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RESEARCH PAPERS - 9TH SPECIAL ISSUE ON GRAPEVINE TRUNK DISEASES

Dissemination of Botryosphaeriaceae conidia in vineyards in the semiarid Mediterranean climate of the Valparaíso Region of Chile

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Summary. The dispersal of Botryosphaeriaceae conidia was studied in two vineyards in the Valparaiso Region of Chile, where semiarid Mediterranean climate conditions prevail. The objective of this study was to record trappings of Botryosphaeriaceae conidia over one year on a weekly basis and to associate these records with weather and agrochemical application data. Two Chardonnay vineyards in Casablanca and two Cabernet Sauvignon vineyards in Panquehue were selected. In each vineyard at each weekly sampling, five microscope slides covered with petroleum jelly (Vaseline) were installed near grapevines affected by *Botryosphaeria* cankers. In addition, Botryosphaeriaceae species associated with cankers in these vineyards were identified. Seasonal peaks of Botryosphaeriaceae conidia were detected among the different grape producing areas. In Casablanca, peaks of conidium dispersal were observed in winter 2013 and in autumn and winter 2014. Peaks of large numbers of conidia were mainly associated with precipitation events equal to or greater than 0.2 mm. The species observed in the spore traps were preliminarily identified as *Diplodia seriata, Spencermartinsia viticola* and *Neofusicoccum* sp., and isolations from adjacent plants confirmed the presence of *D. seriata, S. viticola* and *N. australe* in these vineyards. In Panquehue, peaks of conidium dispersal were observed in winter 2013, with a lower relative abundance of spores detected compared with Casablanca. Conidia dispersal recommenced in autumn 2014 but remained low later in winter. In this location, *D. seriata* was the main species detected, followed by *S. viticola*. No *Neofusicoccum* species was detected in this area. No association was found between conidium dispersal and the volume of water (≤ 1000 L ha^{-1}) used in the application of agrochemical products.

Key words: *Diplodia seriata, Neofusicoccum, Spencermartinsia viticola*, Botryosphaeria dieback, epidemiology.

Introduction

There are approx. 130,400 ha managed for wine grape production at the present time in Chile, and 36% of this area is planted with the most widely cultivated variety, Cabernet Sauvignon (SAG, 2013). In particular, in the Valparaiso Region of central Chile, 9,553 ha are dedicated to wine production, with 63% of this area grown with white grape varieties. The most popular white grape varieties in this region are Sauvignon Blanc and Chardonnay, whereas the red variety Cabernet Sauvignon accounts for only 5% of the total vineyard area.

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Black Dead Arm (BDA) was first described by Lechozcky (1974), and was associated with *Botryosphaeria stevensii* Shoemaker (anamorph *Diplodia mutila* (Fr.) Mont.) as its only causal agent. Cristinzio (1978) reported *Botryosphaeria obtusa* (Schwein.) Shoemaker (anamorph *Diplodia seriata* De Not.) as an additional causal agent for BDA in Italy. Larignon *et al.* (2001) reported *B. dothidea* (Moug.:Fr.) Ces. & De Not (anamorph *Fusicoccum aesculi* Corda) and *D. seriata* as causal agents of BDA in France. To date, at least 21 species belonging to the Botryosphaeriaceae have been described as being capable of infecting grapevines and causing Botryosphaeria dieback. Symptoms of disease include as wedge-shaped stem cankers, necrosis of the wood, progressive bud-break failure and plant dieback (Úrbez-Torres, 2011).

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Several species of Botryosphaeriaceae have been described in Chile, where Botryosphaeria dieback is an important problem in viticulture. Recent studies have reported *D. seriata, D. mutila*, *Neofusiccocum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, *Neofusicoccum australe* (Slippers, Crous & L.J. Wingf.) Crous, Slippers & A.J.L. Phillips and *Spencermartinsia viticola* (A.J.L. Phillips & J. Luque) A.J.L. Phillips, A. Alves & Crous, affecting vine and table grape vineyards (Morales *et al*., 2012; Besoain *et al*., 2013; Díaz *et al.,* 2013). Studies by Torres *et al*. (2011) in the O'Higgins (*c*. 34º S) and Maule (*c*. 35º S) regions of Chile determined the prevalence of dieback disease in these two regions at over 86%, with damage indices of, respectively, 36 and 48% for these two regions.

