PARASITISM OF FISHPOLE BAMBOO ROOTS BY *AFENESTRATA KOREANA*

R. N. Inserra,[1] N. Vovlas,[2] and P. S. Lehman[1]

Florida Department of Agriculture and Consumer Services, DPI, Nematology Section, Gainesville, Florida 32614-7100, U.S.A.,[1] and Istituto di Nematologia Agraria, C.N.R., via Amendola 165/A, 70126 Bari, Italy.[2]

RESUMEN

Inserra, R. N., N. Vovlas y P. S. Lehman. 1999. Parasitismo de *Afenestrata koreana* en la raíz del bambu vara de pesca. Nematrópica 29:105-111.

El nematodo exótico del quiste del bambu, *Afenestrata koreana*, se detectó por primera vez en Florida en el bambu vara de pesca, *Phyllostachys aurea*, propagado a partir de material de reserva proveniente del lejano oriente. Ambos juveniles de *A. koreana*, vermiformes y engrosados, tienen hábitos semi-endoparasíticos como las hembras adultas, y la parte posterior del cuerpo se encuentra sobresaliendo de la superficie de la raíz. Las hembras maduras produjeron masas de hasta 35 huevos. Al final del ciclo de vida, esas hembras pasaron a ser quistes de color carmelitoso claro, los que parecen menos esclerosados que los de *Heterodera*. Todos los huevos contenidos en los quistes, se abrieron cuando el suelo humedo (22%) e infestado con estos se almacenó en bolsas plásticas durante cuatro meses a 22°C. Los cambios anatómicos inducidos por *A. koreana* a las raíces del bambu vara de pesca, nuevo hospedante de este nematodo, consistieron de un sincitio multinucleado, originado a partir de una célula endodermica y la incorporación de parenquima vascular pericíclico y floemático, así como elementos del xylema. Las paredes externas del sincitio se engrosaron más que las de las células sanas. Se observaron protuberancias en la pared celular cerca del sitio de alimentación del nematodo. Las paredes internas del sincitio, las que dividen las unidades de este, estaban perforadas y fragmentadas, debido al enlargamiento del mismo. Durante la observación al microscopio, no se observaron invaginaciones en la pared celular, lo que confirma reportes anteriores sobre la falta de las mismas en los sincitios inducidos por *A. koreana* en bambu comestible (*P. pubescens*) y por *A. africana* en *Panicum maximum*. Los núcleos y nucleolos de los sincitios fueron mayores que los de las células normales. Los núcleos mostraron una mayor indentación profunda. Las características de los sincitios inducidospor *A.koreana* en el bambu vara de pesca fueron similares a los de los sincitios inducidos por *Atalodera* spp. en el cardillo y la madreselva y por *Punctodera chalcoensis* en el maíz.

Palabras claves: biología, Florida, hábitos parasíticos, nematodo enquistador, *Phyllostachys aurea*, respuesta del hospedante, sincitio, sobrevivencia.

In 1997, a cyst-forming nematode new to the United States was detected in regulatory samples collected from fishpole bamboo (*Phyllostachys aurea* Carriere ex A. Riviere and C. Riviere) in a nursery of central Florida (Inserra, 1997). The nematode was identified as *Afenestrata koreana* Vovlas, Lamberti and Choo, 1992, which was described from edible bamboo (*P. pubescens* Mazel ex Houz. De Leh.) in Korea. This species and another, *A. africana* (= *Sarisodera africana*) (Luc, Germani, and Netscher, 1973) Baldwin and Bell, 1985, from Guinea grass (*Panicum maximum* Jacq.) in Ivory Coast, have similar parasitic habits and induce in the roots of their type hosts a syncytium composed of interconnected chambers (Baldwin and Bell, 1985; Vovlas *et al.*, 1993). Three additional species have been reported in distant geographical areas of the world: *A. axonopi* Souza, 1996 on a perennial grass (*Axonopus marginatus* (Trinius) Chase) in Brazil, *A. orientalis* Kazachenko, 1989 also on a perennial grass (*Miscanthus purpureus* = *M. purpurescens* Andersson) in the Maritime Territory in the far-east of Russia, and *A. sac-*

chari Kaushal and Swarup, 1988 on *Saccharum munjo* (= *S. munja* Roxb.) in India. Also, *A. africana* was found on a unidentified grass in South Africa (Kleynhans, 1992). However, in spite of these reports of detection of new and described *Afenestrata* species, there is a lack of information on the biology of these cyst-forming nematodes. The objective of this study was to obtain additional biological data on *A. koreana* parasitizing fishpole bamboo.

