

Symptomatology and Histopathology of Soybean Roots Infected by *Pratylenchus scribneri* and *P. alleni*¹

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Abstract: Size of lesions caused by *Pratylenchus scribneri* on roots of 'Clark 63' soybean was correlated with nematode colony size within roots. A single nematode was capable of causing a detectable lesion. When a root became highly necrotic and shrunk, few nematodes but numerous eggs remained in the tissue. In histological sections made 5, 11, 18, and 45 d after planting, *P. scribneri* was located entirely within the cortex and generally was oriented longitudinally to the vascular cylinder, either outstretched in the same plane or coiled through several cells. Nematodes moved intracellularly, causing extensive rupturing of cell walls, retraction and disappearance of cytoplasm, and thickening of cell walls and necrosis of cells around feeding sites. Depth of penetration within the cortex and necrosis of cells increased with time after infection, eventually resulting in formation of cavities in the cortex and occasional secondary injury to the endodermis. Stele tissue was unaffected by feeding, and damage to the epidermis was limited to nematode entry points. Orientation of *P. alleni* and histopathology of its infection at 45 days were identical to those of *P. scribneri*, except that there was no injury to the endodermis. **Key words:** *Glycine max*, lesion nematodes, mode of parasitism, root lesion development.

Pratylenchus scribneri Steiner and *P. alleni* Ferris are common parasites of soybean (*Glycine max* (L.) Merr.) in the mid-western United States and reproduce readily on this host (2). The histopathology of infection by the monosexual *P. scribneri* has been studied on two species of bean (6) but not on soybean. Lesion formation has been associated with parasitism of soybean by the bisexual *P. alleni* (3), but the pathology was not studied microscopically. This study was undertaken to correlate lesion formation with infection by *P. scribneri*, verify the mode of parasitism of both species, and determine cellular effects of their feeding in roots of soybean.

MATERIALS AND METHODS

For symptomatological study, 'Clark 63' soybean seeds were surface-sterilized in 0.5% NaOCl and germinated in petri dishes. Three-day-old seedlings were planted singly in five 12.5-cm d clay pots of sterile Sparta loamy fine sand that had been uniformly infested with 950 *P. scribneri*/pot obtained from roots of soybean culture plants in a mist chamber. After 45 days of plant growth in a 28 C greenhouse, roots

were washed free of soil and observed under a dissecting microscope. Lesions of various sizes and stages of development, as well as healthy portions of roots, were dissected gently, and the relative abundance of nematodes and eggs within the tissue was noted.

For histological study, twenty 3-day-old soybean seedlings from surface-sterilized seed were planted singly in 7.5-cm d plastic pots of the sterile soil that had been infested with 500 *P. scribneri*/pot. Pots were placed in an incubator at 30 C with 15 h of fluorescent light. Five plants were harvested 5, 11, 18, and 45 d after planting. Roots were washed free of soil, and segments from primary and lateral roots exhibiting typical lesions were fixed in FAA. Root segments then were cut into 1-cm sections, dehydrated in tertiary butyl alcohol (4), and embedded in plastic polymer-supplemented paraffin. Roots were sectioned transversely and longitudinally at a thickness of 15 μ m with a rotary microtome. Sections were mounted on glass slides and stained with safranin O and fast green (4) before microscopic examination. Three-day-old seedlings also were planted singly in three 12.5-cm d clay pots in which *P. alleni* had been cultured on soybean for 6 wk and which averaged 2,000 nematodes/pot. Plants were harvested after 45 d of growth in a greenhouse where temperature averaged 30 C, and roots were processed as described for *P. scribneri*.

