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First Report of the Root-Knot Nematode (*Meloidogyne javanica*) Infecting Hops (*Humulus lupulus*) in Florida, USA

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Humulus lupulus (Cannabaceae), commonly referred to as hops, are perennial, herbaceous climbing plants, native to temperate northern climates. Hops are cultivated for their strobiles or cones, which are often used for flavoring and aroma in food, tea, and beer (Almaguer et al., 2014). Because of the high demand for hops from the micro-brewing industry in the Tampa-St. Petersburg area, it has recently been introduced in Florida. The crop grows rapidly in the early spring to late summer. Plants reach a mature height of 5.5 to 7.6 m in one year and produce cones from mid-summer to early fall. Hop rhizomes were planted in April 2016 at the Gulf Coast Research Station, Wimauma, Hillsborough Co., Florida, USA. In October 2016, several hop plants that exhibited yellowing leaves and stunted growth were uprooted and showed severe root galling (Figs 1,2). Rhizosphere soil samples were collected for nematode extraction and showed high numbers of root-knot nematode second-stage juveniles (J2) (up to 1,500 J2/200 cm^3 soil). Heavily galled root samples were sent to the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Nematology Laboratory in Gainesville, FL. Species identification was performed using morphological analyses of females perineal patterns (n = 22), selected characters of second-stage juveniles (n = 17), and isozyme phenotypes (esterase and malate dehydrogenase) of egg- laying females (n = 26) extracted from the roots. Configuration of the perineal patterns, morphometrics of body, stylet, and tail length of J2 and the esterase phenotype (EST = J3), which is species-specific and malate phenotype (MDH = N1) are consistent with those reported in the original description of *M. javanica* and many other populations of this nematode species collected in Florida and other countries (Esbenshade and Triantaphyllou, 1985; Jepson, 1987; Brito et al., 2008). For molecular analyses, DNA was extracted from individual females and mitochondrial DNA was amplified with MORF (5′-ATC GGG GTT TAA TAA TGG G-3′) and MTHIS (5′-AAA TTC AAT TGA AAT TAA TAG C-3′) primer set (Stanton et al., 1997). A fragment of approximately 740 bp was produced, which has been reported for *M. incognita* and *M. javanica* found in Florida (Baidoo et al., 2016). To further confirm the species identification we use the species-specific SCAR primer set Fjav (5′-GGT GCG CGA TTG AAC TGA GC-3′) and Rjav (5′-CAG GCC CTT CAG TGG AAC TAT AC-3′) (Zijlstra et al., 2000). This primer set yield a fragment of approximately 670 bp, which is identical to that previously reported for *M. javanica* (Zijlstra et al., 2000; Baidoo et al., 2016). Additionally, NADH dehydrogenase subunit 5 gene was amplified using NAD5F2 (5′-TAT TTT TTG TTT GAG ATA TAT

Figure 1: Closeup view of the root system of the *Humulus lupulus* infected with *Meloidogyne javanica* showing galls on primary, secondary and tertiary roots.

Figure 2: Plants of *Humulus lupulus* showing the above ground symptoms induced by *Meloidogyne javanica* in the field.

TAG-3′) and NAD5R1 (5′-CGTGAATCTTGATTTTC-CATTTTT-3′) primers as described by Janssen et al. (2016). The GenBank accession number of the *nad5* gene sequence of *M. javanica* found infecting hops in Florida is MH230176. The obtained *nad5* gene sequence was identical to the reference sequence of *M. javanica* provided by Janssen et al. (2016). To our knowledge, this is the first report of *H. lupulus* as a host of the Javanese root-knot nematode (*M. javanica*) in Florida.

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