Histopathology of Beta vulgaris to Individual and Concomitant Infections by Meloidogyne hapla and Heterodera schachtii[1]

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Abstract: Histological changes in roots of *Beta vulgaris* cultivar 'USH9A' resulting from infection of *Meloidogyne hapla* alone, *Heterodera schachtii* alone, or infection by both species on one feeding site were studied. Anatomical changes caused by *M. hapla* infection were characterized by regions of hypertrophy and hyperplasia. Giant cells were formed within the stele and varied in numbers from 4-7/feeding site; hyperplasia occurred in the form of a large number of relatively small compacted cells generally surrounding the hypertrophied region. *H. schachtii*-induced syncytia became dense and multinucleate. Syncytia were formed in the stele and were limited on the side toward the nematode by endodermis or in part by cortical tissue. Histological changes due to the presence of both parasites on one feeding site were characterized by formation of two distinctive pathological tissues typical of both nematodes. In most infections, xylem elements separated the two pathological tissues. In some sections, a single wall separated the two pathological tissues, and no dissolution of separating wall was noted in any sections. Each nematode developed normally and produced its own characteristic pathological tissue independently. *Key Words:* interrelationships, cyst, root-knot, sugar beet.

Histopathological aspects of nematode-fungus (11, 13, 14) and nematode-bacteria (8) complexes in various plants have been reported. However, studies regarding histopathological responses of plants infected with different nematodes are scarce and warrant attention. The only report of this nature is by Mankau and Linford (10) who found no interactions between *Meloidogyne hapla* and *Heterodera trifolii* on Ladino clover with respect to selection of feeding sites and tissue development. The scope of the present study was to investigate the interrelationships of *M. hapla* Chitwood and *H. schachtii* Schmidt on *Beta vulgaris* L. at the cellular level. A preliminary report of this study has been presented (5).

MATERIALS AND METHODS

Three-week-old seedlings of *Beta vulgaris* cultivar 'USH9A' were inoculated with freshly hatched larvae (selected numbers) of *Meloidogyne hapla* or *Heterodera schachtii* separately or in various combinations. Inoculum combinations included both nematodes inoculated simultaneously, *M. hapla* preceding *H. schachtii*, or *H. schachtii* preceding *M. hapla* by 10 days. Thirty days after the original inoculation, roots were soaked in water and carefully removed from the soil. Root specimens infected with *M. hapla* or *H. schachtii* alone and together on one feeding site were collected from different treatments for histological studies. Root samples were also collected from non-inoculated seedlings.

The roots were placed in vials containing a formalin-aceto-alcohol solution (7) and stored until being processed for study. Root segments were cut into 1-cm sections, dehydrated with tertiary-butyl alcohol, and embedded in tissuemat (7). Longitudinal and cross sections of 12 μm thick were cut with a rotary microtome. Sections were mounted on slides and the preparations were stained with Johansen's quadruple stain (7).

RESULTS AND DISCUSSION

Histopathology of Meloidogyne hapla infected tissue: Anatomical changes due to *M. hapla* infection were characterized by regions of hypertrophy and hyperplasia. Hypertrophied cells containing many nuclei occurred in the form of giant cells filled with a reticulate network of protoplasm. Giant cell formation was associated with the feeding site of nematodes. In one instance, a large number of hypertrophied cells was observed in the vicinity of giant cells (Fig. 1-E). Hyperplastic regions were composed of large numbers of relatively small, compacted cells which surrounded the hypertrophic regions. Extensive hyperplasia was evident

