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Histological Changes in *Gossypium hirsutum* Associated with Reduced Reproduction of *Rotylenchulus reniformis*[1]

Paula Agudelo,[2] Robert T. Robbins,[2] Kyung S. Kim,[2] and James McD. Stewart[3]

Abstract: The reniform nematode (*Rotylenchulus reniformis*) is an important parasite of upland cotton (*Gossypium hirsutum*). Parasitism involves the formation of syncytia to provide nutrition for the female. Events that occur at the feeding site may determine the degree of susceptibility of cotton plants to reniform nematode. The objective of this work was to describe histological modifications associated with reduced reproduction of *Rotylenchulus reniformis* in upland cotton roots. 'Deltapine 50' cotton and a selection from this line with a moderate level of resistance were inoculated with reniform nematode in the greenhouse, and observations on roots were made 3, 6, 9, 12, and 15 days after inoculation. No differences in penetration behavior or in the formation and characteristics of syncytia were observed. Reduced reproduction was correlated with an earlier degeneration and collapse of the syncytial cells, and occasionally, with lack of hypertrophy of the pericycle cells involved. These two mechanisms accounted for 40% to 60% reduction of reproduction of reniform nematode in the plants examined.

Key words: cotton, *Gossypium hirsutum,* histopathology, reniform nematode, reproductive index, resistance, *Rotylenchulus reniformis.*

The reniform nematode (*Rotylenchulus reniformis*) is an important emerging problem in cotton (*Gossypium hirsutum*) production in the southeastern United States (Koenning et al., 2004). Management practices for this nematode in cotton include the use of nematicides and crop rotation (Davis et al., 2003; Gazaway et al., 1992, 2000). Even though upland cotton lines resistant to reniform nematode have been reported (Jones et al., 1988; Yik and Birchfield, 1984), no resistance has been incorporated into commercial cultivars (Robinson et al., 1999) and no studies of the resistant reaction at the cellular level have been published. Previous ultrastructural and histological publications on upland cotton describe only susceptible reactions to the nematode infection (Birchfield, 1962; Carter, 1981; Cohn, 1973, 1976; Rebois, 1980; Rebois et al., 1970). The histopathology of resistant reactions has been documented in soybean (*Glycine max*) (Rebois et al., 1970, 1975) and in tree cotton (*Gossypium arboreum*) (Carter, 1981).

Parasitism of reniform nematode involves the modification of host cells into syncytia that provide nutrition for the female (Birchfield, 1962; Cohn, 1973). Events that occur at these feeding sites are thought to determine the degree of susceptibility of cotton plants to reniform nematode. Early degeneration of syncytia (Carter, 1981; Rebois et al., 1970, 1975), formation of wall deposits (Carter, 1981; Rebois et al., 1970), and lack of hypertrophy of pericycle cells (Carter, 1981) have been proposed as mechanisms for plant resistance to reniform nematode in the plant species studied. The objective of this study was to determine what histological and ultrastructural changes are associated with reduced reproduction of reniform nematode in an upland cotton selection with a moderate level of resistance.

Materials and Methods

Resistance: A single plant of cotton cv. Deltapine 50 (DP50), used as a susceptible check in other research, was observed to support reduced reniform nematode reproduction. All progeny from seed obtained by self-pollinating this plant also supported a reduced number of nematodes. For these experiments, seed from this plant, plus seed from typically susceptible cultivar DP50 plants, was germinated in vermiculite. At the primary leaf stage, 10 seedlings of each cotton line were transplanted to pasteurized fine sand in 500-cm^3 pots and inoculated with 3,000 nematodes/pot. The plants were kept in the greenhouse, where the ambient temperature was maintained between 28 °C and 34 °C. Sixty days after inoculation, the vermiform stages present in the soil were extracted by centrifugal-flotation (Jenkins, 1964), and the reproductive index (R.I. = final population/initial population) was calculated. The number of eggs per egg mass was determined from 20 arbitrarily selected mature females.

