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**DISEASE NOTES** 

### First Report of Meloidogyne arenaria on Lisianthus (Eustoma grandiflorum) in Brazil

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Citation I

Lisianthus (Eustoma grandiflorum) is an ornamental species cultivated in pots or as cut-

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### **ABSTRACT**

flower and it has been growing in Brazil since the 1990s. During summer 2015, lisianthus plants (cv. Mariachi Blue) exhibiting symptoms of stunting, leaf wilting, and multiple galls in the roots associated with root-knot nematode (Meloidogyne sp.) were detected in a commercial field in the municipality of Dois Irmãos (29°35′ S, 51°06′ W), Rio Grande do Sul State, Brazil. Subsequently, individual females (n = 40) were extracted from root samples and submitted to Meloidogyne species identification by a-esterase phenotypes (Carneiro and Almeida 2001), perineal pattern morphological analysis (n = 20), morphometric measurements of second stage juveniles (J2) (n = 20), and amplification of the D2/D3 fragments of the 28S rRNA using universal primers D2A (5'ACAAGTACCGTGAGGGAAAGTTG3') and D3B (5'TCGGAAGGAACCAGCTACTA3'). In addition, root and soil samples were processed to determine the number of eggs and J2. The nematode population density was 534 eggs and J2 per gram of fresh root and 250 J2 per 100 cm<sup>3</sup> of soil. The analyze of polymorphisms of a-esterase phenotypes revealed the A2 phenotype (Rm = 1.26, 1.36) typical of Meloidogyne arenaria (Carneiro et al. 2008). Perineal patterns of females showed a low dorsal arch, with lateral field marked by forked and broken striae; phasmids apart 30.08 μm (27.67 to 33.23 μm). No punctate markings between anus and tail terminus were observed, similar to the M. arenaria description. The measured M. arenaria J2 showed the following morphometric characters: body length =  $483.05 \pm 21.33 \, \mu m$ ; body width =  $15.04 \pm 0.96 \, \mu m$ ; a =  $32.25 \pm 2.6$ ; c =  $8.65 \pm 0.67$ ; DGO =  $3.02 \pm 0.43 \, \mu \text{m}$ ; stylet =  $13.14 \pm 1.24 \, \mu \text{m}$ ; tail length =  $56.20 \pm 5.27 \, \mu \text{m}$ ; hyaline tail terminus =  $9.70 \pm 0.87 \, \mu m$ . The DNA fragment obtained showed a 754 bp length (GenBank accession no. KX151138) that was sequenced and analyzed, revealing more than 99.9% homology with M. arenaria sequence data from United States (EU364889) and South Africa (KC287191, KC287192, and JX987332) populations. The confirmation of nematode species identification was done by PCR species-specific SCAR using the primer set Far (5'-TCGGCGATAGAGGTAAATGAC-3') and Rar (5'-TCGGCGATAGACACTACAAACT-3'). The PCR product of SCAR was ~420 bp, which was identical to that previously reported for M. arenaria (Zijlstra et al. 2000). To verify the nematode pathogenicity on lisianthus

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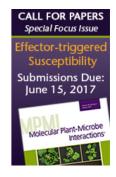
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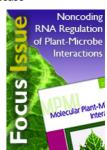
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plants, individual plantlets of cv. Mariachi Blue, maintained in pots with sterilized soil (10

replicates), were inoculated with 5,000 eggs plus J2 of a pure population of *M. arenaria* under greenhouse conditions. A noninoculated control was included in the test (10 replicates). After 70 days, all inoculated plants showed reduced growth compared with control. In addition, root-galling symptoms were similar to those observed in the field, and the mean nematode reproduction factor (final population/initial population) was 9.6. *M. arenaria* is one of the most important root-knot nematodes and causes great losses in many crops around the world (Perry et al. 2009). Although other *Meloidogyne* species, including *M. javanica*, *M. incognita*, and *M. hapla*, have already been described infecting lisianthus (Schochow et al. 2004), this is the first report of *M. arenaria* parasitizing this plant around the world. This finding has great importance to Brazilian flower growers to establish management measures for this nematode in lisianthus.



References:	Section:	Choose	

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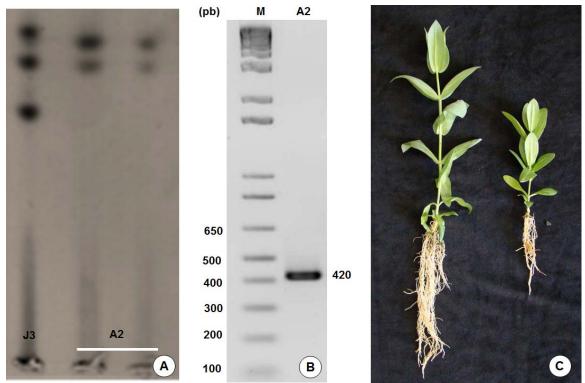
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C. G. Neves and C. Bellé contributed equally to this work.

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**Fig. S1. A,** Esterase phenotypes of *Meloidogyne arenaria* (est. A2) from Lisianthus (*Eustoma grandiflorum*) and *M. javanica* (J3) as known reference. **B,** SCAR-PCR of *M. arenaria* population using DNA extracted from females. Species was characterized with primers Far/Rar (Zijlstra et al. 2000). **C,** Plantlets of lisianthus (cv. Mariachi Blue) noninoculated (left) and inoculated (right) with *M. arenaria*.