Effect of Temperature on Conidial Germination of *Botryosphaeriaceae* Species Infecting Grapevines

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ABSTRACT

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Germination of conidia of eight botryosphaeriaceous fungi infecting grapevines was evaluated after 2, 4, 6, 12, and 24 h incubation under eight different temperatures (5, 10, 15, 20, 25, 30, 35, and 40°C). The effect of temperature on conidial germination was also evaluated in different stages (hyaline versus pigmented conidia) of the species *Lasiodiplodia theobromae*. Conidial germination of *Botryosphaeriaceae* species infecting grapevines was significantly affected by temperature. Overall, conidial germination increased significantly with longer incubation times, especially from 2 to 12 h. In most cases, germination of conidia was not significantly different between 12 and 24 h incubation. Conidia of botryosphaeriaceous species did not germinate (with the exception of *Botryosphaeria dothidea* and *Neofusicoccum parvum*) at 5°C, and only *B. dothidea*, *Diplodia seriata*, and *L. theobromae* showed high levels of conidial germination at 40°C. Optimum conidial germination temperatures (defined as the temperature in which germination reached at least 50% in the shortest incubation time) were 25°C for *B. dothidea* and *Dothiorella iberica*, 25 to 30°C for *Spencermartinsia viticola*, 30°C for *Diplodia corticola*, *D. mutila*, *D. seriata*, *N. parvum*, and hyaline *L. theobromae*, and 40°C for pigmented *L. theobromae* conidia. Successful conidial germination of species of *Botryosphaeriaceae* infecting grapevines was always observed between 10 and 35°C with the exception of *Dothiorella iberica* and pigmented *L. theobromae* conidia, neither of which germinated at 35 and 10°C, respectively. Results of this study show conidia of botryosphaeriaceous species infecting grapevines to be capable of germination under a broad range of temperatures including those considered to be extreme, which may explain the success of these species as grapevine pathogens throughout most of the grape-growing areas in both Northern and Southern Hemispheres.

Botryosphaeriaceae Theiss. & P. Syd. is a species-rich family with 26 genera (www.MycoBank.org). Species in the family *Botryosphaeriaceae* are found worldwide and are saprophytic, endophytic, and parasitic in a wide range of both annual and perennial host plants (2,27). Several botryosphaeriaceous species are well-known pathogens of woody perennial plants causing significant economic losses in important crops including pome and stone fruit trees (5,26), blueberry (7,18), pistachio (15), almond (10), avocado (14), and grapevines (33,41). To date, 15 species in the *Botryosphaeriaceae*, placed in the anamorphic genera *Diplodia* Fr., *Dothiorella* Sacc., *Fusicoccum* Corda, *Lasiodiplodia* Ellis & Everh, *Neofusicoccum* Crous, Slippers & A.J.L. Phillips, and *Spencermartinsia* A.J.L. Phillips, A. Alves & Crous, have been recognized to be pathogenic on grapes (31,33,39–41). Species of *Botryosphaeriaceae* infecting grapevines cause a broad variety of disease symptoms including light brown discoloration of the wood, dark wood streaking, bud necrosis, graft failure, cane bleaching, perennial cankers, and bunch rot (13,20,21,38,44). Significant economic losses to the grapevine industry as a result of *Botryosphaeriaceae* species infection have been reported in the southeastern United States and California (17,25).

Conidia, produced in pycnidia located in different parts of the vine (old pruning wounds, dead tissue of spurs, cordons, and trunk, embedded in the bark and in pruning debris left on the vineyard floor), are the primary propagules of *Botryosphaeriaceae* in vineyards. Additionally, pycnidia of species of *Botryosphaeriaceae* have been reported to occur in a wide range of other hosts surrounding vineyards (4,10). Seasonal patterns of conidial release have been shown to vary from one grapevine-growing region to another; however, conidial release has always been shown to be correlated with rain events (12,24,32). Conidia are rain splash dispersed and infect grapevines primarily through pruning wounds. Úrbez-Torres and Gubler (34) presented evidence that pruning wound infections by species of *Botryosphaeriaceae* occur mainly during the dormant season in California, especially when vines were pruned from November to January. In a similar study conducted in Italy, conidia of *Diplodia seriata* De Not. were shown to infect grapevine pruning wounds up to late spring/early summer (24). Consequently, it is likely that *Botryosphaeriaceae* spores germinate and infect grapevines under a wide range of different environmental conditions including temperature.

Temperature is known to play a critical role in germination of most fungal spores. Consequently, the effect of temperature on spore germination of fungal pathogens has been widely studied in order to determine the necessary conditions for fungal infection. Moreover, knowledge of various spore reactions to temperature has been shown to significantly improve both disease prediction models and management options in many different crops (3,9,11,16,19,29). The effect of temperature on germination of both ascospores and conidia of *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not. and *D. seriata* causing white and black rot, frog-eye leaf spot, and limb canker of apples has been widely studied (1,28). However, the precise influence of temperature on conidial germination of species of *Botryosphaeriaceae* infecting grapevines has not yet been investigated. Knowledge of both optimum and range of temperatures in which *Botryosphaeriaceae* conidia germinate will result in a better understanding of their biology and disease epidemiology. Accordingly, this information will improve the development and implementation of more efficient management techniques for grapevine diseases caused by *Botryosphaeriaceae*. Thus, the objective of this study was to determine the effect of temperature on conidial germination of at least one species of each of the *Botryosphaeriaceae* genera infecting grapevines.

MATERIALS AND METHODS

This study included 20 isolates of eight different species representing all botryosphaeriaceous genera infecting grapevines (Table 1). Isolates were obtained from grapevine cankers from previous studies,

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identified by means of both morphological characters and molecular analyses, and stored at the Plant Pathology Department, University of California Davis, fungal collection (31,34,35,38). Isolates were maintained on 4% potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) mycelial plugs in glass vials with sterile water at 4°C until use. Mycelium of each isolate was recovered from our collection after plating of a mycelial plug on PDA petri dishes amended with tetracycline hydrochloride (0.01%) (Sigma-Aldrich, St Louis, MO) (PDA-tet). Petri dishes were incubated at room temperature (24 ± 2°C) for 10 days. Fungal isolates then were transferred to new 9-cm-diameter PDA petri dishes and incubated at room temperature under a 12-h light-and-dark cycle of cool-white fluorescent light until pycnidia were formed. In order to enhance pycnidia formation of the *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips isolates UCD642So and UCD1125Na, mycelial plugs were additionally placed onto 2% water agar (WA) (Difco) in 9-cm-diameter petri dishes containing autoclaved pine needles.

