RESPONSE OF THE SOYBEAN CULTIVAR PIONEER 9501 TO *HETERODERA GLYCINES*

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Summary. Well-developed syncytia, affecting mainly the central cylinder, were observed in roots of the soybean cv. Pioneer 9501 attacked by *Heterodera glycines* race 1, indicating the high susceptibility of the cultivar to the pathogen.

The soybean cyst nematode, *Heterodera glycines,* is a major pest of soybean in Brazil, Colombia, Canada, Indonesia, Japan, Korea, China, Russia, Taiwan and 27 states of the USA (Riggs and Niblack, 1999). Until 1997, yield losses of soybean caused by nematodes in Argentina had been associated only with species of the genus *Meloidogyne* (Doucet *et al.*, 1997), but late in 1997, cysts of *H. glycines* were detected in fields with patchy growth (Baigorri *et al.*, 1998; Doucet and Lax, 1999).

The relationship between *H. glycines* and a soybean cultivar frequently grown in the central region of the Córdoba province was studied by Lorenzo *et al.* (1999). Since the knowledge of the responses of crop cultivars to nematode infection is very important for the development of disease control programme (Kim and Riggs, 1992), the present work was undertaken to determine the degree of susceptibility of one of the most sown cultivar in Argentina to a population of the soybean cyst nematode belonging to race 1 (Silva *et al.*, 1999). The responses of the plants to the nematode were followed by studying the histological alterations that occurred during the infection.

MATERIALS AND METHODS

Soybeans [*Glycine max* (L.) Merr.], cv. Pioneer 9501, were sown in January, 1998, in a field in Laguna Larga, Province of Córdoba, Argentina infested by *H. glycines* Ichinohe. Plants were collected for analysis in late January, February and April .

As control, seeds with the soybean symbiont *Bradyrhizobium japonicum* E 109, CKC soybean liquid (Laboratory Komag S.R.L. Buenos Aires, Argentina), were sown in pots with sterile soil kept in a glasshouse. Roots with and without nodules were collected after 40 days. For histological studies control and nematode infested roots were washed with tap water to remove soil and portions of up to 5 mm in length were cut and fixed in FAA. They were dehydrated in an ethyl alcohol series and finally embedded in histowax. Serial sections, 7 to 10 µm thick, were cut with a rotary microtome and stained with hematoxylin-safranin-fast green and mounted in DPX (Johansen, 1940; O' Brien and Mc Cully, 1981).

Previously dewaxed sections were submitted to PAS test (Periodic acid-Schiff) to check for total polysaccharides and starch (Harris and Oparka, 1994). Other dewaxed sections were treated with an alcoholic solution of 1% acidic phloroglucinol (D' Ambrogio de Argüeso, 1986) and observed under bright field microscopy. These sections were also examined by fluorescence microscopy to differentiate lignin from suberin (Baayen *et al.*, 1996). Fluorescence and bright field microscopy was performed with an Axiophot Carl Zeiss microscope, equipped with a HBO 100 W mercury lamp.

RESULTS

In the non-infected control root systems, spherical nodules of *B. japonicum* of about 4 mm diameter developed on the principal roots, and of approximately 2 mm diameter on the lateral roots. A single cell layer epidermis with small cells and very thin walls was observed in the absorption area of the primary roots. In the parenchymal cortex, intercellular spaces were small and scarce; in the innermost layer (the endodermis) all the cells were at the Casparian strip stage. The vascular cylinder showed a diarch or triarch arrangement (Fig. 1 B). Secondary root growth was apparent in the numerous secondary roots.

Spherical nodules of *B. japonicum* of approximately 1 mm diameter were observed in the root systems of the nematode infested plants collected in late January and February. No nodules were observed in the roots in April. Juveniles, females and mature cysts occurred in the principal and lateral roots collected at all dates.

