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ISSN: 0191-2917

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Editor-in-Chief: Alison E. Robertson
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[Previous Article](#) | [Next Article](#)January 2017, Volume 101, Number 1
Page 260
<https://doi.org/10.1094/PDIS-07-16-0993-PDN>

DISEASE NOTES

First Report of Root Rot of White Mulberry Caused by Simultaneous Infections of *Phytophthora megasperma* and *P. multivora* in Italy

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ABSTRACT

White mulberry (*Morus alba* L., Moraceae) is a deciduous shrub or tree native to East Asia where it is cultivated for feeding silkworms. In many temperate to subtropical regions of the northern hemisphere, it is wild and cultivated; in Italy, it is cultivated for its edible fruits and for landscaping and gardening. In summer 2015, symptoms of leaf blight, chlorosis, wilting, defoliation, trunk cankers with resin exudates, and root rot were observed on scattered trees in home gardens near Catania (Sicily). Overall, 15 out of 100 trees were symptomatic. Two homothallic *Phytophthora* species were isolated on BNPRAH selective medium singularly from infected roots of three and five trees, respectively, while both species were isolated from roots of seven out of 15 trees; both species were found in the rhizosphere of all 15 symptomatic trees with carob leaf as baits (Jung et al. 2016). Pure cultures were obtained by single-hypha transfer. Isolates of first species formed petaloid colonies on potato dextrose agar (PDA) and had an optimum growth temperature of 25°C (6 mm/day). On V8 juice agar, they produced nonpapillate, noncaducous sporangia and oogonia with paragynous antheridia. Mean diameter of oospores was 37.8 ± 0.4 µm (range 30 to 43 µm). The second species formed petaloid felty colonies on PDA and had an optimum growth temperature of 25°C; it produced ovoid or obpyriform, semipapillate, and persistent sporangia and oogonia with paragynous antheridia. Oospores (mean diameter 22.7 ± 1.3 µm; range 19 to 31 µm) were nearly plerotic with a thick wall (2.6 ± 0.5 µm). Internal transcribed spacer region 1 (ITS1)-5.8S-ITS2 (Van Tri et al. 2015) and a fragment of cytochrome oxidase subunit 1 gene (COI) from mitochondrial DNA (Robideau et al. 2011) of all isolates were sequenced. Sequences of representative

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Article History

Issue Date: 23 Dec 2016

Published: 17 Oct 2016

First Look: 23 Aug 2016

Accepted: 18 Aug 2016

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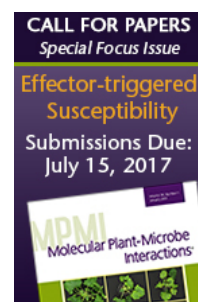
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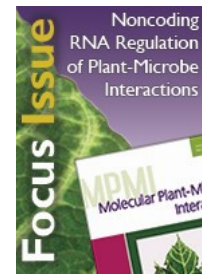
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isolates were submitted to GenBank (*P. megasperma*, accession no. KX370824 and KX370823 for ITS and COI, respectively; *P. multivora*, KX370826 and KX370825 for ITS and COI, respectively). ITS fragments had a 100% similarity with reference isolates of *P. megasperma* (GU259356) and *P. multivora* (KU146526), respectively. COI amplicons of mulberry isolates of two species had 99% similarity with reference isolates of *P. megasperma* (HQ261362) and *P. multivora* (HQ708344) retrieved from GenBank. On the basis of morphological and molecular characters, the species were identified as *P. megasperma* s.s. and *P. multivora*. Pathogenicity of two representative isolates (one per species, separately and in combination) was determined by a soil infestation test using inoculum produced on kernels and 10 18-month-old white mulberry rooted cuttings for each isolate (Salamone et al. 2011). Ten rooted cuttings were used as a control. Cuttings were grown in a greenhouse (20 to 32°C). In combination trials, soil was infested with a mixture of inoculum of both isolates. Within 40 days, all cuttings in infested soil showed leaf chlorosis, wilting, and root rot. Controls remained healthy. The two *Phytophthora* species were reisolated solely from inoculated roots, thus fulfilling Koch's postulates. Presently, the economic significance of this disease is limited as silk production in Italy has been dramatically reduced. However, to our knowledge, this is the first report of *Phytophthora* root rot on *Morus* species worldwide.



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