Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Enhancing epidemiological knowledge of *Botryosphaeriaceae* in Mexican vineyards

Edelweiss A. RANGEL-MONTOYA[1,2], Ofelia CANDOLFI-ARBALLO[3], José Abraham OBRADOR-SÁNCHEZ[4], Cesar VALENZUELA-SOLANO[5], Rufina HERNANDEZ-MARTINEZ[1,*]

[1] *Departamento de Microbiología, Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), Ensenada, Baja California, 22860, Mexico*
[2] *Facultad de Ciencias Químicas e Ingeniería campus Tijuana, Universidad Autónoma de Baja California. Tijuana, Baja California, 22390. Mexico*
[3] *Departamento de parasitología, Universidad Autónoma Agraria Antonio Narro Unidad Laguna (UAAAN-UL), Torreón, Coahuila, 27059, México*
[4] *Facultad de Ciencias de la Salud campus Valle Dorado. Universidad Autónoma de Baja California. Valle Dorado, 22890, Mexico*
[5] *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Campo Experimental Costa de Ensenada, Ensenada, Baja California, 22880, Mexico*
*Corresponding author. E-mail: ruhernan@cicese.mx

Summary. Grapevine cultivation in Mexico is important, especially in the states of Baja California and Coahuila, which are the main wine production regions in the country. Grapevine trunk diseases (GTDs) impact productivity and cause substantial economic losses, with Botryosphaeria dieback being one of the most destructive. This disease is caused by fungi in the *Botryosphaeriaceae*, including species of *Botryosphaeria*, *Diplodia*, *Lasiodiplodia*, and *Neofusicoccum*. To date, *Lasiodiplodia* spp. are the primary *Botryosphaeriaceae* fungi reported in Mexico. The present study aimed to enhance the epidemiological knowledge of *Botryosphaeriaceae* in Mexican vineyards. Samples from grapevine plants exhibiting disease symptoms were collected from the states of Baja California and Coahuila. Of a total of 37 *Botryosphaeriaceae* isolates, six species were identified: *Neofusicoccum parvum*, *N. australe*, *N. vitifusiforme*, *Botryosphaeria dothidea*, *Diplodia corticola*, and *D. seriata*. *Neofusicoccum parvum* isolates were the most virulent, but were less virulent than previously reported *Lasiodiplodia* spp. The optimum growth temperatures for *N. parvum* and *B. dothidea* were from 28 to 30°C, but 25°C for *D. seriata, N. vitifusiforme, and N. australe* isolates. Only *D. seriata* isolates recovered growth when transferred to room temperature after exposure to 37°C or 40°C. This report is the first identification of *B. dothidea* and *N. parvum* as causative agents of Botryosphaeria dieback in the vine-growing regions of Mexico.

Keywords. Trunk disease fungi, Botryosphaeria canker, fungi.

OPEN ACCESS

Citation: Rangel-Montoya, E.A., Candolfi-Arballo, O., Obrador-Sánchez, J.A., Valenzuela-Solano, C., & Hernandez-Martinez, R. (2024). Enhancing epidemiological knowledge of *Botryosphaeriaceae* in Mexican vineyards. *Phytopathologia Mediterranea* 63(2): 191-206. doi: 10.36253/phyto-15292

Accepted: June 3, 2024

Published: July 17, 2024

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: José R. Úrbez Torres, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada.

ORCID:
EAR-M: 0000-0002-2362-9229
OC-A: 0000-0001-8687-2915
JAO-S: 0000-0002-6531-2019
CV-S: 0000-0003-3030-6038
RH-M: 0000-0002-0914-3732

INTRODUCTION

In Mexico, the grapevine cultivation sector is economically important. In 2022, total grape production exceeded 450,000 tons, with Sonora, Zacatecas,

Baja California, Aguascalientes, and Coahuila being the foremost producers (SIAP, 2023). Despite regulations governing importation of grapevine planting materials, Mexico still relies on these imports, posing ongoing threats of introducing pathogens from foreign sources. Many factors may be involved in the emergence of these diseases, so that grapevine trunk diseases (GTDs) have emerged as an international problem with implications for productivity and significant economic losses (van Niekerk *et al*., 2006; Fontaine *et al*., 2016; Gramaje *et al*., 2018; Hrycan *et al*., 2020). Botryosphaeria dieback is one of the most destructive GTDs. This is a degenerative disease caused by fungi within the *Botryosphaeriaceae*. Botryosphaeria dieback has been linked to over 30 species in the genera *Botryosphaeria, Diplodia, Dothiorella, Lasiodiplodia, Neoscytalidium, Neofusicoccum, Phaeobotryosphaeria*, and *Spencermartinsia*. This extensive species range highlights the complexity of this disease, as has been documented in several studies (Úrbez-Torres, 2011; Rolshausen *et al*., 2013; Stempien *et al*., 2017; Gramaje *et al*., 2018).

Botryosphaeriaceae fungi cause necrotic lesions in grapevine vascular tissues, including xylem and phloem occlusions, as well as stunted growth, wedge-shaped cankers in woody trunks, dieback, and over time, plant death (Gramaje and Armengol, 2011; Úrbez-Torres, 2011; Bertsch *et al*., 2013; Hrycan *et al*., 2020). The primary source of inoculum for these pathogens is conidia, which are released and dispersed under conditions of high humidity, rainfall, and wind. Conidia enter plants through pruning wounds (Agustí-Brisach and Armengol, 2013; Gramaje *et al*., 2018; Waite *et al*., 2018). *Botryosphaeriaceae* spp. can exist as endophytes within asymptomatic plant tissues for extended periods, functioning as latent pathogens (Slippers and Wingfield, 2007). This characteristic is important, as infected plants can go undetected in nurseries or vineyards (Hrycan *et al*., 2020). The transition of *Botryosphaeriaceae* species from endophytic to pathogenic has been associated with environmental stress, particularly when host plant undergoes water or heat stresses (Slippers *et al*., 2007; Czemmel *et al*., 2015; Rathnayaka *et al*., 2023). Botryosphaeria dieback was previously primarily associated with older vineyards (Gubler *et al*., 2005), but this disease has also been found in young grapevines (Gramaje and Armengol, 2011; Bertsch *et al*., 2013). In the context of climate change, grapevines are likely to be subjected to consistent heat and water stresses, making them susceptible to trunk diseases (GTDs) (Fontaine *et al*., 2016; Mehl *et al*., 2017). Inadequate cultural practices, such as insufficient protection of pruning wounds and inadequate sanitary care of propagation material, can contribute to GTD proliferation (Graniti *et al*., 2000; Fontaine *et al*., 2016).

GTDs were first reported in Mexico in the late 1970s, when fungi linked to Eutypa dieback were identified in aged and neglected vineyards located in Coahuila, Durango and Aguascalientes (Téliz and Valle, 1979). Subsequently, Úrbez-Torres *et al*. (2008) identified the presence of *L. theobromae* and *D. seriata* associated with Botryosphaeria dieback in Baja California and Sonora. Following this, Candolfi-Arballo *et al*. (2010), found four *Botryosphaeriaceae* spp., *D. seriata*, *D. corticola*, *N. vitifusiforme*, and *N. australe*, in vineyards of Baja California. Later, Paolinelli-Alfonso *et al*. (2015) reported *Eutypella microtheca* associated with Eutypa dieback in Baja California. Most recently, Rangel Montoya *et al*. (2021) described species of *Lasiodiplodia* such as *L. crassispora*, *L. brasiliensis*, *L. exigua*, and *L. gilanensis* associated with Botryosphaeria dieback in Baja California and Sonora.

The status of GTDs is understudied in Mexico, so the present study aimed to broaden understanding of the fungi associated with Botryosphaeria canker present in Mexican vineyards, and compare their pathogenicity in grapevines.

MATERIALS AND METHODS

Isolation and morphological characterization of fungi

Samples of grapevine exhibiting Botryosphaeria dieback symptoms were collected from seventeen vineyards in the primary grape-producing regions of Baja California, and three vineyards from Coahuila. Trained personnel from the plant pathology laboratory of CICESE collected samples of wood pieces exhibiting wedge-shaped cankers. Approximately 100 samples were obtained and processed as follows. Small pieces of tissue were obtained, immersed in 95% ethanol, then quickly flamed, and placed onto potato dextrose agar (PDA, Difco Laboratories) supplemented with 25 $mg{\cdot}mL^{-1}$ chloramphenicol to prevent growth of bacteria. The PDA plates were incubated at 30°C until fungal growth was observed. Fungal colonies that showed fast growth with abundant aerial mycelium were sub-cultured onto PDA plates to obtain pure cultures, and these were preserved at -4°C in 20% glycerol.

Pure cultures were grown on PDA incubated at 30°C for 7 d to determine morphological characteristics of isolates, including their pigmentation and aerial mycelium. Pycnidium production was induced using liquid Minimal Medium 9 (MM9) (10.0 $g{\cdot}L^{-1}$ glucose (FagaLab), 1.0 $g{\cdot}L^{-1}$ NH_4Cl (Sigma), 0.5 $g{\cdot}L^{-1}$ NaCl (Fermont), 3.0 $g{\cdot}L^{-1}$ K_2HPO_4 (Jalmek), and 3.0 $g{\cdot}L^{-1}$ KH_2PO_4 (JT Baker), supplemented with sterile pine needles (5% w/v). Flasks containing medium were incubated for 15 d at room tem-

perature under a near ultraviolet electromagnetic radiation lamp, using 12 h in of light irradiation and 12h of darkness. Formed pycnidia were collected and suspended in 0.5% Tween 20 to obtain conidiospores, which were then observed under a light microscope (AxioVert200 Zeiss). Images of the conidia were captured with a Zeiss AxioCam HRc camera and analyzed using AxioVision 4.8.2. software, and the dimensions (length and width) of 30 conidia per isolate were measured. Statistical analyses of these data were performed using STATISTICA 8.0 to compare conidium spore sizes across species.

