UNIVERSITY OF TALCA
FACULTY OF AGRICULTURAL SCIENCES
Agricultural Science PhD. Program

BOTRYTIS BUNCH ROT RISK INDICATORS: AN APPROACH TO RATIONAL DISEASE MANAGEMENT

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A thesis submitted to the University of Talca in fulfilment of the requirements for the Degree of Doctor in Agricultural Sciences

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Fecha de presentación de Tesis de Grado: 11 de Julio de 2017.

La presente tesis de Grado fue financiada por la Comisión Nacional de Investigación Científica y Tecnológica (CONICYT), mediante el Programa de Formación de Capital Humano Avanzado. Beca de Doctorado Nacional año 2013 N°21130505

DEDICATION

Dedicada a las maravillosas personas que me acompañaron durante este camino.

ACKNOWLEDGMENTS

Termina el doctorado, una etapa "caóticamente hermosa" en mi vida, y no puedo dejar de recordar y agradecer a todas aquellas personas que fueron parte de este camino. En primer lugar, quiero agradecer a mi familia, en especial a mis padres (Verónica De la Fuente y Sergio Pañitrur) y mi hermana (Andrea Pañitrur De la Fuente) por su paciencia y por permitirme cumplir mis sueños. Gracias por comprenderme, por no cortarme las alas y permitirme volar cuando quizás ustedes me necesitaban. Les agradezco infinitamente por estar siempre conmigo, a pesar de la distancia, y por el amor incondicional que siempre me han dado. Agradezco también a mi gran compañero de vida, Nicolás Verdugo, por ser mi gran apoyo y amigo. Juntos hemos sido capaces de cumplir este gran desafío y sé que seguiremos cumpliendo muchos más, pues como cómo tú dices: "estamos hechos para cosas grandes". Aprovecho de agradecer a la familia de Nicolás, mi segunda familia, por el apoyo entregado durante este proceso. Especialmente agradezco a los Tíos (Tía Nancy y Tío Tabo) por sus consejos, conversaciones y oraciones que siempre estuvieron presentes para desearnos lo mejor en este proceso. No puedo dejar de lado a mis "ángeles guardianes", mi lelita y mi tatita, que a pesar de haber partido físicamente, me han seguido acompañando en cada paso que he dado. Gracias también a mi lito ("el cacique"), mi mita jol y koki, por el apoyo y buenos deseos que siempre me han entregado. Todos ustedes han sido y seguirán siendo mi pilar fundamental. Este trabajo, no sólo es fruto de mi esfuerzo, sino que de todo el apoyo y todo lo que ustedes me han enseñado. Si soy quién soy y si he llegado hasta aquí es, en gran parte, gracias a ustedes. Infinitas gracias.

Para continuar con los agradecimientos, y volviendo a la formalidad, agradezco a CONICYT (Beca Doctorado Nacional N° 21130505) por el apoyo económico entregado para desarrollar este estudio de doctorado. Agradezco a mis profesores, Dr. Héctor Valdés y Dr. César Acevedo, por confiar en mí y permitirme trabajar junto a ustedes. Agradezco sus consejos, su apoyo y el facilitar siempre los procesos para poder cumplir con el desarrollo de esta investigación. Gracias también al Dr. Mauricio Lolas, miembro del comité de mi tesis, por su buena disposición a ayudarme siempre que fue necesario. Gracias por facilitarme las dependencias del laboratorio de Sanidad Vegetal de la Universidad y por estar siempre dispuesto a resolver mis dudas. Agradezco también al Dr. Christian Gary, miembro de mi

comité de tesis, por la colaboración en todos los procesos para el desarrollo y buen cumplimiento de esta investigación. Finalmente, agradezco a mis compañeros de trabajo y oficina: Miguel, Paulo, Panchito y Nicolás (otra vez!) por su colaboración, apoyo en terreno y por hacer más amena mi estadía en el laboratorio (CITRA) durante el desarrollo de este trabajo doctoral.

D'autre parte, je ne peux pas laisser de côté à tous les personnes en France qui m'ont aidé et ont été avec moi pendant cette étape. D'abord, je remercie énormément au Dr. Marc Fermaud pour le grand soutien et aide qu'il m'a donné dès que j'ai commencé cette thèse. Ça a été un vrai plaisir pour moi de travailler avec vous. Merci pour votre très bonne disponibilité, pour partager vos connaissances et pour votre patience. Je suis contente d'avoir eu l'opportunité de travailler avec vous et de connaitre votre grande qualité scientifique et humaine. Je veux remercier aussi à Jean Roudet pour tout le soutien qu'il m'a donné pendant les stages que j'ai fait à Bordeaux. Pour m'aider ne seulement avec les mesures au terrain et au labo, sinon aussi pour m'aider avec les « affaires domestiques » qui sont un grand défi lorsque on arrive à un nouveau pays. Un grand merci aussi à tout l'équipe de l'unité UMR 1065 Santé et Agroécologie du Vignoble (SAVE) de l'INRA de Bordeaux pour l'accueil. Sans doute, le laboratoire compte d'un grand staff des personnes, d'une très bonne qualité professionnel et humaine, qui font du laboratoire une grande ambiance de travail. Je suis vraiment contente d'avoir travaillé avec cette grande équipe ! Par ailleurs, je ne peux pas oublier mes amis et ma famille en France ! Merci à tous ces grand personnes (et futures chercheurs !) que j'ai connu pendant mes stages. Merci les amis de la Rouquette 2014 : Julita, Marie, Malick et Ahmed. Les amis de la Rouquette 2015: Awatefita, Noelita, Franklina, Antonio, Gerardo, Nazareth et Eugenia. Les amies de la Rouquette 2016: Phuonguita et Marie. Vous êtes des personnes qui ont laissé une grande empreinte dans mon cœur. Merci pour les bons moments et pour m'avoir appris que l'amitié ne comprends pas de longue ni de culture☺. Finalement, un grand merci à ma petite sœur Eloïse Weill et sa famille pour le grand soutien et accueil pendant mes stages en France. Je porte à tous ces grandes personnes par toujours dans mon cœur…

ABSTRACT

Botrytis bunch rot (BBR), caused by the fungus *Botrytis cinerea*, can reduce both yield and wine quality, leading to substantial economic losses in vineyards worldwide. The control of this disease is still largely based on the use of repetitive synthetic fungicide applications and therefore, disease management must be optimized. Thus, the main objective of this doctoral thesis was to study factors related with BBR development to be used as risk indicators in a rational disease management. Particularly, permanent (cultivar) and variable (berry skin components, vigor and floral calyptra infection) grapevine factors were investigated. Information originating from different field trials performed in Chile (Maule Region) and France (Bordeaux Region) between 2010 and 2016 was used. First, the cultivar susceptibility to *B. cinerea* and its relation to fruit maturity were investigated. For that, BBR incidence and severity were evaluated at harvest, and indices of susceptibility (SI) and maturity (F_{Mat}) were calculated. Also, vine features related to the potential susceptibility to *B. cinerea*, i.e. pectin and tannin content in berry skins and the vegetative growth, were evaluated early in the season and correlated with the disease development at harvest. Additionally, the relationship between floral calyptra infections and BBR development in mature berries was also studied. Results showed a similar cultivar classification according to their susceptibility to *B. cinerea* in the two contrasting conditions of Chile and France. Sauvignon Blanc and Gewürztraminer were the most susceptible cultivars, whereas Petit Verdot, Cabernet Sauvignon, Mourvèdre and Syrah were rather resistant or highly resistant. Moreover, an exponential and positive relationship was established between SI and F_{Mat}. Otherwise, tannin content in berry skins and grapevine vigor, measured via NDVI, were significantly correlated with both BBR incidence and severity at harvest, whereas pectins only showed significant correlations with BBR severity. Lastly, no significant correlation between floral calyptra infections and BBR development in mature berries were observed. The findings of this study showed that grapevine factors could be used as disease risk indicators. Thus, this information was used to propose an improvement to the Decision Support Rule previously developed in France for the rational management of *B. cinerea* in grapevines.

Keywords: *Botrytis cinerea*, Grape maturity, Susceptibility Index, *Vitis vinifera*, Integrated Pest Management (IPM), Decision Support Rule, Disease Risk Indicator, Vegetative Growth, NDVI, pectin content, tannin content.

SCIENTIFIC VALORIZATION

Scientific articles:

1. **Pañitrur-De la Fuente, C.**, Valdés-Gómez, H., Roudet, J., Acevedo-Opazo, C., Verdugo-Vásquez, N., Araya-Alman, M., Lolas, M., Moreno, Y., Fermaud, M. 2017. Classification of wine grape cultivars in Chile and France according to their susceptibility to *Botrytis cinerea* related to fruit maturity. Australian Journal of Grape and Wine Research. Accepted.
2. **Pañitrur-De la Fuente, C.**, Valdés-Gómez, H., Roudet, J., MirabaL, Y., Laurie, F., Goutouly, J.P., Acevedo-Opazo, C., Fermaud, M. Key early risk indicators of Botrytis Bunch Rot development: Berry skin tannin content and grapevine vigor. To be sent to Phytopathology.

National and International Conferences:

1. **Pañitrur-De la Fuente, C.**, Fermaud, M., Vergara, C., Bugueño, D., Araya, M., Verdugo-Vásquez, N., Acevedo-Opazo, C., Valdés-Gómez, H. Evaluación de índices de riesgo relacionados con el daño de *Botrytis cinerea* en cultivares Cabernet Sauvignon y Sauvignon Blanc. XXIII Congreso Sociedad Chilena de Fitopatología. December 3-5, 2014. Talca, Chile.
2. Calvo-Garrido, C., **Pañitrur-De la Fuente,C.**, Davidou, L., Aveline, N., Cestaret, S., Duffau, L., Roudet, J., Valdés-Gómez, H. and Fermaud, M. Epidemiology of Botrytis bunch rot in Bordeaux vineyards and alternative control strategies. OILB Meeting, October 20-22 October 2015; Vienna, Austria.
3. **Pañitrur-De la Fuente, C.**, Valdés-Gómez, H., Acevedo-Opazo, C., Verdugo-Vásquez, N., Araya-Alman, M., Roudet, J., Lolas, M., Moreno, Y. and Fermaud, M. How climate change may affect grapevine susceptibility to Botrytis Bunch Rot? Ollat, N., Garcia de Cortazar-Atauri, I. and Touzard., J.M. eds. ClimWine 2016 International Symposium. Sustainable grape and wine production in the context of climate change; April 10-13 2016; Bodeaux, France (Bordeaux Sciences Agro: Bordeaux, France) pp 83.
4. **Pañitrur-de la Fuente, C.**, Valdés-Gómez, H., Roudet, J., Lolas, M., Acevedo-Opazo, C. and Fermaud, M. Classification of wine grape cultivars according to susceptibility to *Botrytis*

cinerea in Chile and France: effects of fruit maturity and cluster compactness. XVII International Botrytis Symposium. October 23-28 2016; Santa Cruz, Chile.

5. **Pañitrur-De la Fuente, C.**, Valdés-Gómez, H., Roudet, J., Acevedo-Opazo, C., Gary, C., and Fermaud, M. Classification of wine grape cultivars according to their susceptibility to *Botrytis cinerea*: importance of fruit maturity. To be presented in: 20th GIESCO 2017 International Meeting. November 5-10, 2017. Mendoza, Argentina.
6. **Pañitrur-De la Fuente, C.**, Valdés-Gómez, H., Roudet, J., MirabaL, Y., Laurie, F., Goutouly, J.P., Acevedo-Opazo, C.and Fermaud, M. Early evaluation of grape berry susceptibility to *Botrytis cinerea* under contrasting growing conditions. To be presented in: 20th GIESCO 2017 International Meeting. November 5-10, 2017. Mendoza, Argentina.

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calyptras was assessed at the end of flowering. BBR assessment was carried out 30 days after mid-véraison. Residue was calculated as the difference between each severity value and the severity value predicted by the regression line.

