Pathogenicity of *Botryosphaeriaceae* Species Isolated from Grapevine Cankers in California

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ABSTRACT

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Fungal species in the family *Botryosphaeriaceae* have been recently recognized as the most common fungi isolated from grapevine (*Vitis vinifera*) cankers in California. However, the role of these fungi in causing grapevine dieback as well as their status as canker-causing agents was unknown. Therefore, pathogenicity studies were conducted to determine their importance as grapevine pathogens in California. A total of 72 isolates representing all nine *Botryosphaeriaceae* species isolated from grapevine cankers from California were used in five different pathogenicity studies. Overall, experiments showed all nine *Botryosphaeriaceae* species able to infect both young and mature tissues as well as green shoots of the new vegetative growth causing cankers, vascular discoloration, and/or otherwise dark streaking of the wood. However, virulence varied among species. *Lasiodiplodia theobromae* was the most virulent species followed by *Neofusicoccum luteum*, *N. parvum*, and *N. australe*, all categorized as highly virulent. *Botryosphaeria dothidea* was considered intermediately virulent and *Diplodia mutila*, *D. seriata*, *Dothiorella iberica*, and *D. viticola* were shown to be weakly virulent. This study shows species of *Botryosphaeriaceae* to be much more important pathogens on grapevines than originally thought, and some of them, in view of their virulence, should be considered high risk for causing severe and rapid canker and dieback diseases in the grapevine industry in California.

Grapevine (*Vitis vinifera* L.), currently cultivated on more than 8 million hectares worldwide, is the most widely planted and economically important fruit crop in the world. Grapevine canker diseases, caused by fungal pathogens, are some of the primary factors limiting vineyard longevity and productivity as well as increasing management costs. Fungi associated with canker diseases of grapevine invade primarily through pruning wounds on cordons and spurs of grapevines. However, other mechanical or natural injuries on the plant can be entry points used by these pathogens. Grapevine cankers cause dieback and death of spurs, arms, cordons, and trunks, and eventual vine death. Grapevine cankers are responsible for significant economic losses to the grapevine industry worldwide, and in California alone, the overall loss in net income for wine grapes was estimated to exceed US\$260 million annually (26).

Moller and Kasimatis (17) first described *Eutypa lata* (Pers.:Fr.) Tul. & C.

Tul. (syn. *Eutypa armeniaca* Hansf. & Carter) as responsible for canker formation and dieback of grapevines in California (17). Since then, *E. lata*, the causal agent of Eutypa dieback, was considered to be the primary canker-causing pathogen of grapevines in California. However, research conducted in recent years has shown fungal species in the family *Botryosphaeriaceae* to be associated with different grapevine dieback symptoms including cankers in the main grape-growing regions of both Northern and Southern Hemispheres (2,19,28,30,31,34). *Botryosphaeriaceae* species occurring on grapevines are currently placed in the anamorphic genera *Fusicoccum*, *Neofusicoccum*, *Diplodia*, *Lasiodiplodia*, and *Dothiorella* (6,7,20).

Symptom expression caused by *Botryosphaeriaceae* species has been shown to differ from region to region and from different grapevine cultivars (34). As a result, different disease names such as Diplodia cane dieback (8), Macrophoma rot (16), excoiiose (18), bot canker (11,31), and black dead arm (2,9,12) have been given in different countries to different types of symptoms often caused by the same fungus. Confusion also has been compounded because disease symptoms caused by these fungi often resemble those caused by other fungal pathogens such as *E. lata* or *Phomopsis viticola* (Sacc.) Sacc. (5,18). Addi-

tionally, differences in virulence have been reported for the same species within the same and in different grape-growing areas, which has made it difficult to determine the importance of these fungi as grapevine pathogens (25,28,31,34).

Between 2003 and 2006, a survey of California vineyards was conducted to assess the occurrence of fungi associated with grapevine cankers (33). Among other fungi, nine different species in the family *Botryosphaeriaceae* were isolated from cankers, including *Botryosphaeria dothidea*, *Lasiodiplodia theobromae*, *Diplodia seriata*, *D. mutila*, *Neofusicoccum parvum*, *N. luteum*, *N. australe*, *Dothiorella viticola*, and *D. iberica* (29,33). Moreover, isolations from cankers showed *Botryosphaeriaceae* species to be the main fungi associated with cankers from the major grapevine-growing areas surveyed in California (33). *Botryosphaeriaceae* species have been recently reported to be the cause of grapevine cankers in South Africa (34), Chile (2,10), Australia (5,25,28), and Mexico (31). However, no data are available regarding the pathogenicity and virulence of *Botryosphaeriaceae* species in California and thus the role that these fungi play on grapevine health in the state.

The purpose of this study was to evaluate the pathogenicity and virulence of *Botryosphaeriaceae* species isolated from grapevine cankers and therefore determine which species pose the greatest threat to grapevines in California.

MATERIALS AND METHODS

Fungal isolates. Seventy-two fungal isolates, representing the nine different *Botryosphaeriaceae* species isolated from grapevine cankers from different geographical regions in California, were selected to conduct the pathogenicity studies (Table 1). Identity of the different *Botryosphaeriaceae* species was determined based on previous morphological studies and analyses of nucleotide sequences of three genes (29,33). All isolates were maintained in our collection in 4% potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) mycelial plugs in glass tubes with sterile distilled water at 5°C until use. In addition, representative *Botryosphaeriaceae* sequences and isolates from California were deposited in GenBank and in

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Table 1. Mean lesion length caused by *Botryosphaeriaceae* species from California on rooted-potted Crimson dormant cuttings in the greenhouse

