

First report of the Root-Lesion Nematode, *Pratylenchus fallax*, on Soybean in Wisconsin, USA

Kanan Saikai,
Zafar Ahmad Handoo, and
Ann E MacGuidwin

K. Saikai, Department of Plant Pathology, University of Wisconsin-Madison, Madison 53706; Z. A. Handoo, USDA-ARS Mycology and Nematology Genetic Diversity and Biology Laboratory, Beltsville, MD 20705; A. E. MacGuidwin, Department of Plant Pathology, University of Wisconsin-Madison, Madison, 53706.

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Abstract

The root-lesion nematode (*Pratylenchus* spp.) is the most common nematode pest on soybean in Wisconsin. More than 100 root-lesion nematodes per 100 cm³ soil were detected from a sample received for nematode diagnostics from a soybean field in Outagamie County WI in October of 2013. Corn was planted in the previous year. Monoaxenic in vitro cultures were established from single females grown on “IOChief” sweet corn explants on Gamborg’s B5 medium without auxins or cytokinin. Reproduction of the nematode was observed, and after three months there were many adult females and males. Nematodes were extracted from the culture by incubating roots for 48 hours on a Baermann funnel. Morphometrics were determined for 25 females and 25 males: measurements are presented as means (range). The body length of females was 529 μm (438 to 615 μm), with a maximum body width of 20.1 μm (15.3 to 23.6 μm). The a, b, and c ratios were 26.4 (24.1 to 30.1), 5.4 (4.3 to 7.5), and 21.6 (16.3 to 28.3), respectively. The head region was 3.0 μm high (2.1 to 3.9), with two to three lip annules that were obscure in most specimens. The stylet had rounded knobs with a length of 15.0 μm (13.8 to 16.4 μm). The excretory pore was at or posterior to the nerve ring. There were four lateral lines at mid-body. The vulva opening was located at 79 % (77 to 81 %) of the body length from the anterior end. The post-uterine sac was smaller than the body width at the vulva with a length of 15.0 μm (10.6 to 20.0 μm). The female tail shape was conical or slightly irregular with a round or striated tail tip and the tail length was 29 μm (23.5 to 36.7 μm) with 23 annules (20 to 30). The body length of males was 454 μm (381 to 543 μm) and the stylet length was 13.4 μm (11.8 to 14.8 μm). The length and range of the spicule and gubernaculum, respectively, were 16.6 μm (14.0 to 19.7 μm) and 4.2 μm (3.2 to 6.2 μm). The isolate was morphologically identified as *P. fallax* based on species keys (Castillo and Vovlas 2007; Handoo and Golden 1989). Molecular characterization based on 18S rDNA (Chizhov et al. 2006), the

D2-D3 expansion region of 28S rDNA (Subbotin et al. 2008), and cytochrome c oxidase subunit I (COI) (Derycke et al. 2010) were conducted for five individual nematodes collected from the monoaxenic cultures. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Inc). The 18S sequence (GenBank Accession No. MK217948) was 98% identical to *P. fallax* and 97% identical to *P. penetrans*, *P. convallariae*, and *P. pinguicaudatus* according to the Basic Local Alignment Search Tool (BLAST). The COI sequence (GenBank Accession No. MK041095) was 99% identical to *P. fallax* (87% identical to *P. pinguicaudatus*; 86% identical to *P. penetrans* and *P. convallariae*). The 28S sequence (GenBank Accession No. MK217949) was 99% identical to *P. fallax* (97% identical to *P. penetrans* and *P. pinguicaudatus*; 95% identical to *P. convallariae*). The species specific primer Ppen for *P. penetrans* based on the D3 region of 28S rDNA (Al-banna et al.2004) was negative for our *P. fallax* isolate. This species was reported beneath oak in North Dakota (Donald and Hosford 1980) but this is the first molecular information for a U.S. isolate of *P. fallax*. Specimens were sent to the Mycology & Nematology Generic Diversity & Biology Laboratory, USDA, ARS, Northeast Area (Beltsville, MD) for species confirmation, and validated as *P. fallax*. Three soybean plants cv. Corsoy were inoculated with 1000 mixed stages of the isolate and grown at 24°C in pasteurized loamy sand soil. The reproductive factor 3 months after inoculation was 2.94 (0.185 SD), thus confirming soybean as a host of *P. fallax*. Lesions were noted only on plants that were inoculated. To our knowledge, this is the first report of soybean as a host of *P. fallax* as well as the first report of this species in Wisconsin. As *P. fallax* is recognized as an economically important *Pratylenchus* species in Europe, further study of its distribution and economic impact on soybean is warranted.