The aerial spread of *Botryosphaeria* spp. spore inoculum and the influence of environmental conditions on propagule dispersal have been studied in deciduous fruit tree species such as apple, blueberry, peach and pistachio (Holmes and Rich, 1970; Sutton, 1981; Creswell and Milholland, 1988; Pusey, 1989; Michailides and Morgan, 1993; Ahimera *et al*., 2004). Under rainfall conditions and high relative humidity, conidia of Botryosphaeriaceae are released from mature pycnidia in gelatinous exudates into the atmosphere. However, in grapevines, the large number of species associated with Botryosphaeria dieback and the diversity of grape growing environments make epidemiological studies difficult (Van Niekerk *et al*., 2004). New information about the conditions and patterns of spore liberation has been contributed by various authors in support of the epidemiological study of this disease (Amponsah *et al*., 2009; Kuntzmann *et al*., 2009; Úrbez-Torres *et al*., 2010; Van Niekerk *et al*., 2010). The dissemination of Botryosphaeriaceae spores is dependent on meteorological factors, with spores becoming abundant during and after periods of rainfall or high relative humidity (Lehoczky, 1974; Hewitt and Pearson, 1988; Ahimera *et al*., 2004; Kuntzmann *et al*., 2009; Amponsah *et al*., 2009; Úrbez-Torres *et. al*., 2010; Van Niekerk, *et al*., 2010). Additional factors include the effects of wind in carrying spores (Van Niekerk *et al*., 2010) and transport by insects (Holmes and Rich, 1970). Ahimera *et al.* (2004) reported the effect of raindrop impacts on mature pycnidia of *Botryosphaeria dothidea* to release the potentially infectious inoculum on pistachio. Van Niekerk *et al.* (2010) and Úrbez-Torres *et al*. (2010) established that relative humidity and rainfall were positively correlated with the spore release of trunk disease pathogens of grapevines, including spores of *Eutypa lata*, *Phomopsis* and species of Botryosphaeriaceae. Either high relative humidity did not produce liberation or the capture of spores was low, thus demonstrating a strong dependence on rainfall. In contrast, Luque *et al*. (2014) found a strong seasonal effect in grapevines, where more Botryosphaeriaceae species were detected after a late pruning compared with an early pruning.

Copes and Hendrix (2004) found that *B. dothidea*, *B. obtusa* (=*D. seriata*) and *B. rhodina* (anamorph *Lasiodiplodia theobromae*) produced conidia from 6 to 30°C, with maximum sporulation occurring at 18 to 24°C under laboratory conditions. In field studies, Kuntzmann *et al.* (2009) recorded *D. seriata* spore trappings from 9°C and higher as well as clear adaptive differences between *D. seriata* and *D. mutila*, with the latter species being detected late in the season.

The seasonal spore release of Botryosphaeriaceae species can be studied in the field through the periodical monitoring of spore traps. In California, Úrbez-Torres *et al.* (2010) observed marked differences in spore trappings across seasons, and reported that rainfall was strongly correlated with the spore release of Botryosphaeriaceae. More than 60% of conidia were captured in the rainy winter season. Spore release was observed from mid-autumn until spring with peaks in the winter months (Úrbez-Torres *et al*., 2010). However, in other grape growing areas, Kuntzmann *et al.* (2009), in Alsace, France, and Amponsah *et al.* (2009), in Canterbury, New Zealand, reported that spore release occurred throughout the year, with peaks in the summer months. Consequently, the seasonal abundance of spores varies among different geographical regions. Thus, the objective of the present study was to describe the seasonal abundance, dispersal, and release peaks of Botryosphaeriaceae spores in two grapevine areas with the semiarid Mediterranean climate of the Valparaiso Region, central Chile, and to study the association of the aerial inoculum dynamics with climatic events and water volumes applied with agrochemicals.

