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Yield loss estimation and pathogen identification from Botryosphaeria dieback in vineyards of Central Chile over two growing seasons

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Summary. Dieback symptoms have been increasingly reported in Chilean vineyards over recent years. Although there have been studies on *Botryosphaeriaceae* species and associated trunk disease incidence and severity in table grape-producing vineyards, their impacts on 'Cabernet Sauvignon', the most planted red wine grape in Chile, is unknown. This study determines the fungus species, incidence, disease severity, and yield losses associated with Botryosphaeria dieback in Chilean 'Cabernet Sauvignon' vineyards. Nine vineyards were surveyed during two growing seasons (2010 and 2018), and symptomatic wood samples were taken. Total potential production and yield losses were estimated from spur counts (2010) from harvested vines (2018) with different degrees of infection. Overall disease incidence was 87% in 2010 and 84% in 2018. Severity was 49% in 2010 and 47% in 2018. Yield losses were 39% in 2010 and 46% in 2018. *Diplodia seriata* was the most prevalent fungus isolated from symptomatic plants in both growing seasons. This study highlights the impacts of grapevine trunk diseases in vineyards in Central Chile, and indicates the need for improved disease management strategies.

Keywords. *Vitis vinifera*, 'Cabernet Sauvignon' disease incidence, disease severity, yield loss.

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INTRODUCTION

Grapevine (*Vitis vinifera* L.) is the most widely cultivated fruit species in Chile with an area of approx. 190,000 ha for the production of table grapes, spirits and wine. As the leading table grape exporter and the fourth largest exporter of wine worldwide (ODEPA, 2019a, 2019b), Chile exported

846 million liters of wine with a value of US $ 2 billion (ODEPA, 2019c) in 2018. 'Cabernet Sauvignon', which represents 19% of total Chilean wine exports, is the most widely planted nationwide, principally concentrated in Central Chile and particularly in the O'Higgins and Maule regions (SAG, 2019). Reports of generalized grapevine dieback (i.e., vine decay, perennial cankers, wood necroses, progressive sprouting failure, death of cordons and plants) have significantly increased in Chilean vineyards in recent years (Morales *et al*., 2012; Díaz *et al*., 2013; Besoain, 2018). While dieback symptoms in table grape cultivars are commonly associated with *Botryosphaeriaceae* spp. (Latorre *et al*., 1986; Auger *et al*., 2004; Díaz *et al*., 2011; Morales *et al*., 2012) with incidence of up to 69% in adult plants (Morales *et al*., 2012), symptoms have also been described in other Chilean winegrape cultivars (Auger *et al*., 2004; Besoain *et al*., 2013; Díaz *et al*., 2011, 2013). Although 'Cabernet Sauvignon' has been shown to be susceptible to grapevine trunk diseases (GTDs) (Larignon *et al*., 2009; Travadon *et al*., 2013), incidence, severity and impacts caused by *Botryosphaeriaceae* in Chilean 'Cabernet Sauvignon' remains unknown.

Studies of pathogens associated with Botryosphaeria dieback have identified 26 species (Gramaje *et al*., 2018), with prominent representation from *Botryosphaeria, Diplodia, Lasiodiplodia* and *Neofusicoccum* (Úrbez-Torres, 2011; Gramaje *et al*., 2018; Billones-Baaijens and Savocchia, 2019). Botryosphaeria dieback of grapevines is mainly associated with cankers and dead spurs and cordons with no general foliar symptoms and wood damage, with brown streaks, discolouration, and characteristic wedge-shaped perennial cankers (Larignon *et al*., 2001; Van Niekerk *et al*., 2004; Úrbez-Torres, 2011; Spagnolo *et al*., 2014; Fontaine *et al*., 2015; Besoain, 2018; Gramaje *et al*., 2018).

Botryosphaeria dieback symptoms are ubiquitous (Duthie *et al*., 1991; Úrbez-Torres, 2011; Morales *et al*., 2012; Baskarathevan *et al*., 2012; Bertsch *et al*., 2013; Gramaje *et al*., 2018), and the disease causes significant production losses and disease management costs. For example, Hillis *et al*. (2016) reported that Californian vineyards spent an average of $US 477 ha^{-1} per year to prevent and manage GTDs. In Napa, California, cumulative losses due GTDs (including Botryosphaeria dieback) in 'Cabernet Sauvignon' vineyards was estimated at $US 160,000 per acre ($US 400,000 ha^{-1}) over a 25 -year vineyard lifespan (Kaplan *et al*., 2016).

Given the importance of 'Cabernet Sauvignon' to the Chilean wine industry, the present study aimed to: (i) determine the incidence, severity, and yield losses associated with Botryosphaeria dieback in 'Cabernet Sauvignon' vineyards in two wine-producing regions of Chile; and (ii) identify the fungi causing this disease.

