Pathology and Histopathology of Pratylenchus scribneri Infecting Snap Bean and Lima Bean

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Abstract: The pathological effects of *Pratylenchus scribneri* on susceptible snap bean and resistant lima bean were studied. In a pathogenicity test, the nematode increased nearly 75-fold on snap bean and suppressed top growth. On lima bean, *P. scribneri* reproduced slowly and did not significantly affect top growth. Discreet lesions formed on lima bean roots, but no lesions developed on snap bean roots. Paraffin sections taken 2, 5, 13, 25, and 32 days after inoculation showed little cellular necrosis in snap bean roots, whereas cells surrounding the nematode in lima bean were extensively necrotic. Cortical cells of infected snap bean roots were almost completely invaded and killed 25 and 32 days after inoculation. The cortex of lima bean tissues was intact, although localized necrotic areas remained at sites of nematode invasion. *Key Words:* host resistance, lesion nematode, *Phaseolus lunatus, Phaseolus vulgaris.*

Host-range and pathogenicity studies with *Pratylenchus scribneri* Steiner on crop plants have been limited (8). We found only one report on the histopathological effects of this nematode (4). Since *P. scribneri* is widely distributed in California, greenhouse tests were conducted to determine the host range and pathogenicity of this nematode (8). The findings indicated that *P. scribneri* was capable of reproducing well on a number of crop plants and of suppressing the growth of several plants that were tested. In additional host range tests, however, *P. scribneri* reproduced poorly on lima bean (*Phaseolus lunatus* L. 'Fordhook 242') but increased to high numbers on a closely related plant, snap bean (*Phaseolus vulgaris* L. 'Kentucky Wonder'). Further studies of the host-parasite relations of *P. scribneri* on these two plants were initiated to determine pathogenicity and histopathology of the nematode on lima bean and snap bean. A preliminary report on a portion of the results has been published (9).

MATERIALS AND METHODS

Pathology: Pratylenchus scribneri (2,944 ± 50/1,100 cm^3 soil) in tap water was added and mixed in a steamed loamy sand (75% sand, 24% silt, and 1% clay). Controls received only tap water. Soil was then placed into 20-cm diam polyethylene pots and two fungicide-treated lima bean or snap bean seeds were each planted into five inoculated and five control pots. Soil temperature was 30±2 C. After germination, plants were thinned to 1/pot, and 1 week after plant emergence, 2,300 additional nematodes were added to each of the previously inoculated pots. Six additional pots, three each of lima and snap bean, were inoculated as previously described and used for observations on lesion development at intervals during the experiment. Fifty days after planting, roots and soil were analyzed for nematodes, and foliage dry weights were determined. Roots were placed in 1-liter jars containing water and were aerated for 84 h. A 50-cm^3 soil sample was taken, screened, and placed on a Baermann funnel for 48 h. Total soil nematodes/pot were estimated by using a soil volume of 1,100 cm^3.

Histopathology: Fifteen cm^3 of silica sand were placed in each of 24-25 x 200-mm culture tubes. The moisture level was raised to 10% by addition of 3.2 ml Hoagland's solution and distilled water. The culture tubes were autoclaved for 30 min at 2.68 Kg/cm. Seed of 'Ventura' lima bean and 'Wade' snap bean were surface sterilized for 5 min in a 10% Chlorox® solution and were then aseptically transferred to each tube. Seven days after planting, 500 *P. scribneri* treated in ethoxyethyl mercury chloride (Ayerst Laboratories, N.Y., N.Y.) at standard concentration for 2.25 h were added to each tube. At 2, 5, 13, 25, and 32 days after inoculation, 2 inoculated and 2 control plants were harvested and portions of the roots fixed in a Navashin's solution. Selected root segments were dehydrated and cleared in a tertiary butyl alcohol series and embedded in paraffin. Root material was

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sectioned to a thickness of 15-30μm and stained with safranin and fast green for microscopic examination.

RESULTS

Pathology: Pratylenchus scribneri suppressed top growth of snap bean but did not significantly affect top growth of lima bean (Table 1). Reproduction of *P. scribneri* in lima bean was very low, whereas the nematode increased nearly 75-fold in snap bean. Thirty-eight days after inoculation, lima bean roots exhibited numerous discreet brownish-black necrotic lesions, some of which coalesced to form larger areas of necrotic tissue (Fig. 1). Few discreet lesions were observed on snap bean roots, and new feeder roots showed little evidence of injury. Older roots showed a general light brown discoloration and finally a collapse of the cortical tissue.

