Identification and Pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata*, the Causal Agents of Bot Canker Disease of Grapevines in Mexico

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ABSTRACT

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Perennial cankers and consequent grapevine dieback are a major problem in vineyards of Sonora and Baja California, the most important grape-production areas of Mexico. In order to identify the canker-causing agents, symptomatic arms, cordons, and trunks were collected from 13 and 6 vineyards in Sonora and Baja California, respectively. Two *Botryosphaeriaceae* spp., *Lasiodiplodia theobromae* and *Diplodia seriata*, were isolated frequently from infected wood and identified based on morphological and cultural characters as well as analyses of nucleotide sequences of three genes, the internal transcribed spacer region (ITS1-5.8S-ITS2), a partial sequence of the β -tubulin gene, and part of the translation elongation factor 1- α gene (EF1- α). Although both *L. theobromae* and *D. seriata* were isolated from grapevine cankers in Baja California, only *L. theobromae* was found in vines in the Sonora region. Pathogenicity of both species was verified by inoculation of rooted cuttings and green shoots of Thompson Seedless and Chardonnay cultivars. Isolates of *L. theobromae* were more virulent, based on the extent of spread in the secondary wood and green tissue, than those of *D. seriata*. These findings confirm *L. theobromae* and *D. seriata* as the causal agents of dieback and canker formation of grapevines in northern Mexico.

Additional keywords: dead arm, Eutypa dieback, *Eutypa lata*, trunk diseases, *Vitis vinifera*

Grapevine (*Vitis vinifera* L.), cultivated in over 26,000 hectares, is the most important fruit crop in the Mexican States of Baja California and Sonora. These regions are the largest wine and table grape-producing areas of Mexico and generate an annual crop valued of US\$152 million dollars (39). Grapevine decline in Mexico first was observed in the states of Aguascalientes and Coahuila-Durango in 1979, and was associated with *Eutypa lata* (Pers.) Tul. & C. Tul., the causal agent of Eutypa dieback (44). Ten years later, grapevine cankers and subsequent dieback were observed in table-grape vineyards of Hermosillo, Sonora. Disease symptoms were characterized by dead arms and cordons, trunk dieback due to canker formation in the vascular tissue, and the total absence of the stunted chlorotic spring growth which is typical of infections by *E. lata*. Subsequently, these symptoms were associated with *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (23). For many

years, *E. lata* was thought to be the most important canker- and dieback-causing agent of grapevines not only in Mexico but worldwide. However, recent studies also have identified *Botryosphaeriaceae* spp. as important grapevine pathogens causing cankers and other dieback symptoms such as wood streaking, shoot dieback, cane bleaching, bud necrosis, and graft failure in all major viticulture regions throughout the world (20,25,29,43,45,47,48).

Based on a recent study, the genus *Botryosphaeria* has been shown to be restricted only to *Botryosphaeria dothidea* (Moug.) Ces. & De Not. and *B. corticis* (Demaree & Wilcox) Arx & E. Müll. (12). Consequently, the name *Botryosphaeria* is no longer acceptable for most of the species with *Fusicoccum*-like and *Diplodia*-like anamorphs (12,31). Although the genus *Neofusicoccum* Crous, Slippers & A.J.L. Phillips has been described to accommodate *Botryosphaeriaceae* with *Fusicoccum*-like anamorphs; no teleomorph name has been proposed for *Botryosphaeriaceae* spp. with *Lasiodiplodia*- and *Diplodia*-like anamorphs (12). For this reason, fungi such as *B. rhodina* and *B. obtusa* will be named by their anamorphs, *L. theobromae* and *Diplodia seriata* De Not., respectively (12,31). Along with all species of *Botryosphaeriaceae* found in grapevines, *L. theobromae* (= *B. rhodina*),

D. seriata (= *B. obtusa*), *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (= *B. parva*), *B. dothidea* (Moug.) Ces. & De Not., *D. mutila* (Fr.) Mont. (= *B. stevensii*), *N. luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (= *B. lutea*), and *N. australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips (= *B. australis*) are the most commonly found associated with grapevine dieback worldwide (2,20,29,43,45,47,48).

L. theobromae is a pleomorphic and plurivorous Ascomycete, mostly prevalent in tropical and subtropical climate regions (33). *L. theobromae* is recognized as an important wood pathogen and has been reported to cause cankers, dieback, and fruit and root rots in over 500 different hosts, including perennial fruit and nut trees, vegetable crops, and ornamental plants (32). Vascular cankers and grapevine dieback caused by *L. theobromae* first were reported in Egypt in 1972 (13). Fifteen years later, a field study conducted in California showed *L. theobromae* to be an important grapevine pathogen, capable of causing cankers, dieback, and dead arm symptoms on vines (24). The disease has since been referred to as "Bot canker" or "Diplodia cane dieback" and now is known to be prevalent in the warm growing areas of the southern San Joaquin Valley and the Coachella valley in southern California (17,23,45). Recent studies conducted in South Africa and Australia, where *L. theobromae* was isolated primarily from declining grapevines showing perennial cankers in spurs and cordons, have confirmed the significance of this species as a grapevine pathogen (43,48,50).

D. seriata has been observed in temperate areas on most continents and described from over 35 different hosts, including *Vitis* spp. (34). *D. seriata* is recognized as an important pathogen of pome and stone fruit trees, causing cankers, leaf spots, and black rot of the fruit (5,6,22,40,41). Unlike *L. theobromae*, pathogenicity of *D. seriata* in grapevines remains unclear. Whereas studies in Western Australia and Portugal have considered *D. seriata* to be a saprophyte or a weak and secondary pathogen of grapevines, respectively (28,43), other studies report *D. seriata* to be a primary pathogen in grapevines causing vascular

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symptoms such as brown streaking of the wood and cankers in Australia, South Africa, Chile, and France (2,10,20,37,48). Different studies have reported *D. seriata* to be associated with grapevine decline symptoms such as trunk and bark infections in Herzegovina, Yugoslavia (35), xylem necrosis in Italy (3,36), infectious drying of grapes in Ukraine (18), vine decline in Hungary (16,25), perennial cankers in Spain (1,47), "Black dead arm" in Lebanon (11), and darkened pith in New Zealand (4). However, Koch's postulates were not accomplished in most of these studies. Therefore, whether *D. seriata* is acting as a saprophyte or is a pathogen causing grapevine dieback symptoms has not yet been clarified in many grape-growing regions worldwide.

