Downy mildew intensity in tolerant grapes varieties in highlands of southern Brazil

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Abstract. The aim of this study was to evaluate the different degrees of tolerance to infection by *P. viticola* among three genotypes with constitutive resistance in comparison to susceptible varieties *Vitis vinifera*. For this purpose two experiments was conducted at EPAGRI Experimental Station, located in the city of São Joaquim, Santa Catarina State, at 2015/16 cycle. In the first experiment on the field, were quantified the incidence and severity and downy mildew intensity was compared by epidemiological variables, on the tolerant varieties Bronner, Regent, Cabernet Cortis and the susceptible Sangiovese. On the second experiment forty leaf discs for the same tolerant genotypes and the susceptible variety Chardonnay were artificially infected with *P. viticola* sporangia suspension and after seven days of incubation the discs were examined and the degree of infection was estimated based on the intensity of sporangiophore formation. Sangiovese showed highest downy mildew intensity in comparison to the tolerant varieties Cabernet Carbon, Regent and Bronner. Under controlled conditions, the susceptible variety Chardonnay showed higher sporangiophore formation on discs leafs in comparison to the tolerant varieties. All the downy mildew tolerant varieties evaluated showed lower disease development in comparison with *V. vinifera* varieties.

1. Introduction

Santa Catarina State wine industry is gaining strong space on the national scene, especially in highland regions (900 m a.s.l.). The climatic potential of these regions for grape production (*Vitis vinifera* L.) has been proven by several researches [1–3]. These conditions provide distinctive climate compared to other grape-growing regions of Brazil, shifting the vine phenological cycle [1].

The viticulture in highlands of Santa Catarina State, as a new winegrowing region, show some production risks. Among the numerous factors that can derail the production, are phytosanitary problems.

Downy mildew, caused by the obligately biotrophic peronosporomycete *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni., is among of the most destructive grapevine diseases, that occurs worldwide, particularly in warm and humid climates [4]. This condition often occurs in highlands regions of Santa Catarina State during the vine growing cycle.

Some of the American species have been shown to have effective resistance against these diseases, but their wines had unwanted flavors, so vine growers started crossbreeding them with European *Vitis vinifera* varieties to obtain vines with increased resistance to fungi in order to produce high quality wines [5]. A large number of so-called fungus-resistant PIWI varieties (from the German word 'pilzwiderstandsfähig') therefore emerged in the 20th century [6].

The high disease intensity leads to overuse of chemicals in the vineyards of this region. The constant use of pesticides are very prejudicial to the enviroment, field workers and to the final consumer. The use of resistant varieties (PIWI), could be an alternative to cultivation, helpfull to decrease the pesticides level in the vineyards, reducing costs and increasing wine quality.

The aim of this work was to evaluate the different degrees of tolerance to infection by *P. viticola* among three genotypes with constitutive resistance in comparison to susceptible variety *Vitis vinifera* in highlands of Santa Catarina State.

2. Materials and methods

The experiments were conducted in São Joaquim Experimental Station (EPAGRI) Vineyards (28° 17'39" S; 49° 55'56" W, altitude 1,415 m), in 2015/2016 cycle. The evaluated varieties were the tolerants Cabernet Cortis, Bronner, Regent and the suscetible Sangiovese (*V. vinifera*). The plants were three years old, grafted on Paulsen 1103 rootstock, trained in VSP, spaced 3.0 meters between rows and 1.2 meters between plants. The pruning was performed leaving 2 buds per spur, in an unilateral cordon system.

In filed conditions, low rates of Cimoxanyl and Metalaxyl combined with others fungicides were applied to maintain low levels of downy mildew and others foliar diseases, yet still allow sufficient disease to evaluate the different genotypes.

The meteorological data were obtained from a meteorological station located at vineyard. The daily data of precipitation (mm), air relative humidity (%)

and mean temperature (°C) were obtained from CIRAM (Center of Environmental Resources Information and Hydrometeorology of Santa Catarina).

The middle portion of each vine was assessed for incidence and severity of downy mildew during the year 2015–2016 growing seasons and evaluated bi-weekly, beginning first symptom appearance in field and continuing until harvest, using 25 young leaves per branch on 2 medium-height branches of each vine, on five repetitions. Branches with diseased leaves were marked with a plastic tag proximal to the branch tip to ensure the same branch and leaves were surveyed throughout the season. Downy mildew incidence was defined as the number of leaves with downy mildew symptoms divided by the total number of leaves evaluated. Downy mildew severity was assigned using a visual diagrammatic scale based on seven levels of disease severity: 1, 3, 6, 12, 25, 50 and 75% [7].