MATERIALS AND METHODS

Vineyard sampling

Samples were taken from fourteen blocks in nine vineyards distributed across the O'Higgins and Maule regions of Chile, during the growing seasons of 2010-2011 and 2018-2019. The exception was for vineyard number 9, block 14, which was only sampled in the 2010-2011 growing season (Table 1). All the contained

Table 1. Vineyards and blocks 'Cabernet Sauvignon' analyzed, located in Central Chile.

Region/Locality	Vineyard	Block	Planting Year	Sampling season/plant age	Sampling season/plant age
O'Higgins/Chépica	1	1	1996	2010-11/15	2018-19/23
O'Higgins/Palmilla	2	2	1991	2010-11/20	2018-19/28
O'Higgins/Palmilla	2	3	2005	2010-11/06	2018-19/14
O'Higgins/Palmilla	3	4	1995	2010-11/16	2018-19/24
O'Higgins/Peralillo	4	5	1991	2010-11/20	2018-19/28
O'Higgins/Peralillo	5	6	1996	2010-11/15	2018-19/23
O'Higgins/Peralillo	5	7	1996	2010-11/15	2018-19/23
O'Higgins/Peralillo	5	8	1995	2010-11/16	2018-19/24
Maule/Batuco	6	9	1997	2010-11/14	2018-19/22
Maule/Pencahue	7	10	1997	2010-11/14	2018-19/22
Maule/Pencahue	7	11	2002	2010-11/09	2018-19/17
Maule/Pencahue	8	12	1997	2010-11/14	2018-19/22
Maule/Pencahue	8	13	1997	2010-11/14	2018-19/22
Maule/Talca	9	14	2003	2010-11/08	

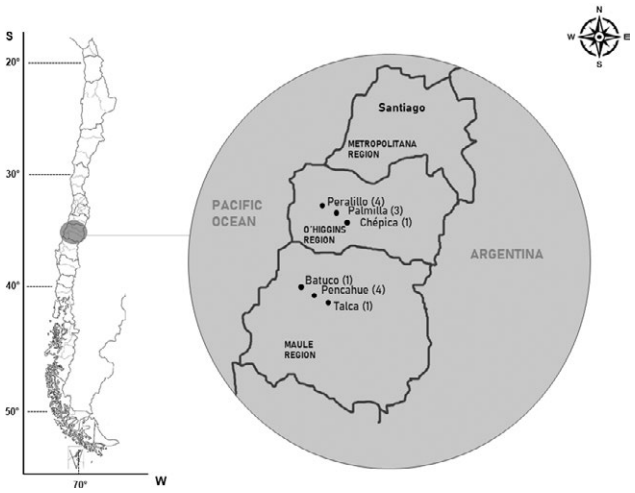

Figure 1. Sampling localities (black dots) in the Maule and O'Higgins Regions, Central Chile. The number of blocks analyzed in each locality is indicated in parentheses.

'Cabernet Sauvignon' non-grafted vines trained using the bilateral cordon system, which were spur pruned to an average of 12 spurs per plant. The majority of the vineyards also used Winter pruning (June-August). The vineyards were located in temperate climate regions in the Colchagua (34° S; O'Higgins Region) and Maule Valleys (35° S; Maule Region) (Figure 1), which generally receive rain in Autumn and Winter months. All nine vineyards were on flat or slightly sloping land. Vineyards in the Colchagua area were mainly on loamy soils with some variations from sandy loam to clay loam soils (Covarrubias *et al*., 2004), and were at altitudes between 133 and 201 m.a.s.l. In the Maule region, the vineyard soils were loamy to clay loamy (Gallardo *et al*., 1994), and between 107 and 131 m.a.s.l. In this part of Chile, altitude is the main factor determining climatic conditions (4 months rainfall season; Tmax = 28.1ºC; Tmin = 2.9ºC; rainfall = 582 mm; pan evaporation = 1,230 mm: Novoa and Villaseca, 1989).

Assessments of Botryosphaeria dieback incidence and severity

Incidence and severity of symptoms were determined during spring season (October-November), in 14 vineyard blocks in 2010, and 13 blocks 2018 respectively (Table 1). Each block included a randomly selected quadrant of 100 plants for determining incidence and severity of decay symptoms. Researchers recorded data on dead cordons of each plants as: wood canker, spur necrosis, dead spurs with grayish colour (non-sprouting spurs), or spurs taken from the plant in the winter pruning period. Leaf symptoms were also recorded. All diseased plants had cankers, mostly wedge-shaped (Figure 2A). Disease incidence was calculated as the percentage of symptomatic plants within the 100 plant quadrant. Severity of the damage was recorded using a rubric severity score of 0 to 4, where: 0 = healthy plant (both cordons complete and asymptomatic); 1 = one asymptomatic cordon and one cordon with up to 50% of symptoms (1-3 dead or missing spurs); 2 = one asymptomatic cordon and an entire symptomatic cordon (4-6 dead or missing spurs), 3 = one symptomatic cordon and the second with up to 50% of symptoms (7-9 dead or missing spurs); and 4 = dead plant (both cordons dead or totally missing) (Figure 2). Disease Index (DI) were calculated using a formula modified from Mc Kinney (1923):

$$DI\ (\%) = \frac{\sum nv}{VN}\ 100 \quad (1)$$

where n = number of plants per score degree; v = score; N = total number of plants evaluated; and V = maximum score.

