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Anthracnose susceptibility for grapevines with resistance loci to downy and powdery mildew in Southern Brazil

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Summary

Anthracnose, downy and powdery mildew are the principal fungal diseases of grapes in tropical and subtropical regions. The pesticide active ingredients that control downy and powdery mildew diseases provide some protection against anthracnose attack. The release of varieties with resistance alleles to downy and powdery mildew results in less pesticide use that can increase anthracnose attack. Thus, the present work aimed to evaluate anthracnose susceptibility of genotypes with resistance loci to downy and powdery mildew under Southern Brazilian conditions. Genotype susceptibility was tested, as well as the influence of the environment (location and crop season) on increased susceptibility to anthracnose infection. To accomplish the objective, a trifactorial design was established that included 20 genotypes, two locations, and two crop seasons. Anthracnose incidence and severity were evaluated under natural infection in the field. Temperature around 16 °C and relative humidity at 84 % increased susceptibility to anthracnose infection compared to temperature around 20 °C and relative humidity at 75 %. All tested genotypes with resistance alleles to downy and powdery mildew presented symptoms of anthracnose. 'Baron', 'Cabernet Cortis' and 'Calardis Blanc' showed the least susceptibility to anthracnose, whereas 'Aromera', 'Felicia', 'Gf.2004-043-0004' and 'Gf.2004-043-0021' were the most susceptible and bore symptoms of anthracnose. Other genotypes showed variable susceptibility during the evaluation period, depending on environmental conditions. Overall, all interactions among the three tested factors were highly significant.

Key words: *Elsinoë ampelina*, mildew, PIWI, *Vitis vinifera*, climatic conditions.

Introduction

The *Vitis* genus comprises approximately 70 species distributed in three origin centers: Asia, the Americas, and Eurasia (Liu *et al.* 2016). The species *Vitis vinifera* is the most grown worldwide because of its high fruit quality (This *et al.* 2006, Chen *et al.* 2018). Varieties of this species produce fruit that provide sensorial characteristics to wine production, accounting for 90 % of wines produced worldwide (Chen *et al.* 2018). Nevertheless, *V. vinifera* is more susceptible to many fungal diseases than other *Vitis* species; consequently, it requires more applications of pesticide during the season to ensure the production of undamaged fruit by disease (This *et al.* 2006). *V. vinifera* varieties have been bred through the introgression of resistance genes against fungal diseases from *Vitis* species, such as *V. amurensis*, *V. rupestris* and *Muscadinia rotundifolia* (Töpfer *et al.* 2011). The focus of several grapevine breeding programs was the introgression of resistance genes against downy (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*) on the *V. vinifera* genome (Töpfer *et al.* 2011). These resistant *V. vinifera* varieties are called 'PIWI', that is the acronym for the German word "Pilzwiderstandfähig" (Siegfried and Temperli 2008, Sivcev *et al.* 2010), which means fungus resistant. Dozens of these varieties have been released in last two decades, mainly in Europe.

However, other major diseases challenge grapevine production around the world. One of these diseases is anthracnose, caused by *Elsinoë ampelina* (de Bary) Shear (Shear 1929) (Kirk 1895, Mortensen 1981, Brook 1992), especially in times of climate changes. This plant pathogen causes severe damage in tropical and subtropical countries in years with high precipitation, relative humidity above 80 % and long periods of wetness of fruit and leaves (Brook 1992, Santos *et al.* 2018). *E. ampelina* infects all green parts of the grapevine, but young and green tissues are the most susceptible (Brook 1973). Most of the *V. vinifera* varieties are susceptible to anthracnose (Mortensen 1981). The available PIWI genotypes with downy and powdery mildew resistant loci were developed and tested in European countries where anthracnose, currently, is not a major disease. Thus, it will be of great importance for breeding programs to make up for the lack of information about anthracnose susceptibility of PIWI varieties. In addition, until now, no resistance loci or linked markers have been mapped to this disease.

In Brazil, Australia, China and India, anthracnose is considered an important disease for viticulture, especially at the beginning of the cycle, owing to favorable weather

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conditions, mild temperature, wind, and rain (Suhag and Grover 1977). In Europe, the largest wine producer of the world, anthracnose is not yet a major threat. However, with the upcoming climate changes and the potential for reduced pesticide use promoted by the PIWI varieties, secondary diseases, such as anthracnosis, may become increasingly costly to producers. In addition, following the precaution principle, it is also recommended to include resistance to anthracnose in grapevine breeding as well. Grapevine breeding is the most sustainable approach to manage diseases such as anthracnose, even though the introgression of genetic resistance from wild relatives is very costly and time demanding. Assuming that resistance genes to anthracnose could be linked to the genomic regions that confer resistance to mildew diseases, a shortcut would involve evaluating the response of PIWI varieties to anthracnose susceptibility. Therefore, this study aimed to evaluate anthracnose infection in grapevine genotypes that carry different resistance loci to mildew diseases using two climatic conditions in Southern Brazil.