Downy mildew disease progress curves were constructed and the epidemic were compared using four epidemiological measures: beginning of symptom appearance (BSA), time to reach maximum disease incidence/severity (TRMDI and TRMDS), maximum disease incidence/severity (Imax and Smax), and area under the incidence/severity disease progress curve (AUDIPC and AUDSPC). AUDPC was calculated according the Eq. (1)

$$AUDPC = \sum((Yi + Yi + 1)/2)(ti + 1 - ti). \quad (1)$$

Where: Y = disease intensity (incidence or severity), t = unit of time,and i = cumulative number of evaluations. This area represented thetrapezoidal integration value of severity [8]. Tests for significance were performed using an analysis of variance and post-hoc comparisons performed using a Tukey test ($\alpha = 0.05$). SAS software, version 9.1 (Cary, NC), was used for the data analysis.

At harvest time the productivity were evaluated by clusters weight (kg. plant^{-1}) of pre-selected plants. The estimated productivity (ton.ha^{-1}) were obtained by the plant density per hectare and by plants production. At that time, 300 berries of each cultivar were randomly collected and sent to perform physical-chemical and phenolic analysis. From grape must were performed analyses of total soluble solids (°Brix), total acidity (meq L^{-1}) and pH, according to the methodology proposed by OIV [9].

The soluble solids (°Brix) were measured using an optical refractometer (model Instrutherm – RTD-45) with temperature correction. The pH was measured with a pH meter (model MP 220 Metler-Toledo). Total acidity was measured by titration method with a 10 mL aliquot of juice with standardized 0.1 N NaOH. Total polyphenols content was quantified by the Folin-Ciocalteu method, with absorbance readings at 760 nm [10].

The second experiment was conducted at Phytopathology Laboratory in São Joaquim Experimental Station (EPAGRI). Leaf disc tests were used to determine the leaf tolerance against downy mildew according to Staudt and Kassemeyer [11] and Rumbolz et al. [12]. Ten leaves from tolerant varieties (Cabernet Carbon, Bronner and Regent) and from the susceptible Chardonnay were collected in the field. Leaves, always obtained from the same position on the shoot (5th to 6th unfolded leaf), were surface sterilized with sodium hypochlorite (1.0% of active chlorine), followed by triple washing with distilled water.

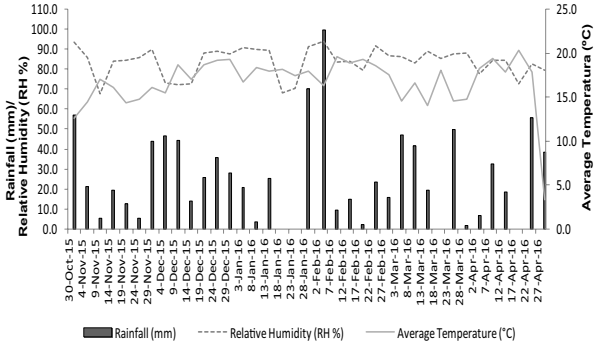

Figure 1. Average monthly accumulated precipitation (mm), air relative humidity (RH%) and mean temperature (°C) at Sao Joaquim, Santa Catarina State, Brazil, across 2015/2016 growing season.

Then four discs from each leave were excise with an 12-mm-diameter metal awl. Were evaluated eight discs per repetition, with five repetition, totalizing 40 discs per treatment/variety. The leaf discs were placed upside-down in Petri dishes containing filter paper. Each disc was artificially infected with 60μL of a *P. viticola* sporangia suspension (80,000 sporangia per ml).

Sporangia were collected from leaves of non-sprayed field-grown plants of different cultivars showing fresh sporulation after incubation overnight in a wet chamber. The infected discs were incubated for seven days at 25 °C with a photoperiod of 12 h and high relative humidity. The disease severity in discs leaves were evaluated on seventh day after inoculation (dai), and during the next ten days.

Infected leaf discs were examined with a stereo microscope at tenfold magnification. This rating on low magnification allowed screening of a large number of leaf discs with a resolution adequate to observe single sporangiophores. The degree of infection was estimated based on the intensity of sporangiophore formation (9: no, 7: one to five, 5: six to twenty, 3: more than twenty, 1: dense sporangiophore carpet), according Schwander et al. [13]. This method is similar to the evaluation protocol given by OIV descriptor 452 [14].