Materials and methods

Experimental plots and spore trap setting and monitoring

In the Valparaiso Region of central Chile, two blocks of 1.5 ha (designated CasB1) and 3.0 ha

(CasB2), from two different Chardonnay vineyards in the area of Casablanca, and two blocks of 3.7 ha (PanB1) and 4.5 ha (PanB2) from two Cabernet Sauvignon vineyards in Panquehue were selected. All vines were trained on vertical trellises. Within each vineyard block, five continuous rows (with at least 20 plants) were selected, and one spore trap was installed in each row. Each trap was placed adjacent to a plant showing Botryosphaeria dieback symptoms, such as dieback of shoots with grayish aspects and necrotic V-shaped cankers (Figures 1A and B). Each spore trap consisted of a glass microscope slide (25.4 × 76.2 mm) coated on the upper side with a thin layer of petroleum jelly (Vaseline) and placed with a paperclip on grapevine cordons (Figure 1C). This was similar to the methodology described by Eskalen and Gubler (2001) and used for trapping Botryosphaeriaceae spores by Úrbez-Torres *et al.* (2010).

The spore traps were changed every week, for 16 months (69 weeks) in Casablanca (from June 2013 to October 2014) and for 12 months (53 weeks) in Panquehue (from June 2013 to June 2014). The spore trap slides were sent to the Phytopathology Laboratory of the Pontificia Universidad Católica de Valparaíso to be examined. Fifty percent lactophenol blue stain (Merck, Darmstadt, Germany) was used for slide mounting, and further observation of the spores was performed using an optical microscope. Conidia were identified at the genus and species levels according to morphological characteristics using the key of Phillips (2007) and Phillips *et al*. (2013). Conidia were counted over the total area of each microscope slide and recorded as the total sum per week. For every vineyard a total of five slides per block per week were examined, and spore count data were recorded.

Meteorological data and foliar spray applications

The meteorological data for weekly accumulated precipitation, average relative humidity, and average cardinal temperatures (minimum, maximum and mean) in the area of Casablanca were obtained using the Meteovid platform, from Adcon Telemetry Livedata, at http://meteovid.cl. For the Panquehue area, data were collected from the national Agromet network, at http://agromet.cl. In addition, the number of foliar spray applications in each vineyard was obtained from the record books required by Good Agricultural Practices (GAP).

Survey of *Botryosphaeriaceae* species in the experimental plots

A survey was performed in each experimental area to determine the Botryosphaeriaceae species mycoflora at each location. In each vineyard, ten wood samples from each block in Casablanca and 15 samples from the two blocks in Panquehue were obtained. The samples were taken from the margins of the lesions associated with V-shaped necrosis or vascular streaking. Five pieces of wood (<1 cm^2) were obtained from each sample. Wood pieces were disinfected for 30 s in 95% ethanol, washed three times in sterile distilled water (SDW), dried on a sterile paper towel, left aseptically for 40 min next to a burner. The sections were then plated onto Potato Dextrose Agar (PDA, Difco Laboratories) amended with 0.01% tetracycline hydrochloride, and incubated for 2 d at 25 ± 1°C. Resulting mycelia were hyphal-tip subcultured onto new PDA plates acidified with 0.5 mL L^{-1} lactic acid (APDA). Isolates were maintained at room temperature as APDA plugs with mycelium (8 mm) placed in tubes filled with SDW. Species were identified by growing fungal colonies for a minimum of 3 weeks at room temperature under 12h/ near ultraviolet light (UVA, λ=320 nm)/12h darkness daily cycles. Preliminary identifications were performed using the key provided by Phillips *et al.* (2013).

Preliminary identifications based on morphological characters were later confirmed by molecular studies. Fungal DNA was extracted from pure cultures of representative isolates using the DNeasy® Plant Mini Kit (Qiagen). PCR was performed using the methodology of Úrbez-Torres *et al*. (2008). Partial sequences of the internal transcribed spacer (ITS) of the ribosomal DNA region and the beta-tubulin gene (BT) were obtained using the ITS4 and ITS5 (White *et al*., 1990), and BT2a and BT2b (Glass and Donaldson, 1995) primer pairs, respectively. The PCR products were purified and sequenced by Macrogen (Macrogen Inc.). The sequences were edited and assembled using CodonCode Aligner version 4.2 (CodonCode Corporation), and compared with the GenBank database using the BLAST software (Basic Local Alignment Search Tool program). All of the sequences obtained in this study were deposited in GenBank.

