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Effect of Y-trellis and Vertical Shoot Positioning Training Systems on Downy Mildew and Botrytis Bunch Rot of Grape in Highlands of Southern Brazil

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Abstract

Downy mildew (*Plasmopara viticola*) and botrytis bunch rot (*Botrytis cinerea*) are important diseases in the highlands of Santa Catarina State, a relatively new wine-growing region in Brazil. Although it is known that training systems can affect microclimate and subsequent disease development, this has not been examined in the highlands of Brazil. Thus, the purpose of this study was to evaluate the influence of Y-trellis (YT) and vertical shoot positioning (VSP) training system on downy mildew and botrytis bunch rot disease development in "Cabernet Sauvignon" cultivar. Experiments were carried out in commercial vineyards in São Joaquim, SC Municipality, southern Brazil, during the year 2012–2013 and 2013–2014 growing seasons. Downy mildew incidence and severity were quantified weekly from the first symptoms appearance on leaves, and botrytis bunch rot incidence was evaluated at harvest. Disease progress curves were constructed compared according to: (a) beginning of symptoms appearance; (b) time to maximum disease incidence and severity; (c) maximum disease incidence and severity; and (d) area under the incidence and severity disease progress curve. Results showed significant differences in downy mildew and botrytis bunch rot intensity among grape training systems, where VSP training system showed significantly lower area under the incidence and severity disease progress curve and intensity of downy mildew and botrytis bunch rot in both 2012–2013 and 2013–2014 growing seasons. Collectively, the results of this study suggest VSP training system should be recommended for grapevine production to reduce both downy mildew and botrytis bunch rot in the highlands regions of southern Brazil.

Keywords: *Vitis vinifera*, *Plasmopara viticola*, *Botrytis cinerea*, training systems, canopy management, epidemiological models

1. Introduction

In the last decade, the highlands of southern Brazil have been a rapidly growing region for wine grape (*Vitis vinifera* L.) production, where local consumer demands and favorable growing conditions make this location ideal. In particular, production sites at altitudes up to 1400 m above sea level allow grapevines to complete a long ripening cycle, which improves ripening and develops better quality wines, especially from *V. vinifera* varieties (Protas et al., 2006). These same climatic conditions are also favorable to many fungal pathogens that can significantly reduce both yield and fruit quality.

Downy mildew [*Plasmopara viticola* (Berk. and Curt) Berl. and de Toni] and botrytis bunch rot (*Botrytis cinerea* Pers.: Fr.) are the main problems affecting grape production in southern Brazil (Chavarria et al., 2007). Downy mildew occurs worldwide and in all wine-producing regions of Brazil, and in southern Brazil it is considered to be the most devastating grape pathogen. Depending on the year, production of grapes in southern Brazil has been estimated to be at a loss of 100% when warm, moist, and humid environment occurs (Garrido et al., 2004; Naves, 2006). Similarly, botrytis bunch rot is a common problem wherever grapes are grown, especially for varieties and cultivars with compact clusters. Unlike downy mildew, botrytis bunch rot primarily affects developing grape clusters, which can cause serious losses in both yield and fruit quality when weather conditions favor the disease. Infection is optimal at 15–20°C when free water is available or when humidity is greater than 90%, which can result in yield loss up to 70% when it coincides with veraison (Chavarria et al., 2007). Grape cultivars with dense canopies, thin skins, and/or tight clusters are more susceptible to botrytis bunch rot (Pezet et al., 2003; Ky et al., 2012; Mundy et al., 2012). Both downy mildew and botrytis bunch rot occurrence are closely related with environmental conditions like humidity, temperature, and light.