Fishpole bamboo roots were collected from the infested site and observed in water with the aid of a stereo microscope to detect nematode life stages present on the root surface. Nematode specimens were removed from the root surface, transferred to water agar (Esser, 1986), and life stages determined with a compound microscope on the basis of body size and molting cuticle. Survival of eggs in the cysts was studied in a sandy soil at two moisture levels, 22% and 4%, which was stored at 22°C in plastic bags for 4 and 12 months, respectively. In order to determine the ability of cysts to withstand air drying, *A. koreana* cysts were placed in dry petri dishes and stored for 4 days. *Heterodera glycines* Ichinhoe, 1952 cysts were stored in the same manner for comparison. Histological examination of nematode-infected roots was conducted on selected fishpole bamboo roots with attached nematode females. The roots were gently washed free of soil, cut into 4-5 mm long pieces, fixed in 2% formaldehyde aqueous solution, dehydrated in a tertiary butyl alcohol series, and embedded in paraffin under vacuum. Embedded root segments were sectioned 10-12 μm thick, stained with safranin fast green, mounted in Dammar xylene and examined with the aid of a compound microscope (Johansen, 1940).

Examination of nematode-infected fishpole bamboo roots indicated that *A. koreana* juveniles have semiendoparasitic habits as do the adult females (Fig. 1A-D). Both vermiform and swollen juveniles were observed with the posterior portion of the body protruding from the root surface (Fig. 1A-C). The juveniles were in clusters and often associated with swollen females in the same portion of the root (Fig. 1A,C). The majority of white adult females had the posterior portion of the body covered by a gelatinous matrix containing up to 35 eggs, some of which embryonated (Fig. 2A, B). These eggs hatched in 24 hours when kept in water in petri dishes at 22°C. White adult females also produced clusters of eggs without gelatinous matrix when kept directly in water. These white females contained eggs in different stages of development. At the end of their life cycle, white females became light brownish cysts. Only empty eggshells remained in cysts stored in soil at 22% moisture for 4 months. Only cyst fragments and no eggs were recovered from soil that was stored for 12 months. Soil moisture of the latter treatment declined from 4% to 1% during storage. These results suggest that the vermiform second-stage juveniles (J2) of *Afenestrata koreana* emerge quickly from the cysts, rather than remaining quiescent in the egg as do the J2 in embryonated eggs of *Heterodera* spp. cysts. Cysts of *A. koreana* were less sclerotized than *Heterodera* cysts and when they were kept in dry petri dishes, they all shrank, whereas hardened and sclerotized *Heterodera* cysts maintained their original shape.

Histological examination of fishpole bamboo roots infected by *A. koreana* showed that the nematode penetrated the epidermis and the cortical parenchyma, and established a permanent feeding site in an endodermal cell (Fig. 3A). Multiple infection sites in the same root section were also observed (Fig. 3B). The nematode remained attached to the wall of the endodermal cell adjacent to the pericycle

