Anatomical and histological alterations induced by *Hemicycliophora poranga* Monteiro & Lordello, 1978 in celery (*Apium graveolens* L.) roots

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Summary. Celery is an intensive horticultural crop. In Argentina, it is grown in several localities of the provinces of Buenos Aires, Santa Fe, Mendoza and San Juan. A population of *Hemicycliophora poranga* has been recently detected infesting celery roots, which showed particular swellings and proliferation of lateral rootlets. Tissues were analysed to describe anatomical and histological alterations caused by the parasite. Infected roots had simple and compound galls containing specimens of the nematode. Simple galls exhibited cellular hyperplasia that affected the pericycle, producing lateral root primordia that were contained within the cortical parenchyma. Simple galls were almost entirely composed of meristematic tissue or poorly differentiated cells of the new lateral roots. Compound galls exhibited similar histological alterations to those of simple ones, but showed a higher amount of lateral roots. The central body of compound galls was occupied by differentiated tissues. *Hemicycliophora poranga*-induced modifications in celery are related to an important proliferation of lateral roots.

Key words: Argentina, gall, *Apium graveolens*, *Hemicycliophora poranga*, histopathology.

Celery (*Apium graveolens* L.) is an intensive biennial crop native to the Mediterranean basin. The United States, Mexico, Chile, Spain, Italy, France and Israel are among the most important producers (ASFE, 2007); data on global production, however, is very scarce. In Argentina, it is cultivated in different localities of the provinces of Santa Fe, Mendoza, San Juan and Buenos Aires (Tiscornia, 1989), the latter being the main producing area in the country (APREA, 2008). Celery is occasionally used as an alternative crop to tomato, pepper or eggplant (Kebat & Riccetti, 2008).

This plant has been cited as a host of different plant-parasitic nematodes, such as *Hemicycliophora arenaria* (Franklin & Stone, 1974), *Longidorus apulus* (Bleve-Zacheo *et al*., 1979; Wyss, 1980), *Meloidogyne incognita* (Incer & López, 1979), *M. incognita* race 1 (Vovlas *et al*., 2008) and *Cactodera cacti* (Esser, 1992). In Argentina, the presence of some nematode species has been detected in celery-cultivated soils, the most important ones being *M. incognita, M. javanica* (Chaves, 2002), *M. arenaria, Pratylenchus* sp., *Paratylenchus* sp., *Nacobbus aberrans* (Doucet, 1999), and *Ditylenchus dipsaci* (Di Benedetto, 2005). A population of *Hemicycliophora poranga* has been recently detected infesting celery roots, which showed particular swellings and proliferation of lateral rootlets.

Little is known about the symptoms of a plant attacked by *Hemicycliophora* spp. (Sofrygina, 1972). Some species of the genus induce galls in the host roots, such as *H. arenaria* in tomato (Van Gundy & Rakham, 1961), *H. poranga* in tomato, bean, pepper, onion, lettuce, cucumber, okra (Chitambar, 1993) and *H. similis* in apricot (Van Gundy & Rakham, 1961). *Hemicycliophora typica* has been reported to generate slight swellings in carrot (Kuiper, 1959) and rice (Bleve-Zacheo *et al.,* 1987) roots. In addition, *H. conida* produced growth

inhibition and root proliferation in forage crops (Kuiper, 1977; Spaull & Mewton, 1982). However, those references were based only on external observations of the root system. Studies on histopathology induced by *Hemicycliophora* spp. are scarce (Chitambar, 1993). The aim of the present work was to characterise alterations induced by *H. poranga* in celery roots.

MATERIAL AND METHODS

Soil previously known to be naturally infested with *H. poranga* was collected from the locality of El Pucará del Aconquija (Department of Andalgalá, Province of Catamarca, Argentina) and placed in pots at the Laboratorio de Nematología (Centro de Zoología Aplicada, Universidad Nacional de Córdoba). Seeds of celery cv. Dulce were placed to germinate in sterile soil. Some seedlings were transplanted to the pots containing contaminated soil and others were transplanted to pots containing sterile soil to obtain healthy control plants. Samples of lateral and main roots were taken. Galled and non-galled tissues were cut into pieces of up to 5 mm in length; they were fixed in FAA and were dehydrated in an ethyl alcohol series and finally embedded in histowax. Serial sections, ranging from 7 and 10 µm in thickness, were cut with a rotary microtome. They were stained with hematoxylin-safranin-fast green and mounted in DPX (Johansen, 1940; O'Brien & Mc Cully, 1981). Photographs were taken with a Carl Zeiss Stemi SV6 stereoscopic microscope equipped with a Canon digital camera and an Axiophot optical microscope, Carl Zeiss; images were captured with an AxioCam HRc camera and processed with AxioVision 4 software.