Disease control is generally achieved by use of triazoles, phenylamide, strobilurin fungicides but is not always possible or effective due to the phenological stage of the plant, pre-harvest spray interval restrictions, and/or environmental conditions. Chemical alternatives, such as shoot thinning and leaf removal before fruit set have been shown to reduce botrytis bunch rot (Sanzani et al., 2012), and pruning has been shown to minimize leaf wetness and reduce downy mildew (Alonso-Villaverde, 2011; Yu et al., 2012). An additional benefit to these cultural practices is that basal leaf removal, shoot positioning, and trellising have been shown to improve fruit composition by altering canopy microclimate (Zahavi et al., 2001).

Over the past two decades, advancements in vineyard design, training systems, and canopy management practices have dramatically improved wine grape productivity and fruit quality in southern Brazil. Prior to this period, a standard "Tendone"/trellised vineyard system was used throughout the region. Little attention was paid to site-specific factors influencing vine vigor, such as climate, growing region, soil type, and rootstock. Now significant effort is made to match vineyard design and trellis system to the site-specific factors that influence potential vine growth. As a result, a wide range of plant densities and training/trellis systems are routinely employed in southern Brazil.

Vertical Shoot Position (VSP) and Y-trellis (YT) are the most common training systems used in southern Brazil because of simplicity, effectiveness, and ability to reduce labor required for canopy management. In the VSP system, vine shoots are trained upward in a vertical, narrow curtain with the fruiting zone below. A VSP trellis system can consist of four to six levels of wire. For vines with small vine size, the VSP trellis is ideal. VSP can ease the work of many cultural practices such as leaf removal, shoot removal, and cluster thinning, while also providing for more efficient spray coverage. It is particularly suited to the natural upward growth of *V. vinifera* vines. VSP also has the advantage of being compatible with vineyard mechanization. The Y-Trellis (YT) or lyre vine training system uses wide rows, an open canopy, and shoot positioning to increase grape maturity and quality while maintaining production levels. "Y" trellis systems usually support arms extended 1.8–2.6 m apart, and the overhead gable systems (Peacock, 1993).

Intensity of disease epidemics depends on initial inoculum pressure and climatic conditions. It is also influenced by canopy architecture and of host receptivity to infection over time, therefore raising three primary questions: (i) can architecture modify inoculum interception, (ii) how does architecture drive the occurrence of microclimatic conditions favorable to disease development, and (iii) can architecture change the dynamics of tissue receptivity? (Tivoli et al., 2013). Leaf density measured through the leaf area index (LAI) had an effect on the physiological barrier increasing with canopy LAI. Similar effects were observed for *Colletotrichum acutatum* on strawberry (Yang et al., 1990; Madden et al., 1993), suggesting that the physiological barrier effect is a major plant architectural phenomenon that influences a wide range of pathosystems. In apple trees, Tivoli et al. (2013) suggested that some trimming systems led to a higher aeration and therefore shorter periods of wetness, hence less apple scab infection (*Venturia inaequalis*). Generally, it can be concluded that plant canopy architecture unfavorable to an aerial epidemic may result in the total avoidance of disease, but more frequently reduces disease severity rather than preventing infection completely. Thus, Bregaglio et al. (2011) suggested that in addition to genetic resistance, the use of a plant architecture that produces less favorable microenvironmental conditions for fungal infection could significantly reduce disease. In the case of grapevine canopy architecture, although it is known that vine trellising and training systems may have a direct impact on disease development, direct comparison of different canopy management systems on intensity of downy mildew and botrytis bunch rot has not yet been examined.

This study was undertaken to test the effects of two training systems: Y-trellis (YT) and vertical shoot positioning (VSP) of Cabernet Sauvignon cultivar on the intensity of downy mildew and botrytis bunch rot in a vineyard in the highlands regions of Santa Catarina State, southern Brazil, during the year 2012–2013 and 2013–2014 growing seasons.

