Infectivity of Pratylenchus penetrans on Alfalfa[1]

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Abstract: The infectivity of *Pratylenchus penetrans* on alfalfa seedlings cv. Du Puits was studied. The dense root-hair zone was the preferred zone of penetration by females, males, and third-stage larvae. A lesion initially appeared as a water-soaked area at the root surface, becoming yellow and elliptical as the nematode entered the cortex, with dark-brown cells later appearing in the centre as the nematode fed. At 20 C, females penetrated roots earlier, faster, and in greater numbers than either males or third-stage larvae. Females penetrated roots at temperatures from 5 to 35 C, with maximum penetration between 10 and 30 C, while males and third-stage larvae penetrated roots only between 10 and 30 C with maximum penetration at 20 C. Penetration of roots by females, males, and third-stage larvae increased after storage of 5 C for 35 days, but decreased after storage of 140 days or more. Combinations of the three life stages in pairs neither enhanced nor inhibited penetration of roots by individual life stages; males were not attracted to females. Increasing inoculum density up to 20 nematodes/seedling did not affect penetration.
Key Words: root-lesion nematode, penetration, lesion, *Medicago sativum*.

Few quantitative studies have been made of *Pratylenchus* since the study by Mountain (7). Females of *P. penetrans* were shown to be more infective on alfalfa than are males and second- or fourth-stage larvae (10). Penetration of alfalfa seedlings in soil by adult *P. penetrans* increased arithmetically with nematode numbers in the inoculum over the range of 25 to 1,000/pot (5). On water agar, penetration of red clover seedlings by *P. penetrans* also increased arithmetically at inoculum densities from 2 to 40 per seedling, and the preferred site of penetration was 3–13 mm posterior to the root tip (6). Penetration of corn by *P. brachyurus* and *P. zeae* increased arithmetically with increasing inoculum density only up to 200 nematodes per root, then decreased rapidly as inoculum density increased to 1,000 nematodes per root (8). The optimum temperature for penetration by these two species was 20 C. *P. thornei* had no preferred sites of penetration on roots of wheat (1, 2).

This paper further details some of the extrinsic and intrinsic factors that affect the infectivity of *P. penetrans* on alfalfa.

MATERIALS AND METHODS

The Cornell population of *P. penetrans* originated from a single gravid female reared on alfalfa callus tissue since 1963. Nematodes were extracted aseptically from callus tissue in miniature Baermann pans (11), usually overnight. Life stages for experimentation were hand-picked under a dissecting microscope.

Seed of alfalfa (*Medicago sativum* L cv Du Puits) were surface-sterilized in 37N sulphuric acid, washed in sterile water, and germinated at 20 C in inverted Petri dishes of sterile water agar. Only 72-h-old seedlings with a root 15-22 mm long were used.

Five drops of water, each containing a single nematode or combination of two nematodes of the desired life stage were placed equidistant on the surface of water agar in a 90-mm Petri dish. The root of each seedling was covered with silica sand and moistened with additional water when needed. Aseptic procedures were used. Each replicate was a single dish of five inoculated seedlings. Each root system was examined for a lesion at the conclusion of each experiment.

A randomized block design was used in each experiment and the compiled data were subjected to analyses of variance. An angular transformation was used when necessary.

Some details of individual experiments are described briefly with the results.

RESULTS

The lesion: A clear water-soaked area appeared on the surface of the root within hours as the nematode penetrated the epidermis. A yellow-coloured lesion, elliptical in shape and 1 mm in length, then developed as the nematode fed in the cortex. The long axis of the lesion was parallel to that

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of the root. Later, dark brown cells appeared in the centre of the lesion, and these occasionally developed near or at the margin as the nematode moved to adjacent undamaged cells, thereby altering the shape of the lesion.

A nematode was consistently dissected from each of the many hundreds of lesions examined. Observation on the location of lesions and their number provided the data for a quantitative study of the infectivity of *P. penetrans*.

Zones of penetration on the root: Nematodes penetrated all along the root from behind the root-cap to the junction of the root and hypocotyl. The preferred zone of penetration was the dense root-hair zone, where 61% of the lesions developed. Thirty-two percent of the lesions developed in the area above the root-hair zone, where the root hairs were sparse. Only 4% of the penetrations occurred between the root-hair zone and the root cap, and only 3% in the area immediately below the junction of the root and the hypocotyl. Females, males, and third-stage larvae all preferred the same zones of penetration. Penetration of the root tip by the nematode occasionally impeded root growth. Initiation of lateral roots did not appear to be stimulated by the penetration of a nematode, but such initiation may facilitate nematode entry. Nematodes and lesions were sometimes seen near ruptures of the cortex by secondary roots.

