# COMPARATIVE HISTOLOGY OF FEEDING SITES INDUCED BY *CACTODERA ROSAE, GLOBODERA MEXICANA,* AND *MELOIDODERA ASTONEI* (NEMATODA: HETERODERIDAE)

Y. Gómez Hernandez[1], I. Cid del Prado Vera*[1], P. Yáñez Jiménez[2], and A. García Esteva[2]

[1]Programa de Fitopatología, Colegio de Postgraduados-Campus Montecillo, km 36.5 Carretera México Texcoco, Estado de México, C.P. 56230. [2]Posgrado en Botánica, Colegio de Postgraduados-Campus Montecillo, km 36.5 Carretera México Texcoco, Estado de México, C.P. *Corresponding author: icid@colpos.mx

## ABSTRACT

Hernández Gómez, Y., I. Cid del Prado Vera, P. Yáñez Jiménez, and A. García Esteva. 2017. Comparative histology of feeding sites induced by *Cactodera rosae*, *Globodera mexicana*, and *Meloidodera astonei* (Nematoda: Heteroderidae). Nematropica 47:114-119.

Histological studies were conducted on host plants infected by three species of nematodes of the Heteroderidae (*Cactodera rosae*, *Globodera mexicana*, and *Meloidodera astonei*). *Cactodera rosae* established a syncytium, which extended into the cortex, pericycle, phloem, and cambium of *Amaranthus hybridus* roots. The syncytial cells showed dense cytoplasm and hypertrophied nuclei and nucleoli. In *Solanum rostratum* roots, *G. mexicana* induced a syncytium that expanded from the pericycle into the stele incorporating phloematic elements and interfascicular cambial cells. The affected cells had dense cytoplasm, hypertrophied nuclei and nucleoli, and were surrounded by hyperplastic vascular parenchyma tissue. Females of *M. astonei* induced an uninucleate giant cell in the pericycle of *S. rostratum* roots. The giant cell, which had a dense cytoplasm and a hypertrophied nucleus, expanded in the tissues of the stele and especially in the secondary phloem. The cells of vascular parenchyma and secondary phloem tissues were hyperplastic. These anatomical changes are in agreement with those of other species of these genera reported in the literature.

*Key words:* *Amaranthus hybridus*, Heteroderidae, histopathology, *Solanum rostratum*, syncytium, uninucleate giant cell.

## RESUMEN

Hernández Gómez, Y., I. Cid del Prado Vera, P. Yáñez Jiménez, y A. García Esteva. 2017. Histología comparativa de sitios de alimentación inducidos por *Cactodera rosae*, *Globodera mexicana* y *Meloidodera astonei* (Nematoda: Heteroderidae). Nematropica 47:114-119.

Estudios de histología se realizaron en algunas especies de nematodos de la familia Heteroderidae con el objetivo de comparar y describir los cambios en sus hospedantes causados por (*Cactodera rosae*, *Globodera mexicana*, y *Meloidodera astonei*,). *Cactodera rosae* forma un sincitio con citoplasma denso y núcleos y nucleólos hipertrofiados en el periciclo y expanden en las células de la corteza y cambium de *Amaranthus hybridus*. En *Solanum rostratum*, *Globodera mexicana* induce un sincitio con denso citoplasma y núcleos y nucleólos hipertrofiados e hiperplasia en las células del periciclo y en el tejido del cambium interfascicular. Finalmente las hembras de *M. astonei* inducen una célula gigante uninucleadas en el periciclo y expanden en el floema secundario de *S. rostratum*. La célula gigante tiene citoplasma denso, un núcleo hipertrofiado con nucléolo prominente esta circundada de tejidos vasculares del floema secundario. Estos cambios anatómicos están relacionados con los reportados en la literatura con otras especies.

*Palabras clave: Amaranthus hybridus* Heteroderidae, histopatología, *Solanum rostratum,* sincitio, célula gigante uninucleada.

## INTRODUCTION

Sedentary plant-parasitic nematodes cause serious problems in global agriculture. In particular, species of the cyst-forming genera *Globodera*, *Heterodera*, and *Punctodera* adversely affect the production of agronomic and vegetable crops (Wieczorek and Gundler, 2006). A characteristic feature of the parasitic habits of these sedentary plant-parasitic nematodes is their ability to induce the formation of specialized feeding sites in the roots of their hosts (Siddiqi *et al*., 2012). These specialized feeding sites consist of a syncytium for the cyst-forming nematodes of the genera *Globodera* and *Heterodera* and of an uninucleate giant cell for the cystoid nematodes of the genus *Meloidodera*, which do not form cysts. The nature, structure, and physiological function of the modified cells are the product of the plant's response to infection and the long coevolution that has resulted in a relationship between the parasite and its host (Subbotin, 1993; Subbotin *et al*., 2010). In Mexico, various species and populations of Heteroderidae have been described, including: *Cactodera rosae* Cid del Prado and Miranda, 2008, *Globodera mexicana* (Campos Vela, 1967) Cid del Prado, 1991, and *Meloidodera astonei* Cid del Prado and Rowe, 2000. The anatomical changes induced by these nematode species on their hosts are not known. In the present study, the nematode feeding sites and histological alterations observed in the roots of their host plants are described and compared.

