Morphological Characteristics of Syncytia in Susceptible Hosts Infected by the Soybean Cyst Nematode

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ABSTRACT

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Morphological characteristics of syncytia induced by *Heterodera glycines* (race 3) were compared in soybean, cleome, and 'Kobe' lespedeza. The syncytia formed in cleome were confined to the stele, where they replaced large regions of the vascular bundle. The nematode penetrated into the vascular bundle to initiate and feed on these syncytia. Syncytia in lespedeza were initiated in the inner cortical region, then developed centripetally ultimately to involve the phloem and xylem with only a small part of the vascular bundle invaded by syncytia. The nematodes that fed on such syncytia were located in the cortex. In soybean, syncytia were formed inside or outside the stele or both. The major characteristic features of syncytia, such as cell-wall perforation and hypertrophy of syncytium component cells, were similar among the various host plants. However, the cell-wall ingrowths, another characteristic of syncytia, were more prominent in soybean than in lespedeza, and much more than in cleome.

Additional key words: cytopathology, soybean, susceptibility and tolerance.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, induces syncytia and reproduces in susceptible hosts. The syncytium induced by *H. glycines* is a multinucleate unit, termed a transfer cell, that is characterized by cell-wall dissolutions (3,5) and cell-wall ingrowths (6). The maturation of the nematode is directly related to the development and persistence of syncytia (1,4). However, the efficiency of nematode reproduction is known to vary among compatible host species. Riggs (10) demonstrated considerable differences in nematode reproduction between susceptible soybeans and wild hosts as well as among soybean cultivars. Nevertheless, very little is known about the mechanisms that cause such variations in host response.

Nematode maturation and reproduction is known to be dependent on the successful development of syncytia. An attempt has been made to determine the nature and structural variations that exist in the development of syncytia in the following susceptible hosts, 'Lee' soybean (*Glycine max* (L.) Merr.), 'Kobe' lespedeza (*Lespedeza striata* L.) and 'Pink Queen' cleome (*Cleome spinosa* L.).

MATERIALS AND METHODS

Cultivars Lee soybean, Kobe lespedeza, and Pink Queen cleome were grown and inoculated with *H. glycines* race 3. The inoculation procedures were similar to those described by Gibson et al (5) in which they used plants at the primary leaf stage.

For electron microscopy, root segments were fixed with a modified Karnovsky's fixative consisting of 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.2, for 2 hr at room temperature. After being rinsed several times with the same buffer, the tissues were postfixed in 1% OsO_4 for 2 hr, then prestained in bulk overnight in 0.5% uranyl acetate at 4 C. The tissues were dehydrated in an ethanol series and embedded in Spurr's medium (11). For all the plants examined, nematode-infected root segments were taken 20–22 days after inoculation.

Thick sections (0.5–2.0 μm) for light microscopy were serially cut near a mature female nematode from a tissue block and the sections stained with 1% toluidine blue in 30% ethanol. Subsequently, thin sections (about 800 Å) were cut from the same tissue block for electron microscopy. The sections were double stained in 2% aqueous uranyl acetate for 5 min and lead citrate (9) for 2 min before examination with a JEOL 100 CX electron microscope.

RESULTS

Location of syncytia in the root tissues. Syncytia were induced by *H. glycines* in all three susceptible host plants. Syncytial cells were well developed and exhibited very dense cytoplasm 20–22 days after inoculation. The structural features of syncytia under the light microscope were similar in that all hosts had cell-wall perforations, irregularly shaped hypertrophied nuclei and an absence of central vacuoles. The size of syncytial cells varied within each plant and precise comparisons of syncytia among various hosts were not possible. However, there appeared to be no significant differences in sizes of syncytial cells among the hosts.

The sites of syncytial formation differed among the host plants. In cleome, syncytia were formed inside the xylem, occupying the central portion of the vascular tissue (Figs. 1 and 2). In transverse sections of cleome roots, syncytial component cells were located at the central portion of the stele and were surrounded by xylem elements. Nematodes apparently penetrated deeply into the central xylary region where the anterior portion of the nematode was often located (Fig. 1). In longitudinal sections, such syncytia appeared to be sandwiched between two layers of xylem cells (Fig. 2).

