Journal of Nematology 18(3):392–397. 1986.

Population Dynamics, Root Penetration, and Feeding Behavior of *Pratylenchus agilis* in Monoxenic Root Cultures of Corn, Tomato, and Soybean[1]

R. V. Rebois and R. N. Huettel[2]

Abstract: Population dynamics, rate of root penetration, and external root feeding behavior of *Pratylenchus agilis* (Pa) in monoxenic cultures of intact corn seedlings and root explants of corn, tomato, and soybean were studied. In descending order of suitability as hosts were I. O. Chief corn, Rutgers tomato, and Williams soybean. Soybean entries Kent, Pickett 71, PI 90763, and Essex were poor hosts. Numbers of eggs and vermiform Pa in the agar medium indicated total fecundity and host suitability. Agar, sand, or soil as support media did not appear to affect Pa root penetration, but the rate of corn root growth did. Whereas most vermiform Pa and eggs were in roots, substantial numbers appeared able to feed and complete their life cycle as ectoparasites on root epidermal cells and root hairs.

Key words: corn, ectoparasite, feeding behavior, *Glycine max*, host, lesion nematode, *Lycopersicon esculentum*, population dynamics, *Pratylenchus agilis*, resistance, root population, soybean, technique, tissue culture, tomato, *Zea mays*.

Pratylenchus agilis Thorne and Malek, 1968 (Pa) has been associated with soybean yield suppressions of 30% (4,7). In microplot studies (8,9), the Pa population development from good to poor hosts was as follows: corn (*Zea mays* L. cv. I. O. Chief) > tomato (*Lycopersicon esculentum* Mill. cv. Marglobe) > soybean (*Glycine max* (L.) Merr. cv. Williams) > soybean cv. Essex. Three years of continuous cropping to Essex in microplots did not suppress yields. However, continuous cropping to corn or tomato, or 2 years of corn followed by Essex, resulted in damage in year 3 when the initial Pa infestation was one nematode per 150 cm^3 soil.

Our objectives were 1) to determine in vitro population dynamics of Pa on various hosts under controlled conditions, 2) to determine if the feeding behavior was primarily ectoparasitic or endoparasitic, and 3) to compare the influence of sand, soil, and agar as support materials for root penetration by Pa.

Materials and Methods

Unless otherwise stated all operations were performed aseptically using sterile materials and demineralized water. All cultures were incubated at 28 C in the dark. Nematodes used in these studies were

Received for publication 3 July 1985.

[1] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products or vendors that may also be suitable.

[2] Nematologist and Microbiologist, Nematology Laboratory, Plant Protection Institute, USDA ARS NER, Beltsville, MD 20705. Present address of senior author: 2701 Muskogee Street, Adelphi, MD 20783.

The authors thank B. L. Craddock for technical assistance.

TABLE 1. *Pratylenchus agilis* eggs observed in agar or recovered from roots of corn, tomato, and soybean root cultures grown in the dark at 28 C.

Cultivar	In agar medium				In roots after 6 wk		
	2 wk	4 wk	6 wk	N*	Per dish	Per g root	N*
I. O. Chief	15.8 a	44.0 a	508.3 a	8	7,197 a	18,393 a	5
Rutgers	18.8 a	82.0 a	352.0 a	9	880 b	2,516 b	5
Williams	4.2 b	8.7 b	48.4 b	8	1,117 b	1,232 c	5
Kent	4.6 b	6.1 bc	3.8 c	9	482 bc	372 cd	5
Pickett 71	2.1 bc	5.8 bc	13.0 c	6	378 bc	307 cd	5
PI 90763	1.2 cd	1.7 cd	5.3 c	10	228 bc	220 d	5
Essex	1.6 c	3.2 d	8.0 c	9	159 c	328 cd	5

Column numbers followed by different letters are significantly different ($P = 0.05$) according to Duncan's new multiple-range test using transformed data, $\log_{10}(\text{No.} + 1)$.
* Number of replications observed at 6 weeks.

progeny of single Pa females cultured on root explants of I. O. Chief corn (5,10).

