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# First report of root-knot nematode, *Meloidogyne javaniva*, infecting *Stachys byzantina* on São Paulo, Brazil

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## Abstract

Stachys byzantina belongs to the Labiatae and is known by the names "peixinho-da-horta" (Brazil) and "lamb's ear" (USA). Its importance is associated with its medicinal properties (Bahadori et al. 2020) and nutritional aspects (Milião et al. 2022). Root-knot nematodes cause severe damage to plants and suppress production. In January 2021, plants of S. byzantina in the municipality of Jaboticabal (21°14'38.7"S, 48°17'10.6"W) showed symptoms of reduced growth, yellowed leaves and the presence of galls in the roots. Initially, samples of roots from a S. byzantina were analyzed at the Nematology Laboratory (LabNema/UNESP), Jaboticabal, Brazil, estimating 20,000 eggs and juveniles of Meloidogyne sp. in 10 g of roots. To confirm the host ability of the species, a pathogenicity test was performed using Koch's postulate. For this purpose, the test was conducted in a greenhouse where 3,000 eggs and second-stage juveniles (J2) were inoculated onto three plants (n=3) of S. byzantina. After 90 days, the inoculated plants showed the same symptoms as those observed in the field. No symptom or nematode was detected in the uninoculated plant (control). Nematodes were extracted from the roots of inoculated plants and quantified. The perineal pattern of females (n=10) (Netscher and Taylor, 1974) and the labial region of males (n=10) (Eisenback and Hirschmann, 1981) were analyzed and compared with the morphological characteristics of the original description of the species (Chitwood, 1949). For analysis based on esterase isozyme phenotype, the α-method of Esbenshade and Triantaphyllou (1990)

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DNA from an adult female (n=1) was extracted using the Qiagen DNeasy® Blood & Tissue Kit and this sample was used for both genetic sequencing and the sequence-characterized amplified region techniques (SCAR). PCR amplifications were performed for the 18s rRNA gene using primers 988F and 1912R from Holterman et al (2006). Our sequence was deposited in GenBank (NCBI) under the identifier OP422209. Finally, species-specific SCAR primers (Fjav/Rjav, Me-F/Me-R, and Finc-F/Finc-R) designed by Zijlstra (2000) were used to identify Meloidogyne spp. Koch's postulate analysis yielded the following results: (n=1) 9,280 eggs and J2 (Reproduction factor, RF = 33.09); (n=2) 111,720 eggs and J2 (RF = 37.24); (n=3) 59,700 eggs and J2 (RF = 19.9) (RF mean = 30.08). The following characteristics were observed in the perineal region of females: Low and rounded trapezoidal dorsal arch with two distinct lateral lines clearly separating the dorsal and ventral arch regions, similar to the morphological features of the species description by Chitwood (1949). Males had a convex labial plate with a non-raised labial disk joining the submedial labia, a non-rugged labial region, the basal tubercles were usually wider than high, and a rounded tail tip (Eisenback and Hirschmann 1981). The α-esterase enzyme profile showed the J3 phenotype typical of M. javanica (Rm [×100] = 46.0, 54.5, and 58.9). The 18s rRNA sequences grouped Meloidogyne sp. with species such as M. enterolobii, M. incognita, and M. javanica. A DNA fragment of about 700 bp was amplified with Mj (Fjav/Rjav) primers, but not with Me (Me-F/Me-R) and Mi (Finc-F/Finc-R) primers, which confirmed the identification of M. javanica. Accurate identification and characterization of the occurrence of new hosts of M. javanica will allow us to determine the range and geographic distribution of the species. This is the first report on the occurrence of M. javanica on S. byzantina in Brazil. This report is important so that management strategies can be applied to prevent the spread of the pest to other areas.

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