Histopathology: Paraffin sections of plants harvested 5 days after inoculation revealed *P. scribneri* scattered throughout cortical root cells of lima bean. Nematode movement was intracellular and resulted in cell-wall breakage (Fig. 2-A, C). The nematodes were usually oriented longitudinally to the vascular tissue, but occasionally a *P. scribneri* was found coiled in one or two cortical cells (Fig. 2-A, B). The nematodes were usually found surrounded on all sides by one or two darkly stained, necrotic cells. Initial

TABLE 1. Influence of *Pratylenchus scribneri* on growth of lima bean and snap bean, and reproduction in these plants.

Treatment[y]	Dry weight of foliage (gm)[z]	Nematode densities (1000's)
Lima bean		
Control	6.68 a	—
Inoculated	6.50 a	6.8
Snap bean		
Control	4.46 a	—
Inoculated	1.90 b	384.5

[y]Inoculum 5,200 nematodes/1,100 cm^3 of soil; plants grown for 50 days.
[z]Grouped means followed by the same letter not significantly different ($P = 0.05$) according to the analysis of variance test.

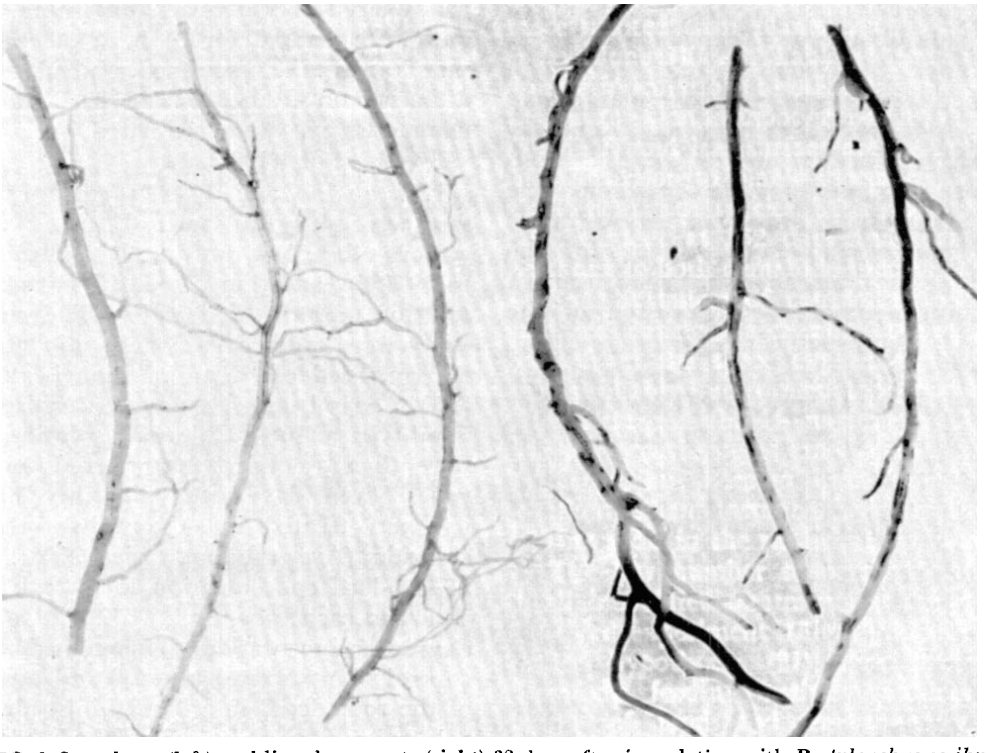

FIG. 1. Snap bean (left) and lima bean roots (right) 38 days after inoculation with *Pratylenchus scribneri*. Little evidence of necrosis on snap bean roots but extensive lesion formation and coalescence of lesions on lima bean roots.