Histology and ultrastructure: Plants of the susceptible (DP50-HR: Deltapine 50 with higher reproduction) and the resistant selection (DP50-LR: Deltapine 50 with lower reproduction) were inoculated in the same manner described above for the reproduction tests. Infected root segments were collected 3, 6, 9, 12, and 15 days after inoculation. Roots were fixed with Karnovsky's fixative (Karnovsky, 1961) and rinsed in 0.05 M cacodylate buffer. The segments were then fixed with 1% osmium tetroxide, stained with 0.5% uranyl acetate overnight, and dehydrated in an ethanol series. The segments were further dehydrated with 100% propylene oxide and embedded in Spurr's epoxy resin. Thick sections (1 µm) were stained with toluidine blue O (1%) and examined with a phase-contrast light microscope. Thin silver-gold sections (800 Å) were stained

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[2] Graduate Research Assistant, Professor and Emeritus Professor, Department of Plant Pathology, University of Arkansas, Nematology Lab, 2601 N. Young Ave., Fayetteville, AR72704. Current address of senior author: 210 Long Hall, Department of Entomology, Soils, and Plant Sciences, Clemson University, Clemson, SC 29631.

[3] Professor, Department of Crop, Soil, and Environmental Sciences, University of Arkansas, Plant Science Bldg., Fayetteville, AR 72701.

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E-mail: rrobbin@uark.edu

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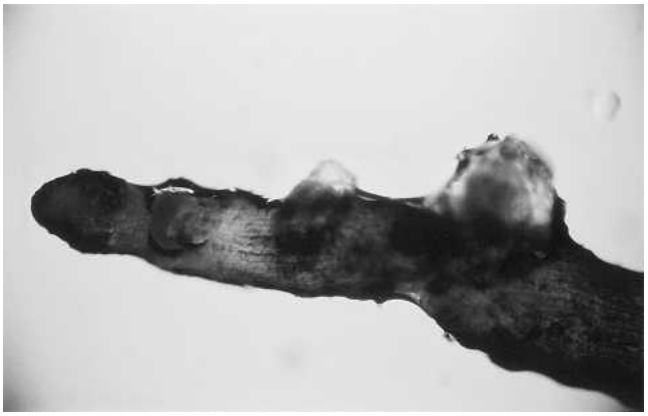


FIG. 1. Three successive stages of reniform nematode female maturation indicating a preference for penetration near root tips. The nematode has no specificity for age of root tissue and can be found penetrating other areas of the root.

with uranyl acetate (2%) and lead citrate (1.76 g sodium citrate and 1.33 g lead nitrate in 50 ml of H_20; pH 12.0), and examined with a transmission electron microscope.

RESULTS

Resistance: Penetration behavior of females was the same in DP50-LR and DP50-HR roots. Immature females showed no specificity for age of root tissue, but a preference for penetration of young succulent roots near root tips was observed (Fig. 1) in both plant lines. Infective females appeared to mature equally on DP50-LR and DP50-HR roots, but the host affected fecundity ($P \leq 0.05$). Eggs per egg mass ranged from 12 to 63 (average = 32.9) in DP50-HR, and from 5 to 46 (average = 19.5) on DP50-LR (Table 1). This difference in fecundity (≈40%) was consistent with the observed difference in the soil population 60 days after inoculation. The reproductive index of reniform nematode on DP50-LR was 58% lower than on DP50-HR (Table 1). No difference in fertility was noticed between the females on DP50-LR and those on DP50-HR, and appearance of females, egg masses, and eggs was the same on roots from both hosts.