Some of the pieces containing galls were fixed in FA4/1, dehydrated in a graded series of alcohol solutions and critical-point dried using CO_2. Root portions were mounted on aluminum stubs with double adhesive tape, coated with a 300 Å layer of gold-palladium and observed with a Jeol SM-U3 microscope at an accelerating voltage of 15 kV.

Species verification was based on morphology and ribosomal DNA sequence. Adult nematodes were picked out from other inoculated plants, killed and processed to anhydrous glycerin (Seinhorst, 1962) before mounting on slides for light microscopy. Other nematode specimens were immediately preserved in DESS (Yoder *et al*., 2006) for PCR. Nuclear DNA was extracted as described in Tandingan De Ley *et al.* (2007) and the D2-D3 expansion segment of the rDNA was amplified from 3 µl of the genomic template DNA for a 25 µl PCR reaction using Illustra PuReTaq Ready-To-Go™ PCR beads (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA) following the manufacturer's protocol with the following amplification conditions: initial denaturation at 94°C for 5 minutes, 35 cycles of 94° C for 30 s, 60° C for 1 min, 72° C for 2 min, and a final extension of 72° C for 7 min.

PCR products were cleaned with QIAquick® PCR Purification Kit (QIAGEN Inc., Valencia, California, 91355, USA) following the manufacturer's protocol. Nucleotide sequences were determined using dye-terminator sequencing chemistry on a 96-capillary ABI 3730xl (Applied Biosystems, Foster City, CA, USA) at the UCR Core Instrumentation Facility. Contigs were assembled and compared with published sequences in GenBank using CodonCode (CodonCode Corp. Dedham, MA, USA). The nematode species identity was confirmed by morphology and sequence of the D2-D3 domains of the rDNA that was a complete match of AY780975, a population of *H. poranga* from Venezuela (Subbotin *et al*., 2005).

RESULTS

The analysis of root tissues allowed us to differentiate two types of galls based on their shape: 'simple' and 'compound'. The former were somewhat cylindrical, about 2.5 mm long and 1 mm wide (Fig. 1 A). Some of these simple galls had few lateral roots. Compound galls were isodiametric (Fig. 1 B) or exhibited various shapes, with the major axis of about 5 mm and the minor axis of about 2 mm (Fig. 1 C), and abundant lateral roots developing in different directions. Both simple and compound galls contained adult and juvenile specimens of the nematode (Fig. 1 D), with their anterior region embedded in the root tissues. In some cases, up to seven individuals were observed in the same gall.

The anatomical and histological analysis of control roots showed primary growth of triarch structure (Fig. 2 A) and, occasionally, development of secondary vascular tissues.

Simple galls. An apical zone with meristematic cells protected by 4-5 layers of parenchymal tissue was detected. The meristematic cells were isodiametric, of about 15 µm diam. (Fig. 2 B); cytoplasm was very dense; the nuclei were generally big and spherical (approximately 7 µm diam.) with prominent nucleoli. These cells progressively differentiated from the periphery to the interior of the gall. At certain sectioning levels, the epidermis and subepidermic parenchyma cells appeared already differentiated. In addition, in the central region some meristematic cells remained

undifferentiated (Fig. 2 B); later in the differentiation zone these cells formed the vascular tissues and the pericycle. Close to the apex, immediately after differentiation of adult tissues was complete, the pericycle cells underwent periclinal and anticlinal divisions at points adjacent to one another. This evident cellular hyperplasia affecting the central zone of the gall (central cylinder of the modified root) produced protuberances of meristematic tissue that developed through the cortex and advanced in different directions, forming lateral root primordia (Fig. 2 C). Up to four primordia, very close to one another, have been observed inside a gall of only 1.1 mm in length (Fig. 2 D). With such important cell proliferation, galls were almost entirely composed of meristematic tissue or, occasionally, of poorly differentiated cells of the new lateral roots. The primordia were therefore contained inside the cortical parenchyma (Fig. 2 E, F). Parenchymal cells of cortex exhibited small intercellular spaces. On occasions, cortical cells in some zones were crushed. Cell hypertrophy was also observed in this zone (Fig. 2 G). In one case, notably, cells differentiated from the rest by particular characteristics, such as a greater cytoplasmic density, slightly larger nuclei (7-8 µm) and prominent nucleotides. In some of those cells the cytoplasm was separated from the cell walls (Fig. 2 H). Vascular tissues occupying the central region of these galls were disorganised, displaced and their cells atypically arranged.

