Botryosphaeriaceae Species Spore-Trapping Studies in California Vineyards

J. R. Úrbez-Torres, Department of Plant Pathology, University of California, Davis 95616; **M. Battany**, University of California Cooperative Extension, San Luis Obispo County, San Luis Obispo 93401; **L. J. Bettiga**, University of California Cooperative Extension, Monterey County, Salinas 93901; **C. Gispert**, University of California Cooperative Extension, Riverside County, Indio 92201; **G. McGourty**, University of California Cooperative Extension, Mendocino County, Ukiah 95482; **J. Roncoroni**, University of California Cooperative Extension, Napa County, Napa 94559; **R. J. Smith**, University of California Cooperative Extension, Sonoma County, Santa Rosa 95403; **P. Verdegaaal**, University of California Cooperative Extension, San Joaquin County, Stockton 95206; and **W. D. Gubler**, Department of Plant Pathology, University of California, Davis

ABSTRACT

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The seasonal abundance of *Botryosphaeriaceae* spp. spores was studied in California vineyards by using glass microscope slides covered with petroleum jelly placed on grapevine cordons and Burkard volumetric spore traps at seven and two different locations, respectively. Correlation analysis was used to determine which meteorological variables (precipitation, relative humidity, temperature, and wind speed) influenced *Botryosphaeriaceae* spp. spore release. Among all variables, regression analysis resulted in a strong relationship between spore release and precipitation. Additionally, a positive relationship between irrigation and spore release was also observed in the Riverside County vineyard. During the study period, spore discharge of *Botryosphaeriaceae* spp. occurred from the first fall rain through the last spring rains, coinciding with September to April. However, based on the results obtained from the spore traps, most spores (over 60%) were trapped following rain events during the winter months of December, January, and February, which coincides with the grapevine pruning season. *Botryosphaeriaceae* spp. spore release was much lower in fall and early spring (22%) and very few or no spores were trapped in late spring and summer (3%). This work suggests that a delay of pruning time in California may be warranted to reduce grapevine infection because the current timing coincides with the greatest period of spore discharge.

Fungal species in the family *Botryosphaeriaceae* are cosmopolitan endophytes, pathogens, and saprophytes on a diverse range of dicots, gymnosperms, and monocots (2,27,42). Although long known as vascular pathogens causing canker diseases and dieback in numerous woody perennial hosts (41), species of *Botryosphaeriaceae* have only recently been recognized as important pathogens of grapevines (*Vitis vinifera* L.) (29,35,36,41). To date, 15 different members of the *Botryosphaeriaceae* family, placed in the anamorphic genera *Diplodia*, *Dothiorella*, *Fusicoccum*, *Lasiodiplodia*, and *Neofusicoccum*, have been reported to be pathogenic on grapevines and to cause different grapevine dieback symptoms worldwide (29,34,37,39,41). Grapevine diseases caused by species of *Botryosphaeriaceae* include black dead arm (11),

bot canker (10,36), Diplodia cane dieback (7), excoriosis (19), and macrophoma rot (13).

In California, *Botryosphaeriaceae* spp. have been reported to be the most prevalent fungi isolated from grapevine cankers, and nine specific fungi are known to be responsible for one particular disease known as bot canker (10,35,38,39). Symptoms of the disease are characterized by dead cordons, spurs, and trunks, and the total absence of spring growth due to canker formation in the vascular tissue (10,38). In general, cankers are wedge shaped, typically begin in pruning wounds, and primarily grow basipetally from the initial point of infection (6,38). Dark vascular streaking of the wood is also commonly observed in grapevines infected by species of *Botryosphaeriaceae* such as the pathogens *Botryosphaeria dothidea*, *Lasiodiplodia theobromae*, and *Neofusicoccum parvum* in California (35,38,39). Members of the *Botryosphaeriaceae* family have been reported to cause bot canker in grape-growing regions of Australia (4,29), Chile (9), Mexico (36), and Spain (40) and in Arizona, Arkansas, Connecticut, Maryland, Massachusetts, Missouri, Nevada, New Hampshire, New York, Oregon,

Pennsylvania, Rhode Island, Texas, Vermont, and Virginia in the United States (23,34,37).