Species	Isolate	Origin	ATCC ^a	GenBank ITS	Mean lesion length (mm) ^b ± SE	Reisolation ^c
<i>Lasiodiplodia theobromae</i>	UCD196Co	Riverside County	MYA-3687	DQ233592	315.3 ± 2.7 * ^d	3
<i>L. theobromae</i>	UCD1962SB	Santa Barbara Co.	-	DQ233597	290 ± 2.2 *	3
<i>L. theobromae</i>	UCD526Kr	Kern Co.	-	DQ233595	282.6 ± 6 *	3
<i>L. theobromae</i>	UCD205Co	Riverside Co.	MYA-3689	DQ008310	264.1 ± 1.1 *	3
<i>L. theobromae</i>	UCD220Co	Riverside Co.	MYA-3691	DQ008312	245.4 ± 8.6 *	3
<i>L. theobromae</i>	UCD206Co	Riverside Co.	MYA-3690	DQ008311	207.3 ± 6.6 *	3
<i>L. theobromae</i>	UCD256Ma	Madera Co.	-	DQ233594	184.5 ± 5.1 *	3
<i>L. theobromae</i>	UCD1814Md	Madera Co.	-	DQ233596	177.3 ± 5 *	3
<i>Diplodia seriata</i>	UCD244Ma	Madera Co.	MYA-3692	DQ008314	148 ± 6 *	2
<i>D. seriata</i>	UCD465Fr	Fresno Co.	-	DQ008316	128.6 ± 5.5 *	2
<i>D. seriata</i>	UCD352Mo	Monterey Co.	MYA-3693	DQ008315	112.3 ± 5.9 *	3
<i>D. seriata</i>	UCD602Kr	Kern Co.	MYA-3694	DQ008317	93.3 ± 2.2 *	3
<i>D. seriata</i>	UCD645So	Sonoma Co.	-	DQ008319	61 ± 3.1	2
<i>D. seriata</i>	UCD614Tu	Tulare Co.	-	DQ008318	59.1 ± 2.8	1
<i>D. seriata</i>	UCD710SJ	San Joaquin Co.	MYA-3695	DQ008321	28 ± 9.1	1
<i>D. seriata</i>	UCD666Na	Napa Co.	-	DQ008320	17.3 ± 2.3	1
<i>Diplodia mutila</i>	UCD288Ma	Madera Co.	-	DQ008313	128.7 ± 5.9 *	3
<i>D. mutila</i>	UCD1965SB	Santa Barbara Co.	MYA-3697	DQ233599	125.5 ± 4.5 *	1
<i>D. mutila</i>	UCD1953SB	Santa Barbara Co.	MYA-3696	DQ233598	122 ± 8.6 *	3
<i>D. mutila</i>	UCD1951SB	Santa Barbara Co.	-	-	95.3 ± 1.4 *	1
<i>D. mutila</i>	UCD1955SB	Santa Barbara Co.	-	-	85 ± 4.4 *	1
<i>D. mutila</i>	UCD300Ma	Madera Co.	-	-	84.1 ± 6.4 *	3
<i>D. mutila</i>	UCD289Ma	Madera Co.	-	-	67.6 ± 5.1 *	2
<i>D. mutila</i>	UCD1966SB	Santa Barbara Co.	-	-	61.6 ± 3.7	3
<i>Neofusicoccum australe</i>	UCD1313So	Sonoma Co.	-	-	319 ± 7.2 *	2
<i>N. australe</i>	UCD1467So	Sonoma Co.	MYA-3699	DQ233610	273 ± 8.2 *	3
<i>N. australe</i>	UCD1498So	Sonoma Co.	-	-	248.5 ± 5.2 *	3
<i>N. australe</i>	UCD1469So	Sonoma Co.	-	-	226.2 ± 9.3 *	3
<i>N. australe</i>	UCD1468So	Sonoma Co.	-	-	215.3 ± 8.9 *	3
<i>N. australe</i>	UCD1490So	Sonoma Co.	-	-	188.6 ± 12.6 *	3
<i>N. australe</i>	UCD1314So	Sonoma Co.	MYA-3698	DQ008323	177.6 ± 8.1 *	3
<i>N. australe</i>	UCD1315So	Sonoma Co.	-	-	139.7 ± 7.8 *	3
<i>Neofusicoccum luteum</i>	UCD2070Te	Riverside Co.	-	-	313.6 ± 6.8 *	3
<i>N. luteum</i>	UCD2103Te	Riverside Co.	MYA-3703	DQ233608	289.7 ± 7.1 *	3
<i>N. luteum</i>	UCD2089Te	Riverside Co.	-	DQ233605	281 ± 6.3 *	3
<i>N. luteum</i>	UCD2057Te	Riverside Co.	MYA-3700	DQ233625	216.3 ± 8.9 *	3
<i>N. luteum</i>	UCD2118Te	Riverside Co.	MYA-3704	DQ233609	208 ± 5.6 *	3
<i>N. luteum</i>	UCD2090Te	Riverside Co.	MYA-3701	DQ233606	205.6 ± 6.8 *	3
<i>N. luteum</i>	UCD2069Te	Riverside Co.	-	-	205.3 ± 8.5 *	3
<i>N. luteum</i>	UCD2098Te	Riverside Co.	MYA-3702	DQ233607	73.1 ± 2.2 *	3
<i>Neofusicoccum parvum</i>	UCD646So	Sonoma Co.	MYA-3706	DQ008329	335.7 ± 4.9 *	3
<i>N. parvum</i>	UCD759St	Stanislaus Co.	-	DQ233611	310.2 ± 4 *	3
<i>N. parvum</i>	UCD642So	Sonoma Co.	MYA-3705	DQ008328	278.3 ± 4.8 *	3
<i>N. parvum</i>	UCD1844Me	Mendocino Co.	-	-	268 ± 1.1 *	3
<i>N. parvum</i>	UCD650So	Sonoma Co.	-	-	253.3 ± 7.1 *	3
<i>N. parvum</i>	UCD649So	Sonoma Co.	-	-	238.4 ± 2.1 *	3
<i>N. parvum</i>	UCD1349So	Sonoma Co.	-	DQ008330	195.3 ± 1.6 *	3
<i>N. parvum</i>	UCD1125Na	Napa Co.	-	DQ233612	178 ± 4.5 *	3
<i>Botryosphaeria dothidea</i>	UCD1181Me	Mendocino Co.	MYA-3709	DQ008325	277.8 ± 8.4 *	2
<i>B. dothidea</i>	UCD1672Yo	Yolo Co.	MYA-3710	DQ233603	158.6 ± 9.8 *	1
<i>B. dothidea</i>	UCD1065So	Sonoma Co.	MYA-3707	DQ233601	136.7 ± 6.3 *	2
<i>B. dothidea</i>	UCD1156Me	Mendocino Co.	-	DQ233602	134.3 ± 4.6 *	2
<i>B. dothidea</i>	UCD1213Me	Mendocino Co.	-	DQ008326	118.3 ± 4.3 *	2
<i>B. dothidea</i>	UCD1064So	Sonoma Co.	-	DQ233600	108.5 ± 4.3 *	3
<i>B. dothidea</i>	UCD1333So	Sonoma Co.	-	DQ008327	94.5 ± 6.8 *	3
<i>B. dothidea</i>	UCD1066So	Sonoma Co.	MYA-3708	DQ008324	86 ± 3.2 *	3
<i>Dothiorella viticola</i>	UCD2100Te	Riverside Co.	-	-	103 ± 8.5 *	2
<i>D. viticola</i>	UCD1962SB	Santa Barbara Co.	-	-	79.1 ± 2.2 *	3
<i>D. viticola</i>	UCD1435SLO	San Luis Obispo Co.	MYA-4112	EF202007	63.6 ± 5.1	2
<i>D. viticola</i>	UCD1958SB	Santa Barbara Co.	MYA-4114	EF202011	61.3 ± 7.7	1
<i>D. viticola</i>	UCD2073Te	Riverside Co.	MYA-4115	EF202012	51.6 ± 2.5	1
<i>D. viticola</i>	UCD1642Yo	Yolo Co.	MYA-4113	EF202010	46 ± 9.1	1
<i>D. viticola</i>	UCD1360So	Sonoma Co.	-	-	34.3 ± 1	1
<i>D. viticola</i>	UCD3So	Sonoma Co.	MYA-4116	EF202013	30 ± 6.1	1
<i>Dothiorella iberica</i>	UCD1396SLO	San Luis Obispo Co.	-	-	81.3 ± 5.1 *	2
<i>D. iberica</i>	UCD1413SLO	San Luis Obispo Co.	-	-	43.6 ± 3.3	2
<i>D. iberica</i>	UCD1439SLO	San Luis Obispo Co.	MYA-4110	EF202008	35 ± 1.6	1
<i>D. iberica</i>	UCD1410SLO	San Luis Obispo Co.	-	-	34.8 ± 1.8	2
<i>D. iberica</i>	UCD1381SLO	San Luis Obispo Co.	-	-	24.5 ± 1.1	1
<i>D. iberica</i>	UCD1388SLO	San Luis Obispo Co.	-	-	22.1 ± 7.8	2
<i>D. iberica</i>	UCD1399SLO	San Luis Obispo Co.	-	-	19 ± 0.6	3
<i>D. iberica</i>	UCD1448SLO	San Luis Obispo Co.	MYA-4111	EF202008	16.6 ± 0.3	3
Control	Control	-	-	-	14.7 ± 0.3	0