The purpose of this study was to identify and characterize, by means of morphological features, multigene phylogeny, and pathogenicity studies, the causal agents of grapevine dieback in Mexican vineyards.

MATERIAL AND METHODS

Field survey, disease symptoms, and fungal isolation. Field surveys were conducted in 2004 in 13 and 6 vineyards in Sonora and Baja California regions, respectively (Fig. 1). Diseased plants were characterized by dead arms and spur positions with poor or no shoot development. Depending on the disease progress, death of the entire part of the cordon or different portions could be observed while other parts of the vine showed normal and healthy growth. Canker development was observed to grow basipetally from putative points of infection. In all, 55 and 180 cankers were collected from infected arms, cordons, and trunks from different wine-grape cultivars (Cabernet Sauvignon, Carignane, Chenin Blanc, Sauvignon

blanc, and Petit Sirah) in Baja California and from table-grape cultivars (Flame Seedless, Perlette, Sugar One, Rubi Seedless, and Superior) in Sonora, respectively, and brought to the laboratory. Longitudinal and transversal cuts from symptomatic arms, cordons, and trunks were made to observe any internal symptom. Vascular symptoms were characterized by wedge-shaped cankers, which appeared to be associated with pruning wounds (Fig. 2a).

In addition to sectioning, diseased samples were surface sterilized in 10% sodium hypochlorite for 10 min. Surface tissue was cut away to expose the canker, and wood chips of approximately 1 cm² were cut from the margin of the dead tissue and cultured on 4% potato dextrose agar (PDA; Difco, BD Micro Biology Systems, Franklin Lakes, NJ) amended with 0.01% tetracycline hydrochloride (PDA-tet; Sigma-Aldrich, St. Louis). Cultures were incubated at 25°C until fungal colonies were observed. *Botryosphaeriaceae* isolates first were separated based on colony morphology by examination of the cultures after 5, 10, and 14 days. Fungal mycelium from *Botryosphaeriaceae* colonies was transferred to fresh PDA-tet petri plates and incubated at room temperature, and hyphal tips from 2-day-old cultures were aseptically placed on PDA petri plates using a dissecting microscope in order to obtain pure cultures. Isolates were incubated at 25°C in a 12-h fluorescent light-and-dark cycle. Based on colony and pycnidia morphology formed in culture of all *Botryosphaeriaceae* isolates inspected, 20 were selected for detailed identification to species level (Table 1).

Morphological characterization. In all, 15 isolates from both the Baja California and Sonora grapevine regions were used for morphological characterization

(Table 1). *Botryosphaeriaceae* spp. first were identified based on colony and conidial morphology and by comparing with previous published studies (29,33,34,43,45,47). Colony morphology of *Botryosphaeriaceae* isolates from Mexico was compared in the laboratory with previously identified *Botryosphaeriaceae* spp. from California (45) (Table 2). In order to enhance sporulation, cultures were placed on 2% water agar (Difco, BD Micro Biology Systems) containing autoclaved grapevine wood chips and incubated at 25°C under intermittent light (12 h). Isolates were examined weekly for formation of pycnidia and conidia. Conidial morphology (cell wall, shape, color, and presence or absence of septa) from pycnidia was recorded using a compound microscope (Nikon Inc. Instruments Group, Elville, NY). The length and width of 50 conidia per isolate were measured using the imaging device SPOT RT software (v3.5.1; SPOT; Diagnostic Instruments Inc., MI). Minimum, maximum, mean, standard deviation, and 95% confidence intervals were calculated from measurements using summary statistics in SAS (SAS System, version 8.1; SAS Institute, Cary, NC). Optimum growth temperature and mycelium growth rates were determined in nine isolates (Table 1) by placing a 4-mm-diameter plug from the growing margin of a 3-day-old colony in the center of an 85-mm-diameter PDA petri dish. Three replicates of each isolate were incubated separately in the dark at 5 to 40°C at 5°C intervals. Colony diameter was measured at 24-h intervals, and data were converted to daily radial growth in millimeters. The experiment was conducted twice. Isolates used in this study are maintained in the collection in the Department of Plant Pathology at the University of California, Davis, and representative isolates of each *Botryosphaeriaceae* sp. were deposited in the American Type Culture Collection.

DNA extraction, polymerase chain reaction amplification, and multigene phylogenetic analysis. Total genomic DNA from 20 *Botryosphaeriaceae* isolates (Table 1) was extracted from pure culture mycelia using an AquaPure Genomic DNA Kit (Bio-Rad Laboratories; Hercules, CA). Oligonucleotide primers ITS4 and ITS5 were used to amplify the ITS region of the nuclear ribosomal DNA, including the 5.8S gene (49). Oligonucleotide primers Bt2a and Bt2b were used to amplify a portion of the β -tubulin (BT) gene (15). Amplification of part of the translation elongation-factor (EF) gene was done with the primers EF1-728F and EF1-986R (8). Each polymerase chain reaction (PCR) contained 5 μ l of 10 $\times$ PCR buffer containing 15 mM MgCl₂, 2 μ l of 25 mM MgCl₂, 1 μ l of 10 mM dNTPs, 2 μ l of 0.5 mM of each primer, 0.25 μ l of *Taq* DNA polymerase (*Taq* PCR core kit; Qiagen, Valencia, CA) at 5 units/ μ l, and 2 μ l of template

Fig. 1. Map of Mexico indicating the wine-grape region of Baja California and the table-grape region of Sonora where field surveys were conducted.