Results

In Casablanca, Botryosphaeriaceae spore dispersal was detected primarily during the autumn and

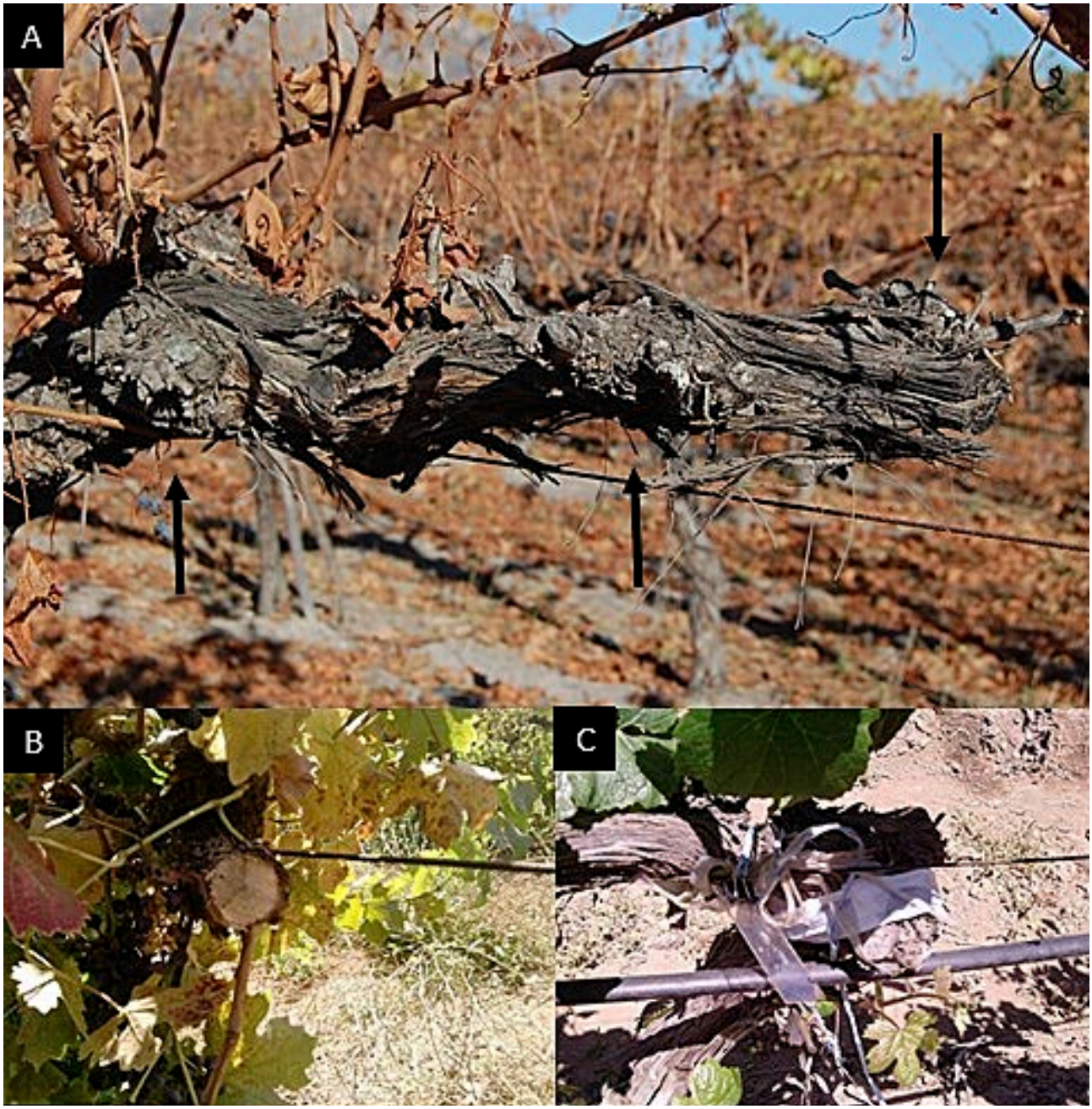

Figure 1. A. Symptoms of Botryosphaeria dieback in grapevine: black arrows indicate dead grayish-brown shoots. B. Transverse cut showing V-shaped necrosis in Cabernet Sauvignon in Panquehue. C. Spore trap placed on a Chardonnay cordon in Casablanca.