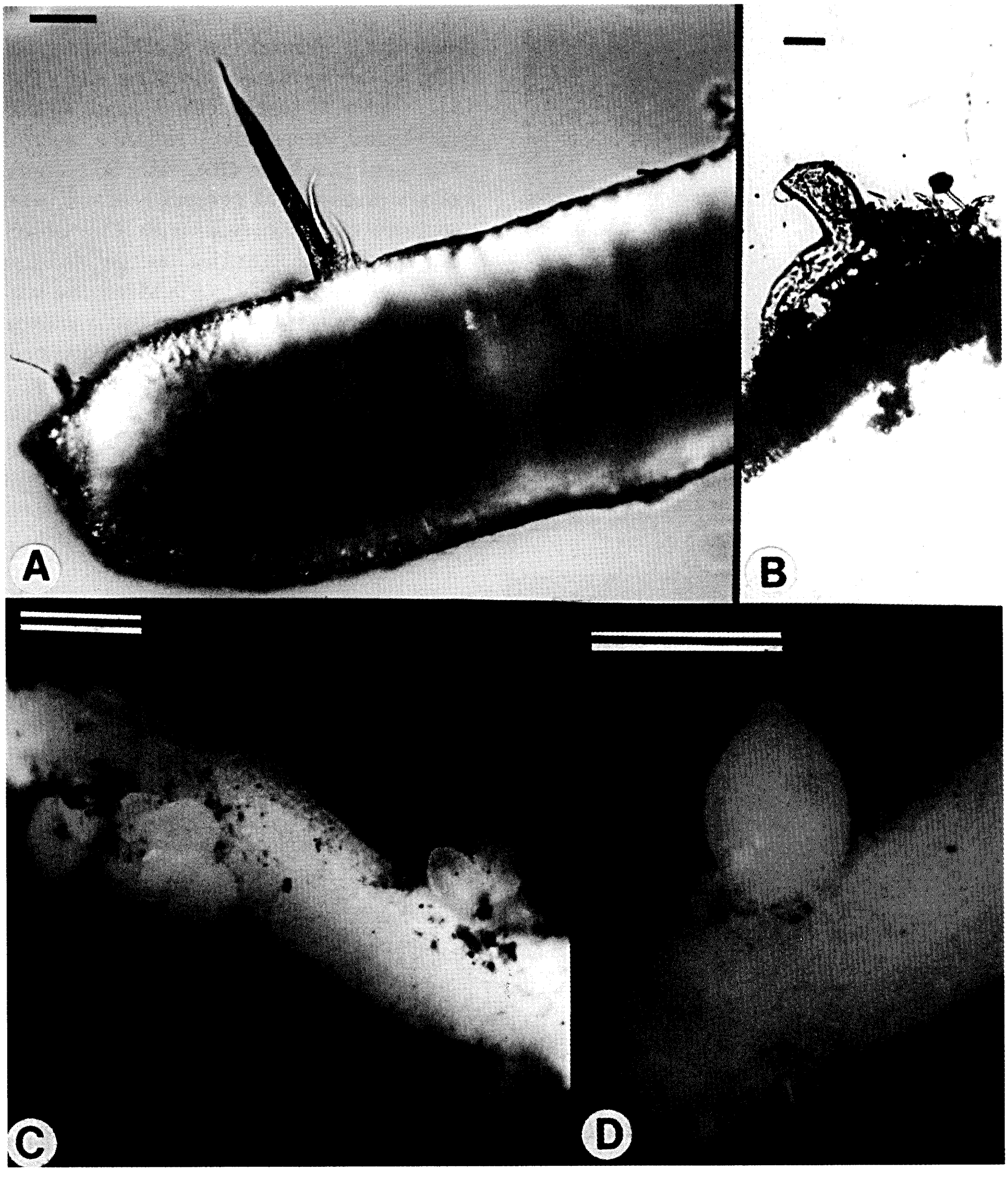

Fig. 1. Photomicrographs of *Afenestrata koreana*. Semiendoparasitic habits of juvenile and adult females on *Phyllostachys aurea* roots. A) Cluster of second-stage juveniles (J2). B) Swollen J2. C) Clusters of fourth-stage juveniles. D) Adult swollen female. Scale bars = 70 µm in A, 43 µm in B, 320 µm in C, and 380 µm in D.

and started feeding in the pericycle after perforating the endodermal cell wall with the stylet (Fig. 4A, B). Nematode feeding caused enlargement of pericyclic cells and subsequent dissolution of cell walls and fusion of these cells to form a syncytium,