Temperature and penetration: The influence of temperature on the penetration of roots of alfalfa by the three life stages of *P. penetrans* was determined over a temperature range from 5 to 35 C. Seedlings were inoculated singly with females, males, and third-stage larvae. Sufficient plates of seedlings were inoculated to study penetration at seven temperatures with ninefold replication. Incubation was for 72 h.

Females were able to penetrate roots over the entire range of temperatures from 5 to 35 C (Fig. 1). Penetration was maximum (70 to 80%) between 10 and 30 C, dropping sharply at lower and higher temperatures, with only 13% of the females entering the roots at 5 C, and 9% at 35 C. The patterns of penetration of roots by males and third-stage were almost identical (Fig. 1). Males and larvae were able to penetrate roots only between 10 and 30 C,

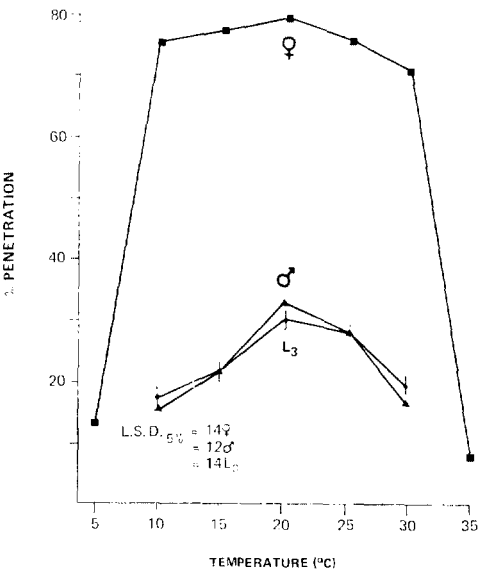

FIG. 1. Infectivity of females, males, and third-stage larvae of *Pratylenchus penetrans* on alfalfa at temperatures from 5 to 35 C using an incubation period of 72 h.

with maximum penetration (32%) at 20 C; at 10 and 30 C only 18% of males and third-stage larvae entered the roots.

Rate of penetration: Penetration of roots by the three life stages of *P. penetrans* was studied over 96 h at 20 C. Alfalfa seedlings were inoculated singly with females, males, and third-stage larvae. Sufficient plates were inoculated to permit sampling of eight replicates every 12 h for 96 h.

Eighty percent of females penetrated the roots in 96 h, but only 43% of males and 30% of larvae. Penetration of roots by the three life stages increased logarithmically with time (Fig. 2). Rates of penetration differed, however, in the order females > males > third-stage larvae. The percentage of penetration of roots began to plateau after 48 h by females, after 60 h by males and after 84 h by third-stage larvae.

Size of subventral oesophageal lobe: Nematodes in a water suspension were killed by raising the temperature to 60 C for 2 min in a hot-water bath. Upon cooling the nematodes were fixed overnight in a 3% formaldehyde solution by adding an equal volume of a 6% formaldehyde solution to the nematode suspension. Fixed specimens of females, males, and third-stage larvae were mounted on slides in lactophenol.

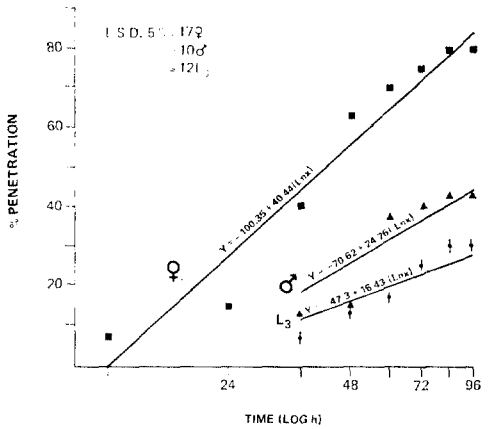

FIG. 2. Penetration of roots of alfalfa by females, males, and third-stage of *Pratylenchus penetrans* over 96 h at 20 C.

The area of the longitudinal section of the subventral oesophageal lobe was determined. The perimeters of the oesophageal lobe and of 10 × 10-μ squares were outlined on heavy bond paper at ×1,800 magnification with a drawing tube. The lobes and squares were cut out and each weighed in mg. Area was calculated as follows:

$$\text{Area in } \mu^2 = \frac{\text{wt of longitudinal section x 100}}{\text{wt of 10 x 10 } \mu \text{ square}}$$

The area of the longitudinal section of the female subventral oesophageal gland was 338 μ^2, over twice that of the male (157 μ^2) and the third-stage larvae (146 μ^2) (LSD 5% = 23).