Material and Methods

Experimental design and location: The experiments were designed in randomized blocks with 5 replications and 10 plants per replication. The evaluations were done in two experimental vineyards, one located at the Agricultural Experimental Station of the Federal University of Santa Catarina - UFSC, Curitibanos (latitude 27°27'36" S, longitude 50°50'31" W and at an altitude of 1,000 m), and the other was located at the Agricultural Research and Rural Extension Company of Santa Catarina - EPAGRI, Videira (latitude 27°00 "29"S, longitude 51°09"8" W and at an altitude of 840 m) in the State of Santa Catarina, both in Southern Brazil. According to the Köppen-Geiger climate classification, the two locations are humid mesothermic, or Cfb (Peel *et al.* 2007).

For anthracnose evaluation, 14 PIWI varieties and six advanced breeding lines (ABL) were used (Tab. 1). The vineyards were planted from September 2015 to September 2017. The varieties were grafted onto Paulsen 1103 rootstock and trained by a vertical shoot positioning trellis system (VSP) at a spacing of 3.0 m between rows and 1.20 m between plants.

Experimental vineyard management: Pruning was carried out in September of each crop season, leaving from 2 to 3 buds per cane. The chemical fertilizers used as a nutrient source were urea (45 % N), potassium chloride (60 % K) and simple superphosphate (46 % P). The amount of each fertilizer was calculated using soil analysis (0-40 cm), according to recommendations of the State of Santa Catarina (Tedesco 2004). The total amount of fertilizers was applied in two halves, using the phenological stage scale created by Eichhorn and Lorenz (1984). Accordingly, the first half was used when the first green tip appeared (stage 5), and the second half was used during full bloom (stage 23). Fungicides were sprayed to protect plants against grapevine diseases, allowing natural infection of anthracnose at low level. Registered commercial products against anthracnose disease containing the active ingredients Dithianon, Diphenoconazole or Methyl Thiophanate were used.

Table 1

Description of the genotypes evaluated for the response to anthracnose under natural infection in Southern Brazil

Variety	Genealogy (Crossings)	Resistance Locus(i)[1]	Color of Berry skin	Origin (Institute)
Aromera[2]	Eger 2 x Muscat Ottonel	*Rpv3.1*	White	InnoVitis
Baron	Cabernet Sauvignon x Bronner	*Rpv3.3, Rpv10*	Black	WBI
Bronner	Merzling x Geisenheim 6494	*Ren3, Ren9, Rpv3.3, Rpv10*	White	WBI
Cabernet Cantor	Chancellor x Solaris	*Ren3, Ren9, Rpv3.1, Rpv3.3, Rpv10*	Black	WBI
Cabernet Carbon	Cabernet Sauvignon x Bronner	*Rpv10*	Black	WBI
Cabernet Cortis	Cabernet Sauvignon x Solaris	*Ren3, Ren9, Rpv3.3, Rpv10*	Black	WBI
Calandro	Domina x Regent	*Ren3, Ren9, Rpv3.1*	Black	JKI
Calardis Blanc	Calardis Musque x Seyve Villard 39-639	*Ren3, Ren9, Rpv3.1, Rpv3.2*	White	JKI
Felicia	Sirius x Vidal Blanc	*Ren3, Ren9, Rpv3.1, Rpv3.3*	White	JKI
Gf.2004-043-0004[3]	Advanced breeding line	*Run1, Ren3, Ren9, Rpv1, Rpv3.1*	White	JKI
Gf.2004-043-0010[3]	Advanced breeding line	*Run1, Ren3, Ren9, Rpv1, Rpv3.1*	black	JKI
Gf.2004-043-0013[3]	Advanced breeding line	*Run1, Ren3, Ren9, Rpv1, Rpv3.1*	black	JKI
Gf.2004-043-0015[3]	Advanced breeding line	*Run1, Ren3, Ren9, Rpv1, Rpv3.1*	white	JKI
Gf.2004-043-0021[3]	Advanced breeding line	*Run1, Ren3, Ren9, Rpv1, Rpv3.1*	Black	JKI
Gf.2004-043-0024[3]	Advanced breeding line	*Run1, Ren3, Ren9, Rpv1, Rpv3.1*	White	JKI
Helios	Merzling x Freiburg 986-60	*Ren3, Ren9, Rpv3.1*	White	WBI
Johanniter	Riesling Weiss x Freiburg 589-54	*Ren3, Ren9, Rpv3.1*	White	WBI
Prior	Freiburg 4-61 x Freiburg 236-75	*Ren3, Ren9, Rpv3.1, Rpv3.3*	Black	WBI
Regent	Diana x Chambourcin	*Ren3, Ren9, Rpv3.1*	Black	JKI
Souvignier Gris	Cabernet Sauvignon x Bronner	*Ren3, Ren9, Rpv3.2*	Rose	WBI