Grapevine trunk disease pathogens infect grapevines primarily through pruning wounds. Previous studies carried out in California have reported that inoculum of *Phaeoacremonium chlamydospora* and *Phaeoacremonium* spp., the causal agents of petri disease and esca, respectively, as well as *Eutypa lata* and *E. leptoplaca*, the causal agents of eutypa dieback, are airborne following rainfall events and typically enter grapevines from late fall through early spring (5,18,21,32,33). However, little is known about the epidemiology and mode of inoculum dispersal of *Botryosphaeriaceae* spp. on grapevines. Bot canker (along with eutypa dieback) is responsible for important economic losses to the California grapevine industry, which are estimated to be over \$260 million annually (26). Knowledge of *Botryosphaeriaceae* spp. inoculum availability during timing of inoculum dispersal, as well as the environmental factors that favor spore release, is critical to determine periods of low infection risk and, therefore, potential preventative measures, including appropriate timing for pruning and pruning wound treatments. The purpose of this study was to determine when and under what environmental conditions spores of *Botryosphaeriaceae* spp. are released in California vineyards.

MATERIALS AND METHODS

Glass microscope slide spore trapping. Glass microscope slides provided a rapid and easy means of quantifying spores from within vineyards. Spore traps consisted of glass microscope slides (25 by 76 mm) coated on both sides with a thin layer of white petroleum jelly and placed on grapevine cordons at approximately 80 cm above the ground as described by Eskalen and Gubler (5) (Fig. 1a). Traps were placed in seven different vineyards where bot canker had previously been reported to occur by field surveys (38,39). Vineyards were located throughout the major grape-growing regions of California, including Mendocino and Napa Counties in the North Coast, Colusa County in the Sacra-

Corresponding author: W. D. Gubler
E-mail: wdgubler@ucdavis.edu

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mento Valley, San Joaquin County in the north San Joaquin Valley, Monterey and San Luis Obispo Counties in the Central Coast, and Riverside County in Southern California (Fig. 1b). Ten spore traps were placed randomly on separate vines in the middle of a block (each block contained at least 45 rows of vines) in each vineyard. Spore traps were changed weekly and individually collected in preautoclaved 50-ml screw-cap tubes (Sarstedt, Inc., Newton, NC) and sent to the laboratory. Spores were removed by adding 10 ml of approximately 20°C distilled water into the screw-cap tube and then shaken by hand for 60 s. Two aliquots of 200 µl each were collected per spore trap and placed on two replicate 85-mm-diameter petri plates containing potato dextrose agar (PDA; Difco Laboratories, Detroit) amended with tetracycline hydrochloride (0.01%; Sigma-Aldrich, St. Louis) (PDA-tet). The liquid solution was spread over the petri plates using a sterilized curved glass rod and plates were dried for 10 min inside a laminar flow hood. Petri plates were incubated on the laboratory bench at room temperature (25 ± 1°C) and *Botryosphaeriaceae* fungal colonies were recorded after 7 days (Fig. 1d and e). *Botryosphaeriaceae* colonies were subcultured onto fresh PDA-tet in an attempt to morphologically identify to genus or species level. Spore-trapping studies were conducted from September 2006 through May 2009 in Napa, San Joa-

quin, San Luis Obispo, and Riverside Counties; from September 2006 through October 2008 in Colusa County; and from September 2007 through May 2009 in Mendocino and Monterey Counties.