Figure 3. Efficacy of natural products applied to control *Botrytis* bunch rot in two organic vineyards near Bordeaux in 2015. Incidence (black bars) and severity (grey bars) were assessed at commercial harvest in St. Yzan (a) and Montagne (b) field sites. Treatments consisted on 5 or 6 spray applications (St. Yzan and Montagne, respectively) at key phenological stages. ES (Early Season): only three sprays before véraison; Model: only three post véraison sprays, following a decision rule based on a Disease Risk Index. For upper and lower case, values linked by the same letter are not significantly different (p = 0.05) according to Newman-Keuls test. 70

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GENERAL INTRODUCTION

1. General Background: Viticulture in Chile and France.

Chile has been globally positioned as a major exporting wine country, due to its good quality and not expensive wines (Moguillansky 2006). The valorization of their wines by foreign markets has become the national wine industry into one of the main agricultural sectors (Lacoste 2005). Currently, Chile is the principal wine exporter country in the southern hemisphere and fourth in the world, with 8% of world exports (OIV 2016). As a result of the market growth, the area planted with vineyards for winemaking in the country has been increasing in recent years, reaching 138.355 hectares. Among them, 40% are in the Maule Region (ODEPA 2015). On the other hand, France is the first wine exporter country in value in the world and the second wine producer country, producing 17% of the total wine worldwide (OIV 2016). Thus, the wine is the second export industry in France (LARVF, 2017). The total area planted with wine-grapes in this country reach 754.473 ha (Ministère des finances et des comptes publics 2016), among them, 17% are planted in the Bordeaux Region, considered one of the most important regions in terms of production and prestige (Vin et vigne 2017). Therefore, both countries have a great importance in the wine world sector: Chile as country of the "new wine world" and France as the "main world wine power".

In order to maintain the competitiveness of both countries in the wine world sector, several strategies and technologies should be implemented to optimize the vineyard managements. These management tools should mitigate the increase in production costs, mainly due to increased price of fungicides and labour. Furthermore, these strategies should help vine-growers to face the continuous pressure from international markets demanding healthier products, produced under sustainable conditions and with low environmental impact. One of the main vineyards managements is the control of fungal diseases among which Botrytis Bunch Rot (BBR), caused by the fungus *Botrytis cinerea Pers.:Fr. (teleomorph: Botryotinia fuckeliana (de Bary) Whetzel)*, is considered as one of the most important and harmful diseases in grapevines (Latorre 2004; Lipsa et al., 2012).

2. Botrytis Bunch Rot (BBR): Characteristics and damage.

The term bunch rot refers to the disintegration of ripening grape clusters due to infection by different pathogens, with *B. cinerea* as the most important causal agent (Keller 2015). This pathogen is a polyphagous fungus that infects more than 1400 species of cultivated plants, including grapevine (*Vitis vinifera* L.) (Elad et al. 2016). In fact, *B. cinerea* is one of the most ubiquitous plant pathogens in the world. Its dispersal spores, called conidia, can survive temperatures as low as -80 ° C for several months, be dispersed by wind at great distances and can germinate in a temperature range between 1 and 30°C when the relative humidity is

higher than 90% (Pezet et al., 2004). Then, most of the vineyards in the world are under permanent *B. cinerea* conidia pressure, which is considered part of the environmental microflora in vineyards (Keller, 2015).

On grapevine this fungus can reduce both the yield and quality of wine (Ribéreau-Gayon et al. 1998), especially sensory qualities such as colour, taste and odour (Pszczolkowski et al. 2001). The enzymes secreted by the pathogen, e.g. polyphenol oxidase and termed lacasse, can oxidise the phenolic compounds in grapes and wine, turning them into quinones. This process can form brown polymers, which caused the discoloration of red wines and browning of white wines (Pezet et al. 2004; Ribéreau-Gayon et al. 1998; Pszczolkowski et al. 2001). Furthermore, the pathogen is able to reduce amino acid concentrations, degrade aroma compounds and produce volatiles, leading to undesirable aromas in wines (La Guerche et al. 2006). These changes in sensory qualities are perceived in the wine from a threshold of 5% fruit severity at harvest (Ky et al. 2012). Thus, substantial economic losses in grapevines due to BBR have been estimated to be approximately 2 billion $US per annum (Elmer and Michailides 2004).

3. Epidemiology of *B. cinerea* in grapevines.

Understanding *B. cinerea* epidemiology is essential for designing rational disease management strategies. The epidemic of this pathogen includes a sequence of different processes, which are influenced by the host and the environment. Thus, even a life cycle can be described for the fungus (Figure 1), there are variations depending on agricultural practices, environmental conditions and the geographical region (Carisse 2016). In general, the *B. cinerea* epidemic involves a sequence of chronological events as following: i) production and dispersal of initial inoculum, ii) primary infection and production and dispersal of secondary inoculum and finally, iii) production of survival structures (mycelium and sclerotia) (Elmer and Michailides 2004). Each of these stages in BBR development are highly influenced by factors related with the host and the environment, as described below:

3.1. Host factors.

3.1.1. Genetic and morphological features.

Several genetic features, which are highly dependent on grapevine cultivar, has been described to predispose grape berries to *B. cinerea* infections. For example, thin berry cuticles (Commenil et al. 1997; Zoffoli et al. 2009; Marois et al. 1986; Rosenquist and Morrison 1989), high berry porosity (Blaich et al. 1984; Mlikota Gabler et al. 2003) and lower number and thickness of the skin cell layers of the berry (Mlikota Gabler et al. 2003). Furthermore constitutive berry compounds, such as pectins and phenols, have been related to susceptibility and resistance to *B. cinerea*, respectively (Deytieux et al. 2009). Lastly,

cluster compactness has been shown to be a major morphological factor affecting the disease development (Marois et al. 1986, Vail and Marois 1991, Percival et al. 1994, Fermaud et al. 2001).

3.1.2. Grapevine phenological stage.

Grapevine is more prone to be infected by *B. cinerea* at flowering and veraison, considered as two critical phenological stages in BBR development. Grape flowers are particularly vulnerable to infection, which has been mainly associated to low resveratrol, i.e. antifungal compound, synthesized during this period (Keller et al 2015). These infections can be favoured by the presence of senescent anthers and calyptras which remain in the inflorescence (Bulit and Dubos 1982; Pearson and Goheen 1998) and by the abundance in pollen over the period (Chou and Preece 1968). After flowering infection, *B. cinerea* rests in a latency state until veraison, at which time grape clusters becomes again susceptible to the pathogen (Deytieux et al. 2009). This increase in susceptibility is mainly due to modification of the berry cuticle and cell walls and the modification of constitutive defense compounds (Keller 2015).

3.1.3. Management factors

Different grapevine management has been described to affect BBR infections. These managements include the cultivar susceptibility (Mlikota Gabler et al. 2003), the use of specific rootstocks (Delas et al. 1984; Ferrerira and Marais 1987), water and mineral nutrition (Mundy 2007; Valdés-Gómez et al. 2008) and canopy managements like winter pruning (Savage and Sall 1984), grape training system (Pereira de Bem et al. 2015) and leaf removal (English et al. 1989; Gubler et al. 1987; Molitor et al. 2011; Zoecklein et al. 1992; Elmer 2016). Among these factors, the cultivar is considered one of the most important variables affecting BBR epidemics and thereby, its selection should play a major role in disease management strategies (Elmer and Michailides 2004). On the other hand, the canopy management has also been described as an important control practice due to high grapevine vigor favoured the disease development (Valdés-Gómez et al. 2008).

3.2. Environmental factors:

3.2.1. Climate and microclimate:

It is widely accepted that climatic and microclimatic conditions are the main factors governing *B. cinerea* infections (Latorre et al. 2015). Specifically temperature and relative humidity within the cluster zone are key factors for disease development, since they favoured the presence of free water, which is essential for the fungal germination, penetration and sporulation (Thomas et al. 1988; Broome et al. 1995; Nair and Allen 1993; Coertze and Holz 1999; Latorre and Rioja, 2002; Steel et al. 2011; Ciliberti et al., 2015a, b; Ciliberti et al.

2016). In addition to temperature and relative humidity, wind speed and rainfall are other crucial factors in disease infections, because they affects aerial mycelia and conidia production; and also contribute to the presence of free water at the fruit surface (English et al. 1989; Thomas et al. 1988). In general, optimal climatic conditions for BBR infections occurred at 15-20°C in the presence of free water, or relative humidity above 90%, for at least 4 hours (Carisse 2016).

3.2.2. Interaction with other organisms:

Several microorganisms have been identified as vectors favoring *B. cinerea* conidial dispersion. Specifically, insects such as the vinegar fly (*D. melanogaster*) (Louis et al. 1996), the grape berry moth (*Lobesia botrana*) (Fermaud and Le Menn 1992), the Nez Zealand flower thrips (*Thrips obscuratus*) (Fermaud and Gaunt 1995) and the Mediterranean fruit fly (*Ceratitis capitate*) (Engelbrecht 2002) can disperse the conidias, which are trapped in their corps and/or are ingested by them. Moreover, organisms like birds, snails and other plant pathogens, e.g. powdery mildew, can induce wounds in berry skins favouring the penetration of *B. cinerea* (Latorre et al. 2015).

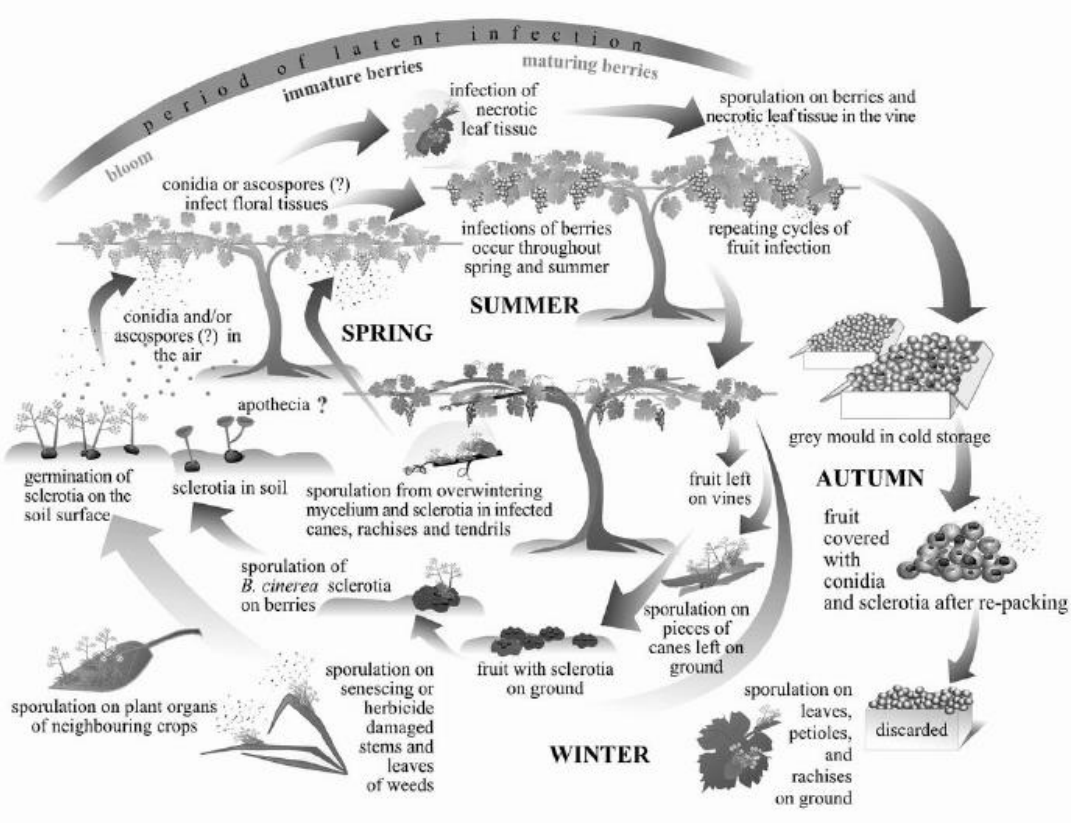

Figure 1: Life cycle of *Botrytis cinerea* in vineyard. (Source: Elmer and Michailides, 2004).

4. Disease control.

In general, the control of *B. cinerea* is extremely difficult because of the ability of the pathogen to attack crops at almost any phenological stage and to affect all the plant organs (Guillino 1992). In grapevines, due to important damages caused on this crop by the fungus, the disease control is crucial and it can be achieved by different methods as described below.

