PARASITISM OF THE ROOT-KNOT NEMATODE *MELOIDOGYNE HAPLA* ON PEONY IN NORTHERN ITALY

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Summary. A root-knot nematode was frequently detected on the roots of peony (*Paeonia lactiflora* Pall.) in northern Italy, inducing typical spherical galls with large egg masses (each averaging 320-350 eggs). According to its typical cuticular perineal pattern, esterase and malate dehydrogenase phenotypes, and morphological observations on adults and juveniles, the nematode was identified as *Meloidogyne hapla*. Galls induced by the nematode on the roots of peony were variable in size but relatively small (only two to three times the healthy root diameter), located mostly along the root axis and less frequently at the root tips. Numerous lateral roots arising from galled main roots were also galled. Comparative histopathological observations of healthy and *M. hapla*-infected roots of peony showed cellular alterations induced by the nematode in the cortex, pericycle and vascular parenchyma. Permanent feeding sites of the nematode within root tissues consisted of 3-8 giant polynucleate cells surrounding the lip region of females.

Keywords: Histopathology, northern root-knot nematode, *Paeonia lactiflora*.

During the winter growing season of 2009-2010, plants of peony [*Paeonia lactiflora* Pall. (syn. *P. albiflora* Pall.)], exhibiting slightly stunted growth and leaf yellowing, were observed in northern Italy. The plants were growing in a nursery in the Province of Vercelli, and were propagated by plant division, grafted with scions of *Paeonia suffruticosa* Andrews and other *Paeonia* spp. and transplanted into open fields in a drip-irrigated sandy soil. They were uprooted to be sold 2-4 years after transplanting. At harvest, 60 to 70% of the uprooted plants showed severely deformed root systems with a gall rating of 4, according to a 1-4 rating scale, where 1 = no galls; 2 = 1-10 galls; 3 = 11-100 galls; and 4 = >100 galls per root system (LaMondia, 1995). In galled fresh rootlets, nematode population densities ranged from 31 to 256 eggs and second-stage juveniles per g of fresh root. Dissection of the infected roots under a stereomicroscope revealed the presence of nematode females belonging to the genus *Meloidogyne* Göldi. For identification, the perineal patterns of a number of mature females were studied and the females were also characterized by isozyme electrophoretic patterns, in particular esterase (Est) and malate dehydrogenase (Mdh), which have been proved to be valuable for precise identification of *Meloidogyne* species (Orton Williams, 1974; Esbenshade and Triantaphyllou, 1990).

Observations of morphometrical and morphological data from eighteen second-stage juveniles showed the following features: body length = 335 ± 26 (312-352) µm, stylet length = 10 ± 0.8 (9-11) µm; tail length = 45 ± 1.0 (32-47) µm. Adult females showed perineal patterns with distinct punctuations between anus and tail terminus, and an excretory pore/stylet length ratio (Ep/st) = 2.3. Esterase (Est) and malate dehydrogenase (Mdh) phenotypes of *Meloidogyne* sp. infecting peony in the nursery in the Province of Vercelli were compared with those of a reference population of *M. javanica* from IAS-CSIC, Córdoba, Spain. For this, five young egg-laying females of both nematode species were macerated in microtubes containing 5 µl of 20% (wt/vol) sucrose, 1% (vol/vol) Triton X-100 and 0.01% (wt/vol) of bromophenol blue. Electrophoresis was carried out in 7 × 8-cm separating (pH 8.8) and stacking (pH 6.8) homogeneous gels, 7% and 4% polyacrylamide, respectively, 0.75 mm thick, with Tris-glycine buffer in a Mini Protean II electrophoresis unit (BioRad, Madrid, Spain). Gels were stained with the substrate α-naphthyl acetate for Est and with Fast Blue RR (Sigma-Aldrich, Madrid, Spain) for Mdh (Esbenshade and Triantaphyllou, 1990). The isozyme electrophoretic analysis revealed one H1 Est band and an H1 Mdh phenotype (Fig. 2B) in the population from peony that did not occur in the Est and Mdh phenotypes of *M. javanica*, which showed a J3 and N1 phenotypes (Fig. 2B). All the above morphometrics and isozyme characterizations of the peony population conformed to the description of *Meloidogyne hapla* Chitwood, 1949 (Orton Williams, 1974; Esbenshade and Triantaphyllou, 1990).

