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Differences in Egress of Male and Female *Pratylenchus penetrans* from Pea Roots[1]

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Pratylenchus spp. are migratory endoparasites of plants. Despite being obligate parasites, however, a significant proportion of the population can be recovered outside of roots throughout the year (7,8). *Pratylenchus agilis* Thorne & Malek (10), *P. crenatus* Loof (5), and *P. penetrans* (Cobb) Filipjev & Schuurmans Stekhoven (Wixted, unpubl.) can feed ectoparasitically, indicating that movement out of roots does not necessarily prohibit feeding.

Our objective was to determine if movement out of roots differs between the sexes of *P. penetrans.* Differences in the rate of root penetration among the sexes were previously reported (13). Under certain conditions, males of *P. penetrans* comprised a larger proportion of the total soil population than did other stages (9). However, whether this was due to more males than other stages having left roots, or whether males comprised most of the total soil and root population, was not clear from their studies. Sex-specific differences in movement within hosts do occur with *Nippostrongylus brasiliensis* (Travassos) Travassos & Darriba in the mouse intestine (4), where males disperse over a larger percentage of the intestine than do females in single-sex inoculations.

Two experiments were conducted using an in vitro system. *Pratylenchus penetrans* from monoxenic corn (*Zea mays* L. cv. IO Chief) root explants grown in Gamborg's medium (2) were used. Inoculum consisted of 40 males or 40 females which had been axenically transferred into a sterile BPI dish containing 0.5 ml sterile water.

Pea seeds (*Pisum sativum* L. cv. Early Perfection 8221) were surface sterilized by immersing them in 70% ethanol for 5 minutes, followed by 20 minutes in 10% NaOCl, and then rinsing in sterile water. Seeds were germinated in petri dishes containing 1.2% water agar and incubated at 24 C in the dark for 5 days. Roots from seedlings of uniform size were then cut 3 cm from the root tip and the explants were placed on 1.2% water agar, one root per petri dish. Roots were incubated in the dark at 24 C for 24 hours before nematodes were added.

All nematodes from one BPI dish were pipetted in a 10-μl drop of water onto the surface of the agar 1 cm from a root and counted to ensure that all 40 had been delivered. Additional nematodes were transferred into dishes if needed. Roots were incubated in the dark at 24 C.

Seventy-two hours after inoculation, each root was removed from its petri dish and rinsed in sterile water to remove nematodes from the surface, thus ensuring that only nematodes inside roots remained. At this time, some roots were stained (1) and nematodes inside the roots were counted to confirm infection rates. One root inoculated with males was placed in the center of a petri dish containing 1.2% water agar, with one female-inoculated and one uninoculated root placed 1 cm on either side of, and parallel to, the male-inoculated root. The three roots were then covered with additional water agar so that they were totally submerged. Ten replicate petri dishes were prepared.

Dishes were incubated in the dark at 24 C for 5 days and then viewed under a dis-

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TABLE 1. Effect of *Pratylenchus penetrans* on pea root lengths and lesions per root 5 days after transferring roots to fresh petri dishes containing 1.2% water agar.

Inoculated sex	Experiment 1		Experiment 2	
	Root length (mm)	Root lesions	Root length (mm)†	Root lesions
Control	44.2	0.0	33.6	0.0
Male	43.9	0.2	29.2	0.8
Female	41.3	10.0	36.4	9.2
$P =$	0.52	0.01	0.01	0.01

Data are means of 10 replicates in experiment 1 and 5 in experiment 2.

Parameters with mean of zero were not included in statistical analyses.

† Roots inoculated with males were significantly shorter than control or female-inoculated roots (LSD = 3.9) in experiment 2.

TABLE 2. Location of *Pratylenchus penetrans* 5 days after transferring root explants infected with males, females, or no nematodes (control) to fresh petri dishes containing 1.2% water agar.

Location†	Experiment 1		Experiment 2	
	Males	Females	Males	Females
Away from root	10.1	0.0	4.0	0.6
In female root	10.4	29.6	4.4	22.4
On female root	0.3	0.2	0.0	0.4
In male root	6.5	0.4	9.0	0.2
On male root	0.1	0.0	0.2	0.0
In control root	0.3	0.0	0.2	0.0
On control root	0.1	0.0	0.0	0.0
LSD ($P = 0.05$)	3.1	1.9	2.5	5.3

Data are means of 10 replicates in experiment 1 and 5 in experiment 2.

Parameters with mean of zero were not included in the statistical analysis.

