HISTOPATHOLOGY OF TWO TOBACCO CULTIVARS INFECTED BY *GLOBODERA TABACUM* IN ARGENTINA

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Summary. The histological alterations induced by *Globodera tabacum* in the tobacco cvs K 326 and 493 were investigated in Argentina. Syncytia were observed in the cortex and central cylinder of both cultivars. Their presence produced a reduction in the vascular tissues varying from moderate to large without causing significant disorders.

Among nematodes attacking tobacco, *Globodera tabacum* (Lownsbery) Behrens, is particularly dangerous in different parts of the world (Barker *et al.*, 1984; Shepherd *et al.*, 1990). In Argentina cysts of this nematode were found in the soils of the provinces of Salta, Santa Fe, Catamarca and Buenos Aires (Chaves, 1984, 1987, 1993). The detection of tobacco plants attacked by *G. tabacum* in north Argentina suggested to investigate the histological alterations induced by the nematode in the roots of this crop plant.

MATERIALS AND METHODS

Roots of *Nicotiana tabacum* L. "cv. K326" and "cv. 493", from El Chucupal, Departament El Carmen, province of Jujuy, were selected. Roots infected and non infected (used as a check) by *G. tabacum* were separated using a stereoscopic microscope. Roots segments of 5-8 mm from both cultivars were fixed in FAA. They were then dehydrated in an ethyl alcohol series and finally embedded in histowax. Seriated cross sections of 8 to 10 µm of thickness were cut with a rotary microtome, triple-colored stained (hematoxylin-safranin-fast green) and mounted in DPX (Johansen, 1940; O' Brien and McCully, 1981). Photomicrographs were taken with an Axiophot Carl Zeiss microscope.

RESULTS AND DISCUSSION

In the cv. K 326 the establishment of the nematode produced modifications in the root tissues because of the formation of syncytia occupying part of the vascular cylinder and part of the cortex (Fig. 1A and D). Depending on the nematode development, it was possible to recognize two stages of syncytia: functional and non-functional.

Functional syncytia were associated to preadult stage. They were formed by parenchymatic cells that could belong to the cortex, the pericycle, the xylem and the phloem. These cells usually confronted the protoxylem poles. All of them showed hypertrophia, increase of cytoplasmic density and decrease of the central vacuole, which was replaced by many smaller vacuoles (Fig. 1E).

Cellular hipertrophia was variable, being frequently greater in the cortex cells (Fig. 1A and E). The cytoplasm of syncytial cells was dense, with fibrous aspect and abundant starch grains. There was usually one hypertrophic nucleus in each cell, with an approximate size of 25 µm (Fig. 1C). The shape of the nucleus varied from spherical to ameboidal, with borders slightly or moderately lobular. In some cases the presence of two nucleoles could be observed. The degree of cell vacuolization was variable; the cytoplasm appeared more vacuolated in the later stages of functionality of the syncytium.

The cell walls of syncytial cells were cellulosic and slightly thicker than those of the cortical parenchyma; the outer walls showed lignin depositions that thickened and strengthened them. The internal walls remained cellulosic, showing openings typical of the structure of a syncytium (Fig. 1A, B and E). These openings allowed the convergence of the cytoplasm and the migration of organelles to neighboring cells; for this reason, on various occasions cells with more than one nucleus were found. The observation of these openings in longitudinal sections revealed that they were not uniform in all the syncytium. In more hypertrophic cells they were wider, while in the less hypertrophic cells they were poorly developed and more uniform in structure, indicating that they had originated from primary pit fields (Fig. 1B). The cell wall where the female inserted the stylet appeared irregularly thickened mainly due to the deposition of cellulose. However, the color of some sectors allowed to infer the presence of callose and lignin. Structures of fibrous aspect could be observed in the cytoplasm.

The syncytial walls that were in contact with the conducting elements, both of the xylem and the phloem,

