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October 2016

ISSN: 0191-2917
e-ISSN: 1943-7692

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Editor-in-Chief: Alison E. Robertson

Published by The American Phytopathological Society

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October 2016, Volume 100, Number 10

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<https://doi.org/10.1094/PDIS-03-16-0405-PDN>

DISEASE NOTES

First Report of Bark Canker Disease of Poplar Caused by *Lonsdalea quercina* subsp. *populi* in Spain

I. M. Berruete, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), 50059 Zaragoza, Spain; **M. A. Cambra** and **R. Collados**, Centro de Sanidad y Certificación Vegetal (CSCV), 50059 Zaragoza, Spain; **A. Monterde** and **M. M. López**, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113, Moncada, Valencia, Spain; **J. Cubero**, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), 28040 Madrid, Spain; and **A. Palacio-Bielsa**, Unidad de Sanidad Vegetal, Centro de Investigación y Tecnología Agroalimentaria de Aragón. Instituto Agroalimentario de Aragón-IA2 (CITA-Universidad de Zaragoza), 50059 Zaragoza, Spain.

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Symptoms of a bacterial disease were observed in nine plantations of hybrid poplar clones (*Populus* × *interamericana* 'Beaupré,' and *Populus* × *euramericana* 'I-214' and 'MC') in five localities of Castilla y León and Aragón (north and northeastern Spain, respectively) in summer 2002, 2014, and 2015. Affected trees were from 9 to 26 years old and disease incidence was up to 30% in some poplar stands. The bark of symptomatic trees was vertically cracked and copious white frothy fluid and creamy slime was observed. Some severely affected trees even died after few years. Isolations from exudates on King's B medium yielded colonies light cream colored, round, slightly convex, and not fluorescent under UV light. A selection of 10 purified isolates was further characterized and compared with the reference strain DSM25466^T of *Lonsdalea quercina* subsp. *populi* (Tóth et al. 2013). All bacterial isolates were Gram-negative, facultative anaerobic, produced levan positive colonies, and were esculin hydrolysis positive, but negative for oxidase, urease, and tobacco hypersensitivity. Results in API 20E tests, incubated at 25 and 37°C, showed that biochemical characteristics of the studied strains were consistent with those described for *L. quercina* subsp. *populi*. PCR amplification using specific primers for *L. quercina* LqfF/LqfR and LqgF/LqgR (Shang et al. 2015) resulted in the expected 382- and 286-bp amplicons, respectively. Partial 16S rDNA sequencing was used for identification of 10 strains after amplification (Martinez-Murcia et al. 1992). Sequences were aligned and compared with those available in the GenBank database for species of the genera *Lonsdalea* and *Brenneria* and other phylogenetically related species. The program MEGA version 6.06 (Tamura et al. 2013) was used to construct a dendrogram using the maximum-likelihood method based on p-distance or the Tamura-Nei models. Results of 16S rDNA sequencing showed 99.8 to 100% sequence identity to the sequence of the

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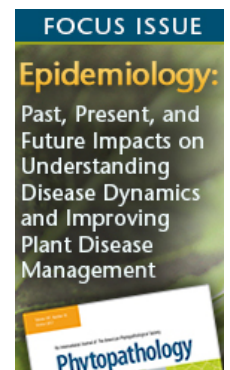
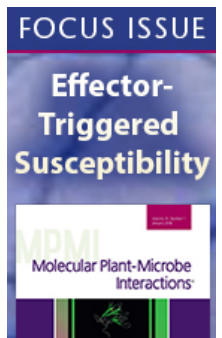
Issue Date: 14 Sep 2016

Published: 21 Jul 2016

First Look: 10 May 2016

Accepted: 3 May 2016

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strain DSM25466^T of *L. quercina* subsp. *populi* obtained in this work, or NY011 and NY041 from the database. All sequences obtained were submitted to GenBank under accession nos. KU531470 to KU531480. Pathogenicity tests were carried out on excised segments of poplar stems (*P. × euramericana* 'I-214') inoculated with bacterial suspensions (10⁷ CFU/ml) (Li et al. 2014). Three stem segments were inoculated per bacterial strain and after 4 to 5 days incubation at 28°C, typical symptoms observed in the field were reproduced in 100% of the inoculated stems, whereas no symptoms were observed on negative controls. Similar bacteria were reisolated from lesions of inoculated stems and resulting colonies were confirmed by biochemical tests and PCR. The recently described subspecies *L. quercina* subsp. *populi* had been previously reported in Hungary (Tóth et al. 2013) and China (Li et al. 2014). To our knowledge, this is the first report of this bacterium causing bark canker disease of poplar in Spain and further surveys will help to assess its precise distribution. The disease could have a potential significant economic impact on susceptible poplar clones.



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Yong Li, Han Xue, Li-min Guo, András Koltay, Ana Palacio-Bielsa, Jupu Chang, Shoujiang Xie, and Xuqi Yang
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