Population dynamics: Treated seed (95% ethanol for 1–2 minutes followed by 5.25% NaOCl : water [1:9] for 10–15 minutes) were germinated in the dark at 30 C for 2–3 days on water agar. Two excised root tips of corn or soybean and three of tomato, 2–3 cm long, were then transferred to 100 × 15-mm plastic petri dishes containing 35 ml of Gamborg's B-5 media minus auxins or cytokinins (GB-5) with 2% sucrose and 1.5% Difco Noble agar (3,10).

I. O. Chief corn, Rutgers tomato, and soybean cultivars Williams, Kent, Pickett 71, PI 90763, and Essex were used. Treatments were replicated 10 times and arranged in a randomized complete block design. After 2 weeks at 28 C, each root culture was infested with ca. three eggs and 80 mixed stages of Pa juveniles and adult females (collected from 10–12-week-old stock cultures) in 0.5 ml aqueous suspension.

Eggs and vermiform nematodes observed outside of roots were counted 2, 4, and 6 weeks after infestation (WAI). At 6 WAI, intact roots from five replications of each treatment were removed from the medium after softening the agar in the dishes placed in a hot water bath. Roots were blotted dry, weighed, and fixed for 45 seconds in boiling glycerol, 85% lactic acid, and water (1:1:1) solution containing 0.05% acid fuchsin (2). Roots were destained in the same solution lacking acid fuchsin for up to a week at 22 C. The fixed roots were macerated at high speed for three 10-second intervals in 25 ml water using a 50-ml stainless steel jar on a Waring Blender. Between maceration intervals, any tangled roots were freed from the blender blades. Nematodes and eggs in three 5-ml aliquots from 50 ml homogenate were counted to determine total root populations.

Root penetration: GB-5 was used as the nutrient source in the three support materials tested including 1.5% agar; acid washed, rinsed, and neutralized quartz sand; or soil (22% sand, 21% clay, 57% silt, pH 6.8). The following treatments were used: 1) petri dish (PD) with 80 g sand, 18 ml GB-5, and an excised corn root (ER); 2) PD with 35 ml GB-5 in 1.5% agar and ER; 3) 2.3 × 19.5-cm culture tubes (CT) with 30 g sand, 6.5 ml GB-5, and one intact corn seedling (IS); 4) CT with 30 g soil, 6.5 ml GB-5, and IS; 5) CT with 25 ml GB-5 in 1.5% agar and IS. Treatments were replicated 10 times and incubated for 3 or 9 days after infesting (DAI) in culture dishes and tubes with a ca. 52 eggs and 1,170 vermiform Pa in 0.5 ml water. Five replications were removed at 3 and 9 DAI. Roots were weighed, fixed, and macerated as described for population dynamics.

Feeding behavior: Ectoparasitic nematode feeding was observed directly in root cultures using a dissecting microscope at 10–40 × and an inverted microscope equipped with Hoffmann interference optics, television monitor, camera, and time lapse recorder at magnification of 40–400 ×.

Statistics: Data were analyzed by ANOV and Duncan's new multiple-range test (11). Nematode and egg numbers data were log transformed before statistical analysis. Mean numbers of nematodes penetrating roots per culture and root weights data

TABLE 2. Vermiform *Pratylenchus agilis* observed in agar or recovered from roots of corn, tomato, and soybean root cultures grown in the dark at 28 C.

Cultivar	Nematodes in agar				Nematodes in roots after 6 wk		
	2 wk	4 wk	6 wk	N*	Per dish	Per g root	N*
I. O. Chief	8.3 a	62.3 a	389.8 a	8	3,647 a	8,700 a	5
Rutgers	11.0 a	77.2 a	281.1 a	9	435 b	1,251 b	5
Williams	5.5 a	38.7 ab	67.8 b	8	627 b	688 bc	5
Kent	7.4 a	30.3 bc	22.8 c	9	318 bc	232 c	5
Pickett 71	5.1 a	24.2 bc	21.2 c	6	215 bc	187 c	5
PI 90763	7.5 a	22.6 bc	21.9 c	10	221 bc	216 c	5
Essex	6.2 a	12.2 c	22.9 c	9	103 c	252 c	5

Column numbers followed by different letters are significantly different ($P = 0.05$) according to Duncan's new multiple-range test using transformed data, $\log_{10}$(No. + 1).
* Number of replications observed at 6 weeks.

were subjected to regression analysis. The equation for the linear regression was $Y = a + bx$ and for the power curve $y = ax^b$, where a and b are regression coefficients.