^a ATCC, American Type Culture Collection. ITS, internal transcribe spacer accession numbers from sequences deposited in the GenBank.^b Mean values correspond to the sum of both upward and downward extent of vascular discoloration measured from the point of inoculation.^c Number of samples from which the fungus was reisolated out of 3 samples inoculated.^d *, Significantly different than the uninoculated controls at the 0.05 level.

the American Type Culture Collection (ATCC), respectively (Table 1).

Inoculum preparation. Pathogenicity studies were conducted using either fungal mycelium or a conidial suspension. Fungal mycelium of each isolate was recovered from our collection after plating of a mycelial plug on PDA and incubating it at room temperature ($23 \pm 2^\circ\text{C}$) for 10 days. Spore suspensions were prepared as follows: 7- to 10-day-old mycelial plugs for each of the isolates used were placed onto petri plates containing 2% water agar (Difco) and autoclaved pine needles. Plates were incubated at 25°C under intermittent (12 h) cycle fluorescent light until pycnidia and sporulation were observed. Mature pycnidia of each *Botryosphaeriaceae* anamorph were collected and separately placed in 1.5-ml sterile microcentrifuge tubes. Pycnidia were gently crushed in sterile distilled water using a sterile plastic pestle. Water containing the conidia was filtered through four layers of cheesecloth in order to remove both mycelial and pycnidia fragments. Spore suspensions were adjusted to a final density of approximately 1×10^5 conidia/ml using a hemacytometer.

Pathogenicity studies. Five different pathogenicity tests were conducted.

Greenhouse-potted grapevine inoculations. Eight different isolates of each of the nine *Botryosphaeriaceae* species were used in this experiment (Table 1). The experiment was conducted using 1-year-old dormant grapevine cuttings of cv. Crimson. Dormant cuttings were cut to a uniform length of five internodes (50 cm), rooted, and planted in May 2007 as described by Úrbez-Torres et al. (31). Inoculations were made by wounding the tissue between the fourth and fifth internode using a 5-mm-diameter cork borer and placing a 5-mm-diameter mycelial plug from a 7-day-old culture into the wound. Then, wounds were sealed with petroleum jelly and wrapped with Parafilm (Pechiney, Chicago, IL). Noncolonized agar plugs were used for the negative control. After inoculation, potted vines were placed in a greenhouse. Three cuttings per fungal isolate or control treatment were used, and plants were arranged in a completely randomized design on the greenhouse bench. Plants were maintained under controlled temperature ($30 \pm 2^\circ\text{C}$) and relative humidity (40%) and watered as needed. Six months after inoculation, cuttings were surface disinfected in 10% sodium hypochlorite for 5 min. After air-drying, canes were sectioned longitudinally in half through the point of inoculation, and the extent of vascular discoloration was measured both upward and downward from the point of inoculation (Fig. 1d). In order to fulfill Koch's postulates, small pieces of necrotic tissue from the edge of each lesion were cut and placed on PDA amended with tetracycline hydrochloride (PDA-tet,

0.01%) (Sigma-Aldrich, St. Louis, MO) to recover the inoculated fungus.

Spur inoculations. Three different isolates of each of the nine *Botryosphaeriaceae* species collected from California vineyards were used in this experiment (Table 2). The field trial was conducted in a 14-year-old Syrah vineyard located at the University of California, Kearny Agricultural Center in Parlier. Vines were cordon trained and spur pruned. Grapevines were pruned 8 to 10 buds in January 2006. Fresh pruning wounds were inoculated immediately by applying 50 μl of a 1×10^5 conidia/ml suspension from each isolate prepared as previously described. Control treatments were inoculated by applying 50 μl of sterile distilled water. One 1-year-old cane per grapevine on each of 10 grapevines per fungal treatment or control was inoculated, and treatments were applied in a completely randomized design. Canes were collected 13 months after inoculation and brought to the laboratory where surface disinfection was conducted as previously described. Canes were sectioned longitudinally in half and the extent of vascular discoloration was measured from the point of infection. Koch's postulates were fulfilled by isolation from infected tissue.

Inoculation of green shoots. Three different isolates of each of the nine *Botryosphaeriaceae* species were used in this study (Table 3). The field trial was conducted in a 7-year-old Red Globe vineyard at the University of California Field Station in Davis. The experimental design and number of replicates were the same as in the spur inoculation pathogenicity test. Green shoots of the new vegetative growth were inoculated with mycelial plugs by wounding between the fourth and fifth internodes in June 2007 as described in the greenhouse-potted grapevine pathogenicity test. Shoots were collected 5 months after inoculation and brought to the laboratory. Shoots were then disinfected, sectioned longitudinally in half through the point of inoculation, upward and downward vascular discoloration was recorded from the inoculation point, and fungal reisolation was conducted as described above.

Cordon inoculations. One isolate of each of the nine *Botryosphaeriaceae* species was used in this experiment (Table 4). Field trials were conducted in different grapevines of the same Syrah and Red Globe vineyards previously described. Inoculations were made on mature tissue at the end of the cordon between the last two spur positions by drilling a hole 10 to 15 mm deep using a 5-mm-diameter electric drill. Cordons were immediately inoculated by placing a mycelial plug in the hole. Wounds were sealed and wrapped as previously described. Ten cordons per fungal isolate were inoculated in each Syrah and Red Globe vineyard in January and February of 2006, respectively. Fungal treatments also included one isolate of *E.*

lata (Table 4), as well as noncolonized agar plugs for a negative control. Cordons were collected 24 months after inoculation. Cordons were disinfected, sectioned longitudinally in half through the point of inoculation as shown in Figure 1h, and vascular discoloration recorded both upward and downward from the point of inoculation. Fungal reisolutions were made as previously described.

