which *H. glycines* was not observed, there were relatively high densities of one or more other plant-parasitic genera. Three fields were chosen for establishment of research plots, with respective infestations of *Pratylenchus hexincisus* Taylor and Jenkins, a mixture of *Paratylenchus projectus* Jenkins (91%) and an unidentified *Paratylenchus* sp. (9%), or *H. pseudorobustus* (Steiner) Golden. Soil characteristics were determined by the University of Missouri Regional Soil Test Laboratory.

The *P. hexincisus* site was a field in which soybeans had been grown in 3 of the previous 10 years in rotation with corn, with a preplant density of 80 adults and juveniles/100 cm^3 soil. Soil characteristics were 16.4% sand, 57.0% silt, and 26.6% clay; 2.2% organic matter; and pH 6.0. Low densities (less than 10 per 100 cm^3 soil) of *Helicotylenchus* sp., *Hoplolaimus* sp., *Paratylenchus* sp., *Tylenchorhynchus* sp., and *Tylenchus* spp. were also detected in the preplant sample.

The *P. projectus* site was a field in which soybeans had been grown continuously for the previous 3 years, following 2 years of wheat. The preplant density of adult and juvenile *P. projectus* was 400 per 100 cm^3 soil. Soil characteristics were 18% sand, 51.4% silt, and 30.6% clay; 2.0% organic matter; and pH 5.9. Low densities (less than 10 per 100 cm^3 soil) of *Pratylenchus* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., and *Tylenchus* spp. were also detected in the preplant sample.

The *H. pseudorobustus* site was a field with a 7-year continuous soybean history, with a preplant density of 150 *H. pseudorobustus*/100 cm^3 soil. Soil characteristics were 15.2% sand, 46.6% silt, and 38.2% clay; 2.0% organic matter; and pH 6.1. Low densities (less than 10 per 100 cm^3 soil) of *Hoplolaimus* sp., *Paratylenchus* sp., *Tylenchorhynchus* sp., and *Tylenchus* spp. were also detected in the preplant sample.

Two approaches were taken to determine the relationships between soybean and the dominant plant-parasitic nematodes in each field: application of aldicarb to reduce initial nematode densities and use of cultivars with previously reported differential reactions to species of the genera tested. The cultivars chosen were from maturity groups (MG) III and IV: Williams 82 (MG III), a popular cultivar from Williams, which was susceptible to three *Pratylenchus* species (20) and a host for *H. pseudorobustus* (18); BSR 301 (MG III), a better host for *P. hexincisus* than either Pickett or Peking in a greenhouse test (25); Fayette (MG III), a popular *H. glycines*-resistant cultivar that was a good host for *P. projectus* in greenhouse tests (Niblack, unpubl.); Pickett (MG IV), and Peking (MG IV), previously used in tests of all three nematode species (15,25); and Sherman (MG III), a poor host for *P. projectus* in greenhouse tests (Niblack, unpubl.). Chamberlain (MG III) was substituted for BSR 301 at one site due to limited seed availability.

The treatment design was a 3 (sites) × 5 (cultivars) × 2 (with and without nematicide) factorial. Within sites, treatments were arranged in five randomized complete blocks. Treated plots had 5.43 kg/ha aldicarb applied in-furrow at planting. Plots were four rows spaced 76 cm apart, 6.1 m long in the *P. hexincisus* and *H. pseudorobustus* sites and 4 m long in the *P. projectus* site due to limited space. The *P. hexincisus* and *H. pseudorobustus* tests were each conducted for 1 year, planted on 25 May 1989. The *P. projectus* test was conducted for 2 years, planted on 25 May 1989 and 6 June 1990. Plant heights at late midseason (R5-R6 growth stage) and harvest, maturity date, and seed yields were recorded for each plot. Plots were harvested with a plot combine after all cultivars reached harvest maturity. Seed yield data were adjusted to 13% moisture and standardized to g/m row to account for the differences in plot sizes.

