Effect of Age of Alfalfa Root on Penetration by *Pratylenchus penetrans*

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Abstract: Penetration by all migratory life stages of *Pratylenchus penetrans* into roots of alfalfa (*Medicago sativa* L. cv. Du Puits) was inversely proportional to tissue age. Two-day-old tissue in the root hair zone was penetrated twice as much as 10- or 20-day-old sections of the tap root. Age-related differences were also observed in branch roots; these differences were not affected by increasing the number of nematodes from 1 to 10 per inoculation site, nor by increasing the length of the incubation period from 6 to 96 h. Age-related differences were only significant with 3-wk-old plants, not with 2- and 1-wk-old seedlings. Nematodes entered roots at temperatures from 5 to 30 C with maximum entry at 20 C and minimum at 5 C. At all temperatures, except 5 C, penetration into young tissue (2 days) was significantly greater than into medium (10 days) and old (20 days) tissue. Females and third-stage larvae entered the different-aged root sections 122% and 83%, respectively, more than did males. Two-day-old seedlings of the alfalfa cultivars Vernal, Saranac, and Du Puits were penetrated equally by *P. penetrans*. Perhaps the inverse relationship between penetration and age of root is, in part, responsible for the increasing resistance or tolerance of plants to nematode damage as they grow older. *Key words:* root-lesion nematode.

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Nematode damage to plants often is inversely proportional to seedling age at time of inoculation (1,2,7,10,11,16,17,19,20,21, 25). Some workers (10,11,21) attribute this to rapid root growth that results in a decrease in nematode density in the infected plant. Jaffee and Mai (10) speculated that host physiological or anatomical changes associated with maturation increased resistance or tolerance in older plants or that the deep roots in older plants are not infected and may compensate for damaged shallow roots. Ogbuyi (17) suggested that resistance in mature plants might result from thickening or suberization of cell walls.

Adults and migratory larval stages of *P. penetrans* are capable of penetrating all parts of the host root (14,22,23). However, it is not known what effect root age (stage of development) has on the rate of penetration. The existence of host age-related differences in penetration could help explain the increase in tolerance associated with plant age.

This study was undertaken to (i) determine the effect of age of alfalfa root tissue on penetration by different life stages of *P. penetrans* and (ii) determine the influence of substratum used for growing roots, length of incubation time, and temperature on age-related differences in nematode penetration.

MATERIALS AND METHODS

The nematode: The origin and method of rearing *P. penetrans* has been reported (18). Nematodes were extracted from heavily infested corn (*Zea mays* L.) roots in a mistifier and stored in water for periods of up to 4 wk at 5 C.

Growth of alfalfa seedlings, nematode inoculation and staining, and experimental design: Alfalfa (*Medicago sativa* L. cv. Du Puits) seeds were surface-sterilized for 30 min. in 37 N sulphuric acid, rinsed in sterile cold water, germinated, and grown at 21 C and 65 hlx in diSPo-pouches (Scientific Products, AMSC, 1210 Leon Place, Evanston, Ill.) moistened with Hoagland's solution. One day prior to inoculation seedlings were removed from the pouches and placed on the surface of 1% water agar in a 140-mm Petri dish. Some trimming of the top and lateral roots was required to fit five seedlings into a dish. Immediately before inoculation, a drop of water (5–8 µl, applied with a 26-gauge needle attached to a syringe) was placed on the agar next to the tap root. One nematode was placed in the water drop with a No. 1 root canal file and the inoculated root segment covered with about 50 mg of glass beads (approx. 125 µm in diam. delivered with a vibrating spatula). After incubation at 21 C for 72 h

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sections of inoculated roots were excised, washed with water to remove the beads, and immersed in boiling lactophenol for approx. 30 s. The inoculated root segments were then stained in Poirrier Blue (0.0005%) in cold lactophenol (8) for 5–7 days and examined under the microscope for the presence of nematodes. In most experiments, 10 Petri dishes (replicates), each with five seedlings, were used per treatment. Data were subjected to a split plot analysis or X^2 – test.

Rate of growth of alfalfa roots and anatomical studies: Lengths of tap roots of 62 alfalfa seedlings, grown for 25 days in pouches, were measured at regular intervals and data plotted against time. Fresh free-hand sections of different-aged parts of tap roots were mounted in lactophenol on microscope slides and diameters of root and stele determined with an image-splitting eyepiece.