4.1. Traditional disease control.

In general, BBR control is still largely based on the use of periodic synthetic fungicide spraying, either or not is necessary (Sthienberg 2004). These repetitive fungicide applications aims to prevent the contamination of the plant tissues and/or to remove almost completely the fungus (Dubos 2002). The traditional or standard control of BBR is based on the method proposed by Agulhon (1966). This method consist in the application of specific fungicides at four key phenological stages: i) end of flowering, ii) cluster closure, iii) veraison and iv) one month before harvest. In rational management strategies the first and third treatments are always performed whereas the second application is carried out depending on the cultivar susceptibility or the conditions for disease development (Dubos 2002). Also, fungicide spraying carried out before harvest can also be repeated two or three times depending of climatic conditions and on the time elapsed between veraison and harvest. Thus, suppression of *B. cinerea* in vineyards is usually achieve by a programme of three to six fungicides, applied between flowering and harvest (Sthienberg 2004). Nevertheless, in Chile, the total treatments during the grapevine season could be higher and rich up to ten fungicide spraying per season. In this country, the critical flowering stage can be subdivided into two or three fungicide application moments, depending on the area and the history of the sector; and one to five extra fungicide treatments can be supplied in late crop cultivars, which are exposed to early autumn rains and/or over maturity conditions (Lolas, personal communication).

These practices based on the use of periodic pesticide spraying, have been used for many years by vinegrowers in worldwide, applying up to 28 kg / ha of pesticides per year to keep vineyards free of pests and diseases (INRA, 2004). The repetitive application of fungicides has become increasingly unacceptable mainly due to their negative effects on health, the environment and by the fungicide residues that can remain on harvested grapes (Fenner et al. 2013; Verger and Boobis 2013). An example is revealed by a study carried out in Europe, which showed that all bottles of conventional wines, from different countries, contained pesticide residues. The majority of these residues were classified as carcinogens, mutagens or endocrine disrupts and 93.1 % corresponded to fungicides. The case of Chile wines was particularly worrying, since the mean pesticide concentration founded in wine bottles was very high (591.3 ug/l). On the other hand, the French wine bottles evaluated in the study showed a mean pesticide concentration of 97.2 ug/l (PAN Europe 2008). Due to this worrying

situation, restriction in fungicide applications have become necessary and alternative approaches for rational disease management are urgently needed.

4.2. Integrated disease control.

A methodology for rational management of BBR can be achieved by applying fungicide only when needed, or by integrating chemical and no chemical measures (Sthienberg 2004), which is accomplished by considering the main principles of Integrated Pest Management (IPM). According to the guidelines proposed by the International Organization for Biological and Integrated Control (IOBC), the integrated disease management should consider indirect (preventive) and direct (control) strategies. Among the indirect strategies, choose a resistant cultivar to disease is one of the most important factors. Also, optimal mineral and water nutrition should also be considered in order to avoid excessive vine vigour. On the other hand and concerning the direct strategies, they should consider cultural, biological and as a last option, chemical control to minimize the use of pesticides. Furthermore, the use of these control strategies, should be based on economic thresholds or risk indices (IOBC 2007).

In the last decades many initiatives pointing to an integrated disease control have used tools, such as warning systems, to apply fungicides only when needed. These warning systems are based on forecasting models and attempted to recognize environmental conditions highly conducive to disease infections, and to schedule fungicide applications accordingly (Sthienberg 2004, Latorre et al. 2015). In the case of vineyards, it is important to highlight the models proposed by Strizyk (1985), Nair and Allen (1993) and Bromme et al. (1995), which calculates the probability of infection by *B. cinerea* on the basis of climatic conditions. Moreover, in recent years, research continues and new forecasting models, based on weather data, have been proposed to predict favorable conditions for disease development (Ciliberti et al. 2015 a,b; González-Domínguez et al. 2015; Ciliberti et al. 2016).

The understanding of the host-pathogen interactions and their relation with the environment is fundamental in a methodology for rational disease management (Sthienberg 2004). In all systems above mentioned, disease warning is based on weather forecasts, leaving out other important variables related with the host susceptibility to *B. cinerea*, such as vigor and clusters and/or berries features. Therefore, new methodologies that integrate the complex interaction between the host, the pathogen and the environment, must be developed.

4.2.1. Decision support system

Integrated disease management may involve the use of decision-support systems (DSSs) to orient management strategies (Elad 2016). DSSs are tools that elaborate and link existing information conceived and previously applied into a comprehensive system for supporting effective managements (Léger et al. 2010). In the last decade, such kind of tools has been proposed by plant pathologists in France to rationalize fungicide applications against several grapevine pathogens (Delière et al., 2008, 2015). These systems, called in this case Decision Support Rules (DSRs), translates the epidemiological and expertise knowledge available into rules easily understandable by phytosanitary practitioners to facilitate the management decisions. In general, the decision to pesticide spraying using a DSR is made according to a set of disease risk indicators such as: climatic models, cultivar susceptibility, vine phenology, symptom monitoring, fungicides characteristics, among others (Delière et al., 2008, 2015). This kind of strategy has been very effective allowing to decrease the number of fungicide applications by up to 50% in France and Chile, in the control of diseases as powdery mildew (Delière et al., 2013, Valdés-Gómez et al. 2017).

Among the DSRs, it is worth emphasizing the one proposed in France for the control of BBR (Figure 2) in the recent years. As a DSR, this tool is based on risk indicators which consider the main factors governing disease development, i.e. the pathogen, the environment and crop features. So far, this DSR is conceptual rather than operational, being implemented only by their developers with some success in experimental vineyards of the cultivar Merlot in France (Fermaud, personal communication). However, there are some information gaps needed to be solved before its implementation at the commercial vineyard level: i) no clear information is available for some disease factors, e.g. cultivar susceptibility, ii) there are not enough risk indicators to be used at key early phenological stages and iii) no threshold values have been identified for them. Therefore, this doctoral thesis aimed to investigate factors related with BBR development that could be used as risk indicators in a rational disease management, specifically in the DSR. The questions or knowledge gaps that this study tried to answer are presented below:

i. Grapevine cultivar: How is the susceptibility of different grapevine cultivars to BBR? Does it change under different climatic and cropping conditions?

As was stated before, the cultivar is one of the most important factors affecting BBR epidemics and it should play a major role in *B. cinerea* management strategies. Although different cultivar classifications according to their susceptibility to the pathogen are available in the literature, they sometimes differ greatly from one another. This situation is due to such classifications are based mostly on professional experience rather than experimental data. Additionally, the proposed classifications do not consider contrasting climatic and cropping

conditions that could affect the cultivar susceptibility. Taking it into account, the **first chapter** of the present study aimed to compare and classify the susceptibility to *B. cinerea* between different grapevine cultivars in two contrasting climatic and cropping conditions, i.e. in central Chile and western France. Furthermore, in this chapter, the maturity of cultivars was modelled and proposed as a major factor determining the susceptibility classification.

ii. Berry skin features and grapevine vigor: Can these factors, measured early in the season, explain BBR at harvest? Could they be used as early disease risk indicators?

In grapevine, early infections play a key role in disease development and therefore, fungicide applications are performed early in the season to reduce the fungal inoculum. Despite the importance of early infections, few studies have investigated the relationships between the disease development at harvest and early grapevine features. Consequently, no forecasting tools and/or no disease risk indicators are available to evaluate early grapevine susceptibility to the fungus. Therefore, the main objective of the **second chapter** was to evaluate early plant features related to the potential susceptibility to *B. cinerea.* In this section pectins and tannins in berry skin and the vegetative growth, measured via NDVI (Normalized Difference Vegetation Index), were studied. Furthermore, in this chapter, the climatic conditions before harvest were also studied to better understand the importance of the early grapevine features in BBR development during the season.

iii. Floral calyptra infection rate: Can floral calyptra infections be related with disease development in mature berries? Could it be used as an early disease risk indicator?

Latent infections initiated in floral tissues have been sometimes associated with final BBR severity in berries. Nevertheless, the importance of flower infection in the epidemiology of *B. cinerea* in grapevine is not widely recognized and the quantitative relationship between floral infection and final disease expression in mature berries has not been established clearly. Therefore, a main objective of a study presented in the "IOBC-WPRS Meeting Integrated Protection and Production in Viticulture" (**annex 1**) was to evaluate the infection of floral tissues, to possibly use it as an early indicator of BBR epidemic. Additionally, the efficacy of natural products to control the pathogen in vineyards was also quantified in this study. Nevertheless, this last point (biological control) is not part of the doctoral thesis.

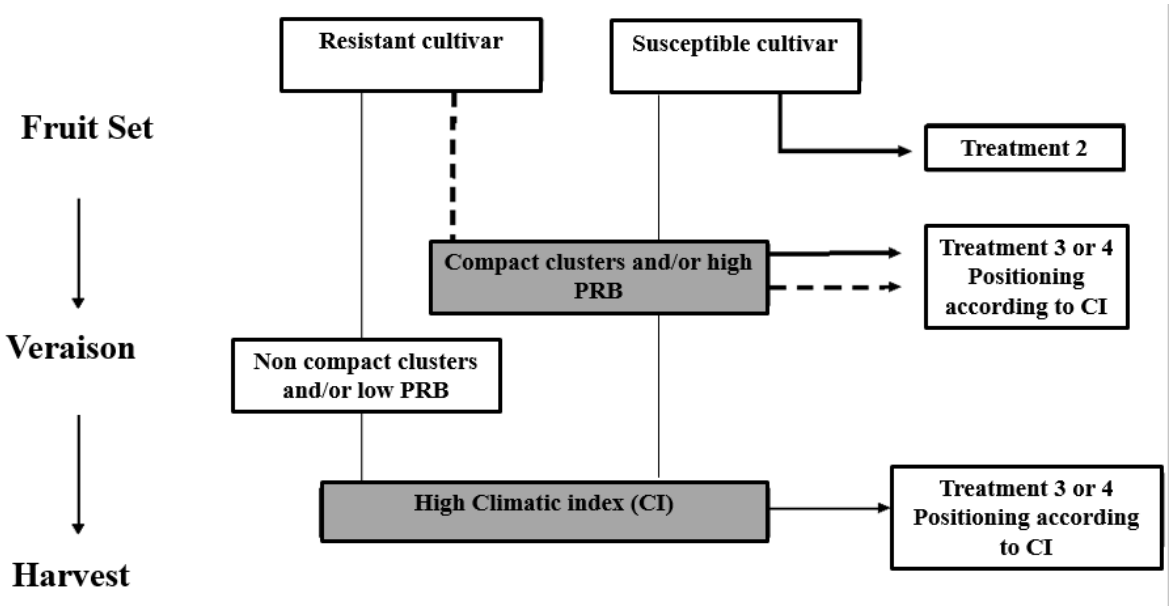

Figure 2: Decision Support Rule to control *B. cinerea* in vineyards. Where PRB= Potential receptivity of berries (index calculated by dividing pectin by phenolic compounds in berry skin), CI= Climatic index. (Source: INRA, France).

CHAPTER 1

Classification of wine grape cultivars in Chile and France according to their susceptibility to *Botrytis cinerea* related to fruit maturity.

(Accepted in: Australian Journal of Grape and Wine Research).

Abstract

Background and Aims: The susceptibility of wine-grape cultivars (cvs) to *Botrytis cinerea* is a debated topic, and the available classifications are based on professional experience rather than experimental data. The main aim of this study was to compare and classify the susceptibility of different wine-grape cvs to *B. cinerea* and its relation to fruit maturity under two contrasting climatic and cropping conditions.

Methods and Results: Between 2011 and 2015, three field trials were performed in Chile and France, including 13 common cvs. Both the incidence and severity of the disease were evaluated at harvest, and indices of susceptibility (SI) and maturity (F_{Mat}) were calculated on a per site basis. The significant differences in incidence and severity observed among cvs led to a similar susceptibility classification in both countries. Cabernet Sauvignon, Cabernet Franc, Grenache Noir and Petit Verdot were the most resistant cvs, whereas Gewürztraminer and Sauvignon Blanc were the most susceptible ones. Moreover, an exponential and positive relationship was established between SI and maturity.

Conclusions: The cultivar classification according to the susceptibility to *B. cinerea* was similar in both countries, despite the contrasting climatic conditions and cropping practices.

Significance of the Study: These findings might be of interest for choosing cvs that are more resistant to *B. cinerea* to reduce the number of fungicide applications.