The microscope observations revealed that the cortex was often greatly damaged by penetration and migration of the juveniles and the subsequent establishment of the females of the nematode, causing necrosis of some cells and total or partial damage of others. The re-

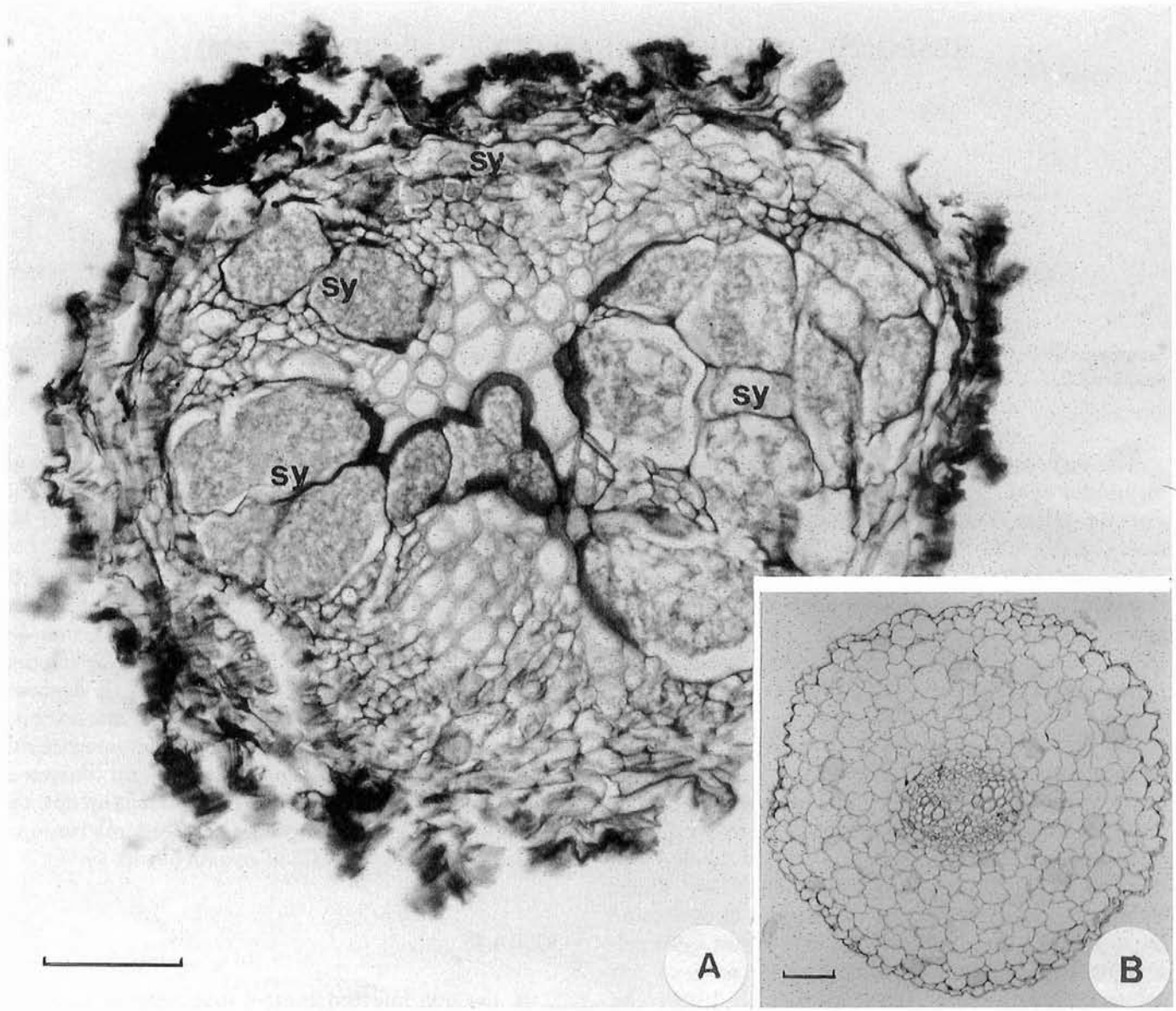

Fig. 1. Relationship between soybean cultivar Pioneer 9501 and *Heterodera glycines*: A, cross-section of root infested with functional syncytia (sy) in the central cylinder; B, non-infested control root with primary growth. Scale bars: 25 µm in A and 100 µm in B.