In lespedeza, the major body of the syncytium, unlike that in cleome, was usually located outside the central portion of the vascular bundle. Therefore, in either transverse (Fig. 3) or longitudinal (Fig. 4) sections of root, the central vascular area was devoid of syncytia. A fully developed syncytium usually extended from the epidermis to the outer region of vascular bundle (Figs. 3 and 4). Thus, only the small portion of the syncytium that faced toward the vascular bundle was associated with the xylem elements. The nematodes feeding on this type of syncytium were located at an area of the syncytium that faced toward the epidermis (Fig. 3).

In soybean roots, the location of syncytia was varied and depended on the root segments observed. The syncytia were observed both inside and outside the vascular bundle and also in the cortical region (Fig. 5).

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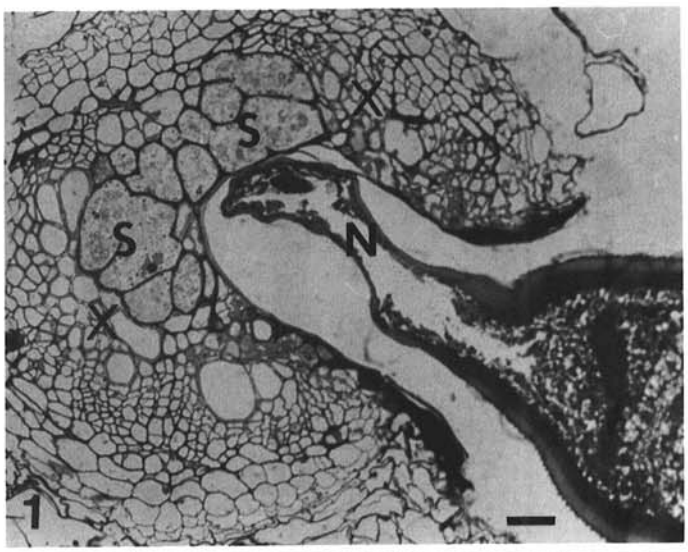
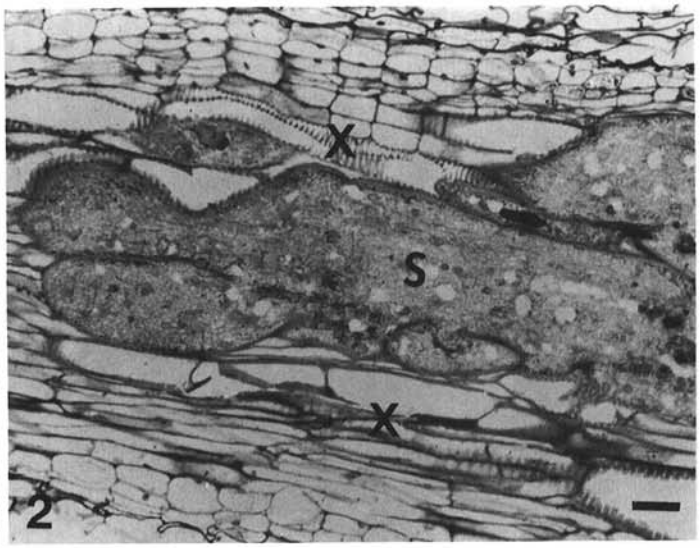
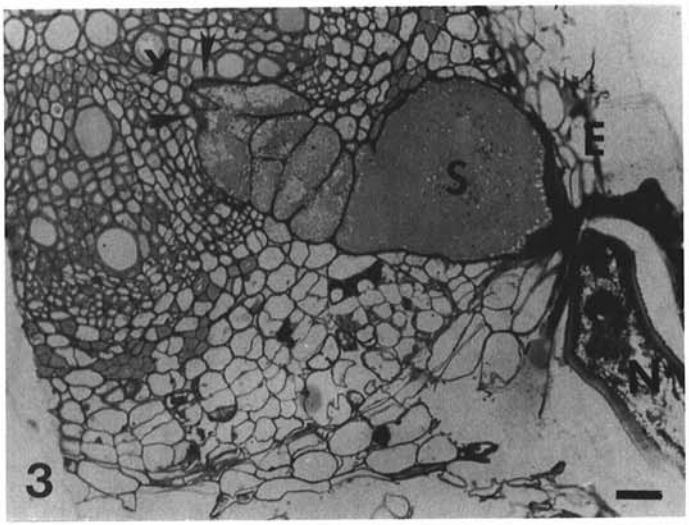
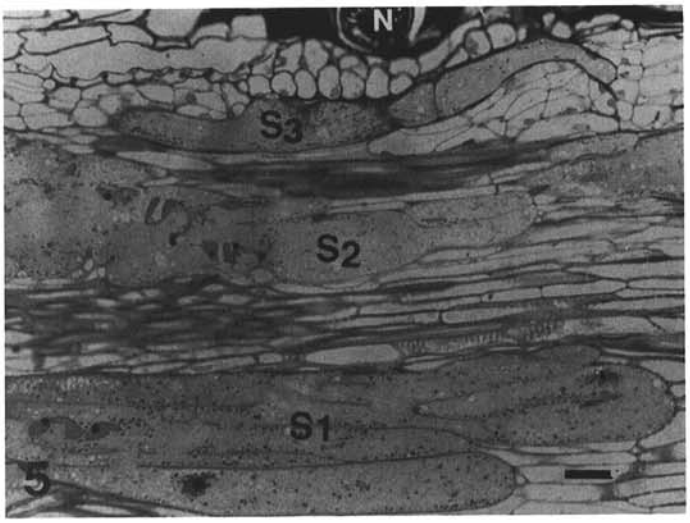