winter months (Figure 2). Peaks of conidial dispersal were detected in the months of April (2014) and at the end of August (2013 and 2014) (Figure 2). In Casablanca, considering both monitored grapevine blocks, spores of Botryosphaeriaceae were detected in 41 of the 69 analyzed weeks. In 16 of these weeks, peaks of dispersal with ≥ 15 conidia per slide were detected. In 15 of these peak weeks, precipitation was

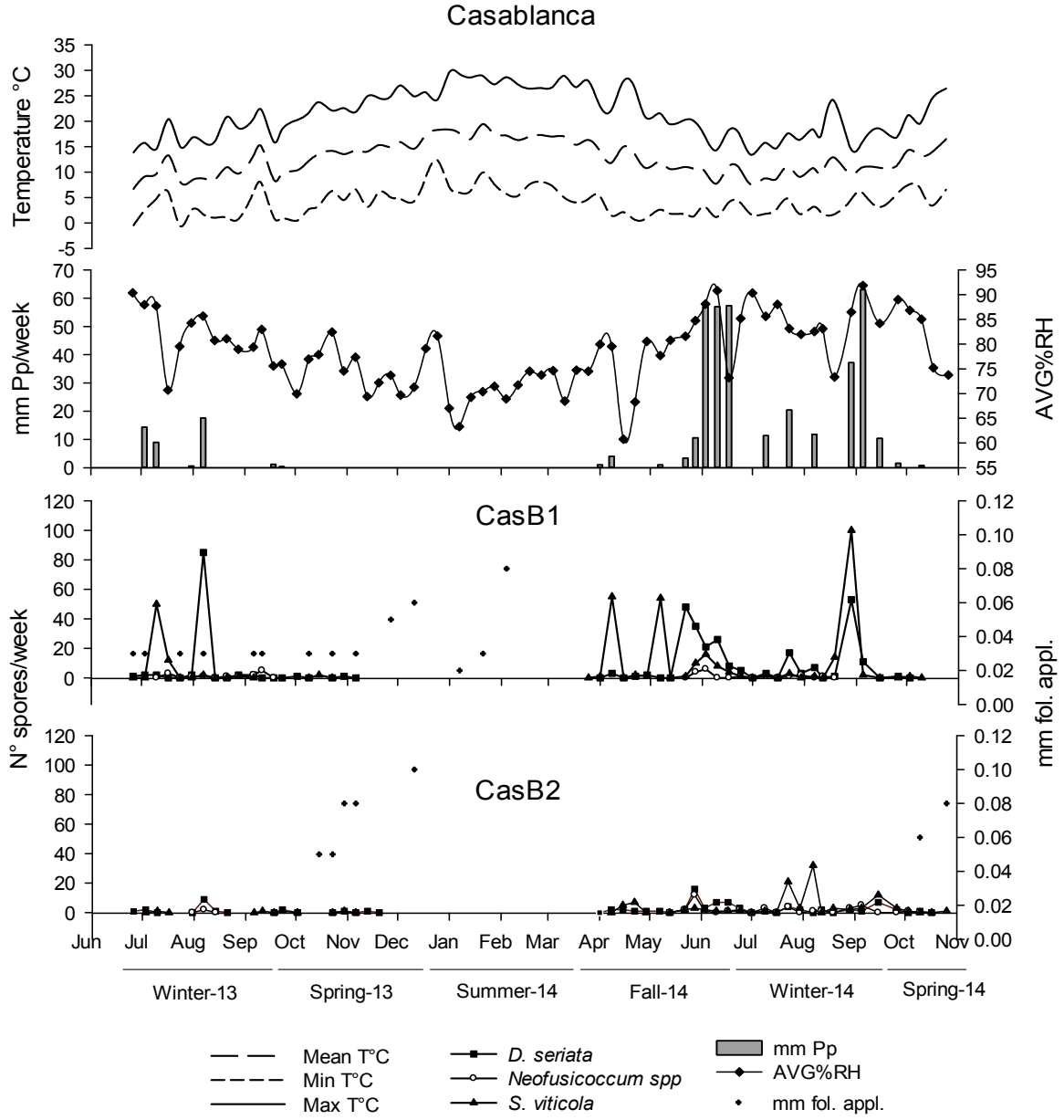

Figure 2. Weekly records of numbers of spore-trapped conidia of *Diplodia seriata*, *Spencermartinsia viticola* and *Neofusicoccum* sp. in two vineyards (CasB1, CasB2) in Casablanca, Valparaíso Region, Chile. Cardinal temperatures, relative humidity (diamond symbols) and accumulated precipitation (histogram bars) recorded during the experimental period are plotted at the top of the figure.

greater than or equal to 0.2 mm. In the week where a peak of dispersal was detected with no precipitation, 9 mm of rainfall had occurred on the day before the trap change. There were also clear differences between the sampled vineyard blocks, with a greater total abundance of spores in CasB1, which had 80% of the spores detected compared with CasB2. In Panquehue, the first peak of conidium dispersal was observed in August 2013 in the PanB1 block after of 16.3 mm of rainfall, and in the next year, the first peak