maining walls of the partially damaged cells were thickened and lignified, and in some parts they were suberized. Females were found near the vascular cylinder, which was the most affected region due to the development of syncytia (Fig. 1A). From the region where the anterior portion of the nematode was located, the syncytium extended both in an acropetal and a basipetal sense along the root, advancing also towards the centre of the vascular cylinder. In all samplings, the regions of the primary and secondary xylem, the vascular cambium, secondary phloem and pericycle were affected by the development of syncytia. These reached the cortex in some cases. Syncytial cells showed marked cytological differences with respect to adjacent, non-affected cells, and were much enlarged, with increased cytoplasm density and hypertrophied nuclei. The internal walls of syncytia that had completed their differentiation appeared irregularly fragmented. The number and size of syncytia that became established influenced the extent of root damage.

Functional syncytia with associated females with egg masses and nonfunctional syncytia associated with mature cysts were observed in roots from January.

Syncytia developed in the vascular cylinder and reached a maximum length of 1 mm. They initiated development in pericycle cells, which increased in size and cytoplasm density. Syncytia also incorporated the initial or derivative cells of the vascular cambium as they formed, as well as parenchymal cells of the secondary vascular tissues. They advanced towards the central region of the root facing and surrounding the protoxylem poles (Fig. 2A and B). Syncytia were demarcated externally by the suberized endodermis, although once completely formed, they could advance towards the cortex occupying an important part of it. At this stage, they became surrounded by parenchymal cells with lignified

walls (Fig. 2A and B). Cells forming the syncytia were of varied shapes and different degrees of hypertrophy; the cellular walls had gaps that became large enough to allow movement of cytoplasm, which showed different degrees of vacuolation (Fig. 2A and B). In some cases the cytoplasm was very dense and of granular aspect, whereas in other cases density decreased. Some cells showed partial plasmolysis in sectors where the wall had not developed intercellular connections or where it was bordered with nonfunctional vascular cells (Fig. 2 B). Nuclei were hypertrophic (about 18 µm in diameter), spherical, ovoid, or of lobed outline and with an obvious nucleolus (Fig. 2B).

Nonfunctional syncytia were formed by cells with cytoplasmic domains at different stages of breakdown. No cellular organelles could be identified, although the cytoplasm sometimes showed formation of numerous small vesicles (Fig. 2 C). The internal cellular walls were thickened and cellulose, whereas the external walls, which were also thickened, were reinforced with lignin. Occluded xylem vessels and occluded fibres with lignified walls were observed very close to the syncytia, probably due to the presence of fungi in the xylem, which also developed in the syncytium cells (Fig. 2C).

The analysis of seven nodules revealed only one functional syncytium at an early stage of differentiation with an associated juvenile. The syncytium was located in the cortical zone of the nodule, very close to the conductive tissue. The syncytium was made up of slightly hypertrophied cells, with thin and partially fragmented walls. The cytoplasm was granulose, slightly dense and with large vacuoles in some cells. The slightly hypertrophied and spherical nuclei had prominent nucleoli (Fig. 2D).

Functional syncytia were found in primary and secondary roots collected in February. The cytological characteristics of the cells were similar to those described for the previous sampling. Females with associated egg mass were found, as in the first sampling. Nodules of *B. japonicum* were not infested.

Functional syncytia associated with females, and nonfunctional syncytia associated with cysts occurred on the roots collected in April. The functional syncytia were ini-