Keywords: Botrytis bunch rot, Grape maturity, Resistant, Susceptibility Index, *Vitis vinifera*.

Introduction

Botrytis cinerea is a polyphagous fungus that infects more than 1400 species of cultivated plants (Elad et al. 2016). On grapevine, this fungus causes one of the most serious diseases, namely, Botrytis Bunch Rot (BBR). The pathogen can reduce drastically both the yield and quality of wine (Ribéreau-Gayon et al. 1998), especially sensory qualities such as colour, taste and odour (Pszczolkowski et al. 2001). Important organoleptic negative consequences are perceived in the wine from a threshold of 5% fruit infection at harvest (Ky et al. 2012). Thus, this fungus causes substantial economic losses in grapevines, which have been estimated to be approximately 2 billion $US per annum (Elmer and Michailides 2004).

To control this disease, fungicides have long been used (Rosslenbroich and Stuebler 2000), leading to the generation of site-specific fungicide resistant strains (Hahn 2014) and harm to both human health and the environment (Damalas and Eleftherohorinos 2011). Therefore, new control strategies that allow growers to reduce the application of pesticides should be developed based on the principles of Integrated Pest Management (IPM) (IOBC 2007). In this context, some cropping practices aiming at BBR control shouled contribute to decrease the favourable conditions for the pathogen's development. This development depends on three major factors: i) climatic and microclimatic conditions, ii) the presence/amount and characteristics of the pathogen inoculum, and iii) the susceptibility of the host, i.e., grapevine. Climatic and microclimatic conditions, specifically temperature and humidity, are key factors for *B. cinerea* infection, notably in grapevine (Savage and Sall 1984, Thomas et al. 1988, English et al. 1989, Nair and Allen 1993, Broome et al. 1995, Fermaud et al. 2001, Valdés-Gómez et al. 2008, Ciliberti et al. 2016). Favourable climatic conditions are temperatures between 15 and 25°C and wetness duration between 12 and 24 h (Thomas et al. 1988). Population genetic structure of the pathogen is also a key factor in the epidemiology of grey mould (Giraud et al. 1997, 1999, Levis et al. 1997, Beever and Weeds 2004, Martinez et al. 2003, 2008, Walker 2016). Regarding the host, the disease development depends on various genetic and phenotypic traits, such as the cluster compactness and morphological, anatomical, and chemical features of the berry skin (Latorre 2015), which are highly dependent on the grapevine cultivar.

Grapevine cultivar susceptibility to *B. cinerea* can be considered an essential management indicator in IPM. Although different cultivar classifications according to their susceptibility

to the pathogen are available in the literature (Orffer 1979, Brocuher-ACTA-ITV 1980, Robinson 1986, Jackson and Schuster 1987, Galet 1988, Dry and Gregory 1990, Marois et al. 1992, Dubos 2002), they sometimes differ greatly from one another (Table 1). This situation may have come to be because the proposed classifications are based mostly on professional experience rather than experimental data. Additionally, there are some gaps in these classifications: i) few studies compare the cultivars under the same environmental and management conditions, and ii) no study has proposed a cultivar susceptibility ranking that considers contrasting climatic and cropping conditions, e.g., northern *vs* southern hemisphere.

The cropping conditions include agronomic factors, such as the canopy and/or foliar density, water and mineral nutrition, grape training systems and winter pruning, which also predispose grapevine berries to *B. cinerea* infection (Latorre 2015). Several studies have investigated the relationship between *B. cinerea* development and these factors (Barbetti 1980, Savage and Sall 1984, Marois et al. 1986, Gubler et al. 1987, English et al. 1989, Vail and Marois 1991, Zoecklein et al. 1992, Percival et al. 1994, Ferree et al. 2003, Mundy 2007, Valdés-Gómez et al. 2008, Hed et al. 2009, Molitor et al. 2011, Pereira de Bem et al. 2015), but most often by taking into account and investigating only one model cultivar. Similarly, some works have studied the correlation between maturity and disease infection (Kosuge and Hewitt 1964, Blakeman 1975, Coley-Smith et al. 1980, Doneche 1986, Padgett and Morrison 1990, Vercesi et al. 1997, Mikota et al. 2003, Deytieux-Bellau et al. 2009), but none of them have related a classification of many cultivars with an explanatory factor of sensibility to the pathogen, such as the grape maturity.

Thus, the main objective of this work was to compare and classify the susceptibility to *B. cinerea* between different grapevine cultivars in two contrasting climatic and cropping conditions, in Central Chile and Western France. Additionally, the fruit maturity was modelled, and we analysed the extent to which this factor may account for the susceptibility rankings.

Table 1. Susceptibility to *B. cinerea* of 13 grapevine cultivars according to different literature sources.

Cultivar	a	b	c	d	e	f	g	h
Grenache Noir	4	3	-	-	4	-	3	4
Cabernet Franc	3	-	-	-	-	-	4	1
Petit Verdot	0-1	-	-	-	-	-	1	1
Cabernet Sauvignon	2	-	0	1	1	0	1	1
Mourvèdre	-	-	-	-	-	-	1	-
Merlot	3	-	-	-	-	-	3	3
Syrah	2	-	1	3	3	-	-	2
Cot	3	-	-	-	-	-	3	3
Roussanne	4	-	-	-	-	-	-	4
Chardonnay	4	-	2	2	3	-	3	3
Pinot Noir	3	4	2	3	4	-	-	3
Gewürztraminer	4	-	-	-	-	-	1	4
Sauvignon Blanc	4	-	4	3	4	-	1	4

a = Dubos (2002), b = Dry and Gregory (1990), c = Orffer (1979), d = Jackson and Schuster (1987), e = Robinson (1986), f = Marois et al. (1992), g = Galet (1988), h = ACTA (1980); 0 = highly resistant, 1 = resistant, 2 = intermediate, 3 = susceptible, 4 = highly susceptible.

Materials and methods

This study evaluated the susceptibility to Botrytis Bunch Rot (BBR) of different *Vitis vinifera* L. cultivars under contrasting conditions. The analysis was performed in three grapevine collections, two of them located in France and one in Chile. A total of 33 and 22 cultivars were evaluated in both grapevine collections located in Aquitaine Region in France, in the sites “Tour Blanche” (Bommes 44°32′33.81″ N, 0°21′02.17″ W, 57 m.a.s.l) and “Grande Ferrade” (Villenave d’Ornon 44°47′15.4″N, 0°34′37.43″W, 22 m.a.s.l), respectively (Table 2). In contrast, 19 cultivars were evaluated in Maule Region in Chile, in the site “Panguilemo” (Panguilemo, 35°22.24’ S, 71°35.62’ W, 125 m.a.s.l). A total of 13 common cultivars were evaluated in both countries. The experimental trials were performed during three seasons in the “Tour Blanche” site (2011, 2012, 2014), one season in the “Grande Ferrade” site (2011) and two seasons in Panguilemo site (2013-14, 2014-15).

Table 2. Cultivars evaluated at each experimental site in France and in Chile.

Tour Blanche (France)	Grande Ferrade (France)	Panguilemo (Chile)	Common cultivars (France and Chile)
Alicante Bouschet	Cabernet Franc	Cabernet Franc	Cabernet Franc
Cabernet Franc	Cabernet Sauvignon	Cabernet Sauvignon	Cabernet Sauvignon
Cabernet Sauvignon	Carignan	Carménère	Chardonnay
Carignan	Chardonnay	Chardonnay	Cot
Chardonnay	Chenin	Cot	Gewürztraminer
Chenin	Cot	Gewürztraminer	Grenache Noir
Cinsault	Gamay	Grenache Noir	Merlot
Colombard	Grenache Noir	Marsanne	Mourvèdre
Cot	Marselan	Merlot	Petit Verdot
Folle Blanche	Merlot	Mourvèdre	Pinot Noir
Gamay	Mourvèdre	Petit Verdot	Roussanne
Gewürztraminer	Muscadelle	Pinot Gris	Sauvignon Blanc
Grenache Blanc	Petit Verdot	Pinot Noir	Syrah
Grenache Noir	Pinot Noir	Roussanne	
Gros Manseng	Riesling	Sangiovese	
Melon	Roussanne	Sauvignon Blanc	
Merlot	Sauvignon Blanc	Sauvignon Gris	
Mourvèdre	Semillon	Syrah	
Muscadelle	Tempranillo	Tempranillo	
Muscat Petit Grain	Touriga Nacional		
Negrette	Ugni Blanc		
Petit Manseng	Viogner		
Petit Verdot			
Pinot Noir			
Riesling			
Rolle			
Roussanne			
Sauvignon Blanc			
Semillon			
Syrah			
Tannat			
Ugni Blanc			
Viogner			

Climatic characterization

The climatic conditions are different in the two regions. The sites located in France are characterized by an Oceanic climate with mild temperatures and annual rainfall of 890 mm,

with approximately 55 and 45% falling during the autumn-winter and spring-summer periods, respectively. In contrast, the site in Chile has a Dry Mediterranean climate with an annual rainfall of 600 mm, with more than 500 mm (80%) falling during the autumn-winter period. To characterize the climatic conditions for the study seasons of both sites, an automatic weather station (AWS) (Adcon Telemetric, A730, Klosterneuburg, Austria in Chile and Cimel Electronique S.A.S, CimAGRO, Paris in France) were installed 50 m from the trial plots and provided data about the air temperature, relative humidity and precipitation at 15-min intervals.

Since Chilean climatic conditions were not favourable to *B. cinerea* development, we moistened the vines during the second season (2014-15) to promote the pathogen development. For this, the vines were water sprayed using a knapsack sprayer (Solo 435). At two consecutive days, close to harvest (approximately 25°Brix), a total of 2 L of water was applied per vine, every 2 hours from 8 pm (day 1) to 9 pm (day 2), resulting in the fruit being moistened for a period of 36 hours.

Experimental conditions

The characteristics of the experimental fields are summarized in Table 3. The main differences between experimental sites are the irrigation and rootstock. The use of irrigation is typical in vineyards in central Valley in Chile but not in Western France. In contrast, vines were grafted in French sites, but in Chile, the vines were planted on their own roots. Concerning disease management and with the aim to study the cultivar susceptibility to *B. cinerea*, no fungicide was applied to control this pathogen. For the others crop managements, conventional agricultural practices as used in commercial vineyards in Central Chile and Western France were used throughout the study period. Neither in Chile nor in France were leaf removal and/or cluster thinning performed during the studied seasons. The vineyards were protected against European Grapevine Moth, and sulphur sprays were applied to control Powdery Mildew in both countries. Additionally, one application of quinoxyfen (Legend ®), one of tebuconazol (Corail ®) and one of trifloxystrobin (Natechez ®) were used to control Powdery Mildew in France, whereas one application of flusiolazol (Nustar ®) and one of penconazol (Topas ®) were performed in Chile. Downy Mildew was controlled only in

France with four fungicide applications per season, corresponding to two applications of cymoxanil (Option ®) and two copper applications. In Chile, due to the unfavourable conditions for grapevine Downy Mildew, no sprays were applied in any season and site.

Regarding the experimental design at both sites, in the "Tour Blanche" site (France), each cultivar was replicated two times in a random design, and each replication consisted of a total of 6 adjacent vines. For the site "Grande Ferrade" (France), the cultivars were repeated in a randomized block design (4 blocks), and each block consisted of a total of 10 vines. Finally, in "Panguilemo" (Chile), each cultivar was replicated four times in a randomized block design (to remove the effect of the soil slope), and each block consisted of a total of 15 vines.

Table 3. Field characteristics of the experimental fields.

	France		Chile
Property	**Tour Blanche**	**Grande Ferrade**	**Panguilemo**
Experimental Period	2011, 2012, 2014	2011	2013-14, 2014-15
Vineyard planting year	1995	2009	2006
Rootstock	3309	SO4	Own-rooted
Location (WGS84)	44°32′ N, 0°21′ W	44°47′N, 0°34′ W	35°22' S, 71°36' W
Vine Spacing (m x m)	1.8 x 0.9	1.8 x 1.0	2.0 x 1.0
Trellis system	Vertical Shoot Positioning System		
Pruning system	Two bilateral spur cordon		
Irrigation system	Non-irrigated	Non-irrigated	Drip irrigation (one dropper per plant with a flow rate of 4 L / h)

Disease susceptibility assessment

To determine the susceptibility of the different cultivars, the incidence and severity of BBR were evaluated at harvest (approximately 25° Brix) in each study season. In France, the surface of all clusters from 3 vines per cultivar, corresponding to environ 70 clusters, was visually evaluated. In Chile, 5 and 20 vines per cultivar, corresponding to approximately 110 and 500 clusters, were evaluated in 2013-14 and 2014-15, respectively. BBR was assessed by observing the surface of the clusters because this methodology has been used in most published works (e.g., Valdés-Gómez et al., 2008, González-Domínguez et al., 2015), thus allowing more direct comparisons of the results from different sources. The incidence was

obtained by dividing the number of clusters infected by the total number of clusters. The severity was calculated in each cluster as the percentage of the rotted and/or sporulating area. Both the incidence and severity were expressed as percentages.