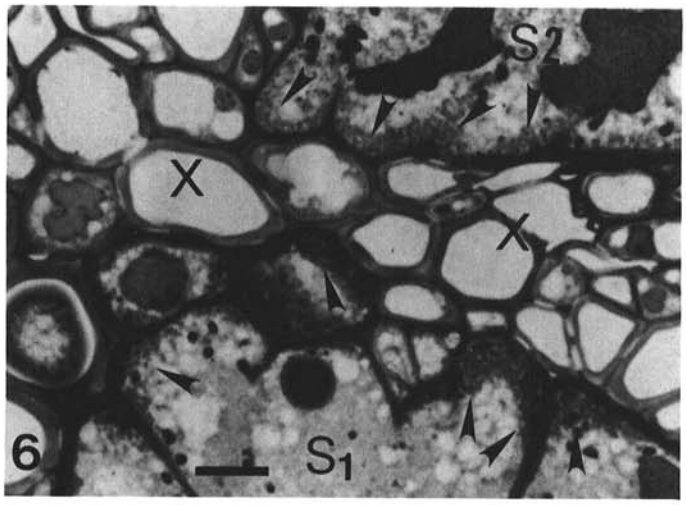


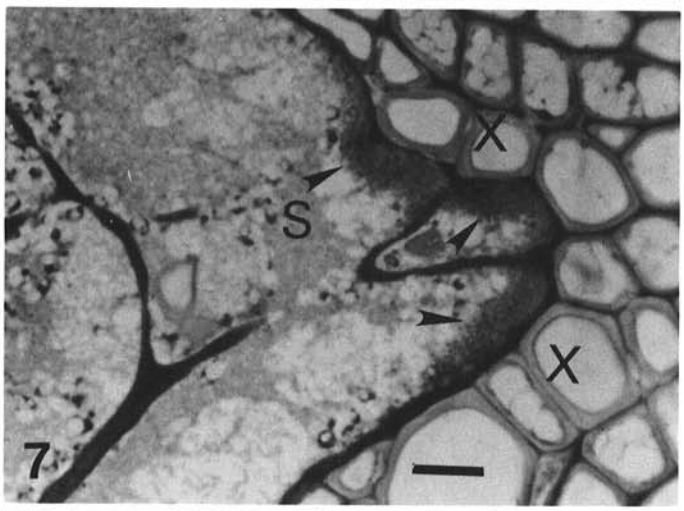


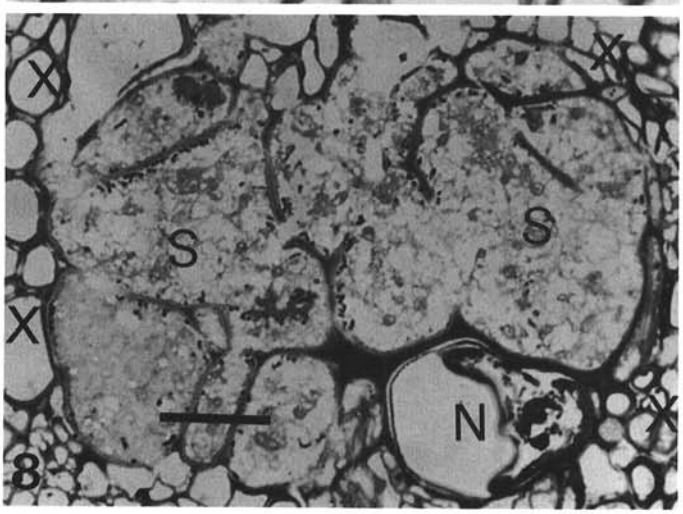
Figs. 1–5. Light micrographs of root tissues showing syncytia (S) formed 20–22 days after inoculation with *Heterodera glycines* race 3. Scale bars = 10 μm. **1,** Transverse section of cleome root showing S located in the central portion of vascular bundle being surrounded by xylem (X). A nematode (N) that penetrated deeply into the vascular bundle is also shown. **2,** Same as Fig. 1, but the root was sectioned longitudinally. The syncytium (S) is sandwiched between xylem vessels (X). **3,** Transverse section of lespedeza root with syncytium (S). The major body of the syncytium is located outside the vascular bundle and only a small area (arrowheads) of the syncytium is interfaced with xylem vessels (X). A nematode (N) feeding on the syncytium is located at an area of the syncytium that faces toward the epidermis (E). **4,** Same as Fig. 3, but the root is sectioned longitudinally. The central portion of vascular bundle, is devoid of syncytium, which extends from the epidermis (E) to the outer portion of vascular bundle. **5,** Longitudinal section of soybean root containing three syncytia (S) located at different areas. Two (S_1, S_2) large syncytia appeared to be in the vascular bundle and the other (S3) in the periphery of the root. A nematode (N) is shown near the epidermis.