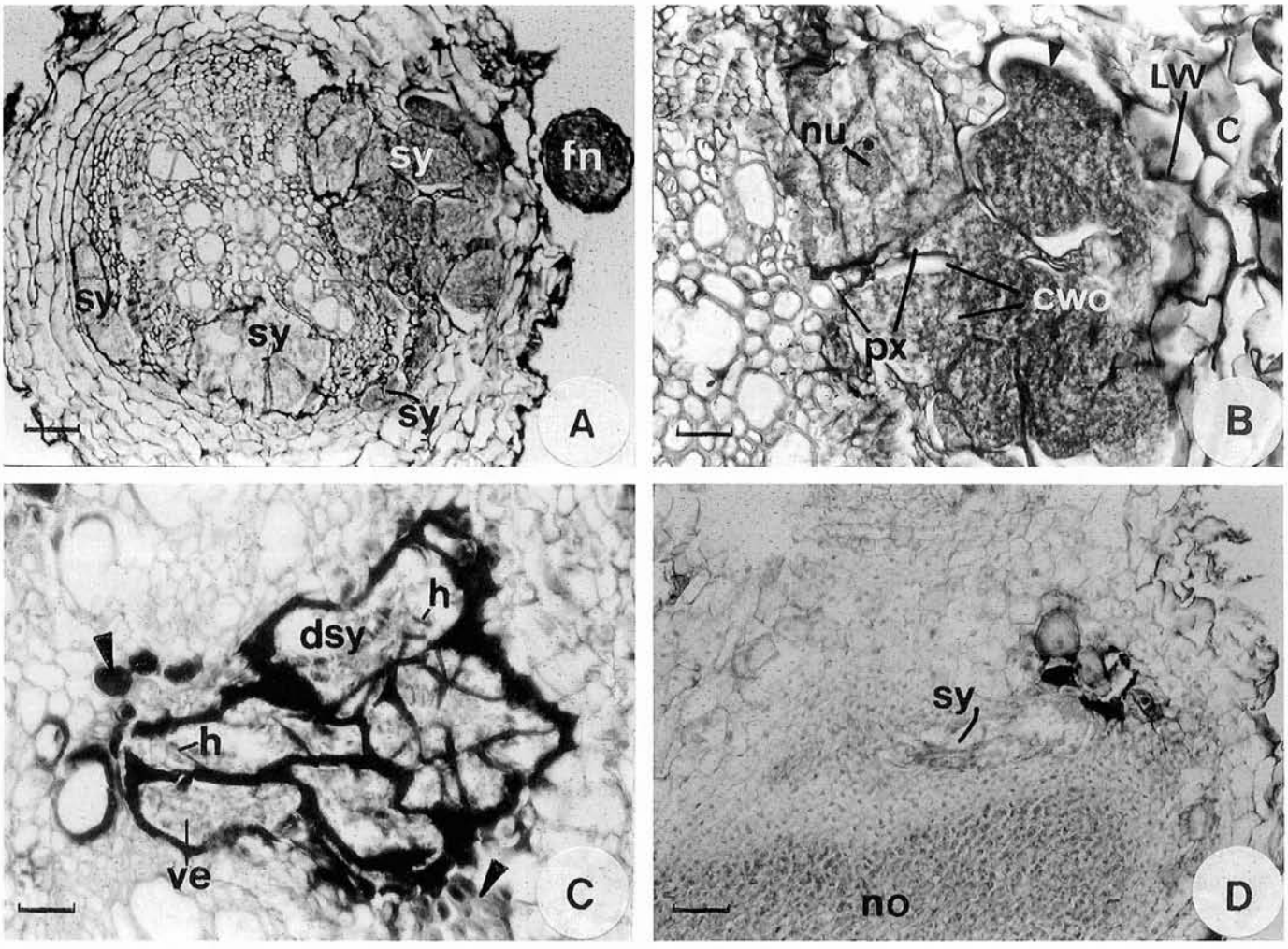

Fig. 2. Relationship between soybean cultivar Pioneer 9501 and *H. glycines*. Cross sections of infested roots and nodule collected in January: A, functional syncytia (sy) at the central cylinder one of them extending to the cortex, female nematode (fn); B, functional syncytium in detail, cortex (c), cell wall openings (cwo), nucleus (nu), lignified walls (lw), protoxylem (px), plasmolysed cells (arrow heads); C, nonfunctional syncytium (dsy), hypha (h), vesicle (ve), occluded cells of xylem and modified fibres [arrow]; D, functional syncytium (sy) at a nodule (no). Scale bars: 50 µm in A, D, and 25 µm in B, C.

