Pathogenicity of *Diatrypaceae* Species in Grapevines in California

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ABSTRACT

Trouillas, F. P., and Gubler, W. D. 2010. Pathogenicity of *Diatrypaceae* species in grapevines in California. Plant Dis. 94:867-872.

Diatrypaceous fungi have been isolated from cankered wood of grapevines (*Vitis vinifera*) in California. However, the pathogenicity of these fungi and their potential to cause diseases in grapevine was unknown. A series of pathogenicity tests were conducted to determine if these fungi were grapevine pathogens. In all experiments, species of the family *Diatrypaceae* were re-isolated from the margins of developing lesions, although frequency of re-isolation and average length of vascular discolorations varied among isolates. *Cryptovalsa ampelina*, *Diatrype stigma*, and *Eutypa leptoplaca* were capable of colonizing dormant canes and causing vascular necrosis. *Cryptosphaeria pullmanensis*, *C. ampelina*, *D. stigma*, *Diatrype whitmanensis*, and *E. leptoplaca* infected and caused lesions in green shoots of the new vegetative growth. *Diatrype oregonensis* and *Diatrypella verrucaeformis* isolates did not produce lesions that were significantly different from those produced in the control shoots and canes. This suggests that *D. oregonensis* and *D. verrucaeformis* fungi may be saprophytic rather than pathogenic to grapevine.

Grapevine canker diseases in California are responsible for severe economic losses to the grapevine (*Vitis vinifera* L.) industry, estimated at up to US\$260 million per annum (15). Among them, *Eutypa dieback*, caused by *Eutypa lata* (Pers.:Fr.) Tul. & C. Tul. (Syn.: *E. armeniacae* Hansf. & Carter) (*Diatrypaceae*), constitutes one of the most important canker diseases in California, causing death of arms, cordons, and eventually entire grapevines (9). Several species of fungi belonging to the family *Botryosphaeriaceae* also have been isolated from grapevine cankers in California, and their pathogenicity has been recently documented (20,21). Furthermore, 11 species of the family *Diatrypaceae* in addition to *E. lata* have been reported from diseased wood of grapevines in California (18). The occurrence of diatrypaceous fungi isolated from the margins of active cankers in grapevine in California suggested their participation in causing canker diseases. However, few studies have been conducted to understand their biology and status as canker pathogens of grapevine. *Eutypa leptoplaca* (Mont.) Rappaz occurs in grapevine in California and is pathogenic to this host (17). Additional diatrypaceous species have been shown to cause disease in cultivated grapevines around the

world. *Cryptovalsa ampelina* (Nitschke) Fuckel was isolated from grapevine canes in South Africa and Australia, and its pathogenicity to grapevine was established (10). *C. ampelina* also was reported from *V. vinifera* in northeastern Spain, and its pathogenicity to grapevine was confirmed, although the authors suggested moderate virulence for this fungus (8). Recently, Úrbez-Torres et al. (19) proved the pathogenicity to grapevine of the diatrypaceous fungi *Eutypella vitis* (Schwein.:Fr.) Ellis & Everh. (Syn.: *E. aequilinearis* (Schwein.:Fr.) Starb.) and *Diatrypella* sp., although the authors suggested a low virulence for these fungi.

Despite the abundance of *Diatrypaceae* species in grapevine cankers in California, the status of these fungi as pathogens remained unknown. The objective of the present study was to determine the pathogenicity in grapevine of the most common diatrypaceous species found in California.

MATERIALS AND METHODS

Twenty-eight isolates of diatrypaceous fungi were used in this study. Isolates were collected from diseased grapevine wood and native tree species in six California counties (Table 1). Fungal isolates were obtained from ascospores as well as from hyphae in infected grapevine wood (18). Pure cultures were obtained by isolation of hyphal tips. Species were identified morphologically and separated using phylogenetic analyses of the complete sequence of the internal transcribed spacer (ITS) of the rDNA and β -tubulin gene (18).

Three pathogenicity tests were conducted in commercial vineyards on *V. vinifera* grapevines. Inoculations were done in green shoots as well as in dormant shoots of mature grapevines using an asco-

spore suspension or mycelium of the various diatrypaceous fungi.