Penetration of roots by life stages in combination: Alfalfa seedlings were inoculated with single females, males, and third-stage larvae of *P. penetrans* and in all combinations of the three life stages. Inoculated seedlings were incubated 72 h at 20 C; each treatment consisted of 25 seedlings.

The percentage of *P. penetrans* entering the roots of alfalfa when seedlings were inoculated with single nematodes of the three life stages fell within the expected range for an incubation of 72 h at 20 C (Table 1). Paired combinations of the life stages neither enhanced nor inhibited the penetration of roots by each life stage. Both members of a nematode pair were seldom found in a single lesion of the paired combinations; the female and male combination was found most frequently in a single lesion.

Attraction between adult stages: The "Mickey Mouse" maze (9) was used to determine attraction between male and female nematodes. Ten females were placed in one "ear" of the maze, and 10 males in the "nose". Similarly, in a second treatment 10 males were placed in the "ear" and "nose" of the maze, and in a third treatment 10 females in the "ear". Each treatment was replicated 10 times, and the dishes were incubated at 20 C. Observations were made periodically for 8 h.

Males were not attracted to the females. As evidenced by the numerous tracks on the agar surface, however, in all life stage combinations there was considerable movement back and forth between the inoculated "ear" and "nose" before the nematodes penetrated the agar and went their separate ways. The nematodes appeared to home on one another's tracks. Occasionally a nematode would wander into the uninoculated "ear".

Inoculum density and penetration: Two inoculum densities of *P. penetrans* were

TABLE 1. Infectivity to alfalfa roots of three life stages of *Pratylenchus penetrans,* alone and in combination.

Life stages & number of nemas	No. of lesions containing:		% nemas penetrating		
	1 nema	2 nemas	♀	♂	L_3
25 females	18	–	72	—	—
25 males	8	–	—	32	—
25 larvae (L_3)	6	–	—	—	24
25 females & 25 females	30	2	68	—	—
25 females & 25 males	24	3	80	40	—
25 females & 25 larvae	23	2	76	—	32
25 males & 25 males	18	1	—	40	—
25 males & 25 larvae	13	2	—	36	32
25 larvae & 25 larvae	11	1	—	—	26

used to determine the effect of competition for entry sites on the infectivity of *P. penetrans*. Alfalfa seedings were inoculated with a single female or twenty females. The two treatments were replicated eight times, and the inoculated seedlings incubated for 72 h at 20 C.

Respectively 70 and 62% of the females penetrated roots when seedlings were inoculated with one nematode and 20 nematodes. The difference was not statistically significant. Again the preferred zone of penetration was the dense root-hair zone.

Storage and penetration: Throughout this study, nematodes extracted from alfalfa tissue cultures of *P. penetrans* were stored at 5 C in a 0.1% streptomycin solution.

Two experiments were performed to determine the effects of prolonged storage on the infectivity of *P. penetrans*. In the first, seedlings were inoculated singly with females, males, and third-stage larvae picked from nematode suspensions that had been stored for 0, 18, 35, 80, and 141 days. Each treatment was replicated 10 times, and the plates of seedlings were incubated at 20 C for 72 h.

The percentage penetration of roots of alfalfa by the three life stages of *P. penetrans* was greatest after the nematodes had been stored 35 days at 5 C (Fig. 3). For stored nematodes the percentage of females, males, and third-stage larvae was generally similar to that obtained with nematodes which had not been stored. However, less than 15% of the males and third-stage larvae entered the roots after the nematodes had been stored 141 days. The percentage penetration of roots by females after 141 days of storage was the same as that of females after no storage.

In the second experiment, concentrated suspensions of nematodes stored for 0 and 141 days were pipetted onto cores of silica sand (5 × 20 mm long), and the nematodes were allowed to migrate through the sand (150–250 μ particles) into watch glasses for 48 h. (3). Nematodes were collected after 8 and 48 h; those collected after 24 h were discarded. Then, seedlings were inoculated singly with each of the three life stages picked from the 8-h and 48-h collections made from the nematode suspensions stored for 0 and 141 days. The four treatments were replicated 10 times; incubation was for