Conidia of the different species were obtained by collecting pycnidia formed in cultures and separately placing them in 1.5-ml sterile microcentrifuge tubes. Pycnidia were crushed in sterile distilled water using a sterile plastic pestle. Water containing conidia was filtered through four layers of cheesecloth to remove mycelial and pycnidia fragments. The resulting conidial suspension was quantified with a hemacytometer, and thereafter was diluted to a final concentration of approximately 50 conidia per microliter. For each isolate, four 1-µl drops of conidial suspension were placed onto 9-cm-diameter PDA petri dishes as described by Inman et al. (11). Inoculated petri dishes were placed in temperature-controlled incubators set at 5, 10, 15, 20, 25, 30, 35, and 40°C and removed at 2, 4, 6, 12, and 24 h thereafter to quantify spore germination. As soon as petri dishes were removed from the incubators, 2 µl of aniline blue dye was deposited onto each group of conidia, and plates were stored at 4°C until the stain was fully absorbed. Afterward, conidia were examined microscopically at ×40 magnification to determine the percentage of germinated conidia. Percent spore germination was determined by observing 50 conidia selected randomly from each drop. Conidia were counted as germinated when the length of the germ tube was at least one-half the length of the spore. The experiment was repeated twice.

A separate experiment was conducted to determine the effect of temperature on germination of hyaline conidia of *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. In this study, *L. theobromae* conidia from the different isolates were harvested from pycnidia when hyaline (before development of septa, pigmentation, and striations), when cultures were approximately 14 days old. Spore germination of hyaline *L. theobromae* conidia was determined as described above. The experiment was repeated twice.

All statistical analyses were performed using SAS (Version 9.2; SAS Institute, Cary, NC). Spore germination percentages were arcsine square root transformed prior to analyses. The significance of differences on conidial germination among species and isolates was tested with analysis of variance (ANOVA) using the SAS PROC Mixed procedure. The effects of isolate, time, and temperature on germination were assessed using a linear mixed effects model for each species separately. Isolate, time, temperature, and all their interactions were treated as fixed effects. Experiment and experiment crossed with all fixed effects were treated as random effects. Replication was treated as a random effect, nested in the four-way interaction of experiment, isolate, time, and temperature. This resulted in a conservative analysis for the main effects. Model fit was assessed via graphical analysis of residuals and the Shapiro-Wilk test for normality. The effects of outliers were assessed by performing the analysis with and without residuals. Pairwise comparisons between times within temperatures were made using the Tukey-Kramer correction test at the 5% significance level. Germination data of each *Botryosphaeriaceae* species were analyzed using linear regression to determine optimum conidial germination temperature. Optimum temperature was defined as the temperature in which germination reached 50% in the shortest incubation time. First- and second-order polynomials were fit to the data, and R^2 values were examined to find curves that best fit the data.

RESULTS

Spore germination data from two separate experiments were not significantly different ($F = 1.07$, $P = 0.3157$), and thus data from both experiments were combined in the analyses. There were no sig-

Table 1. *Botryosphaeriaceae* species isolates used in this study

Isolate	Species	Geographic origin	GenBank accession no.			
			ITS[a]	β-tubulin[b]	EF1-α[c]	Collection no.[d]
UCD1064So	*Botryosphaeria dothidea*	Sonoma County, CA	DQ233600	DQ233621	GU294733	UCD1064So
UCD1333So	*Botryosphaeria dothidea*	Sonoma County, CA	DQ008327	DQ008350	GU294736	UCD1333So
UCD1672Yo	*Botryosphaeria dothidea*	Yolo County, CA	DQ233603	DQ233624	GU294737	MYA-3710
UCD1260So	*Diplodia corticola*	Sonoma County, CA	GU799470	GU799464	GU799467	UCD1260So
UCD1275So	*Diplodia corticola*	Sonoma County, CA	GU799471	GU799465	GU799468	UCD1275So
UCD2397TX	*Diplodia corticola*	Gillespie County, TX	FJ790842	GU294724	GU294710	UCD2397TX
UCD288Ma	*Diplodia mutila*	Madera County, CA	DQ008313	DQ008336	EU012411	UCD288Ma
UCD1965SB	*Diplodia mutila*	Santa Barbara County, CA	DQ233599	DQ233620	EU012414	MYA-3697
UCD244Ma	*Diplodia seriata*	Madera County, CA	DQ008314	DQ008337	EU012406	MYA-3692
UCD352Mo	*Diplodia seriata*	Monterey County, CA	DQ008315	DQ008338	EU012407	MYA-3693
UCD1439SLO	*Dothiorella iberica*	San Luis Obispo County, CA	EF202008	EF202015	EF202022	CBS121001
UCD1448SLO	*Dothiorella iberica*	San Luis Obispo County, CA	EF202009	EF202016	EF202023	CBS121002
UCD191Co	*Lasiodiplodia theobromae*	Riverside County, CA	DQ008308	DQ008331	EU012397	UCD191Co
UCD205Co	*Lasiodiplodia theobromae*	Riverside County, CA	DQ008310	DQ008334	EU012398	MYA-3689
UCD256Ma	*Lasiodiplodia theobromae*	Madera County, CA	DQ233594	DQ233615	GU294742	UCD256Ma
UCD642So	*Neofusicoccum parvum*	Sonoma County, CA	DQ008328	DQ008351	GU294741	MYA-3705
UCD1125Na	*Neofusicoccum parvum*	Napa County, CA	DQ233612	DQ233633	GU294740	UCD1125Na
UCD3So	*Spencermartinsia viticola*	Sonoma County, CA	EF202013	EF202020	EF202027	CBS120999
UCD1435SLO	*Spencermartinsia viticola*	San Luis Obispo County, CA	EF202007	EF202014	EF202021	MYA-4112
UCD1642Yo	*Spencermartinsia viticola*	Yolo County, CA	EF202010	EF202017	EF202024	CBS121000

[a] Internal transcribed spacer region (ITS1-5.8S-ITS2).
[b] Partial beta-tubulin (BT) gene.
[c] Partial translation elongation factor 1-alpha gene.
[d] Acronyms of cultures collections: UCD: University of California Davis, Plant Pathology Department Culture Collection; MYA: American Type Culture Collection, Manassas, VA, USA; CBS: Centraalbureau Schimmelcultures, Utrecht, Netherlands.