The average of eight discs leaves per repetition were used for statistical analyses. Tests for significance difference of downy mildew infection between the varieties were performed using an analysis of variance and comparisons performed using a Tukey test ($\alpha = 0.05$). SAS software, version 9.1(Cary, NC), was used for the data analysis.

3. Results and discussion

Mean temperature, relative humidity and total precipitation between full bloom (November) and maturity (April) were 16.8 °C, 83.6% and 953.4 mm, respectively (Fig. 1). In this period December (238 mm) and March (175 mm) were the months with higher rainfall. A combination of frequent rain and sustained humidity, particularly in spring and summer, provided conditions for leaves and berries remain wet, enabling infections by *P. viticola*. Previous studies have shown that for *P. viticola*, optimal growth is at temperatures of 20–25 °C [15, 16].

Table 1. Characteristics of downy mildew disease: beginning of symptom appearance (BSA), time to reach the maximum disease incidence (TRMDI) and severity (TRMDS), maximum value of disease incidence (Imax) and severity (Smax) and area under the disease incidence progress curve (AUDIPC) and severity (AUDSPC). Disease was evaluated in three tolerant varieties and one susceptible during 2015–2016 growing seasons at Sao Joaquim, Santa Catarina State, Brazil.

Epidem. Variable	Cabernet Carbon	Regent	Bronner	Sangiovese	C.V. (%)
BSA (days)	44.8 ab*	51.8 a	58.8 a	30.8 b	10.4
Imax. (%)	56.3 b	52.0 b	54.4 b	85.8 a	22.6
TRMID (days)	84 a	84 a	81.2 a	58.8 b	4.8
Smax.[a] (%)	1.7 c	4.4 b	3.2 b	39.2 a	36.7
TRMSD (days)	84 a	84 a	84 a	84 a	0.0
AUPIC[b]	1194 b	1195 b	1226 b	3500 a	25.0
AUDSPC[b]	22.3 b	52.1 b	53.6 b	895.1 a	37.8

C.V. – Coefficient of variance. *Means followed by the same letter in the same row are not significant different (Tukey test, $P < 0.05$). [a] Percentage of leaf area infected by a diagrammatic scale of Buffera et al. [7]. [b] Area calculated by trapezoidal integration value according to Campbell and Madden [8].

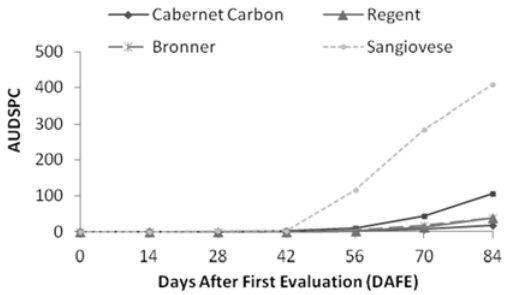

Figure 2. Downy mildew disease progress curves for area under disease severity (AUDSPC) for tolerant varieties Cabernet Carbon, Regent and Bronner and for susceptible variety Sangiovese during 2015–2016 growing season in Sao Joaquim, Santa Catarina State, Brazil.

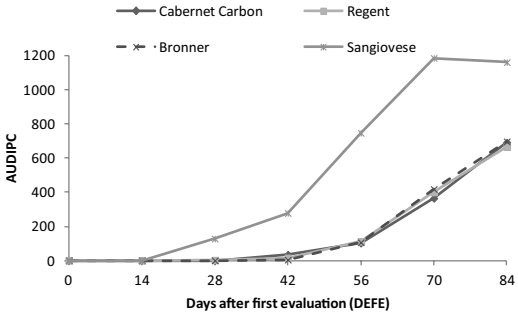

Figure 3. Downy mildew disease progress curves for area under disease incidence (AUDIPC) for tolerant varieties Cabernet Carbon, Regent and Bronner and for susceptible variety Sangiovese during 2015–2016 growing season in Sao Joaquim, Santa Catarina State, Brazil.