[1] Resistance Loci - *Rpv* - Resistance to *Plasmopara viticola*, *Ren* - Resistance to *Erysiphe necator*, *Run* - Resistance to *Uncinula necator*. InnoVitis, Italy; JKI - Intitute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany; WBI - Staatliches Weinbauinstitut Freiburg, Germany. Sources: Maul *et al.* (2020). [2] Stefanini, pers. comm.; [3] Trapp, pers. comm.

Climate monitoring: Climate data were monitored daily by meteorological stations installed close to each vineyard during the crop seasons of 2018/2019 and 2019/2020. Rainfall, relative humidity, and hourly temperatures were obtained from the Santa Catarina Environmental Resources and Hydrometeorology Information Center - Santa Catarina Agricultural Research and Rural Extension Company/EPAGRI.

Assessment of anthracnose incidence and severity: Anthracnose incidence and severity was assessed in December of each crop season using two branches of three plants with five replications (n = 30). The incidence was calculated using the percentage of branches showing anthracnose symptoms per grapevine. Anthracnose severity was measured using the OIV scale (1: very low; 3: low; 5: medium; 7: high; 9: very high). In addition, the scale 0 (zero) was included to the describe grapevines with no visible symptoms (Tab. 3). Average, minimum, and maximum values were calculated for incidence and severity. Moreover, the causal agent, *E. ampelina*, was confirmed using fungal isolation from typical symptoms of anthracnose (Fig. 1A), according to Santos *et al.* (2018), and Koch's postulate was followed (Fig. 1B).

Statistical analysis: Statistical analyses were performed using variance analysis to evaluate the interaction among factors (genotype, location and year), followed by Scott-Knott, a hierarchical clustering algorithm used in the application of ANOVA, to group the treatments. Analyses were performed using the packages Agricolae (De Mendiburu 2009) and Scott-Knott (Jelihovschi *et al.* 2018) in 'R' software, v. 3.4.5 (R Core Team, 2017). In addition, genotypes were classified according to their response to anthracnose using maximum severity: 1) slight symptoms, when genotypes display maximum severity up to 3; 2) moderate symptoms, when genotypes with different maximum severity in different locations and/or years were classified as having intermediate anthracnose susceptibility, and 3) severe symptoms, when genotypes have maximum severity ranging from 5 to 9.

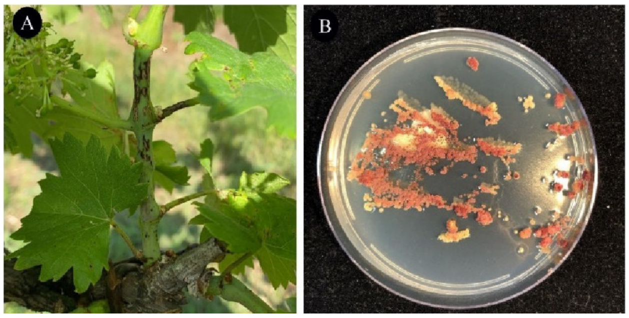

Fig. 1: Anthracnose symptoms on field (**A**) and isolated of *Elsinoë ampelina* from the anthracnose symptoms (**B**).

Results

Climatic conditions of experimental stations: In Curitibanos during spring (from September to November), before disease evaluation, the average temperature was 15.4 and 16.2 °C; the average rainfall was 40.9 and 1.7 mm per month, and average relative humidity was 92.8 and 75.8 % during crop seasons 2018/2019 and 2019/2020, respectively (Fig. 2 a-d). In contrast to the conditions in Curitibanos, Videira's average temperature was