DNA extraction and PCR amplification of selected isolates

All *Botryosphaeriaceae* isolates were grown in potato dextrose broth (PDB, Difco Laboratories) at 30°C for 3 d, and the resulting mycelium was recovered by filtration. Total genomic DNA was extracted using the CTAB protocol (Wagner *et al*., 1987). To characterize *Botryosphaeriaceae* spp., the primers ITS1 and ITS4 were used to amplify the ITS region of the nuclear ribosomal DNA, including the 5.8S gene (White *et al*., 1990). In addition, EF1-728F and EF1-986R were used to amplify part of the translation elongation factor-1a (*tef*-1a) gene (Carbone and Kohn, 1999). These methods were carried out following the recommendations on TrunkDiseaseID.org (Lawrence *et al*., 2017), accessible at http://www.grapeipm.org/d.live/.

Each PCR reaction consisted of the following components: 2.5 µL of 10× PCR buffer with 15 mM $MgCl_2$, 0.5 µL of 20 mM dNTPs, 0.625 µL of 10 µM of each primer, 0.125 µL of Taq DNA polymerase (GoTaq® DNA polymerase, Promega) at 5 units·µL^{-1}, and 1 µL of template DNA at 30 ng·µL^{-1}, adjusted with purified water to reach a final volume of 25 µL. Amplification reactions were carried out in a Bio-Rad T-100 thermal cycler under the following conditions: for tef-1α, an initial cycle of 95°C for 3 min, followed by 35 cycles each of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min; for the ITS region, an initial cycle of 94°C for 2 min was followed by 35 cycles each of 94°C for 1 min, 58°C for 1 min, and 72°C for 1.5 min. Both programs concluded with a final cycle of 72°C for 10 min. After visualizing the amplicons by gel electrophoresis, they were purified using the GeneJet PCR purification kit (Thermo Scientific). The purified products were subsequently sequenced by Eton Bioscience Inc.

Phylogenetic analysis of Botryosphaeriaceae

Before the phylogenetic analysis, the sequence quality was evaluated, and noise was removed using BioEdit v.7.0.5.3 (Hall 1999), and a BLASTn analysis was conducted. Sequences with the greatest similarity were retrieved from GenBank (Table 1) and aligned using ClustalW. For pairwise alignment, the parameters used were a gap opening of 10 and a gap extension of 0.1. For multiple alignment, the parameters used were a gap opening of 10, a gap extension of 0.2, a transition weight of 0.5, and a delay for divergent sequences set to 25% (Thompson *et al*., 1994). The alignment was manually adjusted where necessary. The alignments of ITS and tef-1α were imported into BioEdit v.7.0.5.3 to create the concatenated matrix.

Maximum Likelihood (ML) analysis and Maximum Parsimony (MP) analysis were performed using MEGA-X (Kumar *et al*., 2018) based on the concatenated sequence alignment. The best model of nucleotide substitution was selected according to the Akaike Information Criterion (AIC). For the ML analysis, the K2+G+I model was used (Kimura, 1980). Parameters for Maximum Likelihood were set to Bootstrap method using 1000 replicates. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. Gaps were treated as missing data. The tree was visualized in MX: Tree Explorer. New sequences were deposited in the GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Determination of optimal growth temperatures for Botryosphaeriaceae *spp.*

Optimal growth temperatures were assessed by selecting at least two isolates of each identified species, where possible. The isolates were grown on PDA plates by inoculating each plate with a 3-mm diam. plug of a 2-d-old colony on the edge of the plate. The plates were incubated at 20, 25, 28, 30, 35, 37, or 40°C. Resulting colony radii were measured every 24 h for 4 d. The optimal growth temperature at which maximum mycelial growth rate (mm d^{-1}) was determined following the method of Rangel-Montoya *et al*. (2021). This determination was made using the formula: $GR = R_f - R_i/T_f - T_i$ (where GR = growth rate; R_i, = initial radius (mm); R_f, = final colony radius (mm); T_i, = initial time (day 1), and T_f, = final time where fungal growth was measured. For each temperature, three replicate plates of each isolate were included. Statistical analyses of data obtained were carried out using STATISTICA 8.0, to compare the growth rates of each isolate.

Pathogenicity tests of selected isolates

One-year-old grapevine plants of the 'Merlot' cultivar were used to evaluate the pathogenicity of dif-

Table 1. List of GenBank and culture accession numbers of *Botryosphaeriaceae* spp. used for phylogenetic analyses in the present study.

Species	Isolate	Host	Origin	GenBank accession number	
				ITS	*tef*1-
Botryosphaeria agaves	MFLUCC11-0125	*Agave* sp.	Thailand	JX646791	JX646856
B. agaves	MFLUCC10-0051	*Agave* sp.	Thailand	JX646790	JX646855
B. dothidea	CMW8000	*Prunus* sp.	Switzerland	AY236949	AY236898
B. dothidea	CBS 110302	*Vitis vinifera*	Portugal	AY259092	AY573218
B. dothidea	**MXRJM2**	***V. vinifera***	**Mexico**	**MZ312534**	**MZ397922**
B. dothidea	**MXRJM9A**	***V. vinifera***	**Mexico**	**MZ312535**	**MZ397923**
B. dothidea	**MXRJM19**	***V. vinifera***	**Mexico**	**MZ312536**	**MZ397924**
B. dothidea	**MXRJM22**	***V. vinifera***	**Mexico**	**MZ312537**	**MZ397925**
B. dothidea	**MXRJM23**	***V. vinifera***	**Mexico**	**MZ312538**	**MZ397926**
B. dothidea	**MXRJM25**	***V. vinifera***	**Mexico**	**MZ312539**	**MZ397927**
Diplodia corticola	CBS 112549	*Quercus suber*	Potugal	AY259100	AY573227
D. corticola	CBS 112547	*Quercus. ilex*	Spain	AY259110	DQ458872
D. corticola	**MXSASI12-3**	***V. vinifera***	**Mexico**	**PP150458**	**PP377623**
D. mutila	CBS 112553	*V. vinifera*	Portugal	AY259093	AY573219
D. mutila	CBS230.30	*Phoenix dactylifera*	USA	DQ458886	DQ458869
D. sapinea	CBS393.8	*Pinus nigra*	Netherlands	DQ458895	DQ458880
D. sapinea	CBS109725	*Pinus patula*	South Africa	DQ458896	DQ458881
D. seriata	CBS 112555	*V. vinifera*	Portugal	AY259094	AY573220
D. seriata	CBS119049	*Vitis* sp.	Italy	DQ458889	DQ458874
D. seriata	**MXRF05**	***V. vinifera***	**Mexico**	**MZ312540**	**MZ397928**
D. seriata	**MXRF07**	***V. vinifera***	**Mexico**	**MZ312541**	**MZ397929**
D. seriata	**MXBY06**	***V. vinifera***	**Mexico**	**MZ312542**	**MZ397930**
D. seriata	**MXSASI19**	***V. vinifera***	**Mexico**	**MZ312543**	**MZ397931**
D. seriata	**MXER1**	***V. vinifera***	**Mexico**	**MZ312544**	**MZ397932**
D. seriata	**MX16P2**	***V. vinifera***	**Mexico**	**MZ312545**	**MZ397933**
D. seriata	**MXSASI15-01**	***V. vinifera***	**Mexico**	**PP150447**	**PP320328**
D. seriata	**MXSB01**	***V. vinifera***	**Mexico**	**PP150450**	**PP343114**
D. seriata	**MXSASI01**	***V. vinifera***	**Mexico**	**PP150448**	**PP377618**
D. seriata	**MXSASI08-01**	***V. vinifera***	**Mexico**	**PP150474**	**PP343113**
D. seriata	**MXCCBM09-2**	***V. vinifera***	**Mexico**	**PP150451**	**PP377620**
D. seriata	**MXR02-3**	***V. vinifera***	**Mexico**	**PP150452**	**PP377621**
D. seriata	**MXCCBM08-1**	***V. vinifera***	**Mexico**	**PP150453**	**PP343115**
D. seriata	**MXSACH29-2**	***V. vinifera***	**Mexico**	**PP150457**	**PP343118**
D. seriata	**MXSACH16**	***V. vinifera***	**Mexico**	**PP150456**	**PP343117**
D. seriata	**MXCT01**	***V. vinifera***	**Mexico**	**PP150455**	**PP343116**
D. seriata	**MXCT10**	***V. vinifera***	**Mexico**	**PP150454**	**PP377622**
D. seriata	**MXSACH19-1**	***V. vinifera***	**Mexico**	**PP150449**	**PP377619**
D. scrobiculata	CMW 189	*Pinus resinosa*	USA	AY253292	AY624253
D. scrobiculata	CBS109944	*Pinus greggii*	Mexico	DQ458899	DQ458884
Lasiodiplodia theobromae	CBS 164.96	Fruit along coral reef	PNG	AY640255	AY640258
L. theobromae	CBS111530	Unknown	Unknown	EF622074	EF622054
Neofusicoccum australe	CMW6837	*Acacia* sp.	Australia	AY339262	AY339270
N. australe	CMW6853	*Sequoiadendron giganteum*	Australia	AY339263	AY339271
N. australe	**MXBT10**	***V. vinifera***	**Mexico**	**MZ312546**	**MZ397934**
N. australe	**MXBT12**	***V. vinifera***	**Mexico**	**MZ312547**	**MZ397935**
N. australe	**MX5P5**	***V. vinifera***	**Mexico**	**MZ312548**	**MZ397936**
N. eucalypticola	CMW6539	*Eucalyptus grandis*	Australia	AY615141	AY615133

(Continued)

Table 1. (Continued).