Estimation of production loss per plant

Total production losses for the 2010-2011 harvest season were estimated using the procedure of Munkvold *et al*. (1994). The number of diseased spurs from all plants (n = 100) from each quadrant were compared to healthy plants, which were shown to have an average of 12 spurs per plant (data obtained in the field). At the end of the summer, when fruit ripened, average production per plant was calculated by harvesting ten healthy plants from each block. These provided potential yield (PY) and estimated yield loss (EYL), as follows:

$$PY_1 = \bar{P}_x\, NP_{ha} \quad (2)$$

$$EYL_1 = \left(\frac{S_d S_p N}{100}\right) NP_{ha} \quad (3)$$

where: PY_1 = potential yield (kg ha^{-1}); $\bar{P}_x$ = the mean of fruit weight (kg) from ten score 0 samples (healthy plants); NP_{ha} = number of plants ha^{-1}; EYL_1 = estimated yield loss (kg ha^{-1}); S_d = total number of dead spurs per quadrant; and S_p = mean fruit weight (kg) per spur.

Total production losses for the 2018-2019 harvest season were estimated using the following procedure. Grapes from three plants per disease severity score (1–4) were harvested in each quadrant (the same quadrants used to determine disease incidence and severity). For score 0, bunches from ten healthy plants were randomly

Figure 2. Damage and degrees of disease severity associated with dead arms in 'Cabernet Sauvignon' grapevines: A) Cross-section and old cordon, exhibiting double V-shaped dark brown necrosis; B) Severity score 0, healthy plant (both cordons complete and asymptomatic); C) Score 1, one asymptomatic cordon and one cordon with up to 50% of symptoms (one to three dead or missing spurs); D) Score 2, one asymptomatic cordon and an entire symptomatic cordon (four to six dead or missing spurs); E) Score 3, one symptomatic cordon and the second with up to 50% of symptoms and seven to nine dead or missing spurs; F) Score 4, dead plant (both cordons dead or totally missing).

selected and harvested from each quadrant, or at least in the same block. A calculation was made of average weight (kg) of fruit per severity score, multiplied by the number of plants with the same score in the quadrant (determined in spring severity assessments). Potential yields and yield losses were calculated as:

$$EFW = \frac{\sum \bar{P}_{xGi}\, n_{Gi}}{100}\, NP_{ha} \quad (4)$$

$$PY_2 = \bar{P}_x\, NP_{ha} \quad (5)$$

$$EYL_2 = PY_2 - EFW_2 \quad (6)$$

where: EFW = estimated fruit yield (kg ha^{-1}); $\bar{P}_{xGi}$ = mean fruit weight (kg) from three plants per severity score (based on *DI*) in the quadrant (for score 0, i.e., healthy plants, average fruit weight per plant was determined from ten plants); n_{Gi} = number of plants per severity score in each quadrant; PY_2 = potential yield (kg ha^{-1}); and EYL_2 = estimated yield loss (kg ha^{-1}).

Linear regression analyses were performed for estimated production loss for each year *versus* GTD severity measured in the corresponding Spring.

Fungus isolates

Samples were randomly chosen from diseased cordons from each evaluated block in Autumn and Spring of 2010 and 2018. A total of 108 samples were analyzed, 56 samples obtained in 2010 and 52 in 2018. Wood samples (<1 cm^2) were taken from the margins of canker lesions, disinfected in 70% ethanol for 30 s, rinsed in sterile distilled water, dried and then added to two different media in Petri plates: potato dextrose agar acidified with 0.5 ml of 96% lactic acid (APDA) and malt extract agar (Difco Laboratories). The plates were incubated for 3–7 d at 24°C until fungal colonies developed. Hyphal tip subcultures were the incubated on APDA for 5 d at 24°C. *Botryosphaeriaceae*-like colonies were subcultured and left in an incubation chamber with near ultraviolet light (λ = 320 nm) at room temperature (19–21°C) to stimulate pycnidium production and conidium development. From conidia of each isolate, monospore subcultures (on water agar and APDA subcultures) were made for morphological and molecular identifications.