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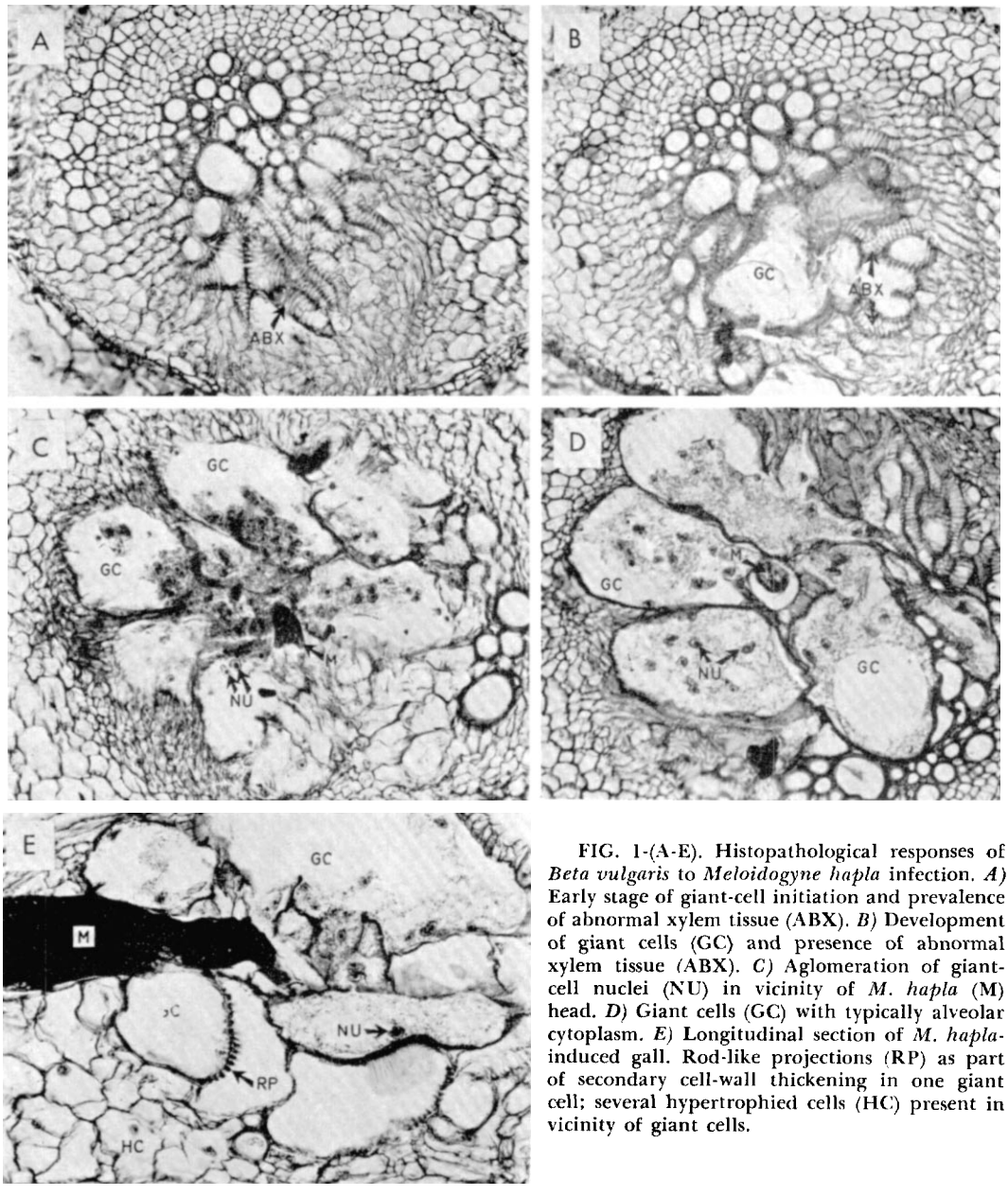

FIG. 1-(A-E). Histopathological responses of *Beta vulgaris* to *Meloidogyne hapla* infection. *A)* Early stage of giant-cell initiation and prevalence of abnormal xylem tissue (ABX). *B)* Development of giant cells (GC) and presence of abnormal xylem tissue (ABX). *C)* Aglomeration of giant-cell nuclei (NU) in vicinity of *M. hapla* (M) head. *D)* Giant cells (GC) with typically alveolar cytoplasm. *E)* Longitudinal section of *M. hapla*-induced gall. Rod-like projections (RP) as part of secondary cell-wall thickening in one giant cell; several hypertrophied cells (HC) present in vicinity of giant cells.