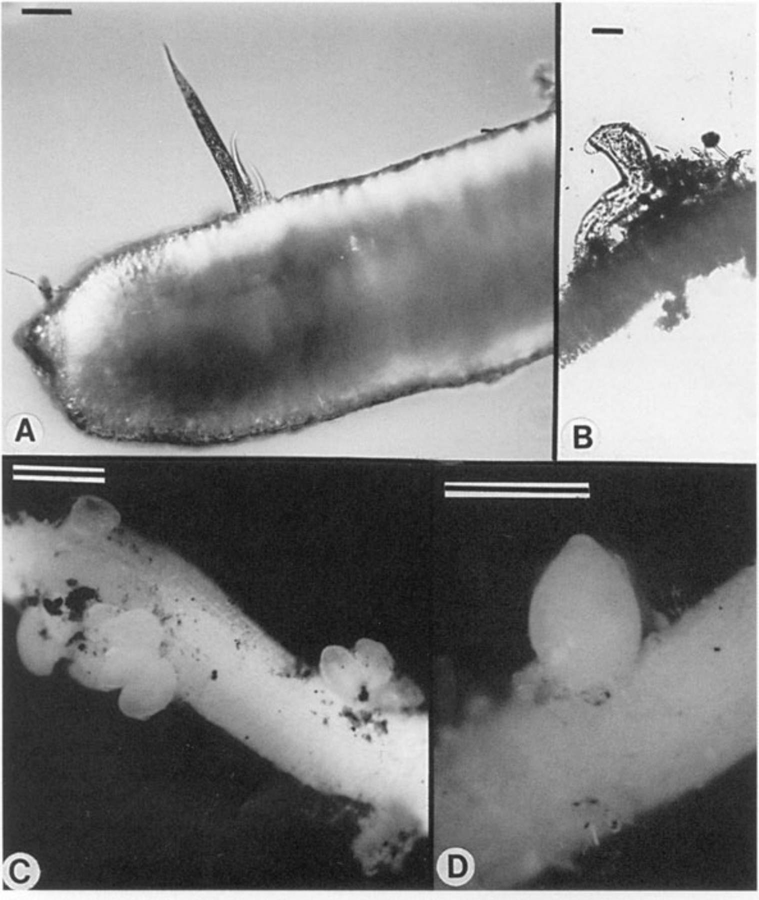

Fig. 1. Photomicrographs of *Afenestrata koreana*. Semiendoparasitic habits of juvenile and adult females on *Phyllostachys aurea* roots. A) Cluster of second-stage juveniles (J2). B) Swollen J2. C) Clusters of fourth-stage juveniles. D) Adult swollen female. Scale bars = 70 µm in A, 43 µm in B, 320 µm in C, and 380 µm in D.

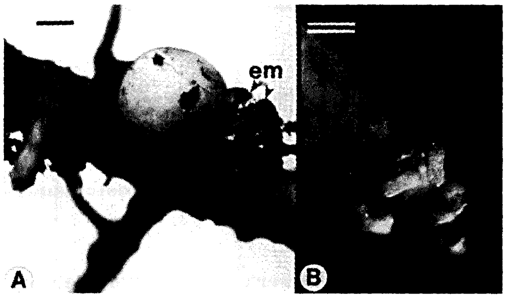

Fig. 2. Photomicrographs of *Afenestrata koreana* on *Phyllostachys aurea* roots. A) Swollen adult female with egg mass (em). B) Eggs in the egg mass. Scale bars = 204 µm in A and 102 µm in B.

which enlarged in time to incorporate vascular parenchymatic and phloematic cells. Cell wall fragments were scattered in the densely stained cytoplasm of the syncytium, which showed very thick walls especially at the nematode feeding site (Fig. 4B). Nuclei of fused cells became hypertrophic and showed deep indentation and prominent nucleoli (Fig. 4C, D). There was no evidence of presence of cell wall ingrowths in the syncytial walls. Our observations with the light microscope did not allow the detection of pit fields in the syncytium walls. Transmission or scanning electron microscope observations are necessary to detect these wall ultrastructures.

Production of gelatinous matrix containing eggs by *A. koreana* females, as observed in this study, has been reported also for females of *A. axonopi* (Souza,