Volumetric spore trapping. Volumetric spore traps allowed us to look at spore release timing during each 24-h period and observe whether or not spores were captured following cessation of rainfall events. Two Hirst-Burkard 7-day volumetric spore traps (Burkard Mfg. Co. Ltd., Rickmansworth, Herts, England) were operated for 20 weeks (1 December 2008 through 1 May 2009) in Monterey County and for 25 weeks (1 November 2008 through 8 May 2009) in Sonoma County. Volumetric spore traps were located in the middle of selected commercial vineyards where bot canker was reported to occur (38,39) (Fig. 1c). Spore traps were operated at an air sampling rate of 10 liters/min with the intake orifice 0.6 m above the ground. Spore trap tape preparation and mounting was done according to the instructions for the Burkard sampler. Spore trap tape was changed weekly and returned to the laboratory. For analyses purposes, the tape was cut to fit on a reading microscope slides measuring 25 by 76 mm (1 day per slide). Spores were counted by transversely scanning trap tapes in 1-h segments (2 mm wide) with a ×10 wide-field ocular and a ×20 objective lens of a compound light microscope as described by Trese et al.

(31). Species spores of *Botryosphaeriaceae* were identified based on morphological characters (shape, size, color, and absence or presence of septa) as previously described (20,38).

Environmental monitoring and data analysis. Total number of *Botryosphaeriaceae* spores captured from glass microscope slides and Burkard volumetric spore traps was recorded weekly and hourly during the study period, respectively. Results were superimposed with weekly and hourly records of total precipitation, average temperature, average relative humidity, and average wind speed. Weekly values were obtained by the sum of daily precipitation and by averaging daily mean temperature, daily mean relative humidity, and daily mean wind speed. Meteorological data were collected from weather stations within or adjacent to vineyard sites. Off-site weather stations were a part of the California Irrigation Management Information System, Department of Water Resources, Office of Water Use Efficiency. Hourly values were obtained from the private weather stations (Adcon International Inc., Davis, CA) located within the vineyard where the Burkard volumetric spore traps were placed and programmed to read sensors at 15-min intervals and to record average values at 1-h intervals. Weekly and daily total numbers of *Botryosphaeriaceae* spp. spores were correlated with the environmental variables recorded on the same week or day at each location by using simple linear regression analysis in PROC GLM in SAS 9.1 (SAS Institute, Cary, NC). In addition, a correlation analysis was performed to determine the correlation between overhead sprinkler irrigation and spore release at the Riverside County vineyard.

RESULTS

Glass microscope slide spore trapping. Regression analysis indicated a positive relationship between spore release and precipitation ($r^2 = 0.7$; $P < 0.0001$; $n = 847$) (Fig. 2) and, as precipitation increased, so did spore release. Weekly relative humidity averages and spore release were also positively correlated ($r^2 = 0.08$; $P < 0.0001$; $n = 847$; data not presented). Overall results present a yearly pattern of *Botryosphaeriaceae* spp. spore release coinciding with rain events that follow the dry summer period. Spores were captured from September to October through March to April following rainfall events at all locations (Figs. 3 and 4) and also by overhead sprinkler irrigation in the Monterey and Riverside vineyards (Figs. 3B and 4D). At the Riverside location, *Botryosphaeriaceae* spp. spores were first trapped the third week of September in 2006, the first week of October in 2007, and the third week of October in 2008, which coincided with the first day of the overhead sprinkler irrigation schedule for

Fig. 1. a, Glass microscope slide spore trap coated in both sides with white petroleum jelly on a grapevine cordon. b, *Botryosphaeriaceae* spore-trapping vineyard sites throughout California (● = microscope glass slide spore traps and ▲ = Hirst-Burkard volumetric spore trap). NC: north coast, SV: Sacramento Valley, SJV: San Joaquin Valley, CC: Central Coast, SC: Southern California grape-growing regions. c, Hirst-Burkard volumetric spore trap placed in Monterey County. d and e, Black arrows show d, *Lasiodiplodia theobromae* colonies from Indio (Riverside County) and e, *Diplodia seriata* colonies from Napa County growing on potato dextrose agar amended with tetracycline hydrochloride.

each respective year. *Botryosphaeriaceae* spp. spores were subsequently captured every week during that period (Fig. 4D). Regression analysis indicated a positive

relationship between spore release and irrigation ($r^2 = 0.49$; $P < 0.0001$; $n = 141$; data not presented) at the Riverside County vineyard.