Species	Isolate	Host	Origin	GenBank accession number	
				ITS	*tef*1-
N. eucalypticola	CMW6217	*E. rossi*	Australia	AY615143	AY615135
N. luteum	CBS110299	*V. vinifera*	Portugal	AY259091	AY573217
N. luteum	CBS 110497	*V. vinifera*	Portugal	EU673311	EU673277
N. mediterraneum	PD312	*Eucalyptus* sp.	Greece	GU251176	GU251308
N. mediterraneum	CBS121558	*V. vinifera*	USA	GU799463	GU799462
N. parvum	CMW9081	*P. nigra*	New Zealand	AY236943	AY236888
N. parvum	CBS 110301	*V. vinifera*	Portugal	AY259098	AY573221
N. parvum	**MX14P4**	***V. vinifera***	**Mexico**	**MZ312549**	**MZ397937**
N. parvum	**MX24P4**	***V. vinifera***	**Mexico**	**MZ312550**	**MZ397938**
N. parvum	**MXRJM6**	***V. vinifera***	**Mexico**	**MZ312551**	**MZ397939**
N. parvum	**MXRJM15**	***V. vinifera***	**Mexico**	**MZ312552**	**MZ397940**
N. parvum	**MXRJM16**	***V. vinifera***	**Mexico**	**MZ312553**	**MZ397941**
N. parvum	**MXCHP08E**	***V. vinifera***	**Mexico**	**MZ312554**	**MZ397942**
N. viticlavatum	STE-U 5044	*V. vinifera*	South Africa	AY343381	AY343342
N. viticlavatum	STE-U 5041	*V. vinifera*	South Africa	AY343380	AY343341
N. vitifusiforme	STE-U 5252	*V. vinifera*	South Africa	AY343383	AY343343
N. vitifusiforme	STE-U 5050	*V. vinifera*	South Africa	AY343382	AY343344
N. vitifusiforme	**MXSACH23**	***V. vinifera***	**Mexico**	**MZ312555**	**MZ397943**
N. vitifusiforme	**MXSACH24**	***V. vinifera***	**Mexico**	**MZ312556**	**MZ397944**
N. vitifusiforme	**MXCNA1**	***V. vinifera***	**Mexico**	**MZ312557**	**MZ397945**
Mycosphaerella pini	CMW14822	*Pinus ponderosa*	USA	AY808300	AY808265

Isolates from the present study are highlighted in bold text.

ferent *Botryosphaeriaceae* isolates in green and woody host tissues. Green shoots were each inoculated by inserting a mycelial plug of the respective fungal isolate into a 2 mm diameter wound created with a drill. The plants were then kept in a greenhouse for 15 d, The woody tissue was inoculated in a similar manner, but the plants were left in a greenhouse for 2 months. Two isolates previously reported as strains of *Lasiodiplodia*, namely *L. brasiliensis* MXBCL28 and *L. gilanensis* MXCS01 (Rangel-Montoya *et al*., 2021), were used for comparisons. Each selected isolate was inoculated into five plants for the green and woody tissue assessments, while sterile PDA plugs were used for experimental inoculation control plants. All wounds were covered with Parafilm® to prevent desiccation. Samples were collected after the respective times to measure lengths of the resulting necrotic lesions caused by the *Botryosphaeriaceae* isolates. To determine fulfillment of Koch's postulates, tissues from all inoculated plants was retrieved, flamed, inoculated onto PDA, and incubated at 30°C. The experiments with the plants were carried out twice. The virulence of each fungus was compared through statistical analyses carried out using STATISTICA 8.0.

RESULTS

Molecular and morphological characterization of Botryosphaeriaceae *isolates*

From plants displaying wedge-shaped cankers and necrotic lesions in the vascular tissues, a total of 37 fungal isolates of similar phenotype to *Botryosphaeriaceae* spp. were obtained. Out of these, 31 isolates were collected from Baja California, and six were from Coahuila. The isolate colonies were whitish to gray or olivaceous, with moderate aerial mycelium. Some of the isolates exhibited yellow pigmentation at the colony centre within the first 24 h of incubation, a characteristic associated with particular *Neofusicoccum* species (Phillips *et al*., 2013). Based on their morphological characteristics, these isolates belonged to the genera *Botryosphaeria*, *Diplodia*, or *Neofusicoccum*. Seven strains of *Lasiodiplodia* were found in Baja California, but none in Coahuila. These *Lasiodiplodia* spp. were reported separately (Rangel-Montoya et al., 2021).

Statistically significant differences in conidium size were observed among the analyzed species (Table 2 and Figure 1). Isolates identified as *Botryosphaeria dothidea*

had narrow hyaline conidia with fusiform bases and granular contents. Some of these conidia had a single septum and were larger compared to conidia of other *Botryosphaeriaceae* species, with average size of 26.6 × 5.0 µm. Isolates of *D. seriata* had dark brown, aseptate and septate, ovoid and wide conidia, averaging 23.3 × 10.05 µm. Some isolates had smaller conidia 17.2 ± 2.8 × 9.3 ±1.1 µm. The *D. corticola* isolate had oblong to cylindrical hyaline and aseptate conidia with granular contents and thick walls, as well as brown and septate conidia. The conidia were of average size 25.3 × 14.1 µm. There were no discernible differences in sizes compared to *D. seriata*, although Phillips *et al*. (2013) suggested that *D. corticola* generally had larger conidia than other *Diplodia* species. Isolates of *N. australe* had hyaline conidia with fusiform bases and granular contents, which lacked septa, and had average size of 19.4 × 5.7 µm. Isolates identified as *N. parvum* had ellipsoidal conidia with flat apices and bases, most of which were hyaline, with an average size of 21.3 × 5.2 µm. Some older conidia of *N. parvum* were light brown and had 1 to 2 septa, with the middle cells being darker coloured. *Neofusicoccum vitifusiforme* isolates had hyaline, ellipsoid conidia with wide apices and subtruncate bases, with average size of 20.7 × 5.5 µm.

Sequences obtained from the ITS regions and *tef*1-α loci were, respectively, approx. 550 and 300 bp. The concatenated dataset comprised 924 characters, including gaps after alignment (578 corresponding to ITS gene and 346 corresponding to *tef*1-α gene), and 68 taxa. *Mycosphaerella pini* (CMW14822) was used as the outgroup taxon. The maximum likelihood analysis using Kimura 2-parameter model resulted in a tree with a log likelihood value of -2038.86, and estimated base frequencies were as follows: A = 0.20968, T = 0.23324, C = 0.29582, and G = 0.26139. A discrete Gamma distribution was used to model evolutionary rate differences among sites [five categories (+G, parameter = 0.4676)]. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 32.28% of sites). The maximum parsimony analysis yielded one most parsimonious tree of length = 269, CI = 0.691943, RI = 0.946765, and RC = 0.655107 for parsimony-informative sites.

Based on the phylogenetic analysis, the Mexican isolates were categorized into six species (Figure 2). Eighteen isolates belonged to *D. seriata*, six belonged to *B. dothidea*, six belonged to *N. parvum*, three to *N. australe*, three were *N. vitifusiforme*, and one isolate belonged to *D. corticola*. *Botryosphaeria dothidea* and