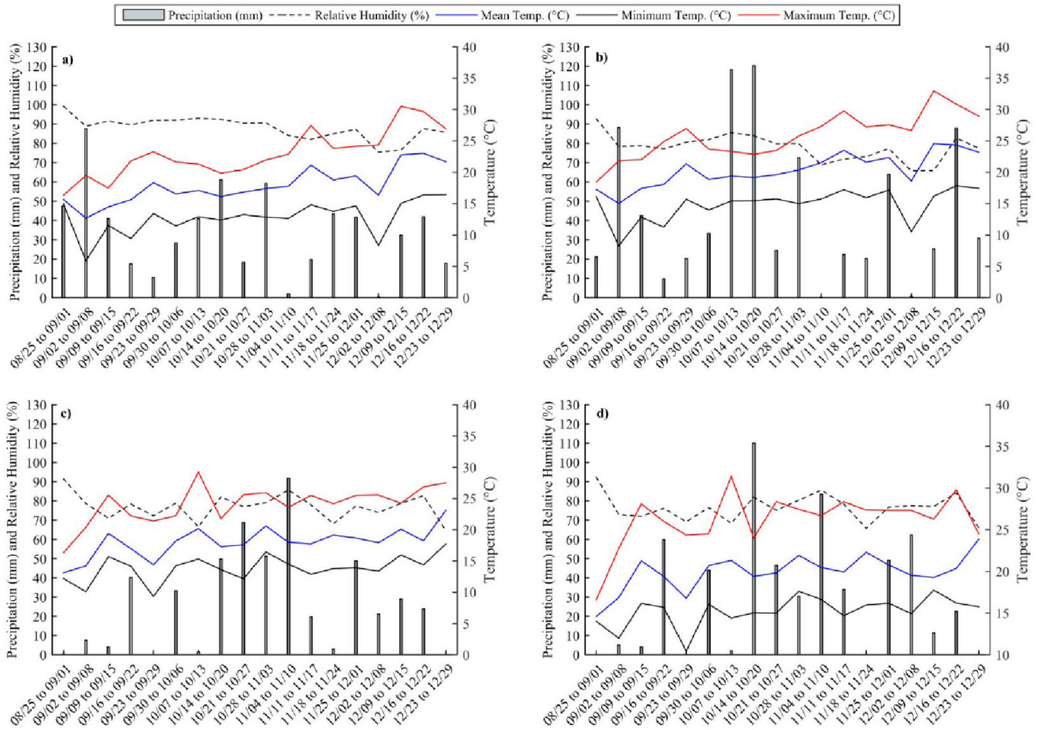

Fig. 2: Climatic conditions from the beginning of the crop season (August) until anthracnose evaluation (December). Grey bars: Precipitation (mm); green, blue and red lines: minimum, average and maximum mean temperature (°C), respectively; black line: relative humidity (%). **a**) 2018/2019 and **c**) 2019/2020 crop seasons in Curitibanos; **b**) 2018/2019 and **d**) 2019/2020 crop seasons in Videira, Santa Catarina, Brazil. Source: CIRAM-Epagri.

about two degrees higher (17.8 and 18.5 °C), rainfall per month was similar (36.4 and 2.3 mm), and the relative humidity was lower (79.4 and 73.2 %) during the crop seasons of 2018/2019 and 2019/2020, respectively.

Anthracnose infection: The incidence and severity of anthracnose infection are influenced by the interactions between genotype and environmental conditions, locations, and crop seasons (Tab. 2). Accordingly, the average anthracnose severity (Smean) ranged from 0 ('Gf.2004-043-0024' in Curitibanos, 2019), which indicates no symptoms, to 6.2 ('Gf.2004-043-0004' in Curitibanos, 2019), which indicates low and moderate susceptibility, respectively (Tab. 3).

Table 2

Degree of freedom and mean squares of the analysis of variance for severity and incidence of anthracnose in 20 genotypes of grapevine

Causes of Variation	DF	MQ	
		Severity	Incidence
Genotype	19	82.90*	151.09*
Location	1	3.47*	146.84*
Year	1	5.87*	31.89*
Genotype x Location	19	21.37*	86.7*
Genotype x Year	19	14.51*	84.63*
Location x Year	1	1.52*	1.73*
Genotype x Location x Year	19	0.495*	81.6*

*Significant differences for sum of square in ANOVA test ($p > 0.01$). **MQ medium square.

For instance, in Curitibanos, the variety 'Johanniter' obtained high Smax (9) in the 2019 crop season, while in the 2018 crop season, the severity was 1.4. In Videira this variety presented Smax values of 1.3 and 1.0, in the 2018 and 2019 crop seasons, respectively. Therefore, this variety in this location under the prevailing environmental conditions experienced low severity symptoms of anthracnose. The genotypes that obtained the highest Smax were 'Gf.2004-043-0004', 'Gf.2004-043-0021' and 'Johanniter' in Curitibanos during crop season 2019 (Tab. 3).