Although *Botryosphaeriaceae* spp. spores were generally trapped from mid-fall to early spring, the total number of spores trapped varied depending on the season. Glass microscope slides detected the greatest number of *Botryosphaeriaceae* spp. spores (68.9%) to have been trapped during the winter months of December, January, and February from all seven locations. *Botryosphaeriaceae* spp. spore release was lower (30.1%) in fall and early spring than in winter, and very few or no spores (1%) were trapped in late spring and summer (May through September) (Figs. 3 and 4).

Regarding the different *Botryosphaeriaceae* spp. spore types trapped, colony and spore morphological characteristics allowed us to identify five isolates to species (*B. dothidea*, *Diplodia seriata*, *D. mutila*, *L. theobromae*, and *N. parvum*) and one to genus level (*Dothiorella*). The incidence

Fig. 2. Scatter plot of the relationship between total spores trapped per week using glass microscope slides and precipitation. Linear regression line and equation are also shown.

Fig. 3. Total number of *Botryosphaeriaceae* spores trapped per week using glass microscope slides and rainfall in **A**, Mendocino; **B**, Monterey; and **C**, Colusa Counties. Symbols: * = pruning dates, s = overhead sprinkler irrigation, and † = vineyard removed.

and distribution of species of *Botryosphaeriaceae* varied by the geographic region. *D. seriata* spores were trapped in all vineyards studied and were the most abundant (59% of the total spores trapped). Species

of *Dothiorella* (15% of the total *Botryosphaeriaceae* spp. spores trapped), followed *D. seriata* in incidence and were only trapped at the vineyard sites located in the Central and North Coast; however, none

were trapped at the Mendocino site. *B. dothidea* spores (9.8% of the total *Botryosphaeriaceae* spp. spores trapped) were trapped at the North Coast, Sacramento Valley, and northern San Joaquin Valley

Fig. 4. Total number of *Botryosphaeriaceae* spores trapped per week using glass microscope slides and rainfall in **A**, Napa; **B**, San Joaquin; **C**, San Luis Obispo; and **D**, Riverside Counties. Dotted line under x axis shows daily overhead sprinkler irrigation period at the Riverside vineyard (8 h day⁻¹ and 34 liters h⁻¹); * = pruning dates.

vineyards. *L. theobromae* spores (9.6% of the total *Botryosphaeriaceae* spp. spores trapped) were only trapped at the vineyard in Riverside County in Southern California

and were the most abundant spore type trapped at that location (88% of the total spores trapped). *D. mutila* and *N. parvum* were the least abundant spores trapped (1 and 5.6% of the total *Botryosphaeriaceae* spp. spores trapped, respectively) and were restricted to vineyards located in the North Coast and northern San Joaquin Valley, respectively.

Volumetric spore trapping. Regression analysis indicated a positive relationship between spore release and precipitation at the Sonoma ($r^2 = 0.58$; $P < 0.0001$; $n = 191$) and Monterey ($r^2 = 0.76$; $P < 0.0001$; $n = 152$) vineyards (Fig. 5). A daily summary of *Botryosphaeriaceae* spp. spores trapped at the Monterey and Sonoma vineyard sites from 1 December 2008 through 1 May 2009 and from 1 November 2008 through 8 May 2009, respectively, is represented in Figure 6. The daily total of *Botryosphaeriaceae* spp. spores was obtained by summation of the spores trapped hourly per 0.6 m³ of air per day. *Botryosphaeriaceae* spp. spores were released at both vineyard sites after at least 2 mm of rainfall (Fig. 6). *Botryosphaeriaceae* spp. spore release was also triggered by overhead sprinkler irrigation at the Sonoma vineyard on both 26 and 27 February 2009 (Fig. 7C). Overhead sprinkler irrigation was used on those dates to lower the level