Additionally, to classify the 13 common cultivars in both countries, a susceptibility index (SI) was calculated using the severity data. The SI was calculated using as reference the index calculated by Boso et al. (2014). Thus, the SI values were calculated for all cultivars at each season and site as specified in equation (1):

$$SI = \frac{Severity\ (\%) for\ cultivar\ in\ question}{Highest\ severity\ (\%) recorded\ in\ the\ season\ and\ in\ the\ most\ rotted\ cultivar} X\ 100 \quad (1)$$

The cultivars were then classified into 5 categories of susceptibility: Highly Resistant (HR) = 0-3.5%; Resistant (R) = 3.51-10%; Intermediate (I) = 10.1-25%; Susceptible (S) = 25.1-50% and Highly Susceptible (HS) = 50.1-100%.

Maturity assessment

A maturity index (F_{Mat}) was calculated to relate the berry maturity to the disease susceptibility of the 13 common cultivars in France and Chile. The index was calculated for each season and site using the Grapevine Flowering Veraison model (GFV) of Parker et al. (2011, 2013) and weather data for each study season, as indicated in equation (2). This phenological model was chosen because it was developed under similar conditions as observed in France and it was calibrated at the Panguilemo site, Chile (data not shown).

$$F_{Mat} = F_{B.c\ assessment} - F_{veraison} \qquad (2)$$

where $F_{B.c\ assessment}$ is the timing of the *B. cinerea* assessment in each study season and $F_{veraison}$ is the timing of veraison for each cultivar, using the model proposed by Parker et al. (2011, 2013). Both variables were estimated as the critical degree-day sum (above 0°C) calculated from the 60th and 242th day of the year in France and Chile, respectively, to the dates of *B. cinerea* assessment ($F_{B.c\ assessment}$) and veraison ($F_{veraison}$). In Chile, the $F_{veraison}$ was corrected according to the results of calibration process by subtracting 100 from the $F_{veraison}$ value proposed by Parker et al. (2013).

Finally, to prevent the effect of the different dates of assessment depending on the season, the F_{Mat} was adjusted (F_{Mat_adj}) in both countries by removing the value of F_{Mat} of the latest cultivar, i.e., Petit Verdot, among the 13 cultivars studied, as shown in equation (3):

$$F_{Mat\_adj} = F_{Mat} for\ each\ cultivar - F_{Mat}\ Petit\ Verdot \quad (3)$$

Statistical analyses

To determine differences of disease incidence and severity among the cultivars, an analysis of variance (ANOVA) was performed using the PROC GLM procedure for each experimental site. The variable "Cultivar" was considered as a fixed factor, whereas the variable "season" was considered as a random factor. When significant differences were found, a least significant difference (LSD) test at a significance level of 95% (p = 0.05) was used to compare cultivars. Additionally, a cluster analysis was performed for each site using the disease severity data. In this analysis, the furthest neighbour method and the squared euclidean distance metric were used. Furthermore, to establish a classification for the 13 common cultivars according to their susceptibility to *B. cinerea*, a box plot analysis was performed using together the SI data from all sites and all studied seasons. Moreover, a Kruskal-Wallis analysis and a Student-Newman-Keuls test at a significance level of 5% (p = 0.05) were performed on the SI data to compare the cultivar susceptibility. Finally, for the 13 common cultivars, the relationship between maturity of cultivars and their susceptibility to the pathogen was studied and modelled using the SI, F_{Mat} and F_{Mat_adj} data in all sites and study seasons. To build this relationship, a nonlinear model based on the equation $SI = a*(F_{mat\_adj})^b$ was chosen. In both analyses using SI data (Box Plot and modelling), we did not include the values of cv. Roussanne in 2011 because the disease was difficult to assess due the presence of sour rot. All statistical analyses were performed using the Statistical Software Statgraphics Plus 5.1 (StatPoint Inc., Warrenton, Virginia, USA).

Results

Climatic conditions

In all years studied in France, spring and summer were characterized by humid and temperate conditions, which favoured the growth and development of *B. cinerea* (Figure 1a, c). From budbreak to harvest, the mean air temperature fluctuated between 8 and 27 °C and was rather similar in all seasons, except in 2011, which was characterized by slightly higher temperatures. From April to October, i.e., during spring and summer in France, a total rainfall of 418 mm and 439 mm were recorded in 2012 and 2014, respectively, whereas a total rainfall of only 240 mm was registered in 2011. However, in the last year, half of this total rainfall fell from veraison to harvest, notably in August and September (124 mm), leading to favourable conditions for disease development. Chilean conditions were characterized by dry and temperate spring and summer periods, in both studied seasons, which were not conducive to disease development (Figure 1b, d). From budbreak to harvest, the mean air temperature in both seasons ranged from 10 to 27 °C, similar to France. However, the total rainfall was much lower than in France: from October to April, only 22 and 36 mm were recorded in 2013-14 and 2014-15, respectively (Figure 1b). In the 2014-15 season, the rain periods were mostly concentrated before veraison.

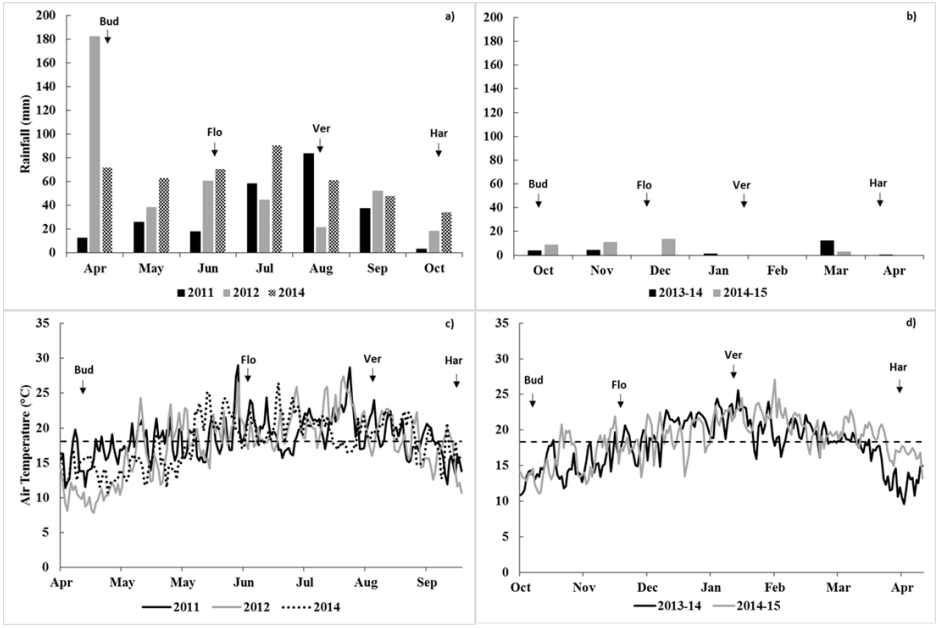

Figure 1. Monthly mean rainfall (mm) in France (a) and Chile (b) and mean air temperature (°C) in France (c) and Chile (d) during all seasons. The horizontal dotted lines in (c) and (d) represent the mean air temperature (°C) in each season. Bud = Budbreak; Flo = Flowering; Ver = Veraison; Har = Harvest.

Disease incidence and severity under field conditions

Experiments in France

In the "Tour Blanche" site for the different *Vitis vinifera* cultivars evaluated, the mean values of disease incidence and severity for the three studied years fluctuated from 0 to 98% and from 0 to 66%, respectively (Table 4). In contrast, for disease incidence, in 2011, the cultivars Riesling, Semillon, Muscat Petit Grain, Chenin, Folle Blanche, Roussanne and Negrette showed the highest values (> 83%). In contrast, Gros Manseng, Petit Verdot, Petit Manseng and Cabernet Franc showed the lowest values (< 16%). In 2012, the cultivars Sauvignon Blanc, Chardonnay, Folle Blanche, Riesling, Muscadelle, Muscat Petit Grain, Grenache Blanc and Semillon showed the greatest incidence values (> 84%). However, Grenache Noir, Carignan, Tannat, Cabernet Sauvignon, Petit Verdot, Merlot, Cabernet Franc and Petit Manseng showed the lowest values (< 18%). In 2014, the cultivars Semillon, Folle Blanche and Pinot Noir showed the highest incidence values (> 74%), whereas Cabernet Franc, Syrah, Grenache Noir, Gros Manseng and Petit Manseng showed the lowest values (< 14%).

In contrast, for disease severity, in 2011, Riesling showed the highest value (66%), followed by Semillon and Chenin (39%), consistent with the incidence levels. Moreover, the cultivars Gros Manseng, Petit Manseng, Cabernet Franc, Colombard, Cabernet Sauvignon, Tannat, Merlot and Petit Verdot showed the lowest severity values (< 1.3%). In 2012, Riesling again was the most rotted cv, with a severity value reaching 47%, followed by Folle Blanche and Sauvignon Blanc (approximately 31%). Grenache Noir, Petit Verdot, Gros Manseng, Carignan, Cabernet Sauvignon, Petit Manseng, Cabernet Franc, Rolle, Tannat, Mourvèdre, Colombard, Ugni Blanc and Merlot were the least attacked, showing the lowest severity values (< 1.2%). In 2014, Folle Blanche showed the highest disease severity (30%), followed by Pinot Noir (22%). Gros Manseng, Petit Manseng, Cabernet Franc, Grenache Noir, Petit Verdot, Tannat, Cabernet Sauvignon, Carignan, Mourvèdre and Alicante Bouchet showed the lowest severity values (< 1.2%).

In the "Grande Ferrade" site, mean incidence and severity values, for the studied season, fluctuated from 65 to 100% and from 5 to 51%, respectively (Table 5). The cultivars Cabernet Franc, Cot, Muscadelle, Petit Verdot, Roussanne, Sauvignon Blanc, Semillon, Tempranillo and Touriga Nacional showed the highest disease incidence, greater than 98%. However,

Mourvèdre showed the lowest value (65%). The cultivar Roussanne showed the highest disease severity value (51%), whereas the cultivars Marselan and Mourvèdre showed the lowest values (< 8%).

Table 4. Mean disease incidence and severity values (%) for each cultivar under field conditions in the "Tour Blanche" site (France) over three seasons.