## MATERIALS AND METHODS

### *Plant material*

Roots of *Amaranthus hybridus* L. parasitized by *C. rosae* were collected at Rancho Los Sauces in Tecoac, Huamantla, Tlaxcala State. Roots of *Solanum rostratum* Dunal colonized by *G. mexicana* were obtained from the type locality Rancho Los Sauces, Tecoac Tlaxcala State. Additional samples of *S. rostratum* roots were collected in La Purificación, Texcoco, Mexico State, where this plant is parasitized by *M. astonei*.

### *Histopathology*

Parasitized fibrous (secondary and tertiary) roots were selected, washed free of soil, cut into 4 to 5 mm long segments, fixed in FAA, dehydrated in a xylol-alcohol series and embedded in paraffin under vacuum. Embedded root segments were sectioned 8 to10 µm thick, stained with safranin fast green, mounted in synthetic resin, examined and photographed using a compound photomicroscope (Karl Zeiss) with a PAXcam3digital camera and Rossbach Microscope with a Canon EOS50D camera (Johansen, 1940).

## RESULTS

In *A. hybridus* roots, *C. rosae* caused necrosis of the cells of the cortex and induced a feeding site in the pericycle initiating the formation of a syncytium in the stele as a consequence of fusion of pericyclic, vascular parenchyma, and phloematic elements and cambial cells (Fig. 1). Cross sections of nematode-infected roots showed a large syncytium consisting of enlarged syncytial cells with thin cell wall and dense cytoplasm, hypertrophied nuclei, and prominent nucleoli (Fig. 1 B, C). Longitudinal sections showed the anterior portion of the nematode penetrated into the phellogen and cortex and attached to a stelar syncytium consisting of enlarged syncytial cells (Fig. 1 D). The syncytium caused asymmetry of the stele and expanded in about 50% of the stele, which was obliterated in large portion (Fig. 1 B, C). In cross sections, the dimensions of the syncytia were 40 to 185 µm long × 35 to 95 µm wide with nuclei 13 to 25 µm long × 8 to 20 µm wide. In longitudinal root sections, syncytium dimensions were 105 to 700 µm long × 30 to 183 µm wide (Fig. 1 D).

Observations of cross sections of *S. rostratum* roots infested by *G. mexicana* revealed that the nematode female remained embedded in the root cortex and induced formation of a stelar syncytium, which partially obliterated and fragmented the stele (Fig. 2). The examination of these cross sections indicates that the syncytium initiated in the pericycle and expanded in the stele by incorporating vascular parenchyma, phloem, and cambial cells. Large syncytial elements with hypertrophied and amorphous nuclei and nucleoli were also observed in both cross and longitudinal sections of the roots (Fig. 2 C, D). The dimensions of the syncytium were 28 to 198 µm long × 18 to 150 µm wide in cross sections (Fig. 2 C) and 50 to 560 µm long × 38 to 200 µm wide in longitudinal sections.

Examination of nematode infected *S. rostratum* fibrous roots indicated that the adult females of *M. astonei* have semiendoparasitic habits. Swollen females were observed attached to the roots with the anterior portion of the body while the posterior and swollen portion of the body was protruding from the root surface. Observations of root cross sections indicated that *M. astonei* penetrated the epidermis and cortex where they caused cell breakdown and necrosis. The nematode established a permanent feeding site in the pericycle inducing the formation of an uninucleate giant cell expanding in the vascular