2. Materials and methods

Experiments were carried out in two nearby commercial vineyards located in São Joaquim municipalities, State of Santa Catarina (SC), southern Brazil, during the year 2012–2013 and 2013–2014 growing seasons. São Joaquim/SC municipality is located at 28°17′39″S and 49°55′56″W at an altitude of 1430 m above sea level. The climate of the region is humid mesothermic according to Köppen classification, and soil type is cambisol, which is characterized as having high clay (492 g kg^{-1}) and organic matter (71 g kg^{-1}). In this region, high precipitation occurs from October to March, during which time rainfall averages approximately 138 mm per month. Daily rainfall, relative humidity, and hourly temperatures were recorded at the Santa Catarina Hydrology and Environmental Resources Center–Epagri (fig. 1). The soil is a cambisol with high values of clay (521 g kg^{-1}) and organic matter (83 g kg^{-1}). Vineyards consisted of approximately 1500 vines (30 rows of approximately50 vines) of 10-year-old *V. vinifera* cv. "Cabernet Sauvignon" cultivar grafted onto rootstock "Paulsen 1103" and either trained to a Y-trellis (YT) or vertical shoot positioning (VSP) at distances of 3.0 m × 1.2 m. In both training systems, vines were pruned to one directional horizontal cordon at 1.0 m height. This cultivar is susceptible to downy mildew, and disease was present in vineyards in previous years. 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 training systems. Vineyards were irrigated and maintained as recommended to commercial growers in this region (Brasil, 2011). Irrigation was usually unnecessary because of adequate rainfall, where annual rainfall in this region was between 1.520 and 1.620 mm and regularly distributed throughout the year. Annual relative humidity averaged 80% and temperatures averaged 13.4°C, with a temperature range of 9.4°C–18.9°C.

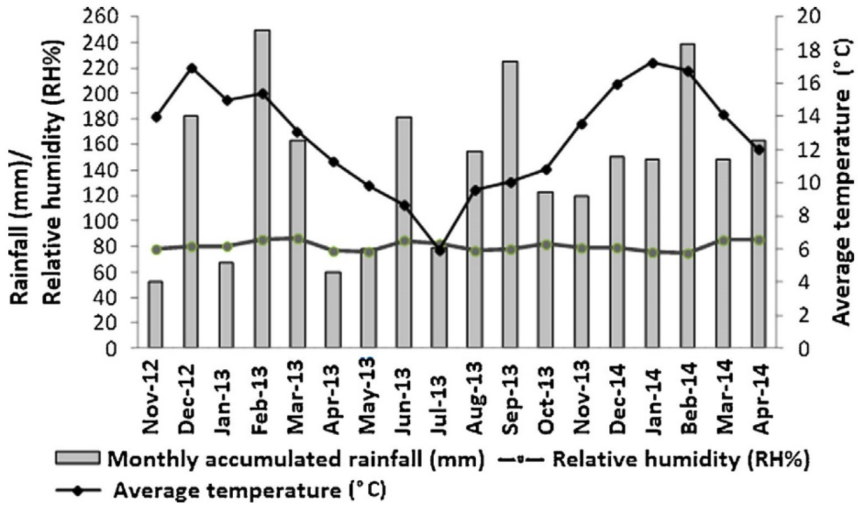

Figure 1. Average monthly accumulated rainfall (mm), relative humidity (RH%), and average temperature (°C) at São Joaquim municipality, southern Brazil, across both the 2012-2013 and 2013-2014 growing seasons.

Vineyards consisted of 90–100 rows of "Cabernet Sauvignon," the first half were YT and second half were VSP trained, where each row consisted of approximately 50 individual vines. Selection of five replicate plots within each training system was done in a complete randomized fashion, where each plot was defined as five consecutive vines that formed a continuous canopy. The three vines on each end of the vines that formed a plot were necessarily excluded, as were vines at each end of each row, and all vines in border rows.