Compound galls. Histological alterations observed in compound galls were similar to those described for simple galls. However, lateral roots were found to be already differentiated. Therefore, these galls were composed mainly of differentiated tissues: parenchyma and vascular tissues oriented in different directions (Fig. 3 A, B). Proliferation of lateral roots produced an extensive disorganisation of the elements of vascular tissue (Fig. 3 C, D). Grouping of the members of sieve tubes and vessel dispersed and embedded in the parenchyma were detected on some occasions (Fig. 3 D). The formation of new simple galls was observed in some of the lateral roots associated with these compound galls.

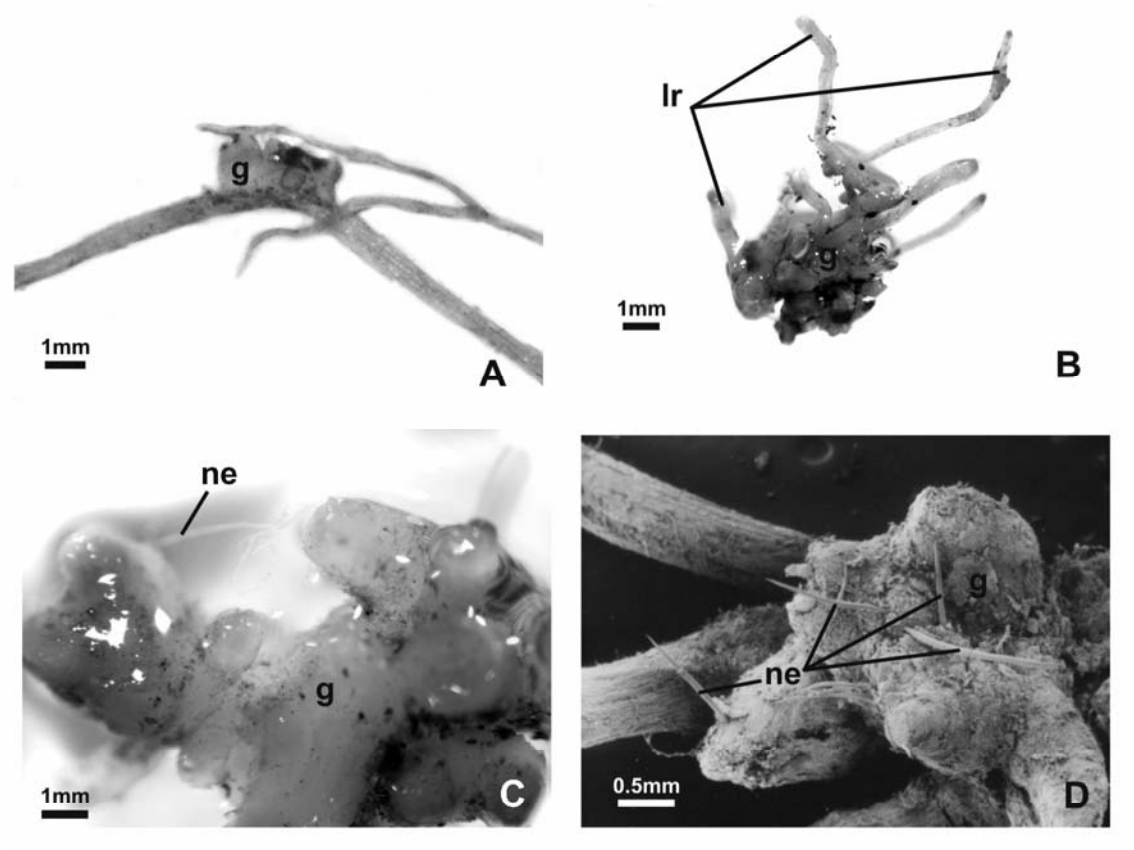

Fig. 1: Celery-*Hemicycliophora poranga* association. A: Exomorphological view of a simple gall. B, C: View of compound galls. D: Photomicrograph (SEM) of a gall containing nematodes. Abbreviations: **g**: gall; **ne:** nematode; **lr:** lateral roots.