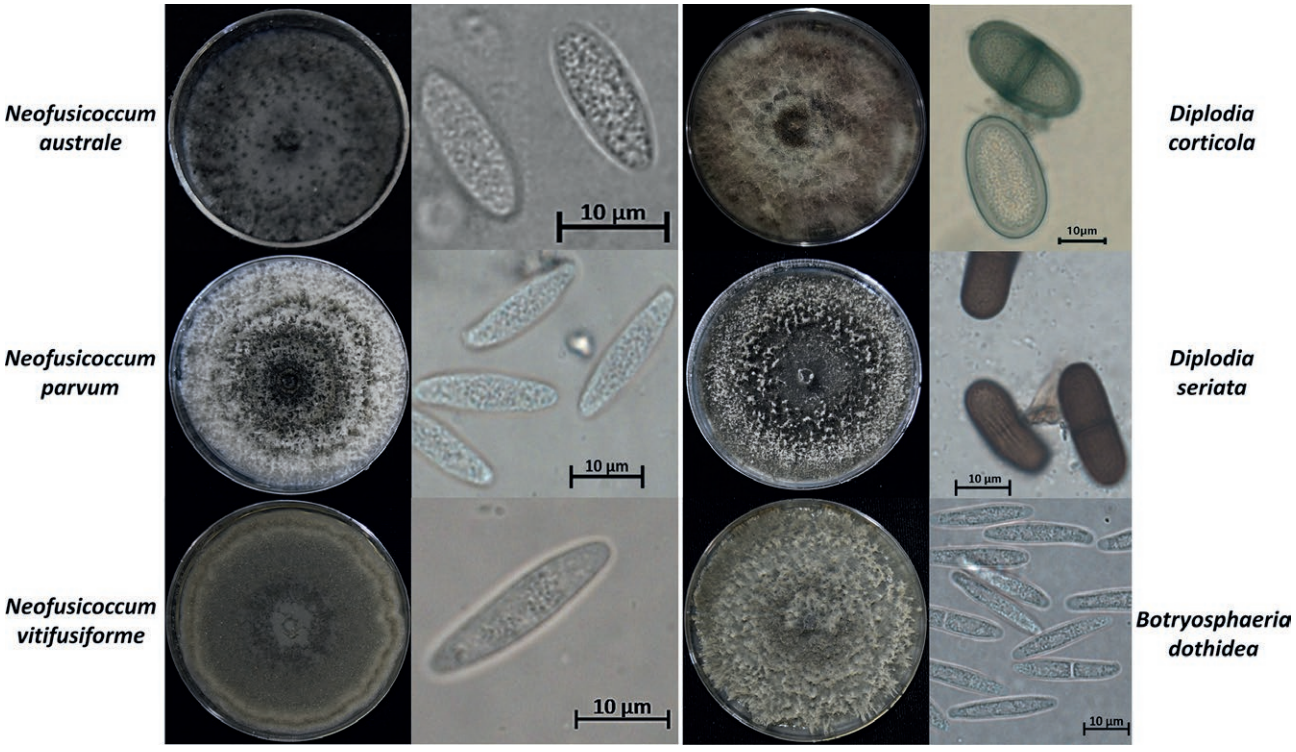

Figure 1. Macroscopic and microscopic characteristics of *Botryosphaeriaceae* spp. isolated from grapevine tissues and grown on PDA at 30°C for 7 d. *Neofusicoccum australe* MX5P5, *Neofusicoccum parvum* MX14P4, *Neofusicoccum vitifusiforme* MXSACH24, *Diplodia corticola* MXSASI12-3, *Diplodia seriata* MXBY06, and *Botryosphaeria dothidea* MXRJM25. Left panel, colony, right panel, conidia.

Table 2. Conidium dimensions of the isolated *Botryosphaeriaceae* spp. from this study.

Isolate	Origin	Conidium size (µm)*	Mean ± SD**
Neofusicoccum australe[a]			
MXBT10	Baja California	(17.8-)18.7-20.1 × (5.7-)6.3-7.2	18.9±1.3 × 5.6 ±1.6
MXBT12	Baja California	(17.9-)18.5-20.3 × (6.8-)6.4-7.5	18.6±1.2 × 6.7±0.6
MX5P5	Coahuila	(17.1-)21.1-24.0 × (4.3-)5.0-6.4	20.9±1.9 × 5.0±0.5
Neofusicoccum parvum[b]			
MX14P4	Coahuila	(19.5-)20.3-24.0 × (4.5-)5.1-5.5	21.9±1.3 × 5.0±0.2
MX24P4	Coahuila	(19.0-)21.9-23.9 × (4.9-)5.2-6.3	21.4±1.4 × 5.5±0.4
MXRJM6	Baja California	(17.9-)21.7-23.8 × (4.4-)5.3-5.8	21.8±1.3 × 5.0±0.4
MXRJM15	Baja California	(18.5-)20.3-22.9 × (4.8-)5.3-5.8	21.0±1.3 × 5.3±0.2
MXRJM16	Baja California	(16.9-)19.6-23.6 × (4.4-)5.8-7.1	20.3±1.9 × 5.6±0.8
MXCHP08E	Coahuila	(18.1-)21.1-24.4 × (4.4-)5.1-6.2	21.7±1.6 × 5.2±0.5
Neofusicoccum vitifusiforme[a]			
MXSACH23	Baja California	(19.4-)21.2-23.9 × (5.2-)5.9-6.8	21.5±1.2 × 5.8±0.5
MXSACH24	Baja California	(16.7-)20.5-23.0 × (5.1-)5.3-6.5	21.0±1.5 × 5.6±0.4
MXCNA1	Coahuila	(16.8-)19.2-23.6 × (4.7-)5.1-5.8	19.7±1.4 × 5.3±0.3
Diplodia seriata[c]			
MXRF05	Baja California	(21.0-)23.9-27.5 × (8.4-)9.6-10.0	23.6±1.4 × 9.5±0.6
MXRF07	Baja California	(21.3-)23.4-25.7 × (8.6-)10.3-10.9	23.4±1.1 × 10.0±0.5
MXER1	Baja California	(20.1-)22.9-28.9 × (8.0-)8.9-11.2	24.1±2.0 × 9.2±0.8
MXBY06	Baja California	(21.9-)24.9-27.2 × (9.3-)11.7-13.4	24.7±1.2 × 11.8±1.0
MXSASI19	Baja California	(19.0-)20.0-22.8 × (8.0-)9.3-11.2	20.3±1.1 × 9.7±0.9
MXSASI15-01	Baja California	(14.0-)27.0-33.0 × (0.5-)10.0-13.0	26.4±3.0 × 9.0±1.5
MXSB01	Baja California	(16.0-)22.0-24.0 × (7.0-)8.0-10.0	20.6±2.2 × 8.2±0.8
MXSASI01	Baja California	(21.0-)27.5-32.0 × (8.0-)10.0-13.5	27.0±2.0 × 9.9±1.0
MXSASI08-01	Baja California	(20.9-)27.7-32.0 × (8.9-)9.0-16.0	26.5±2.3 × 11.4±1.6
MXCCBM09-2	Baja California	(10.8-)22.6-27.0 × (7.9-)10.0-13.0	21.1±2.1 × 9.7±0.8
MXR02-3	Baja California	(13.1-)23.0-27.6 × (5.3-)8.5-12.0	22.1±2.8 × 8.3±1.2
MXCCBM08-1	Baja California	(18.4-)22.8-32.0 × (8.7-)11.0-13.0	22.8±2.3 × 10.7±0.7
MXSACH29-2	Baja California	(16.6-)21.2-27.6 × (8.0-)9.4-20.0	21.4±2.6 × 10.9±2.9
MXSACH16	Baja California	(24.0-)27.8-34.0 × (9.0-)9.0-17.0	28.2±1.8 × 12.0±1.5
MXCT01	Baja California	(20.0-)22.3-26.0 × (7.9)8.0-11.0	22.2±1.3 × 8.8±0.8
MXCT10	Baja California	(19.0-)23.7-29.0 × (8.0-)10.0-15.0	23.6±2.0 × 10.9±1.3
MXSACH19-1	Baja California	(11.0-)16.0-25.0 × (7.0)10.0-14.5	17.2±2.8 × 9.3±1.1
MX16P2	Coahuila	(20.5-)23.2-28.6 × (8.2-)9.8-12.1	23.7±2.2 × 10.1±0.9
D. corticola[c]			
MXSASI12-3	Baja California	(24.0-)17.5-37.0 × (11.0-)14.0-17.5	25.3±3.9 × 14.1±1.3
Botryosphaeria dothidea[d]			
MXRJM2	Baja California	(24.0-)26.3-31.2 × (4.2-)5.6-6.4	26.3±1.9 × 5.5±0.5
MXRJM9A	Baja California	(24.7-)26.1-31.1 × (4.1-)5.3-6.0	26.8±1.6 × 5.0±0.5
MXRJM19	Baja California	(23.5-)26.1-32.4 × (4.1-)4.8-5.5	27.0±2.1 × 4.7±0.3
MXRJM22	Baja California	(24.3-)28.1-30.0 × (4.1-)5.1-6.1	26.9±1.6 × 5.1±0.5
MXRJM23	Baja California	(24.1-)24.6-27.4 × (4.1-)4.8-5.4	25.7±1.1 × 4.6±0.4
MXRJM25	Baja California	(24.2-)27.8-31.9 × (4.3-)5.5-6.1	27.1±1.5 × 5.3±0.4

* Minimum size, most repetitive value and maximum size for length and width of 30 selected conidia.
** SD = standard deviation.
[a,b,c,d] Indicate differences between fungi in conidium size. Species accompanied by the same letters are not significantly different ($\alpha < 0.05$).