Pruning wound inoculation. An initial pathogenicity test was conducted using 10-year-old Merlot grapevines in a vineyard in Napa County, CA. In this trial, one 1-year-old cane per grapevine and 10 grapevines per fungal inoculation were used. Fresh pruning wounds were made above the node at the tip of approximately eight buds on 1-year-old dormant shoots in December 2001. Shoots were inoculated following pruning by applying 10 μ l of an ascospore suspension (200 ascospores μ l⁻¹) directly onto the surface of fresh pruning wounds. Ascospores were obtained from perithecia that were sliced opened with a blade and wetted with sterile water (18). Treatments included inoculations of *Eutypa leptoplaca* isolates DRSN200 and DCA400, *E. lata* isolates DCA700 and DCA200, *Cryptovalsa ampelina* isolates DHB600 and DCA1100, *Diatrype oregonensis* (Wehm.) Rappaz isolates DHB200 and DCA600, and *Diatrypella verrucaeformis* (Ehrh.:Fr.) Nitschke isolate DHB500.

In the absence of perithecia, isolate DCHR5 of *Diatrype stigma* (Hoffm.:Fr.) Fr. was inoculated onto shoots with mycelium placed on the surface of fresh pruning wounds. Mycelium was obtained by scraping the margin of a 10-day-old colony cultured on potato dextrose agar (PDA) amended with 100 ppm tetracycline (PDA-tet), grinding the scrapings using an Eppendorf tube and pestle, and suspending in sterile water. There was no control inoculation in this experiment.

Twelve months after inoculation, the percentage of recovery (positive infection) was estimated for each isolate after culturing of necrotic tissues on PDA-tet. Tissue samples for isolations were taken from along the length of vascular streaking to estimate the extent of colonization by each of the tested isolates. Results are represented as the average distance of recovery from the inoculation point and along the necrotic lesions. Fragments of necrotic tissue were cultured on PDA-tet, and characteristic mycelial growths of the various *Diatrypaceae* species were transferred to fresh PDA-tet petri dishes in an attempt to obtain clean cultures for identification using colony morphology and growth rate.

Dormant cane inoculation. Another pathogenicity test was performed on mature Shiraz and Ruby Cabernet grapevines at the Kearney Agricultural Center of the University of California in Parlier. Inoculations were made in February 2005 in dormant canes using eight isolates represent-

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*The e-Xtra logo stands for "electronic extra" and indicates that Figures 5 and 6 appear in color online.

Accepted for publication 5 February 2010.

ing five species of *Diatrypaceae* (*E. lata* isolates DCA900 and DCA700, *E. leptoplaca* isolate DCA500, *Diatrype stigma* isolate DCASH200, *C. ampelina* isolates DRO101 and DHB600, and *Diatrypella verrucaeformis* isolates DHB500 and DCH500). Ten shoots per fungal isolate were inoculated. Inoculations were made by wounding canes at internodes 3 or 4 using a knife. An agar plug with fresh mycelium obtained from the margin of a 10-day-old colony was placed on the surface of each wound. Control shoots were inoculated with noncolonized agar plugs. Inoculated wounds were covered with Vaseline and wrapped with Parafilm. After 2 years, shoots were collected from the field for laboratory examination of canker formation, extent of vascular discoloration, and recovery of fungal isolates.

Green shoot inoculation. Ten-year-old Chardonnay grapevines planted at the viticulture experimental station of the University of California, Davis were used in this pathogenicity test. Green shoots were inoculated on 12 June 2006 using 15 isolates representing eight species of *Diatrypaceae* (*Eutypa lata* isolates UCD746St and UCD782St, *E. leptoplaca* isolates DHB800 and DCA300, *Diatrype stigma* isolates DCASH200 and DBEY600, *D. oregonensis* isolates DVIT200 and DBEY300, *Diatrype whitmanensis* J.D. Rogers & Glawe isolates DDES300 and DCA1300, *Cryptosphaeria pullmanensis* Glawe isolates HBPF24 and UCD730SJ, *Cryptovalsa ampelina* isolates UCD779St

and DCA1100, and *Diatrypella* sp. isolate UCD1268So). Inoculations were made at internodes 4 to 6 using a 4-mm cork borer to remove bark and placing an agar plug from the growing margin of an 8-day-old colony directly on the fresh wound. In this experiment, six shoots per fungal isolate were inoculated. Control shoots were inoculated with noncolonized agar plugs. Inoculated wounds were covered with Vaseline and wrapped with Parafilm. Shoots were collected

after 10 weeks for laboratory examination. Fungi obtained from re-isolation were identified based on colony size, shape, color, and growth rate.