Effect of alfalfa cultivar, substratum, lateral root sites, and inoculum density: To determine the effect of alfalfa cultivar on penetration, 2-day-old seedlings of the cultivars Du Puits, Vernal, and Saranac, grown on agar, were inoculated in the root-hair zone with 1 ♀ *P. penetrans*/root and incubated for 96 h. To determine whether percentage penetration of seedlings grown in paper pouches differed from those grown on water agar, 2-day-old Du Puits seedlings grown on either substratum were inoculated with 1 ♀ *P. penetrans*/root and penetration determined after 96 h of incubation. To study the effect of soil as a substratum, alfalfa was grown for 3 wk in Vineland loam in the greenhouse. After careful washing, the root systems were placed on agar and young, medium, and old root segments of each tap root inoculated with 1 ♀ *P. penetrans*/segment.

To compare penetration into different-aged segments of lateral and tap roots, alfalfa seedlings were grown for 3 wk in pouches, then placed on a layer of water agar in plastic boxes of 25 × 30 cm. Tip (young) and mid-region (medium) segments of either root type were inoculated with 1 ♀ *P. penetrans*/segment. To determine the effect of inoculum density, young, medium, and old root segments of 3-wk-old pouch-grown seedlings were inoculated with 10 ♀ *P. penetrans*/segment.

Age of seedling and penetration: To further define the effect of seedling age, alfalfa seedlings were grown either in pouches or on water agar for periods of 3, 2, and 1 wk and placed on the surface of agar. Each tap root was then inoculated near the tip (young), in the middle (medium), and near the crown (old) with 1 ♀ *P. penetrans*/segment.

Effect of incubation period, temperature, and life stage: Young, medium, and old root segments of 3-wk-old pouch-grown alfalfa seedlings were each inoculated with 1 ♀ *P. penetrans* and incubated for 6, 12, 24, 36, 48, 72, and 96 h. Similarly grown and inoculated seedlings were incubated for 72 h at 5, 10, 15, 20, 25, and 30 C. To determine the effect of nematode life stage and sex, young, medium, and old root segments of 3-wk-old pouch-grown seedlings were each inoculated with a single specimen of females, males, or third-stage larvae and incubated for 72 h at 21 C.

RESULTS

Rate of growth of alfalfa roots and anatomical studies: Alfalfa tap roots increased in length at an exponential rate, but growth in pouches ceased after 20 days (Fig. 1). Measurements of cross-sections of roots which were 2 days (young), 10 days (medium), and 20 days (old) showed that the proportion of the area occupied by the cortex was much higher ($P = 0.05$) in the young root segments than in medium and old segments (Table 1).

Effect of alfalfa cultivar, substratum, lateral root sites, and inoculum density: No differences in percentage penetration of *P. penetrans* into the alfalfa cultivars Du Puits (66%), Vernal (62%), and Saranac (64%) were observed. Similarly, percentage penetration of seedlings grown in paper pouches (52%) was not statistically different from that of seedlings reared on water agar (66%). With soil-grown seedlings, penetration of young tissue (42%) was highest but did not differ statistically from that of medium (30%) or old root tissue (28%). Entry of the tip (35%) and mid-region of lateral roots (28%) did not differ ($P = 0.05$) from penetration of similar sites on the tap root (respectively, 44% and 28%).

Table 1. Comparison of alfalfa root tissue of different ages.

Root age (days)	Diameter of root (μ)	Diameter of stele (μ)	Area of root (μ^2)	Area of stele (μ^2)	Area of cortex (μ^2)	Area of cortex (% of total)
Young (2)	334 a*	106 a	87,600 a	8,800 a	78,800 a	90 a
Medium (10)	506 b	300 b	201,100 b	70,700 b	130,400 b	64 b
Old (20)	684 c	412 c	367,500 c	133,300 c	234,200 c	64 b

*Means in columns followed by different letters differ significantly at the 5% level according to the Student-Neuman-Keuls multiple-range test.

With 10 ♀ *P. penetrans*/segment, percentage penetration of the young tissue (52%) was similar to that where only one nematode/segment was used, about twice that of medium (22%) or old (27%) root tissue ($P = 0.05$).

Age of seedlings and penetration: With 3-wk-old pouch-grown seedlings, penetration into the young root tissue (1 day) was about twice that in medium (10 day) or old (20 day) root segments; respectively, 60%, 23%, and 33% ($P = 0.01$). With agar-reared 3-wk-old seedlings similar results were obtained; i.e., 47% penetration into young tissue, 16% into medium, and 21% into old root segments. With 2- and 1-wk-old seedlings, whether pouch- or agar-reared, where inoculated root segments were, respectively, 1, 6, or 13 days and 1, 3, or 6 days old, age-related differences were no longer significant (Fig. 2A).