The middle portion of each vine was assessed for incidence and severity of downy mildew during the year 2012–2013 and 2013–2014 growing seasons and evaluated weekly, beginning first symptom appearance and continuing until harvest, using 25 young leaves per branch on 4 medium-height branches of each vine. 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 described by Buffera et al. (2014) based on seven levels of disease severity: 1, 3, 6, 12, 25, 50, and 75%. Botrytis bunch rot incidence was evaluated at harvest by counting disease clusters per five replicates per treatment, and severity was determined by counting the number of rotted berries per cluster. For each plot, 30 random clusters were evaluated per treatment.

Downy mildew disease progress curves were constructed and epidemics were compared in both growing seasons 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 as $\Sigma((Y_i + Y_i + 1)/2)(t_i + 1 - t_i)$, where: Y = disease intensity (incidence or severity), t = unit of time, and i = cumulative number of evaluations. This area represented the trapezoidal integration value of severity (Campbell and Madden,1990).

Downy mildew incidence and severity progression data were fit with three empirical models, monomolecular ($y = 1 - (1 - y_0)^{-rt}$), logistic ($y = 1/(1 + ((1/y_0) - 1)^{-rt}$), and Gompertz ($y = \exp(- (-\ln(y_0))^{-rt}$), where: y = incidence or severity(relative proportion from 0 to 1) over time t, y_0 = initial disease level, and r = rate of disease increment for each empirical model. Since the monomolecular model is typically meant to fit monocyclic disease progression data, it was included in the current analysis as a form of negative control. Fit of each model to the data was evaluated using the coefficient of determination (R^2) and residual error (Jesus Junior et al., 2004). Estimated parameters from each model were compared according to training system using a t test (α = 0.05) (Madden et al., 2007). Tests for significance were performed using an analysis of variance and post-hoc comparisons performed using a Duncan test ($\alpha = 0.05$). SAS software, version 9.1 (Cary, North Carolina) was used for the data analysis.

3. Results and discussion

Average temperature, relative humidity, and total rainfall during both seasons (November–April) were 14.75°C, 81.0%, and 8.75 cm, respectively (fig. 1), where weather conditions were very similar in each season 2012–2013 (14.3°C, 80.2%, and 7.70 cm) and 2013–2014 (15.2°C, 81.8%, and 9.80 cm). 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* and *B. cinerea*. Previous studies have shown that for *P. viticola*, optimal growth is at temperatures of 20–25°C (Madden et al., 2000; Kennelly et al., 2005) and *B. cinerea* growth is optimal at 15–23°C (Wilcox, 2005; Hed et al., 2009).

First symptoms of disease appeared by November 27, 2012, and November 4, 2013, which corresponded to the phenological stage of fruit set in 2012 and full bloom in 2013. The latent period (time from infection to spore production) is expected to take several hours for *B. cinerea* (Pearson and Goheen, 1988; Wilcox, 2005) and 5–18 days for *P. viticola* (Madden et al., 2000; Kennelly et al., 2005). Thus, it is estimated that infection by *P. viticola* may have occurred as early as mid to late October, whereas *B. cinerea* infection likely did not occur until early November. Infections by *B. cinerea* early in the season may remain dormant (latent infections) while berries are green, but under favorable conditions, may resume growth and cause bunch rot as the grapes approach veraison. In this situation, the disease can spread rapidly from berry to berry within clusters and among clusters (Holz et al., 2003; Keller et al., 2003; Wilcox, 2007). Harvest occurred March 15, 2013, and April 2, 2014, meaning that disease evaluations for 2013-2014 were carried out a total of 40 days longer than in 2012-2013.

Incidence and severity of downy mildew increased 14 days after first evaluation (DAFE) in the 2012–2013 season and 56 DAFE in the 2013–2014 growing season (fig. 2). This corresponded to January 29, 2013, and January 20, 2014, when rainfall increased (fig. 1) and optimal environmental conditions for polycyclic disease development were reached (table 1). It is hypothesized that the increase of rainfall during the 2013–2014 growing season was the major contributing factor for an overall increase in downy mildew severity. This hypothesis is supported by the model proposed by Lalancette et al. (1988), which gives greater weight to leaf wetness duration than to average temperatures for estimating disease severity. Thus, duration of leaf wetness can be viewed as the factor that allows infection to take place, whereas temperature determines the rate and extent of infection (Madden et al., 2000; Gindro et al., 2006).