Morphological and molecular identification of isolates

Lengths and widths of 30 conidia were measured for each *Botryosphaeria*-like isolate. For molecular characterizations, total DNA was extracted from each monospore culture using the DNeasy Plant Mini Kit (Qiagen). For all isolates obtained in 2010 and 2018, the ITS1-5.8S-ITS2 region was amplified using primer ITS4/ITS5 (White *et al.,* 1990), and the β-tubulin (BT) region using primer Bt2a/ Bt2b (Glass and Donaldson, 1995). Part of the translation elongation factor gene (*EF1-α*) was amplified for isolates obtained in 2018, using EF1-728F/ EF1-986R primers (Carbone and Kohn, 1999). Amplification reactions were each prepared in a final volume of 22 µL following manufacturer instructions (SapphireAmp Fast PCR Master Mix). A 1% electrophoresis gel was prepared and PCR products in TAE buffer were visualized (Winkler) by staining with GelRed (Biotium) and viewing with UV transilluminator (Vilber Lourmat). Amplified products were purified and sequenced in both directions by Macrogen (South Korea), and were assembled and edited using Geneious 10.0.6 software. The products were compared against reference sequences from the National Center of Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov), using the BLAST tool. All sequences were deposited in the GenBank Database.

A multi-locus phylogenetic analysis was performed using Maximum Parsimony (MP) in MEGAX (Kumar *et al*., 2018). Bootstrap values were calculated using 1,000 replicates yielding the MP tree using Tree Bisection and Reconnection algorithms. The tree was rooted with *Lasiodiplodia gonubiensis* strain CBS 115812 and other reference isolates of different *Botryosphaeriaceae* were used from GenBank (Table 2). Tree length, consistency index, retention index, rescaled consistency index, and homoplasy index were calculated using MEGA X.

RESULTS

Estimation of incidence and severity of Botryosphaeria dieback in 'Cabernet Sauvignon' grapevines

Incidence and severity were measured in Spring 2010 and 2018. In 2010, the mean incidence of Botryosphaeria dieback was 77.6% for blocks in the O'Higgins Region and 98.7% in the Maule Region. In 2018, mean incidence in the O'Higgins Region increased to 82.4%, and decreased to 87.2% in the Maule Region. The average severity in 2010 was 40.1% in the blocks sampled from the O'Higgins Region and 60.1% in the Maule Region. In 2018, mean severity was 43.6% in the blocks sampled in the O'Higgins Region, and 51.4% in the Maule Region (Table 3). Furthermore, incidence and severity of the disease in the O'Higgins Region were related to the age of the vineyards, increasing with the increasing age of the plants (Figure 3).

Table 2. Isolates of *Diplodia seriata*, *D. mutila*, *Neofusicoccum parvum*, *N. australe*, and *Spencermartinsia viticola* obtained from the GenBank Database included in this study for phylogenetic analyses.

Species	Strain	Host	Reference	GenBank accession number[a]	
				ITS	BT
Diplodia seriata	CBS112555	*Vitis vinifera*	Alves *et al*., 2004	AY259094	DQ458856
	CBS119049	*Vitis* sp.	Alves *et al*., 2006	DQ458889	DQ458857
	CMW7774	*Ribes* sp.	Slippers *et al*., 2004	AY236953	AY236931
	CMW7775	*Ribes* sp.	Slippers *et al*., 2004	AY236954	AY236932
	CMW8230	*Picea glauca*	De Wet *et al*., 2003	AY972104	AY972119
	UCD244Ma	*Vitis vinifera*	Úrbez-Torres and Gubler, 2009	DQ008314	DQ008337
	UCD352Mo	*Vitis vinifera*	Úrbez-Torres and Gubler, 2009	DQ008315	DQ008338
	UCD614Tu	*Vitis vinifera*	Úrbez-Torres and Gubler, 2009	DQ008318	DQ008341
	UCD710SJ	*Vitis vinifera*	Úrbez-Torres and Gubler 2009	DQ008321	DQ008344
	USD770St	*Vitis vinifera*	Úrbez-Torres *et al*., 2008	DQ008322	DQ008345
	UCD1010BC	*Vitis vinifera*	Úrbez-Torres *et al*., 2008	EU012377	EU012429
	UCD1015BC	*Vitis vinifera*	Úrbez-Torres *et al*., 2008	EU012378	EU012430
	UCD1035BC	*Vitis vinifera*	Úrbez-Torres *et al*., 2008	EU012379	EU012431
	UCD1038BC	*Vitis vinifera*	Úrbez-Torres *et al*., 2008	EU012380	EU012432
	UCD1052BC	*Vitis vinifera*	Úrbez-Torres *et al*., 2008	EU012381	EU012433
	UCD1061BC	*Vitis vinifera*	Úrbez-Torres *et al*., 2008	EU012382	EU012434
Diplodia mutila	CMW7060	*Fraxinus excelsior*	Slippers *et al*., 2004	AY236955	AY236933
	CBS230.30	*Phoenix dactylifera*	Alves *et al*., 2006	DQ458886	DQ458849
	CBS112553	*Vitis vinifera*	Alves *et al*., 2006	AY259093	DQ458850
	CBS112554	*Pyrus communis*	Alves *et al*., 2006	AY259095	DQ458851
	JL375	*Fraxinus excelsior*	Alves *et al*., 2006	DQ458887	DQ458852
	UCD288Ma	*Vitis vinifera*	Urbez-Torres *et al*., 2006	DQ008313	DQ008336
	UCD1953SB	*Vitis vinifera*	Urbez-Torres *et al*., 2006	DQ233598	DQ233619
	UCD1965SB	*Vitis vinifera*	Urbez-Torres *et al*., 2006	DQ233599	DQ233620
Neofusicoccum parvum	CBS110301	*Vitis vinifera*	Alves *et al*., 2004	AY259098	EU673095
	CCA189	*Ferula communis*	Lopes *et al*., 2016	KX871879	KX871766
	CMW9081	*Populus nigra*	Lopes *et al*., 2016	AY236943	AY236917
Neofusicoccum australe	CAA723	*Tilia platyphyllos*	Lopes *et al*., 2016	KX871862	KX871747
	CAA741	*Acacia longifolia*	Lopes *et al*., 2016	KX871863	KX871748
Spencermartinsia viticola	PD285	*Vitis* sp.	Inderbitzin *et al*., 2010	GU251166	GU251826
	UCP105	*Citrus* (cv. *Parent Washington)*	Adesemoye and Eskalen, 2011	JF271748	JF271766
Lasiodiplodia gonubiensis	CBS 115812	*Syzygium cordatum*	Alves *et al*., 2006	DQ458892	DQ458860