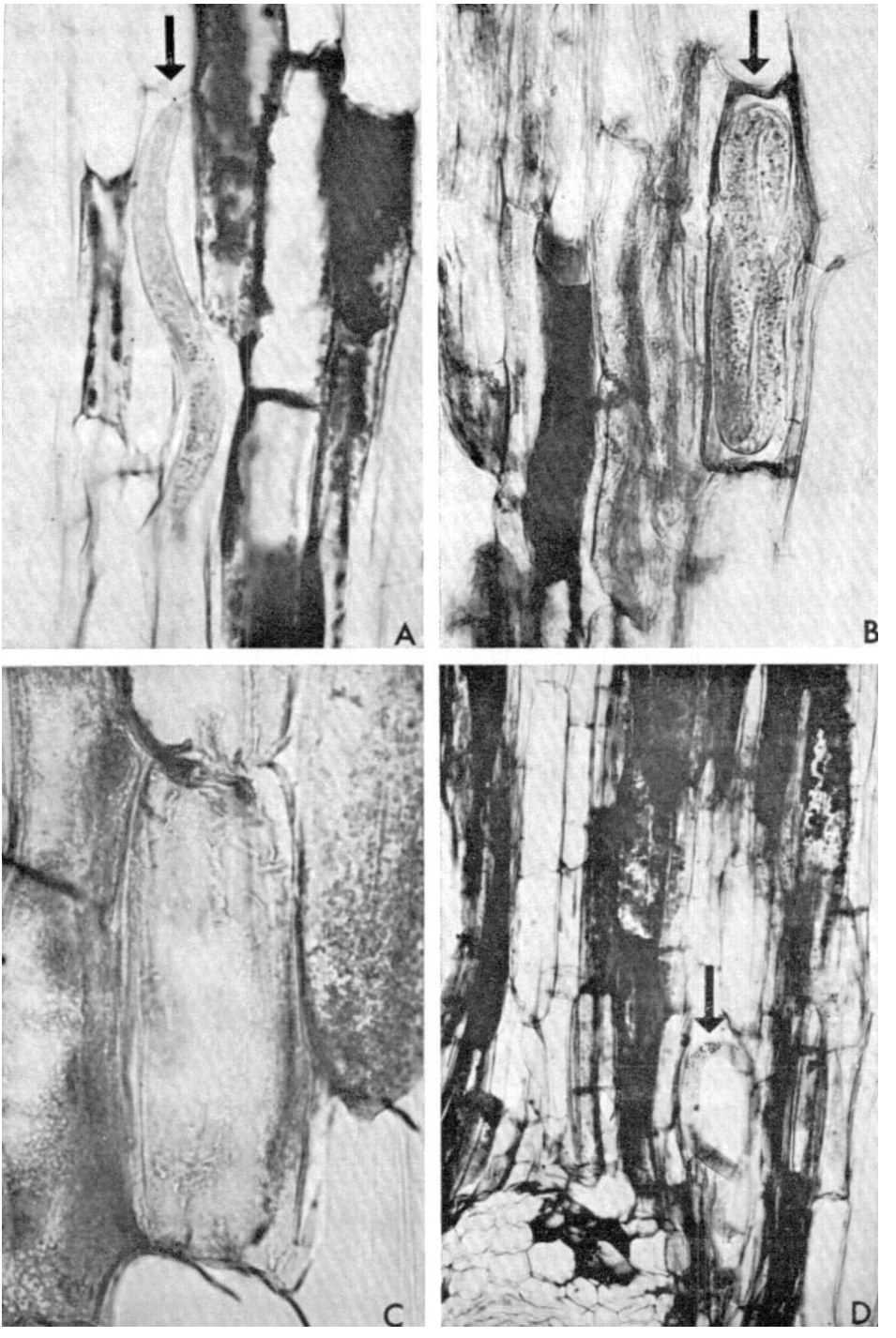

FIG. 2-(A-D). Paraffin sections of lima bean roots infected with *Pratylenchus scribneri. A)* Longitudinal orientation of *P. scribneri* to the root axis and cellular granulation 5 days after inoculation. *B)* Coiling of a nematode in one cell and necrosis of root cells in the area where nematode penetrated. *C)* Early stage of cellular response to *P. scribneri* indicated by presence of nuclei and slight cellular granulation; note apparent nematode breakage of cell and wall. *D)* Extensive cellular necrosis probably resulting from invasions of several nematodes; note nematode surrounded by necrotic tissue.

invasion resulted in a granular appearance of cytoplasm and nuclear disintegration while cells later became completely granulated and stained darkly (Fig. 2-C, D). Cell wall thickening was rare.

Extensive epidermal and cortical necrosis was present in sections of lima bean roots 5 days after inoculation, and endodermal discoloration occurred after 13 days. Necrosis of epidermal, cortical, and endodermal cells increased with time after infection. Twenty-five and 32 days after inoculation, all tissues except stellar tissue were necrotic when invaded by nematodes. The nematodes, however, were not parasitizing endodermal cells, although many appeared necrotic. Few gravid females or eggs were observed in the sectioned tissue.