RESULTS

Population dynamics: Two weeks after infection, the numbers of eggs found in the agar (Table 1) in relation to the type of host were different ($P = 0.05$) but the numbers of vermiform nematodes in agar were not (Table 2). The numbers of eggs observed in the agar 2 WAI in corn and tomato cultures were greater than in any of the soybean treatments. The treatments ranked in about the same order 4 and 6 WAI on the basis of numbers of eggs and vermiform nematodes in the agar.

The numbers of eggs and vermiform nematodes recovered from the roots of cultures were several times greater than those found in agar medium 6 WAI (Tables 1, 2). I. O. Chief was the best host when only numbers of eggs or vermiform nematodes per culture were considered, but the relative ability of Pa to reproduce in root cultures of other plants was not obvious until total numbers (Table 3) and weight of host tissue were considered together (Tables 1–3). The linear regression correlation coefficient ($r = 0.69$, $a = 25.4$, $b = 519.9$) for total numbers of eggs and vermiform nematodes per gram of root tissue for Kent, Pickett 71, PI 90763, and Essex soybeans was highly significant ($P = 0.01$) (Table 3).

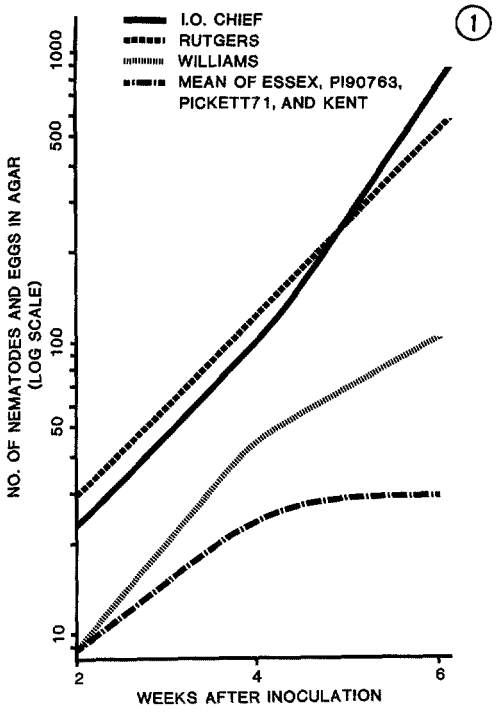
Plots of time against the log of numbers of eggs plus vermiform nematodes observed in the agar from cultures of I. O. Chief, Rutgers, and Williams were relatively linear between 2 and 6 WAI (Fig. 1). Rates of nematode population growth tended to slow between 4 and 6 WAI in the less suitable soybean hosts Essex, PI 90763, Pickett 71, and Kent (Fig. 1, Tables 1–3).

Root penetration: Root growth was significantly ($P = 0.05$) better in agar cultures than in the corresponding sand cultures (Table 4). Roots of intact seedlings in culture tubes grew faster than corresponding excised roots cultured in petri dishes. Root invasion 3 DAI averaged less than 1% of the number of nematodes in the inoculum. Nine days after infection, nematode root penetration and root growth were significantly greater ($P = 0.05$) in cultures with intact seedlings than in corresponding excised root cultures. The number of nematodes and eggs per culture was directly proportional to root weight. When the numbers of nematodes inside roots were

TABLE 3. Total numbers of eggs and vermiform *Pratylenchus agilis* from corn, tomato, and soybean root explant cultures 6 weeks after infestation with ca. three eggs and 80 vermiform nematodes.