of the reservoir following a period of high rainfall. Sprinkler irrigation was also used at the Sonoma vineyard on 23 March and on 4, 5, 16, 26, 28, and 29 April 2009 for frost protection but no significant amount of *Botryosphaeriaceae* spp. spores were captured during 26 and 27 February 2009 (Fig. 6B). High numbers of *Botryosphaeriaceae* spp. spores were first trapped on 14 December 2008 and 1 November 2008 at the Monterey and Sonoma sites, respectively. *Botryosphaeriaceae* spp. spores were then successfully trapped during each rain event at each location until the end of the study (Fig. 6). *Botryosphaeriaceae* spp. spores were last trapped on 7 April and 2 May 2009 at Monterey and Sonoma County, respectively, which coincided with the last rain event of the season at both sites. The highest number of *Botryosphaeriaceae* spp. spores were trapped in February at the Monterey (50.4%) and Sonoma (65%) vineyard sites, which coincided with the maximum amount of precipitation for both sites during the study period (Fig. 6). In the Monterey vineyard, 16.1% of the total spores captured were trapped in December, 24.1% in January, 8.1% in March, and 1.3% in April. In the Sonoma vineyard, 3.8% of the total spores captured were trapped in November, 19.5% in December, 1% in January, 9.8% in March,

Fig. 5. Scatter plot of the relationship between total spores trapped per day using a Burkard volumetric spore trap and precipitation in Monterey and Sonoma vineyards.

Fig. 6. Rainfall and total number of *Botryosphaeriaceae* spores detected daily by sampling 0.6 m³ of air each hour with a Burkard spore trap in A, Monterey and B, Sonoma Counties; s = overhead sprinkler irrigation.

and 0.9% in April. Results of our trapping studies indicated that Sonoma had the greatest amount of inoculum throughout the duration of the study. *Botryosphaeriaceae* spp. spores triggered by both rainfall and overhead irrigation usually began to appear less than 1 h after rainfall or irrigation started, continued through the wet period, and ceased after rainfall or irrigation ended (Fig. 7A–C). *Botryosphaeriaceae* spp. spores were not usually trapped more than 2 h after rainfall or irrigation ended.

DISCUSSION

Grapevine trunk diseases in California primarily include bot canker, esca, eutypa

dieback, and petri disease (10,15,25,38). Spore-trapping studies previously conducted in vineyards in Australia and in California, Michigan, and New York in the United States showed that *E. lata* ascospores spread as airborne inoculum during and immediately following rainfall events (3,14,17,21,31,33). Furthermore, similar studies have also demonstrated that spores of *P. chlamydospora*, *Phaeoacremonium aleophilum*, and *P. inflatipes* are released during and following rain events throughout the year in California (5,24). Results of our study conclusively demonstrated that *Botryosphaeriaceae* spp. spores are also released during rainfall and irrigation

events throughout the different grapevine-growing regions.

California weather conditions are marked by two distinct seasons, a rainy season (October to April) and a dry season. The present study determined that *Botryosphaeriaceae* spp. spores were generally trapped from mid-fall to early spring, which corresponds to the rainy season. However, maximum numbers of *Botryosphaeriaceae* spores were recorded during the winter months of December, January, and February, when the majority of the precipitation occurs in the northern and southern regions of the state. A spore-trapping study was recently conducted in

Fig. 7. Rainfall and total number of *Botryosphaeriaceae* spores detected hourly in **A**, Monterey County from 10:00 A.M. on 23 January 2009 to 11:00 P.M. on 24 January 2009 and **B**, Sonoma County from 6:00 P.M. on 31 October 2009 to 7:00 A.M. on 2 November 2009. **C**, *Botryosphaeriaceae* spores detected during overhead sprinkler irrigation (6.9 mm ha⁻¹ h⁻¹) in Sonoma County from 8:00 A.M. on 26 February 2009 to 4:00 A.M. on 28 February 2009.

the Alsace grape-growing region in eastern France (8). As observed in our study, high numbers of *Botryosphaeriaceae* spores (specifically, *D. seriata* and *D. mutila*) were trapped in France during rain events. However, contrary to what was observed in our study, *Botryosphaeriaceae* spp. spores were mainly captured during the vegetative period (spring through fall) (8). Climatic conditions vary dramatically between California and the Alsace region of France, where the main rainy season occurs from spring to fall, and potentially explains the main differences observed in seasonal patterns of spore release.

