Pathogenicity and Reproduction of *Pratylenchus agilis* in Field Microplots of Soybeans, Corn, Tomato, or Corn-Soybean Cropping Systems

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ABSTRACT

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Pratylenchus agilis-infested microplots were established in a fumigated field. Three-year cropping rotations of continuous corn, soybeans, or tomatoes; alternate corn and soybeans; 2 yr of soybeans followed by corn; or 2 yr of corn followed by soybeans were used to evaluate their effects on *P. agilis* populations and crop yields. Crops were rated according to their host suitability for increasing nematode populations as follows: I.O. Chief (corn) > Marglobe (tomato) > Williams (soybean) > Essex (soybean). Essex was a poor host, and continuous cropping to soybeans did not result in yield losses. Continuous cropping to corn and tomatoes, or 2 yr in corn followed by soybeans, resulted in crop damage in the third year. In excised root tissue cultures, *P. agilis* is capable of ectoparasitic as well as endoparasitic feeding. Possible plant-parasitic relationships between *P. agilis, P. scribneri*, and *P. hexincisus* are discussed.

Additional key words: feeding behavior, lesion nematodes

Thorne and Malek (10) described *Pratylenchus agilis* from prairie soil in North Dakota but presented no information about its hosts or host preferences. Rebois et al (5) reported significant soybean yield increases after nematicide treatments where *P. agilis* occurred along with other root-lesion and spiral nematodes. The fields used in that study had been maintained in a corn-soybean rotation. *P. agilis* was also found in several soybean fields in Maryland that showed signs of severe root damage (4). This study was done to determine the effects of *P. agilis* on soybean, corn, and tomato yields and to determine the host status of those crops. A preliminary report was published (6).

MATERIALS AND METHODS

In May 1975, single seeds of either soybean (*Glycine max* (L.) Merr. cv. Essex) or corn (*Zea mays* L. cv. Golden Bantam) were germinated in 10-cm plastic pots. After seed germination, 25 pots of each crop were infested with a single *P. agilis* female. The *P. agilis* used in these studies were taken from an infested field near the Wye River in Maryland and increased on corn in the greenhouse. Specimens are deposited with the USDA Nematode Collection in Beltsville, MD. To reduce the chances of leaching nematodes, saucers were placed under the pots and they were watered from below. Eleven days later, soil and plant from each infested pot were transferred to a 20-cm (3.8-L) clay pot in the greenhouse. Greenhouse temperatures ranged from 18 to 38 C. Sixteen to 20 mo later, about 100 ml of soil was removed from each of the single-female-infested pots, and the nematodes were extracted by the flotation-seiving method. Nematodes from each infested pot were killed and fixed to verify species. In order to increase the *P. agilis* population to infest the microplots, 10–20 progeny from each of the single-female cultures were subcultured on their respective hosts in 20-cm clay pots that were maintained in the greenhouse for an additional 2.5 yr.

Microplots were established in a sandy loam field, pH 5.9–6.1, that had been deep-plowed, rototilled, leveled, and fumigated. When fumigated in late May 1979, the soil moisture in the top 40 cm varied from 9 to 12%. Methyl bromide (98%) and chloropicrin (2%, Dowfume MC-2) was applied to the field at the rate of 1.46 kg/10 m^2 (3 lb/100 ft^2). The fumigant was deposited 30 cm deep with tractor-drawn chisels spaced 30 cm apart. Immediately after fumigation, the field was completely covered for 3 days with a polyethylene tarp. Before use in the fumigated field, all machinery and equipment was steam-cleaned or dipped in one part Clorox to three parts water (1.3% sodium hypochlorite), and personnel wore protective covering to help prevent the introduction of new organisms. A total of 112 fiberglass microplots, 78 cm in diameter and centered 3 m apart, were established after the method of Barker et al (2).