Cultivar	Average no./ culture	Root wt. (g)/ culture	Average no./ g root tissue
I. O. Chief	11,973 a	0.39 cd	30,700 a
Rutgers	2,010 b	0.34 cd	5,911 b
Williams	1,864 bc	0.84 bc	2,207 c
Kent	835 bc	1.36 a	614 d
Pickett 71	634 cd	1.18 ab	539 d
PI 90763	486 cd	1.04 ab	468 d
Essex	292 d	0.56 cd	525 d

Column figures followed by different letters are significantly different ($P = 0.05$) according to Duncan's new multiple-range test using transformed data, $\log_{10}$(No. + 1). Means of five replications.



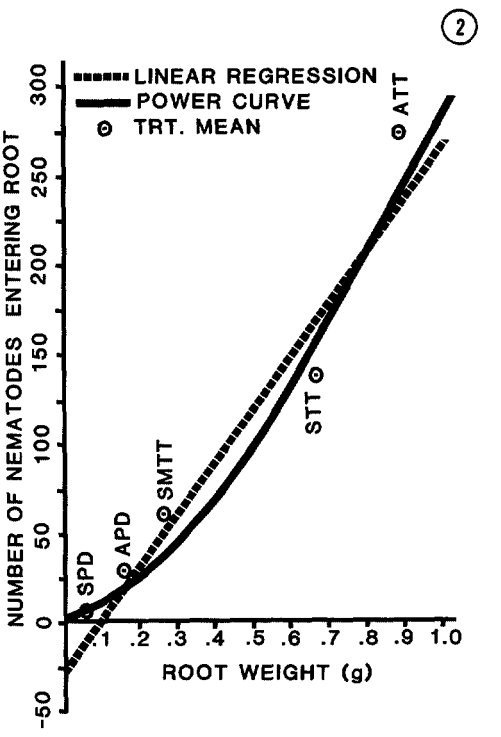


FIG. 1. Numbers of vermiform *Pratylenchus agilis* and eggs observed in agar 2, 4, and 6 weeks after infesting root explant cultures of I. O. Chief corn, Rutgers tomato, and five soybeans—Williams, Kent, Pickett 71, PI 90763, and Essex—with ca. three eggs and 80 vermiform nematodes.

FIG. 2. Correlations between *Pratylenchus agilis* root penetration and fresh root weights as related to five different culture types (linear regression coefficient $r = 0.90$ and power curve correlation coefficient $r = 0.84$). Type cultures: SPD = sand in petri dish. APD = agar in petri dish. SMTT = soil mix in test tube. STT = sand in test tube. ATT = agar in test tube.

compared to root weights, the data fit for the linear regression curve was $r = 0.90$ (a = −24.2, b = 287.4) and for the power curve $r = 0.84$ (a = 347, b = 1.9), which are highly significant ($P = 0.01$) positive correlations (Fig. 2). The total numbers of eggs and vermiform nematodes per gram of root 9 DAI also had highly significant ($P = 0.01$) correlation coefficients of $r = 0.85$ (a = −19.7, b = 563.6) for linear regression and $r = 0.83$ (a = 695.5, b = 1.7) for the power curve.

Feeding behavior: All stages of Pa fed ectoparasitically on root epidermal cells and root hairs. To locate an external feeding site, the nematode browsed along the root, often stopping and positioning its head perpendicular to the root surface and making contact with its labia and oral aperture. This was followed by probing a spot with the stylet on the outer wall of an epidermal cell or root hair. Often several sites on a cell or cells were probed before feeding was initiated. The stylet probing rate was ca. 1–2 thrusts per second. Penetration of the outer cell wall by the stylet tip occurred with as few as 10 stylet thrusts, but often the stylet failed to penetrate the cell wall. The feeding process was initiated when an estimated 1–2 μm of the stylet tip had penetrated the cell wall and membrane and contacted the cytoplasm. The nematode's body remained immobile throughout feeding. When the stylet tip contacted the cytoplasm, the nematode secreted some material into the cell causing a rapid surge of cytoplasmic components away from the stylet tip. During the next 1–3 minutes, a small hyaline area or feeding structure (FS) appeared to envelop the stylet tip inserted into the cell or root hair. Formation of the FS was followed by a rapid pumping (350 ± 50 pulses per minute as observed in 10 specimens) of the metacorpus valve as ingestion began. During feeding the rate of cytoplasmic streaming at the feeding site

TABLE 4. Effect of culture support media on root growth and *Pratylenchus agilis* (Pa) root penetration 3 and 9 days after infesting (DAI) corn root explant cultures with ca. 52 eggs and 1,170 vermiform nematodes, replicated five times.

