Early evaluation indices of berry susceptibility to botrytis cinerea in Chile and France

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EARLY EVALUATION INDICES OF BERRY SUSCEPTIBILITY TO *BOTRYTIS CINEREA* IN CHILE AND FRANCE

EVALUACIÓN TEMPRANA DE LA SUSCEPTIBILIDAD DE BAYAS A BOTRYTIS CINEREA EN CHILE Y FRANCIA

Carolina PAÑITRUR-DE LA FUENTE[1], Héctor VALDÉS-GÓMEZ[3*], Jean ROUDET[4], Yaneris MIRABAL[2], V. Felipe LAURIE[1], César ACEVEDO-OPAZO[1], Marc FERMAUD[4]

[1]*Universidad de Talca, Facultad de Ciencias Agrarias, Talca, Chile;*

[2]*Universidad de Talca, Instituto de Química y Recursos Naturales, Talca, Chile;*

[3]*Pontificia Universidad Católica de Chile, Departamento de Fruticultura y Enología,Santiago, Chile;*

[4]*INRA, UMR 1065 Santé et Agroécologie du VignoblE (SAVE) – ISVV, Centre INRA de Bordeaux, France.*

Abstract

Substantial economic losses occur in grapevines due to Botrytis Bunch Rot (BBR). The control of this disease is still largely based on the periodical use of specific anti-Botrytis chemical pesticides. Therefore, protection strategies require to be optimized in order to reduce pesticides applications. Our main objective was to propose and evaluate disease risk indicators at early grapevine phenological stages related to the potential susceptibility of berries to BBR. The relationships between grapevine features, i.e. pectins and tannins content in berry skins, measured at berry pea-size stage, and BBR intensity at harvest were investigated. Between 2010 and 2016, two experimental plots of Merlot and Sauvignon Blanc were studied in Chile and France. Analyses of pectins and tannins compounds were performed to characterize berry susceptibility to BBR, and both the incidence and severity of the disease were evaluated at harvest. Furthermore, a potential berry susceptibility (PBS) index was calculated using pectins and tannins data. The results obtained showed positive and linear relationships between BBR intensity and pectins and PBS index, whereas negative and linear relationships between BBR intensity and tannins contents were observed. All these relationships were statistically significant, except for the correlation between pectins and BBR incidence. The Merlot cultivar evaluated in Chile showed the lowest PBS and pectins values, and the highest tannins content, which was related with a low disease development. This work points out key disease risk indicators, which could be useful before applying direct chemical-based control measures against BBR.

Keywords: Botrytis bunch rot, pectins, tannins, sustainable management.

Resumen

Pérdidas económicas sustanciales ocurren en los viñedos debido a Botrytis Bunch Rot (BBR). El control de esta enfermedad se basa en gran medida en la aplicación periódica de pesticidas químicos específicos. Así surge la necesidad de optimizar las estrategias de control con el fin de reducir las aplicaciones de pesticidas. Nuestro principal objetivo fue proponer y evaluar indicadores de riesgo relacionados con la potencial susceptibilidad de las bayas a BBR, medidos en estados fenológicos tempranos de la vid. Relaciones entre el contenido de pectinas y taninos en la piel de las bayas, medidos en el estado fenológico "tamaño de arveja", y la intensidad de BBR a la cosecha fueron investigadas. Entre 2010 y 2016, dos cuarteles experimentales de los cvs. Merlot y Sauvignon Blanc fueron estudiados en Chile y Francia. En estos se midieron las pectinas y taninos en la piel de las bayas para caracterizar la susceptibilidad a BBR, además de evaluar la incidencia y la severidad de la enfermedad a la cosecha. Un índice de potencial susceptibilidad de bayas (PBS) fue calculado usando los datos de taninos y pectinas. Los resultados mostraron relaciones positivas y lineales entre la intensidad de BBR y las pectinas y el índice PBS, mientras que relaciones negativas y lineales fueron observadas entre la intensidad de BBR y el contenido de taninos. Todas las relaciones fueron estadístiamente significativas excepto la correlación entre pectinas e incidencia de BBR. El cultivar

Merlot evaluado en Chile, mostró los valores más bajos del índice PBS y de pectinas, y el mayor contenido de taninos, lo cual fue relacionado con un menor desarrollo de la enfermedad. Este estudio deja en evidencia indicadores de riesgo de la enfermedad, que podrían ser útiles antes de realizar medidas de control químico directo contra BBR.