Lath-house rooted grapevine inoculations. One isolate of each of the nine *Botryosphaeriaceae* species was used in this experiment (Table 5). Two-year-old rooted Thompson Seedless grapevines were individually planted in 3.8-liter pots containing sterile Yolo fine sandy loam soil. Plants were placed in a lath-house under ambient environmental conditions and watered as needed at the University of California Experimental Station in Davis in April 2006. After 2 months, the midpart of the trunk of each grapevine was wounded and inoculated using mycelium plugs as previously described. Noncolonized agar plugs were used for negative control. Ten plants per isolate or control were inoculated in a completely randomized design. Plants were collected 12 months after inoculation, sectioned longitudinally in half through the point of inoculation, and extent of upward and downward vascular discoloration was measured. Koch's postulates were fulfilled as previously described.

Data analyses. Data from the different assays were analyzed using SAS (Version 9.1.3; SAS Institute, Cary, NC). Homogeneity of variance was tested using Levene's test. Residuals were visually inspected for each experiment, and when necessary the $\log_{10}$ transformation was used to improve homogeneity of variance. Within each of the five assays, difference in length of discoloration caused by each *Botryosphaeriaceae* isolate was determined by one-way analyses of variance. Treatment means were compared using Fisher's least significant difference (LSD) test at the 5% significance level. A two-way ANOVA was performed to determine significant differences between Syrah and Red Globe grape cultivars in the cordon pathogenicity test. Additionally, virulence of *Botryosphaeriaceae* species across all pathogenicity assays was evaluated. Due to the heterogeneity of the different experimental designs (incubation time, inoculum used, and age of tissue inoculated), a ranking approach was used. The final virulence rank for each *Botryosphaeriaceae* species was determined by averaging the rank of each species across all six pathogenicity experiments (Syrah and Red Globe cordon inoculations were treated as separate trials). Species rank within each trial was determined by ranking all isolates from most virulent (rank 10) to least virulent (rank 2) including control (rank 1) depending on the discoloration they caused. Species virulence ranks obtained across all six

Fig. 1. Symptoms caused by *Botryosphaeriaceae* species on inoculated grapevines. **a**, Greenhouse-potted dormant Crimson cuttings 6 weeks after inoculation. Cuttings inoculated with highly virulent species (2 = UCD196Co, 3 = UCD646So, 4 = 2103Te, 5 = UCD1314So) showed poor or no new growth in comparison with the normal growth of cuttings inoculated with weakly pathogenic species (1 = control, 6 = control, 7 = UCD465Fr, 8 = UCD1065So, 9 = UCD3So, 10 = UCD14395Lo). **b**, Dark wood streaking caused by *Neofusicoccum parvum* (UCD646So). White arrow shows point of inoculation. **c**, Pycnidia of *Lasiodiplodia theobromae* (UCD220Co) formed under wax on Crimson cuttings 6 weeks after inoculation. **d**, Longitudinal section through point of inoculation (dash arrow) showing downward and upward vascular discoloration (white arrows) in a Crimson cutting. **e**, Cross section showing a characteristic wedge-shaped canker. **f**, Lesions caused by *Botryosphaeriaceae* species on Red Globe green tissue 5 months after inoculation (1 = UCD220Co, 2 = UCD220Co, 3 = UCD1314So, 4 = UCD1314So). Lignified canes showed necrotic areas around point of inoculation and flat canker surface. **g**, Vascular discoloration caused by *Botryosphaeriaceae* species on Red Globe green tissue 5 months after inoculation (1 = UCD220Co, 2 = UCD1498So, 3 = UCD2118Te, 4 = UCD642So, 5 = UCD1333Na, 6 = UCD352Mo, 7 = UCD1965SB, 8 = UCD1439SLO, 9 = UCD2073Te, 10 = control). **h**, Cross and longitudinal section showing a wedge-shaped canker caused by *L. theobromae* (UCD205Co) on cv. Syrah mature tissue (cordon). **i**, Wedge-shaped canker caused by *N. parvum* (UCD642So) on Red Globe mature tissue (cordon). Arrows indicate points of inoculation.

trials were analyzed using a Kruskal-Wallis test (nonparametric one-way ANOVA) where total sample size (N) = 60, treatments (k) = 10, nine spp. + control, and replicates (n) = 6.

RESULTS

Greenhouse-potted grapevine inoculations. Mean lengths of vascular discoloration caused by the 72 *Botryosphaeriaceae* isolates tested and the controls are shown in Table 1. Five to six weeks after inoculation, plants inoculated with *L. theobromae*, *N. parvum*, *N. australe*, and *N. luteum* isolates showed poor bud development and stunted green shoot growth (Fig. 1a). Plants inoculated with *B. dothidea*, *D. seriata*, *D. mutila*, *D. iberica*, and *D. viticola* showed normal green shoot development and did not differ from controls (Fig. 1a). Six months after inoculation, dark, rotting lesions and/or streaking of the wood developing both upward and downward from the point of inoculation were observed in all inoculated plants (Fig. 1b). Pycnidia on the surface of the bark of all 9 *Botryosphaeriaceae* species were commonly observed several weeks after inoculation (Fig. 1c). Wedge-shaped cankers were observed in cane cross sections of all plants inoculated with *L. theobromae*, *N. parvum*, *N. australe*, and *N. luteum* (Fig. 1e), and lesions often reached the bottom of the canes (root area). Fifty-five of 72 *Botryosphaeriaceae* isolates produced significantly longer lesions compared to the control (14.7 mm) (Table 1). However,

lesion lengths varied among species and within isolates of the same species. All *L. theobromae*, *N. parvum*, *N. luteum*, *N. australe*, and *B. dothidea* isolates tested in this experiment were highly virulent (Table 1). Vascular discoloration of at least one isolate of *D. seriata*, *D. mutila*, *D. viticola*, and *D. iberica* was significantly longer than that of the control (Table 1). *N. parvum* (UCD646So), *N. australe* (UCD1313So), *L. theobromae* (UCD196Co), and *N. luteum* (UCD2070Te) isolates were the most virulent on Crimson potted grapevines, and produced lesions over 300 mm in length (Table 1). On the other hand, although *D. seriata* (UCD710St), *D. viticola* (UCD3So), and most of the *D. iberica* isolates were reisolated from the observed cankers, the extent of vascular discoloration they produced was smaller than 30 mm in length and not significantly different from the noninoculated controls (Table 1). *N. luteum* isolates showed the highest variability in virulence within species, causing vascular discoloration ranging from 73.1 to 313.6 mm. No fungi were reisolated from the slightly discolored tissue of the controls.