† Away from root refers to nematodes in agar but not on the surface of a root; nematodes detected by staining a root were considered to be in the root, whereas those collected by rinsing a root were considered to be on the root.

secting microscope. Nematodes that had moved out of the roots and into the agar (away from the surface of a root) were counted and sexed. Lesions on roots also were counted. Each root was removed, measured, and rinsed in a separate tube of water to collect nematodes from the root surface. These nematodes were categorized as on the root and were not included in counts from agar. The roots were then stained (1) and nematodes were counted. All nematodes recovered outside the explant to which they were originally added were considered to have egressed.

The experiment was repeated as described above except that roots of 4-day-old pea seedlings cut 2 cm from the root tip were used and were arranged differently on petri dishes. As before, one uninoculated, one male-inoculated, and one female-inoculated root were placed in each petri dish, but they were arranged radially so that all roots were equidistant from each other. The distance between root explants ranged from 3.5 cm at the tips to 7.0 cm at the cut ends. The petri dishes were prepared, incubated, and harvested as described for the first experiment. Ten replicate petri dishes were prepared, but because of contamination, data were collected from only five.

The proportions of nematodes that moved away from roots, entered roots inoculated with the opposite sex, or remained within initial roots were compared among the sexes using one-way analysis of variance (12). Parameters with means of zero were not included in the analyses. Within each sex, the numbers of nematodes recovered from the various habitats also were compared using one-way analysis of variance.

During the course of the experiments, roots grew approximately 50% of their initial length (Table 1) and were suitable hosts for the nematodes. Infection, based on the number of nematodes recovered per dish (27.8 males and 30.2 females in the first experiment and 17.8 males and 23.6 females in the second) was comparable to that reported on intact seedlings (13), and some nematodes infected adjacent roots after exiting the root to which they were originally inoculated (Table 2). Eggs were present in roots inoculated with females (data not shown). Also, lesions developed on inoculated roots (Table 1), suggesting that nematodes were feeding. Thus, the behavior of *P. penetrans* in these experiments probably was not altered by lack of suitable feeding sites.

More males ($P = 0.0001$) exited than remained in roots initially inoculated with

males in the first experiment, whereas in the second experiment numbers of males that exited the root did not differ from those that remained (Table 2). In each experiment, the estimate of remaining males included two that probably left and re-entered the same root, as indicated by their position within that portion of the root that grew after being transferred. Of the males that exited roots in both experiments, about half were located in agar away from roots. Interestingly, of those males that were associated with roots, nearly all migrated to the female-inoculated root, with less than 5% infecting the uninoculated root. Approximately 90% of males in the female roots were less than 1 mm from at least one female. This contrasts with earlier work with *P. penetrans* which found neither attraction of males to females nor an effect of females on penetration of roots by males (13).

Unlike males, fewer females ($P = 0.0001$) exited roots than remained therein (Table 2). If females collected from new root growth (i.e., tissue greater than 3 cm from the cut end) were considered to have exited and re-entered, then the average number that exited would increase from 0.6 to 3.0 in the first experiment and from 1.2 to 2.2 in the second experiment.

Under the conditions and duration of this experiment, males were more likely ($P = 0.0001$) than females to exit host roots and move to the root inoculated with the opposite sex. Similarly, Glassburg et al. (4) noted that females of *N. brasiliensis* tended to remain at suitable feeding sites, whereas males often left such sites if females were not present. Thus, as with other nematode species (3,11), males of *P. penetrans* seem to be attracted to females. However, considering only those nematodes that left roots in the first experiment, 48.7% of males and 70% of females ($P = 0.20$) migrated to the root inoculated with the opposite sex. In the second experiment, 51.6% of males and 16.7% of females ($P = 0.04$) that left roots migrated to the root inoculated with the opposite sex. It seems then that although fewer females leave roots, the behavior of those that do can be affected by males.

The experiment was repeated in sterile soil to confirm that the observed behavior of *P. penetrans* was not peculiar to in vitro conditions. The same methods were used except that intact seedlings rather than root explants were transferred from petri dishes into soil, plants were harvested at 10 days as opposed to 5, and data are the means of 5 instead of 10 replications. No nematodes moved from inoculated to uninoculated (control) roots. One seedling inoculated with males contained one female, whereas males (range = 3–12) were recovered from four of the five seedlings inoculated with females.

The observed egress of males from roots in our studies may have resulted from their attraction to females. However, roots infected with females developed lesions, indicating physiological changes in those roots. Males of *Heterodera glycines* Ichinohe are attracted to several amino acids which may increase in roots infected with nematodes (6). Such physiological changes may attract males of *P. penetrans* to nematode-infected roots, which may be an early step in location of receptive females. Also, movement of males out of a root may be independent of conditions in an adjacent root, but such conditions may influence the behavior of nematodes once they egress.

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