Statistical analyses. Statistical analyses were performed using analysis of variance (ANOVA) in SAS (Version 9.1.3; SAS Institute, Cary, NC). Log transformations were performed to reduce heteroscedasticity. Dunnett's *t* test was used to assess significant differences in the extent of vascular discoloration between the control

Fig. 1. Mean of distance of re-isolation from pruning wound 1 year after inoculation of various diatrypaceous isolates in Merlot grapevine shoots ($P < 0.2153$). Inoculations were made in the field using 200- μ l⁻¹ ascospore suspensions. Isolate DCHR5 was mycelial inoculated. Bars represent standard error of the mean.

Table 1. Origin and source of *Diatrypaceae* isolates used in pathogenicity studies

Species	Collection no.	Host	County	ATCC accession no.	ITS ^a rDNA GenBank accession no.
<i>Cryptosphaeria pullmanensis</i>	UCD730SJ	<i>Vitis vinifera</i>	San Joaquín	MYA-4416	GQ293929
<i>C. pullmanensis</i>	HBPF24	<i>Populus fremontii</i>	Sonoma	MYA-4414	GQ293931
<i>Cryptovalsa ampelina</i>	DCA1100	<i>V. vinifera</i>	Napa	MYA-4399	GQ293903
<i>C. ampelina</i>	UCD779St	<i>V. vinifera</i>	Stanislaus	MYA-4400	GQ293907
<i>C. ampelina</i>	DRO101	<i>Prunus armeniaca</i>	Stanislaus		GQ293902
<i>C. ampelina</i>	DHB600	<i>V. vinifera</i>	Sonoma		
<i>Diatrype oregonensis</i>	DBEY300	<i>Umbellularia californica</i>	Yolo		
<i>D. oregonensis</i>	DHB200	<i>V. vinifera</i>	Sonoma	MYA-4412	GQ293937
<i>D. oregonensis</i>	DVIT200	<i>Vitis californica</i>	Yolo		GQ293943
<i>D. oregonensis</i>	DCA600	<i>V. vinifera</i>	Napa		GQ293942
<i>Diatrype stigma</i>	DBEY600	<i>Quercus</i> sp.	Napa		
<i>D. stigma</i>	DCHR5	<i>V. vinifera</i>	Yolo		GQ293945
<i>D. stigma</i>	DCASH200	<i>Quercus</i> sp.	Yolo	MYA-4419	GQ293947
<i>Diatrype whitmanensis</i>	DCA1300	<i>V. vinifera</i>	Napa		
<i>D. whitmanensis</i>	DDES300	<i>Acer macrophyllum</i>	El Dorado		
<i>Diatrypella verrucaeformis</i>	DHB500	<i>V. vinifera</i>	Sonoma	MYA-4407	GQ293925
<i>D. verrucaeformis</i>	DCH500	<i>Quercus</i> sp.	Napa	MYA-4408	GQ293926
<i>Diatrypella</i> sp.	UCD1268So	<i>V. vinifera</i>	Sonoma	MYA-4403	GQ293924
<i>Eutypa lata</i>	UCD746St	<i>V. vinifera</i>	Stanislaus		
<i>E. lata</i>	UCD782St	<i>V. vinifera</i>	Stanislaus		
<i>E. lata</i>	DCA200	<i>A. macrophyllum</i>	Napa		
<i>E. lata</i>	DCA700	<i>V. vinifera</i>	Napa		
<i>E. lata</i>	DCA900	<i>V. vinifera</i>	Napa		GQ293948
<i>Eutypa leptoplaca</i>	DHB800	<i>V. vinifera</i>	Sonoma	MYA-3379	AY684231
<i>E. leptoplaca</i>	DRSN200	<i>V. vinifera</i>	Sonoma		AY684237
<i>E. leptoplaca</i>	DCA300	<i>A. macrophyllum</i>	Napa	MYA-3382	AY684229
<i>E. leptoplaca</i>	DCA400	<i>A. macrophyllum</i>	Napa		
<i>E. leptoplaca</i>	DCA500	<i>U. californica</i>	Napa	MYA-3381	AY684230

^a Internal transcribed spacer.

group mean and the remaining treatment group means.