[a] ITS = internal transcribed spacer region; BT = β-tubulin gene.

Production loss estimations

In the 2010-2011 harvest season, average fruit production losses were 4.5 t ha^{-1} in the O'Higgins Region and 6.2 t ha^{-1} in Maule (Table 4). Production loss estimates represented 30.7% of total production potential in the O'Higgins Region and 52.9% in the Maule Region. For the 2018-2019 harvest season, average fruit production loss of 10.7 t ha^{-1} was estimated for the O'Higgins Region, and 11.9 t ha^{-1} for the Maule Region (Table 4). Production loss estimates represented 42% of total production potential in the O'Higgins Region and 52.3% in Maule Region.

Linear regressions had the best model fit for the relationship of disease severity (x) and production losses (y) (Figures 4A and 4B).

Table 3. Incidence and severity of Botryosphaeria dieback in Chilean vineyards of 'Cabernet Sauvignon' in O'Higgins and Maule Regions.

Region/Locality/Vineyard-Block	Planting year	Spring 2010		Mean number of dead spurs	Spring 2018	
		Mean Incidence (%)	Mean Severity (%)		Mean Incidence (%)	Mean Severity (%)
O'Higgins/Chépica 1-1	1996	64	27.3	2.5	73	29.0
O'Higgins/Palmilla 2-2	1991	71	36	3.6	94	66.0
O'Higgins/Pamilla 2-3	2005	22	7.8	0.6	63	18.5
O'Higgins/Palmilla 3-4	1995	99	62.3	6.5	84	52.0
O'Higgins/Peralillo 4-5	1991	100	70.5	7.7	83	47.0
O'Higgins/Peralillo 5-6	1996	83	31.3	2.8	94	49.3
O'Higgins/Peralillo 5-7	1996	84	30.3	2.8	88	48.3
O'Higgins/Peralillo 5-8	1995	98	55.0	5.6	80	38.5
O'Higgins average		77.6	40.1	4.0	82.4	43.6
Maule/Batuco 6-9	1997	100	66.8	6.9	82	43.0
Maule/Pencahue 7-10	1997	100	60.3	6.2	88	46.5
Maule/Pencahue 7-11	2002	99	61.3	6.5	92	59.0
Maule/Pencahue 8-12	1997	99	54.0	5.7	86	54.5
Maule/Pencahue 8-13	1997	94	47.0	4.6	88	54.0
Maule/Talca 9-14	2003	100	71.3	7.7		
Maule average		98.7	60.1	6.3	87.2	51.4