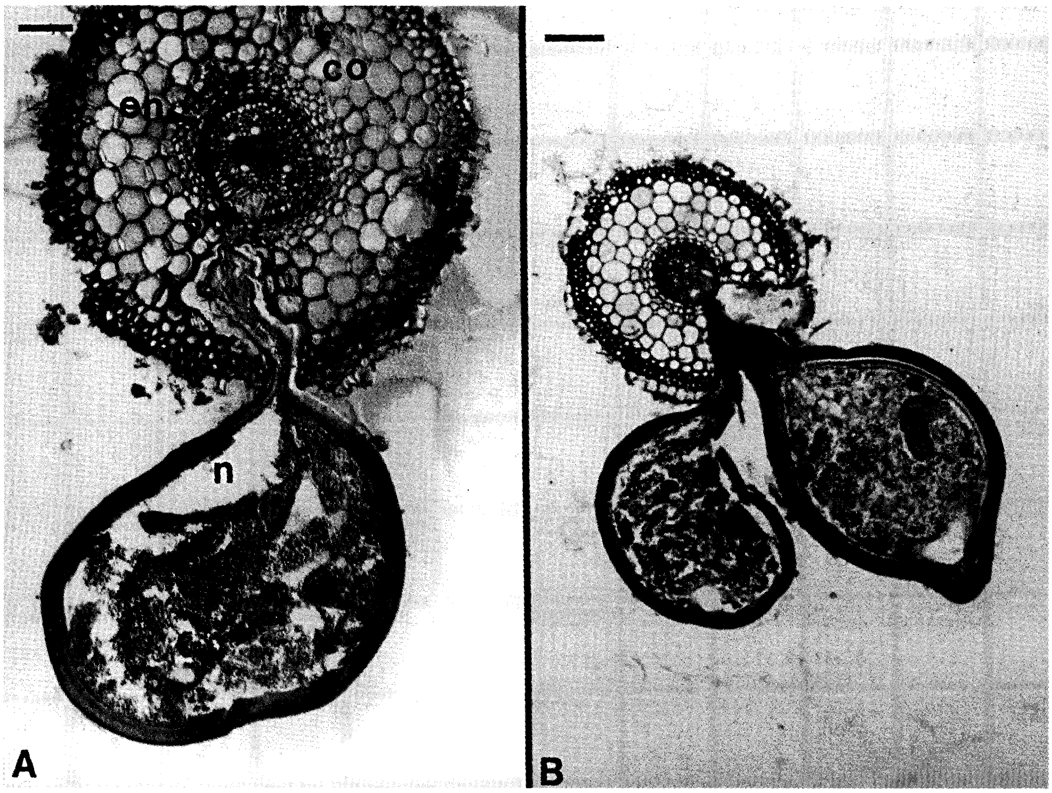

Fig. 3. Cross sections of *Phyllostachys aurea* roots infected by *Afenestrata koreana*. A) Nematode (n) feeding on a syncytium (s) in the root stele (st); co = cortical parenchyma, en = endodermis. B) Multiple infection by two nematodes in the same root section. Scale bars = 45 µm in A and 90 µm in B.

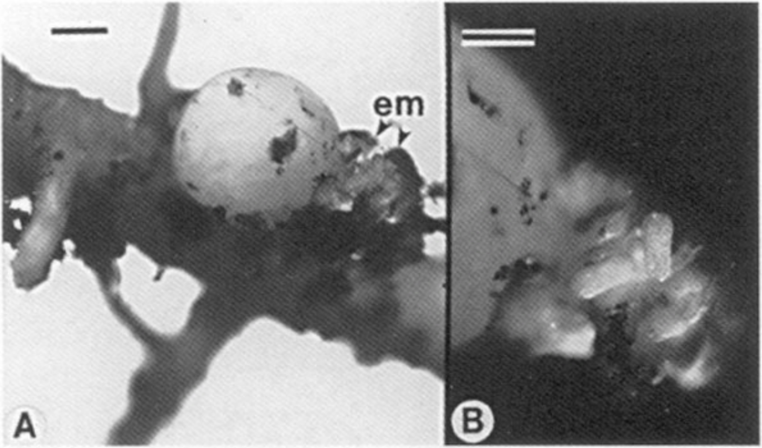

Fig. 2. Photomicrographs of *Afenestrata koreana* on *Phyllostachys aurea* roots. A) Swollen adult female with egg mass (em). B) Eggs in the egg mass. Scale bars = 204 µm in A and 102 µm in B.

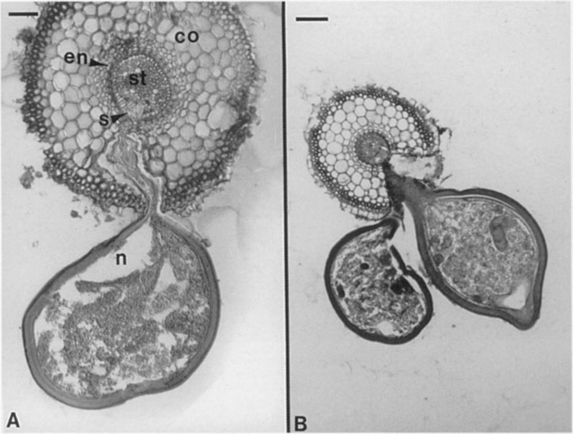

Fig. 3. Cross sections of *Phyllostachys aurea* roots infected by *Afenestrata koreana*. A) Nematode (n) feeding on a syncytium (s) in the root stele (st); co = cortical parenchyma, en = endodermis. B) Multiple infection by two nematodes in the same root section. Scale bars = 45 µm in A and 90 µm in B.

