First Report of *Meloidogyne morocciensis* Infecting Hops (*Humulus lupulus*)

Eduardo France Oza¹*, José Olívio Lopes Vieira Júnior¹, Millena Monteiro dos Santos², Mariana Zandomênico Mangêro¹, Renata Cunha Pereira¹, Ricardo Moreira de Souza¹*, Antônio Fernando de Souza³ and Silverior de Paiva Freitas¹

¹Universidade Estadual Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, Brazil
²Centro Universitário Norte do Espírito Santo, Universidade Federal do Espírito Santo, São Mateus, Espírito Santo, Brazil
³Instituto Federal de Ciência e Tecnologia do Espírito Santo, Santo Teresa, Espírito Santo, Brazil

*E-mail: eduardo.franceoza@hotmail.com; ricmsouza@censanet.com.br

This paper was edited by Peter DiGennaro.

Received for publication February 06, 2022.

### Abstract

*Humulus lupulus* (Cannabaceae) is a climbing herbaceous plant with perennial production, intended mainly for the brewing industry. *H. lupulus* is widely cultivated in temperate regions; hop cultivars have shown good adaptation in regions of Brazil. In a hop-growing area in São Mateus, the state of Espírito Santo, leaf wilting and galling of the root system was observed. Soil and root samples were taken to the laboratory and processed, and the nematodes extracted from the *Meloidogyne* genus were identified by morphology, morphometry, and biochemical analysis. According to the results, the species identified in the hop roots was *Meloidogyne morocciensis*. This is the first report of *H. lupulus* as host of *M. morocciensis*.

### Keywords

diagnosis, first occurrence, nematodes.

Hop (*Humulus lupulus* L.) is a climbing herbaceous plant (Fig. 1A) belonging to the Cannabaceae family, native to temperate regions. Flowers of this plant are mainly used in the brewing industry for conferring aroma and flavor to beer, but since it has secondary metabolites with significant biological activities (Astray *et al*., 2020), the plant is also applied in medicine and can be used in the treatment of diseases, such as insomnia, stress, and anxiety (Kyrou *et al*., 2017). Although Brazil is a country with a tropical climate, *H. lupulus* has adapted in regions with a mild climate and shows good plant development (Durello *et al*., 2019).

Seedlings of *H. lupulus* of the Cascade variety obtained through vegetative propagation of branches of a healthy hop plant were planted in the municipality of São Mateus, in the northern region of the state of Espírito Santo (18° 40’ 25” S, 40° 51’ 23” W; altitude of 77 m) in July 2019. Eight months after transplanting, the plants showed symptoms of leaf yellowing, reduced development evolving to leaf wilting, and senescence of the aerial part. After observation of the presence of root galls (Fig. 1B,D,E), soil and root samples were collected from the rhizosphere of five hop plants. Four soil subsamples were collected from each plant and homogenized in plastic bags to form five composite soil samples (*n* = 5). To sample the roots, five plants were collected from the soil, taking care to keep the root system intact, and placed in a plastic bag. The soil and root samples were taken to the Nematology Laboratory of the Universidade Estadual do Norte Fluminense Darcy Ribeiro, the Nematology Laboratory of the Universidade Federal
Meloidogyne morocciensis parasiting Humulus lupulus: Oza et al.

Nematodes were extracted from soil using the method of Jenkins (1964) and from roots using the method of Coleen and D’Herde (1972). For the identification of Meloidogyne species, the morphology of female perineal patterns was used \((n = 10)\), morphometry of juveniles \((n = 20)\), and by esterase phenotypes obtained by electrophoresis gel \((n = 8)\). Eggs and juveniles were counted on Peter’s slides to quantify the population found in the samples.

According to the results of the analyses, it was possible to identify the species as Meloidogyne morocciensis Rammah and Hirschmann, 1990 and as a parasite of the hop plants. The estimated population of eggs and juveniles was 864/100 cm\(^3\) in soil and 2,763/g root. The perineal patterns found exhibit oval-to-square shapes, with moderately separated dorsal arches and continuous and separate coarse striations, similar to that found by Machaca-Calsin et al. (2021) (Fig. 1G).