Several authors emphasize the importance of the leaf wetness, which represents the time that the leave is covered with a water film, provided by dew, rain or irrigation for the occurrence of epidemic in plants. It is helpfull to the formation of optimal conditions for spore germination and penetration [17]. In the case of downy mildew this time is two hours, and zoospores are biflagellate and require water for getting the host and cause primary infections, and its penetration in vines occurs through the stomata [18]. The high relative humidity and rainfall seems to be more important to promote *P. viticola* infections than the temperature. The temperature it is not a limiting factor to spore germination and infections by *P. viticola*, due to the large range of non restrictive temperature for fungus growth (5–25 °C) [19]. So, the occurrence of intense rainfall in 2015–2016 season, during the vegetative growth of vines in São Joaquim region (Fig. 1), could be the main factor to downy mildew successful infections.

The quantification of the epidemic variables are presented in Table 1. The beginning of symptoms appearance (BSA) occurred on average 44 days after first evaluation (DAFE), which corresponded to February 2nd, 2016 and the phenological stage of *véraison*. The latent period (time from infection to spore production) is expected to take 5–18 days for *P. viticola* [15,16]. Thus, it is estimated that infection by *P. viticola* may have occurred as early as mid to late January. Sangiovese variety showed an anticipation in average of 22 days to appearance of first disease symptoms, compared to Regent and Bronner.

All varieties took the same time to reach maximum severity of disease (TRMSD) (84 days), but the susceptible variety Sangiovese reached the maximum incidence of downy mildew (TRMDI) on average 24 days earlier than the resistant varieties, differing statistically from them.

The resistance in plants can be classified in horizontal or vertical by delaying the onset of the epidemic by reducing the initial infections, or making it slower after it is start, by reducing the rate of infection or progress (r) [20]. The Regent and Bronner varieties provided a delay at the beginning of the epidemic through the BAS. Varieties with constitutive resistance made the rate to reach the maximum incidence slower than the susceptible variety Sangiovese.

The Sangiovese variety presented the maximum severity (Smax), 39.2% superior from resistant varieties. To the maximum values of incidence Sangiovese showed higher values, Cabernet Carbon and Bronner were intermediated and Regent showed significant differences, appearing to be more resistant with a value of maximum incidence (Imax) 34% lower than the susceptible variety Sangiovese (Table 1).

In the comparative epidemiology, the parameter used to compare different degrees of susceptibility of plants to pathogens is the disease progress rate. Quantification of a variable that expresses the incidence and severity (intensity) of the disease is important to describe the progress of epidemics over time and its relationship with the weather or with different forms of management [21].

There were significant differences between the varieties by area under the curve progress severity and incidence of disease (AUDSPC and AUDIPC) (Figs. 2 and 3). There was a significant increase in AUDSPC of Sangiovese variety compared with other varieties evaluated (Fig. 2). The AACPSD of Sangiovese variety with an average of 408, was higher than the average of the curves of the disease of Regent (37.9) Bronner (37.8) and

Table 2. Maturity indices for three tolerant varieties and one susceptible obtained from juice physical-chemical analysis at the harvest on 2015–2016 growing season, in Sao Joaquim, Santa Catarina State, Brazil.

Variety	TSS (° Brix)	TA (meq L^{-1})	pH	Total Polyphenols (mg L^{-1})
Cab. Carbon	21.5	95.9	3.06	1138.00
Regent	20.6	57.6	3.49	4084.03
Bronner	19.0	70.3	2.96	425.97
Sangiovese	18.7	90.9	3.10	1502.92

Cabernet Carbon (17.04), statistically different by Tukey test ($p < 0.05$) (Fig. 2).

The AUDIPC was superior for variety Sangiovese (1161). Cabernet Carbon (692), Bronner (697) and Regent (666) had lower AUDIPC values and differed significantly from the susceptible variety evaluated (Fig. 3).

A significant increase in disease intensity occurred on the 42nd day after the first evaluation (DAFE) in varieties evaluated in the 2015/2016 growing season (Figs. 4 and 5). Possibly, the increasing intensity of disease is related to the increase in temperatures and rainfall in the region, in association with *P. viticola* polycyclic characteristic and the specific conditions of cultural vineyard management.

Maturity indices obtained by physical-chemical analysis of grape juice from different varieties at harvest, was appropriate for the elaboration of high quality wines in the season 2015/2016 (Table 2). Appropriate parameters for winemaking are pH between 3.1 and 3.6; titratable acidity below 110 meq L^{-1} for white varieties and 100 meq L^{-1} for red ones and total soluble solids above 19° Brix to avoid artificially juice sugaring [22].

