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RESEARCH NOTE

Susceptibility of different grapevine (*Vitis vinifera* L.) cultivars to *Diplodia seriata* and *Diplodia mutila*

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Abstract

M. Ramírez, L.M. Pérez, and J. Montealegre. 2018. Susceptibility of different grapevine (*Vitis vinifera* L.) cultivars to *Diplodia seriata* and *Diplodia mutila*. Cien. Inv. Agr. XX: 93-98. Canes obtained from one-year-old stems of the grapevine cultivars Cabernet Franc, Malbec, Merlot, Sauvignon Blanc and Syrah (all non-grafted) were used as models to test their susceptibility to *Diplodia seriata* and *Diplodia mutila*. The results showed that the mean length of lesions caused by *D. mutila* was 3.2 times larger than that produced by *D. seriata*. In addition, the Cabernet Franc and Syrah cultivars were the most susceptible to *D. mutila*, while Merlot and Malbec were the least susceptible. No significant differences were observed between cultivars inoculated with *D. seriata*. The results are discussed in terms of the susceptibility of the different grapevine cultivars to both pathogens.

Keywords: Botryosphaeriaceae, botryosphaeria die-back, grapevine, susceptibility.

Introduction

Wine and table grapes (*Vitis vinifera* L.) are important crops covering diverse climate zones in Chile where they are cultivated on an estimated 182,000 ha, of which approximately 130,400 ha are managed for wine grape production (ODEPA, 2015). Botryosphaeria dieback is one of the predominant grapevine trunk diseases worldwide (Bertch *et al.*, 2013). The frequency of symptoms associated with fungal attack on grapevine trunk wood diseases increased significantly worldwide during the last decade, including in Chile. Fungal species belonging

to the Botryosphaeriaceae are responsible for the death of part of the plant, reducing the productivity and profitability of this crop (Gubler *et al.*, 2010). Both *D. seriata* and *D. mutila* are associated with the Botryosphaeria die-back in Chile (Díaz *et al.*, 2013; Morales *et al.*, 2012). These fungi have been detected in table grape vineyards where no differences were observed among the Thompson Seedless, Red Globe and Flame Seedless cultivars, which were equally susceptible to infection by *D. mutila*, *D. seriata* and *Spencermartinsia viticola* (Morales *et al.*, 2012). In addition, experiments carried out with axenic plants, trunks and shoots of the Carmenère cv. showed that there were no differences in pathogenicity between *D. mutila* and *D. seriata* (Díaz *et al.*, 2013). However, the susceptibility of different wine grape cultivars

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to *D. seriata* and *D. mutila* has not been reported to date. Thus, the objective of this work was to compare the susceptibility of the grapevine cultivars Cabernet Franc, Malbec, Merlot, Sauvignon Blanc and Syrah (all nongrafted) to *D. seriata* and *D. mutila*, using detached grapevine canes as a model system.

Materials and methods

Fungal isolates

Diplodia seriata (strain 1009) and *D. mutila* (strain 894), both pathogenic to grapevine plants and well characterized using morphological and molecular tests (Dr. Josep Armengol, Instituto Agroforestal Mediterraneo, Universidad Politécnica de Valencia, España, personal communication), were obtained from a microbiology laboratory fungal collection (Departamento de Sanidad Vegetal, Universidad de Chile). Both strains were cultured in potato-dextrose-agar (PDA, Difco) at 25 °C in the dark for seven days (Úrbez-Torres and Gubler, 2009).

Plant material

One-year-old healthy canes from the wine grapevine cultivars Cabernet Franc, Malbec, Merlot, Sauvignon Blanc and Syrah (all non-grafted) were collected from local vineyards in the O'Higgins and Metropolitan Regions, Chile. The canes were cut from grapevine plants two weeks before pruning (June 2014 and 2016). Then, the canes were cut in 20 cm pieces and were maintained at 5 °C and 20% humidity for two months before use.

