# Histopathology of Pea Roots Axenically Infected by *Pratylenchus penetrans*[1]

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*Abstract:* The histological changes in pea roots axenically infected by *Pratylenchus penetrans* were studied and described. Roots of pea seedlings growing aseptically on the surface of nutrient agar slants were inoculated with axenized nematodes. Six hours after inoculation most of the nematodes introduced were probing the root epidermis, but none had completely entered though a few were observed with their anterior section already in the root. Most of the nematodes penetrated the roots after 12 hr inoculation. From 18 to 24 hr after inoculation the nematodes were mostly in the mid-cortex. Invaded regions of the cortex often showed orange discoloration. As incubation continued, the number of nematodes in these roots increased, and feeding and reproductive activities extended deeper into the cortex. These activities resulted in extensive breakdown of the cortex. No nematodes were observed within the stele of infected roots; however, the endodermis of infected roots stained dark-brown. Gravid female nematodes probed the root endodermis and some endodermal cells appeared to collapse after prolonged probing by the nematode. All stages in the life cycle of the nematode were observed in infected roots; the female to male ratio inside the root was about 5:1. *Key Words: Pisum sativum* L.

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Histopathology of roots of several major crops infected under sterile conditions by *Pratylenchus penetrans* (1, 2, 6, 8, 9, 10, 11) has shown that changes induced in the roots of various species of plants differ. In roots of apple (8), celery (10), alfalfa (2), and carrot (9), the root lesion nematode was found only in the cortex. Browning of the cortical and endodermal cells occurred and was associated with presence of high concentration of phenolic compounds in the affected tissues. In peach (6), specimens of *P. penetrans* were also only in the

root cortex. However, mechanical wounding of the root cells by the nematodes was believed to result in a reaction between amygdalin, a glucoside in peach roots, and the enzymes of host or nematode origin. Toxic products from the reaction caused rapid necrosis of the root tissue of peach. In strawberry, browning and necrosis of the infected cortical and endodermal tissues were accompanied by hyperplasia and discoloration in the stelar tissue (11). The invasion of the stele and the severe damage to vascular vessels of cabbage roots by *P. penetrans* were unique (1). In all other hosts infected by this nematode under monoxenic conditions (2, 8, 9, 10, 11), the root endodermis prevented the nematode from invading the stele. Knowledge of the histological changes in pea roots grown monoxenically with the nematode is essential to the understanding of the disease caused by the nematode. In order to understand the role of this nematode in certain disease interactions a study was made of the histopathology of pea roots growing monoxenically with *P. penetrans*.

## MATERIALS AND METHODS

The isolate of *P. penetrans* employed in this investigation was obtained from Dr. W. F. Mai, Cornell University, and propagated on alfalfa (*Medicago sativa* L., cultivar Ranger) callus tissues produced by the method of Krusberg (5). Surface-sterile nematodes were obtained from a monoxenic test tube culture in which a large number of nematodes had moved out of the infected callus to the surface of the agar slant. Ten ml of cold sterile distilled water was added to the tube after removing the callus debris. The test tube was shaken for about 3 seconds to suspend the nematodes. The volume of the resulting suspension was adjusted with sterile distilled water to provide an inoculum density of about 30 nematodes/ml.

To obtain sterile seedlings, seeds of pea, *Pisum sativum* L., cultivar Wisconsin Perfection, were soaked until fully swollen in sterile distilled water, surface-sterilized for 10 minutes in 1% sodium hypochlorite, and rinsed once with sterile distilled water. The seeds were germinated on potato dextrose agar. Uncontaminated germinating seedlings were aseptically transferred to the surface of nutrient agar in 500-ml Erlenmeyer flasks. The medium was the same as that used for growing callus (5) but without coconut milk or 2,4-D.

One ml of a suspension containing about 30 axenic nematodes was pipetted around the roots of each seedling. Sterile distilled water (1 ml) was added to seedlings serving as control. The flasks and their contents were incubated at room temperature in a laboratory with a daily illumination of 15 hr. Infected and noninfected roots were selected daily for histological study for the first week of incubation and at 7, 14, and 21 days.

Roots of infected and noninfected plants were cut into small segments about 8-12 mm long. The root segments from each plant were killed by immersion in hot (50 C) Flemming solution for 30 minutes. Killed roots were fixed overnight in chromacetic fixative and washed in running water for 6-12 hr. Washed roots were dehydrated in a tertiary butyl alcohol series and cleared in cedar wood oil. Root sections that contained nematodes were mounted in Canada balsam on Cobb's slides for microscopic studies.

When thin root sections were required, dehydrated and cleared root segments from inoculated and check plants were embedded in tissue wax and sectioned on the rotary microtome at 12 $\mu$. The strips were mounted on clean slides coated with Cobb's adhesive. The sections were stained with safranin and Light Green according to the procedures of Johansen (4).