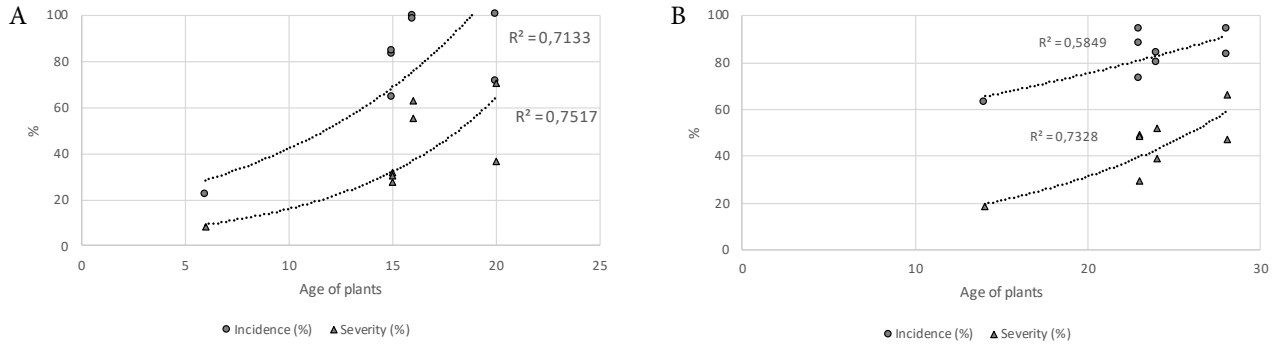

Figure 3. Relationships between vineyard age ('Cabernet Sauvignon') in the O'Higgins Region and the incidence and severity of Botryosphaeria dieback in A) spring 2010; and B) spring 2018.

Morphological and molecular identification of fungus isolates

Seventy *Botryosphaeriaceae* isolates were obtained from 108 diseased vine samples collected from nine vineyards during two growing regions. Isolates were obtained in autumn and spring of 2010 and 2018. Thirty-seven *Botryosphaeriaceae* isolates were obtained in 2010 and 33 in 2018. The study found that colonies of *Diplodia mutila*, *D. seriata*, *Neofusicoccum australe*, *N. parvum* and *Spencermartinsia viticola* developed pycnidia after 20-30 d growth on APDA. The conidia identified as *D. seriata* were unicellular, initially hyaline, and then brown, each with a rounded apex and truncated base, measuring 22.9 ± 1.3 × 11.9 ± 0.4 µm. Conidia of *D. mutila* were aseptate, hyaline, ellipsoid, each with rounded apex and base, measuring 24.7 ± 1.1 × 12.9 ± 0.1 µm. Conidia of *Neofusicoccum* were unicellular, hyaline, ellipsoidal, and thin-walled, measuring 16.1 ± 0.9 × 5.9 ± 0.6 µm. Conidia of *S. viticola* were initially hyaline but still attached to the conidiogenic cells, turning brown, and each developing a central septum; they were ellipsoid, with thick walls, rounded at both ends, or in some cases with truncated bases, and measuring 22.2 ± 0.2 × 9.2 ± 0.1 µm. These conidium characteristics have been respectively described for these species.

Table 4. Estimated yield losses in 'Cabernet Sauvignon' vineyards, sampled in two years (2010 and 2018) in O'Higgins and Maule Regions, Central Chile.

Vineyard block	Planting year	Potential yield (kg ha^{-1}), 2010	Estimated yield loss (kg ha^{-1}), 2010[a]	Potential yield (kg ha^{-1}), 2018	Estimated yield loss (kg ha^{-1}), 2018[b]
O´Higgins Region					
1-1	1996	23,011	4,756	34,257	4,185
2-2	1991	18,264	5,433	18,785	13,529
2-3	2005	14,337	753	9,198	1,441
3-4	1995	12,402	6,666	16,118	8,446
4-5	1991	8,087	5,156	24,769	14,765
5-6	1996	11,822	2,788	31,811	15,554
5-7	1996	15,058	3,564	33,325	15,493
5-8	1995	15,058	7,077	35,688	12,303
Average		14,755	4,524	25,494	10,715
Maule Region					
6-9	1997	11,208	6,445	15,571	6,458
7-10	1997	14,184	7,376	35,947	16,800
7-11	2002	14,188	7,626	26,346	19,617
8-12	1997	10,163	4,794	17,278	7,170
8-13	1997	8,916	3,447	18,681	9,488
9-14	2003	11,666	7,525	-	-
Average		11,721	6,202	22,765	11,907

[a] Estimated yield losses based on dead spurs per evaluated quadrant.
[b] Estimated yield losses based on the harvest of plants with different degrees of GTD damage per evaluated quadrant.