Table 1. *Botryosphaeriaceae* isolates from *Vitis vinifera* from Mexico used in this study

Isolate	Identity ^b	Host (<i>V. vinifera</i> cvs.)	Origin ^c	GenBank accession number ^a			
				ITS	β -tubulin	EF1- α	ATCC ^d
UCD1010BC ^{e,f,g,h}	<i>Diplodia seriata</i>	Cabernet Sauvignon	Ensenada	EU012377	EU012429	EU012400	MYA-4188
UCD1015BC ^{e,g}	<i>D. seriata</i>	Carignane	Ensenada	EU012378	EU012430	EU012401	MYA-4189
UCD1035BC ^g	<i>D. seriata</i>	Chenin Blanc	Ensenada	EU012379	EU012431	EU012402	MYA-4190
UCD1038BC ^{e,f,g,h}	<i>D. seriata</i>	Chenin Blanc	Ensenada	EU012380	EU012432	EU012403	MYA-4191
UCD1052BC ^{e,g,h}	<i>D. seriata</i>	Sauvignon Blanc	Ensenada	EU012381	EU012433	EU012404	MYA-4192
UCD1061BC ^{e,f,g}	<i>D. seriata</i>	Petit Sirah	Ensenada	EU012382	EU012434	EU012405	MYA-4193
UCD1012BC ^{e,g}	<i>Lasiodiplodia theobromae</i>	Carignane	Ensenada	EU012372	EU012424	EU012392	MYA-4194
UCD1014BC ^{e,f,g,h}	<i>L. theobromae</i>	Carignane	Ensenada	EU012373	EU012425	EU012393	MYA-4195
UCD1028BC ^{e,g}	<i>L. theobromae</i>	Chenin Blanc	Ensenada	EU012374	EU012426	EU012394	MYA-4196
UCD1030BC ^{e,f,g,h}	<i>L. theobromae</i>	Chenin Blanc	Ensenada	EU012375	EU012427	EU012395	MYA-4197
UCD1060BC ^{e,f,g,h}	<i>L. theobromae</i>	Petit Sirah	Ensenada	EU012376	EU012428	EU012396	MYA-4198
UCD810SN ^{e,g}	<i>L. theobromae</i>	Flame Seedless	Hermosillo	EU012363	EU012415	EU012383	MYA-4199
UCD881SN ^g	<i>L. theobromae</i>	Perlette	Hermosillo	EU012364	EU012416	EU012384	MYA-4200
UCD914SN ^{e,f,g,h}	<i>L. theobromae</i>	Rubi Seedless	Hermosillo	EU012365	EU012417	EU012385	MYA-4202
UCD916SN ^g	<i>L. theobromae</i>	Sugar One	Hermosillo	EU012366	EU012418	EU012386	MYA-4203
UCD917SN ^g	<i>L. theobromae</i>	Sugar One	Hermosillo	EU012367	EU012419	EU012387	MYA-4201
UCD918SN ^{e,f,g,h}	<i>L. theobromae</i>	Sugar One	Hermosillo	EU012368	EU012420	EU012388	MYA-4204
UCD919SN ^{e,g,h}	<i>L. theobromae</i>	Sugar One	Hermosillo	EU012369	EU012421	EU012389	MYA-4205
UCD921SN ^g	<i>L. theobromae</i>	Sugar One	Hermosillo	EU012370	EU012422	EU012390	MYA-4206
UCD923SN ^{e,f,g}	<i>L. theobromae</i>	Sugar One	Hermosillo	EU012371	EU012423	EU012391	MYA-4207

^a ITS = internal transcribed spacer and EF1- α = elongation factor.^b *Botryosphaeriaceae* spp. from grapevine from Mexico were determined based on morphology and phylogenetic analyses.^c Isolates from Ensenada, Baja California, Mexico collected by G. Leavitt and J. Guevara in July 2004 and isolates from Hermosillo, Sonora, Mexico collected by J. R. Úrbez-Torres, G. Leavitt, and J. C. Guerrero in February 2004.^d ATCC = American Type Culture Collection.^e Isolates used for colony and conidial morphology.^f Isolates used for temperature studies.^g Isolates used for phylogenetic analyses.^h Isolates used for pathogenicity tests.**Table 2.** *Botryosphaeriaceae* isolates used in the phylogenetic study

Isolate ^b	Identity	Host ^c	Origin	Collector ^d	GenBank accession number ^a		
					ITS	β -tubulin	EF1- α
UCD191Co*	<i>Lasiodiplodia theobromae</i>	Vv cv. Flame Seedless	California, USA	J. R. Ú.-T. & G. L.	DQ008308	DQ008331	EU012397
UCD205Co*	<i>L. theobromae</i>	Vv cv. Thompson Seedless	California, USA	J. R. Ú.-T. & G. L.	DQ008310	DQ008333	EU012398
UCD206Co*	<i>L. theobromae</i>	Vv cv. Perlette	California, USA	J. R. Ú.-T. & G. L.	DQ008311	DQ008334	EU012399
CAA006	<i>L. theobromae</i>	<i>Vitis vinifera</i>	California, USA	T. J. Michailides	DQ458891	DQ458859	DQ458876
CMW9074	<i>L. theobromae</i>	<i>Pinus</i> sp.	Mexico	B. Slippers	AY236952	AY236930	AY236901
CMW10130	<i>L. theobromae</i>	<i>V. donniana</i>	Uganda	J. Roux	AY236951	AY236929	AY236900
CBS124.13	<i>L. theobromae</i>	Unknown	United States	J. J. Taubenhhaus	DQ458890	DQ458858	DQ458875
CBS115812	<i>L. gonubiensis</i>	<i>Syzygium cordatum</i>	South Africa	D. Pavlic	DQ458892	DQ458860	DQ458877
UCD244Ma*	<i>Diplodia seriata</i>	Vv cv. Thompson Seedless	California, USA	J. R. Ú.-T. & G. L.	DQ008314	DQ008337	EU012406
UCD352Mo*	<i>D. seriata</i>	Vv cv. Cabernet Sauvignon	California, USA	J. R. Ú.-T. & G. L.	DQ008315	DQ008338	EU012407
UCD614Tu*	<i>D. seriata</i>	Vv cv. Muscat	California, USA	J. R. Ú.-T. & G. L.	DQ008318	DQ008341	EU012408
UCD710SJ*	<i>D. seriata</i>	Vv cv. Zinfandel	California, USA	J. R. Ú.-T. & G. L.	DQ008321	DQ008344	EU012409
UCD770St*	<i>D. seriata</i>	Vv cv. French Colombard	California, USA	J. R. Ú.-T. & G. L.	DQ008322	DQ008345	EU012410
CMW7774	<i>D. seriata</i>	<i>Ribes</i> sp.	New York, USA	B. S. & G. H.	AY236953	AY236931	AY236902
CMW7775	<i>D. seriata</i>	<i>Ribes</i> sp.	New York, USA	B. S. & G. H.	AY236954	AY236932	AY236903
CMW8230	<i>D. seriata</i>	<i>Picea glauca</i>	Canada	J. Reid	AY972104	AY972119	DQ280418
CMW8232	<i>D. seriata</i>	<i>Malus domestica</i>	South Africa	W. A. Smith	AY972105	AY972120	DQ280419
CBS119049	<i>D. seriata</i>	<i>Vitis</i> sp.	Italy	L. Mugnai	DQ458889	DQ458857	DQ458874
UCD288Ma	<i>Diplodia mutila</i>	Vv cv. Thompson Seedless	California, USA	J. R. Ú.-T. & G. L.	DQ008313	DQ008336	EU012411
UCD1953SB	<i>D. mutila</i>	Vv cv. Chenin Blanc	California, USA	J. R. Ú.-T. & G. L.	DQ233598	DQ233619	EU012412
UCD1965SB	<i>D. mutila</i>	Vv cv. Chardonnay	California, USA	J. R. Ú.-T. & G. L.	DQ233599	DQ233620	EU012413
CMW7060	<i>D. mutila</i>	<i>Fraxinus excelsior</i>	The Netherlands	H. A. van der Aa	AY236955	AY236933	AY236904
CBS112554	<i>D. mutila</i>	<i>Pyrus communis</i>	Portugal	A. J. L. Phillips	AY259095	DQ458851	DQ458870
CBS230.30	<i>D. mutila</i>	<i>Phoenix dactylifera</i>	California, USA	L. L. Huillier	DQ458886	DQ458849	DQ458869
JL375	<i>D. mutila</i>	<i>Fraxinus excelsior</i>	Catalonia, Spain	J. Luque	DQ458887	DQ458852	DQ458871
CBS112547	<i>Diplodia corticola</i>	<i>Quercus ilex</i>	Córdoba, Spain	M. E. Sánchez	AY259110	DQ458854	DQ458872
UCD2057Te	<i>Neofusicoccum luteum</i>	Vv cv. Cabernet Sauvignon	California, USA	J. R. Ú.-T. & G. L.	DQ233604	DQ233625	EU012414
CBS110299	<i>N. luteum</i>	<i>V. vinifera</i>	Portugal	A.J.L. Phillips	AY928043	DQ458848	AY573217