Cultivar	Disease incidence (%)			Disease severity (%)		
	2011	2012	2014	2011	2012	2014
Alicante Bouchet	30.7cdef	35.8bcd	33.4bcdefg	3.3ab	3.1abc	1.2a
Cabernet Franc	13.2abc	16.4ab	7.1a	0.4a	0.4a	0.2a
Cabernet Sauv.	27.6bcde	10.7ab	26.6abcde	0.9a	0.3a	0.5a
Carignan	37.9def	10.5ab	25.8abcde	1.9ab	0.3a	0.6a
Chardonnay	79.5klmn	93.1j	51.9fghijk	11.5abcde	26.4fgh	10.2defg
Chenin	94.5mn	49.4cdef	37.0cdefgh	39.0i	7.4abcd	1.7ab
Cinsault	54.9fghijk	29.8abc	55.6ghijk	5.9abcd	2.3ab	3.2abc
Colombard	18.8abcd	29.2abc	36.4cdefgh	0.7a	1.0a	1.3ab
Cot	41.2defg	46.3cde	40.5defghi	4.7abc	2.5ab	2.0abc
Folle Blanche	89.2lmn	92.8j	81.8lm	29.3ghi	32.6h	29.7i
Gamay	51.0efghi	25.6abc	51.7fghijk	13.7bcdef	3.9abc	11.1efg
Gewürztraminer	64.8ghijkl	63.5efghi	68.4jklm	19.3efg	23.3efgh	11.7fg
Grenache Blanc	65.8hijkl	86.0ij	33.4bcdefg	17.1def	17.1def	2.9abc
Grenache Noir	34.9cdef	5.6a	11.8ab	4.0abc	0.2a	0.2a
Gros Manseng	0a	15.1ab	12.4ab	0a	0.3a	0.1a
Melon	42.9defgh	73.7fghij	67.6jklm	4.5abc	10.3abcd	14.5g
Merlot	33.1cdef	15.6ab	51.3fghijk	1.2a	1.2a	3.3abcd
Mourvèdre	21.8abcd	22.5abc	26.4abcde	1.8ab	0.9a	0.7a
Muscadelle	75.9jklmn	88.2ij	51.4fghijk	17.7def	14.4bcdef	5.3abcdef
Muscat petit grain	97.2n	86.7ij	46.9efghij	29.8ghi	12.0abcde	4.4abcde
Negrette	83.8lmn	57.3defg	58.1hijk	24.4fgh	8.6abcd	7.0abcdef
Petit Manseng	12.6abc	18.1ab	13.6abc	0.3a	0.3a	0.2a
Petit Verdot	3.3ab	13.4ab	22.3abcd	1.3a	0.2a	0.4a
Pinot Noir	77.8jklmn	70.2efghij	74.0klm	32.7hi	15.6cdef	21.7h
Riesling	97.7n	91.2j	61.5ijkl	65.7j	47.1i	5.1abcdef
Rolle	48.5efghi	24.3abc	31.0bcdef	3.3ab	0.9a	2.7abc
Roussanne	88.6lmn	63.2efghi	43.1defghi	31.2ghi	7.3abcd	2.1abc
Sauvignon Blanc	71.3ijklm	96.2j	61.8ijkl	15.3cdef	30.6gh	8.3bcdefg
Semillon	96.2n	84.6hij	86.7m	39.2i	19.3defg	11.6fg
Syrah	37.0cdef	58.0defgh	11.5ab	2.6ab	11.8abcde	1.4ab
Tannat	22.4abcd	10.5ab	24.2abcde	1.1a	0.9a	0.4a
Ugni Blanc	43.3defgh	32.5abcd	56.1ghijk	2.8ab	1.1a	1.9abc
Viogner	53.5fghij	80.4ghij	53.6fghijk	8.4abcde	13.2abcde	8.7cdefg

Table 5. Mean disease incidence and severity values (%) for each cultivar under field conditions in the "Grande Ferrade" site (France) in the 2011 season.

Cultivar	Disease incidence (%)	Disease severity (%)
Cabernet Franc	100.0e	36.8efg
Cabernet Sauvignon	83.3bc	15.8abc
Carignan	96.3de	25.9bcde
Chardonnay	92.4cde	39.5efg
Chenin	96.4de	33.9def
Cot	100.0e	37.1efg
Gamay	93.7cde	28.8cde
Grenache Noir	91.7cde	10.1ab
Marselan	71.3ab	7.3a
Merlot	97.7de	28.6cde
Mourvèdre	65.0a	5.1a
Muscadelle	100.0e	47.7fg
Petit Verdot	98.8e	34.6def
Pinot Noir	85.4cd	18.9abcd
Riesling	95.9cde	26.0bcde
Roussanne	98.6e	51.2g
Sauvignon Blanc	98.8e	40.5efg
Semillon	100.0e	30.3cde
Tempranillo	100.0e	48.0fg
Touriga Nacional	98.8e	33.8def
Ugni Blanc	93.8cde	14.8abc
Viogner	97.5de	42.1efg

Experiments in Chile

The *V. vinifera* cultivars evaluated showed disease incidence and severity values lower than in France in both years (Table 6). The cultivars Cabernet Franc, Cabernet Sauvignon, Cot, Merlot, Mourvèdre and Petit Verdot did not develop any BBR symptom in any year, even when the vines were sprayed with water in the 2014-15 season in Chile. Thus, these cultivars are considered not susceptible to the pathogen under Chilean conditions. In addition to these cultivars, Carménère, Grenache, Syrah and Tempranillo were not affected by the disease in 2013-14. In this season, the cultivars Gewürztraminer and Sauvignon Blanc showed the highest incidence values, reaching 5 and 8%, respectively. In 2014-15, the cultivars Sauvignon Gris, Sauvignon Blanc and Gewürztraminer exhibited the greatest incidence, with values fluctuating between 12 to 38%.

Regarding the disease severity, in 2013-14, the cultivars Gewürztraminer and Sauvignon Blanc showed the highest values (approximately 0.2%), followed by Pinot Gris (0.12%). In 2014-15, the cultivar Sauvignon Gris exhibited the highest disease severity (9.8%), followed by Sauvignon Blanc and Gewürztraminer, with 3.9 and 2.3%, respectively.

Table 6. Mean disease incidence and severity values (%) for each cultivar under field conditions in Chile over two seasons.

Cultivar	Disease incidence (%)		Disease severity (%)	
	2013-14	2014-15	2013-14	2014-15
Cabernet Franc	0a	0a	0a	0a
Cabernet Sauvignon	0a	0a	0a	0a
Carménère	0a	0.3a*	0a	0a
Chardonnay	1.07a	2.7ab	0.05ab	0.30a
Cot	0a	0a	0a	0a
Gewürztraminer	8.11c	12.0cd	0.24d	2.25ab
Grenache Noir	0a	0.25a*	0a	0a
Marsanne	0.01a	0.18a*	0.01ab	0a
Merlot	0a	0a	0a	0a
Mourvedre	0a	0a	0a	0a
Petit Verdot	0a	0a	0a	0a
Pinot Gris	2.33ab	9.75bcd	0.12bc	0.78a
Pinot Noir	0.72a	3.93ab	0.06ab	0.30a
Roussanne	0.47a	0.98a	0.03ab	0.23a
Sangiovese	0a	6.05abc	0a	0.8a
Sauvignon Blanc	4.72bc	16.88d	0.19cd	3.85b
Sauvignon Gris	1.28a	37.7e	0.048ab	9.80c
Syrah	0a	0.25a	0a	0.03a
Tempranillo	0a	2.53ab	0a	0.10a

*When there is a value for the incidence but the severity is 0, it is because the severity value is less than 0.001.

Classification of cultivars according to the disease severity

Situation in France

In the “Tour Blanche”, the cluster analysis classified the cultivars tested into 7 groups according to the disease severity (Figure 2a). The groups obtained were classified as follows: resistant-intermediate "R-I" (group 1), susceptible "S" (groups 2 to 4) and highly susceptible "HS" (groups 5-7) cultivars. The first group comprised 17 cultivars (Alicante Bouschet to Syrah) that showed a mean severity value of 1.6% for all of the three seasons. The disease

severity for these cultivars was stable between seasons, i.e., the mean severity fluctuated from 0.1 to 5.3% through the 3 years. The second group from the cluster analysis included 3 cultivars (Gamay to Viogner) presenting a mean severity value of 9.8%. The third group was composed of 6 cultivars (Chenin to Negrette) presenting a mean severity value of 13.8% for the three seasons. The severity values for these cultivars were similar in 2011 and 2012 but lower in 2014. The fourth group, with a mean severity value of 17.4%, included 3 cultivars (Chardonnay through Gewürztraminer). The fifth group comprised 2 cultivars (Pinot Noir and Semillon), which showed a mean severity value of 23.3%. Finally, the cultivars Folle Blanche and Riesling were classified in the sixth and seventh categories showing mean severity values of 30.7 and 39.3%, respectively. A particular case was the cultivar Riesling, which was classified in the most susceptible category and presented a very high severity for the 2011 and 2012 seasons but a relatively low severity value in 2014.

Furthermore, a classification was established based on all the databases from France. A cluster analysis was performed with the common cultivars present in La Tour Blanche and Grande Ferrade sites. The groups obtained in this analysis were classified as follows: resistant-intermediate (group 1), susceptible (group 2) and highly susceptible (groups 3 and 4) cultivars (Figure 2b). The first group was composed of 9 cultivars (Cabernet Franc through Mourvèdre), with a mean severity value of 6.8%. The disease severity for these cultivars was similar in the "Tour Blanche" site during all the three seasons but higher at the "Grande Ferrade" site. The second group included 8 cultivars (Chardonnay through Roussanne), which were characterized by a mean disease severity value of 21%. Similarly, the severity results were higher at "Grande Ferrade". Finally, the cultivars Pinot Noir and Riesling were classified in the third and fourth categories, showing mean severity values of 22.2 and 36%, respectively.

Situation in Chile

In Chile, the cultivars were grouped into 6 groups (Figure 3) according to disease severity. The groups obtained were classified as follows: resistant-intermediate (group 1), susceptible (groups 2 to 5) and highly susceptible (group 6) cultivars. The first group was composed of 12 cultivars (Cabernet Franc through Sangiovese). Within this group, 6 cultivars did not present any rot symptom in any season. However, the other cultivars showed a very low mean

severity value of 0.1%. The second group comprised 3 cultivars (Chardonnay through Roussanne) that presented a mean rot severity value of 0.2%. The cultivars Pinot Gris and Gewürztraminer were classified in the third and fourth groups with mean disease severity values of 0.4 and 1.3%, respectively. Finally, the cultivars Sauvignon Blanc and Sauvignon Gris were ranked in the fifth and sixth groups with mean severity values of 2.0 and 4.9%, respectively.

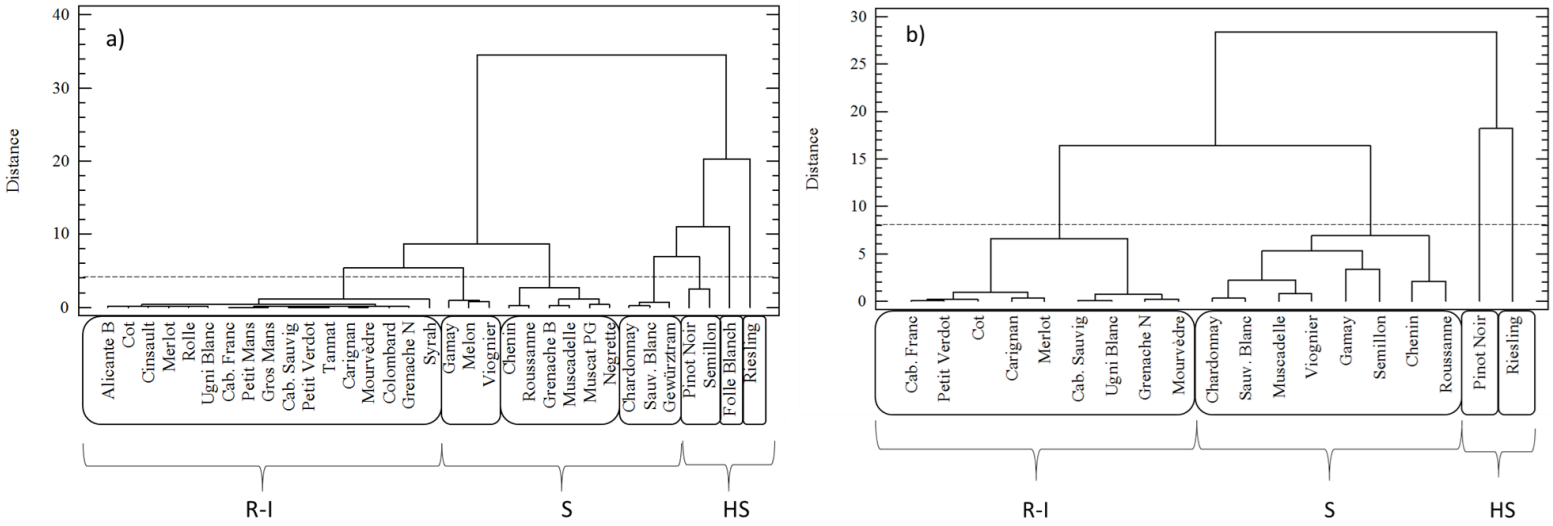

Figure 2. Cluster classification of cultivars in France in the sites "Tour Blanche" (a) and both "Grande Ferrade and "Tour Blanche" (b) according to their severity values.