## RESULTS AND DISCUSSION

Few nematodes were found to have penetrated the roots of peas within 12 hr after inoculation with *P. penetrans*. After this time, adult and juvenile nematodes penetrated the root and the gravid female specimens began to lay eggs once inside the root cortex. For the first 2 days after inoculation, nematodes were found mostly in the mid-cortex of the root. Eggs were few and scattered widely in the cortex. Occasionally, groups of cortical cells surrounding a nematode or its eggs appeared yellowish. A halo of light staining cells with deformed nuclei often surrounded the head of the nematode as it migrated into virgin areas of the root cortex. The track left by a nematode was often followed by other nematodes in a gregarious manner. Movement was mostly along the main axis of the root, especially in roots with small diameters, and was mostly intracellular. The nematode appeared to penetrate the cortex parenchyma with relative ease, but the endodermal cells were apparently impenetrable. Nuclei of cells disrupted by nematodes disintegrated, while those in intact

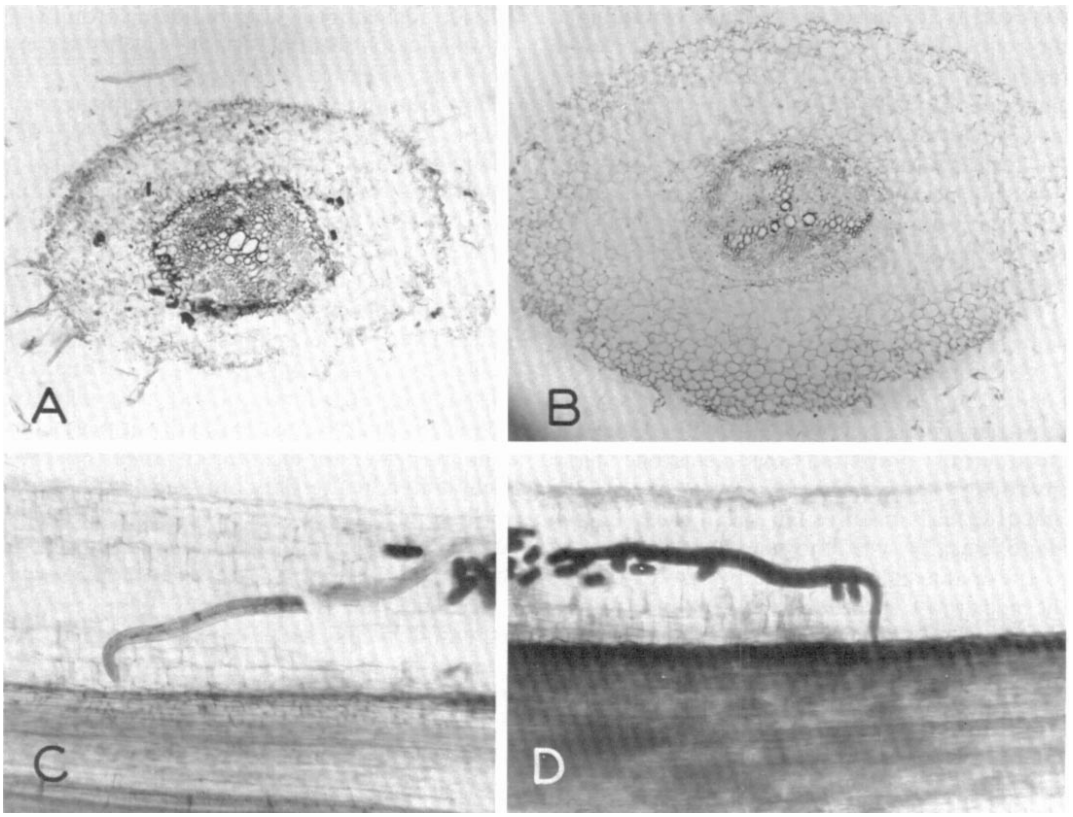


FIG. 1 (A) Transverse section of pea roots infected by *P. penetrans* under sterile conditions. Note the extensive damage in the cortex and the discoloration of the endodermis. (B) Transverse section of a noninfected pea root. (C & D) Root mounts showing individual eggs with nematodes probing the endodermis.

cells surrounding the nematode appeared to be swollen.

Browning of the endodermis did not start until nematodes had probed on this tissue; the discoloration was evident only in sections of the root invaded by nematodes. Necrotic cavities from the breakdown of cortex parenchyma cells were evident in cross sections of infected roots one week after inoculation. Two weeks after inoculation, the number and sizes of such cavities had increased (Fig. 1-A), as well as the number of nematodes in the root. Juvenile and male specimens apparently migrated out of the necrotic cortex while the females remained and continued prolific egg-laying. The female to male ratio inside the root three weeks after inoculation was not less than 5:1. The gravid females were always observed with their heads directed towards the endodermis (Fig. 1-C, D). Although pea roots infected by *P. penetrans* under aseptic conditions became brownish about three weeks after inoculation, definite lesions, comparable to those of infected roots grown in soil under nonsterile conditions, were not evident. At this stage, most of the cortex had been destroyed, but the stele was still intact, although the endodermis was severely discolored. There was little or no breakdown of the cortex or discoloration of the endodermis of the noninfected control roots.

The response of pea roots to infection by *P. penetrans* was similar to those reported for alfalfa, apple, carrot and celery (2, 8, 9, 10). In all of these crops, the endodermis acted as a barrier that prevented the nematode from invading the stele. The absence of any serious damage to the stele of infected pea roots comparable to that observed in strawberry and cabbage infected by *P. penetrans* (1, 11) may explain why the growth of peas was only slightly reduced while supporting a high population of the nematode (3, 7). The chemical reaction in pea roots infected by *P. penetrans* appeared not to result in phytotoxic products similar in action to those reported in

peach roots (6). Damage from physical activities of the nematodes seemed to be more important than that from a chemical reaction. This may explain why the reduction in the growth of peas caused by *P. penetrans* became significant only at a high nematode level (7).

It can be concluded from these observations that *P. penetrans* was unable to seriously damage the vital vascular tissues of the pea root.

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