Results of our study do confirm that, in addition to rainfall, overhead sprinkler irrigation can trigger *Botryosphaeriaceae* spp. spore release. This was particularly true in the vineyard site located in Riverside County (Indio, CA), where *Botryosphaeriaceae* spp. spores were trapped during each irrigation event. Indio is located at the southwestern end of the Coachella Valley, a table-grape production area characterized by its warm, dry climate, with less than 100 mm of annual precipitation and average daily temperatures of 21 and 38°C in winter and summer, respectively. Grapevines growing under these warm conditions year-round exhibit a different agronomic behavior than those growing in temperate climates (30). Table grape growers in the Coachella Valley practice early-winter hydro-cooling with overhead sprinkler irrigation using a drip line (43). Although the irrigation schedule and amount of water applied varies between growers, sprinkling is generally carried out between 9:00 A.M. and 4:00 P.M. for approximately 45 days (1 November to mid-December) to 61 days (1 November to end of December) (43). Evaporative cooling of vineyards by sprinkler irrigation is also used in the Coachella Valley for leaching soil salinity in the rootzone (43). Consequently, we have shown that, even in a dry area where high spore release was not expected due to excessively low precipitation, the use of overhead irrigation was sufficient to trigger the release of *Botryosphaeriaceae* spp. spores. These results corroborate previous studies in which release and dissemination of *B. dothidea* pycnidiospores was reported to be primarily triggered by sprinkler irrigation in pistachio orchards throughout the San Joaquin Valley region in California (12). On the other hand, low numbers of *Botryosphaeriaceae* spp. spores were captured when sprinkler irrigation was used for frost protection in the Sonoma vineyard. Temperatures close to or below 0°C may limit *Botryosphaeriaceae* spp. spore release from pycnidia. However, the effect of freezing temperatures on *Botryosphaeriaceae* spp. spore release requires further investigation. Additionally, weather events such as snow and fog have been reported to induce release of *Botryosphaeriaceae* spp. spores in

France (8). Moreover, snow events can also trigger discharge of *E. lata* ascospores in Michigan and New York vineyards (17,31). Although release of *Botryosphaeriaceae* spp. spores in vineyards by means of snow is less likely under California weather conditions, fog could play a role in spore release, particularly in spring and summer in the coastal grape-growing areas. Results from our sites in Monterey and San Luis Obispo (Central Coast) showed slight captures of *Botryosphaeriaceae* spp. spores in summer, which could be a response to fog events in those areas. However, research would be necessary to categorically confirm that fog is associated with *Botryosphaeriaceae* spp. spore release in California vineyards.

Although there are similarities in seasonal patterns of species of *Botryosphaeriaceae* and *E. lata* in California, spore release timing coinciding with a rainfall or irrigation event differs between species of fungi. *Botryosphaeriaceae* spp. and *E. lata* both release spores shortly after the commencement of a rain or irrigation event (generally within 1 h) (3,6,12). However, the end of the spore release period differs between these fungi; *Botryosphaeriaceae* spp. spores continued to be trapped within 2 h following the termination of rainfall or irrigation, whereas *E. lata* ascospores are reported to be released until drying of the stromata, which in some instances can require up to 36 h (3,6,17,33). *E. lata* ascospores are released within asci from perithecia buried in a stroma which are capable of continuous release while hydrated, even in the absence of rain (3,32). Ascospores of *E. lata* are ejected into the air and disseminated by wind, and some studies have reported that they may travel up to 100 km from the inoculum source (21). Although the sexual stage of *B. dothidea* has been reported from giant sequoia (*Sequoiadendron giganteum*) and coastal redwoods (*Sequoia sempervirens*) in California (45), perithecia of *Botryosphaeriaceae* spp. have been rarely observed in perennial crops, including grapevines (12,38,39). Therefore, the lack of species of *Botryosphaeriaceae* spores captured in our study shortly after the cessation of rainfall or irrigation may be a consequence of pycnidia and not perithecia as the main source of inoculum for *Botryosphaeriaceae* spp.