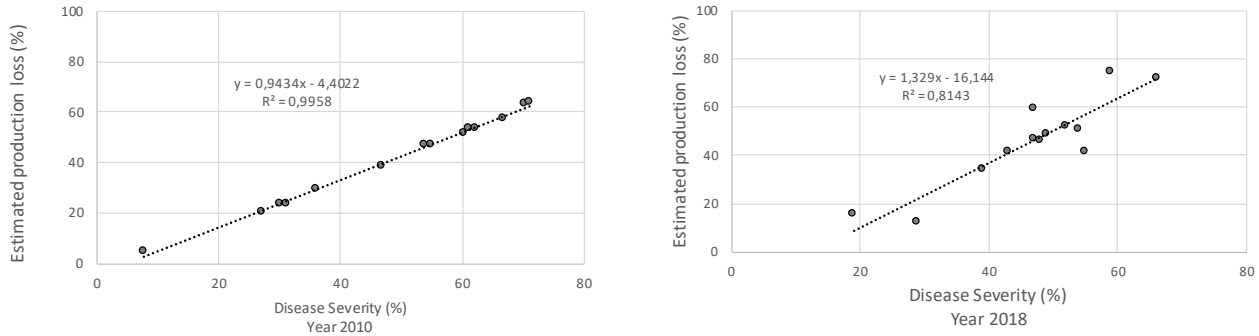

Figure 4. Regression analyses between severity of Botryosphaeria dieback (measured in spring) in 'Cabernet Sauvignon' vineyards located in the O'Higgins and Maule Regions, Central Chile. A) Production losses (%), estimated in 2010 by number of dead spurs; and B) Production losses (%), estimated in 2018 by harvesting plants with different degrees of disease severity.

Comparison of DNA sequences identified in 2010 using BLASTn (NCBI) showed *D. seriata* to be the most prevalent species (68%), followed by *D. mutila* (13%), *N. parvum* (11%), *N. australe* (5%), and *S. viticola* (3%) (Supplementary Table 1). DNA sequences showed ≥98% similarity matches with previously deposited sequences for these species in GenBank. All the species were isolated in autumn and spring, except for *S. viticola*, which was obtained only in autumn. Isolates collected in 2018 were identified as *D. seriata* (91%), *D. mutila* (6%) and *N. parvum* (3%) (Supplementary Table 2). Sequence similarities for these species were also ≥98% with those in GenBank database. *Diplodia seriata* was obtained in samples taken in autumn and spring, while *D. mutila* and *N. parvum* were detected only in spring. In both sampling years (2010 and 2018), isolates obtained from

vines showed dieback in cordons with canker lesions (Figure 2A).

The concatenated ITS and BT phylogenetic analyses included 68 *Botryosphaeriaceae* sequences. The analyses contained 799 nucleotides of which 621 were constant and 111 were parsimony-informative. The analyses yielded the ten most parsimonious trees (one is shown in Figure S1), with tree length (TL) = 236, consistency index (CI) = 0.7965; retention index (RI) = 0.9721, recalled consistency index (RC) = 0.7743, and homoplasy index (HI) = 0.2035. MP analyses showed two main branches, with 68 isolates from Chile grouping into five different clades, including *D. seriata*, *D. mutila*, *N. parvum*, *N. australe*, and *S. viticola* (Figure S1).

DISCUSSION

This study reports estimates of Botryosphaeria dieback incidence and severity, as well as production losses, in 'Cabernet Sauvignon' vineyards in Central Chile over two different growing season sampled 8 years apart. In both growing seasons, the incidence of Botryosphaeria dieback (average by region) varied between 78% and 99%. Morales *et al*. (2012) previously reported incidence of *Botryosphaeriaceae*-associated dieback between 22% and 69% for Chilean table grape vineyards. Although this was less than in the present study, the vineyards assessed by Morales *et al*. (2012) were located in warmer, drier regions (Valparaíso and Metropolitan Regions) than those assessed here. The previous study used the same methodology for determining disease incidence as the present study.

Between 2010 and 2018, average incidence of Botryosphaeria dieback was similar (respectively, 87% and 84%). However, between regions, average incidence in the O'Higgins Region increased by 5% from 2010 to 2018, but decreased 12% in the Maule Region, although average incidence in the Maule Region (93%) was greater than in the O´Higgins Region (80%) (Table 3). This could be due to differences in rainfall. During 2015 to 2018, the O'Higgins Region (San Vicente Meteorological Station, https://agrometeorologia.cl) averaged 384 mm p.a., while the Maule Region (San Clemente Meteorological Station, https://agrometeorologia.cl) averaged 669 mm p.a., 74% more rainfall than in the O'Higgins Region.

Some vineyards in both regions have used of potassium phosphite in attempts to decrease the incidence and severity of Botryosphaeria dieback. Between 2010 and 2018, the use of pruning paste with DMI fungicide additives also increased. Pruning wound treatments have been effective under laboratories conditions for Chilean *Botryosphaeriaceae* isolates (Torres *et al*., 2013), or under field conditions (Díaz and Latorre, 2013). The use of benzimidazole-, QoL- or and DMI-containing pastes was suggested to provide better disease control than spray applications (Díaz and Latorre, 2013).