Formation of cell-wall ingrowth. Another difference in structural features of syncytia was in the formation of cell-wall ingrowths, a characteristic feature of syncytia. In light microscopy, the cell-wall ingrowths appeared as darkly stained fuzz consisting of tightly packed filaments along the inside of the syncytial walls that interface with xylem vessels (Figs. 6 and 7). In syncytia of both soybean (Fig. 6) and lespedeza (Fig. 7), the cell-wall ingrowths were prominent wherever syncytial walls adjoined xylem vessels. Therefore, syncytia in soybeans, especially those occurring in the vascular bundles, had abundant wall ingrowths. In lespedeza, where only a small area of a syncytium interfaces with xylem vessels (Fig. 7), cell-wall ingrowths were scarce. In cleome, wall ingrowths were rarely observed, even though syncytia were practically surrounded by xylem vessels (Fig. 8).

With electron microscopy, the cell-wall ingrowths of all hosts (Figs. 9–11) were structurally indistinguishable from reports of nonsyncytial transfer cells of plants (7). However, the degree of cell-wall ingrowths varied among the hosts. In soybean (Fig. 9) and lespedeza (Fig. 10), the fingerlike cell-wall ingrowths were so densely meshed that they appeared as a thick mat. In cleome, cell-wall ingrowths were not evident using light microscopy (Fig. 8), but were observable with the electron microscope (Fig. 11). The wall ingrowths were very sparse and shorter than those that occur in soybean and lespedeza (Fig. 11). Furthermore, the syncytial wall with ingrowths formed in cleome were much thicker than those of soybean and lespedeza (Fig. 11).