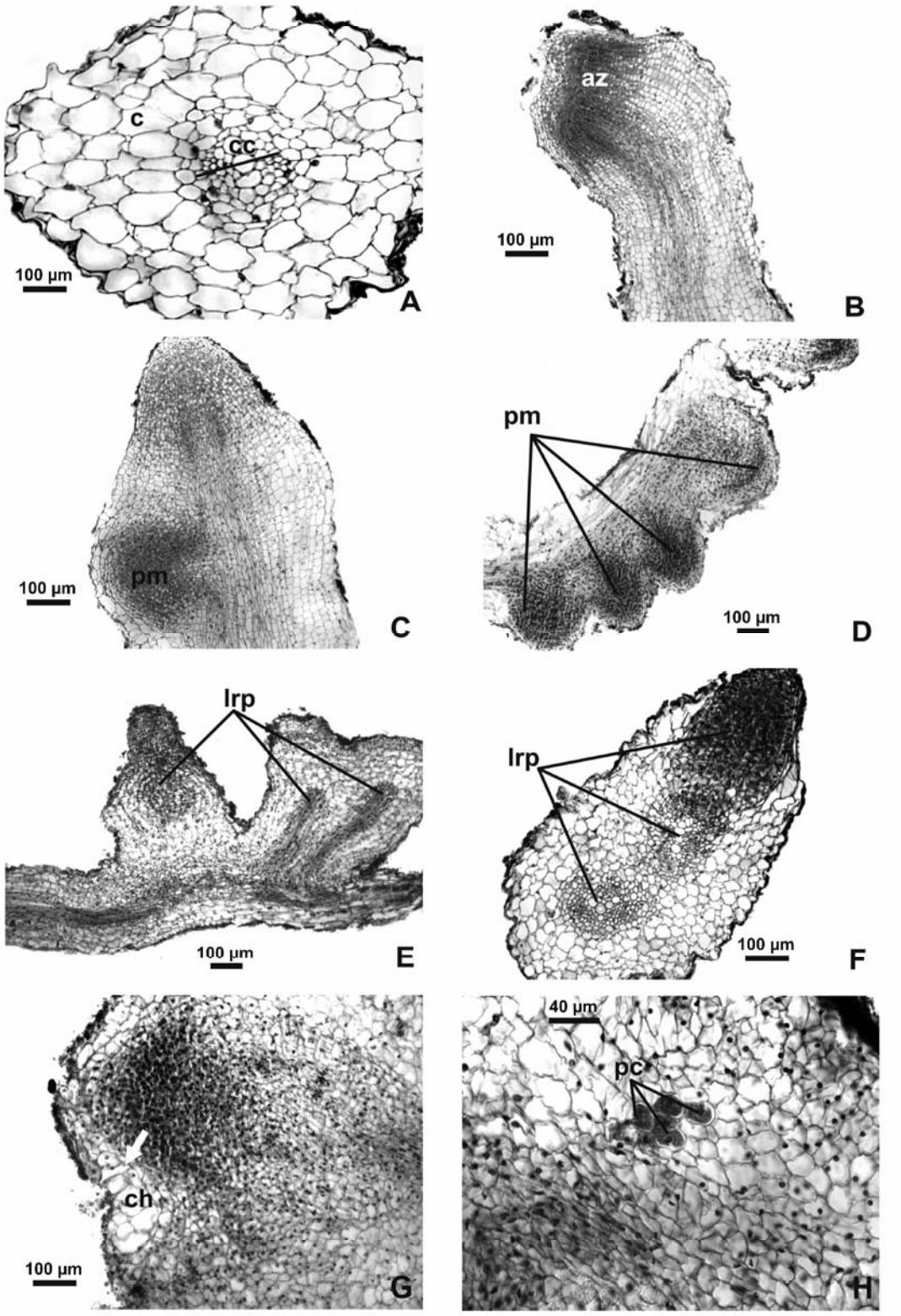

Fig. 2: Celery-*Hemicycliophora poranga* association. Photomicrographs of histological sections. A: healthy control root. B - H: simple galls. B: longitudinal section of simple gall. C: detail of apical region. D: longitudinal section with four very close protuberances of meristematic tissue. E and F: primordia of lateral roots surrounded by a cortical parenchyma. G: cortex with crushed parenchyma cells (arrow) and cell hypertrophy. H: parenchyma cells with high cytoplasmic density. Abbreviations: **az**: apical zone; **c**: cortex; **cc**: central cylinder; **ch**: cell hypertrophy; **lrp**: lateral root primordium; **pc**: parenchyma cells; **pm**: protuberance of meristematic tissue.

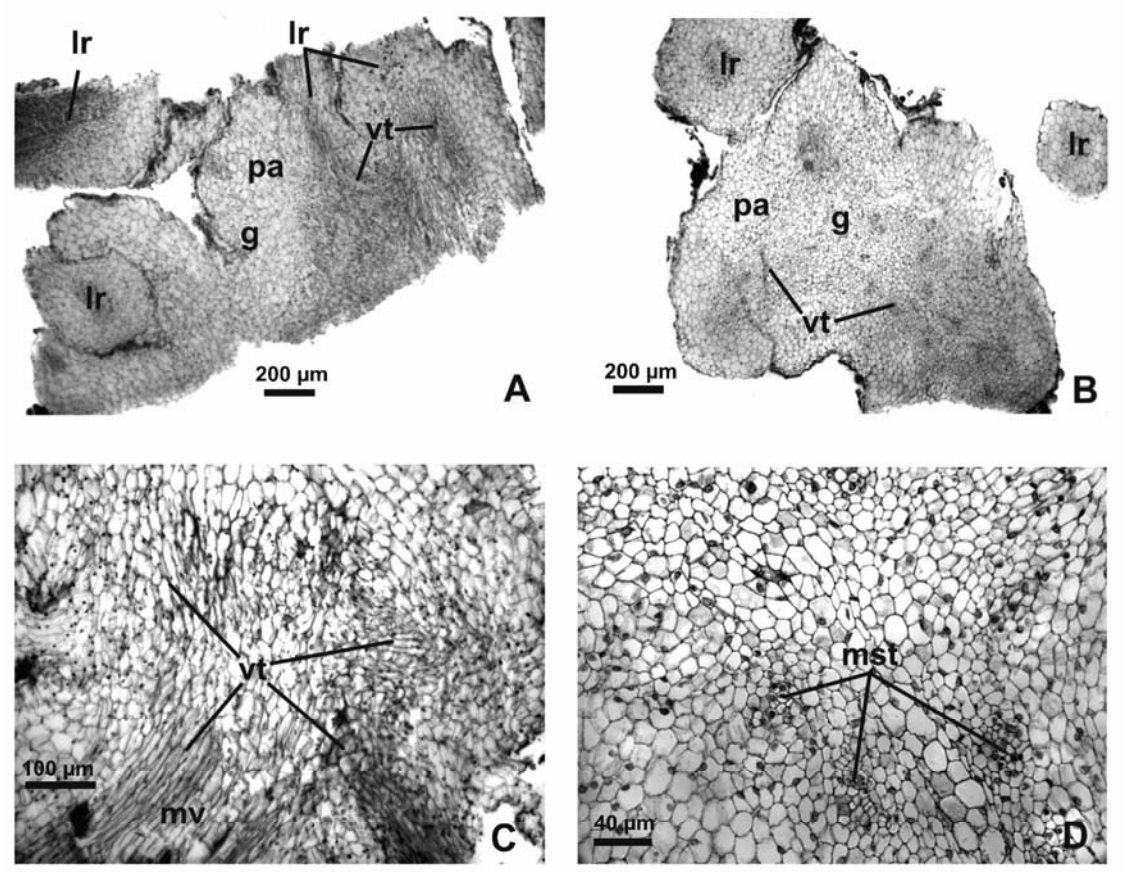

Fig. 3: Celery-*Hemicycliophora poranga* association. Photomicrographs of histological sections of compound galls. A and B**,** View of cross section. C and D**,** details showing the disorganization of vascular tissues. Abbreviations: **g**: gall; **lr**: lateral roots; **mst**: members of sieve tube; **mv**: members of vessel; **pa**: parenchyma; **vt**: vascular tissues.