The high contents of polyphenols, especially on Regent berries may be related to higher levels of resistance. Polyphenols have the function of protecting plants from physical (such as the sun UV radiation) and biological (fungi, viruses, bacteria) attacks. Polyphenols from the group of non-flavonoids, as the stilbenes are phytoalexins, i.e. compounds synthesized by vine in response to a stressful situation, such as pathogen attack [23].

The results of the test conducted under controlled conditions are shown in Table 3. The highest intensity of sporangia formation can be observed in the susceptible variety Chardonnay, with a significantly increase of 61.8% in comparison to the tolerant varieties Cabernet Carbon and Bronner. Regent showed an intermediate tolerance to the pathogen under controlled conditions statistically different from the other varieties evaluated. Cabernet Carbon, and Bronner were more resistant to infection of downy mildew under controlled conditions.

For the beginning of the symptoms appearance (BSA) all varieties studied took on average of 2,8 days after the seventh day of inoculation to present the formation of sporangia under controlled temperature and humidity conditions, and did not differ significantly from each other. None of the varieties caused a delay in the epidemic through BSA, possibly because they are under the same ideal conditions for infection and inoculated with a proper spore solution.

The severity of downy mildew through area under the curve progress severity were different in the varieties evaluated, which resulted higher intensity in

Table 3. Area Under Disease Progress Curve (AUDPC) and Beginning of Symptom Appearance (BSA) on leaf discs of three tolerant varieties and one susceptible under controlled conditions, during 2015–2016 growing season in São Joaquim, SC.

Variety	AUDPC[a]	BSA
Cabernet Carbon	66.9 A[b]	2.5 A
Regent	53.2 B	2.4 A
Bronner	62.3 A	3.2 A
Chardonnay	39.2 C	3.0 A
C.V.[c](%)	8.6	34.9

[a]Area calculated by trapezoidal integration value according to Campbell and Madden (1990). [b]Means followed by the same capital letter in the same row are not significant different (Tukey test, $P < 0.05$). [c]Coefficient of variance.

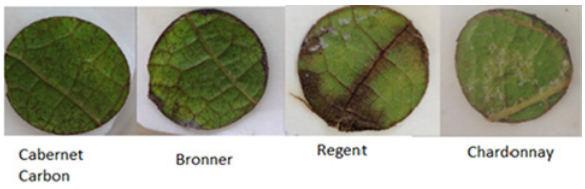

Figure 4. Representative leaf disks of each variety on the end of the evaluation after downy mildew inoculation under controlled conditions, during 2015–2016 growing season in Sao Joaquim, Santa Catarina State, Brazil.

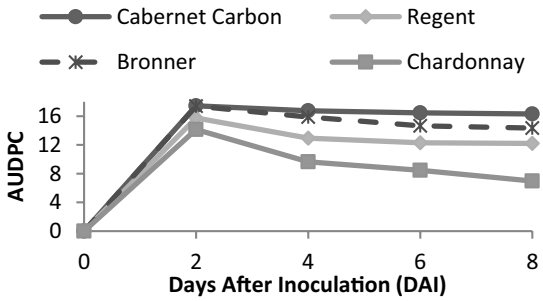

Figure 5. Area under disease progress curve (AUDPC) for tolerant varieties Cabernet Carbon, Regent and Bronner and for susceptible variety Chardonnay during 2015–2016 growing season in Sao Joaquim/SC municipality, southern Brazil.

'Chardonnay', the susceptible variety (Figs. 4 and 5). In Cabernet Carbon and Bronner varieties could be observed visually hypersensitivity reaction (HR) analyzed in leaf discs (Fig. 4).

The appearance of necrotic cells near invasion sites is termed hypersensitive reaction and is an example of localized programmed cell death that stops the pathogen, which as a biotroph depends on live host cells, from spreading to healthy tissues [24].

4. Conclusion

Sangiovese (*Vitis vinifera*) showed superior intensity of downy mildew in comparison to tolerant varieties evaluated (Bronner, Cabernet Carbon and Regent) conducted in field test during 2015–2016 growing season in São Joaquim/SC municipality, southern Brazil. Under controlled conditions, the variety Chardonnay (*Vitis vinifera*) showed higher sporangia formation on leaf discs in relation to the same tolerant varieties.

Based on maturity indices, the PIWI varieties Bronner, Cabernet Carbon and Regent have strong potential for production of quality wines in highlands of Southern Brazil.

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