Histology and ultrastructure: In both DP50-HR and DP50-LR, immature females penetrated the root cortex intracellularly, and penetration generally stopped when the female reached the endodermis (Fig. 2). At the infection site, epidermal cells were destroyed and necrosis of surrounding cells appeared with the collapse of the first layers of cortical parenchyma. The outermost stelar cells and innermost cortical cells were frequently deformed or necrotized. Breakdown and collapse of endodermal cells near the infection site was also common. Thickening of an altered endodermal cell wall, darkly stained with toluidine blue, indicating lignin-type deposits, along with the necrosis and collapse of endodermal and cortical cells were observed in both hosts (Fig. 2). The stylet was generally inserted into an endodermal cell that became the initial syncytial cell, and stylet secretions forming the feeding tube (Fig. 3) were observed.

Syncytia on both hosts formed through cell wall dissolution and coalescence of cytoplasmic contents of adjacent cells (Fig. 4). Dense cytoplasm, containing numerous vacuoles and organelles, filled the cells forming the syncytia. The number of mitochondria and the number and size of plastids increased, along with the elaboration of endoplasmic reticulum. Syncytia generally involved a single layer of cells in the pericycle. However, two layers of hypertrophied cells that involved mostly pericycle, but sometimes also endodermal tissue, were occasionally observed (Fig. 5).

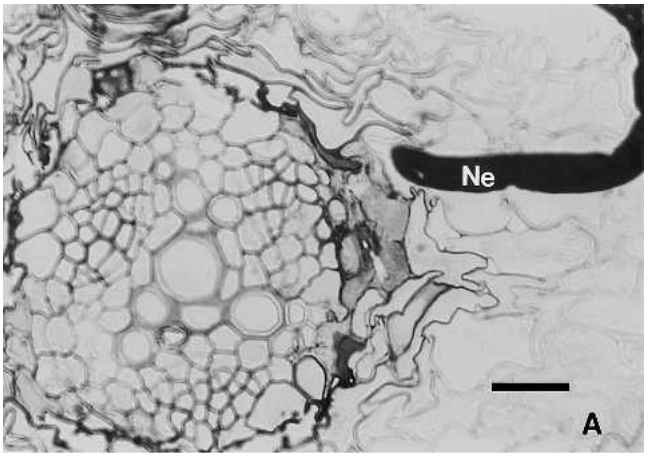
Nine days after inoculation, various stages of cell wall lysis and cytoplasmic disorganization were observed in the DP50-LR roots (Fig. 6). Lysing cells had few or no recognizable organelles. Twelve to 15 days after inoculation, the generalized resistant reaction was a darkened necrotic syncytial cavity filled with amorphous osmophilic material (Fig. 6). Following degeneration, separation of plasmalemma from the cell wall occurred, breaking the cytoplasmic connections through the plasmodesmata and preventing further development of the syncytium. In contrast, 15 days after inoculation, the more susceptible DP50-HR still supported active syncytia (Fig. 4).

In some cases, a resistant reaction characterized by the lack of pericycle hypertrophy was observed (Fig. 7). The syncytia in these reactions did not appear to prevent the female from maturing but apparently reduced

TABLE 1. Number of eggs per egg mass, final population in soil, and reproductive index of *Rotylenchulus reniformis* on upland cotton DP50-HR (susceptible) and DP50-LR (resistant), 60 days after inoculation.

	Eggs per egg mass[a]		Final population[b] (Pf)[c]		Reproductive index[b] (Pf/Pi)[c]	
Host	Range	Mean	Range	Mean	Range	Mean
DP50-HR	12–63	32.9a[d]	105,600–189,000	139,667a	35.2–63.0	46.6a
DP50-LR	5–46	19.5 b	40,800–78,000	58,080b	13.6–26.0	19.4b

[a] Data from 20 arbitrarily selected females.
[b] Data from 10 replications.
[c] Final population (Pf) = population recovered from soil by centrifugal-flotation 60 days after inoculation (individuals/500 cm^3); Initial population (Pi) = 3,000 nematodes/500 cm^3.
[c] Means within a column followed by a different letter differ according to LSD test ($P \leq 0.05$).



