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Detection of *Meloidogyne graminicola* parasitising *Cyperus rotundus* in Rio Grande do Sul, Brazil

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Abstract

Cyperus rotundus plants showing symptoms of root knot were observed in the municipality of Santa Maria, Rio Grande do Sul state, Brazil. Based on the morphological, esterase phenotypes and molecular analyses of rDNA-ITS and D2-D3 regions of 28S rDNA, the causal agents of the observed symptoms were identified as *Meloidogyne graminicola*, pathogenicity was confirmed by fulfilling Koch's postulates. To our knowledge, this is the first report of *M. graminicola* in *C. rotundus* in Rio Grande do Sul State, Brazil.

Keywords Occurrence · Identification · Root-knot nematodes · Purple nutsedge

Weeds reduce agricultural productivity by competing for environmental resources, allelopathic effects and serving as alternative hosts for pests and pathogens (Webster and Nichols 2012; Bellé et al. 2017). *Cyperus rotundus* (purple nutsedge) is a cosmopolitan weed species found in tropical and temperate areas, occurring as in many countries worldwide. The purple nutsedge is a persistent and herbaceous weed, being perennial of multiple crops throughout the world with the primarily reproduces vegetatively via rhizomes and tubers, though it will flower and produce seed.

The *C. rotundus* is among one of the most difficult weeds to control in main summer crops such as sugarcane, pulses, cotton, maize, rice and horticultural crops due to its tolerance of many control practices, including many herbicides (Webster and Nichols 2012). The absence to control of purple nutsedge occurs because of the longevity of tubers, capability to germinate many times, modes of multiple promulgation and unavailability of herbicides for season long control (Iqbal et al. 2018). *C. rotundus* may serve as an alternative host for many crop pests including arthropods, diseases and nematodes (Peerzada 2017).

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In April 2018, samples of purple nutsedge plants (Fig. 1a, b) exhibiting many galls on the roots (Fig. 1) were observed, with representative samples taken from areas in the Santa Maria (29°45'S; 53°38'W; 43 m) county, Rio Grande do Sul, Brazil.

The identification to species of the *Meloidogyne* population was carried out using esterase phenotypes ($n = 40$ females) (Carneiro et al. 2000; Carneiro and Almeida 2001), morphological measurement of second-stage juveniles (J2) ($n = 50$), females ($n = 20$) and males ($n = 10$), and perineal patterns (n = 20) and via the amplification and sequencing of the ITS1–5.8S-ITS2 rRNA region (primer set: forward 5′-TTGATTACGTCCCTGCCCTTT-3′ and reverse 5′-TCCTCCGCTAAATGATATG-3′) and the D2–D3 fragment of the 28S rRNA gene (primer set: forward 5′-ACAAGTACCGTGAGGGAAAGTTG-3′ and reverse 5′-TCGGAAGGAACCAGCTACTA-3′) (Schmitz et al. 1998; De Ley et al. 1999).

After the extraction of adult females, the total nematode population was estimated per gram of purple nutsedge roots (Hussey and Barker 1973). Microscope slides of perineal patterns were deposited (LB0012) in the Universidade Federal de Santa Maria (UFSM) Collection, Rio Grande do Sul state, Brazil.

The nematode population density observed in the sample was 785 J2 s/g of *C. rotundus* root. The J2 s had the following morphometric characters: length (L) = 482.5 ± 30.0 (385.0–479.5) μm, a = 27.1 ± 1.3 (24.4–29.0), c = 5.5 ± 0.5 (5.0–7.3), stylet length = 14.5 ± 0.4 (13.1–15.9) μm, dorsal oesophageal gland orifice (DGO) = 3.5 ± 0.7 (3.2–4.7) μm, tail length = 71.6 ± 4.1 (62.0–75.9) μm and hyaline tail terminus = 18.5 ± 1.4 (15.6–24.7) μm. Morphological