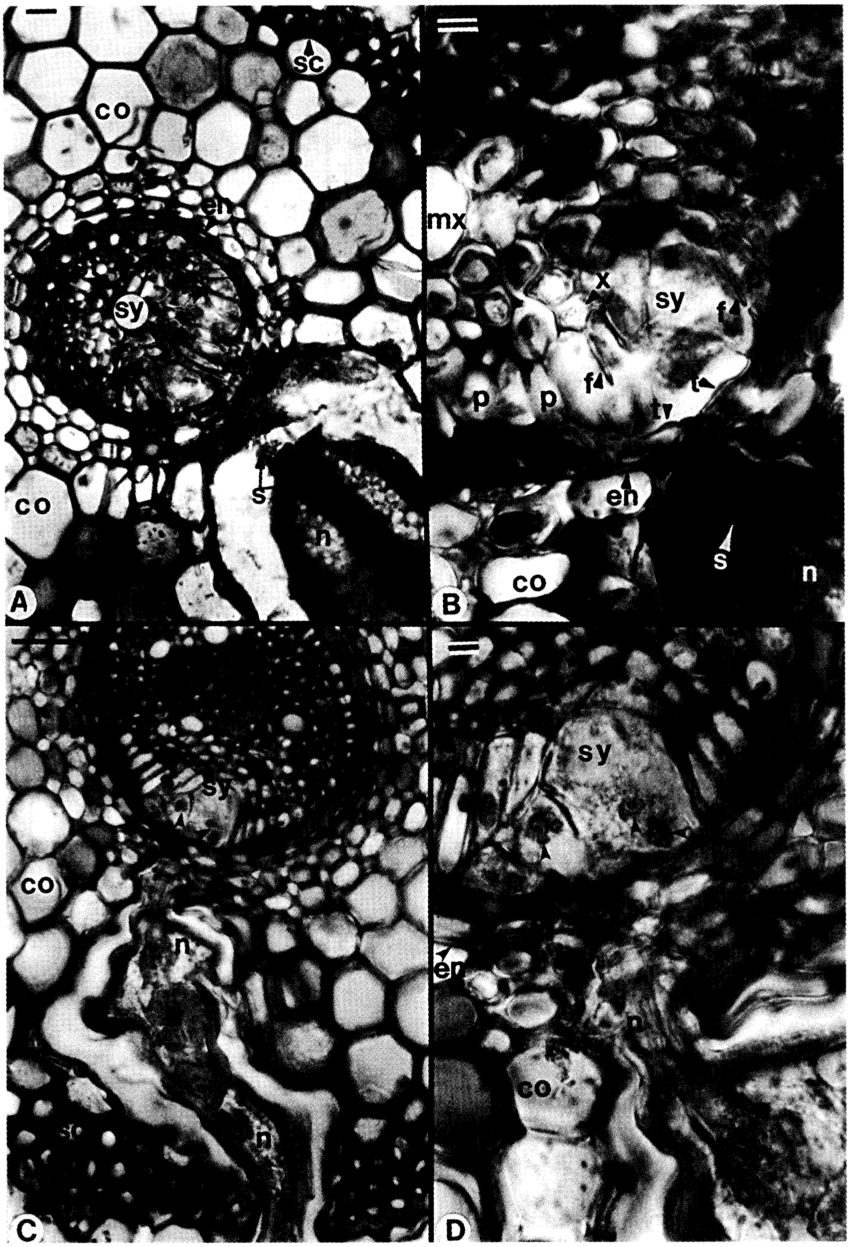

Fig. 4. Cross sections of *Phyllostachys aurea* roots infected by *Afenestrata koreana*. A) Nematode (n) feeding on a syncytium (sy) which occupies a large portion of the stele. Note enlarged pericyclic cells (p) that are becoming incorporated in the syncytium. Nematode is slightly detached from the feeding site due to tearing of the root tissue during sectioning. B) Nematode (n) feeding on a syncytium (sy) showing cell wall fragments (f), enlarged pericyclic cells (p) with partially dissolved walls, and thickened cell walls (t) especially at the nematode feeding site. C) Nematode (n) feeding on a syncytium (sy) with enlarged nuclei (arrows). D) Figure C at higher magnification to show hypertrophied nuclei (arrows) with deep indentation and prominent nucleoli. Other symbols for figure 4 only: co = cortical parenchyma, en = endodermis, mx = metaxylem, s = stylet, sc = sclerenchyma, and x = xylematic element. Scale bars = 12 μm in A, 6.5 μm in B, 25 μm in C, and 5 μm in D.