RESULTS

Roots of plants observed under the dissecting microscope 45 d after planting in

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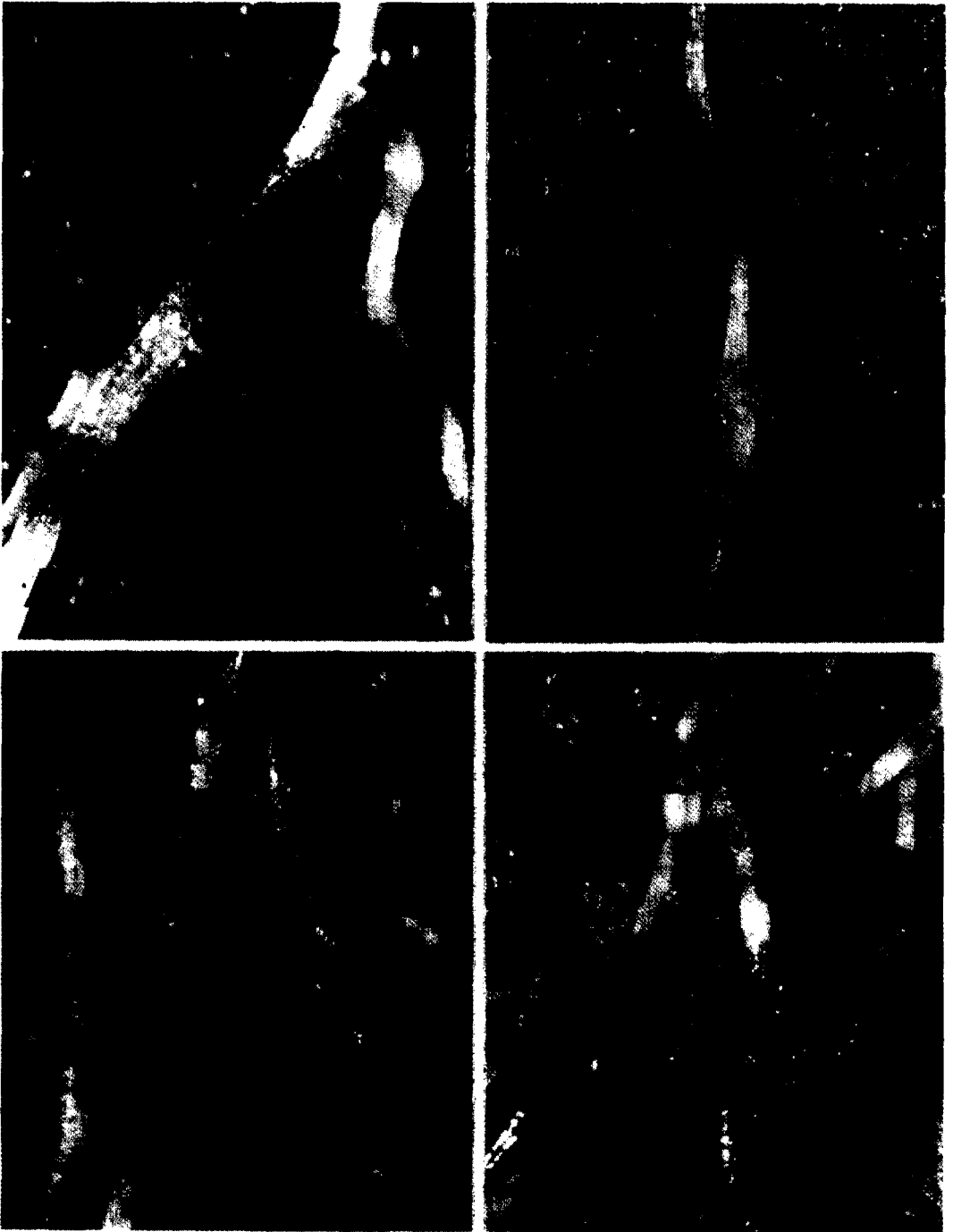


Fig. 1. Stages of lesion development on roots of soybean 45 d after planting in soil infested with *Pratylenchus scribneri*. A, B) Young roots with tiny lesions involving four or five cells and containing a single nematode (a), lesions 1-2 mm in length and containing 3-6 nematodes (b), lesions 3-10 mm in length and containing up to 50 nematodes (c), and healthy portions containing no nematodes (d). C, D) Older roots with necrotic areas of coalesced lesions involving considerable root length and containing an abundance of nematodes and eggs (a) and highly necrotic, shrunken sections containing few nematodes but numerous eggs (b).