Figs. 6–8. Light micrographs showing the cell-wall ingrowths in the areas of syncytia that adjoin xylem vessels. **6,** Portions of two syncytia (S_1, S_2) surrounded by xylem (X) in soybean. The cell-wall ingrowths (arrows), which appeared as densely stained bands, are prominent. Scale bar = 2.5 μm. **7,** The cell-wall ingrowths (arrowheads) along the syncytial wall facing toward the xylem (X) are also prominent in lespedeza. Scale bar = 2.5 μm. **8,** An entire syncytium (S) surrounded by xylem (X) in cleome root. Cell-wall ingrowths as shown in soybean and lespedeza are not evident in this host. A nematode (N) is shown adjacent to the syncytium. Scale bar = 10 μm.

DISCUSSION

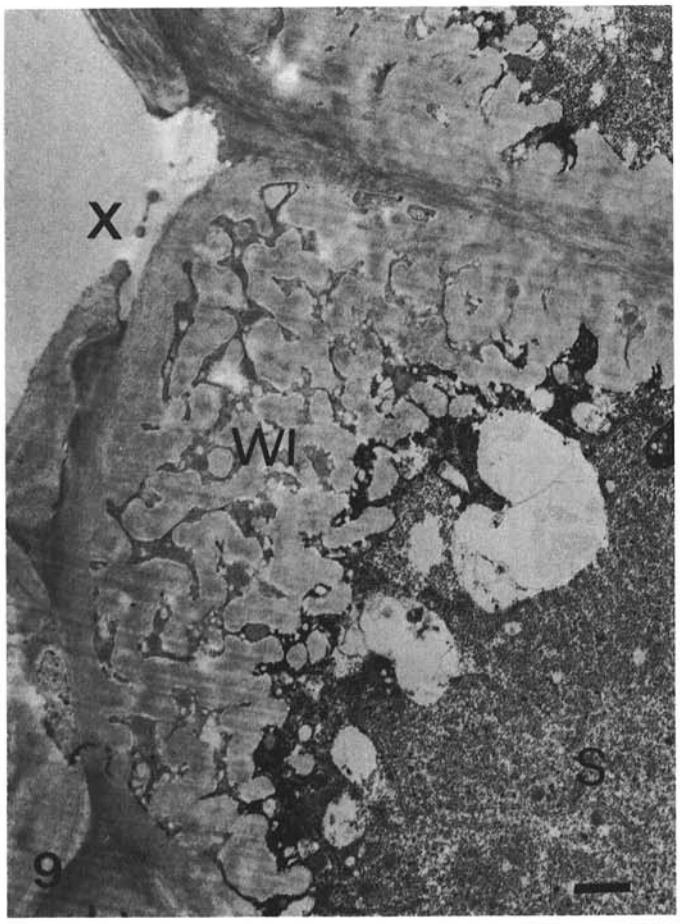
Syncytia were formed in various regions of the root tissues and the detailed cytological features of syncytial cells varied among the compatible host plants. The syncytia that occur inside the vascular bundle in cleome and soybean appear to be initiated in the xylary region and progress in a centrifugal direction ultimately to involve some phloem. In lespedeza the syncytium appears to begin in the cortical cells and then proliferate into the secondary xylem and phloem. Thus, initiation of syncytium begins outside the stele and progresses in a centripetal manner. Accordingly, the nematodes that induce intravascular syncytia in soybean and cleome penetrate deeply into the stele (Figs. 1 and 5) and those that induce the cortical type of syncytia in lespedeza stay at the periphery of the root (Fig. 3).