Palabras claves: Botrytis bunch rot, pectinas, taninos, manejo sustentable.

Introduction

Substantial economic crop losses (~ 2 billion $US per annum) occur worldwide in vines due *to Botrytis cinerea* (Elmer and Michailides 2004). In this crop, the pathogen can reduce both yield and quality of wine (Ribéreau-Gayon et al. 1998). The control of this disease, still largely based on the use of periodic fungicide spraying. However restriction of fungicides have become necessary in order to reduce the negative effects on both, health and environment and to limit residues on harvest (Damalas and Eleftherohorinos 2011). Therefore, protection strategies aiming at the *B. cinerea* control require to be optimized in order to reduce pesticides applications. Under this context, and considering the main principles of Integrated Pest Management (IPM), risk disease assessment must be applied before direct control measures are used (IOBC 2007).

Risk disease assessment should consider factors related with the climate, the crop susceptibility and critical periods of disease development. The climate is considered the main factor involving in *B. cinerea* development and therefore several indices have been proposed. The susceptibility of grapevines to disease depends on various genetic and phenotypic traits, such as chemical features of the berry skin (Latorre, 2015). The cell walls is among the first structures that *B. cinerea* encounters when colonizing the berry skin and it can contribute to susceptibility as wells as resistance to the pathogen. An important component of cell walls are the pectins, considered a potential source of nutrients for the pathogens and the main cell wall targets during *B. cinerea* infections (Blanco-Ulate et al 2014). In contrast to pectins, other components including tannins, provide a protective barrier to *Botrytis* sp. The tannins are able to inhibit the cell wall-degrading fungal enzymes (Goetz et al., 1999), giving resistance to berries against the pathogen.

Regarding critical periods of disease development, a critical moment to disease control on grapevine is between flowering and bunch closure. Although *B. cinerea* appears and develops late in the growing season, early infections plays a key role in disease development (Elmer and Michailides, 2004). Then, fungicide applications are used during this period, in order to eliminate the fungal inoculum. Despite the importance of this critical stage, few studies have investigated the relationship between grapevine features evaluated at early vine phenological stages and the disease infection at harvest. Consequently, no risk assessment indices are available to evaluate early, grapevine susceptibility to the fungus. Thus, the main objective of this work was to evaluate early indices related to the potential susceptibility of grapevine to *Botrytis cinerea*. For that, the relationships between berry skin components (pectins and tannins) and BBR attack at harvest were investigated.

Materials and methods

Experimental field site: Experiments were carried out in two experimental fields of Merlot cultivar, one of them located in Aquitaine Region (France) and the other one situated in Maule Region (Chile). A second plot with the cultivar Sauvignon Blanc was also included in the Chilean experimental field. The experiments were carried out during seven seasons in France (2010 to 2016) and two seasons in Chile (2014-15, 2015-16).

Experimental conditions: In order to evaluate the grapevine susceptibility to *B. cinerea* at harvest, no fungicide with known activity against this pathogen was applied in any site and season.

Disease susceptibility assessment BBR incidence and severity: Berry incidence and severity were visually evaluated at harvest in environ 250 and 300 clusters in France and Chile, respectively. Disease incidence was obtained by dividing the number of clusters infected by the total number of clusters evaluated by replicate. Disease severity was calculated in each cluster as the percentage of the rotted and/or sporulating area. Both incidence and severity were expressed as a percentage.