Trials

In vivo trials were performed using detached canes previously disinfected for three minutes with 70% ethanol and washed with sterile distilled water. A V-shaped wound was made with a scalpel in the internodes region. Mycelial plugs (6 mm diameter) obtained from pure cultures of *D. seriata* or *D. mutila*

were used in inoculations, and PDA plugs were used for controls. The inoculated wounds were covered with Parafilm, and the detached canes were placed inside plastic boxes with a translucent cover. They were incubated in the dark at 25 °C and 95% humidity. Visual observation of the canes was performed weekly to determine the disease's development and to prevent its progress into the nodes. Each independent trial (2014 and 2016) consisted of 10 replicates per each of the five grapevine cultivars tested and was repeated twice. The length of necrotic lesions observed at the internode region after six weeks of incubation was measured on both sides of the inoculation point with calipers after careful removal of the bark (Savocchia *et al.*, 2007; Úrbez-Torres and Gubler, 2009). After the length measurement, transversal sections of canes were obtained to visualize the lesion. The results were analyzed by ANOVA followed by Tukey's test ($p < 0.05$).

Results

Necrosis was observed in the internodes region of all the detached grapevine canes inoculated with *D. seriata* (strain 1009) or with *D. mutila* (strain 894). Additionally, a V-shaped lesion (a typical symptom of *Botryosphaeria* die-back) was observed in the transversal sections of the canes, an observation that was used only to confirm that necrosis was a consequence of the inoculation with *D. seriata* or with *D. mutila*. Oxidation of the tissue was observed in the control inoculated canes as a consequence of wounding. An example of the observations is shown in Figure 1.

The mean length of lesions produced by *D. seriata* or by *D. mutila* in grapevine canes is shown in Figure 2. Significant differences in lesion length were observed among the different grapevine cultivars inoculated with *D. mutila*, while no significant differences were observed among the same cultivars inoculated with *D. seriata*, except for the Malbec cultivar, where significant differences were observed with the controls. The length of oxidation due to mechanical damage

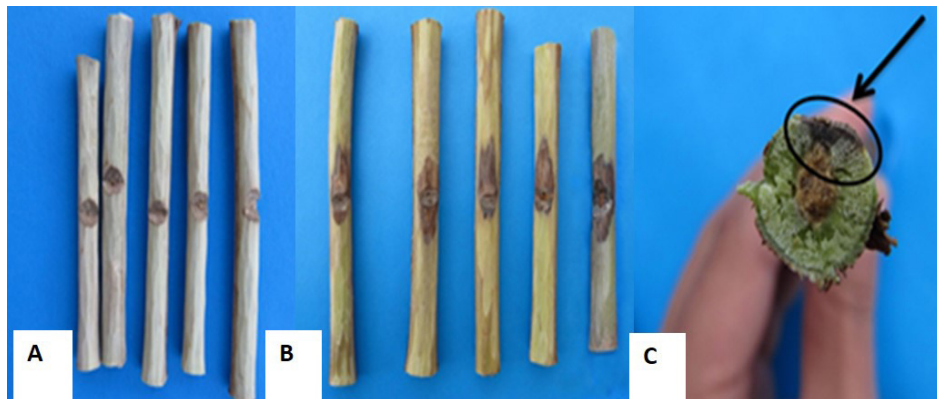


Figure 1. Example of detached canes from different non-grafted cultivars after six weeks incubation in darkness at 25°C and 95% humidity. A) Control canes, B) Canes inoculated with *Diplodia seriata* (strain 894) and C) V-shaped lesion in transversal section of a cane indicated by the arrow and a circle. Grapevine cultivars (left to right): Cabernet Franc, Syrah, Sauvignon Blanc, Malbec and Merlot.

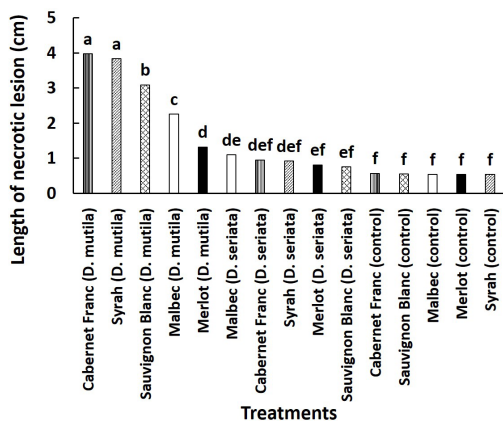


Figure 2. Length of necrotic lesions (cm) caused by *Diplodia mutila* (strain 1009) and by *Diplodia seriata* (strain 894) in detached canes from different non-grafted grapevine cultivars (Cabernet Franc, Syrah, Sauvignon Blanc, Malbec and Merlot). Different letters indicate significant differences after ANOVA followed by Tukey’s test ($p < 0.05$).

did not exceed 10.6% of the length of lesions of inoculated ones.