^a ITS = internal transcribed spacer and EF1- α = elongation factor.^b Acronyms of cultures collections: UCD: University of California, Davis, Plant Pathology Department Culture Collection; CMW: Culture Collection Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CBS: Centraalbureau Schimmelcultures, Utrecht, Netherlands; * indicates isolates used for morphological comparison.^c Vv = *Vitis vinifera*.^d J. R. Ú.-T. & G. L. = J. R. Úrbez-Torres & G. Leavitt and B. S. & G. H. = B. Slippers & G. Hudler.

DNA adjusted with purified water (Mili-Q Water Systems; Millipore, Billerica, MA) to a final volume of 50 μ l. Amplification reactions were carried out in a thermal cycler (PTC 200; M. J. Research Company, Watertown, MA). ITS and BT temperature profiles were as follows: an initial preheat for 2 min at 94°C, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 58°C for 60 s, and extension at 72°C for 90 s. Translation EF temperature profiles were one cycle of initial denaturation at 95°C for 3 min; followed by 35 cycles of denaturation at 95°C for 30 s,

annealing at 55°C for 30 s, and extension at 72°C for 60 s; with a final extension at 72°C for 5 min. The PCR amplification products were separated by electrophoresis in 1.5% agarose gels in 1.0x Tris-boric acid-EDTA (TBE) buffer and photographed under UV light after staining with ethidium bromide for 15 min. PCR products were purified using a QIAquick PCR purification kit (Qiagen). ITS, BT2, and EF1- α regions were sequenced in both directions by the University of California, Davis, Division of Biological Sciences sequencing facility. Sequences were edited

and assembled using Sequencer (version 4.1; Gene Codes, Ann Arbor, MI). Sequences were aligned using the computer software BioEdit Sequencer Alignment Editor (version 7.0.0; Tom Hall, Isis Pharmaceuticals, Inc, Carlsbad, CA) and alignment gaps were treated as missing data. Sequences of *Botryosphaeriaceae* from Mexico were compared with those of *Botryosphaeriaceae* from previous studies available in GenBank (Table 2). The alignment was corrected by visual inspection, and any ambiguously aligned characters were deleted using BioEdit. A partition

Fig. 2. Disease symptoms and *Lasiodiplodia theobromae* and *Diplodia seriata* colony and conidial morphology. **a**, Cross section of a 14-year-old Thompson Seedless grapevine cord. Wedge-shaped canker was the primary vascular symptom observed in grapevines in both Baja California and Sonora grape-growing regions. **b**, Colony morphology of 21-day-old *L. theobromae* (UCD919SN). **c**, Conidiogenous cells and young hyaline and thick-walled *L. theobromae* (UCD918SN) conidia. Nonseptate paraphyses are indicated by black arrows. **d**, Dark-brown mature *L. theobromae* (UCD918SN) conidia. Longitudinal striations and central septum can be observed. **e**, Colony morphology of 21-day-old *D. seriata* (UCD1061BC). **f**, Light-brown mature *D. seriata* (UCD1061BC) conidia. Conidial photographs were taken at $\times 100$ (immersion oil) from pycnidia formed on grapevine wood. Scale bar = 10 μ m.

homogeneity test was performed with PAUP (version 4.0b10; Sinauer Associates, Inc., Publishers, Sunderland, MA; 42) to determine whether the ITS, β -tubulin, and EF1- α datasets could be combined together. Separate phylogenetic analyses also were performed for the ITS dataset alone, β -tubulin dataset alone, and EF1- α dataset alone, and tree topologies were compared. Maximum parsimony analyses was performed in PAUP using the heuristic search option branch swapping, and 1,000 random addition sequences replicates. Bootstrap values were calculated using 1,000 replicates to test branch strength. Tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) also were recorded. Resulting trees were printed in PAUP version 4.0b10. *N. luteum* sequences from GenBank were used as outgroup (Table 2). The ITS, β -tubulin, and EF1- α sequences from *Botryosphaeriaceae* from Mexico used in this study were deposited into GenBank.