Spur inoculations. Mean lengths of vascular discoloration caused by 27 *Botryosphaeriaceae* isolates and the controls are shown in Table 2. Thirteen months after inoculation, canes had developed dark, rotting lesions and/or dark wood streaking originating downward from the point of inoculation. V-shaped cankers were observed when cross sections were

made on symptomatic canes (Fig. 1e). All 27 *Botryosphaeriaceae* isolates tested were considered pathogenic in 1-year-old Syrah canes, and produced significantly longer lesions compared to the control (13.4 mm) (Table 2). However, lesion lengths varied among species and within isolates of the same species. *N. parvum*, *L. theobromae*, and *N. luteum* isolates caused significantly longer lesion means (112.3, 107.1, and 79.8 mm, respectively) than the rest of *Botryosphaeriaceae* species and were considered the most virulent species on Syrah spurs (Table 2). *D. seriata*, *D. iberica*, and *D. viticola* isolates caused the smallest lesion means (39.9, 39.8, and 38 mm, respectively) and were considered the least virulent species on Syrah canes. *N. parvum* isolate UCD646So and *L. theobromae* isolate UCD205Co caused the largest lesions (125.7 and 123.9 mm, respectively), and *D. iberica* isolate UCD1448SLO, *D. viticola* isolate UCD1642Yo, and *D. seriata* isolate UCD244Ma caused the smallest lesions (34.4, 33.6, and 32.3 mm, respectively) among all isolates tested (Table 2). *N. australe* isolates UCD1467So and UCD1314So showed the highest variability in virulence within species, and their vascular discoloration varied from 36.8 to 70.5 mm, respectively. Reisolation of any specific isolate, shown in Table 2, was always higher than 60%. No *Botryosphaeriaceae* fungi were reisolated from the slightly discolored tissue from the controls.

Inoculation of green shoots. Mean lengths of vascular discoloration caused by the 27 *Botryosphaeriaceae* isolates tested and the controls are shown in Table 3. Five months after inoculation, lignified canes showed dark flat lesions originating downward and upward from the point of inoculation (Fig. 1f and g). Twenty-one out of 27 *Botryosphaeriaceae* isolates produced significantly longer lesions in green Red Globe shoots compared to the control (3.3 mm) (Table 3). However, lesion lengths varied among species and within isolates of the same species. *L. theobromae* and *N. australe* isolates caused significantly longer lesion means (37.4 and 28.7 mm, respectively) than the rest of *Botryosphaeriaceae* species and were considered the most virulent species on green shoots of cv. Red Globe (Table 3). *D. mutila*, *D. iberica*, and *D. viticola* isolates caused the smallest lesions (11.3, 9.5, and 8.5 mm, respectively) and were considered the least virulent species. *L. theobromae* isolate UCD220Co caused the largest lesions (45.2 mm) among all *Botryosphaeriaceae* isolates (Table 3). *D. viticola* isolates UCD1435SLO and UCD1642Yo, and *D. iberica* isolate UCD1448SLO caused the smallest lesions among all isolates tested (7.2, 6.6, and 6.3 mm, respectively) (Table 3) and were not significantly different from the control. Reisolation of any specific isolate, shown in Table 3, was always higher than 60%, with the exception of

Table 2. Mean lesion length caused by *Botryosphaeriaceae* species from California on Syrah spurs

Isolate	Species	Mean lesion length (mm) $\pm$ SE ^a	Reisolation ^b
UCD646So	<i>Neofusicoccum parvum</i>	125.7 $\pm$ 16.8 a	10
UCD205Co	<i>Lasiodiplodia theobromae</i>	123.9 $\pm$ 16.5 a	8
UCD1125Na	<i>N. parvum</i>	105.7 $\pm$ 11.2 abc	10
UCD642So	<i>N. parvum</i>	105.6 $\pm$ 19.6 abcd	10
UCD256Ma	<i>L. theobromae</i>	104.7 $\pm$ 15.9 abcd	10
UCD1814Md	<i>L. theobromae</i>	92.5 $\pm$ 14.9 abcd	10
UCD2118Te	<i>Neofusicoccum luteum</i>	88.7 $\pm$ 19.3 bcde	10
UCD2103Te	<i>N. luteum</i>	80.2 $\pm$ 15.8 bcde	10
UCD2098Te	<i>N. luteum</i>	70.6 $\pm$ 9.4 bcdef	9
UCD1467So	<i>Neofusicoccum australe</i>	70.5 $\pm$ 11.3 cdef	10
UCD1065So	<i>Botryosphaeria dothidea</i>	58.8 $\pm$ 14.8 efgh	10
UCD288Ma	<i>Diplodia mutila</i>	50.5 $\pm$ 9.8 efghi	8
UCD710SJ	<i>Diplodia seriata</i>	49.1 $\pm$ 13.6 efghi	10
UCD1410SLO	<i>Dothiorella iberica</i>	46.4 $\pm$ 9.3 efghi	9
UCD1066So	<i>B. dothidea</i>	45.9 $\pm$ 6.6 efghi	7
UCD1469So	<i>N. australe</i>	44.8 $\pm$ 10.4 fghi	10
UCD1965SB	<i>D. mutila</i>	42.2 $\pm$ 8 ghi	10
UCD2073Te	<i>Dothiorella viticola</i>	41.5 $\pm$ 7.5 hi	10
UCD1435SLO	<i>D. viticola</i>	38.9 $\pm$ 12.2 hi	8
UCD1439SLO	<i>D. iberica</i>	38.8 $\pm$ 6.3 hi	9
UCD1953SB	<i>D. mutila</i>	38.7 $\pm$ 5.8 hi	7
UCD1181Me	<i>B. dothidea</i>	38.4 $\pm$ 8.1 hi	10
UCD352Mo	<i>D. seriata</i>	38.1 $\pm$ 7.7 hi	8
UCD1314So	<i>N. australe</i>	36.8 $\pm$ 8.4 hi	7
UCD1448SLO	<i>D. iberica</i>	34.4 $\pm$ 5.3 i	6
UCD1642Yo	<i>D. viticola</i>	33.6 $\pm$ 4.9 i	7
UCD244Ma	<i>D. seriata</i>	32.3 $\pm$ 7.2 i	10
Control	Control	13.4 $\pm$ 1.2 j	0

^a Means with the same letter are not significantly different at the 0.05 level. Mean values correspond to the extent of vascular discoloration measured downward from the point of inoculation.

^b Number of samples from which the fungus was reisolated out of 10 samples inoculated.

isolates UCD1435SLO and UCD1448SLO, which showed only 40% reisolation. No *Botryosphaeriaceae* fungi were reisolated from the slightly discolored tissue from the controls.