Root galls containing more than one nematode female associated with their separate feeding sites were frequently observed. Therefore, comparative histopathological observations were made on healthy and *M. hapla*-infected peony roots. For this, healthy and galled roots of

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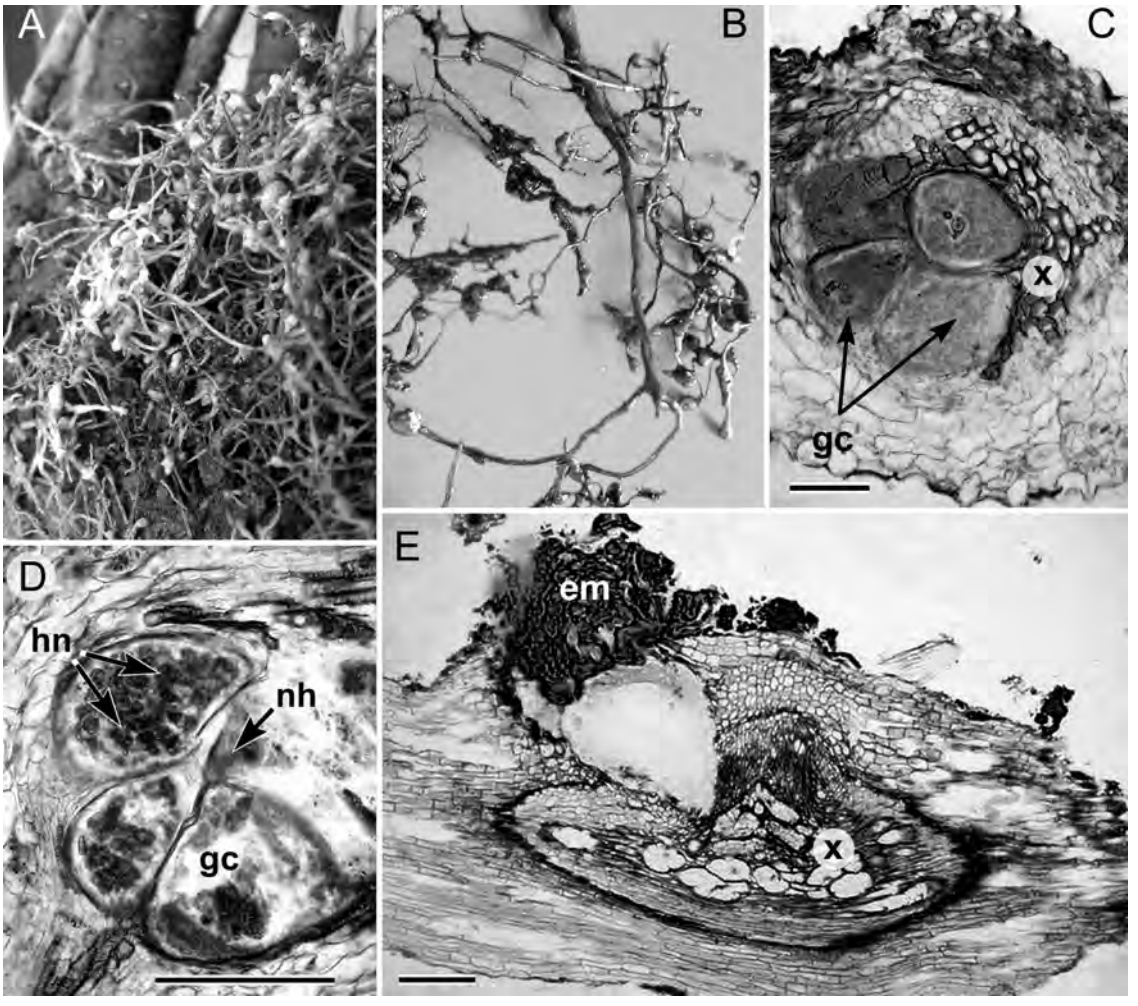

Fig. 1. Severe infection of *Meloidogyne hapla* on peony. A and B, peony roots with numerous galls induced by *M. hapla*. C-E, cross (C, D) and longitudinal (E) sections of galled roots showing the specialized host-parasite relationship at the feeding site level. (Abbreviations: gc = giant cells; x = xylematic elements; hn = hypertophied nuclei; em = eggs in an egg-mass structure); nh = nematode head (Scale bars: C, D = 100 µm; E = 200 µm).