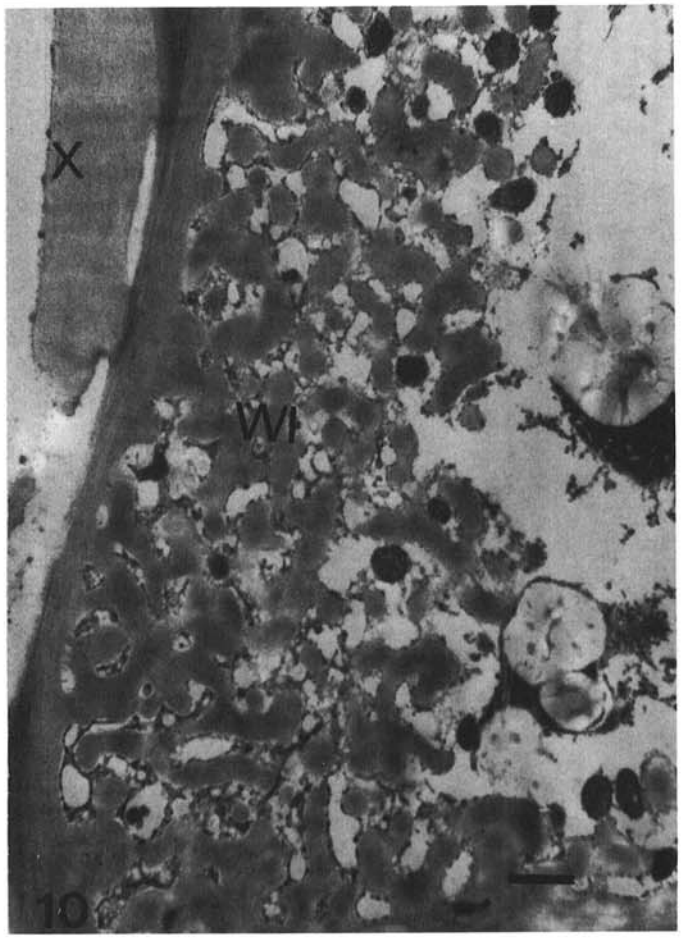
The variation in syncytial location appeared to be related to the degree of damage to the plants or to plant tolerance. Extensive syncytia in the stelar region appear to displace vascular elements so extensively that longitudinal transport of metabolites would be inhibited. In this respect, lespedeza seems to be more tolerant to damage by *H. glycines* than the other hosts of this study. However, the degree of tolerance between different plant species is difficult to measure. Nevertheless, in greenhouse conditions, stunting and/or chlorotic symptoms were more readily observed in soybean and cleome than in lespedeza. More study is needed to confirm these relationships.

The difference in the number of cell-wall ingrowths appeared to be related to the degree of susceptibility of the plant, which was also associated with the persistence of syncytia. Cell-wall ingrowths are known to be responsible for transferring solutes into syncytia from surrounding host tissue (7,8). This suggests that soybean is more susceptible to the nematode than lespedeza and cleome because well-developed cell-wall ingrowths were more prevalent in soybean than in the other two hosts. This study, therefore, supports the findings of Riggs (10) who reported that the efficiency of nematode reproduction was far superior in Lee soybean than in other hosts.

Jones and Dropkin (6) suggested that cell-wall ingrowths might be formed through a stimulus related to depletion of cytoplasmic contents caused by nematode feeding. If so, the variations in susceptibility of the plant may be due to the nutritional quality of the host plants. The variations may, therefore, be applicable to compatible soybean cultivars in which inconsistent nematode reproduction may occur because of environmental settings and other factors affecting host nutrition.

Of interest is the location of syncytia in the root and the degree of cell-wall ingrowths that appeared not to be related to each other because syncytia in cleome, which always formed in the conducting tissue, and were interfaced with many neighboring xylem vessels, had the poorest or fewest cell-wall ingrowths. Thus, tolerance and





Figs. 9–11. Electron micrographs showing cell-wall ingrowths in syncytia formed in different hosts. Scale bars = 1 μm. **9,** Prominent cell-wall ingrowths (WI) in a syncytium (S) formed in soybean. X = xylem. **10,** Cell-wall ingrowths formed in a lespedeza syncytium. X = xylem. **11,** Poorly developed cell-wall ingrowths formed in a cleome syncytium. X = xylem.

resistance may be governed by different genetic factors in the plants. In soybean cultivars, no relationships were found between nematode reproduction and plant tolerance (2).

The results of our study suggest that the differences in morphological characteristics of syncytia are related to the degrees of tolerance and susceptibility of plants. These morphological differences may be used for selection of soybean cultivars that are tolerant and less susceptible to *H. glycines* in breeding programs.

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