To infest microplots, individual 1-mo-old seedlings of corn cultivar I.O. Chief, tomato (*Lycopersicon esculentum*) cultivar Marglobe, or soybean cultivars Essex or Williams grown in 10-cm plastic pots were infested with about 560 *P. agilis*. Corn plants in a high-infestation treatment each received about 1,120 *P. agilis* per pot. The inoculum for all treatments was a mixture of populations derived from 10 single females subcultured on Golden Bantam. The controls received supernatant water from which nematodes had settled out. Twelve days later, four either infected or control plants were transplanted into each of the respective microplots. Thus the potential infestation of each microplot was 2,200 or 4,400 (high infestation for corn only) *P. agilis* females and larvae.

The following 3-yr crop rotations were used: 1) continuous I.O. Chief corn at low and high infestations; 2) continuous Essex soybean; 3) continuous Williams soybean; 4) continuous Marglobe tomato; 5) 2 yr of Essex followed by I.O. Chief; 6) 2 yr of I.O. Chief followed by Essex; 7) corn, Essex, and corn; or 8) Essex, corn, and Essex. After the first year, weeds were controlled with a 5% glyphosate spray before planting and plots were hand-weeded during the growing season. *Rhizobium japonicum* (strain 110) was added to all plots, and fertilizers were applied at the recommended rates.

In May 1980, 15 seeds of corn or soybean or three seedlings of tomato were planted in each plot to bioassay for nematodes. The germinated seedlings and small tomato transplants were removed from the soil 2 wk later and the roots examined for *P. agilis* (11). Soil counts for nematodes were taken in May 1980 and in November of 1980 and 1981. Five soil cores 30 cm deep × 25 mm in diameter were taken from each plot. The soil from each plot was composited and 150 ml was processed by elutriation (3).

Tomato and soybean yields were determined in 1980 and 1981, but the corn plots were considered too small for reliable corn grain yields. In 1981, corn

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Table 1. Effects of cropping sequences on *Pratylenchus agilis* soil populations

Cropping sequence[x]			Nematodes per 150 ml of soil[y]		
1979	1980	1981	May 1980	Nov. 1980	Nov. 1981
E	E	E	0.0	13 a[z]	151 a
E	C	E	0.1	969 b	166 a
C	C	E	0.8	1,232 b	290 a
W	W	W	0.1	64 a	744 b
C	E	C	1.7	126 a	1,175 b
E	E	C	0.8	22 a	1,239 b
C	C	C	0.5	1,211 b	1,259 b
C	C	C (2X)	7.5	1,663 b	1,337 b
T	T	T	11.5	795 b	1,149 b

[x]C = corn cultivar I.O. Chief; soybean cultivars: E = Essex, W = Williams; T = tomato cultivar Marglobe; 2X = high initial *P. agilis* infestation rate.
[y]Soybean cultivar Essex had significantly fewer *Pratylenchus* than cultivar Williams in 1980 and 1981 ($P = 0.05$, ANOVA comparison of two means). *P. agilis* populations in continuous Essex and Williams increased significantly between 1980 and 1981 ($P = 0.05$, ANOVA).
[z]Column figures followed by different letters are significantly different ($P = 0.05$) according to Duncan's new multiple range test based on the log (soil populations + 0.05).

Table 2. Effects of crop rotations on *Pratylenchus agilis* populations and soybean yields

Cropping sequence[a]				Yield/plot (g)	Nematodes per 150 ml of soil[b]	
1979	1980	1981	Treatment	1981	1980	1981
C	C	E	Infected	187	1,232	290
C	C	E	Control	231*[c]	0	7
			LSD 0.05	37		
E	C	E	Infected	257	968	166
E	C	E	Control	240	0	0
			LSD 0.05	54		
E	E	E	Infected	235	13	151
E	E	E	Control	210	0	0
			LSD 0.05	55		
W	W	W	Infected	163	64	744
W	W	W	Control	155	0	0
			LSD 0.05	48		

[a]C = corn cultivar I.O. Chief; soybean cultivars: E = Essex and W = Williams.
[b]Soybean cultivar Essex had significantly fewer *Pratylenchus* than cultivar Williams in 1980 and 1981 ($P = 0.05$, ANOVA comparison of two means).
[c]* = Significantly different from the respective control ($P = 0.05$, ANOVA).