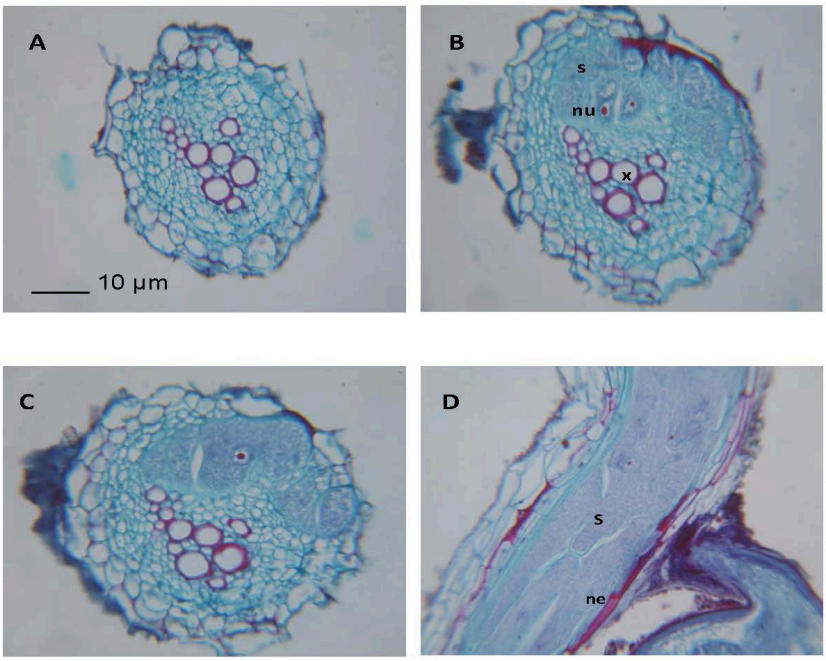


Fig.1. Anatomical alterations induced by *Cactodera rosae* in roots of *Amaranthus hybridus*. A) Cross section of a healthy root. B, C) Cross sections showing syncytia (s) originating from the pericycle and expanding into a large portion of the stele, cortex and reaching the periderm. Elements of vascular parenchyma, phloem and cambial cells are incorporated in the syncytia, which show dense cytoplasm, hypertrophied nuclei (nu) and prominent nucleoli; xylem (x). D) Longitudinal section showing the anterior portion of the nematode body (ne) penetrated into the periderm and cortex and attached to a syncytium, which has obliterated the vascular tissues. (Scale bar for all figures = 10 µm).

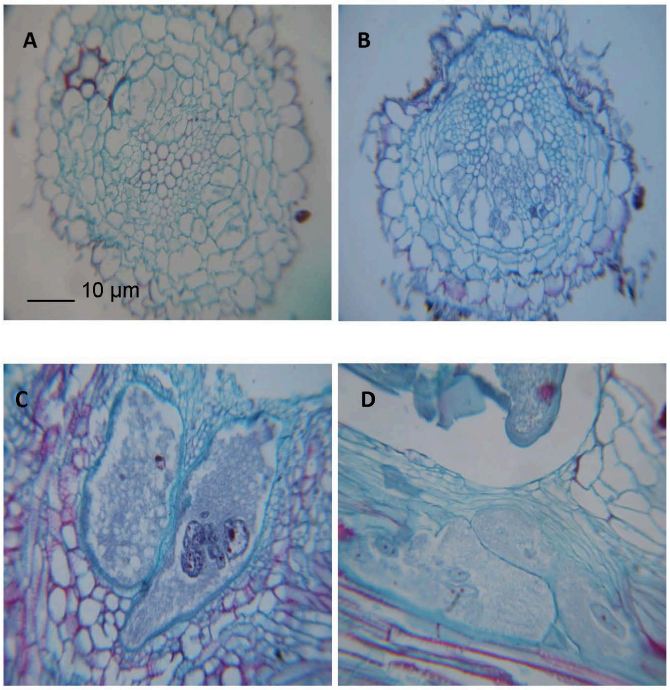


Fig. 2. Histological alterations induced by *Globodera mexicana* in *Solanum rostratum* roots. A) Cross section of a healthy root. B) Cross section showing a syncytium expanding in the stele, incorporating cells of the pericycle, vascular parenchyma, phloem and interfascicular cambium and causing asymmetry of the stele. C) Cross section showing large syncytial cells with hypertrophied amorphous nuclei and prominent nucleoli. D) Longitudinal section showing large syncytial cells with hypertrophied nuclei containing one or two nucleoli. (Scale bar for all figures = 10 µm).