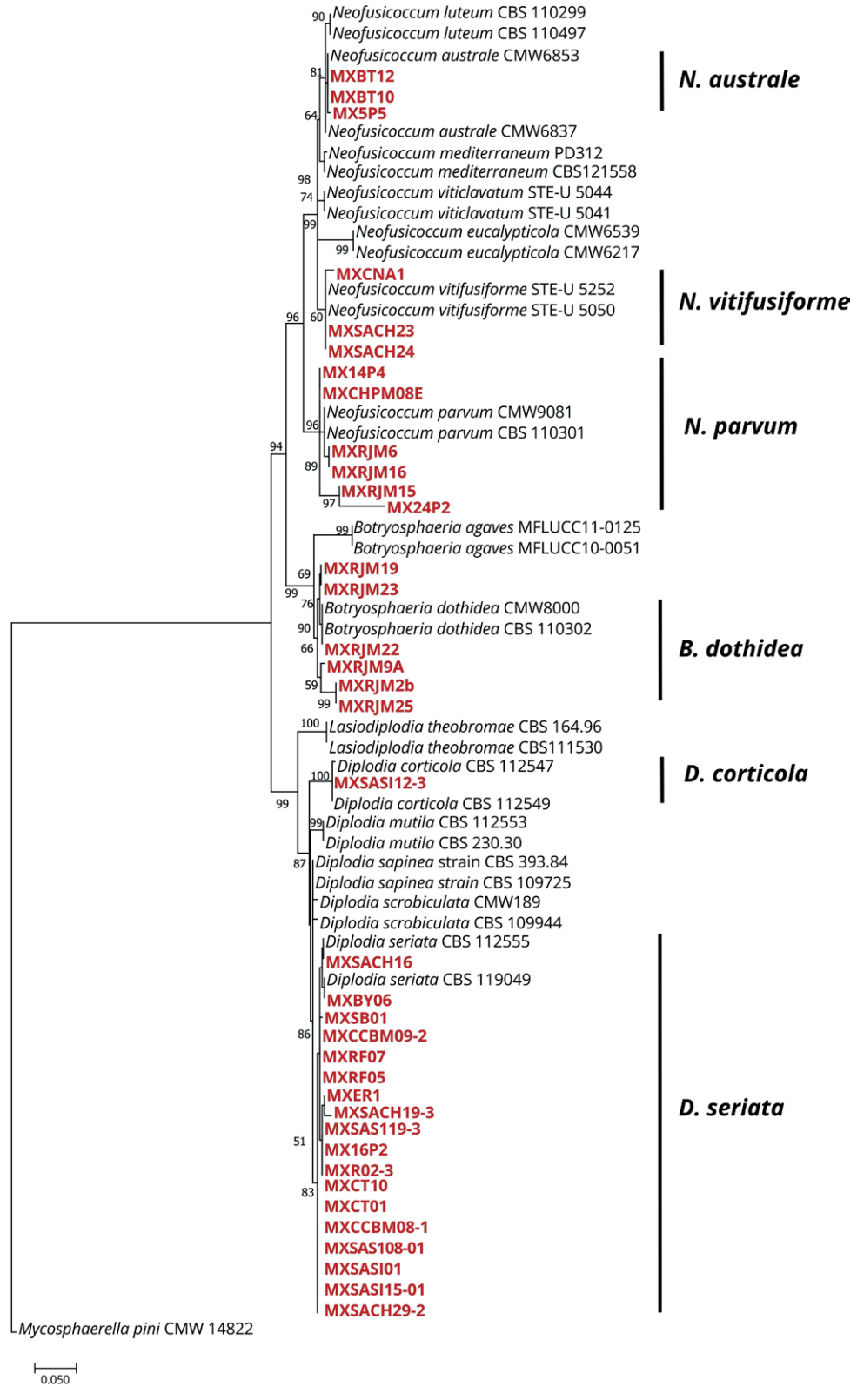

Figure 2. Phylogenetic analyses of *Botryosphaeriaceae* species. Maximum likelihood tree with the greatest log likelihood (-2038.86) obtained from the ITS and tef-1α concatenated dataset. The tree is rooted with *Mycosphaerella pini* (CMW 14822). The isolates from the present study are indicated in bold red font. Bootstrap values from 1000 replicates greater than 50 are indicated at the nodes.

D. corticola were only isolated from samples from Baja California, while *N. australe, N. vitifusiforme, N. parvum* and *D. seriata*, were isolated from samples from Baja California and Coahuila.

Optimum growth temperatures of selected Botryosphaeriaceae *spp.*

Among the selected isolates, *N. parvum* MXRJM6, *N. parvum* MXRJM16, and *B. dothidea* MXRJM22 had optimum growth temperatures within the range of 25 to 30°C (Table 3). Specifically, *N. parvum* MX14P4 grew optimally in the range of 28 to 30°C, while *B. dothidea* MXRJM25 showed optimum growth in the range of 28 to 35°C. *Diplodia seriata*, *D. corticola* and *N. australe* isolates had greatest growth rates at 25°C. *Neofusicoccum vitifusiforme* MXSACH23 had optimum growth at 30°C, while *N. vitifusiforme* MXSACH24 grew best at 25°C. *Botryosphaeria dothidea* RJ22MX had the greatest growth rate, reaching 16.4 ± 1.7 mm d^{-1} at 28°C. For *D. seriata*, isolates MXSASI101 and MXSASI15-01, obtained from Baja California vineyards, showed minimal growth at 37°C, while the remaining isolates did not grow at 37 or 40°C. Nevertheless, all the *D. seriata* isolates were capable of recovering growth when cultures exposed to 37 or 40°C for 4 d were transferred to room temperature.

Pathogenicity assays

In green tissues, plants that were inoculated with *N. parvum* MX14P4, MX24P4, MXRJM6, or MXRJM16, or *B. dothidea* MXRJM22, developed large necrotic lesions 15 d post inoculation (Figure 3A). *Diplodia corticola* did not produce lesions in green tissues (0.4 ± 0.1 cm), so this fungus was not included in the next experiment. The isolates of *B. dothidea*, *N. australe*, *N. vitifusiforme,* and *D. seriata* had mean necrotic lesion lengths that were not significantly different ($\alpha > 0.05$).

Table 3. Mean mycelium growth rates (mm d^{-1}) for Mexican *Botryosphaeriaceae* isolates at different temperatures.

Isolate	Temperature					
	25°C	28°C	30°C	35°C	37°C	40°C
N. parvum						
MX14P4	11.8 ± 0.3 e	12.2 ± 0.4 d	12.7 ± 1.6 cd	3.8 ± 0.2 j	NG*	NG
MX24P4	7.1 ± 1.5 h	10.0 ± 1.3 f	5.9 ± 0.6 i	2.6 ± 0.1 k	NG	NG
MXRJM6	15.8 ± 2.1 a	15.3 ± 2.3 a	15.1 ± 2.2 a	3.1 ± 0.1 k	NG	NG
MXRJM16	15.1 ± 1.5 ab	15.9 ± 0.8 a	15.4 ± 1.0 a	3.3 ± 0.01 jk	NG	NG
N. vitifusiforme						
MXSACH24	15.0 ± 2.5 ab	13.2 ± 3.7 c	13.3 ± 4.4 c	2.2 ± 1.1 kl	NG	NG
MXSACH23	9.3 ± 0.5 g	9.7 ± 0.5 fg	10.0 ± 0.01 f	2.8 ± 0.8 k	NG	NG
N. australe						
MXBT12	9.6 ± 1.8 fg	7.4 ± 1.2 h	4.6 ± 1.0 ij	2.8 ± 0.5 k	NG	NG
MXBT10	14.4 ± 0.3 ab	9.3 ± 0.2 g	4.3 ± 0.3 j	0.9 ± 0.2 l	NG	NG
D. seriata						
MXBY06	14.1 ± 0.6 b	12.8 ± 0.9 cd	11.4 ± 1.0 e	8.9 ± 0.7 g	NG	NG
MXRF05	12.8 ± 0.4 cd	7.1 ± 1.2 h	6.7 ± 0.7 h	3.8 ± 0.3 j	NG	NG
MXSASI101	13.9 ± 0.4 b	12.6 ± 1.2 cd	10.0 ± 0.9 f	5.2 ± 0.5 i	1.7±0.1 l	*NG*
MXSASI15-01	13.7 ± 0.3 b	13.2 ± 1.0 c	8.3 ± 1.4 gh	6.5 ± 1.1 hi	1.2±0.1 l	*NG*
MX16P2	13.6 ± 0.1 bc	13.0 ± 0.4 c	12.4 ± 0.8 d	9.0 ± 0.9 g	NG	NG
D. corticola						
MXSASI12-3	14.0 ± 0.4 b	9.0 ± 2.2 g	4.8 ± 0.8 ij	3.5 ± 0.3 j	NG	NG
B. dothidea						
MXRJM9A	5.5 ± 1.1 i	5.8 ±1.1 i	5.3 ± 1.1 i	5.0 ± 0.6 i	NG	NG
MXRJ22	15.3 ± 2.2 a	16.4 ± 1.7 a	15.6 ± 0.9 a	9.3 ± 0.5 g	NG	NG
MXRJM25	7.3 ± 0.3 h	7.9 ± 0.7 h	8.2 ± 1.5 gh	8.6 ± 0.2 g	NG	NG

NG = no mycelial growth. Means accompanied by the same letters indicate there are not statistically different ($\alpha < 0.05$) based on Fisher's analysis.

Figure 3. Examples of grapevine shoots cv. 'Merlot' inoculated with *Botryosphaeriaceae* spp., showing dark-brown lesions at A) 15 d post inoculation in green tissues, and B) 2-months post inoculation in woody tissues.

In woody tissues, 2 months post inoculation, *N. parvum* isolates MX14P4, MX24P4, MXRJM6, and MXRJM16 were the most virulent (Figure 3B). These plants developed lesions longer than 4 cm, although virulence of these isolates was less than that of the *Lasiodiplodia* isolates used for comparison (Figure 4). *Botryosphaeria*