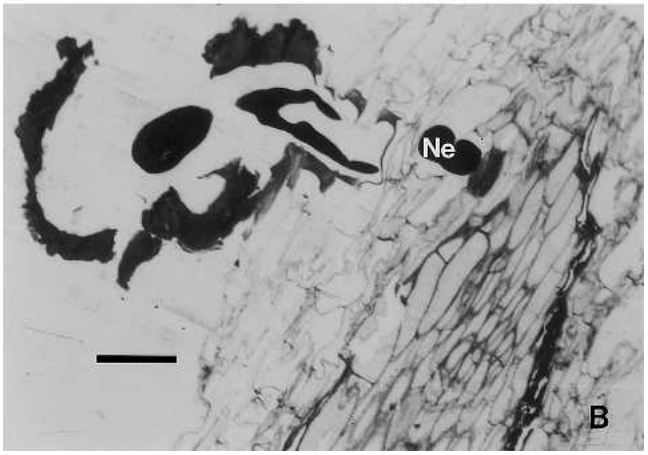


FIG. 2. A) Section of DP50-HR root 6 days after inoculation, stained with toluidine blue, showing collapse and necrosis of cortical and endodermal cells (Ne: nematode; bar = 40 μm). B) Longitudinal section of DP50-HR root 15 days after inoculation, stained with toluidine blue. Mature female body contour, with anterior portion of the body embedded in the root, and dark staining of the initial syncytial cell are visible. Bar = 40 μm.

fecundity, as suggested by the lower number of eggs per egg mass.

DISCUSSION

The cellular alterations characteristic of syncytia induced by reniform nematode, as described in previous studies, including wall perforations, dense cytoplasm, increased number and size of organelles, and hypertrophy, were observed in both DP50-HR and DP50-LR roots. The characteristics of stylet secretions were similar to those described by Rebois (1980), and no differences could be found between the feeding tube in the DP50-LR and DP50-HR roots. Consistent with the observations made on other hosts (Carter, 1981; Rebois et al., 1970, 1975), there were no apparent differences in the early development of syncytia between resistant and susceptible cotton lines.

Birchfield (1962) reported that *R. reniformis* normally penetrated the endodermis and pericycle, before stopping and feeding in the phloem. Cohn (1973) reported that the nematode fed in the pericycle with occasional

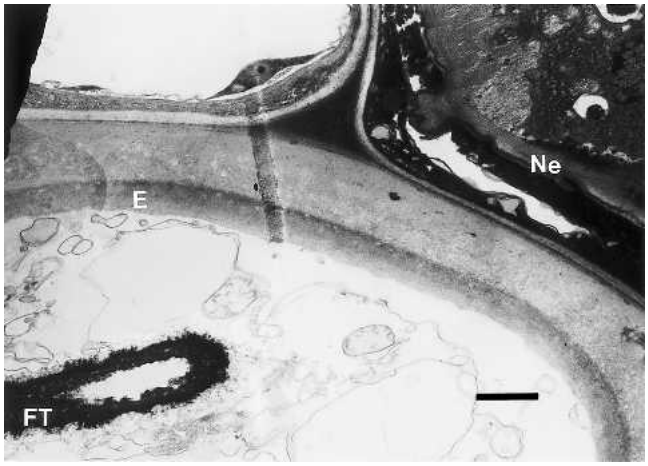


FIG. 3. Electron micrograph of section of DP-HR root, 12 days after inoculation. The mature female lip region (Ne: nematode) is pressed against the endodermal cell (E) that served as the initial syncytial cell. The feeding tube (FT) formed by stylet secretions persists. Bar = 1 μm.

phloem involvement. In soybean, reniform nematode was described as generally stopping against an endodermal cell and then inducing hypertrophy of the adjoining pericycle (Rebois et al., 1970, 1975). Taha and Kassab (1979) reported that reniform nematode stimulated hypertrophy of both pericycle and endodermal cells in cowpea. The results of this study indicated that in upland cotton both pericycle and endodermis may be involved in syncytial formation, but no feeding on phloem cells was observed.

