



DETAILS

RELATIONS



Plant Disease

Volume 101, Issue 8

August 2017

Pages 1345-1561

ARTICLE

A Method to Detect and Quantify *Eutypa lata* and *Diplodia seriata*-Complex DNA in Grapevine Pruning Wounds[View article page](#)Jérôme Pouzoulet, Philippe E. Rolshausen, Marco Schiavon, Sebastiaan Bol, Renaud ... [See all authors](#)

CITE

© 2017 The American Phytopathological Society

<https://doi.org/10.1094/PDIS-03-17-0362-RE>

Publisher Plant Disease

ISSN 0191-2917

Print August 2017

Accepted May 07, 2017

Pages 1470 - 1480

e-Xtra*

A Method to Detect and Quantify *Eutypa lata* and *Diplodia seriata*-Complex DNA in Grapevine Pruning Wounds

Jérôme Pouzoulet, Physiologie Pathologie et Génétique Végétales (PPGV), Université de Toulouse, INP-PURPAN, Toulouse, France; Department of Botany and Plant Sciences, University of California, Riverside; and Loire Viti Vini Distribution (LVVD), Mozé sur Louet, France; **Philippe E. Rolshausen**, **Marco Schiavon**, and **Sebastiaan Bol**, Department of Botany and Plant Sciences, University of California, Riverside; **Renaud Travadon** and **Daniel P. Lawrence**, Department of Plant Pathology, University of California, Davis; **Kendra Baumgartner**, USDA-ARS, Crops Pathology and Genetics Research Unit, Davis; **Vanessa E. Ashworth**, Department of Botany and Plant Sciences, University of California, Riverside; **Gwénaëlle Comont** and **Marie-France Corio-Costet**, INRA, UMR1065 SAVE, ISVV, Villenave d'Ornon, France; **Romain J. G. Pierron**, Physiologie Pathologie et Génétique Végétales (PPGV), Université de Toulouse, INP-PURPAN, Toulouse, France; **Xavier Besson**, Loire Viti Vini Distribution (LVVD), Mozé sur Louet, France; and **Alban Jacques**, Physiologie Pathologie et Génétique Végétales (PPGV), Université de Toulouse, INP-PURPAN, Toulouse, France

Abstract

Trunk diseases are factors that limit sustainability of vineyards worldwide. Botryosphaeria and Eutypa diebacks are caused by several fungi belonging to the Botryosphaeriaceae and Diatrypaceae, respectively, with *Diplodia seriata* and *Eutypa lata* being two of the most common species. Previous information indicated that the traditional isolation method used to detect these pathogens from plant samples could underestimate their incidence levels. In the present study, we designed two sets of primers that target the β -tubulin gene and that are amenable for quantitative real-time PCR (qPCR) Sybr-Green assays for the detection and quantification of *D. seriata*-complex (DseCQF/R) and *E. lata* (ElQF/R) DNA. The design of a species-specific assay was achieved for *E. lata*. For *D. seriata*, a species-specific assay could not be designed. The low interspecific diversity across β -tubulin genes resulted in an assay that could not discriminate *D. seriata* from some closely related species either not yet reported or

presenting a low prevalence on grapevine, such as *D. intermedia*. We validated our technique on grapevine spur samples naturally and artificially infected with *D. seriata* and *E. lata* during the dormant season. Experimental grapevines were located in two counties of northern California where the incidence of both pathogens was previously reported. The qPCR assays revealed that a high frequency of pruning wound infections (65%) was achieved naturally by *E. lata*, while low infection frequency (less than 5%) was observed using the reisolation method. For *D. seriata*-complex, low (5%) to no natural infection frequencies were observed by the qPCR and the reisolation method, respectively. These results also provided evidence that our qPCR detection methods were more sensitive to assess the incidence of *E. lata* and *D. seriata*-complex in plant samples, than traditional isolation techniques. Benefits of molecular methods for the detection of canker pathogens in the field under natural conditions are discussed.

Cultivated grapevine, *Vitis vinifera* L. ssp. *sativa*, is susceptible to a broad diversity of wood-colonizing fungi, including those causing cankers (Bertsch et al. 2013). These diseases are particularly detrimental to grape production by reducing fruit quality, cumulative yield, and vineyard lifetime (Bertsch et al. 2013). Most fungal trunk pathogens disperse by the mean of spores that are airborne after rain (Gubler et al. 2005; Úrbez-Torres et al. 2010a). Trunk pathogens take advantage of either natural openings (e.g., cracks) or pruning wounds to infect plants (Bertsch et al. 2013). Because pruning is a necessary practice to manipulate yields and fruit quality at harvest, trunk diseases are chronic issues of orchards and vineyards. There is

Corresponding authors: Alban Jacques, alban.jacques@purpan.fr; and Jérôme Pouzoulet, jerome.pouzoulet@gmail.com

This project was funded by l'Agence Nationale de la Recherche et de la Technologie (ANRT) (CIFRE grant #129/2009), the American Vineyard Foundation, and the USDA (National Institute of Food and Agriculture, Specialty Crop Research Initiative, grant #2012-51181-19954).

JP designed and performed the experiments, analyzed the data and wrote the manuscript; PR designed and performed the experiments, analyzed the data, supervised and complement the writing; MS analyzed the data and complement the writing; SB and VA performed the experiments and proofread the manuscript, RT, DL, KB, GC and MC performed the experiments; RP contributed to the writing; XB conceived the original project and research plans;

no commercial curative treatment currently available to manage trunk diseases. Consequently, the main management strategies consist in preventing pruning wounds from infections using physical, chemical, or biological protectants (Díaz and Latorre 2013; Rolshausen et al. 2010; Rolshausen and Gubler 2005; Sosnowski et al. 2008).

Two major taxonomic groups of ascomycete are responsible for trunk diseases on grapevine worldwide; the Botryosphaeriaceae (associated with Botryosphaeria dieback) and the Diatrypaceae (associated with Eutypa dieback) (Bertsch et al. 2013; Rolshausen et al. 2014; Trouillas and Gubler, 2010; Úrbez-Torres 2011). Previous epidemiological and pathogenicity studies showed that a large number of species belonging to these two families can be considered pathogenic to grapevine (Rolshausen et al. 2006, 2014; Úrbez-Torres 2011) and to a wide range of trees and shrubs (Chen et al. 2014; Choudhury et al. 2014; Jiao et al. 2014; Munkvold and Marois 1994; Quaglia et al. 2014; Rolshausen et al. 2006; Trouillas et al. 2011; van Niekerk et al. 2006). Within the Diatrypaceae, *Eutypa lata* (Pers.:Fr.) Tul. & C. Tul. (syn. *E. armeniacae* Hansf. and Carter) is the most widespread geographically in grape growing regions worldwide and causes significant impact on grape productivity (Siebert 2001; Travadon et al. 2012). Within the Botryosphaeriaceae, *Diplodia seriata* De Not. (syn. *Botryosphaeria obtusa* (Schwein.) Shoemaker) prevails in many different climates and in several grape growing areas around the globe, although it does not appear to be the most virulent species within this group (Úrbez-Torres 2011).