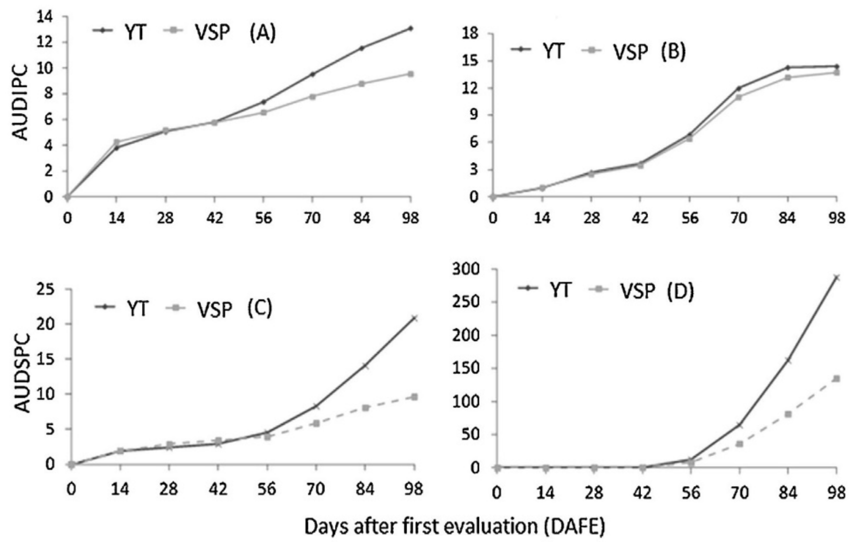

Figure 2. Downy mildew disease progress curves for area under disease incidence (AUDIPC) and severity (AUDSPC) for Y-trellis (YT) and vertical shoot positioning (VSP) in "Cabernet Sauvignon" grape during 2012–2013 (A and C) and 2013–2014 (B and D) growing seasons in São Joaquim/SC municipality, southern Brazil.

Table 1. Characteristics of downy mildew disease estimated from Gompertz model: beginning of symptom appearance (BSA), time to reach the maximum disease incidence (TRMDI) and severity (TRMDS), maximum value of disease incidence (I_{max}) and severity (S_{max}), and area under the disease incidence progress curve (AADIPC) and severity (AADSPC). Disease was evaluated in "cabernet sauvignon" grape under Y-trellis (YT) and vertical shoot positioning (VSP) during the year 2012–2013 and 2013–2014 growing seasons at São Joaquim municipality/SC, southern Brazil.

Epidemiological variable	Growing season					
	2012–2013			2013–2014		
	YT	VSP	CV(%)[a]	YT	VSP	CV(%)
BSA (days)	17.5Ab[b]	18.0Ab	42.4	38.5Aa	36.7Aa	24.3
TRMDI (days)	91.0Aa	86.3Aa	16.5	82.5Aa	84.9Aa	9.0
TRMDS (days)	98.0Aa	92.2Aa	17.4	96.0Aa	97.1Aa	16.8
I_{max} (%)	71.6Aa	40.8Bb	16.2	80.5Aa	66.7Ba	40.5
S_{max} (%)[c]	1.66Ab	0.66Bb	8.2	31.1Aa	7.6Ba	2.5
AADPIC[d]	59.9Ab	48.0Bb	4.4	74.7Aa	51.3Ba	8.7
AADPSC[d]	55.0Ab	35.8Bb	10.2	619.3Aa	226.3Ba	27.5

a. Coefficient of variance

b. Means followed by the same capital letter in the same row and the same lower-case letter in the same column for training system are not significant different (*t* test, $P < 0.05$).

c. Percentage of leaf area infected by a diagrammatic scale of Buffera et al. (2014).

d. Area calculated by trapezoidal integration value according to Campbell and Madden (1990).