nificant differences among isolates of *B. dothidea*, *Diplodia corticola* A.J.L. Phillips, Alves & Luque, *Diplodia mutila* Fr. in Mont., *N. parvum*, and *L. theobromae* for any of the temperature response parameters and incubation times (Table 2). Consequently, germination rates shown in Figures 1 and 2 represent the mean of the different isolates tested for each of those species of *Botryosphaeriaceae*. Statistical analyses showed significant differences among isolates of *D. seriata*, *Dothiorella iberica* A.J.L. Phillips, Luque & Alves, and *Spencermartinsia viticola* A.J.L. Phillips & Luque (Table 2). Consequently, germination rates shown in Figures 3, 4, and 5 represent each isolate tested for those species of *Botryosphaeriaceae*. The effect of temperature and incubation time on conidial germination of each species was highly significant (Table 2). At 5°C, overall results showed no conidial germination for either *Diplodia* or *Lasiodiplodia* species. At 5°C, 4.9 and 13.7% germination was observed after 24 h for *Dothiorella iberica* and *S. viticola* isolates UCD1439SLO and UCD1435SLO, respectively (Figs. 4A and 5B), and 5.2 and 26.9% after 24 h for *B. dothidea* and *N. parvum*, respectively. Conidia from all *Botryosphaeriaceae* species studied germinated to some extent at 40°C. However, percent conidial germination from *B. dothidea*, *D. seriata*, and *L. theobromae* was significantly higher than the rest of the species at 40°C. Percent conidial germination of each species generally increased significantly with longer incubation times when temperatures were equal to and/or above 10°C (Figs. 1 to 5). At 10°C and above and for all species studied, the greatest increase in conidial germination generally was observed between 2 and 12 h incubation time. At low temperatures (10 and 15°C), some species, including *N. parvum*, *L. theobromae* (hyaline), *Dothiorella iberica*, and *S. viticola,* showed a significant increase in conidial germination up to 24 h incubation (Figs. 1 to 3 and 5); however, percent germination in most cases was not significantly different between 12 and 24 h incubation times (Figs. 1 to 5).

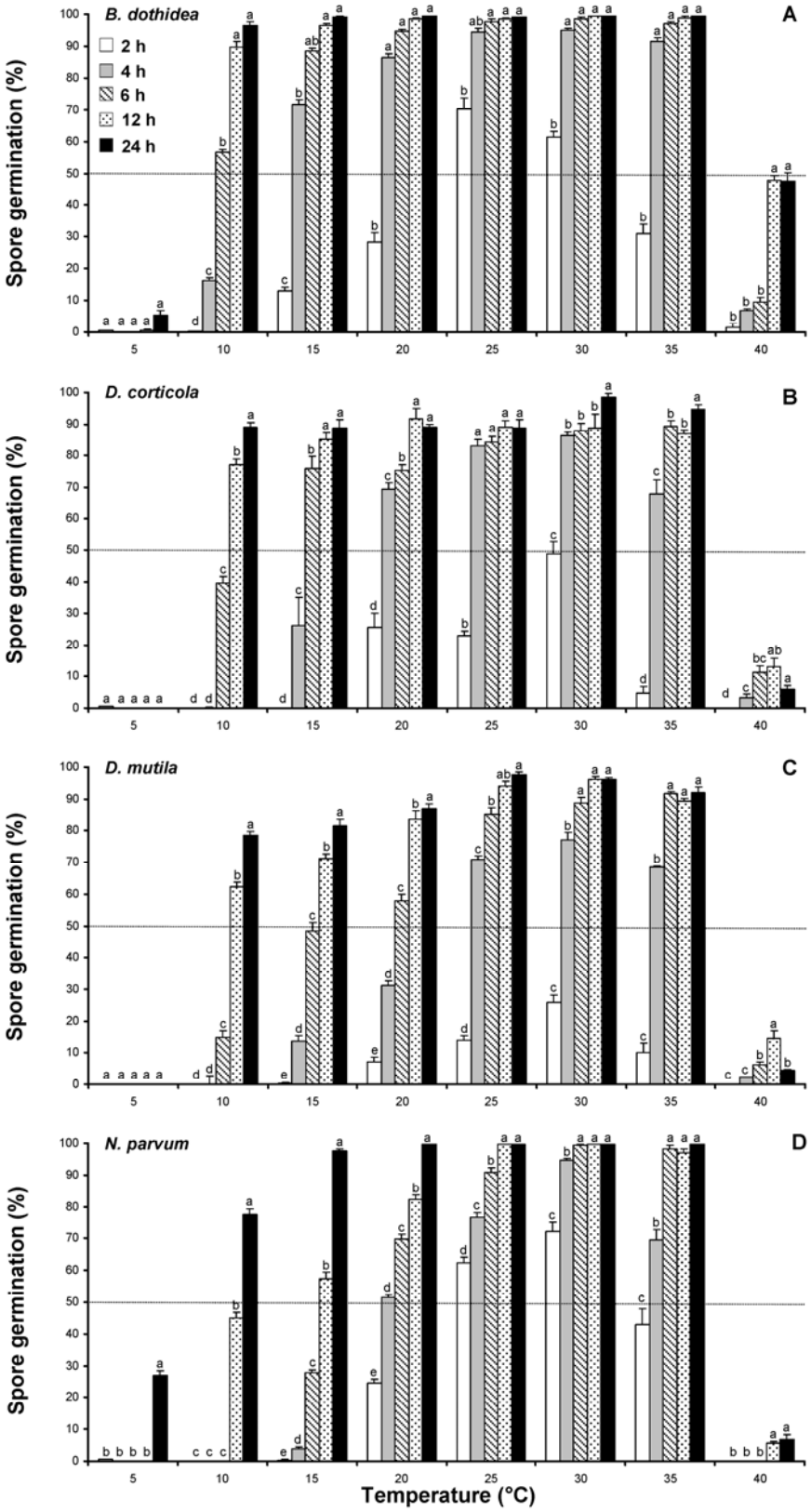

Fig. 1. Effect of temperature on conidial germination of **A,** *Botryosphaeria dothidea*, **B,** *Diplodia corticola*, **C,** *Diplodia mutila*, and **D,** *Neofusicoccum parvum* after 2, 4, 6, 12, and 24 h of incubation. Bars represent standard error of the mean. Means with the same letter are not significantly different at the 0.05 level. Dotted line represents 50% germination.