RESULTS

Pruning wound inoculation. Colonization of dormant grapevine shoot wounds using ascospores was successful for all isolates tested. Fungi obtained from re-isolation were the same as the previously inoculated fungal isolates based on colony morphology and growth rate. Percent recovery was lowest for *Diatrype oregonensis* isolate DCA600 (30%), *C. ampelina* isolate DHB600 (40%), and *D. stigma* isolate DCHR5 (40%). Highest percent recovery was obtained for *E. leptoplaca* isolates DRSN200 and DCA400 (100 and 90%, respectively), *E. lata* isolates DCA200 and DCA700 (100 and 90%, respectively), and *C. ampelina* isolate DCA1100 (80%). *D. oregonensis* isolate DHB200 and *Diatrypella verrucaeformis* isolate DHB500 were recovered from 60 and 70% of the inoculated sites, respectively. The average distance of growth in wood was not significantly different (at $\alpha = 0.05$) among the treatments ($P < 0.2153$) and varied between 21.7 mm (*D. oregonensis* isolate DCA600) and 45.5 mm (*E. leptoplaca* isolate DCA400) (Fig. 1).

Dormant cane inoculation. Results of the 2-year pathogenicity tests are summarized in Figures 2 and 3. In both cultivars, fungal isolates were re-isolated from lesions and identified as being identical to the inoculated isolates. In Shiraz grapevines, best percentages of recovery were found also for *E. leptoplaca* (60%), *D. verrucaeformis* (80%), and *E. lata* (70 and 90%) isolates. Percentage of fungal recovery were lowest for *D. stigma* (20%) and *C. ampelina* (40%). Length of vascular discolorations averaged approximately 39 mm for *D. verrucaeformis* isolates DCH500 and DHB500, 50 mm for *E. leptoplaca* isolate DCA500 and *D. stigma* isolate DCASH200, 50.7 and 59.4 mm for *E. lata* isolates DCA700 and DCA900, respectively, and 47 and 55 mm for *C. ampelina* isolates DHB600 and DRO101, respectively (Fig. 2). Vascular discoloration in the control vines averaged 28.3 mm. ANOVA of the transformed data set showed significant differences among fungal treatment ($P = 0.0129$). The Dunnett's *t* test showed significant differences between treatments with *E. lata* (isolates DCA700 and DCA900) and *C. ampelina* isolate DRO101 from control vines (Fig. 2).

In Ruby Cabernet grapevines, percent fungal recovery from 2-year-old inoculations was highest for *D. verrucaeformis*, *E. lata*, and *E. leptoplaca*, which ranged between 80 and 100%. Percent fungal recovery for *D. stigma* and *C. ampelina* isolate DRO101 was 30%, whereas *C. ampelina* isolate DHB600 was recovered in only 10% of the previously inoculated canes. Extent of vascular discoloration averaged 58.3 mm for *C. ampelina* isolate DRO101,

55.5 mm for *E. leptoplaca* isolate DCA500, 50 and 52.5 mm for *E. lata* isolates DCA900 and DCA700, 50 mm for *D. stigma* isolate DCASH200, approximately 36 mm for *D. verrucaeformis* isolates DCH500 and DHB500, and 33.3 cm for the agar-only inoculated control (Fig. 3). ANOVA of the transformed data set showed highly significant differences (at $\alpha = 0.05$) among the treatments ($P < 0.0004$). The Dunnett's *t* test showed significant differences between treatments with *E. lata* isolate DCA700, *E. leptoplaca* isolate DCA500, *C. ampelina* isolate DRO101, and *D. stigma* isolate DCASH200 from control vines (Fig. 3).

Green shoot inoculation. All fungal isolates were re-isolated from the margins

of developing lesions in the inoculated grapevine shoots. Isolates obtained from re-isolations were identified as being identical to the inoculated isolates. Percentages of fungal recovery ranged between 80 and 100% for all isolates. Extents of vascular discolorations after two and a half months incubation are summarized in Figure 4 and illustrated in Figure 5. Analysis of variance showed significant differences among fungal treatment ($P = 0.0224$). Averages of the extent of vascular discoloration recorded from infections with isolates of *E. lata* isolate UCD782St (26.6 mm), *D. stigma* isolate DBEY600 (25.4 mm), *D. whitmanensis* isolate DDES300 (30.8 mm), and *Cryptosphaeria* isolate HBPF24 (23.8 mm) were significantly different

Fig. 2. Mean of vascular discoloration length 2 years after inoculation of various diatrypeaceous isolates in Shiraz grapevine shoots inoculated in the field using $200\text{-}\mu\text{l}^{-1}$ ascospore suspensions. * Average length of vascular discolorations was significantly different from the agar-only inoculated control according to Dunnett's *t* test ($P = 0.0129$). Bars represent standard error of the mean.