The maximum incidence (Imax) among genotypes ranged from 30 to 100 % of attacked plants (Tab. 3). The highest incidence occurred in 'Aromera' with 66.7 to 83.3 % symptomatic plants during both crop seasons in both locations. The lowest Imax was verified in 'Souvignier Gris', which varied from 30 to 60 % of attacked plants, with a lower anthracnose incidence when grown in Videira (approximately 33 % of attacked plants). The breeding line Gf.2004-043-0024 showed high variation of anthracnose incidence (0 to 93.3 % of attacked plants) during the crop season 2018/2019 in Curitibanos. However, in Videira the same genotype revealed 33.3 % for Imax in both crop seasons, in agreement with the variation in Smax, from 1 to 3. Collectively, these results suggest that the interaction between genotype and environment is significant.

Based on difference between the two locations, genotypes, overall, manifested higher incidence and severity of anthracnose in Curitibanos than in Videira (Tabs 2 and 3). In Curitibanos, the varieties 'Calandro' and 'Cabernet Cortis' reached maximum severity (Smax) 1 in the two crop seasons, including 'Baron' and 'Bronner', 3; 'Felicia', 5; and 'Aromera', 7. The other genotypes varied in severity from 0 to 9. In Videira, Smax occurred in 'Gf.2004-043-0004', 'Gf.2004-043-0015' and 'Regent', ranging from 1 to 3, and Aromera, ranging from 1 to 7. In the other genotypes, severity ranged from 0 to 7 (Tab. 3).

Discussion

In Southern Brazil, grapevines are more exposed to anthracnose attack during the phase from bud break to veraison, or onset of ripening (Naves *et al.* 2006). Since the fruit of all tested genotypes started to ripen in December in the two locations, December was the time when anthracnose scoring was done. In both evaluated crop seasons, the environmental conditions were favorable for natural infection of *E. ampelina*, including temperatures from 12 to 18 °C and rain distributed throughout December with relative humidity (RH) of 75 % or above (Barros *et al.* 2015, Bonin 2018, Murría *et al.* 2018).

In regions of the southern hemisphere, the ideal period for *E. ampelina* infection is during spring, from 7 to 10 h of leaf wetness with an average temperature ±12 °C or from 3 to 4 h of leaf wetness at 21°C during the summer (Brook 1992, Barros *et al.* 2015). The accumulated rainfall from September to December was 644.60 mm in 2018 and 1,202.02 mm in 2019 in Curitibanos, and 818.20 mm in 2018 and 1,485.30 mm in 2019 in Videira. Overall, no frequent rainfall occurred during the crop seasons, since in both years and locations, no rain fell in amounts greater than 2 mm (Fig. 2) for 11 to 16 weeks. Unbiased precipitation distribution favored initial infection and also the progress of disease until the disease was scored in December. In addition, the average RH around 80 % and temperature from 14 to 18 °C in both locations increased anthracnose infection.

In December, when the disease was scored, the average temperature in Curitibanos was 20.7 and 19.4 °C, the accumulated precipitation was 26.7 and 2.4 mm and the RH was 83.0 and 74.9 % during 2018 and 2019, respectively (Fig. 2A-B). Compared to Curitibanos during 2018 and 2019, respectively, Videira during December experienced an average temperature about two degrees higher (22.6 and 21.9 °C), higher accumulated precipitation by about 20-30 % (41.7 and 3.2 mm), and the RH was 73.6 and 75.8 % (Fig. 2C-D). The occurrence of temperatures around 20 °C and higher rain volume during the spring converged to form ideal climatic conditions for disease to attack at beginning of berry maturation.

A rating scale to assess anthracnose severity on grapevine is one of the main tools available for the classification of resistance (Kono *et al*. 2012). The same varieties evaluated under different environmental conditions commonly have different scores of anthracnose severity and/or incidence (Kono *et al*. 2012; Menon 2016; Bonin 2018). For example, in the present study, the incidence ranged from 30 to 90 % and severity scores varied from 1 to 3 for 'Cabernet Cortis', 'Cabernet Carbon', 'Bronner' and 'Regent'. The same varieties

Table 3

Minimum (Smin), maximum (Smax) and mean severity (Smean), quantified with the descriptor OIV, and minimum (Imin), maximum (Imax) and mean incidence (Imean) of anthracnose in PIWI varieties and advanced breeding lines, scored in two years (2018 and 2019) and in two locations [Curitibanos (Cur) and Videira (Vid)] in Santa Catarina, Southern Brazil