Approximately 56% of the conidia of *B. dothidea* germinated at 10°C after 6 h, and more than 80% germination occurred after 12 h (Fig. 1A). Over 80% of conidia of *B. dothidea* germinated between 15 and 35°C after 4 h incubation. Over 50% conidial germination of *B. dothidea* was observed at both 25 and 30°C after only 2 h incubation. Regression analyses showed 25°C to be the temperature at which 50% germination was reached in the shortest incubation time (1.7 h, $r^2 = 0.9267$).

Conidia of *D. corticola* showed 77% germination at 10°C after 12 h. Over 50% germination of conidia of *D. corticola* was observed after 6 h at 15°C and after 4 h between 20 and 35°C. Approximately 98 and 95% of conidia germinated at 30 and 35°C, respectively, after 24 h incubation (Fig. 1B). Regression analyses showed 30°C to be the temperature at which 50% germination was reached in the shortest incubation time (2.2 h, $r^2 = 0.9941$). More than 50 and 80% *D. mutila* conidia germinated between 25 and 35°C after 4 and 6 h,

respectively (Fig. 1C). Based on regression analyses, optimum temperature for conidial germination of *D. mutila* was 30°C. At this temperature, 50% germination was reached in the shortest incubation time (3.12 h, $r^2 = 0.9507$).

Approximately 62 and 72% of the conidia of *N. parvum* germinated after 2 h at 25 and 30°C, respectively (Fig. 1D). After 4 h incubation, over 50% conidial germination of *N. parvum* was observed between 20 and 35°C. Over 98% conidial germination of *N. parvum* was reached after 24 h at 15 and 20°C, after 12 h at 25°C, and after 6 h at 30 and 35°C (Fig. 1D). Regression analyses showed 30°C to be the temperature at which 50% germination was reached in the shortest incubation time (1.9 h, $r^2 = 0.8123$).

L. theobromae pigmented and septate conidia needed temperatures of 20°C and above in order to reach over 50% germination (Fig. 2A). After 4 h incubation, over 50% of pigmented and septate conidia germinated between 25 and 40°C. Over 80% conidial germination was reached after 12 h at 25 and 30°C and after 6 h at 35 and 40°C (Fig. 2A). The shortest incubation time to reach at least 50% of *L. theobromae* pigmented and septate conidial germination was observed at 40°C (3.87 h, $r^2 = 0.9377$) but was not significantly different ($P = 0.2239$) from the incubation time observed at 35°C (3.91 h, $r^2 = 0.9232$). *L. theobromae* hyaline and unicellular conidia reached 50% germination at lower temperatures (10 and 15°C) than the pigmented and septated conidia, and after 4 h incubation 50% germination or more was observed between 20 and 40°C (Fig. 2B). Over 80% hyaline and unicellular conidial germination was reached after 12 h at 15°C and after 6 h between 20 and 40°C (Fig. 2B). The shortest incubation time to reach at least 50% of *L. theobromae* hyaline and unicellular conidial germination was observed at 30°C (2.58 h, $r^2 = 0.9227$).

Conidia of both *D. seriata* isolates UCD244Ma and UCD352Mo showed over 50% germination after 6 h at 10°C (Fig. 3). Over 50% germination was observed after 2 h between 20 and 35°C and between 25 and 35°C for isolates UCD244Ma and UCD352Mo, respectively. Over 80% germination of conidia of *D. seriata* was observed after 4 h between 25 and 35°C and between 20 and 35°C for isolates UCD244Ma and UCD352Mo, respectively (Fig. 3). For isolate UCD244Ma, approximately 63, 85, and 87% of conidia germinated at 40°C after 6, 12, and 24 h incubation, respectively (Fig. 3A). For isolate UCD352Mo, approximately 73, 80, 83, and 75% of conidia germinated at 40°C after 4, 6, 12, and 24 h incubation, respectively (Fig. 3B). Regression analyses showed 30°C to be the temperature at which 50% germination was reached in the shortest incubation time for both UCD244Ma (1.76 h, $r^2 = 0.8789$) and UCD352Mo (1.87 h, $r^2 = 0.7341$).

Conidia of *Dothiorella iberica* needed 24 h incubation to reach more than 50% germination at 10°C and over 12 h at 15, 20, and 30°C (Fig. 4). In both *Dothiorella iberica* isolates studied, conidial germination increased significantly from 2 h (<10% conidia germinated) to 24 h (>90% conidia germinated) between 15 and 30°C. Isolate UCD1439SLO showed slightly higher spore germination at both 35 and 40°C than isolate UCD1448SLO. Regression analyses showed 25°C to be the temperature at which 50% germination was reached in the shortest incubation time for both UCD1439SLO (5.83 h, $r^2 = 0.8633$) and UCD1448SLO (4.75 h, $r^2 = 0.8317$) isolates.

Conidial germination of *S. viticola* varied significantly among the isolates studied. Over 50% conidial germination was observed after 4 h between 25 and 30°C and between 20 and 30°C for isolate UCD3So and UCD1435SLO and UCD1642Yo, respectively (Fig. 5). Isolate UCD3So reached over 80% conidial germination after 24 h at 10 and 15°C, after 12 h at 20°C, and after 6 h at 25 and 30°C (Fig. 5A). Isolate UCD1435SLO reached over 80% conidial germination after 24 h at 10°C, after 12 h at 15 and 20°C, and after 6 h at 30°C (Fig. 5B). Isolate UCD1642Yo reached over 80% conidial germination after 24 h at 10°C, after 12 h at 15°C, and after 6 h at 20, 25, and 30°C (Fig. 5C). Conidial germination did not reach more than 75% at 35°C in all isolates studied, and only isolate UCD1435SLO showed conidial germination at 40°C (Fig. 5B). Based on regression analyses, optimum temperature at which 50% conidial germination of *S. viticola* was reached in the shortest incubation time was not significantly different between 25 and 30°C for all isolates studied: UCD3So (3.03 h, $r^2 = 0.9101$), UCD1435SLO (2.89 h, $r^2 = 0.7342$), and UCD1642Yo (2.6 h, $r^2 = 0.6522$).