The measurements of second-stage (J2) juveniles \((n = 20)\) (Fig. 1C) were as follows: \(L = 408.84 \pm 3.46\) (380.98–434.31) \(\mu\)m; stylet length: \(13.61 \pm 0.17\) (12.48–15.08) \(\mu\)m; dorsal gland opening: \(3.67 \pm 0.08\) (2.99–4.26) \(\mu\)m; tail length: \(48.62 \pm 0.39\) (45.91–51.03) \(\mu\)m; hyaline tail length: \(15.96 \pm 0.25\) (14.73–17.85) \(\mu\)m; \(a: 25.98 \pm 0.42\) (23.2–27.85) \(\mu\)m; and \(c: 7.14 \pm 0.18\) (6.99–7.91) \(\mu\)m. No males were found for the morphological and morphometric analyses. According to Rammah and Hirschmann (1990), the
measurements obtained from the second-stage (J2) juveniles correspond to those of the species *M. morocciensis*. The esterase isoenzyme phenotype was characterized as A3 by PAGE (Fig. 1F) in the esterase identification method (Carneiro et al., 2008).

*Meloidogyne morocciensis* is a species less frequently found worldwide when compared to traditional species such as *M. javanica* and *M. incognita*. In Brazil, this nematode has been found in beetroots (Machaca-Calsin et al., 2021), peach trees (Silva et al., 2020), tomato plants (Barros et al., 2018), and soybean (Dalla Nora et al., 2020). Based on the results of the species identification analyses, this is the first report of *M. morocciensis* on hop roots. We observed that infected hop plants showed developmental damage, such as reduced growth, chlorosis on leaves, and nodules on roots. The disease could reduce hop production and affect other crops reported to be hosts of economic importance in Brazil, such as soybeans and tomatoes. Thus, we recommend hop growers in Brazil, especially in the northern region of Espírito Santo, to take care and avoid the spread of this nematode to other agricultural crops and to other regions of the country.

**Acknowledgments**

We extend our sincere thanks to the farmers Marcelo dos Santos and Ana Maria Monteiro dos Santos for their dedication and enthusiasm in conducting the experiments and to the technician of the Phytopathological Analysis Laboratory of the Universidade Federal do Espírito Santo, Helder P. Marques.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

**Literature Cited**

Astray, G., Gullón, P., Gullón, B., Munekata, P. E., and Lorenzo, J. M. 2020. *Humulus lupulus* L. as a natural source of functional biomolecules. Applied Sciences 10(15):5074.

Barros, A. F., Campos, V. P., Souza, L. N., Costa, S. S., Terra, W. C., and Lessa, J. H. 2018. Morphological, enzymatic, and molecular characterization of root-knot nematodes parasitizing vegetable crops. Horticultura Brasileira 36:473–479.

Carneiro, R. M. D. G., dos Santos, M. F. A., Almeida, M. R. A., Mota, F. C., Gomes, A. C. M. M., and Tygano, M. S. 2008. Diversity of *Meloidogyne arenaria* using morphological, cytological and molecular approaches. Nematology 10(6):819–834.

Coolen, W. A., and D’Herde, C. J. (eds) 1972. A method for the quantitative extraction of nematodes from plant tissue. Ghent, Belgium: State Nematology and Entomology Research Station. 77p.

Dalla Nora, D., Balardin, R. R., Kaspary, T. E., de Souza Júnior, I. T., and Bellé, C. 2020. Host status of soybean genotypes to *Meloidogyne arenaria* and *Meloidogyne morocciensis*. Revista de Ciências Agroveterinárias 19(4):428–433.

Durello, R., Silva, L., and Bogusz, S., Jr., 2019. Química do lúpulo. Química Nova 42:900–919.

Jenkins, W. R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Kyrou, I., Christou, A., Panagiotakos, D., Stefanaki, C., Skenderi, K., Katsana, K., and Tsigos, C. 2017. Effects of a hops (*Humulus lupulus* L.) dry extract supplement on self-reported depression, anxiety and stress levels in apparently healthy young adults: A randomized, placebo-controlled, double-blind, crossover pilot study. Hormones 16(2):171–180.

Machaca-Calsin, C. P., da Silva, W. R., da Silva Grinberg, P., de Araújo Filho, J. V., and Gomes, C. B. 2021. Occurrence of *Meloidogyne morocciensis* parasitizing beetroot in Brazil. European Journal of Plant Pathology 160(1):239–242.

Rammah, A., and Hirschmann, H. 1990. *Meloidogyne morocciensis* n. sp. (Meloidogyninae), a root-knot nematode from Morocco. Journal of Nematology 22(3):279.

Silva, W. R., Machaca-Calsin, C. P., and Gomes, C. B. 2020. First report of the root-knot nematode, *Meloidogyne morocciensis* infecting peach in Southern Brazil. Embrapa Clima Temperado-Nota Técnica/Nota Cientifica.