

First Report of *Fusarium solani* phylogenetic species 25 associated with early stages of Thousand Cankers Disease on *Juglans nigra* and *Juglans regia* in Italy

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Thousand Cankers Disease (TCD) is a disease of *Juglans* spp. resulting from phloem necrosis caused by numerous coalescing *Geosmithia morbida* (*Gm*) branch cankers formed around entrance holes and galleries of its vector, the walnut twig beetle (WTB). Since 2010, TCD has been reported from black walnut (*J. nigra*) in the eastern U.S. (Griffin 2014) and more recently from Italy (Montecchio and Faccoli 2014) as well as from English walnut (*J. regia*) in both countries (Montecchio et al. 2014, Yaghmour et al. 2014). Members of the *Fusarium solani* species complex (FSSC) are associated with later stage TCD in the U.S. (Tisserat et al. 2009), but their role in early WTB colonization is unclear. From 2013-2014, FSSC-like colonies, in addition to *Gm*, were isolated from cankers on symptomatic *J. nigra* and *J. regia* in Italy. FSSC-like fungi were isolated from 82% and 64% of branch cankers from 4 *J. nigra* and 4 *J. regia* and 30% of 186 WTB from *J. nigra*. Symptoms included cankers surrounding WTB galleries as described for *Gm* in Italy (Montecchio and Faccoli 2014, Montecchio et al. 2014). From the necrotic margin of 4 branch cankers from 4 *J. nigra*, wood chips were excised and plated. In addition to *Gm*, a fungus with abundant aerial mycelium and sporodochia containing 2-3 septa macroconidia grew. Oval to kidney-shaped, aseptate microconidia were produced from elongate monophialides consistent with the FSSC. Four isolates were sent to the Fusarium Research Center (Penn State Univ.) for molecular confirmation. Portions of the nuclear ribosomal RNA gene repeat (rDNA), translation elongation factor 1- α (*EF1- α*), DNA-directed RNA polymerase II subunit 1 (*RPB1*), and DNA-directed RNA polymerase subunit 2 (*RPB2*) were PCR amplified and sequenced to resolve placement within the FSSC. Initial GenBank BLASTn searches revealed isolate S1, S2, and S4 *RPB1* sequences (deposited as KP696752 for S1) were all 99% identical to FSSC 25 (GenBank accession HM347154) and 100% to each other but different from isolate S3, which was 99% similar to FSSC 18 (HM347153). Isolate S1 was used for pathogenicity and multi-locus studies. A BLAST search of S1 rDNA sequence (KP696750) was 99% similarity to AB513852, while *EF1- α* and *RPB2* sequences (deposited as KP696749 and KP696751) were 99% similar to DQ247638 and EF469958, respectively. Alignment with known FSSC phylogenetic species confirmed S1 as FSSC 25. Pathogenicity was confirmed by placing 3 mm diam. PDA plugs of FSSC isolate S1, *Gm* isolate LM13GMN, both (split-plug), or sterile plugs into ten cork-borer wounded 3-year-old *J. nigra* and *J. regia* saplings per treatment. Inoculated parafilm trees were maintained in the greenhouse for 100 days. Inoculations with isolates *Gm* and S1 both individually and in combination on *J. nigra* resulted in cankers with similar mean areas of 4.8, 3.1, and 3.1 cm² and greater than ($p < 0.05$) the negative control (0.5

cm²). For *J. regia*, cankers were 3x smaller compared to *J. nigra*, with similar mean areas of 1.5, 1.3 and 1.4 cm², respectively, and greater than ($p < 0.05$) the negative control (0.3 cm²). Isolations from cankers and sequencing confirmed FSSC and/or *Gm*. These results support FSSC 25 as an early colonizer of WTB infested *J. nigra* and *J. regia*, and a contributing pathogen to early stages of TCD.