Genotypes	Location/Year	Severity			Incidence		
		Smean	Smin[1]	Smax[2]	Imean	Imin	Imax
Aromera	Cur/18	4.4 b	1	7	80.0 a	80.0	80.0
	Cur/19	4.4 b	1	7	66.7 b	60.0	80.0
	Vid/18	5.8 a	3	7	83.3 a	80.0	90.0
	Vid/19	3.7 c	3	7	66.7 b	60.0	70.0
Baron	Cur/18	1.2 e	1	3	63.3 b	50.0	80.0
	Cur/19	1.5 f	1	3	76.7 b	70.0	90.0
	Vid/18	1.0 f	1	1	50.0 c	40.0	60.0
	Vid/19	1.0 e	1	3	46.7 c	40.0	60.0
Bronner	Cur/18	2.1 d	1	3	65.9 b	60.0	77.8
	Cur/19	2.5 c	1	3	76.7 b	50.0	100
	Vid/18	3.5 d	1	5	80.0 a	70.0	90.0
	Vid/19	2.9 f	1	5	53.3 c	50.0	60.0
Calandro	Cur/18	1.2 f	1	1	66.7 b	60.0	70.0
	Cur/19	1.0 f	1	1	70.0 b	60.0	80.0
	Vid/18	1.1 e	1	1	40.0 c	30.0	50.0
	Vid/19	1.1 f	1	1	30.0 c	30.0	50.0
Calardis Blanc	Cur/18	1.0 f	1	3	63.3 b	50.0	80.0
	Cur/19	1.0 f	1	1	13.3 d	10.0	20.0
	Vid/18	1.0 f	1	3	56.7 c	30.0	90.0
	Vid/19	1.9 f	1	3	30.0 c	30.0	30.0
Cabernet Cantor	Cur/18	1.3 f	1	3	66.7 b	60.0	80.0
	Cur/19	2.6 d	1	7	66.7 b	60.0	80.0
	Vid/18	1.0 f	1	1	40.0 c	30.0	50.0
	Vid/19	2.4 d	1	7	33.3 c	30.0	40.0
Cabernet Carbon	Cur/18	1.6 e	1	3	86.7 a	80.0	90.0
	Cur/19	3.3 c	1	7	96.7 a	90.0	100
	Vid/18	1.0 f	1	1	30.0 c	30.0	30.0
	Vid/19	2.9 d	1	7	40.0 c	40.0	40.0
Cabernet Cortis	Cur/18	1.0 f	1	1	93.3 a	80.0	100
	Cur/19	1.0 f	1	1	93.3 a	80.0	100
	Vid/18	1.0 f	1	1	43.3 c	40.0	50.0
	Vid/19	1.0 f	1	1	40.0 c	40.0	40.0
Felicia	Cur/18	2.4 d	1	5	70.0 b	60.0	80.0
	Cur/19	3.0 d	1	5	86.6 a	70.0	100
	Vid/18	5.1 a	3	7	76.7 b	70.0	80.0
	Vid/19	2.0 e	1	5	60.0 b	60.0	60.0
Gf.2004-043-0004	Cur/18	3.9 c	1	7	83.3 a	80.0	90.0
	Cur/19	6.2 a	3	9	73.3 b	60.0	100
	Vid/18	1.7 e	1	5	66.7 b	50.0	90.0
	Vid/19	2.5 d	1	5	60.0 b	60.0	60.0
Gf.2004-043-0010	Cur/18	2.7 d	1	7	93.3 a	80.0	100
	Cur/19	2.6 d	1	5	60.0 c	0.0	100
	Vid/18	1.0 f	1	1	33.3 c	30.0	40.0
	Vid/19	1.2 f	1	3	30.0 c	30.0	30.0
Gf.2004-043-0013	Cur/18	2.2 d	1	3	66.7 b	30.0	90.0
	Cur/19	2.9 d	1	5	73.3 b	40.0	100
	Vid/18	1.0 f	1	1	36.7 c	30.0	40.0
	Vid/19	1.9 e	1	3	40.0 c	30.0	40.0
Gf.2004-043-0015	Cur/18	2.3 e	1	7	60.0 b	50.0	70.0
	Cur/19	3.1 c	1	5	66.7 b	40.0	90.0
	Vid/18	1.3 f	1	3	46.7 c	40.0	60.0
	Vid/19	1.7 e	1	3	46.7 c	40.0	60.0