A morphological component of resistance has been proposed in soybean (Rebois et al., 1970, 1975), where apparently optimum infection occurs at protoxylem poles. No evidence in this study suggests that the same occurs in cotton. Active syncytia, supporting mature fe-

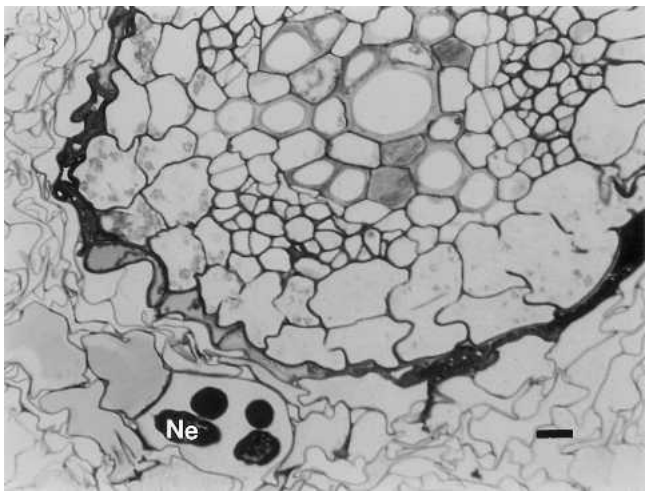


FIG. 4. A) Light micrograph of transverse section of DP50-HR root, 15 days after inoculation. Hypertrophied pericycle cells conforming the syncytium are stained darker, and coalescence of cytoplasm can be noted. (Ne: nematode; bar = 20 μm). B. Electron micrograph of DP50-HR root, 15 days after inoculation, showing partial dissolution of cell walls in the pericycle and coalescence of cytoplasm with numerous organelles and vacuoles. Bar = 25 μm.