around the giant cells as the result of *M. hapla* activities. Head regions of the nematodes were in the vascular tissue, and their swollen bodies were located in the cortex. Cells around the nematode body were either collapsed or compressed due to pressure applied by tissue development or enlargement of the nematode. Head regions of enlarged females were often observed in the vicinity of 4-7 giant cells which engulfed the nematode head (Fig. 1 C-E). Giant cells displayed an irregular pattern of secondary wall thickening with the cytoplasm becoming highly granular and dense. Rod-like projections as part of secondary cell wall thickening, as reported by Krusberg and Nielson (9) and Huang and Maggenti (2), were observed in some giant cells (Fig. 1-E).

Atypical tissues designated "abnormal xylem," as reported by other investigators (9, 15), were observed in many sections (Fig. 1-A, B, D). These tissues were located in vascular tissues around giant cells.

Formation of these tissues was probably due to stimulations by nematode feeding or injury to xylem parenchyma.

Irregularity in shapes of some giant cells was in the form of diffused walls, a factor indicating that dissolution of cell walls gave rise to multinucleation of giant cells. However, Huang and Maggenti (2), in their ultrastructural studies of *M. javanica* infected *Vicia faba,* showed multinucleation was derived from repeated mitosis without cytokinesis. Ultrastructural studies of *Meloidogyne* spp. infections in various hosts may provide an answer to multinucleation phenomena of giant cells.

Histopathology of Heterodera schachtii infected tissue: The syncytium, caused by the feeding of *H. schachtii* in young roots where no secondary growth had occurred, possessed dense and multinucleate cytoplasm (Fig. 2-C). The syncytium was typically formed within the stele and was limited on the side toward the nematode by endodermis or in part by cortical cells (Fig. 2-D). The reticulate hyperchromatic cytoplasm was continuous throughout the syncytium and contained enlarged nuclei with distinct large nucleoli (Fig. 2-C). These findings are in agreement with results of other investigators (1, 10). Mankau and Linford (10) reported the syncytium was formed by a progressive dissolution of adjoining cell walls. Nemec (12) found that dissolution of cell walls first initiated holes and finally caused dissolution of the entire wall. This dissolution resulted in the merging of protoplasts until the syncytium was one continuous mass. Longitudinal sections of roots in our study indicated dissolution of walls of hypertrophied cells in syncytia and subsequent merging of protoplasts to form a large mass of protoplasm (Fig. 2-A, B).

Syncytial cytoplasm in some sections was dense, alveolar, and contained small globules. Nuclei within these syncytia were hypertrophied and pycnotic [with deeply stained membranes (Fig. 2-C)].

The gross effect of the nematode infection was accentuated by extensive cambial damage. This type of cambial damage deprives large regions of expanding roots of normal secondary xylem and phloem tissues.

Histopathology of roots associated with Meloidogyne hapla and Heteroaera schachtii occurring in one feeding site: Histological responses of *B. vulgaris* to *M. hapla* infection in the presence of *H. schachtii* (or vice versa) were characterized by the presence of hypertrophied and hyperplastic cells. Hypertrophied cells occurred in the form of giant cells and syncytia, a factor indicating infection by *M. hapla* and *H. schachtii,* respectively. Hyperplastic cells were abundant around giant cells.

The number of *M. hapla* giant cells varied from 4-7/feeding locus. They exhibited secondary wall thickening and contained highly alveolar cytoplasm with the hypertrophied nuclei clustered irregularly in their centers (Fig. 3 A-C). Rod-like projections as a part of secondary cell wall thickening were not observed. However, abnormal xylem tissues were present and were found in the vicinity of giant cells (Fig. 3-B, C). Sections from roots inoculated simultaneously with both nematodes displayed more numerous abnormal xylem elements than other treatments.