Fig. 2. Histology of roots of soybean during early stages of infection by *Pratylenchus scribneri*. A, B) Longitudinal sections made after 5 d with nematodes outstretched or coiled in the first two or three layers of cortical cells below the epidermis, a single row of destroyed cells devoid of cross walls posterior to a nematode (a), and apparent point of nematode entry (b). C, D) Longitudinal sections made after 11 d with nematodes deeper in the cortex, a gravid female outstretched through three layers of cells, a preadult coiled within a single cell, and limited necrosis of cells surrounding nematodes.

P. scribneri-infested soil showed various stages of lesion development (Fig. 1). On relatively young roots, the smallest detectable lesions, involving light brown discoloration of only four or five cells, contained a single nematode (Fig. 1A, B). Larger, more distinct lesions, 1–2 mm in length, harbored three to six adult females and several eggs (Fig. 1A, B). Lesions 3–10 mm long contained up to 50 nematodes and numerous eggs (Fig. 1A, B). Nematodes and eggs were abundant in necrotic areas of coalesced lesions involving considerable length of the root (Fig. 1C). Numbers of nematodes declined in roots which were highly necrotic and shrunk (Fig. 1C, D), but numerous eggs were still present. No nematodes could be found in light colored, healthy appearing portions of roots (Fig. 1A, B).

Histopathological sections of roots revealed a generally longitudinal orientation of *P. scribneri* and *P. alleni*, entirely within the cortex (Figs. 2–4). Nematodes were either outstretched in the same plane or coiled, usually through several cells. During the early stages of infection, the orientation of *P. scribneri* often involved a single layer of cells (Fig. 2A, B). Later, portions of their bodies were often located in different layers (Figs. 2C, 3A). Nematodes occasionally were coiled within single cells (Fig. 2D). In some sections, the apparent point of entry into the root could be detected by the disrupted and necrotic appearance of the epidermis close to nematodes located near the surface (Figs. 2B, 4A).

Five days after planting, *P. scribneri* was located in the first two or three layers of cortical cells below the epidermis (Fig. 2A, B). All individuals were adult females or advanced juvenile stages. Almost all adults were gravid, and eggs occasionally were found in the tissue. At 11 d, many nematodes had penetrated deeper into the cortex and eggs were more abundant (Fig. 2C, D). The light degree of staining indicated that only a limited amount of necrosis had occurred in the cortical tissue surrounding nematodes within the first 11 d (Fig. 2). Cytoplasm in colonized cells, however, had either disappeared or was limited to the periphery of cells. Cell walls in the longitudinal plane were broken and frequently absent from the area occupied by a nema-

tode (Fig. 2A). Nematodes appeared to have migrated intracellularly, and their paths through the tissue often were indicated by single rows of damaged cells posterior to cells occupied by the nematodes (Fig. 2A).

Root sections at 18 and 45 d after planting showed whole nematodes and sections of nematodes scattered at all depths in the cortical tissue (Fig. 3). Eggs in various stages of embryonic development became increasingly abundant (Fig. 3C). Young juveniles were numerous by 45 d. At 18 d, initial feeding on cells had resulted in nuclear disintegration and a granular appearance of cytoplasm, which stained light brown (Fig. 3A). Where damage was more severe, nematodes were surrounded by darkly stained cells with thickened walls and centrally located, shrunken masses of necrotic cytoplasm (Fig. 3B). Cortical damage was extensive at 45 d (Fig. 3C, D). Cells with broken walls in both longitudinal and transverse planes and large groups of empty or nearly empty cells were common. Cavities had formed in the cortex as a result of total breakdown of walls in multiple layers of cells (Fig. 3D).

Injury to other root tissues was minimal. Occasionally, endodermal tissue near a nematode appeared necrotic (Figs. 3A, D), even though there was no evidence of feeding on its cells. Nematodes or eggs were not detected in this tissue, in or near lateral root initials in the cortex, or in the stele, which was unaffected by cortical feeding. Damage to the epidermis was restricted to apparent entry and reentry points (Figs. 2B, 4A).