Cordon inoculation. Mean extent of vascular discoloration caused by the nine *Botryosphaeriaceae* isolates tested, *E. lata*, and controls are shown in Table 4. Twenty-four months after inoculation, V-shaped canker lesions were the most common symptom observed in the inoculated mature tissue (Fig. 1h and i). Dark wood streaking extending beyond the canker lesion was also observed in cordons inocu-

lated with *L. theobromae* (UCD1814Md), *N. australe* (UCD1324So), *N. parvum* (UCD642So), *N. luteum* (UCD2118Te), and *B. dothidea* (UCD1181Me). Based on length of vascular discoloration and canker formation, the nine *Botryosphaeriaceae* isolates tested in this trial were considered highly virulent, intermediately virulent, or weakly virulent in both cultivars when compared to the control (Table 4). A two-way ANOVA showed no significant differences in the extent of vascular discoloration between the same *Botryosphaeriaceae* species regardless of cultivar ($F = 0.34$, $P = 0.9683$). *L. theobromae*, *N. australe*, *N.*

parvum, and *N. luteum* isolates produced the largest lesions on both cultivars, and the extent of vascular streaking or discoloration was not significantly different from that of the well-documented canker pathogen *E. lata* (Table 4). Thus, the four *Botryosphaeriaceae* species were categorized as highly virulent on mature tissue. Based on the extent of the vascular discoloration and canker formation, *B. dothidea* on Red Globe, and *B. dothidea*, *D. seriata*, and *D. iberica* on Syrah were considered intermediately pathogenic on mature tissue (Table 4). Species that did not cause cankers or lesions that were not significantly different from the negative control included *D. seriata*, *D. iberica*, *D. viticola*, and *D. mutila* isolates on Red Globe, and *D. viticola* and *D. mutila* on Syrah (Table 4). However, these species were reisolated from observed lesions and were thus considered weakly virulent on mature tissue. Reisolation of the specific isolates, shown in Table 4, was accomplished. No *Botryosphaeriaceae* fungi were reisolated from the slightly discolored tissue from the controls.

Lath-house rooted grapevine inoculations. Mean length of vascular discolorations caused by the nine *Botryosphaeriaceae* isolates tested and control are shown in Table 5. Twelve months after inoculation, trunks had developed dark, rotting lesions and black wood streaking starting downward and upward from the point of inoculation. All nine *Botryosphaeriaceae* isolates tested were considered pathogenic and produced significantly longer lesions than the control (7.3 mm) (Table 5). However, lesion lengths varied among the isolates used in this study. The *L. theobromae* isolate caused the average largest lesion size (92.1 mm) (Table 5). The *N. luteum*, *N. parvum*, and *N. australe* isolates used in this assay were also highly virulent and caused lesions significantly longer than the rest of species shown in Table 5. Vascular discoloration caused by *D. mutila*, *B. dothidea*, *D. iberica*, *D. viticola*, and *D. seriata* did not differ significantly among each other and ranged from 19.8 to 24.1 mm

Table 3. Mean lesion length caused by *Botryosphaeriaceae* species on attached green shoots of cv. Red Globe

Isolate	Species	Mean lesion length (mm) $\pm$ SE ^a	Reisolation ^b
UCD220Co	<i>Lasiodiplodia theobromae</i>	42.5 $\pm$ 7.6 a	10
UCD256Kr	<i>L. theobromae</i>	41.6 $\pm$ 8.8 ab	10
UCD1498So	<i>Neofusicoccum australe</i>	36.2 $\pm$ 11.3 abc	9
UCD1467So	<i>N. australe</i>	30.5 $\pm$ 12.7 bcd	10
UCD205Co	<i>L. theobromae</i>	25.5 $\pm$ 3.4 cde	10
UCD2118Te	<i>Neofusicoccum luteum</i>	24.2 $\pm$ 5.1 cdef	10
UCD1333Na	<i>Botryosphaeria dothidea</i>	23 $\pm$ 6.3 cdefg	8
UCD352Mo	<i>Diplodia seriata</i>	21.8 $\pm$ 6.1 defg	10
UCD710SJ	<i>D. seriata</i>	20.6 $\pm$ 3.6 defgh	10
UCD1314So	<i>N. australe</i>	19.6 $\pm$ 3.4 defghi	10
UCD1066So	<i>B. dothidea</i>	17.2 $\pm$ 5.8 defghi	8
UCD1965SB	<i>Diplodia mutila</i>	16.6 $\pm$ 4.1 efghi	10
UCD642So	<i>Neofusicoccum parvum</i>	16.4 $\pm$ 5 efghi	10
UCD2089Te	<i>N. luteum</i>	15.2 $\pm$ 0.9 efghi	9
UCD650So	<i>N. parvum</i>	14.4 $\pm$ 2.1 efghi	10
UCD1063So	<i>B. dothidea</i>	13.8 $\pm$ 1.6 efghi	8
UCD1125Na	<i>N. parvum</i>	11.8 $\pm$ 4.7 efghi	9
UCD2073Te	<i>Dothiorella viticola</i>	11.6 $\pm$ 2.7 efghi	7
UCD1439SLO	<i>Dothiorella iberica</i>	11.4 $\pm$ 2.6 fghi	9
UCD244Ma	<i>D. seriata</i>	11.2 $\pm$ 2.6 fghi	10
UCD1410SLO	<i>D. iberica</i>	10.4 $\pm$ 1.8 fghi	9
UCD1953SB	<i>D. mutila</i>	9.6 $\pm$ 2.6 ghij	9
UCD2103Te	<i>N. luteum</i>	8 $\pm$ 1.1 hij	10
UCD288Ma	<i>D. mutila</i>	7.6 $\pm$ 1.4 hij	6
UCD1435SLO	<i>D. viticola</i>	7.2 $\pm$ 1.6 hij	6
UCD1642Yo	<i>D. viticola</i>	6.6 $\pm$ 1.4 ij	8
UCD1448SLO	<i>D. iberica</i>	6.6 $\pm$ 1.1 ij	4
Control	Control	3.3 $\pm$ 0.2 j	0

^a Means with the same letter are not significantly different at the 0.05 level. Mean values correspond to the sum of both upward and downward extent of vascular discoloration measured from the point of inoculation.

^b Number of samples from which the fungus was reisolated out of 10 samples inoculated.

Table 4. Mean lesion length caused by *Botryosphaeriaceae* species from California on cordons of cvs. Red Globe and Syrah

Isolate	Species	Red Globe		Syrah	
		Mean lesion length (mm) $\pm$ SE ^a	Reisolation ^b	Mean lesion length (mm) $\pm$ SE ^a	Reisolation ^b
UCD1814Md	<i>Lasiodiplodia theobromae</i>	80.2 $\pm$ 5.6 a	10	80.4 $\pm$ 6.7 a	10
UCD1314So	<i>Neofusicoccum australe</i>	77.2 $\pm$ 8.7 ab	7	68.4 $\pm$ 7.5 ab	8
UCD642So	<i>Neofusicoccum parvum</i>	75.1 $\pm$ 9.7 ab	10	63.8 $\pm$ 5.2 ab	10
UCD2118Te	<i>Neofusicoccum luteum</i>	74.9 $\pm$ 6.5 ab	8	72.5 $\pm$ 4.3 a	10
DHB100	<i>Eutypa lata</i>	70.8 $\pm$ 8 ab	8	60.1 $\pm$ 3.2 ab	7
UCD1181Me	<i>Botryosphaeria dothidea</i>	47.8 $\pm$ 4.5 bc	7	42.6 $\pm$ 5.9 cd	6
UCD352Mo	<i>Diplodia seriata</i>	40.2 $\pm$ 5.9 cd	7	35.9 $\pm$ 3.6 de	8
UCD1448SLO	<i>Dothiorella iberica</i>	35.8 $\pm$ 8.8 cd	6	32.8 $\pm$ 2.5 de	7
UCD1642Yo	<i>Dothiorella viticola</i>	34.2 $\pm$ 8.1 d	5	29.2 $\pm$ 3.1 def	6
UCD1953SB	<i>Diplodia mutila</i>	33.1 $\pm$ 2.5 d	6	30.3 $\pm$ 5.8 ef	7
Control	Control	26 $\pm$ 1.4 d	0	21.3 $\pm$ 1.2 f	0

^a Means with the same letter are not significantly different at the 0.05 level. Mean values correspond to the sum of both upward and downward extent of vascular discoloration measured from the point of inoculation.