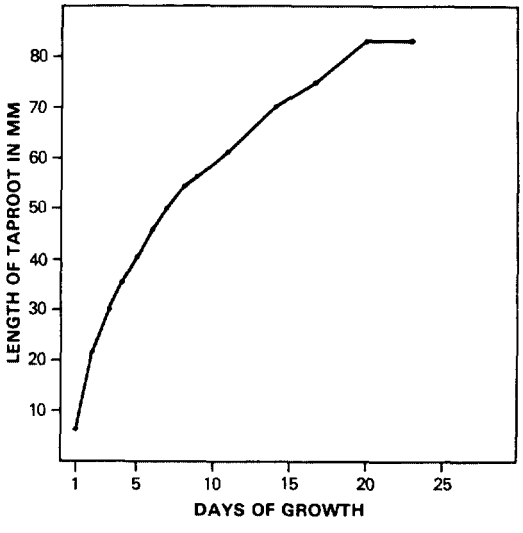

Fig. 1. Length of tap roots of alfalfa cv. Du Puits grown in pouches moistened with Hoagland's solution at 21 C and 65 hlx for 23 days. Mean of 62 plants.

Effect of incubation period, temperature, and life stage: Penetration of nematodes into young root tissue increased from 34% after 6 h to 64% after 48 h of incubation (Fig. 2B). These increases were significantly ($P = 0.05$) greater than the 20% and 38% observed for medium-aged root tissue and the 14% and 40% for old root segments. No increase in penetration was observed between 48 h and 96 h of incubation although the difference between young vs. medium and old root tissue remained significant ($P = 0.05$). Nematodes entered different-aged root segments at temperatures from 5 C to 30 C (Fig. 2C). With both young and old tissue, penetration peaked at 20 C ($P = 0.05$); with medium-aged tissue, penetration did not vary much at 10 C through 30 C. At all temperatures, except at 5 C, penetration into youngest tissue was significantly ($P = 0.05$) greater than into medium and old root segments. All life stages penetrated young root tissue significantly ($P = 0.01$) better than medium-aged or old root segments (Fig. 2D). Penetration into the three different-aged root segments averaged 61% for females, 50% for third-stage larvae, and 27% for males ($P = 0.01$).

DISCUSSION

This study has shown that penetration by *P. penetrans* was inversely proportional to the age of alfalfa root tissue regardless of