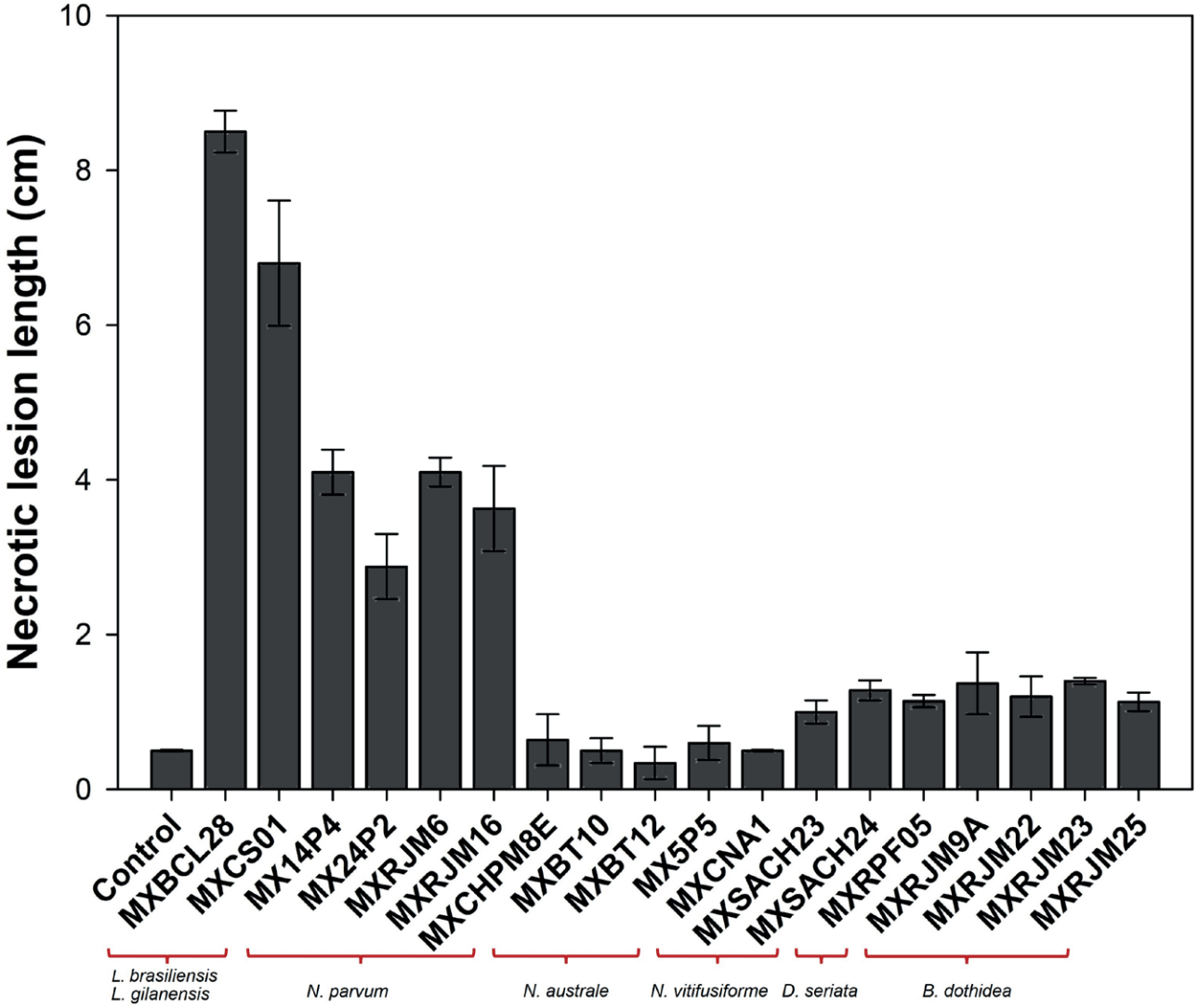

Figure 4. Mean lengths of lesions caused by different *Botryosphaeriaceae* isolates in grapevine plants 'Merlot' observed 2 months post inoculation in woody tissue. letters accompanying the means were assigned based on Fisher's analysis ($P < 0.05$). The bars accompanying each mean represent standard deviations, and means accompanied by the same letters are not significantly different ($\alpha < 0.05$).

dothidea, D. seriata, N. australe, and *N. vitifusiforme* caused lesions shorter than 2 cm. None of the control plants developed necrotic lesions. Koch's postulates were confirmed, as the inoculated fungi were re-isolated from the inoculated plants.

DISCUSSION

This study has identified six *Botryosphaeriaceae* species associated with Botryosphaeria dieback and isolated from vineyards in Baja California and Coahuila. The identified species include *N. parvum*, *B. dothidea*, *D. seriata, N. australe*, *N. vitifusiforme*, and *D. corticola*. Previously, *D. seriata*, *L. theobromae* (Úrbez-Torres *et al*., 2008), *D. corticola*, *N. australe*, *N. vitifusiforme* (Candolfi-Arballo *et al*., 2010), *L. gilaniensis, L. crassispora, L. brasiliensis*, and *L. exigua* (Rangel-Montoya *et al*., 2021) have been reported in Baja California and Sonora. Thus, the isolations *N. parvum* and *B. dothidea* reported in the present study are the first records of these two fungi in vineyards of Mexico.

While this study did not focus on the distribution or abundance of *Botryosphaeriaceae* fungi, among the identified species, *D. seriata* was the most common. This species has previously been documented in Mexico (Úrbez-

Torres *et al*., 2008; Candolfi-Arballo *et al*., 2010). It is a cosmopolitan and plurivorous fungus, broadly prevalent among *Botryosphaeriaceae* species and affecting grapevines in most countries where these plants are cultivated, and causing Botryosphaeria canker (Larignon *et al*., 2001; Úrbez-Torres, 2011). Strains reported from other countries exhibit varying conidium morphologies and dimensions, ranging from 21.5 – 28 × 11 – 15.5 µm, and never exceeding 30 µm in length. Initially hyaline, the conidia darken with time, typically remaining aseptate and ovoid, with smooth external walls that become roughened on the inner surfaces. However, some isolates may develop septa upon germination (Phillips *et al*., 2013). Among the 18 isolates examined, the majority had septate conidia, while for particular isolates (e.g. MXSACH19-1), conidia were smaller (average = 17.2 × 9.3 µm) than the average size. This indicates intraspecific variation, as has been previously highlighted (Elena *et al*., 2015).

Differences in conidium dimensions were observed among species, providing a basis for differentiation. For example, while the conidia of *N. parvum* and *B. dothidea* had similar characteristics, those of *B. dothidea* were longer and narrower; *N. vitifusiforme* (average = 20.7 × 5.5 µm) and *N. australe* (average = 19.4 × 5.7 µm) had similarly sized conidia, but *N. vitifusiforme* had fusoid to ellipsoid conidia widest in the upper thirds, with an obtuse apices and flattened, subtruncate bases. In contrast, the conidia of *N. australe* were non-septate and fusiform, with subtruncate to bluntly rounded bases (Phillips *et al*., 2013). *Diplodia corticola* isolates mainly had hyaline and aseptate conidia, which were oblong to cylindrical with both ends broadly rounded, and gradually became brown and septate with time. This species has the largest conidia of the genus *Diplodia* (average = 29.9 × 13.6 µm) (Phillips *et al*., 2013). However, differentiating among species solely based on their conidium morphology is challenging due to their striking similarities.

Botryosphaeriaceae fungi have cosmopolitan distributions (Úrbez-Torres, 2011), but particular genera within this family tend to prevail in specific climatic regions. For example, *Diplodia* spp. are often found in temperate regions (Burgess and Wingfield, 2002), while species of *Lasiodiplodia* are commonly found in tropical and subtropical regions (Mohali *et al*., 2005; Burgess *et al*., 2006). The optimum growth temperatures for *Neofusicoccum*, *Diplodia*, and *Botryosphaeria* spp. is typically within the range of 25 to 30°C (Phillips *et al*., 2013; Dardani *et al*., 2023). Results from the present study agree with those reports, although two isolates of *D. seriata* (MX16P2 from Coahuila and MXBY06 from Baja California) showed slight growth at 37°C. Furthermore, all the *D. seriata* isolates exposed to 37 or 40°C recovered their growth when cultures were transferred to room temperature. Similarly, Mexican *Lasiodiplodia* spp. did not grow at 40°C but resumed growth once returned to room temperature (Rangel-Montoya *et al*., 2021). Plasticity in temperature tolerance may be linked to broad international distribution of *D. seriata*. On the other hand, the climate of Valle de Guadalupe, where most of the isolates were obtained, is usually warm. Over the course of each year, the temperature varies from 4°C (at night) to 33°C, and is rarely less than 2°C or greater than 34°C (CONAGUA, 2023). This indicates that the Valle de Guadalupe favours occurrence and distribution of *D. seriata*.

Neofusicoccum parvum was initially reported in grapevines in 2002 as *Botryosphaeria parva* (Phillips, 2002). Since then, this fungus has emerged as one of the most frequently isolated species and among the most virulent pathogens affecting grapevines, alongside several species of *Lasiodiplodia* (Úrbez-Torres *et al*., 2006). The isolates of *N. parvum*, specifically MX14P4, MX24P4, MXRJM6, and MXRJM16, exhibited greater virulence in both green and woody tissues. However, previously reported *Lasiodiplodia* isolates from Baja California and Sonora displayed higher levels of virulence than those of *N. parvum* (Rangel-Montoya *et al*., 2021). In contrast, the isolate MXCHP08E, also *N. parvum*, demonstrated weak virulence. As previously indicated, isolates within the same species can vary in virulence (Billones-Baaijens *et al*., 2013; Rangel-Montoya *et al*., 2021). Further investigation is required to determine the reasons for these variations.

Neofusicoccum parvum strains obtained in this study had optimal growth temperatures of 28 to 30°C. This temperature range favours the virulence of *N. parvum*, as it the fungus causes greatest damage at temperatures between 25 and 30°C (Ploetz *et al*., 2009). The isolates of *N. parvum* from Baja California were obtained from a recently planted vineyard, while in older plants, the most isolated species were *D. seriata* and *Lasiodiplodia* spp. It is therefore likely that in regions with low rainfall and high temperatures such as Sonora and Baja California in Mexico, this fungus is not commonly found. Furthermore, conidium germination is also affected by high relative humidity (Amponsah *et al*., 2010), and the optimum conidium germination temperature for this fungus is 30°C (Úrbez-Torres *et al*., 2010b).

Neofusicoccum australe was originally described as *Botryosphaeria australis* and isolated from native Acacia species in Australia (Slippers *et al*., 2004). Presence of this fungus in grapevines was first confirmed in 2004 in South Africa, where it caused lesions in green shoots and mature canes of 'Periquita' plants (van Niekerk *et al*., 2004). In the present study, however, plants inocu-

lated with *N. australe* did not show damage; instead, tissue regeneration was observed at the sites of the mechanical wounds. These differences could be due to the different cultivars used in the tests, as cultivars can exhibit varying levels of susceptibility to this pathogen. Nevertheless, *N. australe* is generally considered a weak pathogen with narrow distribution (Úrbez-Torres *et al*., 2006; Pitt *et al*., 2010).