Syncytia induced by *H. schachtii* were typically formed within the stele and occasionally invaded xylem, phloem, and parenchyma cells. They contained the characteristically reticulate hyperchromatic cytoplasm and hypertrophied nuclei. They did not differ from those which occurred when *M. hapla* was not present. Similarly, the general appearance of *M. hapla*-induced giant cells associated with the presence of *H. schachtii*-induced syncytia did not differ from the appearance of those which occurred in the absence of syncytia. Numbers of *H. schachtii*-induced syncytia varied from 6-16/feeding locus of nematode (Fig. 3 A-C). Cell wall dissolution was observed in some sections (Fig. 3-A, B).

Giant cells induced by *M. hapla* contrasted strikingly with *H. schachtii*-induced syncytia in size and denseness of cytoplasm. The cytoplasm of syncytia was much more dense and granulated than that of giant cells. Individual *M. hapla*-induced giant cells were 2-8 times larger than individual *H. schachtii*-induced syncytia.

Usually there were no connections between *M. hapla*-induced giant cells and *H. schachtii*-induced syncytia as they were formed far from each other with xylem

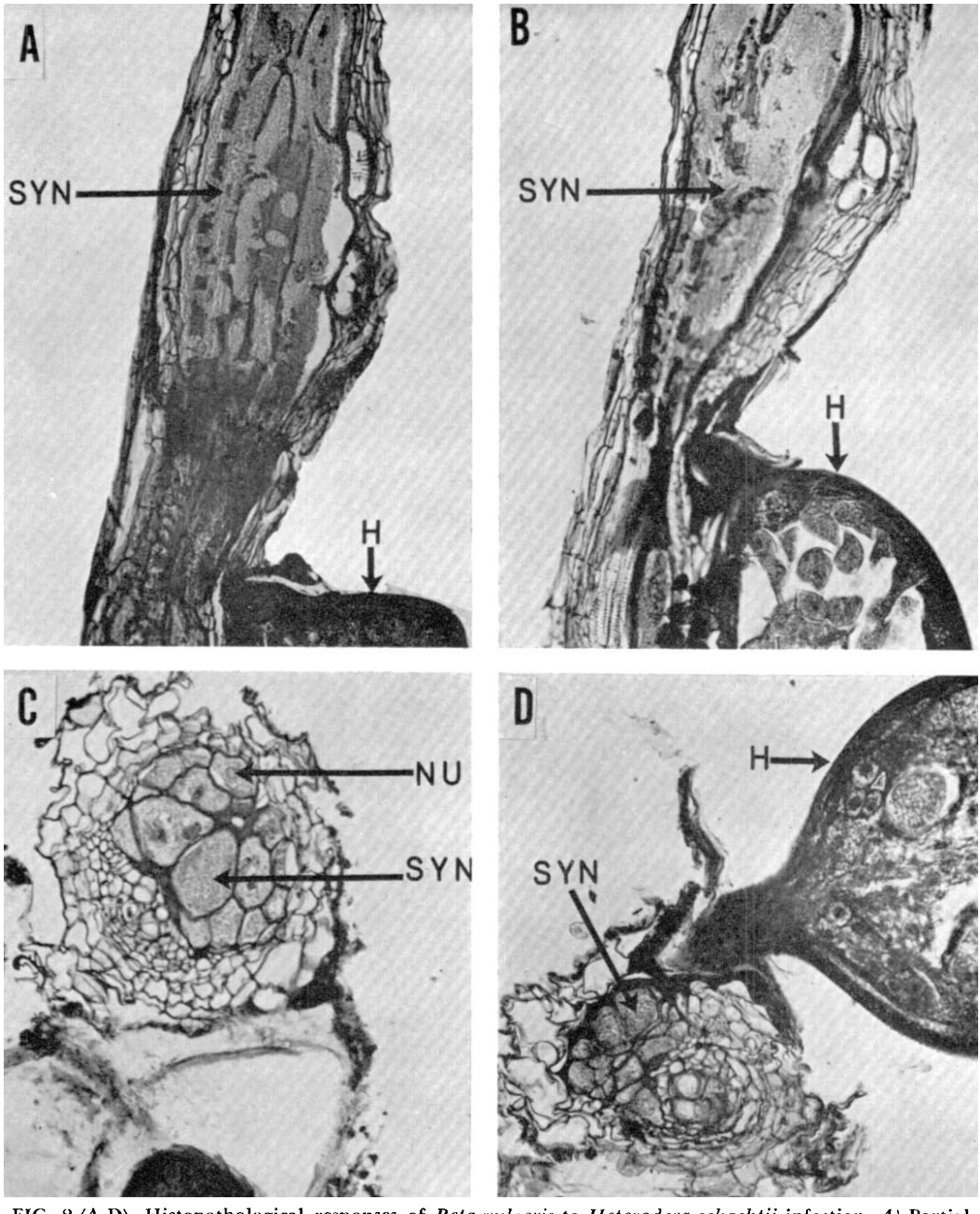

FIG. 2-(A-D). Histopathological responses of *Beta vulgaris* to *Heterodera schachtii* infection. *A)* Partial dissolution of cell walls in syncytia (SYN). *B)* Complete dissolution of cell walls and production of large syncytium (SYN) with cell-wall fragments still present in syncytium. Nematode head (H) in both sections is in contact with partly collapsed tissue. *C)* Syncytium enclosed within stele. Hypertrophied nuclei (NU) in some giant cells. *D)* Syncytium in rootlet of sugar beet with head of gravid female *H. schachtii* (H) in contact with tissue.

elements located between the two pathological tissues. Xylem tissue located between giant cells and syncytia was somewhat deformed in shape (Fig. 3-A). In some sections, when *H. schachtii* preceded *M. hapla* 10 days in inoculation, a single wall separated *M. hapla*-induced giant cells from *H. schachtii*-induced syncytium (Fig. 3-B, C). There was no dissolution of separating wall as reported by Mankau and Linford (10).