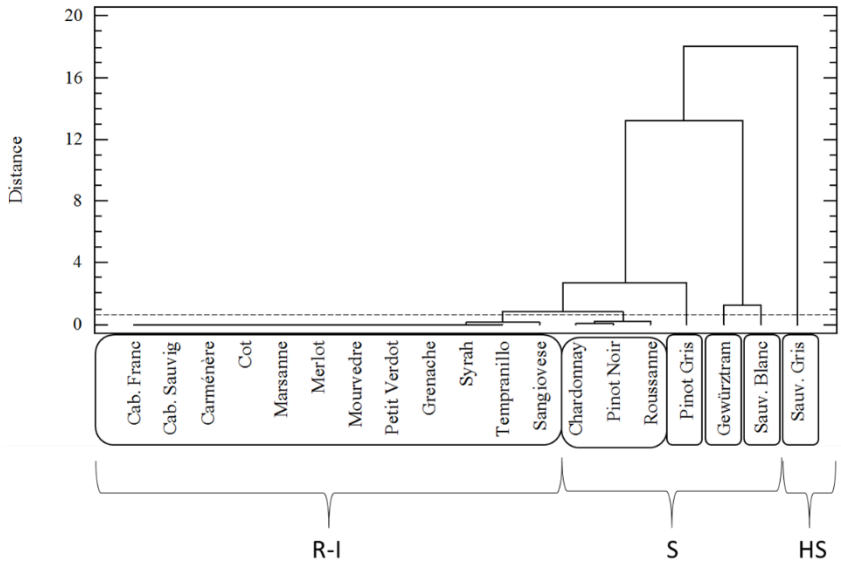

Figure 3. Cluster classification of cultivars in Chile according to their severity values.

Classification of common cultivars in Chile and France according to the susceptibility index

According to the susceptibility index (SI), we classified the common cultivars evaluated in Chile and France in 5 categories: i) highly resistant (HR), ii) resistant (R), iii) intermediate (I), iv) susceptible (S) and v) highly susceptible (HS) cultivars (Figure 4). Five cultivars – Grenache Noir, Cabernet Franc, Petit Verdot, Cabernet Sauvignon and Mourvèdre – were highly resistant (SI ≤ 3.5). Three cultivars were included in the resistant category (Merlot, Syrah and Cot). Only Roussanne was classified as an intermediate cultivar. Finally, the cultivars Chardonnay and Pinot Noir were identified as susceptible, whereas Gewürztraminer and Sauvignon Blanc were highly susceptible (SI > 50). This classification was corroborated with a non-parametric statistical analysis. This analysis demonstrated that the cultivars classified as HR and HS were stable between seasons and sites, in contrast with the R, I and S cultivars, which showed significant variability.

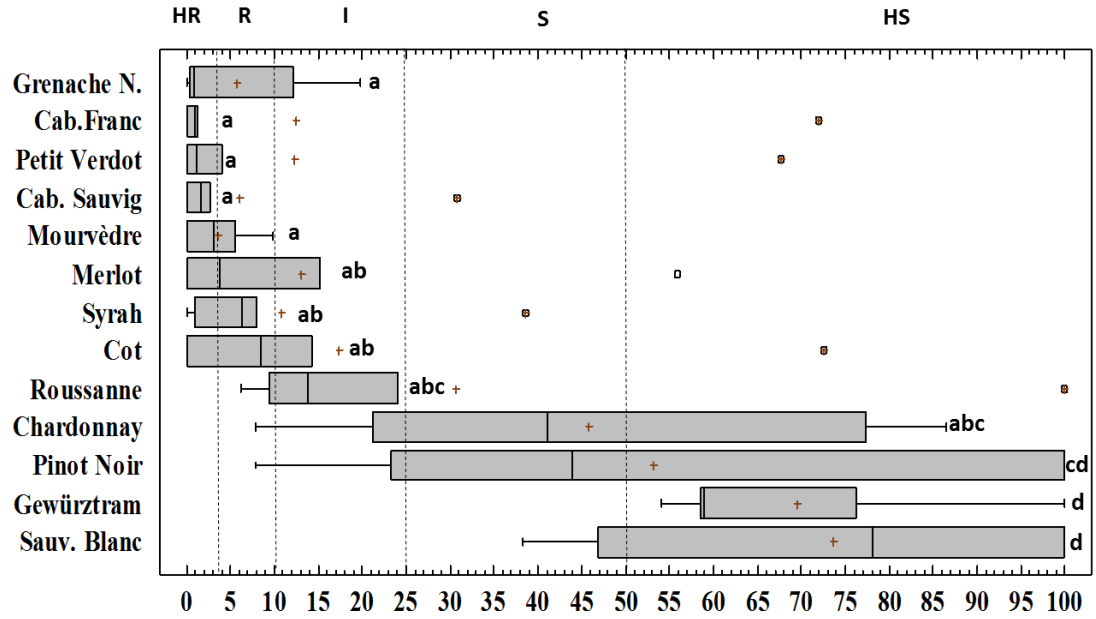

Figure 4. Box plot of cultivars according to the susceptibility index. HR = Highly Resistant; R = Resistant; I = Intermediate; S = Susceptible; HS = Highly Susceptible. The vertical line in each box and the cross represent the median and mean value of the SI, respectively.

Relationship between the cultivar susceptibility ranking and fruit maturity

An exponential relationship between the susceptibility to the pathogen, as indicated by the SI value, and the fruit maturity (F_{Mat}) of cultivars studied in France and Chile was observed (Figures 5 and 6). For every combination "country x season" (experimental condition), the relationship between the two variables was positive, thus showing clearly that the cultivars with more mature berries were the most susceptible. This pattern was very similar in all experimental conditions, but it was noticeable that the F_{Mat} values differed to a large extent from one experimental condition (combination "country x season") to the next (Figure 5).

To prevent the effect of the different dates of assessment depending on the season, the F_{Mat} was adjusted (F_{Mat_adj}) in both countries by removing the value of F_{Mat} of the latest cultivar among the 13 cultivars studied. The relationship between F_{Mat_adj} and the SI value was positive and exponential in both countries (Figure 6). In France ($r^2 = 0.73$), the equation was $y = 3.2\ E\text{-}4 * x^{2.1}$ (Figure 6a), whereas in Chile ($r^2 = 0.55$), it was $y = 4.6E\text{-}11 * x^{4.78}$ (Figure 6b), with “y” representing the SI value and "x" the F_{Mat_adj} value. This pattern was quite similar in both sites, but with a steeper slope in Chile. Note that a change in cultivar susceptibility occurred for adjusted F-Maturity values of greater than approximately 250. In France, for higher F_{Mat_adj} values, the cultivars were classified as susceptible with an SI value higher than 25 (Figure 6a). In Chile, the cultivars with $F_{Mat\_adj} > 250$ corresponded to those developing disease symptoms to some degree, whereas below this value, mostly no disease or very few rot symptoms were recorded (Figure 6b). The Roussanne cultivar was the exception in both sites, presenting a higher disease susceptibility in the 2012 and 2013-14 seasons, despite its low maturity (Figure 6a, b).

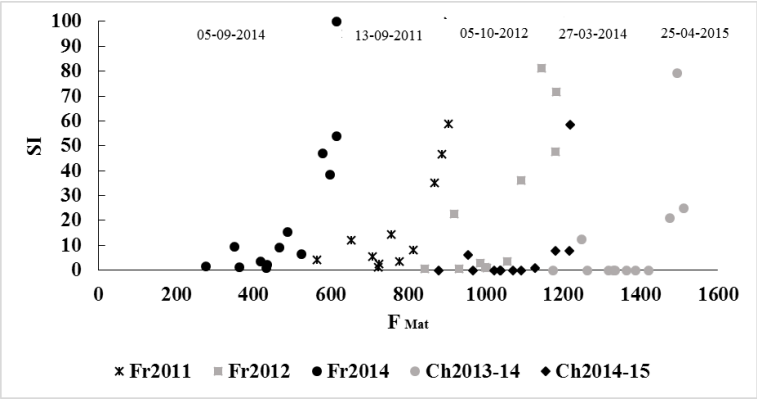

Figure 5. Relationship between the maturity of cultivars (F Mat) and susceptibility to BBR (SI), assessed at different dates, in France and Chile.

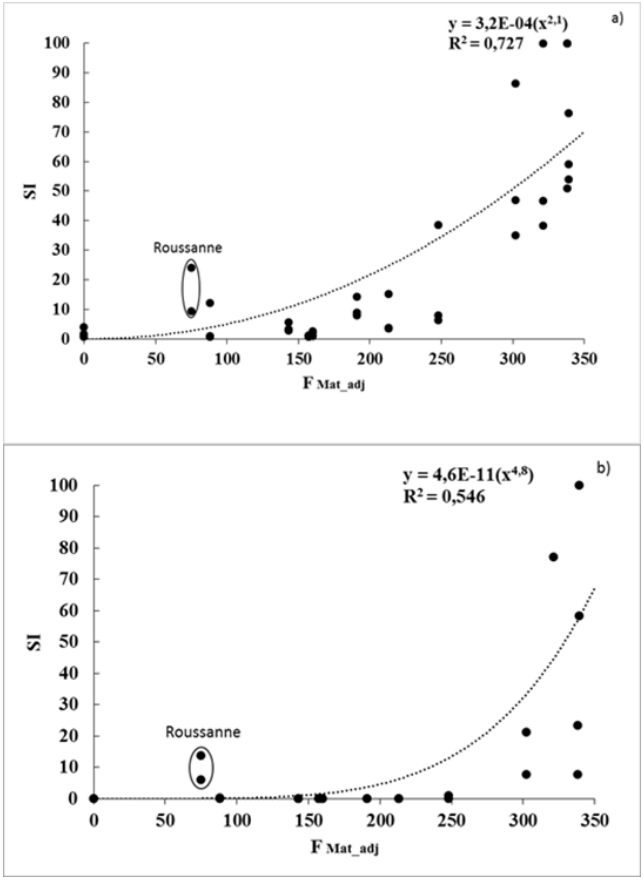

Figure 6. Relationship between the maturity of cultivars (F Mat_adj) and susceptibility to BBR (SI) at both sites, France (a) and Chile (b), during all study seasons.

Discussion

Cultivar classification according to disease susceptibility

The results of this study showed that the cultivar classification according to the susceptibility to *B. cinerea* was generally similar in the two countries, despite the contrasting climatic conditions and cropping practices. Thus, on the one hand, the two *V. vinifera* white cultivars Sauvignon Blanc and Gewürztraminer were classified as the highest-susceptibility cultivars, followed by Chardonnay and Pinot Noir. On the other hand, the four wine black cultivars – Petit Verdot, Cabernet Sauvignon, Mourvèdre and Syrah – were identified as resistant or highly resistant. These classification features confirm various previously published findings (Orffer 1979, Brocuher-ACTA-ITV 1980, Robinson 1986, Jakcson and Schuster 1987, Galet 1988, Dry and Gregory 1990, Marois et al. 1992, Dubos 2002) (Table 7). However, for the

other cultivars tested, our results differ greatly from those published in the literature. We have classified the two black cultivars, Grenache Noir and Cabernet Franc, as highly resistant, yet they were considered as susceptible or highly susceptible by other authors (Robinson 1986, Galet 1988, Dry and Gregory 1990, Dubos 2002). Similarly, both the Merlot and Cot cultivars, which were identified as resistant in this study, appear in the literature as susceptible cultivars. Finally, we classified Roussanne as a cultivar intermediate in susceptibility, whereas it had been identified previously as a highly susceptible cultivar (Table 6).

These differences observed between our results and those from the literature could be accounted for by possible changes in agronomic conditions that could affect the plant, the pathogen, the environment and/or the interactions between these epidemiological factors. Diverse studies have demonstrated the relationship between *B. cinerea* infection and/or BBR development and various environmental/agronomic factors, such as the following: first, climate and microclimate within the canopy (Savage and Sall 1984, Thomas et al. 1988, English et al. 1989, Fermaud et al. 2001, Pieri and Fermaud 2005, Valdés-Gómez et al. 2008, Ciliberti 2015, 2016); second, canopy density and leaf removal after flowering (Gubler et al. 1987, English et al. 1989, Zoecklein et al. 1992, Valdés-Gómez et al. 2008, Molitor et al. 2011); third, cluster compactness and thinning (Barbetti 1980, Marois et al. 1986, Vail and Marois 1991, Percival et al. 1994, Ferree et al. 2003, Hed et al. 2009, Molitor et al. 2011); fourth, mineral and water nutrition (Mundy 2007, Valdés-Gómez et al. 2008); fifth, grape training systems (Pereira de Bem et al. 2015); sixth, winter pruning (Savage and Sall 1984); seventh, cracks caused by biotic (insects, birds, snails, other plant pathogens) and abiotic (rain, hail, frost, sunburn, rapid water intake) factors (Nair et al. 1988, Fermaud and Le Menn 1989, Coertze and Holz 1999, Becker and Knoche 2012a, b); and eighth, clone and rootstock (Bernard and Leguay 1988, Vail and Marois 1991, Derckel et al.1998, Vail et al. 1998).