Treatment*	Support medium	3 DAI	9 DAI			
		Pa + eggs/root	Root wt. (g)	Vermiforms in root/culture (n)	Eggs in root/culture (n)	Total eggs and vermiforms (n)
CT–IS	Agar	2.0 a	0.899 a	273.6 a	249.3 a	522.9 a
CT–IS	Sand	2.0 a	0.680 a	134.3 ab	343.2 a	477.5 a
CT–IS	Soil	1.3 a	0.273 b	59.9 b	52.4 b	112.3 b
PD–ER	Agar	7.7 a	0.167 b	14.4 c†	67.5 b	81.9 b
PD–ER	Sand	1.0 a	0.052 b	3.2 d	4.1 c	7.3 c

Column figures followed by different letters are significantly different ($P = 0.05$) according to Duncan's new multiple-range test using transformed data, $\log_{10}$(No. + 1).

* CT–IS = culture tube with intact seedling. PD–ER = petri dish with excised root.

† Agar culture had significantly ($P = 0.05$) more nematodes penetrating than the corresponding sand culture.

and in adjacent cells increased slightly. Feeding at a single site lasted from less than a minute to several hours. Nematodes appeared to adhere to feeding sites, as many disengaged only with difficulty. Nematodes were often dislodged from feeding sites by other browsing nematodes. The FS remained in the cell when nematodes detached from feeding sites. In heavily parasitized areas, one or more FS were observed in single cells. Nematodes were observed feeding at several sites during all parts of their migratory life cycles. Defecation and egg laying occurred periodically while the nematodes were feeding. Initially, external feeding did not appear to cause major changes or damage to cell structures and organelles. Cell contents in heavily parasitized areas were disrupted, cells appeared flaccid, and nematodes readily migrated in and out of damaged roots. While feeding, some nematodes partially penetrated 2–4 root cortical cells deep in lightly grazed areas; nematodes sometimes backed out of lesions and other nematodes entered the vacated lesions. Nematodes often congregated in small areas to feed on roots.

DISCUSSION

Suitability of hosts for Pa reproduction in monoxenic root explant cultures confirmed field observations (8,9). Stock Pa cultures used as the source of nematode inoculum were maintained on corn root explants; thus these populations are possibly better adapted to I. O. Chief corn than to other plants used through morphological (12) or physiological differences. However, when Pa was first obtained from the field to establish stock cultures in the greenhouse, host suitability for the crops tested ranked the same as that found in the present and other studies (8,9).

I. O. Chief corn on GB-5 (10) is the best combination found to date for culturing Pa because the seeds are easily sterilized, the roots support the largest Pa populations per gram of tissue, populations double about every 6 days at 28 C, and the cultures are relatively maintenance free for up to 3 months. In excess of 500,000 Pa were recovered from single 10–12-week-old excised root cultures.

Numbers of nematodes entering roots of a single cultivar were significantly related to the total root weight, whereas the support media had little or no effect under these conditions. This fact, along with the extensive external feeding observed in cultures, the positive correlation between total root tissues produced, and fecundity on poor soybean hosts (Kent, Pickett 71, PI 90763, Essex), suggests Pa ectoparasitism is a common occurrence and not the result of the agar support media inhibiting penetration. Thus ectoparasitic feeding may be expected to occur under natural growing conditions.