Soil samples were collected from each plot at planting for initial populations (Pi), at late midseason (growth stage R5-R6), and at harvest for final populations (Pf). A 2.5-cm-d soil probe was used to collect 8–10 soil cores to a depth of 20 cm from the center two rows of each plot. Cores were bulked, and 100-cm^3 subsamples and root segments were processed as described above. Extraction efficiency for *Pratylenchus* was approximately 45% but was not determined for *Paratylenchus* or *Helicotylenchus;* nematode data were not adjusted for extraction efficiency. Plant-parasitic nematode individuals were identified to genus at ×40 magnification. Adult female *Paratylenchus, Pratylenchus,* and *Helicotylenchus* were hand picked and identified to species.

Statistical analyses appropriate to the experimental design were performed with SAS/PC (SAS Institute, Cary, NC). The level of significance for testing was $P = 0.05$.

Results

Eighteen plant-parasitic nematode species were identified from samples collected from soybean fields in Missouri (Table 1). In addition, at least five different species of *Tylenchus s.l.* were observed but not identified to species. Mean densities and ranges were not reported herein because no information is available on how samples were taken. In extension samples from fields infested with *H. glycines,* and in profiles prepared for fields used for study of the *H. glycines*–soybean interaction, *H. glycines* was always the dominant plant parasite present. Usually, three to five other plant-parasitic nematode genera were present in low densities (range 10–50 per 100 cm^3).

In the 1989 sites, seed yields were significantly lower in the *P. projectus*-infested site than in other two sites (Table 2); however, because variances associated with sites were heterogeneous, additional analyses were conducted within sites. Harvest maturity dates differed among cultivars, depending on maturity group, and were not

TABLE 1. Plant-parasitic nematode species associated with soybean in Missouri, 1988–1991.

Helicotylenchus pseudorobustus (Steiner) Golden†
H. dihystera (Cobb) Sher
Heterodera glycines Ichinohe†
H. trifolii Goffart
H. schachtii Schmidt
Hoplolaimus galeatus (Cobb) Thorne
Meloidogyne hapla Chitwood†
M. incognita (Kofoid and White) Chitwood†
Paratylenchus projectus Jenkins
Pratylenchus agilis Thorne and Malek
P. alleni Ferris†
P. hexincisus Taylor and Jenkins
P. penetrans (Cobb) Filipjev and Schuurmans-Stekhoven
P. scribneri Steiner
Tylenchorhynchus claytoni Steiner
T. acutus Allen
T. maximus† Allen
Xiphinema americanum Cobb

† Species previously reported from Missouri by V. H. Dropkin (2), not necessarily in association with soybean.

TABLE 2. Effect of nematode infestation and treatment with aldicarb on yields (g/m row) of soybean cultivars at three sites, each infested with lesion (*Pratylenchus hexincisus*), pin (*Paratylenchus projectus*), or spiral (*Helicotylenchus pseudorobustus*) nematodes, respectively, at the Agronomy Research Center near Columbia, Missouri, in 1989.

	Lesion		Pin		Spiral	
Cultivar	T†	NT	T	NT	T	NT
BSR 301	202	180	181	172		
Chamberlain					221	197
Fayette	192	171	197	187	204	172
Peking	54	71	58	59	114	73
Pickett	101	108	107	84	88	117
Sherman	227	228	187	196	201	148
Williams 82	200	189	199	174	215	159
LSD 0.05‡	15		15		33	
Treatment mean§	163	158 ns	158	145 ns	174	144*
Site mean#	160		152*		159	

† T = treated with aldicarb at 5.43 kg/ha, NT = not treated.
‡ Least significant difference ($P < 0.05$) among cultivar × treatment means within sites.
§ Significance of F test ($P < 0.05$) for treatment within sites is indicated by ns = not significant or * = significant.
# Yield mean for the site indicated by * was significantly lower than the other two sites; however, site variances were heterogeneous ($P < 0.01$), and further comparisons did not pool sites.

affected by nematicide treatment. Seed yields within sites were affected by cultivar and nematicide treatment (Table 2); plant heights differed according to the same effects (data not given). In none of the 1989 sites was seed yield significantly correlated with nematode densities, but there was a correlation ($r = -0.34$; $P < 0.05$) between seed yield and *P. projectus* Pi in the 1990 test.