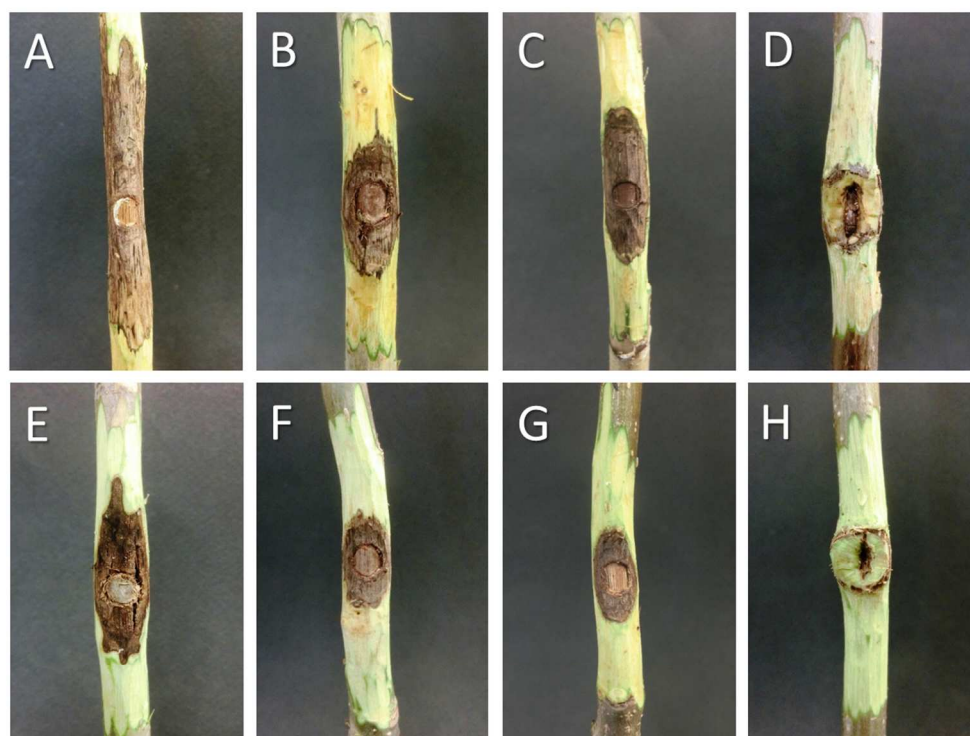
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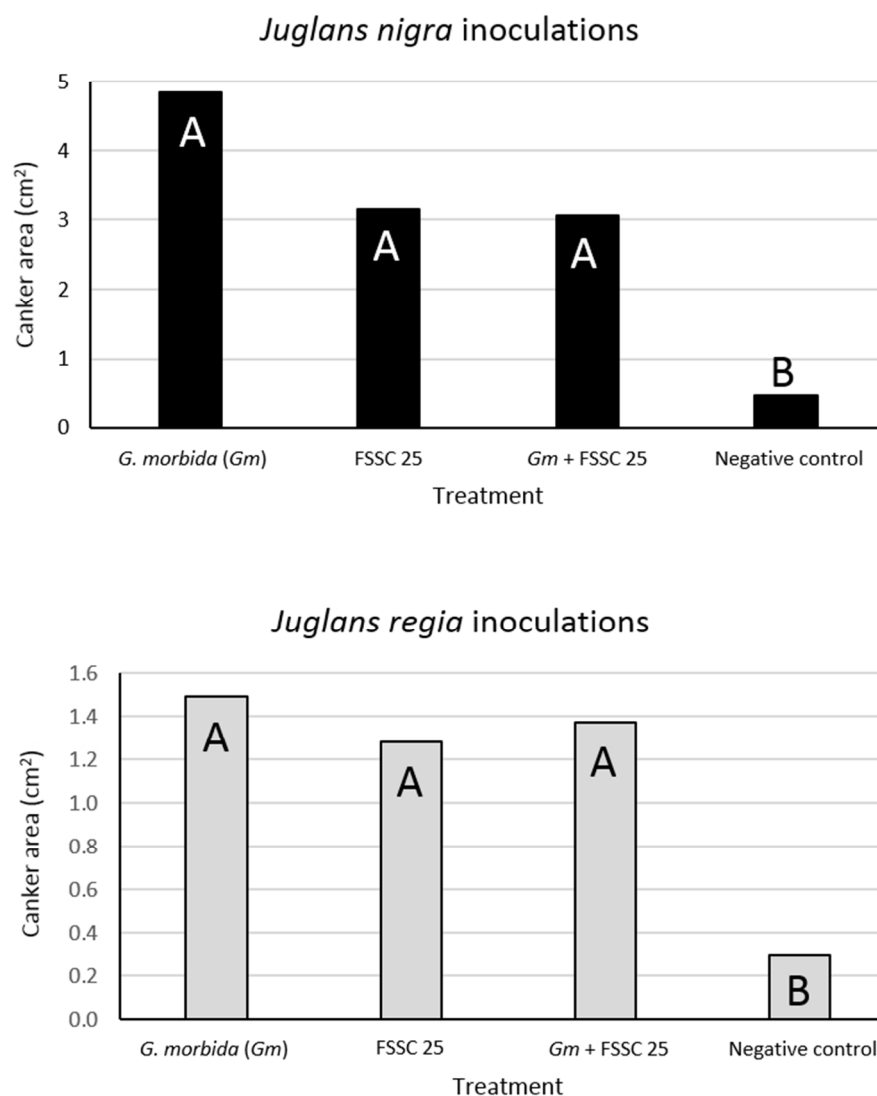
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M. A. Yaghmour et al. 2014. Plant Dis., 98: 1441.



Extent of necrosis assessed 100 days after inoculation on greenhouse grown *Juglans nigra* (A-D) and *Juglans regia* (E-H). Greenhouse conditions were as follows: $21 \pm 2^{\circ}\text{C}$, 80% RH; 12 hd-1 natural light. Treatments are as follows: A and E, *Geosmithia morbida*; B and F, FSSC 25; C and G, *G. morbida* + FSSC 25 (split plug); and D and H, negative control on *J. nigra* and *J. regia*, respectively. Treatment A, B, and C had 2, 1, and 1 dead stems after 100 days, respectively.

211x160mm (150 x 150 DPI)



Comparisons of mean canker area among treatments for *J. nigra* and *J. regia* 100 days after inoculation. Treatments with significantly different mean canker area are indicated by different letters, as determined by one-way analysis of variance (ANOVA, $p < 0.05$; IBM SPSS Statistics for Windows, Version 22.0, 2013).

Canker area for *J. nigra* ranged from 1.6-10.0, 0.9-10.0, 1.5-10.0 and 0.0-0.6 cm² for *Geosmithia morbida*, FSSC 25, *G. morbida* + FSSC 25, and negative control, respectively, whereas canker area for *J. regia* ranged from 1.0-2.5, 0.6-1.8, 1.0-1.6, and 0.0-0.6 cm².

129x164mm (150 x 150 DPI)