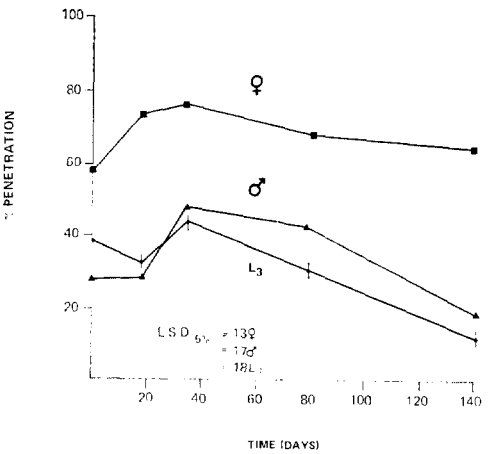

FIG. 3. Infectivity of females, males, and third-stage larvae of *Pratylenchus penetrans* stored at 5C for 0, 18, 35, 80, and 141 days. Infectivity tests on alfalfa were carried out at 20 C for 72 h.

72 h at 20 C.

Females and larvae from nematode suspensions stored 0 and 141 days predominated in collections made after 8 h of migration through cores of sand, while males predominated in collections made after 48 h. Nematodes in the four collections showed various degrees of starvation, with nematodes in the 8-h collection appearing more active than those in the 48-h collection. A much greater percentage of females picked from the 48-h collection made from the stored nematode suspension penetrated alfalfa roots than females picked from the 8-h collection (Table 2). Females picked from both the 8-h and 48-h collections made from the unstored nematode suspension penetrated roots equally well. Males from the four collections also penetrated roots equally well. A greater percentage of third-stage larvae picked from the 8-h collections prepared from unstored and stored nematode suspensions penetrated roots than larvae from the 48-h collections.

A third experiment was performed in which females dissected from infected alfalfa seedlings were placed singly on new alfalfa seedlings. The treatment was replicated eight times; controls were prepared in equal number, in which seedlings were inoculated with females from an unstored suspension of *P. penetrans*. Incubation was for 72 h at 20 C.

Females dissected from lesions on alfalfa

TABLE 2. Percentage penetration of alfalfa roots by three life stages of stored or unstored *Pratylenchus penetrans* which had migrated through sand for 8 and 48 h.

Migration time	Life stages		
	Female	Male	L_3
Unstored			
8 h	74	28	42
48 h	73	30	18
Stored			
8 h	58	43	33
48 h	90	43	15
L.S.D. 5%	15	n.s.	14

roots failed to penetrate new roots, and 80% of these females had died by 72 h.

DISCUSSION

The greater infective capacity of the female of *P. penetrans* is reflected in its ability to penetrate roots earlier, faster, and over a wider range of temperatures than the male and third-stage larvae. Starvation did not appear to inhibit the infective capacity of the female, for the intestinal content was never severely depleted (2). Also, the presence of other life stages was not necessary to assure the females' penetration of a root. The posterior subventral lobe contains the gland for penetrating roots and feeding, and the greater size of this lobe in the female may reflect the maturity of these glands and thus account, in part, for the female's greater capacity to penetrate roots. The glands may produce enzymes in greater quantities and/or at a greater rate than those in the other life stages. The superior infectivity capacity of the females permits *P. penetrans* to escape adverse soil environments (temperature and moisture) and assures its survival in northern climates.

Infectivity of the female, male, and larva is enhanced with prolonged storage up to 35 days at low temperatures above freezing. Less-active females enter roots in greater numbers than more-active females. Perhaps, less-active females settle down on the root surface and start penetrating the root more quickly than do more-active females. In contrast, the more-active third-stage larvae entered roots in greater numbers than less-active larvae. Perhaps starvation, although not observable, may account for the poor infective capacity of less-active larvae (12).

The number of *Pratylenchus* entering roots increases as the inoculum density increases (1, 6, 8). When the inoculum density exceeds 200 nematodes per root, however, the percentage of nematodes entering roots declines (8). Although there is adequate root-surface in a 20-mm length of root to accommodate large numbers of nematodes, the fact that most *Pratylenchus* species, including *P. penetrans,* prefer to penetrate in the root-hair zone may limit the root surface available to the nematodes, thus accounting for the decline in numbers of nematodes entering a root as density exceeds 200 per root. The preference for the root-hair zone may have a biochemical basis involving attraction, or a physical basis allowing easier appression to root surfaces where root hairs are enmeshed with soil particles.

Females dissected from alfalfa roots failed to reenter roots and died after 72 h, apparently of shock; there was no physical injury to these nematodes. Yet, females that have migrated from roots readily reentered roots. The nematode possibly undergoes periods of differing physiological activity that affects its ability to survive sudden adverse conditions.

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