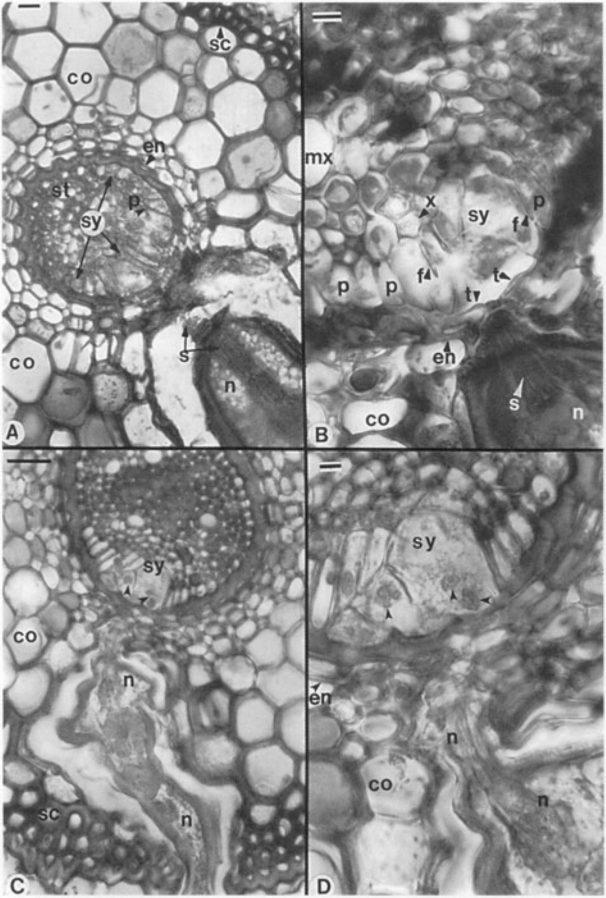

Fig. 4. Cross sections of *Phyllostachys aurea* roots infected by *Afenestrata koreana*. A) Nematode (n) feeding on a syncytium (sy) which occupies a large portion of the stele. Note enlarged pericyclic cells (p) that are becoming incorporated in the syncytium. Nematode is slightly detached from the feeding site due to tearing of the root tissue during sectioning. B) Nematode (n) feeding on a syncytium (sy) showing cell wall fragments (f), enlarged pericyclic cells (p) with partially dissolved walls, and thickened cell walls (t) especially at the nematode feeding site. C) Nematode (n) feeding on a syncytium (sy) with enlarged nuclei (arrows). D) Figure C at higher magnification to show hypertrophied nuclei (arrows) with deep indentation and prominent nucleoli. Other symbols for figure 4 only: co = cortical parenchyma, en = endodermis, mx = metaxylem, s = stylet, sc = sclerenchyma, and x = xylematic element. Scale bars = 12 µm in A, 6.5 µm in B, 25 µm in C, and 5 µm in D.

1996). However, no additional biological information is available on *A. axonopi*, nor on the other three *Afenestrata* species, including *A. africana* maintained in hydroponic cultures of Guinea grass roots in Senegal (Taylor and Luc, 1979). We don't know if the other *Afenestrata* species have semiendoparasitic juveniles. Luc *et al.* (1988) state that "the genus *Afenestrata* possesses a cyst of different type" compared to the cysts produced by *Heterodera* species. The neck region cuticle of *Afenestrata* cysts is brownish-translucent, annulated, and ornamented, which differs from the lace-like patterns on the rest of the cyst body. In contrast, the neck cuticle of *Heterodera* cysts is tanned and similar to that of the globose part of the body. The results of the biological observations on *A. koreana* from this study indicate that *Afenestrata* cysts also differ physiologically from *Heterodera* cysts in that they do not withstand desiccation well. Furthermore, survival of eggs in *A. koreana* cysts is shorter than that of eggs in *Heterodera* cysts. We don't know if the cysts of the other *Afenestrata* species behave as those of *A. koreana.* However, the short survival of *A. koreana* can be explained by the fact that this species, like all the other four of this genus, parasitizes perennial monocots which provide the nematode with new roots to feed on year-round. In these environmental conditions, the long persistence of cysts is not an essential requirement for the survival of the nematode.

The syncytium induced by *A. koreana* on fishpole bamboo roots lacks cell wall ingrowths and was similar to that caused by this nematode on edible bamboo and also to that induced by *A. africana* on Guinea grass roots (Baldwin and Bell, 1985; Vovlas *et al.*, 1993). However, in the syncytium on fishpole bamboo roots there was more accentuated cell wall dissolution and fusion of the pericyclic cells with other stelar elements, whereas in the syncytium of edible bamboo roots there was less cell wall dissolution of pericyclic cells which maintained their individuality.