tiated and differentiated similarly to what was observed in previous samplings. They reached a maximum of 2 mm in length. Syncytia invaded most of the central cylinder causing a significant loss of vascular tissues, which was enlarged by the interruption of the vascular cambium (Fig. 3A). The syncytia appeared to include a greater number of cells than in the previous samplings, although they were greatly hypertrophied (Fig. 3A and B). Compressed cells of the pericycle with thickened and lignified walls externally demarcated these syncytia. Lignifi-

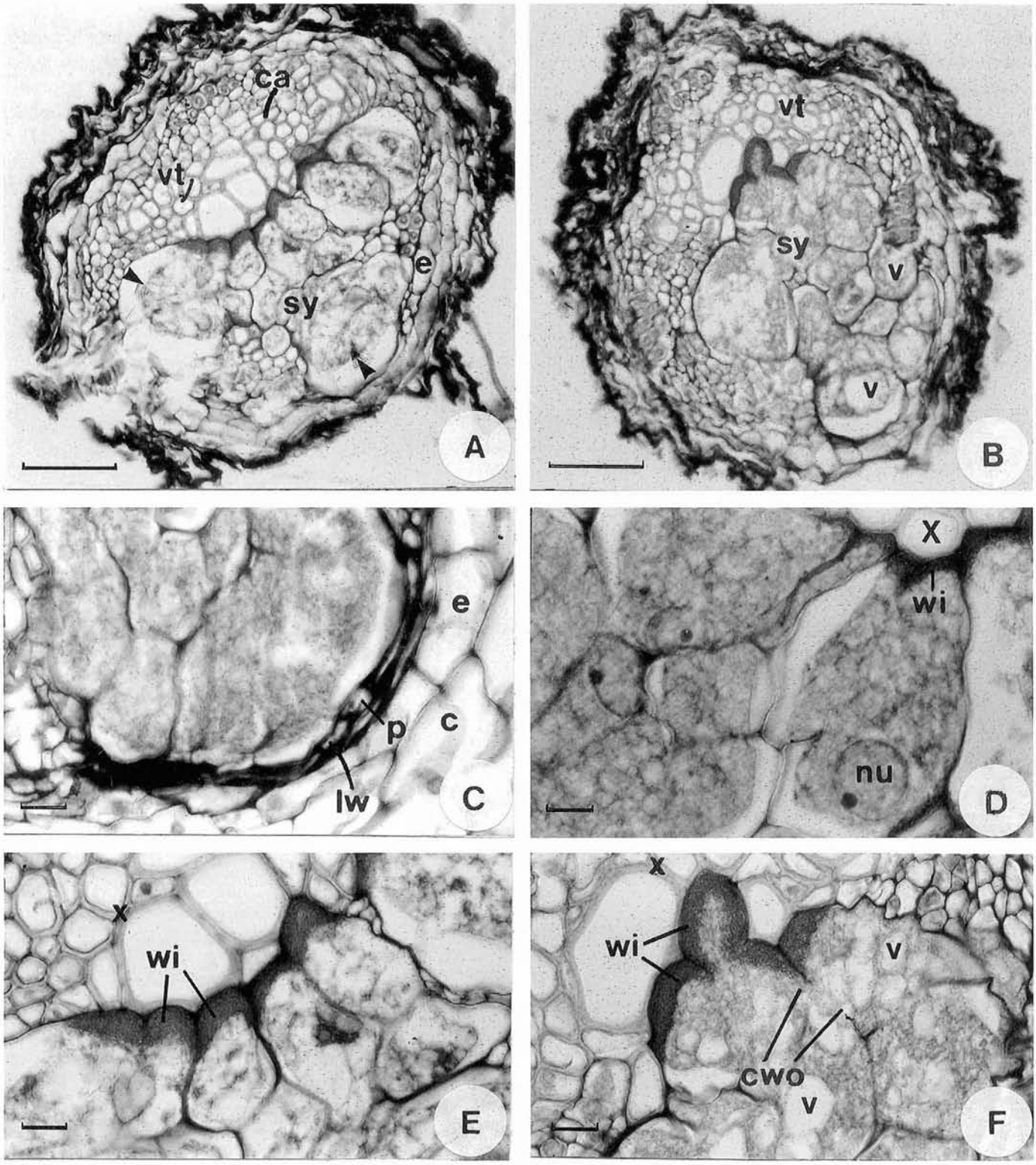

Fig. 3. Relationship between the soybean cultivar Pioneer 9501 and *H. glycines*. Cross sections of infested roots with functional syncytia collected in April: A and B, syncytia (sy) at central cylinder, vascular cambium (ca), endodermis (e), vascular tissues (vt), vacuole (v), plasmolysed cells [arrow]; C, syncytium in detail, endodermis (e), pericycle (p), lignified wall (lw); D, another syncytium in detail, nucleus (nu), wall ingrowth (wi), xylem (x); E and F, A and B in detail, cellular wall opening (cwo), wall ingrowth (wi), vacuole (v), xylem (x). Scale bars: 25 μm in A, B, and 10 μm in C, D, E and F.

cation also affected syncytial cells in contact with this cellular stratum (Fig. 3C). The cells that constituted the syncytia exhibited dense cytoplasm with increasing vacuolation as they differentiated. The nuclei were spherical or ovoid, reaching approximately 20 µm in diameter (Fig. 3D). Plasmolysed cells were frequently observed (Fig. 3A, B and D). This phenomenon was not present in areas were the walls had ingrowths, which were well developed, irregular, granulose and PAS positive (Fig. 3E and F). Only one sample over sixteen analysed presented a syncytium in the cortex; this syncytium was composed of slightly hypertrophied cells with the cytoplasm similar to that previously described. No nodules of *B. japonicum* were found on the roots of the third sampling.

DISCUSSION

The population of race 1 of *H. glycines* considered in this study induced well defined histological changes in the soybean cultivar Pioneer 9501. The changes are characteristic of a susceptible response (Endo, 1992). Lorenzo *et al.* (1999) analyzed the histopathology induced by the same population in the cultivar Asgrow 5401, finding only functional and well developed syncytia. With respect to their origin, location and cytology, the syncytia were similar to those described for the same category in the cultivar Pioneer 9501.