Fig. 3. Mean of vascular discoloration length 2 years after inoculation of various diatrypeaceous isolates in Ruby Cabernet grapevine shoots inoculated in the field using $200\text{-}\mu\text{l}^{-1}$ ascospore suspensions. * Average length of vascular discolorations was significantly different from the agar-only inoculated control according to Dunnett's *t* test ($P < 0.0004$). Bars represent standard error of the mean.

from those measured in the non-inoculated control (8.6 mm) according to Dunnett's *t* test (Fig. 4).

DISCUSSION

Grapevine canker diseases in California have long been attributed mostly to the fungus *E. lata* (9,21). However, other fungal species, particularly in the *Botryosphaeriaceae* family, have recently been associated with these diseases and shown to constitute the main fungi found in cankers in many regions in California (20,21). Additional studies in California have revealed a great diversity of diatrypaceous fungi associated with grapevine canker diseases (14,18). Recently, *E. leptoplaca* was isolated from diseased grapevines in California and its pathogenicity was proven (17).

Identification of *E. lata* has traditionally been conducted based on culture characteristics in agar medium and following isolation of necrotic tissues (1). The majority of fungi in the *Diatrypaceae* grow as white colonies in culture on PDA-tet resembling *E. lata* (Fig. 6), which suggests they may have been misidentified and overlooked in previous studies (18). To date, we have reported 11 species of *Diatrypaceae* from the wood of diseased grapevine in California (18). Fungi in the family *Diatrypaceae* have typically been isolated from necrotic tissue in shoots, at the margins of wedge shaped cankers in cordons and trunks, cankers in spurs, or as fruiting bodies on the surface of decorticated wood or bark of grapevines (18). The occurrence of these fungi on cultivated grapevines has raised questions regarding their role in vineyard ecosystems and the possibility that they may participate in the overall dieback syndrome of grapevine. However, no information has been available regarding the pathogenicity of these fungi in grapevine.

Fig. 5. Lesions caused by *Diatrypaceae* species in Chardonnay 2.5 months after inoculation in green shoots. **A**, Control. **B**, *Diatrype oregonensis*. **C**, *Diatrypella* sp. **D**, *Cryptovalsa ampelina*. **E**, *Eutypa leptoplaca*. **F**, *Cryptosphaeria pullmanensis*. **G**, *Diatrype stigma*. **H**, *Eutypa lata*.

Fig. 4. Mean of vascular discoloration length 2.5 months after inoculation of various diatrypaceous isolates in Chardonnay grapevine green shoots inoculated in the field using mycelium plugs. * Average length of vascular discolorations was significantly different from the agar-only inoculated control according to Dunnett's *t* test ($P = 0.0224$). Bars represent standard error of the mean.

In this study, we performed pathogenicity experiments on *V. vinifera* grapevines using species of *Diatrypaceae* found in California vineyards. Results showed that all species tested were capable of colonizing grapevine wood. In each test, all isolates were re-isolated after a few months to 2 years following inoculations of wounded vegetative or dormant grapevine canes. Isolates of *C. ampelina*, *D. oregonensis*, *D. verrucaeformis*, and *E. leptoplaca* colonized dormant grapevine shoots directly after applying ascospore suspension onto the surface of fresh pruning wounds. Isolates of *C. ampelina*, *D. stigma*, and *E. leptoplaca* were the most virulent in mature wood based on the length of vascular necrosis in the wood and percentage of fungal re-isolation. Isolates of *C. pullmanensis*, *C. ampelina*, *D. stigma*, and *D. whitmanensis* showed the most virulence in green shoots. They produced lesions and vascular discolorations similar to those produced by *E. lata*. *D. oregonensis* and *D. verrucaeformis* produced vascular discolorations not significantly larger than those produced in the control shoots, suggesting they are not pathogenic.

Results of the pathogenicity of *C. ampelina* agreed with those from previous studies (8,10), although some authors have suggested moderate virulence for this fungus based on the low percentage of fungal recovery (8). Similarly, we observed great variability in the re-isolation and average necrosis length for isolates of the same species, indicating there is an intra-specific variation in virulence. Future experiments will be needed to include more isolates of each *Diatrypaceae* species to fully assess species aggressiveness on grapevine.

Only a few cankers were observed in our experiments, and the ability of *Diatrypaceae* to cause perennial cankers similar to those of *Eutypa* dieback remained uncertain. Extended incubation periods will be necessary in future experiments to better characterize disease expression and aggressiveness of these fungi.

