

# HISTOPATHOLOGICAL STUDY IN *SALSOLA KALI* ROOTS INFECTED BY *NACOBBUS ABERRANS*

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## ABSTRACT

Tordable, M. del C., P. Lax, M. E. Doucet, O. Luque, and N. Rojas. 2010. Histopathological study in *Salsola kali* roots infected by *Nacobbus aberrans*. *Nematropica* 40:105-109.

*Nacobbus aberrans* attacks a wide host range of plants that includes not only crop species but also several weeds. Russian thistle plants (*Salsola kali*) were collected from a potato field that had remained uncultivated for five years in the locality of El Pucará del Aconquija (Province of Catamarca, Argentina). The roots had numerous galls induced by *N. aberrans*. Histological alterations of the root tissues were analyzed; samples were fixed in FAA and processed following conventional histological and optical microscopy techniques. The study revealed the presence of syncytia in the central cylinder, occupying an important portion of it, causing breakdown, reduction and displacement of vascular tissues. Functional syncytia, composed of numerous mononuclear cells of variable size with dense, vacuolized cytoplasm, and with starch grains were recognized. Results show this weed ensures pathogen survival in the field when the crop is not present, and at the same time, promote its dispersal and possible damage to neighboring crops. This is the first description of histological alterations induced by *N. aberrans* in this weed of wide distribution in Argentina.

*Key words:* Argentina, histopathology, *Nacobbus aberrans*, Russian thistle, weed.

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## RESUMEN

Tordable, M. del C., P. Lax, M. E. Doucet, O. Luque, and N. Rojas. 2010. Histopatología en raíces de *Salsola kali* atacadas por *Nacobbus aberrans*. *Nematropica* 40:105-109.

*Nacobbus aberrans* ataca una amplia gama de plantas hospedantes que abarca no sólo especies vegetales cultivadas sino también varias malezas. Se recolectaron plantas de cardo ruso (*Salsola kali*) provenientes de un lote de papa inculco durante cinco años en la localidad El Pucará del Aconquija (Provincia de Catamarca, Argentina). Sus raíces mostraron numerosas agallas inducidas por *N. aberrans*. Se analizaron las alteraciones histológicas de los tejidos radicales; las muestras se fijaron en FAA y se procesaron siguiendo técnicas convencionales para microscopía óptica. El estudio reveló la presencia de sincitios en el cilindro central, los cuales ocupaban un importante sector del mismo y ocasionaban la interrupción, reducción y desplazamiento de los tejidos vasculares. Se reconocieron sincitios funcionales constituidos por numerosas células de tamaños variables, uninucleadas, de citoplasma denso, diversamente vacuolizado y con granos de almidón. Por primera vez se describen las alteraciones histológicas inducidas por *N. aberrans* en esta maleza de amplia dispersión en el país.

*Palabras clave:* Argentina, cardo ruso, histopatología, maleza, *Nacobbus aberrans*.

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*Nacobbus aberrans* (Thorne, 1935) Thorne and Allen, 1944 occurs in Argen-

tina, Bolivia, Chile, Ecuador, Mexico, Peru, and United States (Reid *et al.*, 2003). The

species is associated with several crops and native plants in temperate and subtropical areas. The known host range comprises 84 crop and non-crop plant species belonging to 18 families (Manzanilla-López *et al.*, 2002). Among the hosts, weeds play a very important, usually neglected role, because they are effective reservoirs that ensure multiplication and dispersal of the nematode over time.

In Argentina, *N. aberrans* is present in very diverse areas (ranging from sea level up to 4500 m a.s.l.) within a large portion of the territory (Doucet and Lax, 2005). The host range is wide, with records of the species parasitizing several crops, both in field and greenhouse conditions. Among the most important hosts are: tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.) (Lorenzo *et al.*, 2001), sugarbeet (*Beta vulgaris* L.) (Tordable *et al.*, 2007), and potato (*Solanum tuberosum* L.) (Costilla, 1997). In Argentina, several weeds have been cited as effective hosts of *N. aberrans*: *Amaranthus* spp. (Costilla, 1985; Chaves and Sisler, 1980), *Brassica rapa* L. (Costilla and Ojeda, 1985), *Chenopodium album* L. (Doucet, 1989), *Sisymbrium irio* L. (Ponce de León and Doucet, 1989), *Portulaca oleracea* L. (Costilla, 1985; Doucet *et al.*, 2007), and *Datura ferox* L. (Costilla, 1985).