In addition, the lesions produced by *D. seriata* were shorter than those produced by *D. mutila*, reflected in the corresponding ratios (Table 1).

Discussion

Detached canes from different grapevine cultivars (Cabernet Franc, Malbec, Merlot, Sauvignon Blanc

and Syrah) showed differences in their susceptibility to *D. mutila* and *D. seriata* (Table 1). Cabernet Franc and Syrah were the most susceptible, and Merlot and Malbec were the least susceptible. The difference in susceptibility could be related to the vessel dimension, as was previously established when comparing the susceptibility to Esca of the wine grape cultivars Merlot and Cabernet Sauvignon and the table grape cultivar Thompson Seedless (Pozoulet *et al.*, 2014). Those authors showed that the small size of the vessels in the Malbec cv. was related to their decreased susceptibility to Esca, while the Thompson Seedless cultivar showed the largest vessel size. Similar differences were observed by Travadon *et al.* (2013), showing that the Thompson Seedless cultivar was the most susceptible to infection by *Neofusicoccum parvum*, while the least susceptible were the Merlot and Concorde cultivars. Morphological analysis (Pozoulet *et al.*, 2014) showed that vessels from the Merlot cultivar were smaller than those of the Thompson Seedless cultivar. In addition, studies conducted using two-month-old plants of Cabernet Sauvignon, Merlot and Ugni Blanc with 10-12 true leaves showed significant differences in the mean length of the internal necrosis and external canker between the cultivars above mentioned, inoculated with *D. mutila* and *D. seriata* (Bellée *et al.*, 2017). Thus, it appears that a correlation exists between vessel size and susceptibility to Esca or other fungi causing grapevine trunk diseases,

Table 1. Length of lesions produced by *Diplodia seriata* (strain 1009) and by *Diplodia mutila* (strain 894) in detached canes of different non-grafted grapevine cultivars.

	Length of lesion produced by <i>D. seriata</i> ^a (cm)	Length of lesion produced by <i>D. mutila</i> ^a (cm)	Ratio ^b <i>D. mutila</i> / <i>D. seriata</i>	
Merlot	0.27	0.78	2.89	a
Malbec	0.56	1.72	3.07	a
Cabernet Franc	0.38	3.41	8.97	b
Syrah	0.38	3.30	8.68	b
Sauvignon Blanc	0.20	2.54	12.70	c

^aValues correspond to the mean total length of the lesion in treatments minus the total length of the lesion in controls.

^bDifferent letters indicate significant differences after ANOVA followed by Tukey's test ($p < 0.05$).

which could explain the differences observed in the susceptibility to *D. mutila*, among the canes from different grapevine cultivars tested, as well as the difference observed for the Malbec cultivar in response to inoculation with *D. seriata* (Figure 2).

It should also be mentioned that the isohydric characteristic of a specific grapevine cv. could determine a similar susceptibility against different pathogens (Jara-Rojas *et al.*, 2015), as it has been described for the Carménère cultivar using axenic plants, trunks and shoots inoculated with *D. mutila* and *D. seriata* (Díaz *et al.*, 2013). Our results were opposed to those of the Carménère cultivar because they showed different susceptibility to *D. seriata* and *D. mutila* (Table 1), and are consistent with those of the Syrah cultivar, that has been often considered as anisohydric yet shows near-isohydric behavior (Pou *et al.*, 2012). Furthermore, the fact that all the canes used in this work were obtained from non-grafted grapevine cultivars could have affected the difference in susceptibility to *D. seriata* and *D. mutila* (Table 1). Our results also contradict the results of similar susceptibility to *D. mutila* found in vine canes from table grapes cultivars (Flame Seedless, Red Globe and Thompson Seedless), which were all grafted on Harmony (Ramírez, M. personal communication), and those achieved by Morales *et al.* (2012) in which the same cultivars were found equally susceptible to infection by *D. mutila*, *D. seriata* and *S. viticola* at the field level. Nevertheless, it cannot be discarded that the table grape cultivars used in our experiments could

share similar hydraulic properties and similarly sized vessels (Pouzoulet *et al.*, 2014).