^b Number of samples from which the fungus was reisolated out of 10 samples inoculated.

(Table 5). Reisolation of the specific species is shown in Table 5. No *Botryosphaeriaceae* fungi were reisolated from the slightly discolored tissue from the controls.

Ranking analyses. Virulence of the nine *Botryosphaeriaceae* species across all pathogenicity assays was evaluated. According to Kruskal-Wallis test, *L. theobromae* was ranked as the most virulent species among all *Botryosphaeriaceae* species tested from California ($X^2 = 51.09$, $P < 0.0001$) (Table 6). *N. luteum*, *N. parvum*, and *N. australe* were ranked as the second, third, and fourth most virulent species, respectively (Table 2). Based on the overall lesion length, all four species were categorized as highly virulent. *B. dothidea* was considered intermediately virulent based on length of vascular discoloration among all species. *D. mutila*, *D. seriata*, *D. iberica*, and *D. viticola* were all categorized as weakly virulent (Table 6). *D. iberica* and *D. viticola* were considered the least virulent species among all *Botryosphaeriaceae* species tested in this study based on both discoloration length and reisolation percentage.

DISCUSSION

Perennial cankers and consequent grapevine dieback have long been known to occur in California vineyards and have

been thought to be associated with *E. lata*. Even though Leavitt first reported *L. theobromae* (Basionym: *Botryodiplodia theobromae* Pat.) as the causal organism of bot canker disease in California in the late 1980s (11), it was not until recently that members of the family *Botryosphaeriaceae* and not *E. lata* were shown to be the main fungi isolated from grapevine cankers in the state (29,33). This overlooked association between *Botryosphaeriaceae* species and grapevine dieback in California may correspond to the possibility that *E. lata* was considered throughout many years to be the only canker pathogen of grapevines. Furthermore, the inability to distinguish cankers caused by both *E. lata* and *Botryosphaeriaceae* species in the field as well as the lack of characteristic foliar symptoms associated with bot canker could contribute to the failure to identify these species as grapevine pathogens in California. However, the present study has shown clear evidences that all nine *Botryosphaeriaceae* species associated with grapevine cankers in California are pathogenic and capable of causing cankers, vascular discolorations, and dieback individually when inoculated into grapevines.

This study has shown *L. theobromae* to be the most virulent among all *Botryosphaeriaceae* species tested. Although *L.*

theobromae has been consistently shown to be pathogenic on grapes by other authors (1,11,28,31,34), variability in virulence has been observed in different grape-growing regions. For instance, *L. theobromae* isolates from Western Australia (28), Mexico (31), and Spain (1) were shown to be highly virulent and caused the same type of vascular lesions as those reported in the present study. On the contrary, pathogenicity studies conducted in South Africa showed lesions caused by *L. theobromae* to differ little from the noninoculated controls (34). These differences could be due to variability in isolate virulence, type of inoculated tissue, shorter incubation periods, type of inoculum used, age of the host, and/or differences in cultivar susceptibility, among other possible factors. Virulence of *L. theobromae* isolates from California did not vary depending on type of inoculated tissue, cultivar, or with type of inoculum. *L. theobromae* has been reported to be more prevalent in grape-growing regions characterized by high temperatures and very low precipitation (28,31). Our study showed *L. theobromae* isolates from Riverside County (Coachella Valley), an important grape-growing area located in a desert area of Southern California, to be the most virulent among all isolates tested. Whether origins of isolates as well as environmental factors play a direct role in the virulence of *L. theobromae* remains unclear. Therefore, additional investigations considering the use of a broad number of *L. theobromae* isolates from different geographical regions in a multi-variety pathogenicity study under different environmental conditions would be required to further clarify these hypotheses.

In this study, *N. luteum*, *N. parvum*, and *N. australe* followed *L. theobromae* in level of virulence and capability to produce cankers on grapes in California. Although *N. luteum* and *N. parvum* were first reported to be associated with declining grapevines in Portugal in 2002 (19), it was not until recently that their pathogenicity was first assessed on grapevines in South Africa (34). *N. parvum* isolates used in South Africa were considered highly virulent, and the only *N. luteum* isolate used in that study did not cause lesions any larger than the natural dieback associated with the control, and thus it was considered weakly pathogenic (34). The present study has shown *N. luteum* and *N. parvum* as two of the most virulent *Botryosphaeriaceae* species in California and confirms both species as important pathogens of grapes in the state. These results add to recent reports of pathogenicity of *N. luteum* in Eastern Australia (25) and *N. parvum* in Spain (14), where these species were shown to be the most virulent on grapes. Additionally, the present study also showed the association between *N. parvum* and dark wood streaking symptoms from

Table 5. Mean lesion length caused by *Botryosphaeriaceae* species from California on 2-years-old grapevine cv. Thompson Seedless

Isolate	Species	Mean lesion length (mm) $\pm$ SE ^a	Reisolation ^b
UCD220Co	<i>Lasiodiplodia theobromae</i>	92.1 $\pm$ 7.3 a	10
UCD2098Te	<i>Neofusicoccum luteum</i>	71.8 $\pm$ 9.3 b	10
UCD646So	<i>Neofusicoccum parvum</i>	49.7 $\pm$ 6.9 bc	8
UCD1314So	<i>Neofusicoccum australe</i>	40.2 $\pm$ 9.2 c	10
UCD288Ma	<i>Diplodia mutila</i>	25.3 $\pm$ 3.7 d	10
UCD1066So	<i>Botryosphaeria dothidea</i>	23.2 $\pm$ 1.9 d	9
UCD1448SLO	<i>Dothiorella iberica</i>	21.2 $\pm$ 2.7 d	7
UCD2073Te	<i>Dothiorella viticola</i>	19.9 $\pm$ 2.1 d	6
UCD352Mo	<i>Diplodia seriata</i>	19.8 $\pm$ 2.3 d	6
Control	Control	7.3 $\pm$ 1.2 e	0

^a Means with the same letter are not significantly different at the 0.05 level. Mean values correspond to the sum of both upward and downward extent of vascular discoloration measured from the point of inoculation.

^b Number of samples from which the fungus was reisolated out of 10 samples inoculated.