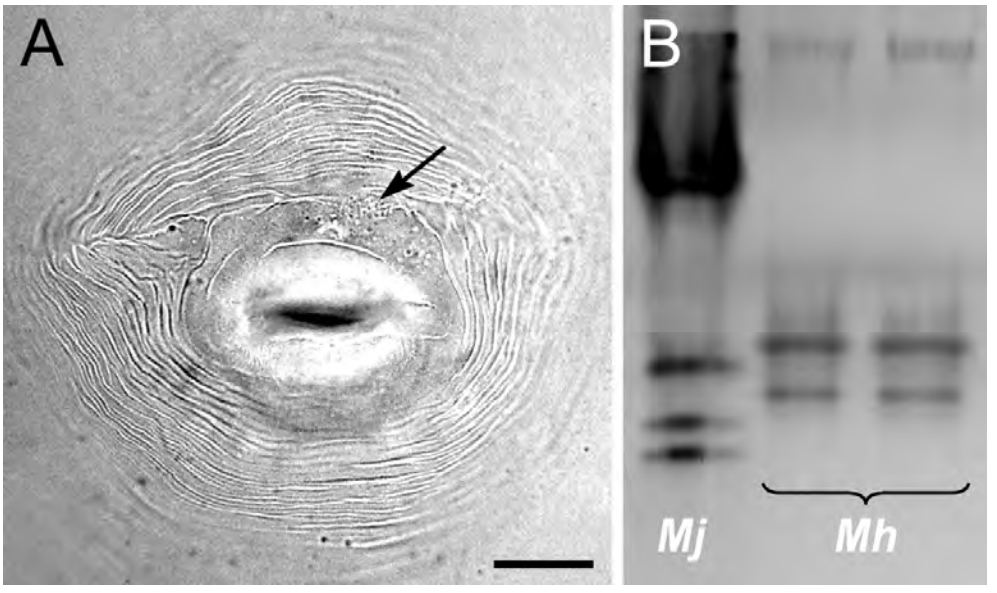

Fig. 2. A. Perineal pattern of adult female of *M. hapla*. Note the ventral striae expanded on the left side ('wing') and the distinct punctation forming the characteristic stippled area between anus and tail terminus (arrow). B. Esterase electrophoretic pattern of protein homogenates of *Meloidogyne hapla* (Mh); *M. javanica* (Mj) is the reference population. (Scale bar: A = 20 µm).

peony were gently washed free of adhering soil and debris. Root tissues were fixed in formaldehyde chromoacetic solution for 48 h, dehydrated in a tertiary butyl alcohol series (40-70-85-90-100 %), embedded in paraffin with a melting point of 58 °C and sectioned with a rotary microtome. Sections 10-12 µm thick were placed on glass slides, stained with safranin and fast-green, mounted permanently in a 40% xylene solution of a polymethacrylic ester (Synocril 9122X, Cray Valley Products, NJ, USA), examined microscopically and photographed (Johansen, 1940). The root tissues of peony revealed marked cellular alterations in the cortex, endodermis and vascular parenchyma induced by the nematode during its feeding activity. In the permanent feeding sites, the nematode induced the formation of large, multinucleate giant cells adjacent to the vascular tissues, leading to disorganization and disruption of xylem elements and primary phloem cells. Nematode feeding sites comprised three to eight giant cells, which surrounded the lip region of a single female (Fig. 1D-F).

A few reports exist of different root-knot nematodes (*Meloidogyne* spp.) parasitizing peonies. Mixed infections by *Meloidogyne hapla* and *M. incognita* (Kofoid *et* White) Chitw. have been reported in the USA on *Paeonia albiflora* Pall. (Eversmeyer and Dickerson, 1966), and *Meloidogyne* sp. was reported by the Connecticut Agricultural Experimental Station in the list of Peony diseases. Furthermore, susceptibility and pathogenicity of *M. hapla* on peony was demonstrated by Park *et al.* (1999, 2004) under greenhouse conditions, showing that plant growth was significantly decreased with inoculum densities of 2.22 nematodes/g of soil.

Meloidogyne hapla is one of the most important nematode pathogens affecting a wide range of flowering herbaceous perennial ornamentals (LaMondia, 1995). The vegetative propagation of many of these plants may result in increased spread and distribution of endoparasitic plant nematodes. *Meloidogyne hapla* is of particular concern for the major flower-producing area of northern Italy. Beside peony, *M. hapla* attacks a number of other perennials, vegetables and field crops, such as carrots, potatoes, sugar-beet and onions. Infested peony was also collected from another field where vegetables had been grown previously. In this field, symptoms of the nematode attack on peony were not severe. However, it must be considered that nurseries sell wholesale uprooted plants to other growers for cut flower production, in Italy and abroad. Such infested plants do not meet Italian and European regulations for marketing plants.

Although infestation of *M. hapla* on peony may not be rare, to the best of our knowledge this is the first report of *M. hapla* in Italy and probably in Europe. However, to limit the spread of the nematode and loss of flower production, and to avoid the rejection of peony stock plants following phytosanitary inspections, we suggest that production of peony plants free of root-knot nematode be considered within the protocols of voluntary European certification programmes.

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