In the subfamily Heteroderinae, the semiendoparasitic habits of *A. koreana* juveniles are similar to those reported for *Atalodera* spp. and *Sarisodera hydrophila* (Mundo-Ocampo and Baldwin, 1983a and 1983b). However, the host response of *A. koreana* differed from that of *S. hydrophila,* which induces a uninucleate giant cell in the stele of the host roots (Mundo-Ocampo and Baldwin, 1983b). The anatomical changes caused by *A. koreana* are similar to those reported for *Atalodera* spp. and also for *Punctodera chalcoensis* on corn (Mundo-Ocampo and Baldwin, 1983a; Suarez *et al.*,1985), which both induce a stelar syncytium lacking cell wall ingrowths. The *A. koreana* syncytium differed from that of *Heterodera* spp. because it lacks cell wall ingrowths which are numerous in *Heterodera* syncytia.

ACKNOWLEDGEMENT

The authors thank Mr. Samuel Fuller, Environmental Specialist, for technical assistance.

LITERATURE CITED

BALDWIN, J. G., and A. H. BELL. 1985. *Cactodera eremica* n. sp. and *Afenestrata africana* (Luc *et al.*, 1973) n. gen., n. comb., and an emended diagnosis of *Sarisodera* Wouts and Sher, 1971 (Heteroderidae). Journal of Nematology 17:187- 201.

ESSER, R. P. 1986. A water agar *en face* technique. Proceedings of the Helminthological Society of Washington 53:254-255.

INSERRA, R. N. 1997. Tri-ology Technical Report 36(5):11. Nematology Section. Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL, U.S.A.

JOHANSEN, D. A. 1940. Plant microtechniques. McGraw-Hill, New York, NY, U.S.A.

KAUSHAL, K. K., and G. SWARUP. 1988. Two new cyst nematode species from India. Indian Journal of Nematology 18:299-306.

KAZACHENKO, I. P. 1989. [A new species of cyst-forming nematodes of the genus *Afenestrata* (Nematoda: Heteroderidae) from the Primore Territory]. Pp. 120-122 *in* B. I. Lebedev ed. Parazity Zhivotnykh i Rastenii: Sbornik Nauchykh Trudov. Biological-Soil Institute, Far-Eastern Branch Academy of Sciences of the U.S.S.R., Vladivostock, Russia.

KLEYNHANS, K. P. N. 1992. Cyst-forming nematodes in South Africa: *Afenestrata africana* (Luc, Germani & Netscher, 1973) Baldwin & Bell, 1985 (Nemata: Heteroderidae). Phytophylactica 24:307-311.

LUC, M., G. GERMANI, and C. NETSCHER. 1973. Description de *Sarisodera africana* n. sp. et considérations sur les relations entre les genres *Sarisodera* Wouts et Sher, 1971 et *Heterodera* Schmidt, 1871 (Nematoda: Tylenchida). Cahiers ORSTOM, Séries Biologie 21:35-43.

LUC, M., R. MAGGENTI, and R. FORTUNER. 1988. A reappraisal of Tylenchina (Nemata) 9. The family Heteroderidae Filipjev and Schuurmans Stekhoven, 1941. Revue de Nématologie 11:159-176.

MUNDO-OCAMPO M., and J. G. BALDWIN. 1983a. Host-parasite relationships of *Atalodera* spp. (Heteroderidae). Journal of Nematology 15:234-243.

MUNDO-OCAMPO M., and J. G. BALDWIN. 1983b. Host-response to *Sarisodera hydrophila* Wouts and Sher, 1971. Journal of Nematology 15:259-268.

SOUZA, R. M. 1996. *Afenestrata axonopi* n. sp. (Nemata: Heteroderidae) from Brazil. Fundamental and Applied Nematology 19:35-42.

SUAREZ, Z., C. SOSA-MOSS, and R. N. INSERRA. 1985. Anatomical changes induced by *Punctodera chalcoensis* in corn roots. Journal of Nematology 17:242-244.

TAYLOR, D. P., and M. LUC. 1979. Observations on *Sarisodera africana* (Nematoda: Heteroderidae): Redescription of anterior end of female and occurrence of juveniles with aberrant tails. Revue de Nématologie 2:111-114.

VOVLAS, N., F. LAMBERTI, and H. Y. CHOO. 1992. Description of *Afenestrata koreana* n.sp. (Nematoda: Heteroderinae), a parasite of bamboo in Korea. Journal of Nematology 24:553-559.

VOVLAS, N., F. LAMBERTI, and H. Y. CHOO. 1993. Host-response of bamboo (*Phyllostachys pubescens*) to the cyst-forming nematode *Afenestrata koreana*. Afro-Asian Journal of Nematology 3:170-172.

Received:
Recibido: *5.II.1999*

Accepted for publication:
Acceptado para publicación: *31.III.1999*