Fig. 1 *Meloidogyne graminicola* root infestation symptoms on purple nutsedge (*Cyperus rotundus*)

measurements of females included L = 650.5 ± 50.6 (510.7–723.8) μm, stylet length = 13.1 ± 0.8 (11.5–14.9) μm, and DGO = 3.8 ± 0.5 (3.2–5.2) μm. The female's perineal patterns (Fig. 2a, b) were oval shape and a low dorsal arch without the presence of a lateral line and the cuticular striations were smooth and thick in the dorsal region of the vulva and phasmids were close together (13,1 to 17.9 μm). The males were cylindroid and vermiform with a robust stylet, arcuate spicule, and short and rounded tail. Measurements of males were L = 1305.8 ± 130.5 (1155.1-1602.1) μm, stylet length = 19.1 ± 0.8 (17.1 to 19.4) μm, DGO = 3.7 ± 0.6 (2.5 to 4.1) μm, tail = 11.2 ± 1.9 (9.5 to 14.7) μm, spicule = 31.5 ± 1.8 (28.1–35.4) μm. The overall morphology and morphometrics of the population of *M. graminicola* correspond well with the original description (Golden and Birchfeild 1965).

The polymorphisms of the esterase bands by electrophoresis revealed the phenotype VS-1 (Rm = 0.70) typical of *M. graminicola* (Carneiro et al. 1996) (Fig. 2c). The sequences of the studied rDNA regions were submitted to GenBank (ITS: MH842692 and D2–D3 of 28S: MH843665). Searches on BLAST showed 99%–100% identity with sequences of *M. graminicola* isolates from Brazil, Taiwan and China.

In greenhouse tests, *C. rotundus* plantlets were maintained in pots with 2000 cm^3 sterilised soil. Six replicates were inoculated with 5000 eggs and J2 s from the original population of *M. graminicola*, in addition to a non-inoculated control. Plants were maintained under greenhouse conditions at 25–30 °C, with watering as needed. After 55 days, the inoculated plants exhibited galled root systems similar to plants observed in the field; the nematode reproduction factor (final population/

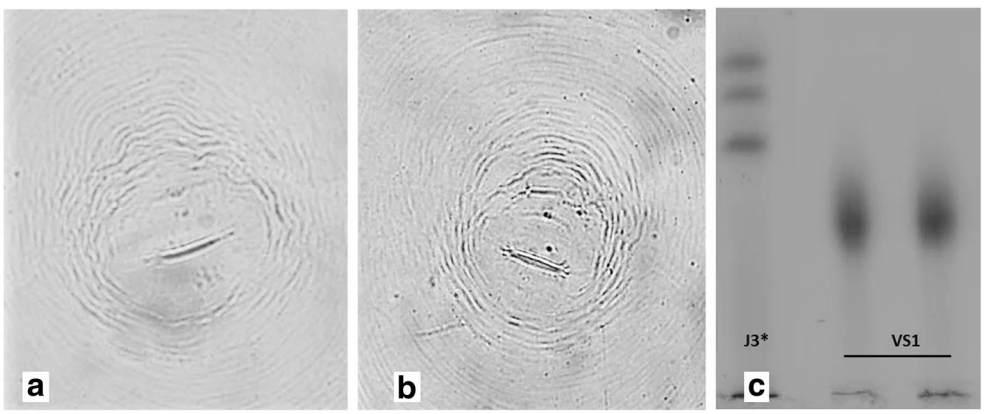

Fig. 2 Perineal pattern (**a** and **b**), and esterase phenotype (**c**) of *Meloidogyne graminicola* detected in *Cyperus rotundus* in Santa Maria, Rio Grande do Sul state, Brazil (VS1: *Meloidogyne graminicola*; Est. J3*: *Meloidogyne javanica* reference isolate)

initial population) was 12.5. The non-inoculated plants did not exhibit any galls. The morphological and molecular characteristics of the re-isolated root-knot nematode were identical those of *M. graminicola*.

To our knowledge, this is the first report of *M. graminicola* parasitising *C. rotundus* in Brazil. Globally, *M. graminicola* is considered an economically important agricultural nematode as it causes severe yield losses to crops such as rice, is reported to infect over 100 plant species, including cereals and grass plants, as well as dicotyledonous plants (Mantelin et al. 2017). This record has significance for rice production in Brazil as the weed *C. rotundus* could act as a potential reservoir for *M. graminicola* in the absence of host crops and necessitate study of its biology to aid the development of appropriate control strategies that minimize its effect on crop production.

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