Little is known about the biodegradation potential and wood decay processes used by *Diatrypaceae*. Pildain et al. (12) found high incidence of cellulase and ligninase within members of the *Diatrypaceae* family and suggested they are physiologically capable of producing wood decay. Rogers (13) argued that the widespread capacity of *Xylariaceae* (a sister group of the *Diatrypaceae*) to destroy lignocellulosic substrates and to degrade tissue would lead to the conclusion that the general lifestyle of these fungi is that of a facultative parasite or facultative saprophyte. Similar concepts may also apply to *Diatrypaceae* fungi parasitizing healthy or decaying grapevine wood. Species such as *D. oregonensis* and *Diatrypella* species occurring in the area of canker but showing low virulence to grapevine could be saprophytic.

Surveys for perithecia of *Diatrypaceae* in northern California vineyards showed the prevalence of *E. lata* and *C. ampelina*, which appear fully capable of completing their life cycle on the grapevine host (18). Alternatively, perithecia of *E. leptoplaca*, *D. oregonensis*, *D. whitmanensis*, and *D. verrucaeformis* on grapevine were rarely observed (17,18). No perithecia of *D. stigma* have yet been found on grapevine. In contrast, most of these diatrypaceous fungi occur commonly in nature, which suggests spread of these fungi from natural ecosystems into vineyards. Grapevine infections by *Diatrypaceae* generally occurred sporadically in vineyards surrounded by natural forests, riparian areas, or ornamental plantings where inocula of these fungi are present.

The role of diatrypaceous fungi in nature is not clear, and most species have

been regarded as saprobes (3). However, several species in the *Diatrypaceae* have been shown to cause disease in forest trees. *Cryptosphaeria lignyota* (Fr.) Auersw. (Syn.: *C. populina* (Pers.) Sacc.) is a major pathogen of *Populus* spp. in the western United States (5,6). *Eutypella* canker caused by *Eutypella parasitica* R.W. Davidson & R.C. Lorenz is a severe disease of sugar maple (*Acer saccharum* Marshall) in the northern United States (2). *Eutypa spinosa* (Pers.:Fr.) Tul. & C. Tul. was associated with elongated bark lesions (strip-cankers) in European beech (*Fagus sylvatica* L.) in the United Kingdom (4). The pathogenicity of *Diatrype* cf. *stigma* on *Quercus suber* has been reported in northeastern Spain (7). The authors reported that this fungus could cause stem cankers and vascular necroses. *D. stigma* as well as *E. leptoplaca* were isolated from

Fig. 6. Fungal colonies of various *Diatrypaceae* species after 21 days on dishes of potato dextrose agar amended with 100 ppm tetracycline incubated at ambient laboratory light and temperature conditions. **A**, *Cryptosphaeria ampelina*. **B**, *Diatrype oregonensis*. **C**, *Diatrype stigma*. **D**, *Diatrype whitmanensis*. **E**, *Diatrypella* sp. **F**, *Eutypa lata*. **G**, *Eutypa leptoplaca*. **H**, *Eutypella* sp.

cankers in California bay laurel (*Umbellularia californica*) (18). *D. whitmanensis* was isolated from cankers of California buckeye (*Aesculus californica*) (18). All these observations suggest that some of these fungi could likely be pathogenic to their host in natural environments.

Little is known about environmental influences on the pathogenicity of *Diatrypaceae* and on the expression of the disease they may cause. Recently, Sosnowski et al. (16) reported that the incidence and severity of foliar symptoms of *Eutypa* dieback vary from year to year and suggested that this variation is more likely to be caused by climatic factors. Similarly, environmental factors could likely influence symptom expression and disease severity caused by *Diatrypaceae*. Future experiments could explore the effect of water stress on canker and disease development by *Diatrypaceae*.

Variation in symptom expression in vineyards affected with *Eutypa* dieback could be partly explained by the differences in the composition and functioning of the fungal community (11). *Diatrypaceae* are widely represented in the overall fungal community of grapevine, and their interaction and competition with major grapevine pathogens such as *E. lata* and the *Botryosphaeriaceae* should be investigated.

Our experiments have proved that several diatrypaceous species can colonize grapevine and cause vascular streaking similar to that of *E. lata*. The ability of these fungi to compete with *E. lata* and *Botryosphaeriaceae* also is uncertain, and more work needs to be done to understand the significance of these fungi in the overall complex of grapevine canker diseases. Diatrypaceous fungi occur commonly on

many woody plants worldwide, and their occurrence in other grape growing regions around the world should be investigated. Also, we anticipate that more species will be shown to be pathogenic to other cultivated or native host plants as more data become available in pathogenicity and identity studies.

ACKNOWLEDGMENTS

We thank the American Vineyard Foundation and the Viticulture Consortium West for financial support.

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