Temporal data collected for downy mildew incidence and severity (fig. 2) were fit with empirical models for each season by year combination. In every case, the Gompertz model showed the best fit, as determined by having the highest coefficient of determination (R^2) and lowest residual error as compared to the other two models (table 3). It was no surprise that the monomolecular model did not fit the data as well, since it is a model most appropriate to describe monocyclic diseases and was used in this study as a form of negative control. In addition, the lack of fit by the monomolecular model confirms that external inoculum sources are not a dominant force in driving the epidemic, which has been known to yield better fit of the model even for polycyclic diseases (Madden et al., 2007). By comparison, both the logistic and Gompertz models fit the data well, but Gompertz always fit the data better, owing to the model's incorporation of a more gradual inflection in disease prior to epidemic. According to this model, the velocity of disease increase was proportional of the preexisting inoculum (initial inoculum)and infection rate (Bergamin Filho, 2011) and is appropriate for polycyclic diseases (Madden et al., 2007).

Downy mildew and botrytis rot diseases incidence as determined by the percentage of infected leaves and clusters were 2–6 times higher in the 2013–2014 growing season (tables 1 and 2). Not surprisingly, disease severity was also significantly higher ($P < 0.05$) on leaves and clusters of YT training system vines in both growing seasons (tables 1 and 2). These differences were more pronounced when disease levels were relatively low; consequently, in the 2013–2014 season that had relatively high levels of downy mildew and botrytis bunch rot, the differences between the two training systems were lower. A similar epidemiological influence was described by Zahavi et al. (2001), examining the effect of VSP and in free-positioned topped grape training system on powdery mildew(table 3).

Table 2. Incidence and severity of botrytis bunch rot in "Cabernet Sauvignon" grape under Y-trellis (YT) and vertical shoot positioning (VSP) during the year 2012–2013 and 2013–2014 growing seasons at São Joaquim/SC municipality, southern Brazil.

Epidemiological variable	Growing season					
	2012–2013			2013–2014		
	YT	VSP	CV(%)[1]	YT	VSP	CV(%)
I_{max} (%)[2]	65.82Ab[3]	48.32Bb	24.5	100Aa	66.67Ba	8.8
S_{max} (%)[4]	2.1Ab	1.18Bb	26.4	20.38Aa	3.47Ba	15.3

1. Coefficient of variance
2. Percentage of infected cluster in relation to the total cluster evaluated.
3. Means followed by the same capital letter in the row and the same lower-case letter in the column for each training system are not significantly different (*F* test, $P < 0.05$).
4. Percentage of rotted berries per cluster.

Table 3. Residual error (Error) and coefficient of determination (R^2) adjusted by Monomolecular, Logistic, and Gompertz models to the Downy Mildew severity in the Y-trellis (YT) and vertical shoot positioning (VSP) at the "Cabernet Sauvignon" grape during the year 2012–2013 and 2013–2014 growing seasons in São Joaquim/SC municipality, southern Brazil.

Training system	Monomolecular[a]		Logistic[b]		Gompertz[c]	
	Error	R^2	Error	R^2	Error	R^2
2012–2013 Growing season						
YT	0.8595	0.87	0.2425	0.98	0.2427	0.99
VSP	0.7296	0.93	0.3429	0.97	0.2426	0.98
2013–2014 Growing season						
YT	0.5795	0.77	0.7196	0.91	0.2421	0.99
VSP	0.7842	0.81	0.9698	0.88	0.2432	0.98

a. Monomolecular $y = 1 - (1 - y_0)\exp(-rt)$
b. Logistic $y = 1/(1 + ((1/y_0) - 1)\exp(-rt)$.
c. Gompertz $y = \exp(-(-\ln(y_0))\exp(-rt)$, where y = severity in proportion of 0–1 in time t and y_0 = initial level of disease and r = disease increment rate for each empirical model.