Tab. 3, continued

Genotypes	Location/Year	Severity			Incidence		
		Smean	Smin[1]	Smax[2]	Imean	Imin	Imax
Gf.2004-043-0021	Cur/18	3.6 c	1	7	80.0 a	70.0	90.0
	Cur/19	4.8 b	1	9	80.0 a	60.0	100
	Vid/18	2.0 e	1	5	60.0 b	50.0	80.0
	Vid/19	2.4 d	1	5	46.7 c	40.0	60.0
Gf.2004-043-0024	Cur/18	1.3 f	1	5	93.3 a	90.0	100
	Cur/19	0.0 g	0	0	0.0 e	0.0	0.0
	Vid/18	1.2 f	1	3	33.3 c	30.0	40.0
	Vid/19	1.6 e	1	3	33.3 c	30.0	40.0
Helios	Cur/18	1.1 f	1	3	73.3 b	60.0	80.0
	Cur/19	1.8 e	1	5	40.0 c	30.0	60.0
	Vid/18	1.1 f	1	3	40.0 c	30.0	60.0
	Vid/19	2.3 d	1	5	30.0 c	30.0	30.0
Johanniter	Cur/18	1.4 e	1	3	93.3 a	90.0	100
	Cur/19	3.0 d	1	9	96.7 a	90.0	100
	Vid/18	1.3 f	1	3	46.7 c	30.0	70.0
	Vid/19	1.0 f	1	1	30.0 b	30.0	30.0
Prior	Cur/18	1.3 f	1	3	63.3 b	40.0	90.0
	Cur/19	2.5 d	1	3	73.3 b	60.0	80.0
	Vid/18	1.0 f	1	1	43.3 c	40.0	50.0
	Vid/19	1.7 f	0	5	40.0 c	40.0	40.0
Regent	Cur/18	2.2 e	1	5	90.0 a	90.0	90.0
	Cur/19	2.5 d	1	3	50.0 c	30.0	60.0
	Vid/18	1.5 e	1	3	40.0 c	30.0	60.0
	Vid/19	1.0 f	0	3	30.0 c	30.0	30.0
Souvignier Gris	Cur/18	1.0 f	1	1	60.0 b	50.0	80.0
	Cur/19	2.1 e	1	7	53.3 c	40.0	70.0
	Vid/18	1.0 f	1	1	36.7 c	30.0	40.0
	Vid/19	0.5 g	0	1	30.0 c	30.0	30.0

[1] = Minimum severity

[2] = Maximum severity

Note: Means accompanied with different letters in the column are significantly different in Scott Knott test ($p > 0.05$).

when evaluated in São Joaquim, also in Southern Brazil, in the crop season 2017-2018, showed incidence fluctuating from 18 to 40 % (Bonin 2018). Despite the differences in incidence and severity, 'Regent' and 'Cabernet Cortis' showed a similar level of attack in the study of Bonin (2018), *i. e.*, moderate and low severity, respectively.

To evaluate fruit development for symptons severity and allow the anthracnose infection, the first fungicide spray was done in the green point stage instead in the cotton stage (Eichhorn and Lorenz 1984), which is used in commercial vineyards. PIWI genotypes responded differently to anthracnose infection, as indicated by the severity and incidence values of the disease, while, at the same time, the environmental variables studied herein favored development of the pathogen (Fig. 2 and Tab. 3). Although the location where grape vineyards are established differentially affected anthracnose incidence, the evaluated genotypes were the major factor underlying the differences of the anthracnose incidence. Environmental conditions highly affected anthracnose incidence, resulting in mean square values for location and year, similar to the value found to genotypes. However, the biggest mean square to severity was observed between the genotypes compared to environmental conditions, location and crop season (Tab. 2). Variation was also observed among the replications, as determined by the range of Smin and Smax and Imin and Imax (Tab. 3). Variations in Smax and Smin depend on the initial amount of inoculum, genotype resistance and the occurrence of favorable climatic conditions (Barros *et al*. 2015).

The differential response from genotypes to environmental conditions was evidenced by the existence of significant interaction among the three factors evaluated: genotypes, locations, and years (Tab. 2). However, the magnitude of environmental influence mostly varied according to genotype. 'Johanniter', for example, manifested an anthracnose severity score from 1.4 to 3.0 in Curitibanos during the crop seasons 2018-2019 and 2019-2020, respectively (Tab. 3). When a variety manifests the same disease severity in two or more locations, it can be classified as more environmentally stable when compared to varieties that respond differentially in different locations with distinct environmental conditions. For example, 'Cabernet Cortis' demonstrated low disease attack in both locations and crop seasons, and the cultivar Aromera was classified as the most attacked genotype in both locations and crop seasons. Genotypes with less environmental influence on specific traits, such as disease resistance, demonstrate the same resistance when grown in another grapevine production region (Delrot *et al.* 2020).

None of the PIWI genotypes evaluated in the present study was immune to anthracnose. That is, all genotypes manifested symptoms of the disease. Accordingly, of the 133 grapevine genotypes tested, including *V. vinifera* and American hybrids, all showed anthracnose symptoms, taking into consideration a wide range of anthracnose spot and symptom size across genotypes (Kono *et al.* 2013). Similar results were obtained by Bonin (2018) who reported that the PIWI variety showed no high resistance or immunity against anthracnose attack. Based on the maximum Smax observed, the genotypes were categorized into three groups according to the symptoms (Tab. 4). Maximum Smax was used assuming that 'Baron', 'Cabernet Cortis' and 'Calardis Blanc' were less susceptible to the disease, manifesting only slight symptoms of the disease in both locations and crop seasons (Tab. 4). In contrast, 'Aromera', 'Felicia', 'Gf.2004-043-0004' and 'Gf.2004-043-0021' manifested severe symptoms and may be considered highly susceptible to the disease.