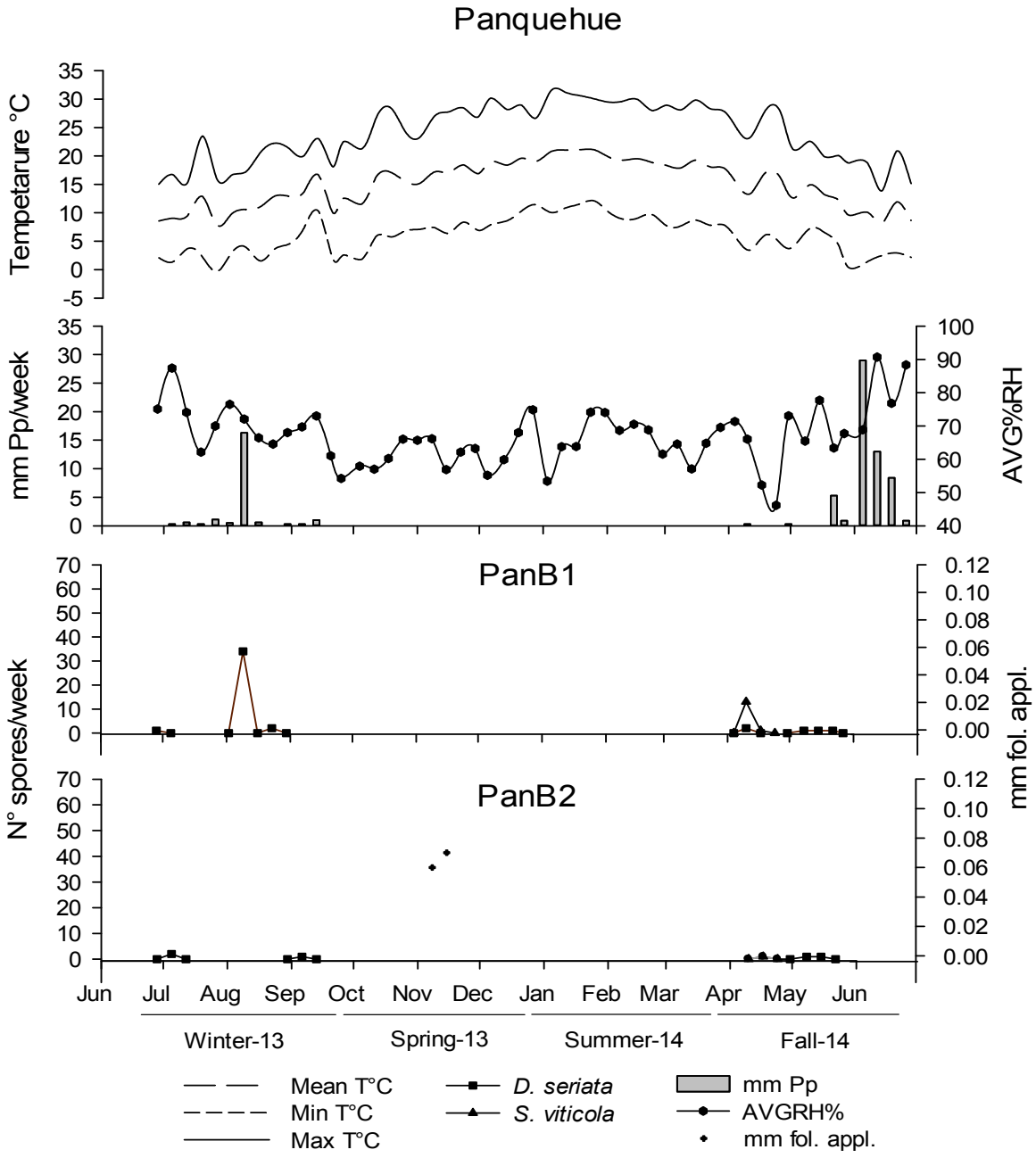

Figure 3. Weekly records of numbers of spore–trapped conidia of *Diplodia seriata*, *Spencermartinsia viticola* and *Neofusicoccum* sp. in two vineyards (PanB1, PanB2) in Panquehue, Valparaíso Region, Chile. Cardinal temperatures, relative humidity (diamond symbols) and accumulated precipitation (histogram bars) recorded during the experimental period are plotted at the top of the figure.

(≥ 15 conidia) was detected in April after precipitation of 0.3 mm (Figure 3). In contrast, in the PanB2 block, no conidial peak was detected throughout the sampling period. No conidia were detected in any of the four sampled locations during the summer (December to March). Median temperature associ-

ated with conidial peaks (≥ 15 conidia) was 10.3°C in both blocks in Casablanca. In Panquehue, the mean median temperature was 12.1°C during the conidial peak periods.

In Casablanca, the Botryosphaeriaceae species detected in the spore traps were identified as *D. seriata*, *S. viticola*, and *Neofusicoccum* sp. (Figure 2). The conidia of *D. seriata* were unicellular, ellipsoidal and truncate and their mean dimensions were 21.1 ± 1.8 × 11.9 ± 1.6 μm. The conidia of *S. viticola* were ellipsoidal and septate, their mean dimensions were 23 ± 1.8 × 11.1 ± 1 μm. The conidia of *Neofusicoccum* spp. were unicellular, fusoid and their mean dimensions