In each site, the nematode of interest was confirmed to be the dominant plant-parasitic species present (Table 3). Correlation coefficients (r) for soil densities of all plant-parasitic nematodes with total (juvenile + adult) *P. hexincisus*, *P. projectus*, and *H. pseudorobustus* were +0.48, +0.96, and +0.99, respectively ($P < 0.0001$ for each r). Total plant parasites at harvest were lower in the *H. pseudorobustus* site (mean = 128 per 100 cm^3, CV = 36%) than in either the *P. hexincisus* of *P. projectus* sites (276 and 245 per 100 cm^3, CV = 73% and 46%, respectively). At no site was Pf affected by cultivar (Table 3) or nematicide treatment, and Pf/Pi ratios for soil densities were not significantly different from 1.0. At midseason, however, *P. hexincisus* were extracted from root segments at a mean of 719/g dry root weight, with a range of 0 to 8,333 (CV = 190%), irrespective of cultivar or nematicide treatment. There was no relationship between root densities of *P. hexincisus* and any of the plant parameters measured. Ratios of juvenile to adult *P. hexincisus* differed between soil and roots.

In 1989, *P. projectus* densities increased from a mean of 400 per 100 cm^3 soil at

TABLE 3. Effect of soybean cultivar on populations of lesion (*Pratylenchus hexincisus*), pin (*Paratylenchus projectus*), or spiral (*Helicotylenchus pseudorobustus*) nematodes at harvest, at the Agronomy Research Center near Columbia, Missouri, in 1989.

	Lesion			
Cultivar	Soil†	Root‡	Pin†	Spiral†
BSR 301	114	0	500	
Chamberlain				100
Fayette	152	160	462	123
Pickett	138	178	320	137
Peking	135	196	422	124
Sherman	101	36	491	110
Williams 82	149	0	417	148
CV%	38	352	73	33
Percentage of total plant-parasitic nematodes	53	67	91	97
Juvenile: adult ratio	1.0	4.8	9.9	nd§

† Density of adults and juveniles per 100 cm^3 soil.
‡ Density of adults and juveniles per g dry root.
§ nd = not determined.

planting to a midseason level of 586, then decreased to 202 at harvest. The non-stylet-bearing fourth-stage juvenile (J4) represented 91% of the population at harvest, compared with 20% at midseason and 46% at planting. About 50% of the population overwintered, with the J4 representing 92% of the population in the 1990 preplant samples. Average soil densities in 1990 increased to 170 per 100 cm^3 at midseason and decreased to 120 at harvest. Meanwhile, detection of *H. glycines* increased from 13% of the plots at planting in 1989 to 67% of the plots at harvest in 1990. Densities of *H. glycines* at harvest in 1990 ranged from 0 to 480 eggs per 100 cm^3 soil but were not significantly correlated with seed yields or *P. projectus* densities.

Discussion

The association of a plant-parasitic nematode species with a particular host through field sampling does not demonstrate a host–parasite relationship; however, all of the nematode species observed from Missouri soybean field samples (Table 1) have previously been reported associated with soybean in other states (12,16, 19,23). The reported association of *Heterodera schachtii* with soybean by Hori (cited in 12) was probably due to "lumping" in the genus *Heterodera* in the first part of the 20th century (13). Various weeds in the Chenopodiaceae and Cruciferae are native to Missouri (1) and can be found in soybean fields, where they could serve as hosts to *H. schachtii* if soybean itself is not a host. Plant-parasitic nematode communities in north Missouri generally fit the taxonomic profile of other north central states (6,10, 14,19,23).