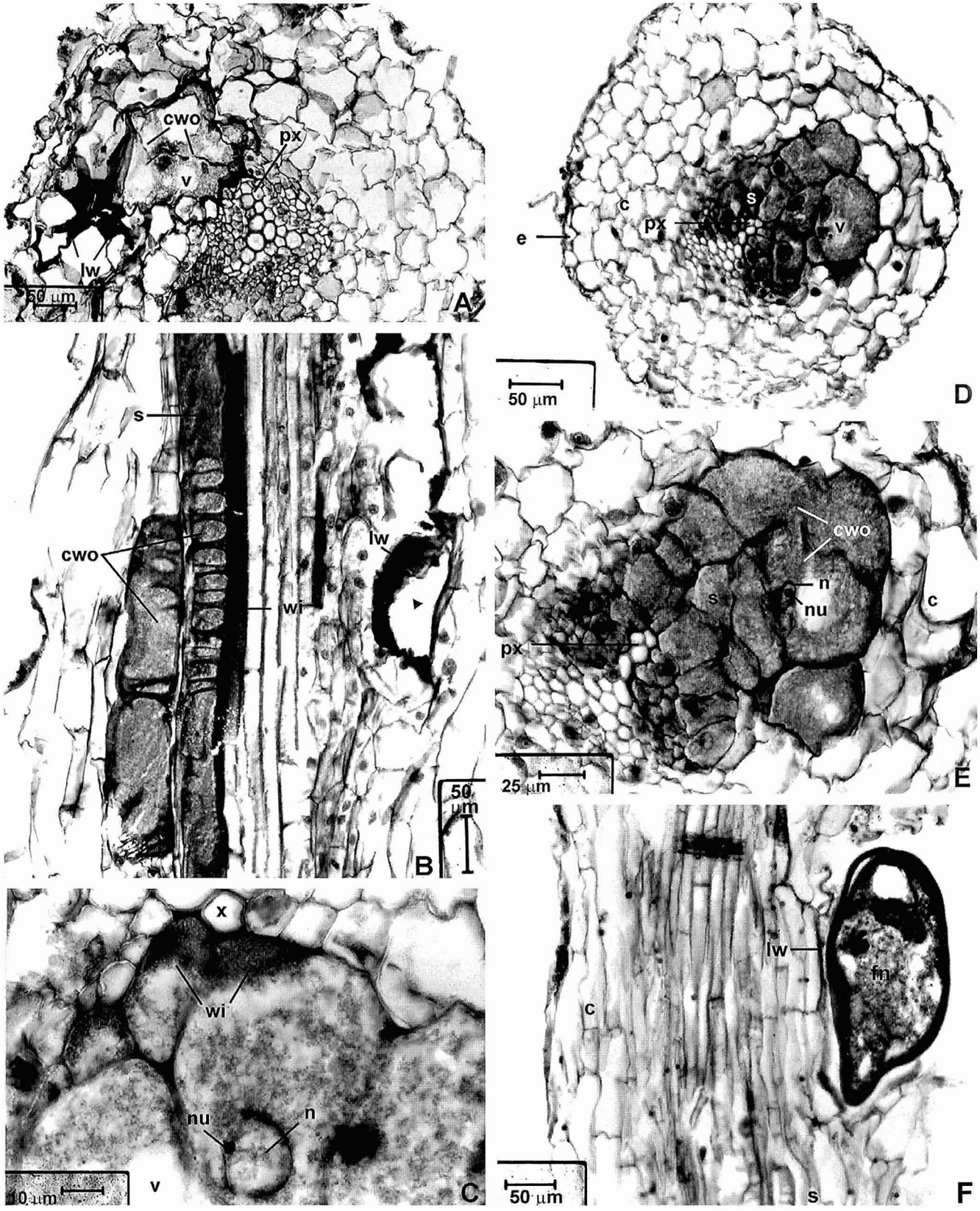

Fig. 1. Relationship between *G. tabacum* and *N. tabacum* Virginia cv. "K326" and cv. "493". A, syncytium located in the cortex with hipertrophic cells; B, funcional syncytium with cell wall openings; C, detail of syncytia cells; rugose ingrowths and a hypertrophic nucleus can be observed; D, functional syncytium in vascular cylinder and cortex; E, detail of the syncytium shown in D; F, location of the female in relation to a functional syncytium.
A, B and C: *N. tabacum* Virginia cv. "K326"; D, E and F: cv. "493".
Abbreviations: c, cortex; cwo, cell wall openings; e, epidermis; lw, lignified wall; n, nucleus; nu, nucleole; px, protoxylem; s, syncytium; v, vacuole; wi, wall ingrowths; x, xylem.
Symbols: arrow head, cavity where the female lodged.

developed rugose ingrowths of approximately 10 µm thick (Fig. 1B and C).

In some cases, the syncytia caused a moderate or important reduction of the conductive tissues, being the phloem the most affected as the differentiation and growth of the feeding site occurred mainly at their expense. There were no significant disorders in the xylem in relation to the disposition of its elements. In some of the samples analyzed it was possible to observe conductive elements both in the xylem and the phloem, distorted due to the pressure of the syncytium. This was particularly evident in the longitudinal sections.

The female caused cellular destruction in the root sector where it was hosted. The extension of the affected zone depended on the position of the nematode. The surrounding walls were impregnated with lignin (Fig. 1F).

Non-functional syncytia were identified in relation to mature females or traces left by the cysts on the root cortex.

The cells conforming them showed different stages of cytplasmic disorganization, not allowing to recognize cell organelles. The cell walls of these syncytia were modified, as they had thickened. The external walls had thickened due to the deposition of lignin and the internal walls had thickened due to a higher deposition of cellulose. Inside some syncytia fungi were found.

Only functional syncytia were observed in the roots of cv. 493. Their origin, localization and cytology were very similar to those of the cv. "K326". However, there was a difference between them related to the development of the rugose wall ingrowths of syncytial cells in contact with the conducting elements: in cv. "493" they were little developed and were present in fewer cells.

The host-parasite relationship observed for *G. tabacum* - *N. tabacum*, was similar in the cv. "K326" and cv. "493". The response of both cultivars was that typical of plants which are susceptible to cyst forming nematodes. The only difference observed between the two cultivars was related to the development of wall ingrowths in cells of the syncytium near the conducting elements of the xylem. In the cv. "K326" they were more developed, which suggets that these cells are more efficient in the short distance transport than the cells of cv. "493".

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