DISCUSSION

Species of *Botryosphaeriaceae* have recently gained importance as grapevine pathogens worldwide, and consequently, interest has risen significantly in understanding their biology, epidemiology, and mode of infection in order to develop and implement efficient chemical, cultural, and organically acceptable control methods. Species of *Botryosphaeriaceae* infect grapevines by means of conidia, primarily through pruning wounds. Although the effect of temperature on mycelium growth has been studied on species of *Botryosphaeriaceae* infecting grapevines (38), little information was available regarding

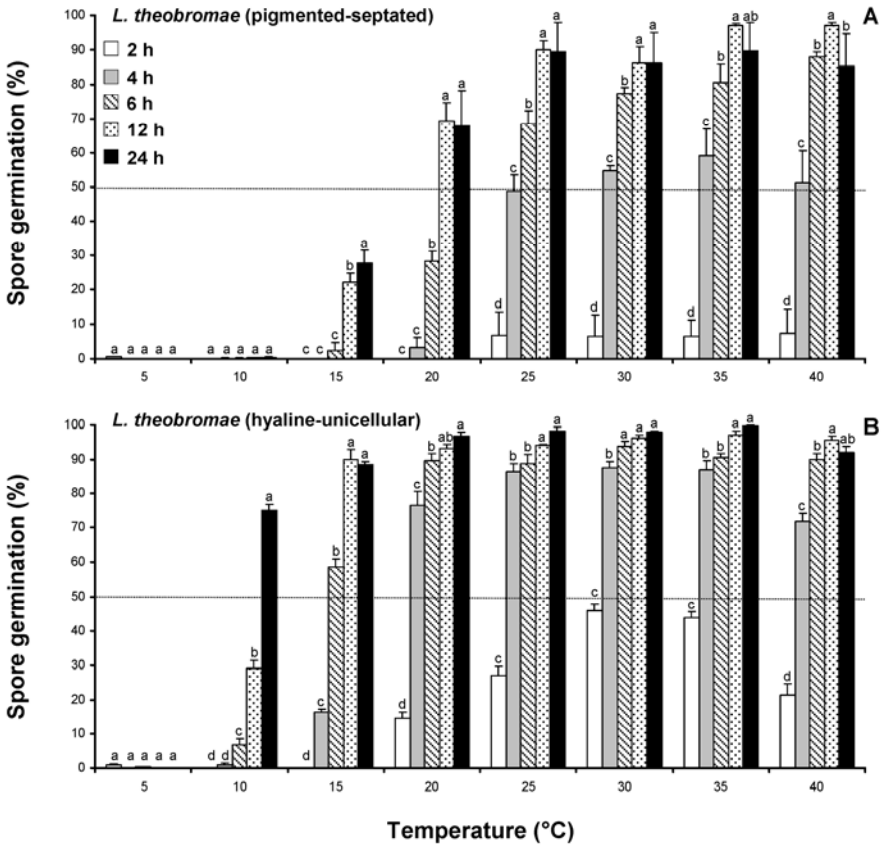

Fig. 2. Effect of temperature on **A,** pigmented-septated and **B,** hyaline-unicellular conidial germination of *Lasiodiplodia theobromae* after 2, 4, 6, 12, and 24 h of incubation. Bars represent standard error of the mean. Means with the same letter are not significantly different at the 0.05 level. Dotted line represents 50% germination.

the role that temperature plays in spore germination. This study represents the first attempt to determine the optimum temperature for conidial germination of *Botryosphaeriaceae* infecting grapevines. Results of this study show conidial germination to be significantly affected by temperature.

The results of this study on the effect of temperature on germination of conidia of *B. dothidea* are similar to those obtained by both Sutton and Arauz (28) and Michailides et al. (16). Sutton and Arauz (28) observed the optimum temperature of conidial germination of *B. dothidea* isolates infecting apples to be between 26.7 and 29.5°C. Michailides et al. (16) showed conidial germination of *B. dothidea* iso-

Table 2. Analysis of fixed effects of isolate, temperature, and time, and their interactions on percentage of conidia germination of each species of *Botryosphaeriaceae* using a mixed procedure[a]

Species	Effect	Num df	Den df	*F* value	*P* value
Botryosphaeria dothidea	Isolate	2	2	14.52	0.0644
	Temperature	7	7	169.55	<0.0001
	Time	4	4	203.22	<0.0001
	Temperature*Time	28	28	10.12	<0.0001
	Isolate*Temperature	14	14	1.72	0.1609
	Isolate*Time	8	8	0.44	0.8679
	Isolate*Temperature*Time	56	56	1.16	0.2867
Diplodia corticola	Isolate	2	2	4.16	0.1939
	Temperature	7	7	3,104.78	<0.0001
	Time	4	4	2,776.22	<0.0001
	Temperature*Time	28	28	206.84	<0.0001
	Isolate*Temperature	14	14	1.88	0.1243
	Isolate*Time	8	8	1.15	0.4218
	Isolate*Temperature*Time	56	56	0.92	0.6145
Diplodia mutila	Isolate	1	1	78.96	0.0713
	Temperature	7	7	2,223.52	<0.0001
	Time	4	4	2,533.35	<0.0001
	Temperature*Time	28	28	131.81	<0.0001
	Isolate*Temperature	7	7	3.47	0.0614
	Isolate*Time	4	4	2.24	0.2272
	Isolate*Temperature*Time	28	28	0.95	0.5543
Diplodia seriata	Isolate	1	1	137.23	0.0542
	Temperature	7	7	1,698.21	<0.0001
	Time	4	4	1,662.57	<0.0001
	Temperature*Time	28	28	100.97	<0.0001
	Isolate*Temperature	7	7	60.58	<0.0001
	Isolate*Time	4	4	24.32	0.0046
	Isolate*Temperature*Time	28	28	15.61	<0.0001
Dothiorella iberica	Isolate	1	1	22.83	0.1313
	Temperature	7	7	917.92	<0.0001
	Time	4	4	1,061.55	<0.0001
	Temperature*Time	28	28	222.05	<0.0001
	Isolate*Temperature	7	7	9.01	0.0048
	Isolate*Time	4	4	12.50	0.0156
	Isolate*Temperature*Time	28	28	9.84	<0.0001
Lasiodiplodia theobromae (mature)	Isolate	2	2	1.75	0.3634
	Temperature	7	7	67.00	<0.0001
	Time	4	4	56.66	0.0009
	Temperature*Time	28	28	6.46	<0.0001
	Isolate*Temperature	14	14	0.43	0.9362
	Isolate*Time	8	8	1.71	0.2318
	Isolate*Temperature*Time	56	56	0.50	0.9948
Lasiodiplodia theobromae (immature)	Isolate	2	2	0.38	0.6903
	Temperature	7	7	62.45	<0.0001
	Time	4	4	54.55	0.0004
	Temperature*Time	28	28	6.13	<0.0001
	Isolate*Temperature	14	14	0.39	0.8991
	Isolate*Time	8	8	2.01	0.2925
	Isolate*Temperature*Time	56	56	0.55	0.9331
Neofusicoccum parvum	Isolate	1	1	11.97	0.1791
	Temperature	7	7	2,522.42	<0.0001
	Time	4	4	2,231.43	<0.0001
	Temperature*Time	28	28	119.85	<0.0001
	Isolate*Temperature	7	7	0.83	0.5950
	Isolate*Time	4	4	4.46	0.0885
	Isolate*Temperature*Time	28	28	0.99	0.5052
Spencermartinsia viticola	Isolate	2	2	502.91	0.0020
	Temperature	7	7	2,831.17	<0.0001
	Time	4	4	2,669.70	<0.0001
	Temperature*Time	28	28	172.83	<0.0001
	Isolate*Temperature	14	14	33.43	<0.0001
	Isolate*Time	8	8	43.86	<0.0001
	Isolate*Temperature*Time	56	56	26.88	<0.0001