The overall average severity of Botryosphaeria dieback was 42% in O'Higgins and 56% in Maule. Morales *et al*. (2012) reported average disease severity 13% across seven table grape vineyards, which are analogous because they used similar analyses to those used in the present study. In 2010 and 2018, overall average severity was similar, at 49% in 2010 and 47% in 2018, although there were differences in incidence between regions and vineyards. Average severity in O'Higgins increased by 4% from 2010 to 2018, but decreased in the Maule by 8.5%. In individual vineyard blocks, average severity increased or decreased between both in O'Higgins and in Maule. Considering differences between vineyards in the same region could be due to effective disease management than to the impacts of climatic conditions.

Gubler *et al*. (2005) showed that the impacts of GTDs were proportional to vineyard age, where the most severe symptoms occurred in older vineyards. Morales *et al*. (2012) also found that Botryosphaeria dieback affecting table grape vineyards increased with the age of the plants. This was also true in the present study (Figure 3), where disease incidence and severity increased with increasing vineyard age, as shown with regression analysis for the O'Higgins Region data. In the Maule region, the surveyed vineyards were of similar age, and were fewer vineyards were sampled, so the regression analysis did not show any correlation between disease and vineyard age.

Grape yield losses for the O'Higgins Region averaged 30.7% in 2010 and 42% in 2018; and were similar in the Maule Region, at 52.9% in 2010 and 52.3% in 2018. A similar study in France estimated yield losses due to Botryosphaeria dieback of 25–30% for 'Cabernet Sauvignon', 'Cabernet Franc', and 'Sauvignon Blanc' (*Úrbez*-Torres *et al*., 2015). Munkvold *et al*. (1994) estimated that production losses caused by Eutypa dieback in Californian vineyards were between 30.1% and 61.9%. Siebert (2001) estimated 14% yield losses due to Eutypa and Botryosphaeria dieback (Bot canker). The estimated yield losses for these GTDs in other vineyards could also be significant, and similar to those determined in the present study.

The analysis of Kaplan *et al*. (2016) (see Munkvold *et al*. (1994), indicated that 'Cabernet Sauvignon' yields in a symptomless San Joaquin Valley vineyard in California was estimated at 25 t ha^{-1}, while in an untreated, infect-

ed vineyard, the estimated potential yield loss was 90% for plants at least 20 years old. These effects are similar to those determined in the present study. In 2018, estimated potential yield was 25.5 t ha^{-1} in O'Higgins Region and 22.8 t ha^{-1} in the Maule Region (Table 4). Average potential loss of both years was 36.5% in O'Higgins and 52.5% in Maule. These losses were potentially of considerable importance, but if no management was carried out, these losses would be greater and up to 90% (Munkvold *et al*., 1994; Kaplan *et al*., 2016).

Disease severity scales were evaluated in this study for Botryosphaeria dieback, and were expressed as severity and linearly associated with losses of fruit production in the vineyards. For every 10% increase in dieback severity, approx. 9% (2010) and 13% (2018) of fruit production was lost, either for estimation of production using dead spurs or from the harvest of plants with varying degrees of disease. The novel severity scale used here is an easier method for detecting losses compared to the spur count method, and is similarly robust. The severity scale also allows damage to be measured before harvest, and to estimate harvest losses (Figure 4). This new method estimates damage and losses by measuring the intensity or severity of losses caused by specific pathogens Chester (1950).

Diplodia seriata has also been reported as the most prevalent *Botryosphaeriaceae* species in vineyards in other parts of the world, including New South Wales (Castillo-Pando *et al*., 2001; Pitt *et al*., 2010; Qiu *et al*., 2011), Western Australia (Taylor *et al*., 2005), United States of America (California) (Úrbez-Torres *et al*., 2006), and South Australia (Pitt *et al*., 2010).

Gubler *et al*. (2005) reported that 36% of cankers in 'Cabernet Sauvignon' vineyards in California were due to *Botryosphaeriaceae* species. Qiu *et al*. (2011) showed that 36% of diseased grapevines in grape growing regions of Australia, among them 'Cabernet Sauvignon', were infected with *Botryosphaeriaceae* species. Proportions of *Botryosphaeriaceae* recovered from diseased plants in a broad California survey found values similar to Díaz *et al*. (2013), at 47%. In New South Wales and South Australia, 56% of diseased plants were infected by *Botryosphaeriaceae*, 68% in New Zealand and 83% in Queensland, Australia (*Úrbez*-Torres *et al*., 2006; Pitt *et al*., 2010; Baskarathevan *et al*., 2012; Sosnowski *et al*., 2013).

The present study contributes to knowledge highlighting the importance of Botryosphaeria dieback for grape production, particularly for the principal wine grape cultivar in Chile. This disease has had severe impacts on yields in Chile during the last 10 years, so these results should encourage implementation of control measures based on estimated potential production losses in each vineyard. The results also indicate the need to implement control measures, assess damage for translation into yield losses, and estimate whether newly implemented management strategies have reasonable cost/benefit outcomes.

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