Disease progress curves for downy mildew incidence exhibited similar shapes in both growing seasons (fig. 2). However, incidence and severity of downy mildew and botrytis bunch rot were both greater for the YT training system in both growing seasons (tables 1 and 2). Epidemiological variables were estimated for each training system according to year and showed the principal variable that differentiated the two training systems was the disease progress rate. Disease progress rate (r) showed downy mildew and botrytis bunch rot development was faster in the YT training system than in VSP training system. In addition, the downy mildew AACPID and AACPSD were significantly higher in the YT training system in both years but showed no significant difference according to BSA, TRMDI, and TRMDS (table 1). Thus, the VSP training system was the most efficient to prevent or suppress disease development, according to AACPID and AACPSD (fig. 2).

Favorable climatic and biological conditions for disease development and pathogen infection are very important and fundamental for disease control strategies. The results of this study showed that vertical shoot positioning (VSP) training system reduced downy mildew and botrytis bunch rot intensity in "Cabernet Sauvignon" grape in the edafoclimatic conditions of Santa Catarina State highlands, southern Brazil, during the year 2012–2013 and 2013–2014 growing seasons. The VSP was the best system when AUDPC was taken into account as a differentiated epidemiological variable. During both the 2012–2013 and 2013–2014 growing seasons, disease incidence and severity was higher in YT training system than VSP. The epidemic development of downy mildew and botrytis bunch rot within the growth cycle of the grape is determined by the initial amount of both diseases and the rate at which downy mildew and botrytis bunch rot increase, described by the apparent infection rate.

The use of the VSP training system may enable a reduction in the number of fungicide applications, compared to that needed in the common YT training system. However, to further reduce the severity of both downy mildew and botrytis bunch rot produced by such polycyclic disease, development of *P. viticola* and *B. cinerea* must be decreased by chemical control. The VSP training system allowed sun, wind, and light penetration to all parts of the canopy zone during most parts of the day, without a marked increase in temperature. The VSP trained vines of "Cabernet Sauvignon" cultivar was also shown (Boso and Kassemeyer, 2008; Boso et al., 2011) to produce a higher annual yield than the YT vines.

This study demonstrates that canopy manipulation in the vineyard can have a significant effect on the development of downy mildew and botrytis bunch rot on leaves and fruit clusters of grapevines, respectively. Further investigations are needed to clarify if lower disease levels in the VSP trained vines was due to a direct effect of wetness duration and temperature on the susceptibility of leaves and berries, or on the fungus by affecting spore germination, hyphal development, or retention of teleomorph stages on bark surfaces (Hed et al., 2009; Alonso-Villaverde, 2011). Since several different cultivars are grown in southern Brazil, additional studies are underway to determine if the VSP training system is the most effective for downy mildew control in all cultivars grown within the region. The promise of developing a fully mechanized vineyard (Zahavi et al., 2001; Boso and Kassemeyer, 2008; Boso et al., 2011), together with our findings in reducing downy mildew and botrytis bunch rot as a result of canopy manipulation, make the VSP training system an important system that should be considered for commercial use in the future.

4. Conclusion

The results of this study showed that incidence and severity of downy mildew and botrytis bunch rot were both greater for the YT training system in both growing seasons evaluated. Disease progress rate (*r*) showed downy mildew and botrytis bunch rot development was faster in the YT training system than in VSP training system. In addition, the downy mildew AACPID and AACPSD were significantly higher in the YT training system in both years evaluated. Thus, the VSP training system was the most efficient to prevent or suppress disease development, according to AACPID and AACPSD. The VSP training system reduced downy mildew and botrytis bunch rot intensity in "Cabernet Sauvignon" grape in the edafoclimatic conditions of Santa Catarina State highlands, southern Brazil, during the year 2012–2013 and 2013–2014 growing seasons.

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