Pathogenicity tests. Nine *Botryosphaeriaceae* isolates were used in two separate tests to determine the pathogenicity of *L. theobromae* and *D. seriata* from Mexico on grapevines (Table 1). An initial pathogenicity test was conducted in 1-year-old grapevine cuttings of cvs. Chardonnay and Thompson Seedless. In all, 100 dormant cuttings of each cultivar were cut into uniform lengths containing six to seven buds. A basal cut was made just below the lower bud and the upper cut 2 cm above the top bud. In order to enhance callusing and rooting, dormant cuttings were buried into

a 3:1 soil:vermiculite mix in plastic boxes, and placed in a callusing room at 35°C and 80% humidity for 4 weeks at the Viticulture Experimental Station of the University of California, Davis. After callusing and rooting, cuttings were wounded at the uppermost internode with a 4-mm cork borer. A 4-mm mycelium agar plug from a 1-week-old culture was placed in the wound. Wounds first were covered with 100% pure Vaseline petroleum jelly (Unilever, Greenwich, CT) and wrapped with parafilm. In all, 10 cuttings per fungal isolate were used for each cultivar. Ten cuttings of each cultivar were inoculated with 4-mm noncolonized PDA agar plugs from two different plates for negative controls. Inoculated cuttings were planted immediately in individual pots and placed in a lathhouse at the University of California Experimental Station in Davis during the last week of April. Plants were arranged in a completely randomized design. Cuttings were maintained under ambient environmental conditions and watered every 3 days or as needed. Cuttings were collected after 20 weeks and inspected for lesion development. Extent of vascular discoloration was measured upward and downward from the point of inoculation. Small pieces (0.5 to 1 cm) of necrotic tissue from the edge of each lesion were cut and placed on PDA-tet petri plates in an attempt to recover the inoculated fungus and complete Koch's postulates. Fungal identity was verified by its colony and conidial morphology.

A second pathogenicity test, using the same nine *Botryosphaeriaceae* isolates

from Mexico (Table 1), was conducted on cvs. Chardonnay and Thompson Seedless green shoots. In all, 100 green shoots of approximately 30 cm in length from each cultivar were cut from vines at the Experimental Station of the University of California, Davis, and immediately inoculated as previously described. Ten green shoots of each cultivar were used per fungal isolate. Ten green shoots of each cultivar were inoculated with 4-mm noncolonized PDA agar plugs from two different plates for negative controls. Green shoots were placed in sterile water into 50-ml screw-cap tubes (Sarstedt, Inc., Newton, NC) and maintained for 10 days at room temperature on the laboratory bench. Afterward, green shoots were sectioned longitudinally, vascular discoloration was recorded, and green tissue reisolations were made as described above.

One-way analyses of variance (ANOVA) in SAS (SAS System, version 8.1; SAS Institute) was performed in order to assess differences in the extent of vascular discoloration induced by *L. theobromae* and *D. seriata* for both hardwood and green tissue pathogenicity tests in both cultivars. To satisfy the assumptions of the ANOVA, the $\log_{10}$ transformation of the data was used. Homogeneity of variance was assessed using Levene's test. The Tukey's test was used for comparison of treatment means at $P < 0.05$. A two-way ANOVA was performed to determine significant differences between cultivars in inoculation of both 1-year-old cuttings and green shoots treatments.

Table 3. Morphological description of *Lasiodiplodia theobromae* and *Diplodia seriata* isolates from Mexico.

Identity, isolate	Conidial dimensions		Temperature study	
	Conidial size (μm) ^a	L/W ratio ^b	Opt. (°C) ^c	Growth rate ^d
<i>D. seriata</i> ^e				
UCD1010BC	(29.2–) 23.6–16.8 (–12.6) $\times$ (12.8) 10.7–8.4 (–7.4)	2.1	25–30	17.5
UCD1015BC	(26.7–) 22.6–17.6 (–14.5) $\times$ (12) 11.4–8.4 (–7.9)	2.03	...	...
UCD1038BC	(26.6–) 23.6–18 (–13.2) $\times$ (12.7) 11.1–8.7 (–7.2)	2.1	25–30	26.3
UCD1052BC	(24.2–) 22.1–18.5 (–16.7) $\times$ (12.9) 10.6–8.3 (–7.1)	2.13	...	...
UCD1061BC	(28.7–) 22.7–17.1 (–13.1) $\times$ (11) 10.1–8.3 (–7.1)	2.16	25–30	19.8
<i>L. theobromae</i> ^f				
UCD1012BC	(28.5–) 26.7–22.5 (–19.9) $\times$ (17.8) 14.6–12.4 (–11.5)	1.8	...	...
UCD1014BC	(37.3–) 28.7–22.1 (–14.9) $\times$ (16.3) 13.9–11.9 (–10)	1.9	30–35	42.5
UCD1028BC	(28.3–) 26.2–22.6 (–19.7) $\times$ (16.4) 14.7–12.3 (–11.4)	1.8	...	...
UCD1030BC	(31.6–) 26.8–22.2 (–17.7) $\times$ (16.6) 14.5–12.3 (–11.2)	1.8	30–35	37.2
UCD1060BC	(29.3–) 27.2–23.4 (–21.7) $\times$ (15.7) 14.1–12.3 (–11.1)	1.9	35	42.5
UCD810SN	(30.1–) 27.8–23 (–20.5) $\times$ (14.9) 13.8–11.8 (–10)	2	...	...
UCD914SN	(30.9–) 27.6–22.8 (–19.8) $\times$ (15.2) 13.8–11.2 (–9.8)	2	35	36.9
UCD918SN	(29.1–) 27.4–24 (–21.8) $\times$ (14.9) 13.9–11.7 (–10.8)	2	30–35	42.5
UCD919SN	(34.5–) 28.3–23.1 (–20.1) $\times$ (16.7) 13.5–10.7 (–9.1)	2.1	...	...
UCD923SN	(34.4–) 28.8–23.6 (–21.4) $\times$ (15) 13.1–10.5 (–9.3)	2.2	35	42.5

^a Data are represented as the lower and upper 95% confidence limits, with maximum and minimum dimensions in parenthesis.

^b L/W = length/width.

^c Optimum temperature is defined as the temperature that produced maximum radial growth after 48 h.

^d Maximum mycelium radial growth in millimeters after 48 h.

^e Colony morphology: Moderate aerial mycelium. Light-green colonies that became dark green with age. Colonies produced many black, single, small (0.5–1 mm in diameter), and ostiolate pycnidia. Conidial morphology: Elliptical-rounded with one of the bases truncated. Immature conidia initially hyaline and thick-walled. Light brown to dark brown when mature. Aseptate conidia.

^f Colony morphology: Abundant aerial mycelium. White colonies with a light-green center. Entire colony became dark-green with age. Colonies produced black, grouped, large (up to 7 mm diameter), ostiolate, and globose pycnidia, embedded in stroma. Conidial morphology: Oval with rounded and pointed ends. Immature conidia hyaline, thick-walled, aseptate and densely granulated. Aseptate paraphyses. Conidia dark brown with longitudinal striations and one septum when mature.