[a] Num df = numerator degrees of freedom, Den df = denominator degrees of freedom.

lates infecting pistachio to germinate within 1.5 to 2 h between 23 and 30°C. This study showed that 50% germination of *B. dothidea* isolates infecting grapevines was reached after only 1.7 h at 25°C and after 2.2 and 2.7 h at 30 and 35°C, respectively. Moreover, *B. dothidea* conidia reached 50% germination after 12 h at 40°C. Additionally, previous studies showed isolates of *B. dothidea* from grapevines to be capable of forming colonies at 40°C which were characterized by very slow growth (38). However, these results differ from the observations reported by Michailides et al. (16) in which germ tubes of *B. dothidea* isolates from pistachio did not grow more than the length of the spore and failed to develop colonies at 36 and 37°C. The reasons why *B. dothidea* isolates infecting grapevines germinate and are viable at higher temperatures than those infecting pistachio are not well understood, but the differences could be due to natural variability of the isolates studied.

Foster (8) and Arauz and Sutton (1) showed favorable temperatures for conidial germination of *D. seriata* to range between 12 and 32°C. Results from our study are similar to those reported by Foster (8) and Arauz and Sutton (1), but *D. seriata* conidia in our experiments showed a high percent germination (>80%) over a broader range of temperatures (10 to 40°C). Arauz and Sutton (1) reported optimum temperature for conidial germination of *D. seriata* isolates from apples to be 24°C. In our study, optimum temperature for conidial germination of *D. seriata* isolates from grapevines was established at 30°C; however, 50% germination was reached within 2 h incubation at 20, 25, and 35°C. Additionally, this study showed the ability of *D. seriata* conidia to reach a high percentage of germination at 40°C, which was unknown from previous studies.

Williamson and Tandon (42) showed mature conidia of *L. theobromae* isolated from banana rot to germinate between 10 and 35°C with an optimum temperature of 25°C. These results differ significantly from the observations recorded in our study. First, optimum temperatures for conidial germination of *L. theobromae* isolates from grapevines in this study were significantly higher (30 to 40°C). Additionally, mature conidia of *L. theobromae* from grapevines did not germinate at 10°C and showed less than 30% germination at 15°C after 24 h incubation. Finally, contrary to what was observed by Williamson and Tandon (42), both hyaline and pigmented conidia of *L. theobromae* from grapevines showed high percent germination (>90%) at 40°C. The *L. theobromae* isolates used in this study were from both different host and geographic location with completely different environmental conditions, which could explain the significant variation that temperature has on spore

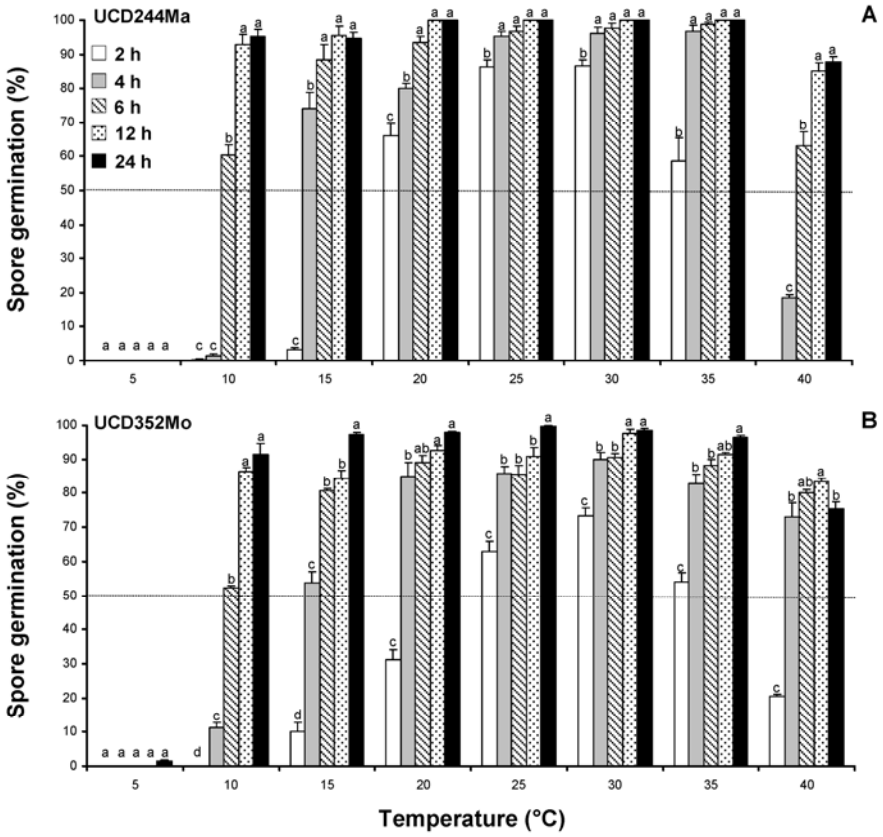

Fig. 3. Effect of temperature on conidial germination of *Diplodia seriata* isolates **A,** UCD244Ma and **B,** UCD352Mo after 2, 4, 6, 12, and 24 h of incubation. Bars represent standard error of the mean. Means with the same letter are not significantly different at the 0.05 level. Dotted line represents 50% germination.