Giant cells induced by *M. hapla*

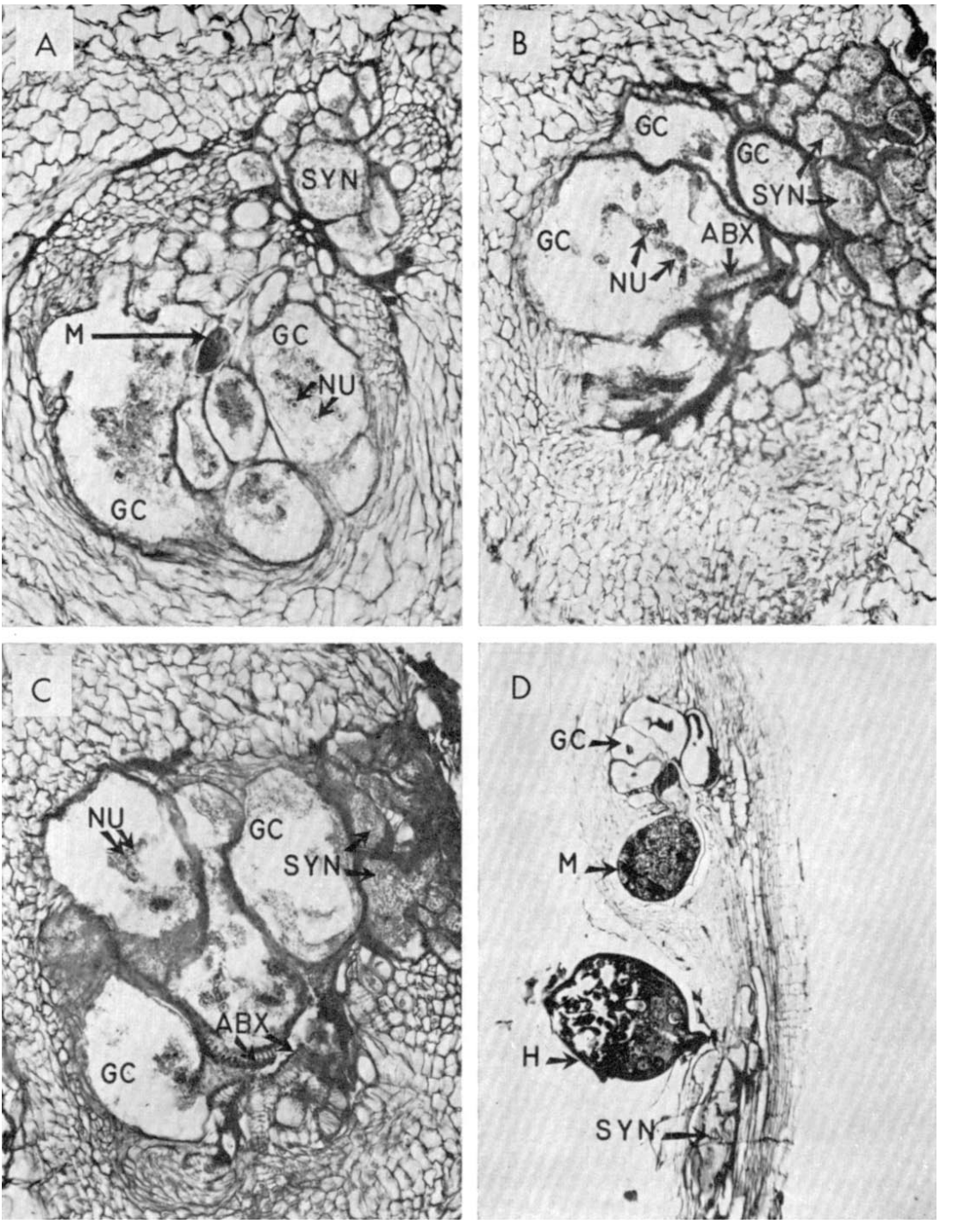

FIG. 3-(A-D). Histopathological responses of *Beta vulgaris to Meloidogyne hapla* and *Heterodera schachtii* occurring in one feeding site. *A* & *B) M. hapla* (M)-induced giant cells (GC), and *H. schachtii* (H)-induced syncytia (SYN) in close vicinity of each other. Hypertrophied nuclei (NU) located at center of giant cells. *B) M. hapla*-induced giant cells in contact with *H. schachtii*-induced syncytia. A single wall separates the two pathological tissues. *C)* Pathological tissues associated with abnormal xylem tissue (ABX). Walls in syncytia partially dissolved. *D)* Longitudinal section of root associated with both parasites feeding in vicinity of each other. The two pathological tissues are not connected.

occupied much of the root stelar region and *H. schachtii*-induced syncytia were pushed out in the cortical tissue. The syncytia were located on the side toward the nematode by cortical cells (Fig. 3 A-C). In some sections, *M. hapla* and *H. schachtii* were observed occurring in close proximity of each other (Fig. 3-D). Each nematode developed normally and produced its own characteristic pathological changes.

In infections following simultaneous inoculations of *M. hapla* with *H. schachtii*, or when either nematode species preceded the other by 10 days, the cytological responses of *B. vulgaris* to dual infection were similar.

Findings of this experiment parallel results of Mankau and Linford (10). They found no evidence of influence of *M. hapla* and *H. trifolii* on each other with respect to selection of feeding site or with respect to development on Ladino clover. They also reported a striking contrast of giant cells and syncytia even when the two types of pathological tissues lay in contact. Our findings indicate that the influence of *M. hapla* on the population shift of *H. schachtii* and vice versa, as reported elsewhere (3, 4, 6), is not at the cellular level. The population shift of either nematode in the presence of the other, as previous studies indicated (3, 4, 6), is probably due to physiological changes of the host plant. However, histopathological studies of these two important parasites on different hosts should provide information on the behavior of two organisms in the presence of each other. Physiological and biochemical investigations of the hosts infected with these two parasites may provide an answer to the population shift of one in the presence of the other and warrant further attention.

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