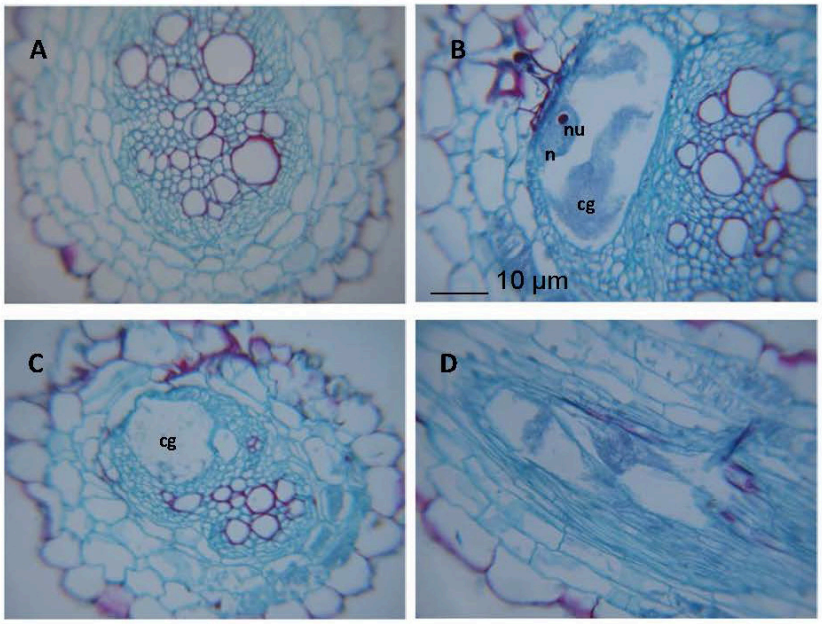


Fig. 3. Histological alterations induced by *Meloidodera astonei* in *Solanum rostratum* roots. A) Cross section of a healthy root. B) Cross section showing a single uninucleate giant cell (cg) expanding in the stele. Note the single hypertrophied nucleus (n) with a prominent nucleolus (nu). The dense cytoplasm of the giant cell is ruptured due to tearing during the sectioning of the root. C) Cross section showing a giant cell expanding from the pericycle into the stele and disorganizing the stelar tissues. D) Longitudinal section showing the single uninucleate giant cell obliterating a large portion of the vascular tissues. (Scale bar for all figures = 10 µm).

parenchyma, phloem and cambium of *S. rostratum* roots (Fig. 3). The vascular parenchyma and phloem surrounding the giant cell were hyperplastic (Fig. 3 B, C). The nucleus of the giant cell was hypertrophic and showed a prominent nucleolus (Fig. 3 B). Displacement and distortion of phloematic and xylematic elements due to the enlargement of the giant cell was observed in both cross and longitudinal section of the roots (Fig. 3 B-D). The dimensions of the giant cells observed in cross sections ranged from 20 to 234 µm long × 13 to 220 µm wide. The dimensions of uninucleate giant cells observed in longitudinal root sections ranged from 63 to 360 µm long × 28 to 158 µm wide.

## DISCUSSION

The anatomical alterations induced by the heteroderids we studied did not differ from those reported for other heteroderid species in the literature. In *A. hybridus*, *C. rosae* induced the feeding site in a cell of the pericycle with formation of a syncytium derived by partial fusion of vascular parenchyma, phloem, and cambial cells causing asymmetry of the stele and obliteration of portion of the cortex. Similar observations were reported by Tovar-Soto *et al*. (2007) in *Hordeum vulgare* where *C. galinsogae* affected the cells of the cortex and the vascular cylinder and led to the disorganization, displacement and rupture of the xylem and phloem. Hernández *et al*. (2006) also observed that the syncytia in *C. galinsogae*, in *Galinsoga parviflora* and *Bidens odorata*, were located mainly in the vascular cylinder and cortex. *Globodera mexicana* induced formation of a syncytium originating from the pericycle of roots of *S. rostratum*. The syncytium induced by *G. mexicana* in the roots of its host expanded in the central vascular cylinder causing displacement of the xylem and phloem like other *Globodera* species (Vovlas *et al*., 1986, Rice *et al*., 1987, Doucet *et al*., 2004). We did not observe expansion of the syncytium from the vascular parenchyma into the cortex as it has been reported for *G. pallida* (Stone, 1973) Behrens, 1975, *G. rostochiensis* (Wollenweber, 1923) Behrens, 1975, and *G. tabacum* (Lownsbery and Lownsbery, 1954) Behrens, 1975.

*Meloidodera astonei* induced an uninucleate giant cell originating from the pericycle in the roots of *S. rostratum* and having characteristics similar to those reported by Cid del Prado and Cardenas (1995)

for the closely related species *M. mexicana* Cid del Prado, 1991 in roots of *Capsicum annuum*, and also to those induced by *M. charis* Hopper, 1960 in the roots of *Peonia californica* reported by Mundo-Ocampo and Baldwin (1983). The results of these studies are in agreement also with other studies reported in literature (Cohn *et al*., 1984; Inserra and Vovlas, 1986).

In conclusion, we would like to emphasize that the heteroderids that we studied induce permanent feeding sites in the pericycle/endodermis. The specialized cells that they induce expand into the central cylinder of the roots and, in the case of the syncytium, can incorporate stelar tissues including cambial cells preventing formation of xylem (Jones, 1981). These specialized feeding sites, which consist of a syncytium or an uninucleate giant cell, disrupt the anatomical structure of the roots preventing the normal flow of water and nutrients from the roots to the canopy of their host plants, which become stunted and unproductive. These feeding structures induced by these parasites have high metabolic activity and serve as a nutrient sink that the nematode female utilizes for its development and reproduction.

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