were 21.7±3.1 × 6.3±1.3 μm. In the two blocks from Casablanca, isolations were performed from surrounding diseased plants and the isolates obtained were molecularly identified as *D. seriata* [nine isolates, accession numbers KP692179 to KP692187 (ITS), and KP692192 to KP692200 (BT)], *S. viticola* [one isolate, accession numbers KP692191 (ITS) and KP692204 (BT)] and *Neofusicoccum australe* [two isolates, accession numbers KP692189 and KP692190 (ITS), and KP692202 and KP692203 (BT)]. In Panquehue, *D. seriata* was detected during the winter of 2013 and autumn of 2014, with peaks that were greater and more pronounced compared with *S. viticola*, which was only detected early in the season. In this location, *Neofusicoccum* sp. was not detected (Figure 3). All of the taxa detected in the spore traps and from the re-isolations agreed with the descriptions given by Philips (2007) and Phillips *et al*. (2013).

In Panquehue, the Botryosphaeriaceae species detected in the spore traps were identified as *D. seriata* and *S. viticola*. The conidia of *D. seriata* were unicellular, ellipsoidal and truncate, and their mean dimensions were 21.3 ± 1.3 × 11.8 ± 1.5 μm. The conidia of *S. viticola* were ellipsoidal and septate, and their mean dimensions were 22.8 ± 1.4 × 10.5 ± 0.6 μm. In the Panquehue location, only *D. seriata* was isolated from vines with dieback symptoms.