However, syncytia developed further in roots of Pioneer 9501, especially by the time of the third sampling, causing a noticeable reduction of vascular tissues. This reduction was not so apparent in Asgrow 5401. Another difference between the two cultivars is the presence of highly hypertrophied cells in Asgrow 5401, related to the anterior portion of the female. This was not observed in Pioneer 9501. Another cytological difference between the cultivars was the development of wall ingrowths in the cells of the syncytia. The wall ingrowths in Pioneer 9501 covered larger areas of the cellular walls, and were thicker and more complex than in Asgrow 5401. Thus, it seems likely that cells of the syncytia in Pioneer 9501 would be more efficient at supplying nutrients to the nematodes.

The presence of phytophagous nematodes in legumes has been reported to cause a reduction in nodulation; this result can be estimated from observations such as the number or mass of nodules (Khan, 1993). Huang *et al.* (1984) concluded that this reduction resulted from the interference of the nematode with soybean lectin metabolism. Nodules found in the roots of Pioneer 9501 in the first and second samplings were smaller than those that developed in the control roots, and no nodules at all were found in the third sampling. This suggests that the reduction in nodule size and the decrease in their number are related to the presence of *H. glycines*. A decrease in the number of nodules has been recorded for the association soybean-*H. glycines* race 1 (Lehman *et al.*, 1971; Huang and Barker, 1983).

The observation of a single nodule of *B. japonicum* infected by *H. glycines* with an associated syncytium shows that these nematodes have the capacity to invade and establish syncytia in the nodular tissues. According to Khan (l.c), the early degeneration of infected nodules is one of the causes of the plant's loss of ability to fix nitrogen.

On the basis of these results, the relationship between the nematode and the cultivar can be considered as that of a highly susceptible host. Cultivation in nematode infested soil thus becomes risky.

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