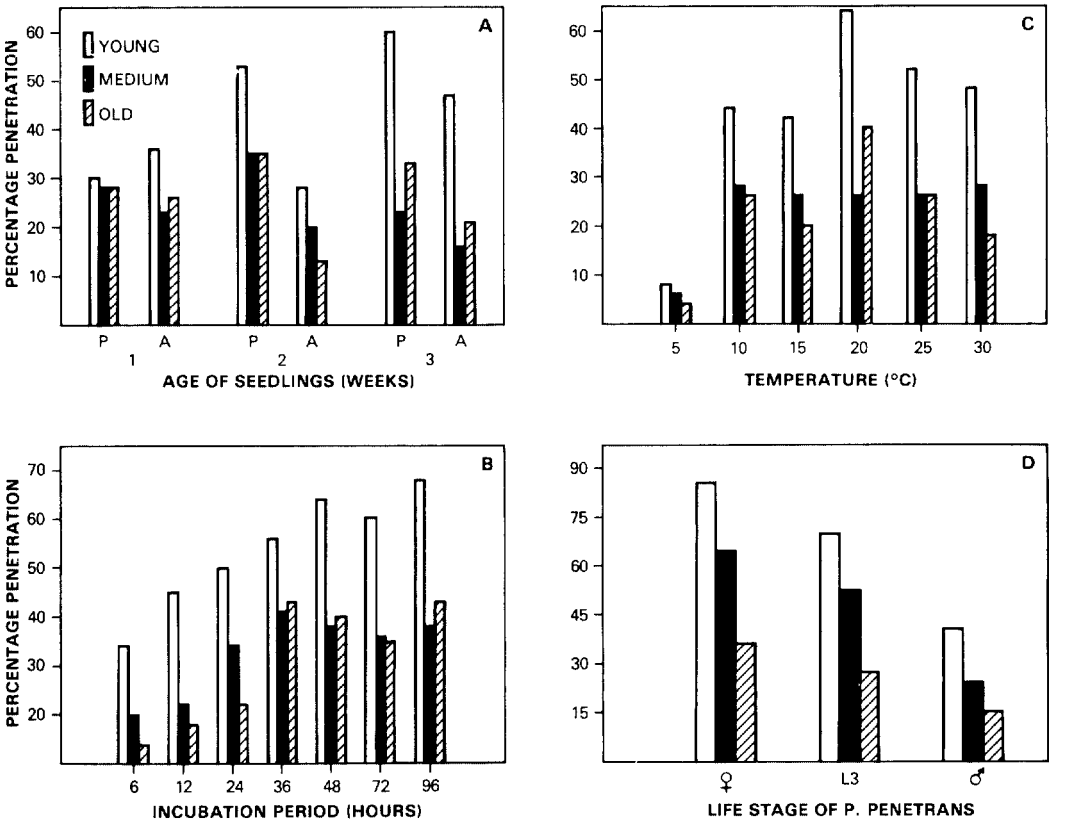

Fig. 2. Percentage penetration into young (2 days), medium (10 days), and old (20 days) sections of alfalfa cv. Du Puits root by one *P. penetrans* per section. A) Effect of age of pouch-grown (P) and agar-grown (A) seedlings. B) Effect of length of incubation period. C) Effect of temperature. D) Effect of life stage and sex.

whether seedlings were grown in paper pouches or on agar. A similar trend was observed with seedlings grown in natural soil. As found by other workers (3,4,6,23), the greatest number of *P. penetrans* penetrated near the root tip.

Klinkenberg (13) showed that penetration of several hosts by *P. penetrans* was not affected by surface sterilizing the nematodes and seedlings; thus, no attempt was made to conduct the present experiments under asepsis. The percentage penetration of *P. penetrans* into young root tissue agrees with that found by other workers (12,23). Differences in percentage penetration of tissue of comparable age between the different experiments are probably due to slight differences in materials and methods.

With both pouch- and agar-grown alfalfa seedlings, significant age-related differences were noted only in 3-wk-old seedlings. Apparently, resistance in root tissue to penetration by *P. penetrans* is negligible in roots of 2 wk or less in age. This agrees with observations made by Jaffee and Mai (10) on apple seedlings.

Young root sections of branch roots, regardless of point of origin, were penetrated at the same high percentage relative to medium and old tissue as young root parts of the tap root. Although occasionally nematodes were observed to penetrate near ruptures of the cortex by branch roots, no consistent relation was found between penetration and branch root initials. This agrees with Turner and Chapman (24).

Increasing the inoculum density from 1 ♀ *P. penetrans* to 10 per segment did not affect the percentage penetration in young roots relative to older tissue. With these numbers there apparently was no competition for entry sites (23).

Townshend (23) noted a positive correlation between rate of penetration and

time. Although a similar correlation was found in the present study, at least during the first 48 h, there was no evidence that penetration into medium or old root sections would eventually equal that of young tissue. It appears that nearly all penetration, at least by females, occurs within 2 days.

The influence of temperature on the penetration of *P. penetrans* followed closely the pattern observed by Townshend (23). At all temperatures tested, except at 5 C, penetration into youngest tissue was significantly higher than into medium or old root tissue. Penetration into root sections of different age at 5 C was too low for any differences to be discernible.

In this study, females of *P. penetrans* were significantly more infective than of larvae or males; this was previously found by other workers (12,15,22,23). Townshend (23) attributed the superior penetration of females to the size of the gland in the posterior subventral lobe, whereas Klinkenberg (14) felt that the stylet of the newly-hatched larvae was still too weak to penetrate the cell walls. Neither proposed mechanism appears to give any life stage an advantage with regards to penetration of different-aged root tissue as all life stages penetrated young root sections better than older tissue.

Why young root tissue is penetrated better than old tissue remains to be investigated. Hollis (9) theorized that plant damage is related to diameter of roots attacked. However, in this study, even though the percentage area of the cortex of young root was 40% greater than that of older roots, the volume of cortex in all three different-aged root sections was ample to accommodate the presence of nematodes. For example, even in the young roots with the smallest absolute area of cortex (78,800 μ^2), there was, theoretically, space for more than 100 nematodes which usually lie more or less parallel to the stele. In alfalfa the expansion of the vascular cylinder by secondary growth causes the rupture and sloughing of the cortex (5). It is likely that as the cortex degenerates in maturing roots, penetration becomes more difficult. Similarly, biochemical and nutritional changes may render the cortex less favourable for nematode colonization (23).

This study has shown that penetration decreases with increase in age of the root tissue. This closely parallels the phenomenon of increased resistance or tolerance of plants to nematode damage as the plant becomes older. Conceivably, the latter phenomenon could be explained, at least partially, by the greater proportion of old root tissue to young root tissue with increased age. This study therefore emphasizes the importance of control of *P. penetrans* during the early phase of seedling development and suggests control through increasing plant tolerance by early seeding or the use of large transplants with some crops.

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