The histopathology of *P. alleni*-infected roots at 45 d (Fig. 4B–D) was identical to that of *P. scribneri*, except that there was no evidence of injury to the endodermis. Both males and females were present in the roots. The population densities of the two species in roots appeared to be similar at this point, but gravid females and eggs of *P. alleni* were rarely encountered.

DISCUSSION

The size of lesions induced by *P. scribneri* on soybean roots in general was correlated with nematode colony size within the affected tissue until a root became highly necrotic. Nematodes apparently migrated

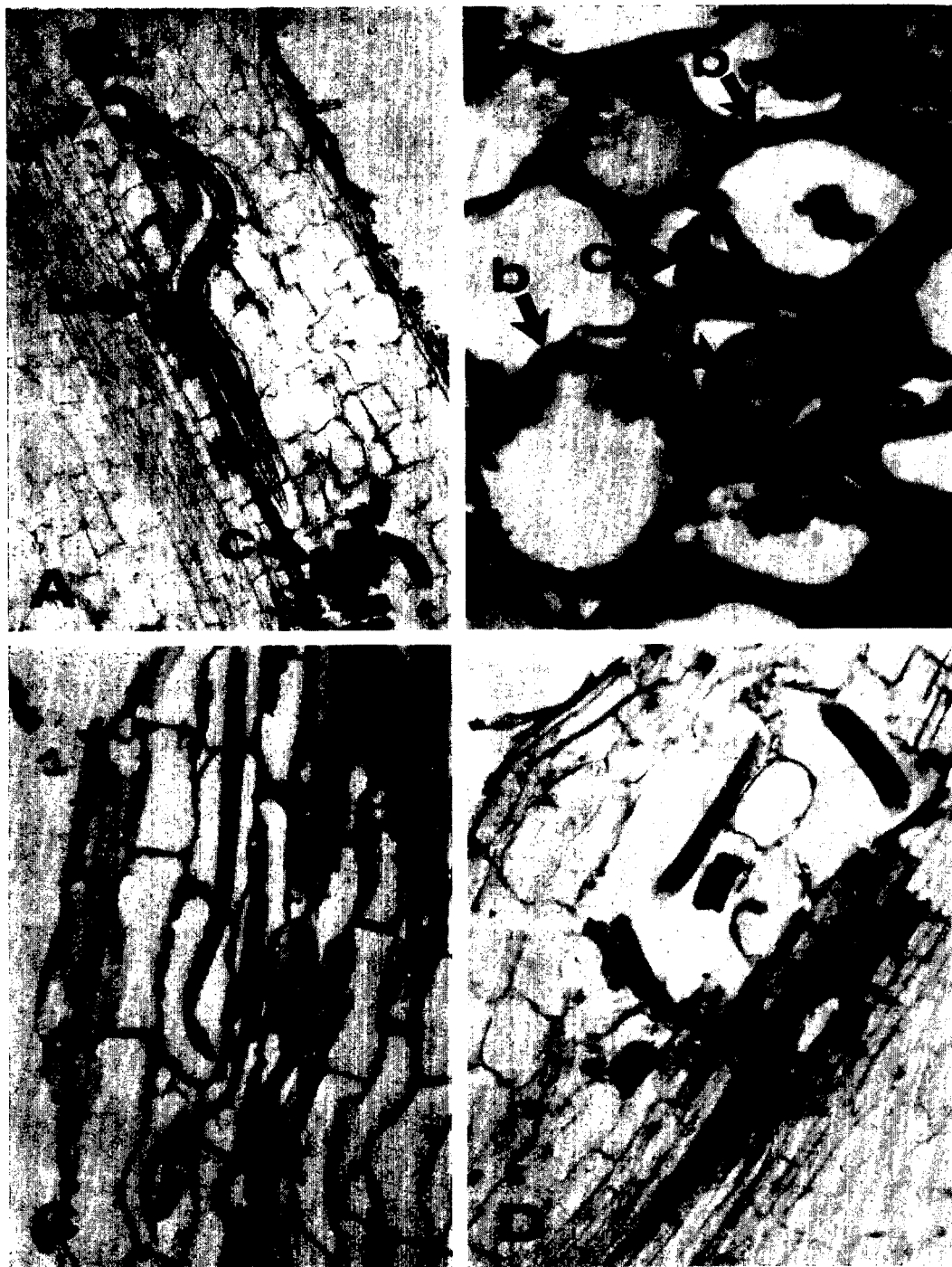


Fig. 3. Histology of roots of soybean during later stages of infection by *Pratylenchus scribneri*. A) Longitudinal section made after 18 d with granulation of cytoplasm around the anterior end of an intact nematode (a), extensive necrosis around its body, necrosis of endodermal cells (b), and sections of nematodes in a multilayer cavity forming in the cortex (c). B) Cross section made after 18 d with a nematode (a) surrounded by darkly stained, necrotic cells with thickened walls (b) and centrally located, shrunken masses of cytoplasm (c). C) Longitudinal section made after 45 d with several nematodes and eggs in cells virtually devoid of cytoplasm. D) Longitudinal section made after 45 d with fragments of nematodes in a multilayer cortical cavity and heavily stained endodermis above the stele.



Fig. 4. Longitudinal sections of roots of soybean made 45 d after planting in soil infested with *Pratylenchus scribneri* or *P. alleni*. A) Disrupted epidermis and necrotic shallow cortical cells caused by late invasion by one or more *P. scribneri* (a) and apparent point of entry (b). B) *P. alleni* lying mostly within a single cell with recently deposited egg. C) Male and juvenile *P. alleni* in a single row of damaged cortical cells and deep multilayer cortical cavity now devoid of nematodes. D) Two-layer cavity indicating the path of *P. alleni* through the cortex.

out of severely damaged roots, leaving an abundance of eggs in the necrotic tissue.