RESULTS

Field survey. *Botryosphaeriaceae* spp. were the most common fungi isolated from grapevine perennial cankers in both Baja California and Sonora grape-growing regions. *D. seriata* was found in all vineyards surveyed in Baja California and was isolated from 21 of 55 perennial cankers (38%). *L. theobromae* was found in four of six vineyards in Baja California and was isolated from 9 of 55 grapevine cankers (16%). In this study, *D. seriata* and *L. theobromae* were not isolated together from the same canker in any of the samples from Baja California. Other sporadically isolated fungi from cankers in Baja California (5%) were species of *Alternaria* and *Aspergillus*. *L. theobromae* was the only fungus isolated from grapevine cankers in Sonora region. *L. theobromae* was found in all vineyards sampled in Sonora and was isolated from 140 of 180 grapevine cankers (80.5%).

Morphological characterization. Isolates of *Botryosphaeriaceae* from Baja California and Sonora were separated into two groups based on their appearance in culture, conidial morphology and size, and optimum growth temperature (Table 3; Fig. 2). Based on the morphological characters observed and by comparing them with those previously reported (2,20,29,33,34,43,45,47), isolates from Mexico were identified as *L. theobromae* (Fig. 2b–d) and *D. seriata* (Fig. 2e and f).

Phylogenetic analysis. ITS, β -tubulin, and EF1- α sequences of *L. theobromae* and *D. seriata* from grapevines from Mexico (Table 1) were aligned with GenBank ITS, β -tubulin, and EF1- α sequences of *Botryosphaeriaceae* spp. from grapevines and other hosts from different countries (Table 2). After alignment, a partition homogeneity test showed a value of $P = 0.09$, indicating that the ITS, β -tubulin, and EF1- α datasets were congruent ($P > 0.05$) and could be combined in a single phylogenetic analysis. The combined dataset consisted of 1,246 characters, of which 954 were constant, 52 were parsimony uninformative, and 240 were parsimony informative. The maximum parsimony analyses yielded one most parsimonious tree (length = 477, CI = 0.786, RI = 0.955, RC = 0.751, and HI = 0.214; Fig. 3). The combined data set phylogenetic tree included two well-separated clades. *L. theobromae* isolates formed a strongly supported clade, with bootstrap value of 100% (Fig. 3). *L. theobromae* isolates from Mexico had nearly identical sequences and were grouped together with *L. theobromae* isolates from California (Fig. 3). *D. seriata* isolates from Mexico resided in a well-supported separate clade with bootstrap value of 100% (Fig. 3). *D. seriata* isolates from Mexico grouped together with *D. seriata* isolates from California, New York, Canada, South Africa, and Italy, showing almost no variation in the DNA sequences

(Fig. 3). Analyses of the ITS, β -tubulin, and EF1- α datasets alone yielded the same tree topology as the combined dataset (*trees not shown*), and the only differences between trees generated from different datasets were changes in the positions of some isolates within one of the main clades. Results from both the combined and single datasets of the ITS, β -tubulin, and EF1- α DNA sequences verified the morphological identifications of *L. theobromae* and *D. seriata* from grapevines from Mexico.

Pathogenicity tests. Mean lengths of the extent of vascular discolorations caused by *L. theobromae* and *D. seriata* isolates from Mexico on inoculated 1-year-old cuttings and green shoots are shown in Figure 4. *L. theobromae* and *D. seriata* produced dark-brown, black necrotic lesions on both 1-year-old cuttings and green shoots, which extended upward and downward from the point of inoculation (Fig. 5b, e, and f). Dark wood streaking or discoloration also was observed in green shoots and 1-year-old cuttings of both cultivars inoculated with *L. theobromae* (UCD914SN, UCD921SN, and UCD1014BC) and *D. seriata* (UCD1015BC, UCD1038BC, and UCD1061BC) (Fig. 5eI). Differences in susceptibility between *L. theobromae* and *D. seriata* in 1-year-old Chardonnay cuttings were evident. Chardonnay cuttings inoculated with *L. theobromae* isolates rarely developed spring growth and, when developed, young shoots, petioles, and leaves died back and dried out very rapidly. Chardonnay cuttings inoculated with *D. seriata* isolates developed healthy spring growth which did not differ from the control (Fig. 5a). Wedge-shaped cankers and necrotic tissue produced by both species were observed when cross sections were made in 1-year-old cuttings and green shoots, respectively (Fig. 5c, d, g, and h). Pycnidia were observed on both wood and green shoot surfaces in plants inoculated only with all *L. theobromae* isolates but not with the *D. seriata* isolates (Fig. 5i).

L. theobromae and *D. seriata* isolates used in this study were pathogenic and caused longer basipetal than acropetal lesions in all treatments (Fig. 4). *L. theobromae* isolates from Mexico were more virulent and produced significantly longer basipetal lesions in all inoculated plants than those of *D. seriata* (Figs. 4 and Fig. 5a and f). *D. seriata* isolates produced substantially smaller lesions than those caused by *L. theobromae* in all inoculated 1-year-old cuttings and green shoots but still differed significantly in symptom expression from control treatments (Fig. 4). However, two *D. seriata* isolates (UCD1010BC and UCD1038BC) did not cause lesions in 1-year-old Chardonnay cuttings (Fig. 4A). *L. theobromae* caused significantly ($F = 40.89$, $DF = 9$, $P < 0.0001$) longer lesions (range from 301.1

to 338.3 mm) in 1-year-old Chardonnay cuttings than those in Thompson Seedless (range from 71.2 to 183.1 mm) (Fig. 4A and B). Lesions caused by *L. theobromae* in Thompson Seedless green shoots ranged from 58.6 to 85.4 mm and differed significantly ($F = 5.1$, $DF = 9$, $P = 0.0254$) from the lesions caused in Chardonnay (range from 20.9 to 45.5 mm) (Fig. 4C and D). *D. seriata* isolates from Mexico showed no significant differences ($F = 0.35$, $DF = 9$, $P = 0.555$) in the extent of vascular discoloration between cultivars in both 1-year-old cuttings and green shoots (Fig. 4A–D). Lesions caused by *D. seriata* in 1-year-old Chardonnay and Thompson Seedless cuttings varied from 21.8 to 50.9 and 36.8 to 49.6 mm, respectively. *D. seriata* caused lesions on green Chardonnay and Thompson Seedless shoots that ranged from 4.2 to 6.2 and 3.7 to 8.7 mm, respectively. *L. theobromae* was reisolated from infected tissue in both 1-year-old cuttings and green shoots in 100% of the samples. *D. seriata* was reisolated in 100% of the samples from 1-year-old cuttings and between 80 to 90% from green shoots. No *Botryosphaeriaceae* fungi were reisolated from the control treatments.