DISCUSSION

In Argentina the genus *Hemicyclipohora* has been reported from soils associated with cultivated and non-cultivated plants belonging to different families (Doucet, 1999). The species *H. poranga* was detected in soils devoted to cauliflower production (Chaves, 1983) and in soil surrounding roots of *Poa* sp., *Cassia aphylla* Cav., and *Salix* sp. (Doucet, 1983). This is the first time that the genus has been detected parasitising roots of a horticultural plant in the country.

Not all species of the genus *Hemicyclipohora* induce galling in the roots of the plants attacked. Histopathological studies conducted up to the present have evaluated species that can induce gall formation in their hosts (Chitambar, 1993). The roots analysed exhibited galls containing more than one (up to seven) individuals of *H. poranga*, with their anterior region embedded in the tissues. This finding agrees with observations of Chitambar (1993), who detected several nematodes per gall in a study of the association of this species with tomato (cv. VF 145B 7879).

Endo (1975) indicated that in the *H. arenaria*-tomato interaction, the nematodes fed on cortical parenchyma cells, including endodermis, as well as on meristematic tissues that could mature and form endodermis or pericycle. This characteristic would be common to the species of the genus (Endo, 1975). As the nematode removes cell content through the stylet, cells near the stylet penetration site are crushed and pushed toward the root surface by new tissue formed by the pericycle, which would be involved in constant supply of cells for nematode feeding (Endo, 1975). In the present study, the characteristics of the gall cells are partially consistent with the description of Endo (1975); crushed parenchyma cells were observed close to the epidermal region and an important meristematic activity of the pericycle, which would replace these cells.

The cell hyperplasia in the cortical layers observed in the present work agrees with observations in roots of citrus and tomato parasitised

by *H. arenaria*, in which galls exhibited an increase of cell divisions, producing enlargement of the cortex (Franklin & Stone, 1974). These authors also indicated the presence of hypertrophic nurse cells, with thick wall, some of which were multinucleate. Cells with these characteristics were not found in the present work. In one case hypertrophic cells with dense cytoplasm were observed but in these cells the cytoplasm was separated from the cell wall and had a single nucleus.

Proliferation of lateral roots was evident in the samples analyzed. In simple galls, those proliferations were generally protuberances of meristematic tissue; these galls were therefore mostly composed of undifferentiated tissue. In compound galls, lateral roots were composed of differentiated tissue; hence, these galls were mainly composed of adult tissues oriented in different directions. Histological alterations induced by the nematode in both gall types show that they do not represent different responses of celery to the nematode infection but different developmental stages; simple galls would precede compound ones. Histological alterations induced by a *H. poranga* population on celery roots are described for the first time.

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Emilse C., A. Oggero, M. del Carmen Tordable, P. Lax, I. Tandigan De Ley and M. Doucet. Анатомические и гистологические изменения, вызываемые *Hemicycliophora poranga* Monteiro & Lordello, 1978, на корнях сельдерея (*Apium graveolens* L.).

Резюме. Выращивание сельдерея относится к интенсивным направлениям сельского хозяйства Аргентины, где эта культура возделывается в некоторых местностях в пров. Буэнос Айрес, Санта Фе, Мендоса и Сан Хуан. Недавно были выявлены популяции *Hemicycliophora poranga*, поражающие сельдерей, что выявляется по характерным вздутиям корней и активному образованию корневых волосков. Был проведен анализ тканей корня сельдерея для выявления характерных анатомических и гистологических изменений, вызываемых паразитированием. Пораженные корни несут сложные и составные галлы, заключающие особей нематод. Простые галлы показывают все признаки клеточной гиперплазии, поражающей перицикл, стимулирующей образование боковых корневых зачатков, содержащихся внутри кортикальной паренхимы. Простые галлы состояли почти исключительно из меристемной ткани или слабо дифференцированных клеток вновь образующихся боковых корней. Составные галлы показывали сходные гистологические изменения, но отличались большим количеством боковых корней. Центральная часть составных галлов занята дифференцирующейся тканью. Вызываемые *Hemicycliophora poranga* изменения всегда сопровождаются значительным развитием боковых корней.