Table 3. Effect of *Pratylenchus agilis* on Marglobe tomato yields

	Yield (g)/Microplot[a]		Nematodes/150 ml of soil	
Year	1980	1981	1980	1981
Infected	8,440	2,780	795	1,149
Control	10,250	4,190*[a]	1	210[b]
LSD 0.05	3,600	1,420		

[a]* = Significant ($P = 0.10$, ANOVA).
[b]One and four of six control plots infested in 1980 and 1981, respectively.

Table 4. Effect of cropping sequence and *Pratylenchus agilis* populations on height of corn plants 6 wk after planting

Cropping sequence[a]				Height/plot (cm)	Nematodes per 150 ml of soil	
1979	1980	1981	Treatment		1980	1981
E	E	C	Control	24.1*[b]	0	71
E	E	C	Infected	22.6*	20	1,239
C	E	C	Control	24.6*	0	20
C	E	C	Infected	22.1	121	1,175
C	C	C	Control	22.8*	0	68
C	C	C	Infected	21.3	1,211	1,259
C	C	C (2X)	Infected	18.5	1,663	1,337
			LSD 0.05	3.8		

[a]C = corn cultivar I.O. Chief, E = soybean cultivar Essex, and 2X = high initial *P. agilis* infestation rate.
[b]* = Significantly better growth than the high-nematode-infestation treatment ($P = 0.05$, ANOVA).

height was recorded 6 wk after planting.

Plots were established in a randomized complete-block design and replicated six times. All treatments were paired with their respective controls. Duncan's new multiple range test and analysis of variance (ANOVA) were used to analyze the data (8). Nematode soil populations were log-transformed for statistical analysis.

Sterile *P. agilis* cultures were established on I.O. Chief, Essex, and Williams root explants grown on Gamborg B-5 (7).

RESULTS

Sixteen months after pots were infested with single females, *P. agilis* were recovered from 18 of 25 pots of corn and 0 of 25 pots of soybeans. After four additional months of incubation, *P. agilis* were recovered from four of 25 infested pots of soybeans. In March 1979, the female soil population density of *P. agilis* on corn was more than 100 times greater than that in pots with soybeans.

In May 1980, nematode soil populations were very low (Table 1). *P. agilis* was not detected in the controls or 80% of the infested plots using the short-term root bioassay. However, all infested plots were *P. agilis*-positive in November.

The cropping system used had a significant effect on *P. agilis* soil populations (Tables 1–4). Tomato and corn were good hosts, whereas *P. agilis* population development in soybean plots was cultivar-dependent. Continuous cropping of corn and tomatoes increased *P. agilis* populations significantly more than did soybeans in 1980 (Table 1). Continuous Essex suppressed nematode populations significantly more than did Williams (Table 2) in the 1980–1981 seasons. At the end of the third growing season, final *P. agilis* populations were significantly lower ($P = 0.05$) in Essex plots regardless of the cropping system used. Although the 1981 *P. agilis* populations were lower in Williams than in corn and tomato plots, the difference was not significant. *P. agilis* populations increased significantly between 1980 and 1981 in both continuous Essex and Williams plots. Crop yields were not significantly affected in 1979 (not reported) and 1980 (Tables 2–4). Two years of corn preceding Essex resulted in the only significant soybean loss in infested plots (Table 2). The high *P. agilis* inoculum level significantly reduced the height of corn plants over that of the controls (Table 4). Two years of Essex preceding corn significantly increased corn height over the high- but not the low-infestation levels.

Tomato yields were lower in *P. agilis*-infested plots (Table 3) in the second year and missed the generally accepted level ($P = 0.05$) of significance in 1981 by less than 1% (10 g).

Observations on monoxenic root explants indicate *P. agilis* can complete its life cycle and feed very effectively without penetrating the roots of corn, soybeans, or tomato (Fig. 1). They may also be found in large numbers in the root cortex.

This experiment was terminated in the third year because *P. agilis* contamination had reached detectable levels in 14 of 48 control plots (Tables 2–4). *P. hexincisus* Taylor & Jenkins was also reaching detectable levels in 14 of 39 infested I.O. Chief, Marglobe, and Williams plots but not in the Essex plots. *Paratylenchus* sp. was the first plant parasite to become reestablished in about 7% of the fumigated plots. A few plots contained non-plant-parasitic Dorylaimidae and Aphelenchidae. Rhabditoid species soon reached detectable levels in all plots.