Biochemical berry skin assessment: Analyses of pectin and tannins compounds were performed to relate berry skin susceptibility/resistance to *B. cinerea*. For this, 20 grapes per experimental plot were randomly collected at berry pea-size stage and immediately stored at -20°C. Once in laboratory, berries were peeled to determine pectins and tannins compounds.

Pectins content in berry skin: No-alcohol-soluble components (NAS fraction) were separated by a fractional process as proposed by Chenet (1997). Skins (5 g) were boiled for 10 min in 250 ml of ethanol 95%, grounded in a blender for 5 min and then centrifuged (10,000 g) for 20 min at 0°C. On a minimum of three occasions, the solid material component was resuspended in ethanol 95% and centrifuged similarly until the liquid supernatant was decolorized. The resulting NAS fraction was dried overnight at 60°C and ground to a fine powder (<100 um). After that, 0.1 g of NAS fraction were diluted in 20 ml of distilled water and 0.2 ml of ethanol 95% and shaken horizontally for 16 h at room temperature. The Water-soluble pectin (WSP) were extracted from the NAS fraction by centrifugation (10,000 g) for 20 min at 0°C. The supernatants were then diluted 1/10 and the concentration in galacturonic acid, expressed as mg g-1 NAS, was measured in three replicates using an adaptation to the colorimetric method described by Robertson (1979).

Tannins content in berry skin: The tannins content was extracted and determined from berry skins (0.5 g) ground in liquid nitrogen. The extraction process was based on two successive macerations of berry skins for 3 h each, at room temperature. For that, berry skin were stirred with 5 ml of methanol containing 0.1% of 12N HCl, according to Gagné et al. (2006). The tannins content was determined by spectrophotometry and expressed as mg g^{-1} skin in three replicates, using the methodology proposed by Ribéreau-Gayon and Stonestreet (1966).

PBS index: A potential berry susceptibility (PBS) index was calculated dividing the pectins by the tannins data.

Statistical analyses: The relationships between BBR incidence and severity with the explanatory variables pectins (WSP), tannins (TAN) and Potential berry susceptibility (PBS) index, were explored by using correlations and linear regressions. To determine if correlations were significant, Pearson's correlations coefficient were calculated based on the P-value = 0.05. All data analyses were performed using the SAS University Edition software.

Results and discussion

Disease incidence and severity: In France, the Merlot cultivar showed mean values of disease incidence and severity for the seven studied years of 57.1% and 8.6%, respectively. Nonetheless, the disease level was different between years depending mainly on climatic conditions. On the other hand, the Merlot cultivar evaluated in Chile, presented disease incidence and severity values of 0 % in all studied seasons and the Sauvignon Blanc cultivar showed a mean disease incidence and severity for both seasons of 8.4 % and 1.2%, respectively (Figure 1). Then, BBR attack was much lower in Chile than in France mainly due to climatic conditions, specifically to rainfall before harvest (data not shown).

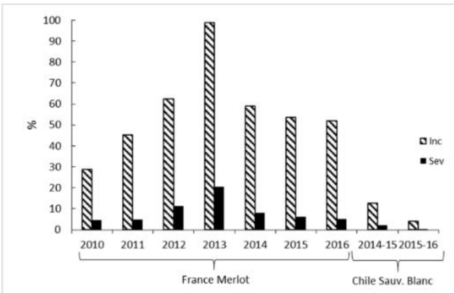

Figure 1: Mean disease incidence (Inc) and severity (Sev) values (%) for each season under field conditions in France and Chile.

Relations between berry skin components and BBR at harvest

Positive and linear relationships were observed between BBR intensity and pectins and PBS, whereas negative relationships between BBR and tannins content were observed (Figure 2). The pectins appear to be the main cell wall targets during *B. cinerea* infections and their growth is enabled by the metabolism of sugars obtained from them. Although pectins were significantly correlated with BBR severity, this berry feature did not present a significant correlation with BBR incidence (Table 1). This could be explained because in addition to pectins, there are other types of polysaccharides presented in the primary cell wall, which are also considered substrates for the pathogen (Kars and van Kan 2004). On the other hand, the tannins are constitutive antifungal compounds of berry skins which play an important role in resistance to *B. cinerea* (Pezet et al., 2004), maintaining the pathogen in a quiescent stage.