DISCUSSION

Botryosphaeriaceae spp. recently have been shown to be a more prevalent and important wood pathogen on grapevines than originally thought. In total, 14 different *Botryosphaeriaceae* spp. have been described associated with grapevine dieback from the most important grape-growing areas of North and South America (2,14,21,24,27,38,45,46), Europe (1,20,25,26,29,47), Africa (13,48), Asia (19), and Oceania (4,10,37,43,50). The present study has shown that two *Botryosphaeriaceae* spp., *L. theobromae* and *D. seriata*, also are associated with grapevine cankers in Mexico.

Perennial cankers and grapevine dieback have been known to occur in Mexico since the late 1970s, when Eutypa dieback was associated with grapevine decline in old and abandoned vineyards in the grape-growing areas of Coahuila-Durango and Aguascalientes (44). However, this association was based mainly on the discovery of *E. lata* perithecia in old pruning wounds, and no confirmation of the fungus isolated from declining vines and cankers was reported. Therefore, future field surveys and canker isolations from those grapevine areas may reveal the presence of other fungal pathogens (in addition to *E. lata*), such as species of *Botryosphaeriaceae* associated with the dieback symptoms reported in the past. Because Eutypa dieback of grapevines has been known and well studied for more than 30 years and because cankers caused by both *E. lata* and *Botryosphaeriaceae* spp. are indistinguishable, *E. lata* has been considered as the primary canker causing agent of grapes

worldwide. Our surveys in Sonora and Baja California have shown cankers, dead spurs with lack of spring growth, and dead cordons as the primary symptoms on declining vines. Characteristic Eutypa die-back symptoms such as stunted shoots, shortened internodes, and chlorotic and abnormal growth of the leaves were not observed during our sample collections in either the Sonora or Baja California regions. Furthermore, *L. theobromae* and *D. seriata* were the main fungi isolated from canker lesions in the current study whereas *E. lata* was not recovered from any of the cankers collected. Cankers produced by *E. lata* are rarely observed in vineyards

younger than 10 years old due to the slow movement of the fungus through the vascular system following infection of a pruning wound (9). In the current study, we isolated *L. theobromae* from cankers collected from vineyards 5 to 7 years old in the Sonora region. This result agrees with a previous study conducted by Leavitt, in which *L. theobromae* commonly was isolated from cankers on 5-year-old grapevines in Southern California (23). These results suggest that *L. theobromae* is a faster wood colonizer than *E. lata*.

The dominance of *Botryosphaeriaceae* spp. and the absence of *E. lata* from grapevines in Baja California and Sonora

may be due to climatic conditions. *E. lata* perithecia commonly are found in old vines from grape regions with an annual precipitation over 600 mm but rarely are found in areas with precipitation lower than 300 mm per year (9). Weather data recorded during the last 50 years show much lower annual precipitation in both the Ensenada (283 mm) and Hermosillo (240 mm) surveyed grapevine areas than in Aguascalientes (537 mm), from which *E. lata* perithecia mainly were reported to occur in Mexico. Similarly, field surveys conducted throughout the warm and desert areas of Southern California and Western and Eastern Australia have reported isola-

Fig. 3. Most equally parsimonious tree with bootstrap values obtained from the combined internal transcribed spacer, β -tubulin, and elongation factor-1 sequence data using 1,000 replicates generated in PAUP 4.0b10. Asterisks show *Botryosphaeriaceae* spp. from *Vitis vinifera*. Isolates from Mexico are indicated in bold.

tion of *Botryosphaeriaceae* spp. but no *E. lata* from cankers and necrotic lesions (10,23,37,43,45). However, other environmental factors, the lack of many of the susceptible hosts to *E. lata* in desert areas, as well as the possibility that *E. lata* has not been yet introduced in Baja California and Sonora also could explain the dominance of *Botryosphaeriaceae* spp. in these regions.

Morphological characteristics combined with analyses of DNA sequences allowed us to identify and characterize *L. theobromae* and *D. seriata* from grapevine cankers from Mexico. Colony and conidial morphology were the most helpful features with which to identify and distinguish *L. theobromae* from *D. seriata*. *L. theobromae* isolates used in this study grew much faster than those of *D. seriata* and were able to fully colonize an 85-mm-diameter petri plate in 48 h. Furthermore, isolates of *L. theobromae* showed a higher optimum growth temperature than those of *D. seriata*. These results are in agreement with a previous temperature study conducted with *Botryosphaeriaceae* isolates from California, in which *L. theobromae* and *D. seriata* isolates achieved maximum growth rates at 31 and 26°C, respectively (45). Conidial shape and color and the presence of septa and longitudinal striations were robust characteristics for identification and separation of *L. theobromae* from *D. seriata*.

The utility of these morphological distinctions is in agreement with previous literature (23,32,43,45,50). Another important morphological characteristic for the identification of *L. theobromae* was the presence of aseptate paraphyses observed in immature pycnidia. This feature was reported in a recent study, in which aseptate paraphyses along with conidial size were the primary morphological characteristics to separate *L. theobromae* from other *Lasiodiplodia* spp. found in the tropics (7).

DNA sequence comparisons allowed us to verify the morphological identification of *L. theobromae* and *D. seriata* from grapevines in Mexico. Results of the single as well as the combined ITS, β -tubulin, and EF1- α phylogenetic analyses clearly separated *L. theobromae* isolates from *D. seriata* isolates. The remaining *Botryosphaeriaceae* isolates with *Diplodia*-like anamorphs that were included in the analyses, such as *D. mutila* and *D. corticola*, clustered together within the *D. seriata* clade. In our study, *L. theobromae* from Mexico grouped together with *L. theobromae* isolates from the desert area known as the Coachella Valley in California, reinforcing the idea that this fungus is prevalent in regions with high temperatures (17,23,32,45). Furthermore, the similarity between *L. theobromae* sequences from grapevines in Mexico and California could support the hypothesis that this species

was introduced accidentally in Mexico from California or vice versa. However, more intensive sampling and, perhaps, also more sensitive measures of interisolate relationships, such as microsatellites or vegetative compatibility studies, would be required to test this hypothesis. *L. theobromae* isolates from Mexico and California differed from isolates CMW9074 and CMW10130 from South Africa and isolate CBS124.13 from an unknown origin in the United States. Host and geographical differences could explain the variation in the DNA sequences between these isolates. In contrast, *D. seriata* isolates from Mexico grouped together with *D. seriata* isolates from other geographical regions and different hosts showing almost no variation in the DNA sequences. This result agrees with previous phylogenetic studies conducted with *D. seriata* isolates from California (45) and may explain the cosmopolitan distribution of *D. seriata* worldwide.