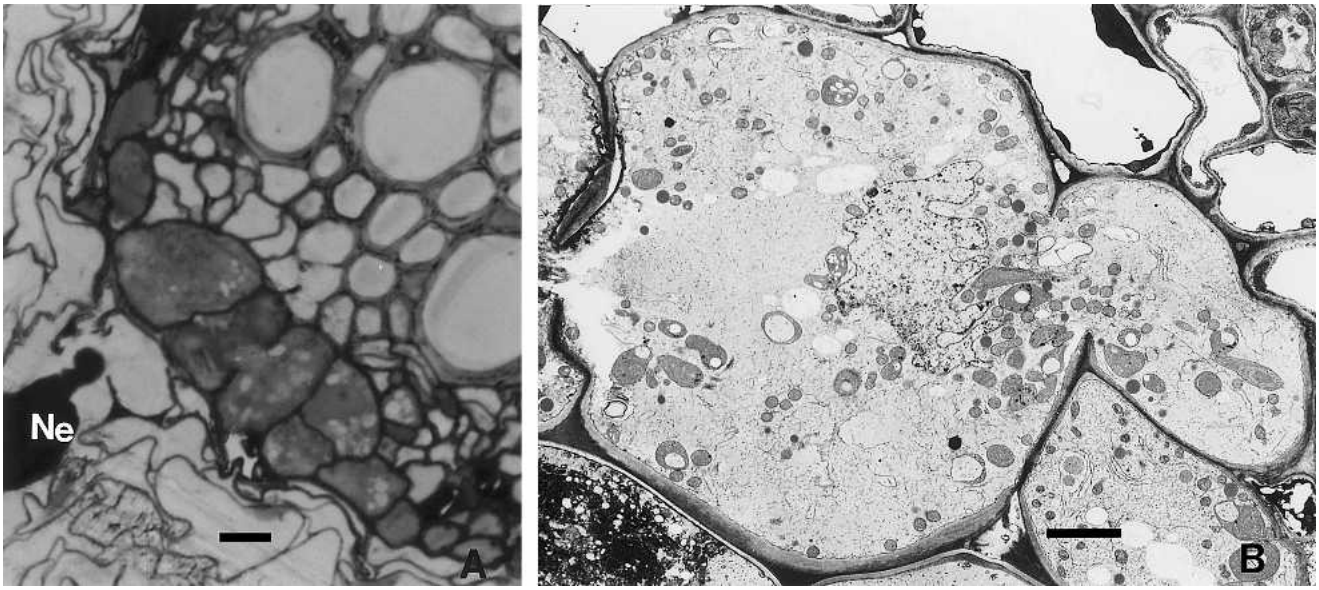


FIG. 5. Transverse section of DP50-HR root, 12 days after inoculation. The syncytium involved two layers of hypertrophied pericycle cells, and the first layer of cortical cells is collapsed (Ne: nematode; Bar = 20 μm).

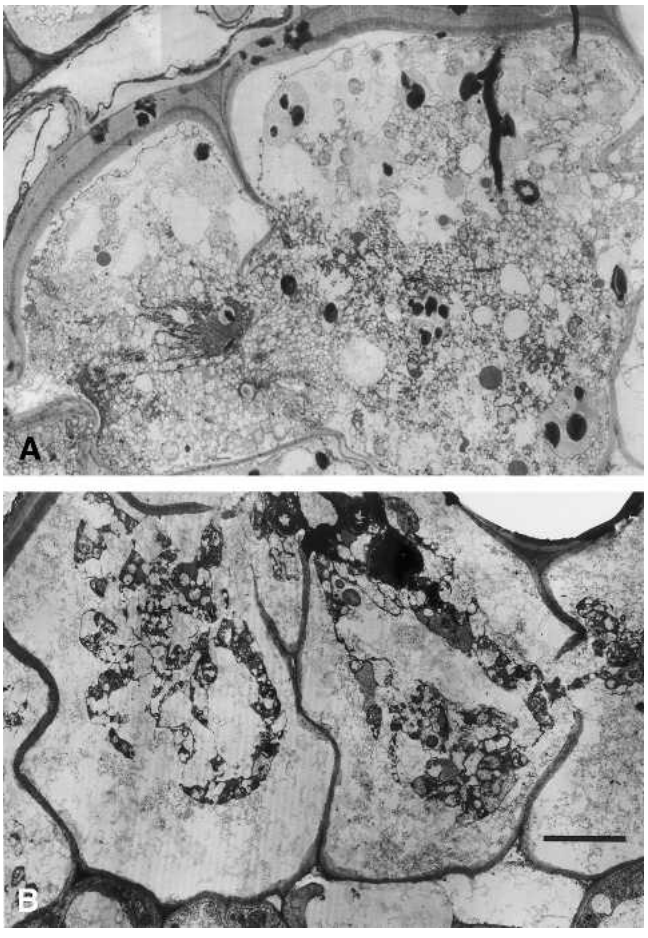


FIG. 6. A) Electron micrograph of DP50-LR root, 9 days after inoculation. Early stage of syncytial degeneration, with separation of plasmalemma from the cell wall. B) Twelve days after inoculation, cytoplasm occurs as an irregular mass in the center of the syncytial cells and organelles are not distinguishable. Bar = 1 μm.

males and normal reproduction, were found on both protoxylem and protophloem poles in DP50-HR and DP50-LR. Also, reniform nematode did not show any specificity for the age of root tissue, although young succulent roots were frequently infected near root tips, consistent with Birchfield's (1962) findings on cotton cultivars Deltapine Smooth-Leaf and Coker 100 Wilt.

The principal mechanism of resistance observed in this study was the more rapid degeneration of syncytia. Carter (1981) documented that in resistant *G. arboreum,* the hypertrophied pericycle cells began to disintegrate 7 to 12 days after penetration. Degeneration of syncytia in DP50-LR was observed at 9 days after inoculation, and in DP50-HR at 20 to 22 days after inoculation. In resistant soybean, lysis of syncytial cells was reported to occur 2 to 4 days after infection (Rebois et al., 1975).