Two days after inoculation, many *P. scribneri* had invaded cortical cells of snap bean. The nematodes, destroying cells but producing little necrosis, moved through the root cortex, and orientation was similar to that in lima bean. The primary cellular reaction to *P. scribneri* was nucleus and nucleolar enlargement (Fig. 3-A). In one or two cells surrounding some invading nematodes, a slight granulation and darkening of cellular contents were observed (Fig. 3-B). Granulation of cell contents, however, was observed to occur only sporadically and was not associated with any specific root tissue. A few cortical cells exhibited cell-wall thickening, but this phenomenon was not widespread. Cortical damage increased with time after infection. Paraffin sections revealed broken cell walls near the nematodes, and in later stages, large cortical cavities (Fig. 3-C). Twenty-five and 32 days after inoculation, numerous nematodes were in the cortex (Fig. 3-D), and almost no intact cortical cells were present. The nematode was not in endodermal or stellar tissue. Numerous gravid females and eggs were found throughout infected tissue.

Preliminary tests indicated no real difference in the rates at which *P. scribneri* penetrated snap or lima bean roots, and significant numbers were in the roots of both hosts in 24 h.

DISCUSSION

Prolific *P. scribneri* reproduction and damage on snap bean indicated a positive pathogenic potential on this plant. Conversely, lima bean showed an apparent hypersensitive reaction that allowed for only minimal reproduction and localized damage by the nematode.

Intracellular movement and cellular damage caused by *P. scribneri* on snap bean was similar to that found for other *Pratylenchus* spp. (1, 6). Lack of cellular necrosis in snap bean and prolific nematode reproduction suggest that *P. scribneri* could move freely into and through epidermal and cortical cells, and thus snap bean appears to possess little or no mechanical or chemical resistance to the nematode. Cells which became necrotic in snap bean stained only a light brown, a reaction indicating that perhaps only small quantities of phenols were present or that little oxidation of these compounds occurred (11). Since cellular response was compatible, these observations suggest lack of phenolic compounds for oxidation or absence of enzymes capable of oxidizing those compounds present. Little cell-wall thickening and no evidence of wound periderm around invaded cells indicated that this defense mechanism was not operative (12). Since *P. scribneri* was not observed in endodermal cells, a physical barrier or poor food source may have prevented colonization. Little to no differential endodermal necrosis occurred, a fact which suggests that this was not a factor in preventing nematode penetration into this tissue. Nematode reproduction and multiple invasions probably accounted for cortical destruction in snap bean 25 or more days after inoculation. Mechanical damage (and thus loss of the functional integrity of the epidermis and cortex) is the major effect of the nematode on snap bean. Since the nematodes do not directly affect snap bean vascular tissues, these findings may explain why large numbers of *P. scribneri* are necessary to suppress plant growth (5; Rich, unpublished).

A hypersensitive reaction apparently limited *P. scribneri* growth and reproduction in lima bean roots. In associated studies, visual lesion formation was observed as early as 1 day after inoculation. Similar observations were made by Mountain & Patrick (3) on peach. Cortical