Pathogenicity tests conducted in 1-year-old cuttings and green shoots of cvs. Chardonnay and Thompson Seedless confirmed that *L. theobromae* and *D. seriata* isolates from Mexico were pathogenic. In these tests, *L. theobromae* isolates produced much larger lesions than those of *D. seriata* in all inoculated 1-year-old cuttings and green shoots. These results are consistent with previous pathogenicity trials

Fig. 4. Pathogenicity study of *Lasiodiplodia theobromae* and *Diplodia seriata* in 1-year-old cuttings and green shoots of cvs. Chardonnay and Thompson Seedless. Mean lesion length is based on 10 replicates per isolate. Means followed by different letters differ significantly ($P < 0.05$) according to Tukey's test. Bars represent standard error of the mean.

Fig. 5. Pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata* on **a**, 1-year-old Chardonnay cuttings 8 weeks after inoculation (aI = *L. theobromae* UCD914SN, aII = *D. seriata* UCD1052BC, and aIII = control). White arrows show the point of inoculation. **b**, Vascular lesion caused by *L. theobromae* (UCD1060BC) in 1-year-old Chardonnay cutting 8 weeks after inoculation. Dark-brown lesion can be observed upward and downward from the point of inoculation causing the death of the wood and leaf dieback at the last internode. Canker continues growing basipetally throughout the second internode as shown by the dashed white arrow. **c**, Cross-section in 1-year-old Chardonnay cutting showing a young wedge-shaped canker caused by *D. seriata* (UCD1052BC) 20 weeks after inoculations. Black arrow shows the edge of the canker. **d**, Cross-section in 1-year-old Chardonnay cutting showing the canker caused by *L. theobromae* (UCD919SN) 20 weeks after inoculations. Light-gray area shows dead wood caused by the total vascular colonization of the fungus. Dark-brown area shows the fungus still growing and colonizing healthy vascular tissue. **e**, Lesions caused in 1-year-old Thompson Seedless cuttings 20 weeks after inoculation (eI = *L. theobromae* UCD914SN, eII = *D. seriata* UCD1052BC, and eIII = control). White arrow shows black wood streaking caused by *L. theobromae* moving downward the point of inoculation. No lesions were observed on the noncolonized inoculated controls. **f**, Lesions caused on Thompson Seedless green shoots 10 days after inoculation (fI = *L. theobromae* UCD918SN, fII = *D. seriata* UCD1038BC, and fIII = control). **g**, Cross-section in a Thompson Seedless green shoot showing the necrotic tissue caused by *L. theobromae* (UCD1060BC) 10 days after inoculation. **h**, Cross-section in a Thompson Seedless green shoot showing a smaller necrotic lesion caused by *D. seriata* (UCD1052BC) 10 days after inoculation. **i**, Pycnidia of *L. theobromae* (UCD914SN) formed on Chardonnay green shoot surface 10 days after inoculation.

conducted in California and South Africa, in which *L. theobromae* was shown to be one of the most virulent species tested on mature grapevine canes (23,48). However, differences in isolates of *L. theobromae* also have been reported from a recent study conducted in Western Australia, where only one of seven *L. theobromae* isolates produced lesions significantly different from the noninoculated controls (43).

In the current study, 1-year-old Chardonnay cuttings were more susceptible to infection by *L. theobromae* than Thompson Seedless. The table-grape cv. Thompson Seedless is recommended in grape regions with more than 4,000 degree-days per year. Consequently, Thompson Seedless has long been planted in the warm and desert grape-growing areas of Southern California and Northern Mexico where *L. theobromae* commonly is found. Similar results were obtained in a pathogenicity study in Western Australia, in which *L. theobromae* isolates were avirulent or only weakly pathogenic in inoculated Red Globe mature canes (43). Red Globe is another table-grape cultivar widely planted in warm regions with more than 4,000 degree-days per year. Thompson Seedless green shoots were more susceptible to infection by *L. theobromae* than were Chardonnay shoots. The reason for this difference in susceptibility is still unknown. Furthermore, the lack of multicultivar *Botryosphaeriaceae* pathogenicity tests limits comparison of our results to those of previous studies. More field inoculations of diverse cultivars are needed to better characterize the range of susceptibilities.

D. seriata isolates from Mexico were much less pathogenic than those of *L. theobromae* but still capable of causing larger lesions than were evident in the noninoculated controls. This result agrees with previous pathogenicity studies conducted in South Africa, the New South Wales region in Australia, Eastern Australia, Chile, and France, in which *D. seriata* has been reported to be pathogenic in grapevines (2,10,20,37,48). In contrast, *D. seriata* isolates from grapevines in Western Australia were found to be nonpathogenic (43). We found *D. seriata* isolates from Mexico to be more pathogenic on 1-year-old cuttings than on green shoots. Differences in pathogenicity and virulence also have been reported among *D. seriata* isolates in South Africa, Eastern Australia, and France (20,37,48). Furthermore, in France, Chile, and Lebanon, *D. seriata* has been associated with a disease called "Black dead arm" characterized by light-brown wood streaking and red patches at the margin of leaves, with large areas of chlorosis and deterioration between the veins (2,11,20). The symptoms observed in Baja California and Sonora were not those associated with Black dead arm. This

agrees with previous studies conducted in California and Portugal (28,45), which found that there were no foliar symptoms associated with this pathogen. Variable reports on the pathogenicity of *D. seriata* from different grape-growing regions throughout the world may reflect differences in (i) virulence of the isolate used, (ii) cultivar susceptibilities, (iii) inoculation methods, or (iv) incubation times, among other possibilities. Results from this study have confirmed *L. theobromae* and *D. seriata* as the primary causal agents of Bot canker of grapevines in Mexico, indicating the important role that these fungi can play in grapevine health, in general.

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