Lack of nematode body movement during external feeding appears to be essential to prevent detachment from the feeding cell and feeding structure. The origin (or mechanisms) of FS formation was not determined, but it appears to be related to feeding tubes, pegs, or plugs associated with many other nematodes (6). Extensive external feeding by vermiform Pa and the occurrence of all life stages simultaneously

and in large numbers without frequent entering or exiting of young roots suggest that this nematode can complete its life as an ectoparasite.

Based on total Pa reproduction per soybean culture, Kent and Williams produced significantly greater populations than Essex. Based on total nematode and egg reproduction per gram of root tissue in culture, Williams was the best soybean host, whereas Kent, Pickett 71, PI 90763, and Essex all supported significantly smaller but similar populations (541 ± 73/g root). Total fecundity for the poor soybean hosts was positively correlated with root weight, as was the number of nematodes found in I. O. Chief corn roots.

One advantage of excised root cultures is that nematode behavior and population dynamics can be observed directly and more easily than under field condition. Using Pa for screening for resistance may have some relevance also to other nematodes such as *P. scribneri* and *P. hexinisus,* since both have been reported (1,13,14) to reproduce well on corn and tomato and better on Williams than on other common soybean cultivars tested as hosts for these species.

Literature Cited

1. Acosta, N., R. B. Malek, and D. I. Edwards. 1979. Susceptibility of soybean cultivars to *Pratylenchus scribneri.* Journal of Agriculture of the University of Puerto Rico 63:103–110.

2. Bridge, J., S. L. Page, and S. M. Jordan. 1981. An improved method for staining nematodes in roots. P. 173. Rothamsted Report for 1981, Rothamsted Experimental Station, Harpenden, Herts., England.

3. Gamborg, O. L., T. Marashige, T. A. Thorpe, and I. K. Vasil. 1976. Plant tissue culture media. In Vitro 12:473–478.

4. Golden, A. M., and R. V. Rebois. 1978. Nematodes on soybeans in Maryland. Plant Disease Reporter 62:430–432.

5. Huettel, R. N., and R. V. Rebois. 1985. Culturing plant parasitic nematodes using root explants. Pp. 155–158 *in* Laboratory manual for nematologist. Amherst, Massachusetts: University of Massachusetts Agricultural Experiment Station.

6. Rebois, R. V. 1980. Ultrastructure of a feeding peg and tube associated with *Rotylenchulus reniformis* in cotton. Nematologica 26:396–405.

7. Rebois, R. V., J. Feldmesser, and R. C. Leffel. 1976. Effects of three nematicides on nematode populations and soybean (*Glycine max* L. Merr) yields in two Maryland fields. Proceedings of the American Phytopathological Society 3:300.

8. Rebois, R. V., and A. M. Golden. 1984. *Pratylenchus agilis,* Thorne and Malek, 1968, population dynamics and pathogenicity on corn, soybean and tomato (Abstr. 200). Proceedings of the First International Congress of Nematology, Aug. 5–10, Guelph, Ontario, Canada. Pp. 75–76.

9. Rebois, R. V., and A. M. Golden. 1985. *Pratylenchus agilis,* Thorne and Malek, 1968, pathogenicity and reproduction in field microplots of soybeans, corn, tomato or corn-soybean cropping systems. Plant Disease 69:927–929.

10. Rebois, R. V., R. N. Huettel, and J. A. Lauritis. 1984. A comparison of media for the monoxenic culture of corn cyst, soybean cyst, reniform, root-knot and lesion nematodes on root explants (Abstr. 201). Proceedings of the First International Congress of Nematology, Aug. 5–10, Guelph, Ontario, Canada. P. 76.

11. Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. New York: McGraw-Hill.

12. Tarte, R., and W. F. Mai. 1976. Morphological variation in *Pratylenchus penetrans.* Journal of Nematology 8:185–195.

13. Zirakparvar, M. E. 1980. Host range of *Pratylenchus hexincisus* and its pathogenicity on corn, soybean and tomato. Phytopathology 70:749–753.

14. Zirakparvar, M. E. 1982. Susceptibility of soybean cultivars and lines to *Pratylenchus hexincisus.* Journal of Nematology 14:217–220.