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DISEASE NOTES

First Report of *Lasiodiplodia viticola*, *Spencermartinsia viticola* and *Diplodia intermedia* Associated With *Vitis vinifera* Grapevine Decline in French Vineyards

G. Comont, **V. Mayet**, and **M. F. Corio-Costet**, INRA, UMR1065 SAVE (Santé et Agroécologie du VignoblE), ISVV, CS 20032, 33882 Villenave d'Ornon Cedex, France.

ABSTRACT

A wide sample campaign involving external symptoms of grapevine decline (Esca/Black Dead Arm) was carried out on *Vitis vinifera* grapevines in 21 different French vineyards in 7 wine producing regions (Alsace, Bordeaux, Burgundy, Champagne, Cognac, Jura, and Languedoc). The sampling was carried out from June to August on different French grapevine varieties (Gewurztraminer, Cabernet-Sauvignon, Auxerrois, Chardonnay, Pinot Meunier, Ugni-Blanc, Pinot noir, Sauvignon blanc). Fungi were isolated from asymptomatic wood, from the border of the necrotic area in wood, or from bark. Small pieces of tissue were placed onto malt extract agar (MEA) and incubated at 25°C. After 3 to 4 days, isolates with mycelial features of Botryosphaeriaceae were transferred to new MEA. More than 600 isolates were collected. Identification of the isolates was based on morphological comparisons along with DNA analyses and with sequences of previously identified isolates. For the first time in French vineyards, three species were identified in grapevines: *Diplodia intermedia*, *Lasiodiplodia viticola*, and *Spencermartinsia viticola*. All isolates described here were molecularly identified with partial sequencing of 28S rDNA, partial sequencing of the 5′ end of the β-tubulin gene, partial sequencing of the EF1-alpha gene, and sequencing of the rDNA internal transcribed spacer region (ITS). All DNA sequences of *D. intermedia*, *S. viticola*, and *L. viticola* showed 99 to 100% homology with GenBank and those obtained with reference species obtained from the Centraalbureau voor Schimmelcultures (CBS): *D. intermedia* (CBS124462), *L. viticola* (CBS122313), and *S. viticola* (CBS 117009). Sequences of each fragment were deposited in GenBank with accessions: *L. viticola* (KP699099, KP699095, KX011349, KP699091), *D. intermedia* (KT595691, KT595692, KX151721, KT595693), *S. viticola* (KT595696, KT595694, KX098285, KT595695) for LSU, ITS, EF1-α, and β-tubulin genes, respectively. Morphological criteria were also measured to compare with species described by Phillips et al. (2013) and with reference species from the CBS. All measurements made for the three species were comparable to those obtained under the same conditions for reference species. Pathogenicity of these isolates was tested on stems of 2-month-old rooted plants of ungrafted cv. Cabernet Sauvignon with 10 to 12 leaves (20 replicates per isolate) and inoculated with an agar plug after drilling. Plants inoculated with only MEA were used as negative controls. All plants were maintained in the greenhouse for 3 months (20 to 28°C), cuttings were sacrificed, and the length of canker and internal necrosis was measured. The

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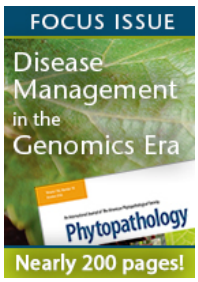

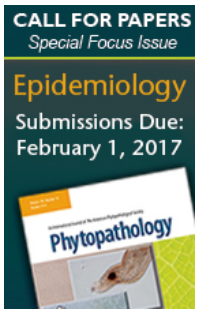

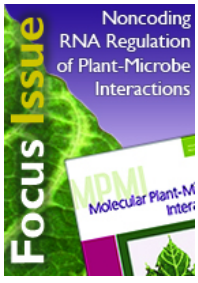

canker and internal necrosis was long for isolates of *L. viticola* (respectively 1.6 and 0.7 cm, $P \leq 0.05$), shorter for isolates of *D. intermedia* (0.3 cm, $P \leq 0.05$), and very short and not significantly different to the control for the isolate of *S. viticola* (respectively 0 and 0.1 cm). All isolates were reisolated from internal brown streaking lesions of inoculated plants. Growth rates of isolates on MEA were also assessed. A 5 mm plug of subculture of each isolate was deposited on three petri dishes. Mycelial growth was measured every day and all growth rates were significantly different ($P \leq 0.05$). At 28°C, isolates of *L. viticola* exhibited the highest growth rate (34 mm/day), followed by *D. intermedia* isolates (12 mm/day), and isolates of *S. viticola* (5 mm/day). Our results combining different tests (length of necroses in plants and growth rate) enabled us to classify the aggressiveness of these new species and groups, isolated in grapevine wood in descending order as *L. viticola*, *D. intermedia*, and *S. viticola.* It should be noted that isolates of *L. viticola* came from a vineyard particularly affected by wood diseases. To our knowledge, this is the first report of *L. viticola* causing necrosis and canker in grapevines in France. For the first time, the species *D. intermedia* and *S. viticola* have been identified in grapevines in France, but they do not appear to be very aggressive.

Reference:

Section: *Choose*

Phillips, A. J. L., et al. 2013. Stud. Mycol. 76:51. 10.3114/sim0021 [CrossRef] [ISI]

Cited by

Life traits of four Botryosphaeriaceae species and molecular responses of different grapevine cultivars or hybrids
A. Bellée, G. Comont, A. Nivault, E. Abou-Mansour, C. Coppin, M. C. Dufour, and M. F. Corio-Costet
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