The second mechanism observed, although it occurred less frequently, was the lack of hypertrophy of the syncytial cells. This was also reported by Carter (1981) in resistant *G. arboreum* but was not observed in resistant soybean (Rebois et al., 1970, 1975). Rebois et al. (1975) documented the collapse of endodermal cells in resistant soybean. In this study, necrosis and collapse of endodermal and cortical cells were observed in both hosts.

The condition causing reduced reproduction, or partial resistance, in DP50-LR is heritable and homozygous for the plant, as evidenced by expression in all the progeny. Although reduced reproduction of the nematode can be correlated with the observed phenomena, the mechanisms for blocking syncytial development and inhibiting nematode fecundity are not known and further studies of the biochemical and genetic basis of the resistance are needed.

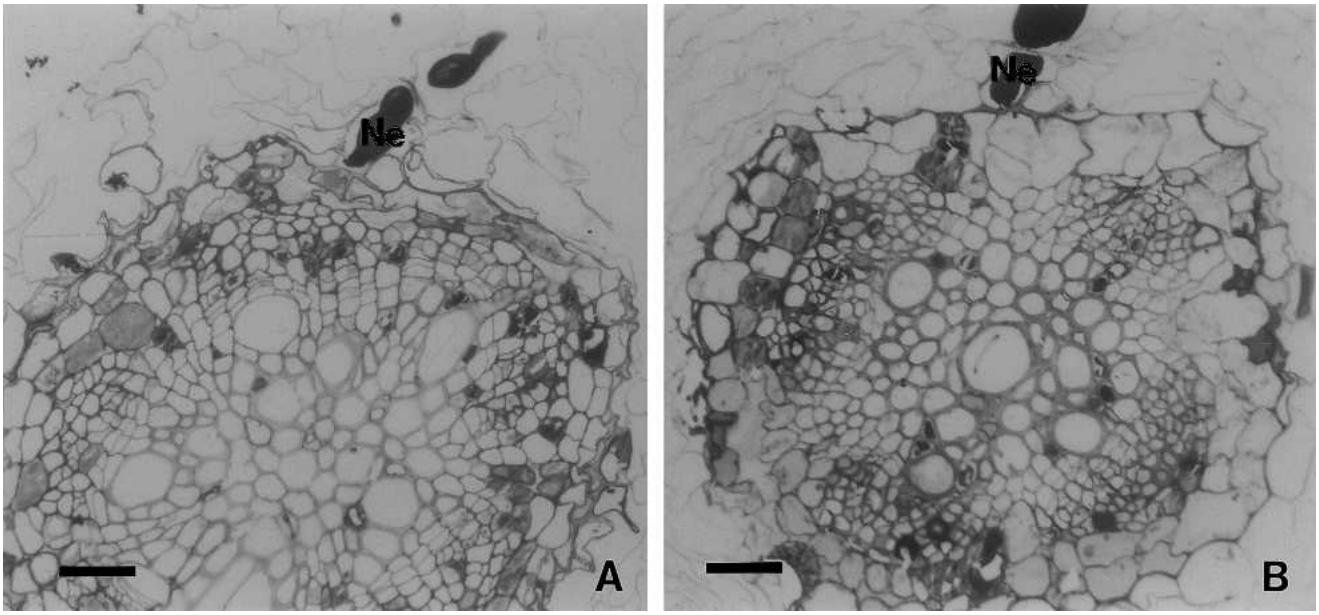


FIG. 7. Light micrographs stained with toluidine blue. A) Cross section of DP50-LR root, 6 days after inoculation. Female is swollen, but the feeding site cells are not hypertrophied. B. Cross section of DP50-HR root, 6 days after inoculation. Female is swollen and the feeding sites are characteristically hypertrophied (Ne: nematode; Bar = 40 μm).

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