The results of the present study suggest that these indicators measured in an early phenological stage, might be used to estimate the potential susceptibility of grapevine berries to *B. cinerea*. Nevertheless, they should remain as trend indicators as there are other factors such as vegetative growth, microclimatic conditions and interactions with microorganisms that may also affect the BBR development. Then, the interpretation of them should always consider environmental conditions as the climate and the vegetative growth, considered the two major important factors affecting *B. cinerea* development (Valdés-Gómez et al. 2008).

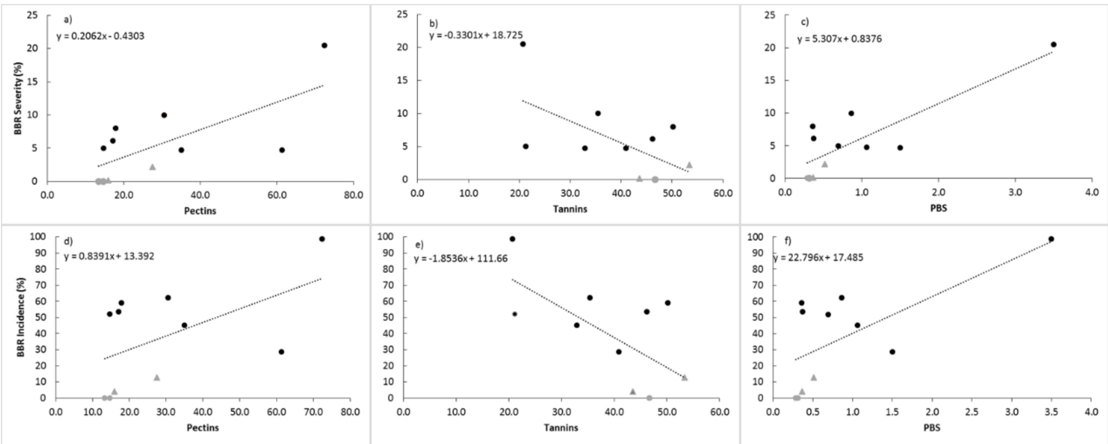

Figure 2: Relationship between BBR Severity (%) with Pectins (a), Tannins (b) and PBS index (c); and BBR Incidence (%) with Pectins (d), Tannins (e) and PBS index (f). Merlot in France (•), Merlot in Chile (•), Sauvignon Blanc in Chile ▲ (   )

Table 1: Statistical parameters for relations between BBR severity and incidence (%) and pectins, tannins and PBS index, in all study cultivars and seasons.

	Pectins			Tannins			PBS Index		
	R^2	**r**	**P- value**	**R^2**	**r**	**P- value**	**R^2**	**r**	**P- value**
Severity	0.49	0.70	0.02	0.38	-0.62	0.04	0.71	0.84	0.00
Incidence	0.29	0.54	0.09	0.42	-0.65	0.03	0.46	0.68	0.02

Conclusion

The results of the present study left in evidence early indices related with the potential susceptibility of grapevine to *Botrytis cinerea*. The pectins and the PBS index showed a positive relationship with BBR severity and incidence at harvest, whereas the tannin content showed a negative and linear relationship with the final disease attack. These indices, evaluated at berry pea-size stage (environ 100 days before harvest), could be used in IPM strategies as risk disease assessment before direct control measures are used. Nevertheless, they should remain as trend indicators as there are other factors such as vegetative growth, microclimatic conditions and interactions with microorganisms that may also affect the BBR development. Then, the interpretation of these indicators should always consider environmental conditions as the climate and the vegetative growth, considered the two major important factors affecting *B. cinerea* development

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