Table 6. Virulence ranking of the nine *Botryosphaeriaceae* species from California on grapevines from most virulent (10) to least virulent (1)

<i>Botryosphaeriaceae</i> species	Rank ^a	Std. error
<i>Lasiodiplodia theobromae</i>	9.67	0.08
<i>Neofusicoccum luteum</i>	8.17	0.18
<i>Neofusicoccum parvum</i>	7.83	0.30
<i>Neofusicoccum australe</i>	7.67	0.13
<i>Botryosphaeria dothidea</i>	6.17	0.15
<i>Diplodia mutila</i>	4.33	0.19
<i>Diplodia seriata</i>	4.17	0.30
<i>Dothiorella iberica</i>	3.50	0.08
<i>Dothiorella viticola</i>	2.50	0.13
Control	1.00	0.00

^a Virulence rank for each *Botryosphaeriaceae* species represents the average rank of each isolate across all six pathogenicity experiments (Syrah and Red Globe cordon inoculations were treated as separate trials) based on a Kruskal-Wallis test. Isolate ranks within each trial was determined from most virulent (rank 10) to least virulent (rank 2) including control (rank 1) based on length of discoloration they caused.

which the fungus was commonly isolated in California and in seven other states (32). *N. australe* is mainly found in native and introduced plants in the Southern Hemisphere and is thought to be native to Australia (27). This species was primarily found associated with grapevine decline in South Africa (34) and Western Australia (28), where it was shown to be the most virulent *Botryosphaeriaceae* species. To date, California is the only grape-growing region from which *N. australe* has been found associated with grapevine dieback in the Northern Hemisphere (33). The present study also has confirmed the high level of virulence of *N. australe* on grapevines in California. Contrary to what has been observed with *N. parvum* in the United States, *N. australe* and *N. luteum* have a very narrow geographical distribution, and their occurrence is restricted to some vineyards in Northern and Southern California, respectively (32,33). However, in view of their high level of virulence on grapes, further investigations are warranted to study its occurrence in other grape-growing regions as well as in other hosts.

Overall, *B. dothidea* isolates from California caused less tissue discoloration than *L. theobromae*, *N. parvum*, *N. luteum*, and *N. australe*, but discoloration was still significantly longer than for *D. seriata*, *D. mutila*, *D. iberica*, and *D. viticola*, and thus *B. dothidea* was categorized as intermediately virulent in the present study. *B. dothidea* is confirmed to be responsible for excoiiose in Portugal (18), and is associated with Macrophoma rot in the southeastern United States (16). Pathogenicity of *B. dothidea* on grapevines in California confirms its capability of causing cankers and agrees with previous reports from France (9) and Chile (10) in regards to its pathogenicity on grapes. In California, *B. dothidea* has been reported from over 90 native and introduced plant species including 30 different plant families (4,15). Therefore, and in view of the important role that *B. dothidea* plays in grapevine health in California, further studies are needed to determine if its isolates from different hosts could act as potential sources of inoculum for grapevines and vice versa.

D. mutila, *D. seriata*, *D. iberica*, and *D. viticola* isolates from California caused small lesions, which occasionally were not different than the noninoculated controls, and thus were considered weakly pathogenic. *D. mutila* was first associated with grapevines as the cause of black dead arm in Hungary (12). However, little information is available in the literature regarding its pathogenicity on grapes. The current study has shown *D. mutila* isolates from California to be weakly pathogenic. The same conclusion was obtained from studies conducted in Portugal (18) and Australia (28). On the contrary, *D. mutila* was shown to be highly virulent in South Af-

rica (34), reinforcing the previous idea that different factors might be involved in virulence variability in species of *Botryosphaeriaceae*. A recent study has shown *D. mutila* to be capable of infecting young grapevines through roots and causing dieback (35). In California, as well as other states surveyed, *D. mutila* was only isolated from cankers from spurs and cordons showing dieback symptoms (32,33). However, only aerial parts of the plants were surveyed.

D. seriata has been shown to be a homogenous, cosmopolitan, and plurivorous species (21,22). Although *D. seriata* has been reported from grapevines in most continents, its pathogenicity on grapes has largely remained unclear (19). The lack of fulfillment of Koch's postulates from many studies associating *D. seriata* with grapevine decline symptoms (3,23,24,30) and discrepancies regarding its virulence from studies where pathogenicity tests were conducted (5,18,25,28,31,34) have added confusion in regards to its pathogenicity on grapevines during the past years. This study represents the first attempt to fully characterize the pathogenicity of *D. seriata* on grapes, and although the overall results showed this species to be a weak pathogen, there was a high level of variability among isolates. For instance, *D. seriata* was more virulent than *N. parvum* and *N. luteum* when inoculated in green tissue. Why green tissue is more susceptible to *D. seriata* isolates from California is unknown, and further investigations under field conditions are needed to determine the importance that this species may have in infection of vegetative growth. *D. seriata* has been commonly associated with black dead arm disease of grapes, which is characterized by light-brown discoloration of the wood and red patches at the margin of leaves and large chlorotic areas and deterioration between the veins (2,9,12). Despite the fact that black dead arm vascular symptoms have been reproduced in this and previous studies (2,9,31), the association between *D. seriata* and foliar symptoms of the disease has not been demonstrated. Therefore, whether black dead arm foliar symptoms are caused by *D. seriata* alone or by a complex of organisms still needs to be determined.

D. iberica and *D. viticola* have been recently described as new species in the *Botryosphaeriaceae* family (13,20). Whereas both species have been isolated from grapevine cankers in California (29), only *D. viticola* has been found associated with grapevines elsewhere (13,34). Based on results of this study, *D. iberica* and *D. viticola* isolates from California were the least virulent among all species tested, but still capable of causing vascular discoloration. *D. viticola* was found mainly on pruning debris and not associated with any decline symptom in Spain, and thus it was thought to act as a saprophyte (13). How-

ever, this study has shown the capability of this species to colonize grapevine tissue to some extent, revealing the pathogenic role it has on grapevines in California.

Botryosphaeriaceae species have been shown to be isolated from grapevine dieback symptoms directly associated with pruning wounds and/or natural openings of the plant. Therefore, these fungi have been thought to infect grapes primarily through wounds (25,28). This notion is true in California, where *Botryosphaeriaceae* species were isolated from cankers primarily developing from pruning wounds (33). Moreover, inoculations of fresh pruning wounds by spore suspensions of nine different *Botryosphaeriaceae* species in this study clearly showed that pruning wounds are susceptible to invasion by these pathogens. However, little information is available regarding the epidemiology of *Botryosphaeriaceae* infecting grapes. Further investigations to determine the environmental conditions that cause spore release and thus establish high-risk infection periods throughout the grapevine growing season in California are needed. This study also showed the capability of *Botryosphaeriaceae* species to infect and cause lesions on green tissue of the new vegetative growth. However, the implications that these results could have in the epidemiology of the disease are unknown at this time.

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