Under humid conditions, pycnidia of *Botryosphaeriaceae* spp. produce pycnidiospores that are generally exuded in a gelatinous matrix forming cirrhi, a ribbonlike mass of spores (1,12,20,38). Consequently, once the impact of water droplets over pycnidia cease following the termination of rain or irrigation, *Botryosphaeriaceae* pycnidiospores may not be dispersed. Previous studies conducted in California pistachio orchards have confirmed *B. dothidea* to be a water-splash-dispersed pathogen with relatively short

distances of spore dispersal (1,12). Consequently, although high relative humidity caused by a fog event, for example, could initiate sporulation in *Botryosphaeriaceae* spp. pycnidia, the impact of water droplets would be necessary for spore dispersal. In addition, there is a lack of information regarding the role that wind plays in dissemination of *Botryosphaeriaceae* spp. conidia, which may affect the distance of conidia dispersal. Moreover, studies have reported conidia of *B. dothidea* to also be disseminated by birds and insects in California pistachio orchards (12). Although birds, insects, and wind could be involved in dissemination of *Botryosphaeriaceae* spp. spores in vineyards, results from this and other studies (1,12) indicate that *Botryosphaeriaceae* inoculum appears to be primarily splash dispersed. Therefore, because *Botryosphaeriaceae* spp. spores appear to be less airborne than those of *E. lata*, we hypothesize that relatively short-distance dispersal also occurs in vineyards. Consequently, it is probable that most of the inoculum leading to new infections originates from within the same vineyard. Research in our laboratory is currently underway to investigate the role of natural ecosystems surrounding vineyards in the epidemiology of *Botryosphaeriaceae* spp.

Pruning of grapevines takes place during the dormant season, which in California coincides with the major rainfall period and, consequently, with the main fungal spore release period. Thus, highly susceptible infection sites are readily available to the fungi during this period. However, both spore trapping and pruning wound susceptibility studies have provided valuable information regarding periods of low risk of infection by *E. lata*, as well as management practices to minimize the risk of infection (3,16,18,21). Successful disease management practices have been developed against eutypa dieback, which include pruning wound treatments (22,28); late pruning (as close as possible to bud-break), when marked reduction of ascospore inoculum and more rapid wound healing occur (6,16,21); and double-pruning, a practical technique to achieve late pruning in large vineyards (44). These strategies have resulted in significant reduction in pruning wound infection by *E. lata* in California. Accordingly, prevention of grapevine infections caused by *Botryosphaeriaceae* spp. may also depend on pruning wound protection with a fungicide and manipulation of the time of pruning. Manipulation of both the time of pruning and of sprinkler irrigation could greatly reduce pruning wound infections by *Botryosphaeriaceae* spp. in dry regions such as the Coachella Valley, where fewer precipitation events typically occur. Based on the overall results of this study, pruning grapevines in California from mid-December to mid-February could be particularly risky because of the high prob-

ability of rain and subsequent high *Botryosphaeriaceae* spp. spore release. An important reduction of *Botryosphaeriaceae* spp. spores was generally observed in late winter and early spring throughout the course of this study, due to decreased rainfall; this suggests that pruning during this period would likely decrease the risk of infection. Because late pruning is not always feasible, mostly in large-acreage vineyards, a fungicide or a biological control application directly on the wounds at the time of pruning may enhance disease control.

The work presented here emphasizes the importance that spore-trapping studies have in understanding the epidemiology of *Botryosphaeriaceae* spp. in California vineyards and provides valuable information regarding the characterization of low-risk infection periods that could be used by growers to improve disease control by alteration in the pruning timing.

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