The finding of no significant differences in susceptibility to *D. seriata* observed among the Cabernet Franc, Malbec, Merlot, Sauvignon Blanc and Syrah cultivars (Figure 2) has agreed with previous studies on the susceptibility of different grapevine rootstocks to a single pathogen or disease, where both different and similar susceptibility to certain pathogens have been observed. For example, material from the Sonoma Grapevine Nursery in California showed differences between the different rootstocks analyzed in relation to susceptibility to *Phaeoconiella chlamydospora* and *Phaeoacremonium* spp. (Eskalen *et al.*, 2001). However, other reports show that depending on the rootstocks used, no susceptibility differences were observed to certain pathogens, as was reported for the infection status of different viruses (Komar *et al.*, 2010) in three clones of the *Vitis vinifera* cultivar Savagnin Rose and rootstock genotype (*Vitis rupestris*, Kober 5BB, or 16149 Couderc). Our results, using detached grapevine canes from wine grape cultivars as a model, are in accordance with those already reported where different and similar susceptibility to certain pathogens could be observed and where no generalization could be deduced.

Comparison of susceptibility to *D. mutila* and *D. seriata* of the different rootstocks tested showed that the majority were more susceptible to *D. mutila*. This can probably be explained due to

the *D. mutila* isolate used in this study, which appears to be more pathogenic than the *D. seriata* one (Figure 1). This agrees with previous reports on Chardonnay grapevines (Pitt *et al.*, 2013), as opposed to the no differences in pathogenicity observed among these pathogens when infecting the table grape cultivars (Morales *et al.*, 2012). Moreover, it is also important to consider that the pathogenicity of a certain pathogen on the same grapevine cultivars could depend on the specific isolate tested, as has been described by Yan *et al.*, (2013).

Therefore, we conclude that different aspects come together in terms of grapevine susceptibility to *D. mutila* and *D. seriata*, and the rootstock and vessel size could partially explain the results observed. Among the different grapevine cultivars tested, Cabernet Franc appeared to be more susceptible to *D. mutila* than to *D. seriata*, Syrah cultivars were the most susceptible to *D. mutila* and Merlot and Malbec were the least susceptible. Field experiments using the same grapevine cultivars will be necessary in order to complement our results.

Resumen

M. Ramírez, L.M. Pérez, y J.R. Montealegre. 2018. Susceptibilidad de diferentes cultivares de vid vinífera (*Vitis vinifera* L.) a *Diplodia seriata* y *Diplodia mutila*. Cien. Inv. Agr. XX: 93-98. La susceptibilidad de los cultivares de vid vinífera Cabernet Franc, Malbec, Merlot, Sauvignon Blanc and Syrah (todos patrón Franco) a *Diplodia seriata* y *Diplodia mutila* se analizó usando estacas como modelo. Las estacas se obtuvieron de sarmientos de un año de edad. Los resultados mostraron que la longitud promedio de las lesiones producidas por *D. mutila* era 3,2 veces mayor que la producida por *D. seriata*. Al comparar las lesiones producidas por *D. mutila* en los diferentes cultivares, se encontró que Cabernet Franc y Syrah fueron los más susceptibles, mientras que Merlot y Malbec fueron los menos susceptibles. Por otra parte, no se observaron diferencias significativas en el tamaño de las lesiones producidas por *D. seriata* en los diferentes cultivares. Los resultados se discuten con relación a la susceptibilidad de diferentes cultivares de vid vinífera al daño producido por ambos patógenos.

Palabras clave: Botryosphaeriaceae, brazo muerto de la vid, susceptibilidad, vid.

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