The genotypes 'Gf.2004-043-0004' and 'Gf.2004-043-0021', which carry at least four loci for downy and powdery mildew, were classified as highly susceptible to anthracnose attack, similar to 'Aromera', which contains resistance alleles in one locus to downy mildew (Tab. 4). The low attacked genotypes by anthracnose, such as 'Baron', 'Cabernet Cortis' and 'Calardis Blanc', have the same resistance alleles in the loci to downy and powdery mildew that are also present in cultivars classified as highly attacked. Thus, these three varieties are putative candidates to be parents in breeding crosses because they carry resistant alleles in genes for downy and powdery mildew and exhibit low susceptibility to anthracnose. In addition, genotypes could be classified as intermediate in terms of anthracnose attack because they exhibited different Smax depending on vineyard location (*i. e.,* 'Bronner', 'Helios' and 'Regent'). However, loci to different grapevine disease with resistance alleles can be linked (Hausmann *et al.* 2018). If resistance is taken to mean the ability to reduce pathogenic infection on plants owing to the effect of distinct genes and mechanisms, such as physical barrier to fungal penetration, increase of incubation period or enzymatic defense (Welter *et al*. 2007, Petit-Houdenot and Fudal 2017, Delrot *et al.* 2020), then the combination of distinct genes and mechanisms against the pathogen will allow for the development of distinct varieties with different level of disease attack (Dodds *et al.* 2020).

There are many sources of resistance to anthracnose, but studies are still needed to identify the genetic basis of resistance, the localization of genomic regions associated with resistance and the development of usable molecular markers for assisted breeding (Li *et al.* 2008, Kim *et al.* 2008). In tropical and subtropical countries, *V. vinifera* varieties are already widely distributed, and the preventive application of fungicides is adopted to avoid the infection and propagation of anthracnose (Wadekar *et al.* 2015). The varietal characteristics associated with climatic conditions determine the number of pesticide sprays required to manage the fungus (Naves *et al.* 2006, Grimalt and Dehouck 2016). The cultivation of the most resilient PIWI varieties allows a reduction in the amount of pesticide required to manage the disease comparatively to the pure *V. vinifera* varieties. In some regions of Southern Brazil, anthracnose is already a major disease. Therefore, some level of resistance to anthracnose, together with the resistance to downy and powdery mildew, is a *sine qua non* condition to allow the reduction of pesticides used, and thus, to decrease costs and to avoid the adverse effects to human and environment health.

Conclusions

PIWI varieties responded differently to natural infection with *Elsinoë ampelina*. The PIWI varieties 'Baron', 'Cabernet Cortis' and 'Calardis Blanc' showed low anthracnose severity, making them a potential source for crossing, in order to develop new varieties. In addition, these varieties need to be further evaluated in other climatic conditions before they can be recommended as grapevines resistant to anthracnose.

Credit authorship contribution statement

AHD, RON and LJW planned and designed the experiments. AHD, LJW, LLDV and ALKS performed field evaluations. AHD, LRM and DRMS isolated anthracnose. LRM and AHD performed data analyses. AHD and LRM created the images. AHD, LRM and RON wrote the first draft of the manuscript. RON and ALKS controlled the funding acquisition. All authors revised and contributed to the submitted version of the manuscript.

Table 4

Classification of 14 PIWI varieties and six advanced breeding lines according to their response to natural infection of anthracnose in two evaluation years and two locations. The genotypes were classified according to the maximum severity observed in the four evaluations

Classification of infection	Genotypes
Slight symptoms[1]	Baron, Cabernet Cortis, Calardis Blanc
Moderate symptoms[2]	Bronner, Calandro, Cabernet Cantor, Cabernet Carbon, Gf.2004-043-0010, Gf.2004-043-0013, Gf.2004-043-0015, Gf.2004-043-0024, Helios, Johanniter, Prior, Regent, Souvignier Gris
Severe symptoms[3]	Aromera, Felicia, Gf.2004-043-0004, Gf.2004-043-0021

[1] Genotypes with maximum severity from 1 to 3 in both locals and years;

[2] Genotypes with different maximum severity in different locals or/and years;

[3] Genotypes with maximum severity from 5 to 9 in both locals and years.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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