The isolates of *N. vitifusiforme* used in the present study caused lesions ranging from 0.5-1.2 cm in length, which were not significantly different from those on the control plants. *Neofusicoccum vitifusiforme* was first reported in grapevine in 2004 as a weak pathogen (van Niekerk *et al*., 2004). Initially, this fungus was believed be restricted to *Vitis* spp. (Phillips *et al*., 2013), until it was isolated in Italy from *Olea europaea*, where it was reported to be an aggressive pathogen (Lazzizera *et al*., 2008). Cross-inoculations followed by histological or transcriptomic analyses using isolates from grapevine and *O. europaea* would help to clarify reasons for this different behaviour.

Botryosphaeria dothidea was initially found as an endophyte in the bark of white cedar (Xiao *et al*., 2014). More recently, this fugus has gained recognition as a latent pathogen of widespread significance in woody plants. This is attributed to its ability to undergo a prolonged endophytic phases before causing decay symptoms in host plants (Marsberg *et al*., 2017). The pathogenicity of *B. dothidea* isolates evaluated in the present study resulted in lesions that were larger than experimental controls. The *B. dothidea* isolates MXRJM9A and MXRJM25 did not have differences in growth within the range of 25 to 35°C, while isolate MXRJM22 exhibited greatest growth at 25 to 30°C and also grew at 35°C. This pathogenicity and ability to grow at different temperatures indicates that *B. dothidea* poses potential challenges for vineyards in Mexico.

Diplodia corticola was first reported as associated with oak (*Quercus suber* L.) dieback in Portugal (Alves *et al*., 2004), establishing this fungus as one of the most important pathogens affecting these trees (Muñoz-Adalia and Colinas, 2021; Muñoz-Adalia *et al*., 2023). In grapevines, *D. corticola* has been isolated in Texas (Úrbez-Torres *et al*., 2009), California (Úrbez-Torres *et al*., 2010a), Spain (Pintos Varela *et al*., 2011), and Italy (Carlucci *et al*., 2015), where it has been assessed to be a moderately virulent pathogen. The isolate obtained from Baja California exhibited no virulence to grapevine and displayed optimal growth at 25°C. The isolate was obtained from an approx. 30-year-old plant growing in a temperate climate. This suggests that *D. corticola* may not be well adapted to the climate conditions of Baja California, but could potentially exist as an endophyte within that region.

The diversity, distribution, wide host range, and the several factors that favour conidium distribution (e.g. wind, rain, and insects), make *Botryosphaeriaceae* important plant pathogens (Slippers and Wingfield 2007; Mehl *et al*., 2017). Reports of the distribution and pathogenicity of *Botryosphaeriaceae* in different countries provide helpful information on the frequency and diversity of hosts of these fungi (Batista *et al*., 2021). The present study has broadened knowledge on the incidence of *Botryosphaeriaceae*, and provides a benchmark for future research on GTD epidemiology and disease management in Mexico.

ACKNOWLEDGMENTS

Edelweiss Airam Rangel-Montoya was supported for the postdoctoral program of El Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT-México). The authors thank the grape growers of Baja California and Coahuila for allowing sampling in their vineyards, and SADER-Baja California, La Junta Local de Sanidad Vegetal de Hermosillo, and CONAHCYT for their support for this research.

LITERATURED CITED

Agusti-Brisach C., Armengol J., 2013. Black-foot disease of grapevine: an update on taxonomy, epidemiology and management strategies. *Phytopathologia Mediterranea* 52: 245–261.

Alves A., Correia A., Luque J., Phillips A., 2004. *Botryosphaeria corticola*, sp. nov. on *Quercus* species, with notes and description of *Botryosphaeria stevensii* and its anamorph, *Diplodia mutila*. *Mycologia* 96: 598–613. https://doi.org/10.1080/15572536.2005.11832956.

Amponsah N.T., Jones E.E., Ridgway H.J., Jaspers M.V., 2010. Effects of solar radiation and relative humidity on germination of *Botryosphaeriaceae* species conidia. *New Zealand Plant Protection* 63: 28–32. https://doi.org/10.30843/nzpp.2010.63.6610

Batista E., Lopes A., Alves A., 2021., What do we know about *Botryosphaeriaceae*? An overview of a worldwide cured dataset. *Forests* 12: 313. https://doi.org/10.3390/f12030313

Bertsch C., Ramírez-Suero M., Magnin-Robert M., Larignon P., Chong J., … Fontaine F., 2013. Grapevine trunk diseases: complex and still poorly understood. *Plant Pathology* 62: 243–265. https://doi.org/10.1111/j.1365-3059.2012.02674.x

Billones-Baaijens R., Jones E.E., Ridgway H.J., Jaspers M.V., 2013. Virulence affected by assay parameters during grapevine pathogenicity studies with *Botryosphaeriaceae* nursery isolates. *Plant Pathology* 62: 1214–1225. https://doi.org/10.1111/ppa.12051

Burgess T., Wingfield M.J., 2002. Impact of fungal pathogens in natural forest ecosystems: a focus on eucalypts. In: *Microorganisms in plant conservation and biodiversity* (K. Sivasithamparama, K.W. Dixon, R.L. Barrett, ed.), Dordrecht: Springer Netherlands, 285–306.

Burgess T.I., Barber P.A., Mohali S., Pegg G., de Beer W., Wingfield M.J., 2006. Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. *Mycologia* 98: 423–435. https://doi.org/10.1080/15572536.2006.11832677

Candolfi-Arballo O., Valenzuela-Solano C., Gubler W.D., Hernández-Martínez R., 2010. *Botryosphaeriaceae* species associated with grapevine decline in Mexico. In: *7th International Workshop on Grapevine Trunk Diseases, Santa Cruz, Chile, 2010*. *Phytopathologia Mediterranea* 49: 103–133 (abstract).

Carbone I., Kohn L., 1999. A method for designing primer sets for speciation studies in filamentous Ascomycetes. *Mycologia* 91: 553–556. https://doi.org/10.1080/00275514.1999.12061051

Carlucci A., Cibelli F., Lops F., Raimondo M.L., 2015. Characterization of *Botryosphaeriaceae* species as causal agents of trunk diseases on grapevines. *Plant Disease* 99: 1678–1688. https://doi.org/10.1094/PDIS-03-15-0286-RE

CONAGUA Comisión Nacional del Agua (2023) Red de estaciones climatológicas, Ciudad de México México: Servicio Meteorológico Nacional. https://smn.conagua.gob.mx/

Czemmel S., Galarneau E.R., Travadon R., McElrone A.J., Cramer G.R., Baumgartner K., 2015. Genes expressed in grapevine leaves reveal latent wood infection by the fungal pathogen *Neofusicoccum parvum*. *PloS One* 10: e0121828. https://doi.org/10.1371/journal.pone.0121828

Dardani G., Mugnai L., Bussotti S., Gullino M.L., Guarnaccia V., 2023. Grapevine dieback caused by *Botryosphaeriaceae* species, *Paraconiothyrium brasiliense*, *Seimatosporium vitis-viniferae* and *Truncatella angustata* in Piedmont: characterization and pathogenicity. *Phytopathologia Mediterranea* 60: 283–306. https://doi.org/10.36253/phyto-14154

Elena G., Garcia-Figueres F., Reigada S., Luque J., 2015. Intraspecific variation in *Diplodia seriata* isolates occurring on grapevines in Spain. *Plant Pathology* 64: 680–689. https://doi.org/10.1111/ppa.12296

Fontaine F., Pinto C., Vallet J., Clément C., Gomes A.C., Spagnolo A., 2016. The effects of grapevine trunk diseases (GTDs) on vine physiology. *European Journal of Plant Pathology* 144: 707–721. https://doi.org/10.1007/s10658-015-0770-0

Gramaje D., Armengol J., 2011. Fungal trunk pathogens in the grapevine propagation process potencial inoculum sources, detection, identification, and management strategies. *Plant Disease* 95: 1040–1055. https://doi.org/10.1094/PDIS-01-11-0025

Gramaje D., Úrbez-Torres J.R., Sosnowski M.R., 2018. Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. *Plant Disease* 102: 12–39. https://doi.org/10.1094/PDIS-04-17-0512-FE

Graniti A., Mugnai L., Surico G. 2000. Esca of grapevine: a disease complex or a complex of diseases. *Phytopathologia Mediterranea* 39: 1000–1005. https://doi.org/10.14601/Phytopathol_Mediterr-1539

Gubler W.D., Rolshausen P.E., Trouillase F.P., Úrbez J.R., Voegel T., 2005. Grapevine trunk diseases in California. *Practical Winery & Vineyard* Jan/Feb: 6–25.

Hall T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium series* 41: 95–98.