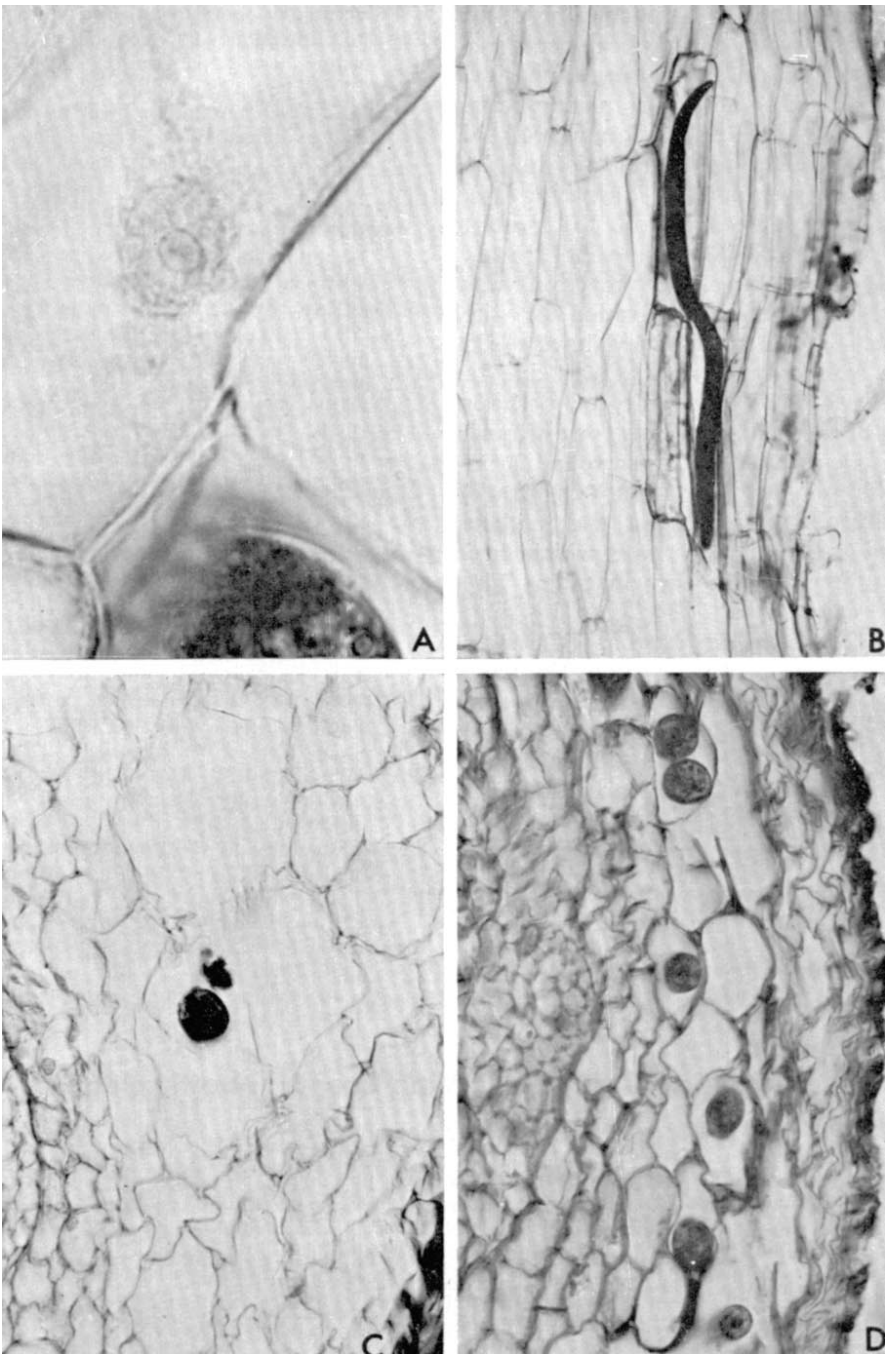

FIG. 3-(A-D). Paraffin sections of snap bean roots infected with *Pratylenchus scribneri*. *A)* Nucleus and nucleolar enlargement in cell near invading nematode. *B)* Only slight granulation and darkening of cells near a *P. scribneri*. *C)* Broken cell walls and cavities around the nematode. *D)* A later stage of infection; large number of *P. scribneri* present in cortical tissue with few cortical and epidermal cells remaining intact.

cell granulation and necrosis and the associated phenolic oxidation probably produced an unsuitable food base for the nematode, and/or chemical constituents may have been directly inhibitory to the nematode (3). Either or both of these mechanisms are supported by limited nematode reproduction and rapid necrosis

of lima bean root cells. As in snap bean, no endodermal invasion of lima bean roots was observed, a reaction indicating a physical barrier or poor food source as in snap bean. The presence of higher quantities of phenols associated with necrosis in the endodermis, as suggested by Townsend (10), would seem unlikely since no differential necrosis was seen between root tissues. Rather, a strong necrotic reaction was apparent in all tissues invaded by the nematode. The necrosis may have been simply a wound reaction that was due to destruction by the nematode, or perhaps diffusion of nematode enzymes, or diffusion of nematode-induced elicitors to adjacent cells. The last two explanations would seem plausible since necrosis was evident in several cells beyond those which had been contacted by the nematode. The different reactions to *P. scribneri* by snap bean and lima bean suggest that differences in phenolic constituents or quantities were involved (2, 7, 11). Further study to determine chemical characteristics is needed in order to elucidate the differences in cellular reaction of lima bean and snap bean to *P. scribneri*.

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