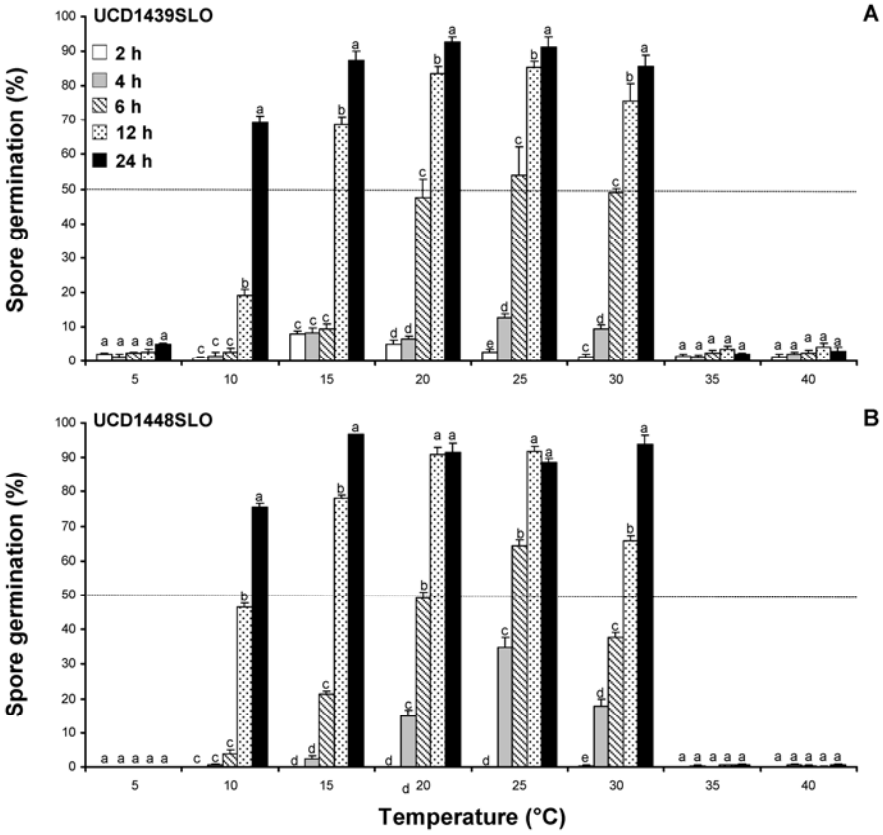

Fig. 4. Effect of temperature on conidial germination of *Dothiorella iberica* isolates **A,** UCD1439DLO and **B,** UCD1448SLO after 2, 4, 6, 12, and 24 h of incubation. Bars represent standard error of the mean. Means with the same letter are not significantly different at the 0.05 level. Dotted line represents 50% germination.

germination between *L. theobromae* isolates infecting grape and banana. Moreover, this study showed the capability of both hyaline-unicellular and pigmented-septated conidia of *L. theobromae* to reach high levels of germination between 10 and 40°C and 15 and 40°C, respectively. *Botryosphaeriaceae* species in the genera *Diplodia* and *Lasiodiplodia* are characterized by having hyaline conidia when young turning light-brown and/or dark-brown in color and developing septa when mature (20,21,30,38). Results of this study showed hyaline-unicellular conidia of *L. theobromae* to reach a significantly higher percentage of conidial germination in a shorter incubation time at the same temperatures than the pigmented-septated conidia. Accordingly, conidia of *L. theobromae* can be considered mature in an early stage when hyaline. Therefore, the development of pigmentation and septa of *L. theobromae* conidia could be more a consequence of aging than of maturity. However, the biological consequences of this finding are not clear at this time, and more research will be needed in order to determine the role that hyaline conidia could play in disease epidemiology.

Although species of *Botryosphaeriaceae* infect grapevines worldwide, their geographical distribution has been associated in some grape-growing regions with climatic conditions, especially temperature (21,30,36,38). Previous studies found some correlation between the optimum temperature for mycelium growth of *Botryosphaeriaceae* species infecting grapevines and their geographical distribution throughout California (38) and Mexico (36). Results of this study reinforce the idea that the geographical distribution of *Botryosphaeriaceae* species infecting grapevines may be strongly associated with temperature. This study showed both mature and immature conidia of *L. theobromae* to have the highest optimum temperatures for conidial germination (30 to 40°C) and the lowest germination rate at low temperatures (5 to 15°C) among all species of *Botryosphaeriaceae* studied. These results are in agreement with those reported by Copes and Hendrix (6) in which high temperatures (30°C) were also necessary to reach optimum conidia sporulation of *L. theobromae* isolates infecting apples and peaches. These results may explain why *L. theobromae* is rarely isolated from cold regions and why it is not only the most prevalent but occasionally the only fungus isolated from diseased vines in the warmest grapevine-growing regions of California (38), Mexico (36), and Western Australia (30). *Dothiorella iberica* conidia showed high germination rate (>70%) at low temperatures (10 to 15°C), needed the longest incubation time (5.86 h) to reach 50% germination at the optimum temperature (25°C), and did not germinate at 35 and 40°C, showing its adaptability to low temperatures. These results, along with those reported by Úrbez-Torres et al. (35) in which *Dothiorella iberica* showed the lowest optimum temperature (22°C) for mycelium growth among all species of *Botryosphaeriaceae* infecting grapevines may explain why this species was mainly isolated from diseased vines in cold grape-growing regions in California. Although this study showed the optimum temperature for conidial germination of *N. parvum* to be 30°C, the capability of this species to reach over 30% germination at 5°C and 80 and 98% germination at 10 and 15°C, respectively, could not only explain why this species is more prevalent in the coldest grape-growing regions in California (38), but also why it was reported to be the main fungal species associated with grapevine trunk diseases in northeastern American vineyards (23). Among all species of *Botryosphaeriaceae* used in this study, conidia of *D. seriata* showed the highest percent germination under the widest range of temperatures (10 to 40°C), which may explain why this species is probably the most cosmopolitan botryosphaeriaceous fungus infecting grapevines. *D. seriata* has been reported from over 35 different hosts in most continents (22) and was reported to be the most prevalent botryosphaeriaceous fungus isolated from grapevine cankers in California. Moreover, it is the only botryosphaeriaceous species present in all grapevine-growing regions surveyed throughout California and the United States (37,38).