Hrycan J., Hart M., Bowen P., Forge T., Úrbez-Torres J.R., 2020. Grapevine trunk disease fungi: their roles as latent pathogens and stress factors that favour disease development and symptom expression. *Phytopathologia Mediterranea* 59: 395–424. https://doi.org/10.14601/Phyto-11275

Kimura M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111–120. https://doi.org/10.1007/BF01731581

Kumar S., Stecher G., Li M., Knyaz C., Tamura K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549. https://doi.org/10.1093/molbev/msy096

Larignon P., Fulchic R., Cere L., Dubos B., 2001. Observation on black dead arm in French vineyards. *Phytopathologia Mediterranea* 40: 336–342. https://doi.org/10.14601/Phytopathol_Mediterr-1629

Lawrence D.P., Travadon R., Nita M., Baumgartner K., 2017. TrunkDiseaseID. org: A molecular database for fast and accurate identification of fungi commonly isolated from grapevine wood. *Crop Protection* 102: 110–117. https://doi.org/10.1016/j.cropro.2017.08.017

Lazzizera C., Frisullo S., Alves A., Phillips A.J.L., 2008. Morphology, phylogeny and pathogenicity of *Botryosphaeria* and *Neofusicoccum* species associated with drupe rot of olives in southern Italy. *Plant Pathology* 57: 48–956. https://doi.org/10.1111/j.1365-3059.2008.01842.x

Marsberg A., Kemler M., Jami F., Nagel J. H., Postma-Smidt A., … Slippers B., 2017. *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. *Molecular Plant Pathology* 18: 477–488. https://doi.org/10.1111/mpp.12495

Mehl J., Wingfield M.J., Roux J., Slippers B., 2017. Invasive everywhere? Phylogeographic analysis of the globally distributed tree pathogen *Lasiodiplodia theobromae*. *Forests* 8: 1–22. https://doi.org/10.3390/f8050145

Mohali S., Burgess T.I., Wingfield M.J., 2005. Diversity and host association of the tropical tree endophyte *Lasiodiplodia theobromae* revealed using simple sequence repeat markers. *Forest Pathology* 35: 385–396. https://doi.org/10.1111/j.1439-0329.2005.00418.x

Muñoz-Adalia E.J., Colinas C., 2021. Susceptibility of cork oak (*Quercus suber*) to canker disease caused by *Diplodia corticola*: when time is of the essence. *New Forests* 52: 863–873. https://doi.org/10.1007/s11056-020-09829-8

Muñoz-Adalia E.J., Uppara A.B., Albó D., Meijer A., Colinas C., 2023. Cork harvest planning and climate: high air humidity favors availability of airborne inoculum of *Diplodia corticola*. *Forest Ecology and Management* 536: 120935. https://doi.org/10.1016/j.foreco.2023.120935

Paolinelli-Alfonso M., Serrano-Gomez C., Hernandez-Martinez R., 2015. Occurrence of *Eutypella microtheca* in grapevine cankers in Mexico. *Phytopathologia Mediterranea* 54: 86–93. https://doi.org/10.14601/Phytopathol_Mediterr-14998

Phillips A.J.L. 2002. *Botryosphaeria* species associated with diseases of grapevines in Portugal. *Phytopathologia Mediterranea* 4:3–18.

Phillips A.J.L., Alves A., Abdollahzadeh J., Slippers B., Wingfield, M.J., … Crous P.W., 2013. The *Botryosphaeriaceae* : genera and species known from culture. *Studies in Mycology* 76: 51–167. https://doi.org/10.3114/sim0021

Pintos Varela C., Fernández V.R., Casal O.A., Vázquez J.M., 2011. First report of cankers and dieback caused by *Neofusicoccum mediterraneum* and *Diplodia corticola* on grapevine in Spain. *Plant Disease* 95: 1315–1315. https://doi.org/10.1094/PDIS-05-11-0429

Pitt W.M., Huang R., Steel C.C., Savocchia S., 2010. Identification, distribution and current taxonomy of *Botryosphaeriaceae* species associated with grapevine decline in New South Wales and South Australia. *Australian Journal of Grape and Wine Research* 16: 258–271. https://doi.org/10.1111/j.1755-0238.2009.00087.x

Ploetz R.C., Pérez-Martínez J.M., Palmateer A.J., Tarnowski T.L., 2009. Influence of temperature, light intensity, and isolate on the development of *Neofusicoccum parvum*–induced dieback of Eugenia, *Syzygium paniculatum*. *Plant Disease* 93: 804–808. https://doi.org/10.1094/PDIS-93-8-0804

Rangel-Montoya E.A., Paolinelli M., Rolshausen P.E., Valenzuela-Solano, C., Hernandez-Martinez R., 2021. Characterization of *Lasiodiplodia* species associated with grapevines in Mexico. *Phytopathologia Mediterranea* 60: 237–251. https://doi.org/10.36253/phyto-12576

Rathnayaka A.R., Chethana K.T., Phillips A.J., Liu J.K., Samarakoon M.C., … Zhao C.L., 2023. Re-evaluating Botryosphaeriales: ancestral state reconstructions of selected characters and evolution of nutritional modes. *Journal of Fungi* 9: 184. https://doi.org/10.3390/jof9020184

Rolshausen P.E., Akgül D.S., Perez R., Eskalen A., Gispert C., 2013. First report of wood canker caused by *Neoscytalidium dimidiatum* on grapevine in California. *Plant Disease* 97: 1511–1511. https://doi.org/10.1094/PDIS-04-13-0451-PDN

SIAP Servicio de Información y Estadística Agroalimentaria y Pesquera, 2023. Ministerio de Agricultura de Mexico, Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA).

Slippers B., Fourie G., Crous P.W., Coutinho T.A., Wingfield B.D., Wingfield M.J., 2004. Multiple gene sequences delimit *Botryosphaeria australis* sp. nov. from *B. lutea*. *Mycologia* 96: 1030–1041. https://doi.org/10.1080/15572536.2005.11832903

Slippers B., Wingfield M., 2007. *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* 21: 90–106. https://doi.org/10.1016/j.fbr.2007.06.002

Slippers B., Smit W.A., Crous P.W., Coutinho T.A., Wingfield B.D., Wingfield M.J., 2007. Taxonomy, phylogeny and identification of *Botryosphaeriaceae* associated with pome and stone fruit trees in South Africa and other regions of the world. *Plant Pathology* 56: 128–139. https://doi.org/10.1111/j.1365-3059.2006.01486.x

Stempien E., Goddard M.L., Wilhelm K., Tarnus C., Bertsch C., Chong, J., 2017. Grapevine Botryosphaeria dieback fungi have specific aggressiveness factor repertory involved in wood decay and stilbene metabolization. *PloS One* 12: e0188766. https://doi.org/10.1371/journal.pone.0188766

Téliz D., Valle P., 1979. Eutypa dieback in Mexican vineyards. *Plant Disease Reporter* 63: 312–314.

Thompson J.D., Higgins D.G., Gibson T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.

Úrbez-Torres J.R., Leavitt G.M., Voegel T.M., Gubler W.D., 2006. Identification and distribution of *Botryosphaeria* spp. associated with grapevine cankers in California. *Plant Disease* 90: 1490–1503. https://doi.org/10.1094/PD-90-1490

Úrbez-Torres J.R., Leavitt G.M., Guerrero J.C., Guevara J., Gubler W.D., 2008. Identification and pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata*, the causal agents of bot canker disease of grapevines in Mexico. *Plant Disease* 92: 519–529. https://doi.org/10.1094/PDIS-92-4-0519

Úrbez-Torres J.R., Adams P., Kamas J., Gubler W.D., 2009. Identification, incidence, and pathogenicity of fungal species associated with grapevine dieback in Texas. *American Journal of Enology and Viticulture* 60: 497–507. https://doi.org/10.5344/ajev.2009.60.4.497

Úrbez-Torres J.R., Peduto F., Rooney-Latham S., Gubler W.D., 2010a. First report of *Diplodia corticola* causing grapevine (*Vitis vinifera*) cankers and trunk cankers and dieback of canyon live oak (*Quercus chrysolepis*) in California. *Plant Disease* 94: 785–785. https://doi.org/10.1094/PDIS-94-6-0785A

Úrbez-Torres J.R., Bruez E., Hurtado J., Gubler W.D., 2010b. Effect of temperature on conidial germination of *Botryosphaeriaceae* species infecting grapevines. *Plant Disease*, 94: 1476–1484. https://doi.org/10.1094/PDIS-06-10-0423

Úrbez-Torres J.R., 2011. The status of *Botryosphaeriaceae* species infecting grapevines. *Phytopathologia Mediterranea* 50: S5–S45.

van Niekerk J.M., Crous P.W., Groenewald J.Z., Fourie P.H., Halleen F., 2004. DNA phylogeny, morphology and pathogenicity of *Botryosphaeria* species on grapevines. *Mycologia* 96: 781–798. https://doi.org/10.1080/15572536.2005.11832926

van Niekerk J.M., Fourie P.H., Halleen F., Crous P.W., 2006. *Botryosphaeria* spp. as grapevine trunk disease pathogens. *Phytopathologia Mediterranea* 45: 43–54.

Wagner D.B., Furnier G.R., Saghai-Maroof M.A., Williams SM, Dancik B.P., Allard R.W., 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. *PNAS* 84: 2097–2100.

Waite H., Armengol J., Billones-Baaijens R., Gramaje D., Hallen F., … Smart R., 2018. A protocol for the management of grapevine rootstock mother vines to reduce latent infections by grapevine trunk pathogens in cuttings. *Phytopathologia Mediterranea* 57: 384–398. https://doi.org/10.14601/Phytopathol_Mediterr-22772

White T.J., Bruns T., Lee S.J.W.T., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a Guide to Methods and Applications* 18: 315–322.

Xiao J., Zhang Q., Gao Y.Q., Tang J.J., Zhang A.L., Gao J.M., 2014. Secondary metabolites from the endophytic *Botryosphaeria dothidea* of *Melia azedarach* and their antifungal, antibacterial, antioxidant, and cytotoxic activities. *Journal of Agricultural and Food Chemistry* 62: 3584–3590. https://doi.org/10.1021/jf500054f