To date, histological alterations in roots induced by this nematode species have been studied mostly in plants of agricultural importance (Inserra *et al.*, 1983; Finetti Sialer, 1990; Doucet *et al.*, 1997; Lorenzo *et al.*, 2001; Vovlas *et al.*, 2007). Analyses of this type in weeds, however, are scarce, with only few reports in: *S. irio* (Ponce de León and Doucet, 1989), *Spergula arvensis* L. (Doucet *et al.*, 1994), *C. album* (Doucet *et al.*, 1997), *C. murale* L., and *Malva parviflora* L. (Tovar *et al.*, 1990).

In the locality of El Pucará del Aconquija (Department of Andalgalá, Province of Catamarca, Argentina) sampling was

conducted in a potato field naturally infected by *N. aberrans*. As suggested by the agricultural technician, the field was left uncultivated for 5 years with the aim of reducing nematode population density in the soil. After that period, plants of the dominant weed present in the plot, *Salsola kali* L. (Family Chenopodiaceae), called 'Russian thistle', were collected; these plants did not show any apparent foliar symptoms. When plants were extracted, however, numerous small galls caused by *N. aberrans* were observed in the roots. The aim of this work was to analyze the host response to *N. aberrans* attack on the basis of the histological alterations induced by the parasite.

Fifteen plants were collected at random across the field. Roots were carefully washed with water to remove adhering soil particles. They were observed under stereoscopic microscope; healthy (without galls) and infected (galled) roots were cut in sections of about 5 mm in length and fixed in FAA. Then they were dehydrated in a graded series of ethyl alcohol-xylene and included in Histowax<sup>TM</sup>. Serial transverse and longitudinal sections 8-10 µm thick were obtained with a rotary microtome. Sections were stained with the triple coloration Hematoxylin-safranin-fast green and final mounting was made with Depex<sup>TM</sup> (Johansen, 1940; O'Brien and McCully, 1981). Photographs of external roots were taken with a stereoscopic microscope DV 6 Carl Zeiss equipped with digital camera Canon PowerShot A 95. Micrographs of the sections were obtained with a microscope AxioPhot Carl Zeiss equipped with an AxioCam HRC camera and the image capture and digitalization device AxioVision 4.3.

Galls were spherical, with maximum diameter of 4 mm and with numerous lateral roots (Fig. 1A), some of which were equally infected by the nematode and

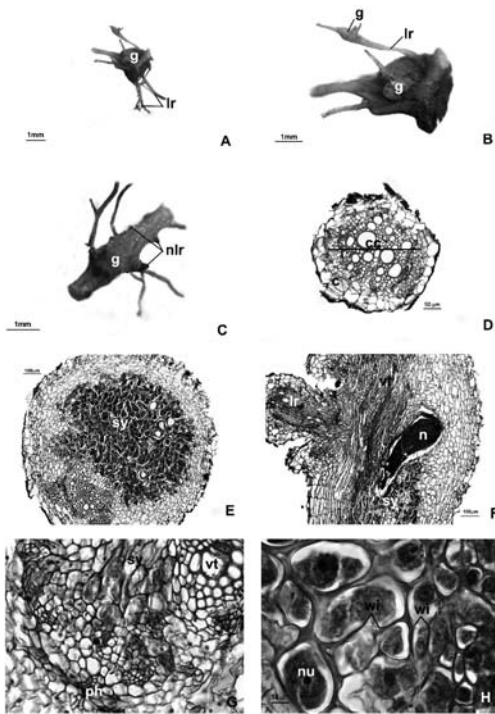


Fig. 1. Roots of *Salsola kali* from a field naturally infected by *Nacobbus aberrans* in Argentina. A: gall with several lateral roots. B: gall development in lateral roots. C: gall with necrosis in lateral root apex. D: transverse section of control (healthy) root. E: general view of transverse section with development of a syncytium. F: longitudinal section of gall with syncytium adjacent to vascular tissues and a female of the nematode. G, H: details of sectors showing syncytial characteristics. Abbreviations. c: cortex; cc: central cylinder; g: gall; lr: lateral root; n: nematode; nlr: necrosis in lateral root; nu: nucleus; ph: phloem; sy: syncytium; vt: vascular tissues; wi: wall interruption.