Neither the foliar spray applications for nutritional management nor those for phytosanitary purposes had any impact on spore liberation (Figures 2 and 3). In CasB1, the applications were sprayed throughout the growing season, beginning with applications at 300 L ha^{-1} (equivalent to 0.03 mm) early in winter 2013, and continuing until the second week of January, without any measureable increase in conidium liberation (Figure 2). Similarly, in CasB2, the spray applications were restricted to October and December 2013 but did not affect conidium liberation. In CasB1 during 2013, the main foliar applications were acaricides, fungicides (thiophanate-methyl and myclobutanil), and nutrient fertilizers. During 2014, foliar applications began in November, so they were not included in this study. In Panquehue, in PanB1, the vineyard management consisted only of dusting applications with sulfur products, and only PanB2 used foliar water application (for foliar nutrients and mancozeb), also without any observed effects on conidium dispersal (Figure 3).

Discussion

In the wine-producing area of central Chile, the predominant climate is semiarid Mediterranean, with rainfall events occurring primarily in the autumn and winter. The main peaks of numbers of Botryosphaeriaceae conidia observed in this study were detected from the middle of autumn until the middle of September and coincided with seasonal precipitation. This is in accordance with the study conducted by Úrbez-Torres *et al.* (2010) in California, where 82% of spore liberation was detected during the autumn and winter seasons. Studies from France and New Zealand detected an abundance of spores throughout the year, but with numerical peaks associated with rainfall during the summer months (Amponsah *et al.*, 2009; Kuntzmann *et al.*, 2009). The effect of temperature on Botryosphaeriaceae sporulation is discussed by Copes and Hendrix (2004), as they observed sporulation from 6°C, with optimum temperatures between 18–24°C under *in vitro* conditions. In the present study, peaks of conidium release were observed with an average of weekly median temperatures over 10°C, and mainly coinciding with rainfall events. Similarly, other field studies have detected a similar trend. Kuntzmann *et al.* (2009) reported liberation from 9°C, and Úrbez-Torres *et al.* (2010) detected liberation during the winter months with median temperatures between 3 and 7°C, which indicates that liberation can occur at low temperatures. Serra *et al*. (2008) found mean temperatures for infection of 9.1°C (2005) and 10.4°C (2006). Thus, Botryosphaeriaceae species can be dispersed over a wide range of temperatures.

In this study, the most commonly detected species in Casablanca were *S. viticola* and *D. seriata* (46.9 and 44.4%, respectively). In Panquehue, the most detected species was *D. seriata*, which appeared in

84.1% of the visual observations, and only 11.9% of the spores were *S. viticola*. A study conducted in Canterbury, New Zealand by Amponsah *et al.* (2009) reported a 59.8% of conidia were *Neofusicoccum* spp., and 40.2% of conidia were *Diplodia* spp. Kuntzmann *et al.* (2009) observed the presence of *D. seriata* and *D. mutila* in French vineyards, where spores of *D. seriata* peaked during the host vegetative period while *D. mutila* released its spores later. This seasonal difference in occurrence among Botryosphaeriaceae species has also been reported in the Valparaiso Region of Chile, in loquat crops (*Eriobotrya japonica* L.), with peaks of conidium detection of *D. seriata* occurring in September and *D. mutila* in October (Cornejo, 1993).

Agrochemical applications equivalent to rain events between 0.03 to 0.1 mm did not play significant roles in conidium liberation in any of the sampled vineyard blocks. Research by Rolshausen *et al.* (2010) in California showed that thiophanate-methyl efficiently controls both *D. seriata* and *Sp. viticola*. Similarly, in Chile, Torres *et al.* (2013) demonstrated that several demthylation inhibitor fungicides (including myclobutanil) inhibit Chilean strains of *D. seriata, D. mutila, Neofusicoccum parvum* and *N. australe* under *in vitro* conditions. In CasB2, only substances permitted for organic production were applied, with application volumes between 0.05 and 0.1 mm; however, in comparison with CasB1, there was lower conidial liberation in CasB1 than CasB2 (Figure 2). It is possible that the lower amount of inoculum present was due to other cultural management practices that were not included in the scope of this study. The lower liberation of spores in Panquehue could be due to the dry conditions that prevailed during the period of this study or to a lack of inoculum present in these vineyards.

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