Diversity of plant-parasitic nematodes in soybean fields tends to be low. In general, it is lower in agroecosystems than in undisturbed systems (17), and in Missouri, diversity is lower under soybean than other crops. In addition, soybean field soil samples in which a pathogenic nematode (such as *H. glycines*) is missing tend to have fewer total plant parasites. For example, in the present study, the *H. pseudorobustus* site had been planted to soybean for at least 7 of the previous 10 years, and had lower total plant parasites compared with the other two sites in which soybean had been planted only 2 or 3 of the previous 10 years (Table 3). *Helicotylenchus pseudorobustus* individuals also represented a higher percentage of total plant-parasitic nematodes, compared with the other two sites (Table 3). This pattern is frequently seen in Missouri extension samples from soybean. The significance of the low diversity and density of plant parasites in soybean is unclear, and is difficult to document from extension samples because the researcher has no control over how the samples are taken. The trend seems clear enough to warrant further study with controlled sampling. The increasing distribution of *H. glycines* (about 60% of soybean fields in Missouri) will present a challenge to such study, however, because *H. glycines* displaces the dominant plant-parasitic nematode in a field. Because of this problem, two of the sites for the studies reported herein were abandoned after one season when *H. glycines* was detected. The third site was abandoned after the second season when the average density of *H. glycines* approached the damage threshold (500 eggs/250 cm^3 soil) established for northern Missouri (Niblack and Smith, unpubl.).

The average number of individuals per plant-parasitic nematode genus in a Missouri soybean field ranged from 10–50 per 100 cm^3 soil; thus, the population densities in the *P. hexincisus, P. projectus,* and *H. pseudorobustus* sites were considered high enough to be useful in exploratory field studies. Nematode densities were not affected by the application of aldicarb, nor did the soybean cultivars used support differential reproduction, in contrast to results reported from greenhouse studies (15,18,25). Furthermore, in 1989, densities of the nematodes were not correlated with seed yields. A pathogenic relationship did not appear to exist in these three fields; however, seed yields in the *P. pro-*

jectus site were lower than those in the other two sites, a difference that could not be explained readily by soil type or other differences among the sites. In 1990, there was a low negative correlation between *P. projectus* Pi and seed yield, but the presence of increasing densities of *H. glycines* made the correlation difficult to interpret, even though there were no discernible relationships between *H. glycines* densities and *P. projectus* densities or seed yields. Attempts to study the *P. projectus*–soybean interaction in the greenhouse failed for various reasons related to difficulty obtaining large quantities of the nematode in culture, but this interaction deserves more study. *Paratylenchus projectus* densities were high in Illinois soybean fields with a continuous soybean history, compared with corn fields or soybean following corn (9). In Iowa, high *P. projectus* densities were associated with reduced seed yield of a *H. glycines*-resistant soybean cultivar in a *H. glycines*-infested field (Niblack, unpubl.). The sensitivity of *P. projectus* to large numbers of another plant parasite, expressed as a reduction in its rate of reproduction, was demonstrated in an interaction study including *Criconemoides simile* (Cobb) Chitwood and *H. pseudorobustus* (15), and may explain the lower densities observed in the 1990 *P. projectus* test.

As noted above, Missouri plant-parasitic nematode communities in soybean reflected those previously reported for north central states. Observations on nematode densities and life histories also agreed with previous report. In an Illinois study (7), *P. projectus* densities varied over 2 years with a proportion of J4 at harvest that was similar to those observed in the *P. projectus* study reported herein. *Helicotylenchus pseudorobustus* occurs frequently in soybean fields, and although it has not been demonstrated as a pathogen on soybean, soybean is a host, and the nematode may build up to a level potentially damaging to corn (18). *Pratylenchus hexincisus* increased on soybean in greenhouse tests (25), and it is also potentially damaging to corn (24). There are conflicting conclusions as to the effect of crop rotation on soybean-parasitic nematodes in the north central region (8, 9,19); however, most of the available evidence, including the present study, confirms the inadvisability of planting soybean continuously and illustrates the potential nematode problems that may result from an exclusive corn–soybean rotation.

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