showed new galls (Fig. 1B). In other roots, the apical zone showed necrosis (Fig. 1C). Histological observations revealed that galls were located in roots with secondary growth (Fig. 1D). Hyperplastic gall tissue was observed occupying part of the central cylinder. Syncytia were located in the same zone of the central cylinder (Fig. 1E) and were composed of hyperplastic tissue cells, parenchymatic cells of vascular tissues, phloem and xylem, and cells of the vascular

lar cambium. In all cases syncytia were adjacent to vascular tissues (Fig. 1F); in large sectors syncytia remained immersed in the xylem, incorporating some of the xylem cells into them (Fig. 1G), which resulted in a notable reduction of the xylem as well as its partial fractioning. In turn, the phloem was displaced to the gall periphery, interrupting the connection of both vascular tissues. Mature females, some of them with their respective egg mass, were observed closely associated with feeding sites (Fig. 1F). In some sectors, the anterior region of the nematode was surrounded by syncytial cells, whereas in other sectors, it was observed within those cells. Each syncytium was composed of numerous cells, with a maximum recorded of about 270 cells counted at the cross-sectional plane. Cells had dense cytoplasm, with small vacuoles and with others of bigger resulting from the fusion of the small ones; abundant amount of starch in some cases. Cell walls were thickened (approximately 7-9  $\mu\text{m}$  vs 2-3  $\mu\text{m}$  in normal cells) with interruptions in wide sectors. Nuclei were hypertrophied (up to 14  $\mu\text{m}$  in diameter vs 6-8  $\mu\text{m}$  in normal cells), spherical or slightly oval (Fig. 1H). In the same sectional plane, those cells that were located within the syncytium and near the anterior region of the nematode appeared of bigger size, with a maximum of about 60-85  $\mu\text{m}$  in diameter, whereas those of the periphery, adjacent to non-modified root tissues, were approximately 20-35  $\mu\text{m}$  in diameter and their cytoplasm was less dense. Another alteration observed was the presence of several primordia of lateral roots, resulting from changes in meristematic activity. Tissues of the central cylinder were even more disorganized as a result of this proliferation. In galls, anticlinal and periclinal division takes place in pericycle cells, forming the root primordia in very close sectors, generating a discontinuity in this cell stratum

and in vascular tissues involved in the development of these new roots.

Some histological characteristics observed in syncytia of *S. kali*, such as: position of the syncytium in the central portion of galls, cellular hypertrophy with interruption of cell walls, increased cytoplasmic density and secondary vacuolization, were in agreement with those already described for other *N. aberrans* populations parasitising weeds (Ponce de León and Doucet, 1989; Tovar *et al.*, 1990; Doucet *et al.*, 1994, 1997) and plants of agricultural importance (Lorenzo *et al.*, 2001; Tordable *et al.*, 2007), some of them belonging to the Chenopodiaceae Family. However, some differential aspects, such as the presence of cellular hyperplasia not only in the central cylinder but also in the cortex, have been indicated in *S. irio* (Ponce de León and Doucet, 1989). Observations of the present work are consistent with the second and third syncytium stages of the four described by those authors (functional syncytia composed of hypertrophied cells, with dense cytoplasm and wide fragmentations in the cell walls). In *S. arvensis* (Doucet *et al.*, 1994) and *C. album* (Doucet *et al.*, 1997) syncytial cells with thick, barely fragmented walls were observed, whereas in the cell walls of *S. kali*, wide, fragmented, although somewhat thickened zones appeared.

*Salsola kali* has cosmopolitan distribution and is considered a pest of agricultural importance. In Argentina, it is present in several provinces (Marzocca, 1993), being harmful to summer crops, fruit plantations, perennial pastures, natural grasslands, as well as all type of modified environments (Tolaba, 2008). This work shows that, although potato had not been cultivated for five crop years in the plot studied, *N. aberrans* continued to develop on plants of *S. kali* present there. Thus, in the absence of a susceptible crop, *S. kali*

provides this polyphagous nematode the possibility to persist in the soil. This shows the importance of controlling weeds, which not only ensure pathogen survival but also, at the same time, promote its dispersal and possible damage to neighboring crops. This shows the scarce information on nematology of technicians advising producers on the management of this important pest.

The species *S. kali* has been previously indicated as a host of *N. aberrans* in the US and Mexico (De la Jara *et al.*, 1990; Manzanilla-López *et al.*, 2002). The results obtained show that the weed is an efficient host for the nematode. This is the first description of histological alterations induced by *N. aberrans* to the plant species.

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