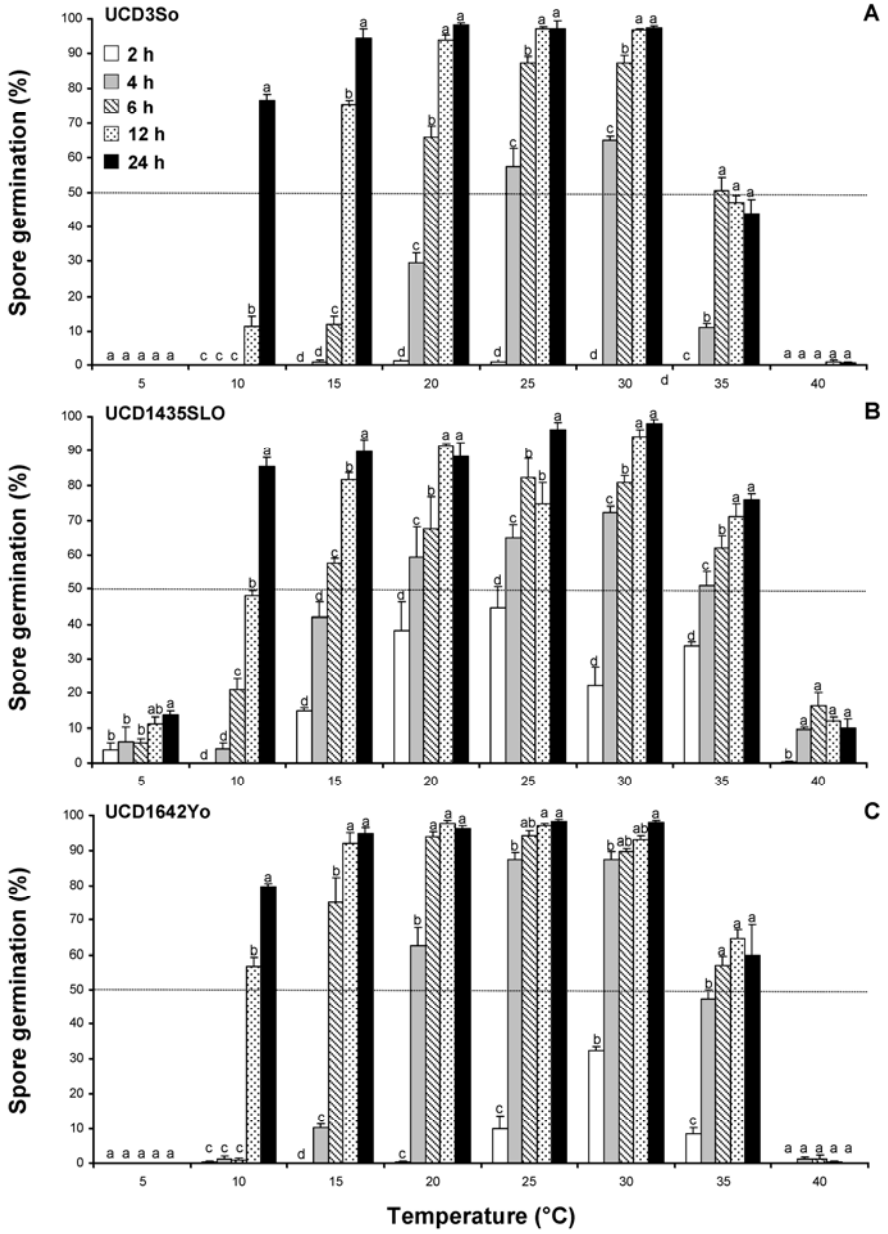

Fig. 5. Effect of temperature on conidial germination of *Spencermartinsia viticola* isolates **A,** UCD3SoMa, **B,** UCD1435SLO, and **C,** 1642Yo after 2, 4, 6, 12, and 24 h of incubation. Bars represent standard error of the mean. Means with the same letter are not significantly different at the 0.05 level. Dotted line represents 50% germination.

Based on both spore trapping (12,24,32) and pruning wound susceptibility studies (34), *Botryosphaeriaceae* were shown to infect grapevines mainly during dormancy coinciding with the pruning season. Based on the results obtained in this study, conidia of *Botryosphaeriaceae* species can germinate under a broad range of temperatures including low temperatures (5 to 15°C). Consequently, it is reasonable to hypothesize that conidia can germinate, colonize pruning wounds, and therefore infect grapevines during winter time (dormant season) when the amount of spores in the environment has been shown to be the highest (32). This can be particularly true in grapevine-growing regions of temperate zones with mild winters. During a 3-year study conducted in California vineyards to determine the environmental factors that trigger *Botryosphaeriaceae* species spore release, average winter temperatures ranged from 8 to 16°C depending on the grapevine-region studied (32). The present and other studies have shown successful germination of *Botryosphaeriaceae* species within the range of those temperatures (1,28). Additionally, Copes and Hendrix (6) showed sporulation of *B. dothidea*, *D. seriata*, and *L. theobromae* to successfully occur over 6 to 30°C. On the other hand, in grapevine-growing regions with significantly colder winters such as the northeastern United States, a dormancy period of *Botryosphaeriaceae* species may play an important role in the infection process. Conidial release at temperatures close to and/or below 0°C would necessarily need a dormant period until temperatures are more favorable to start germinating. However, previous work showed that even on cold winter days with air temperatures below 0°C, the temperature on the bark of aspen trees (*Populous tremuloides*) can be above freezing (43). Consequently, ascospores of the aspen canker pathogen *Hypoxylon mammatum* (Wahl.) Mill. (syn. *H. pruinatum* (Klotz.) Cke.) were discharged and could potentially germinate causing new infections in even cold winter days (43). Similar conditions could occur on grape-growing regions with much colder winters than California, allowing possible sporulation, germination, and infection of grapevines by species of *Botryosphaeriaceae* in cold winter days. Whether or not grapevine bark can present temperatures above freezing in cold winter days and *Botryosphaeriaceae* conidia are released at temperatures close to 0°C, present a dormant period, and/or if in regions with colder winters *Botryosphaeriaceae* infections would occur later in the season (spring) when temperatures are more favorable for conidial germination are still not clear at the present time, and more research is needed in order to clarify these questions. Conidia of *Botryosphaeriaceae* species infecting grapevines are water-splash dispersed, and thus it is logical to think that besides temperature, moisture can also play a critical role in spore germination. The effect of relative humidity on spore germination has been widely studied in many fungal pathogens including *D. seriata* and *B. dothidea* infecting apples, thus enhancing disease prediction models (1,28). Studies on the effect of relative humidity on both conidia germination and infection process of species of *Botryosphaeriaceae* infecting grapevines is currently being investigated in our laboratory.

The results presented in this study have added significant information to better understanding of the biology and geographical distribution of *Botryosphaeriaceae* species infecting grapevines. However, the effect of other environmental factors such as relative humidity and light should not be ignored due to the important role they could play in conidial germination and infection.

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