DISCUSSION

These results clearly indicate that the buildup of *P. agilis* population levels can vary greatly depending on the crop, cultivar, or cropping sequence used. The results also demonstrate that *P. agilis* field populations can reach levels that will damage corn, soybean, and tomato. *P. agilis* populations can increase sufficiently on two successive corn plantings to reduce Essex soybean yields the following year. In designing crop rotations to maintain *P. agilis* populations below damaging thresholds, it will be necessary to take into account the cropping sequence and the particular cultivar.

The data suggest that the rate of *P. agilis* population buildup on the crops tested over the 3 yr ranks (from highest to lowest) as follows: I.O. Chief > Marglobe > Williams > Essex. Our preliminary observations (5) and unreported results on monoxenic root explants support these field observations.

Of interest is the fact that *P. agilis*, *P. scribneri* Steiner, and *P. hexincisus* are often found in the same field in Maryland (4). Morphologically, these three species are closely related and many of their characters overlap. However, certain features can be used to distinguish these forms, and *P. hexincisus* also has two additional incisures in its lateral field. Other similarities are found in their host preferences and method of reproduction. All three species reproduce well on corn and tomato and do better on Williams than on most other soybean cultivars tested (1,6,12). These observations may prove useful in developing some crop resistance and cultural and other control strategies that are common to the three nematode species. The relationship of the three species to each other and to other lesion nematodes needs to be better understood.

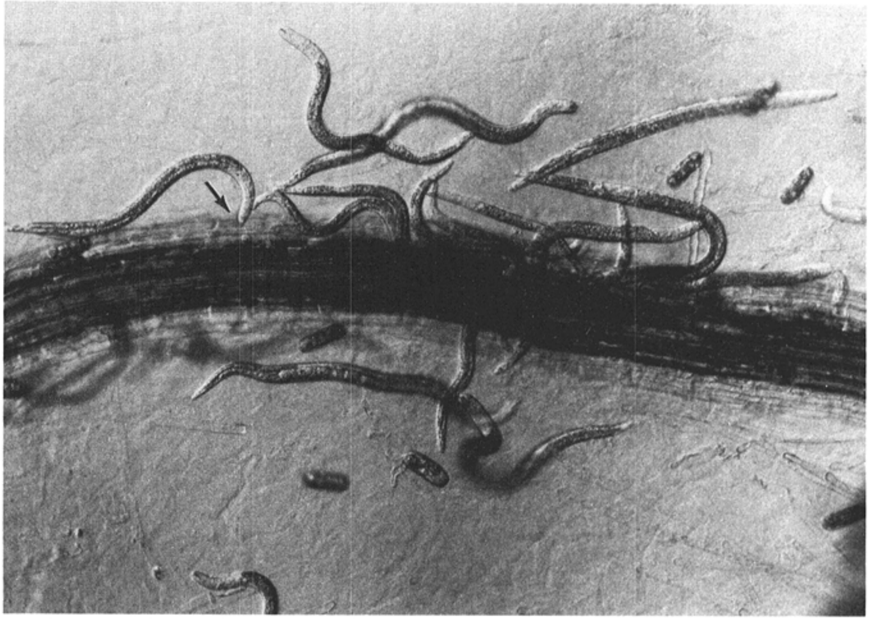

Fig. 1. Ectoparasitic feeding (arrow), migration, and egg laying of *Pratylenchus agilis* on sterile corn root explant.

The initially slow but eventually large population buildup in Williams plots over the years also needs further investigation. Is it just part of a normal sinusoidal population increase or does this parthenogenic species eventually adapt to feed more efficiently on a new host? The latter is morphologically feasible considering the feeding habits of *P. agilis* in root-tissue culture and the potential morphological variations encountered from single-female populations of *P. penetrans* (9).

We believe this is the first report of extensive ectoparasitic feeding on epidermal cells by any lesion nematode (*Pratylenchus* spp.). Since ectoparasitic feeding was observed only in agar cultures on root explants (7), further studies are under way to determine if this feeding behavior occurs on roots grown in soil.

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