The histopathology of both *P. scribneri* and *P. alleni* in roots showed rather typical behavior of lesion nematodes and tissue reaction to feeding in susceptible plants. Nematodes were restricted to the cortex, moved intracellularly causing extensive rupturing of cell walls and primarily were oriented longitudinally to the vascular tissue. Depth of penetration within the cortex and necrosis of cells increased with time after initial infection, resulting eventually in cavities in the cortex and occasional secondary injury to the endodermis. Whether the endodermis itself acts as a physical barrier to the vascular tissues or is unattractive to the nematodes is still unknown.

There was no hyperplasia or hypertrophy of cells or tissue associated with invasion and colonization, but thickening of intact cell walls adjacent to nematodes, also noted in snap bean (5), was a typical reaction to feeding. The light-brown staining of cells, especially around anterior ends of nematodes, probably resulted from the gradual accumulation in phenolic compounds by the plant in that region in response to the initial invasion of the tissue.

Although soybean is highly susceptible to *P. scribneri* and moderately susceptible to *P. alleni* (1), the degree of tissue response to parasitism by both species appeared to be greater than that observed by Thomason et al. (6) in roots of *P. scribneri*-infected susceptible snap bean but less than in the hypersensitive, resistant lima bean. Injury in soybean also was not as severe as that reported by Nong and Weber (5) in amaryllis

roots, nor was there the characteristic "nesting" of *P. scribneri* observed in that host.

Population densities of the two species in soybean roots and degree of cortical injury at 45 d were similar, even though initial numbers of *P. alleni* were much higher than those of *P. scribneri*. Gravid females and eggs of the monosexual *P. scribneri* were abundant in colonized tissue, while those of the bisexual *P. alleni* were scarce. These observations suggest that the more rapid population development of *P. scribneri* recorded in other comparative studies of the two species on soybean (1,2) is due to a faster rate of maturity in this host. Although this study was not conducted under axenic conditions, bacteria or fungi were not seen within the root tissues, indicating that they play little or no role in lesion formation by these species of *Pratylenchus* on soybean.

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