An important source of variation may be the clone effect, which may cause important susceptibility differences within one considered cultivar. From this point of view, Pinot Noir is a model cultivar of interest. Significant differences in susceptibility to *B. cinerea* between Pinot Noir clones have been attributed to variations in cluster compactness (Bernard and Leguay 1988). Additionally, Derckel et al. (1998) also detected differences in susceptibility to *B. cinerea* amongst the four Pinot Noir clones, suggesting that some grape berry defences

may play an important role in this interaction. Similarly, within the Chardonnay cultivar, variability in the susceptibility of different clones to *B. cinerea* has also been shown, although the variability attributable to the clone may be considered lower than the variability explained by the cultivars (Vail and Marois 1991, Vail et al. 1998).

The rootstock may also play an important role in the observed variability in the susceptibility to the pathogen among and within cultivars. For example, the SO4 rootstock induces higher disease infection in Pinot Noir cultivar because it promotes vine vigour, which is conducive to the disease (Dubos 2002). Additionally, the rootstock, by affecting depth of the root system and vine vigour, can influence significantly the cluster compactness, berry size and fruit maturity, which are known factors that modify the susceptibility to *B. cinerea* (Cordeau 1998).

As a first conclusion, despite all the variations and differences possibly due to agronomic factors, the cultivar effect *per se* seems to be the most important for the extreme susceptibility groups of cultivars (highly resistant and susceptible), as defined and demonstrated in the present work.

Table 7. Comparison of the susceptibility to *B. cinerea* of 13 grapevine cultivars according sources and our results.

Cultivar	Mean lit.	Sd lit.	Our res.	Sd res.
Grenache Noir	4	0.5	0	1.0
Cabernet Franc	3	1.5	0	1.6
Petit Verdot	1	0.3	0	1.6
Cabernet Sauvignon	1	0.7	0	1.2
Mourvèdre	1	-	0	0.5
Merlot	3	0	1	1.5
Syrah	2	0.8	1	1.2
Cot	3	0	1	1.5
Roussanne	4	0	2	1.2
Chardonnay	3	0.8	3	1.2
Pinot Noir	3	0.8	3	1.3
Gewürztraminer	3	1.7	4	0
Sauvignon Blanc	3	1.2	4	0.5

0 = highly resistant, 1 = resistant, 2 = intermediate, 3 = susceptible, 4 = highly susceptible; Mean lit = Mean of literature source, Our res = Results of our study; Sdlit = standard deviation of literature sources, Sd res = standard deviation of our results.

Stability of cultivar classification between years, sites and literature

Our results suggest that the susceptibility of some cultivars is not stable and changes depending on environmental, seasonal or management conditions. To compare the differences in susceptibility and to know the stability of the cultivar classification, we calculated the standard deviation corresponding to the literature results (Sdlit) and that from our experimental data (Sdres) (Table 6). The susceptibility classification of Cabernet Franc cultivar was not stable, neither in the literature nor in our study (Sdlit = 1.5; Sdres =1.6). This could be due to the use of different clones because a great variability among Cabernet Franc clones has been demonstrated to be related to key susceptibility factors, notably, maturity, berry size, yield and tannin content (Van Leeuwen et al. 2013). However, in our case, this difference appears to be due to the vegetative growth because this cultivar was classified differently only at the "Grande Ferrade" site, at which the vigour was higher. For the other cultivars, Petit Verdot and Grenache Noir, their susceptibility rank was rather stable in the literature (Sdlit = 0.3 and 0.5), but it differed according to the season and country in our work (Sdres = 1.6 and 1.0). For the cultivars Merlot, Cot and Roussanne, the classification was the same in all other works (Sdlit = 0), but it differed significantly under our conditions (Sdres = 1.5 and 1.2). Interestingly, the four cultivars Grenache Noir, Petit Verdot, Merlot and Cot are susceptible to flower abortion (Reynier 2011); consequently, they may present very different cluster compactness depending on seasonal climatic conditions during bloom, leading to more or less flower abortion (Keller 2015). Such a difference in compactness should account for great variations in the susceptibility to *B. cinerea*, as has been often demonstrated in the literature (Marois et al. 1986, Vail and Marois 1991, Percival et al. 1994, Ferree et al. 2003, Hed et al. 2009, Molitor et al. 2011). Regarding the susceptibility classification, the cultivars Grenache Noir, Cabernet Franc, Merlot, Cot and Roussanne showed significant differences between literature works and our study (Table 6). To understand this difference, further studies about the clone and the vegetative growth related to the rootstock are necessary.

It is important to note the effect of Chilean data, which decrease the average of the Susceptibility Index (SI) in the cultivars classification due to the existence of climatic conditions unfavourable to disease development. Even if the grapevines were water sprayed in Chile, this effect was temporary and did not allow the pathogen to develop to a large extent, as may occur under natural wet conditions such as e.g., under oceanic conditions. Finally, it

may be discussed whether these results could have been affected by the phenotypic variability among *B. cinerea* strains, particularly in terms of difference in virulence. It has been demonstrated that the virulence of the two *B. cinerea* genetic types, *vacuma* and *transposa*, differed significantly in terms of disease incidence and severity, with *transposa* strains being more virulent than *vacuma* ones. This virulence on leaves or on berries was significantly and negatively correlated with the mycelial growth rate (Martínez et al. 2005). Moreover, the mechanism involved in this pathogenicity could be explained by the presence of transposable elements, which is a characteristic feature of *transposa* isolates. Thus, Baulcombe (2013) explained that transposon small RNA (sRNA) molecules are associated with the suppression of host defences, which may have important implications for the pathogen arms race. This idea is supported by Weiberg et al. (2013), who founded that transposon sRNA molecules derived from *B. cinerea* can act as effectors to suppress host immunity and play a positive role in pathogenicity. Thus, although we did not consider the high phenotypic variability in this study, it has been demonstrated that the two major sympatric transposon genotypes (*transposa* and *vacuma*) are present similarly in Chile as in France (Martinez et al. 2003, 2008). They also tend to have similar characteristics in both countries (Muñoz et al. 2002); consequently, this variability should not affect the results to a great extent.

Effect of grape maturity on disease susceptibility

The fruit maturity was identified as a major factor determining the cultivar susceptibility to *B. cinerea*. Several studies, often based on one selected model cultivar, have demonstrated that increasing sugar concentration with the phenological stage in maturing grape berries promotes infection and colonization by *B. cinerea*. Some of these studies also demonstrated that the presence of sugar in berry exudates stimulates the germination and mycelium growth of *B. cinerea* (Kosuge and Hewitt 1964, Blakeman 1975, Coley-Smith et al. 1980, Doneche 1986, Padgett and Morrison 1990, Vercesi et al. 1997, Deytieux et al. 2009). Despite several authors having demonstrated the relationship between sugar concentration and pathogen infection, few works have revealed a correlation between increasing maturity and progress of disease severity, and they mostly used a single cultivar (Fermaud et al 2011), not a set of different cultivars. Studies related to the infection by the pathogen and the solid soluble

contents of grapes have been conducted, in particular by Mundy and Beresford (2007), who established clearly a significant and positive linear regression between berry sugar concentration and the percentage of rotted berries. Furthermore, regarding the maturity effect, the susceptibility of berries increased during ripening (Kretschmer et al. 2007), and, more precisely, a positive, close and sigmoid relationship between maturity variables and *B. cinerea* susceptibility was established by Deytieux-Belleau et al. (2009). This last study demonstrated that severity of *B. cinerea* increases regularly during berry maturity, reaching a maximum at the over-maturity stage: then, this relationship can be represented by a sigmoid curve. In our study, these relationships were exponential, showing that the most mature grapevine cultivars were the most susceptible to the pathogen. These cultivars were mostly white cultivars, in which the sugar content is, generally, higher than in black ones (Doneche 1986). If we had measured the disease severity of cultivars in a more advanced state of maturity, these results may have been similar. Moreover, the most mature cultivars correspond to the earliest cultivars. They could also have been more attacked because they were exposed, in a susceptible, mature stage, for a longer time under favourable conditions for infection and disease development.

In addition to the maturity, other factors may account for the variability in susceptibility. For example, the less-susceptible cultivars, according to the disease incidence and severity, were in both countries black cultivars. In contrast, the most susceptible cultivars were white and pink ones. This relationship between susceptibility and berry colour was expected because it has been shown that the susceptibility of grapes may be affected by the concentration of phenolic compounds in grapes (Frankel et al. 1995, Goldberg et al. 1995), and particularly, the tannin content within the berry skin (Deytieux-Belleau et al. 2009). These results confirmed previous studies (Goetz et al. 1999, Xie and Dixon 2005) that demonstrated that black cultivars are less susceptible to *B. cinerea* than white or pink cultivars. In addition, the compactness of clusters has been shown to be an important morphological feature that affects the susceptibility to *B. cinerea* by affecting the microclimate and the thickness and wax content of the berry cuticle (Marois et al. 1986, Vail and Marois 1991, Percival et al. 1993, Fermaud et al. 2001). In this study, we observed a clear trend in the vineyard conditions that the cvs with more compact clusters were more severely attacked and more susceptible to the pathogen. In contrast, we noted that the less-attacked cvs presented looser clusters and were

classified as less susceptible to *B. cinerea*. This corroborates a previous study that showed a positive correlation between BBR development and cluster compactness (Hed et al. 2009). Lastly, and in addition to the fruit maturity, berry skin colour and cluster compactness, which also may affect the susceptibility to BBR, there are other predisposal factors, such as genetic (morphological, anatomical and chemical features of the berry skin), physical (wounds), environmental (climate and weather conditions) and agronomic (cultural practices) (Latorre et al. 2015). For agronomic factors, after the climate influence, vegetative growth and canopy development are considered the second most important factors favouring *B. cinerea* development (Valdés-Gómez et al. 2008). Then, some morphological factors related to cluster architecture, e.g., the bunch mass and berry number, also have an important influence on BBR epidemics (Vail and Marois 1991, Valdés-Gómez et al. 2008). The bunch mass has been positively and significantly correlated with the BBR incidence and considered more relevant than the yield to account for disease development. This factor contributes largely to cluster compactness; thus, it can be considered as a key morphological feature that increases *B. cinerea* susceptibility (Valdés-Gómez et al. 2008). Although in this work we did not consider any of these factors, they should be further studied in future works addressing cv susceptibility to the pathogen.

Main findings and implications for IPM and climatic change adaptations

As previously reported, our results also confirmed that environmental conditions are a main factor in the disease epidemiological development (Savage and Sall 1984, Thomas et al. 1988, English et al. 1989, Fermaud et al. 2001, Valdés-Gómez et al. 2008, Ciliberti 2015, 2016). The contrasting climatic conditions in the two regions studied led to different levels of disease infection, due principally very different amounts and distributions of rainfall. Rainfall, which is predominantly at the origin of increased relative humidity and wetness duration in the vineyards, was found to be of primary importance in disease development (Ciliberti 2015, 2016). Thus, in France, all cultivars were attacked by *B. cinerea*, and they presented more advanced disease development than in Chile. Although under Chilean conditions, no cultivars seemed to be very susceptible, considering the low disease severity values, it was possible to classify them according to their susceptibility. This classification was similar to that in France, thus demonstrating that climate does not change the

susceptibility of cultivars. However, when the climatic conditions are not favourable to the pathogen development, it is difficult to differentiate resistant from intermediate cultivars because the latter do not develop the disease at all. This situation was observed, in particular, in grapes that were not sprayed with water in Chile (data no shown). Thus, the decision to apply a fungicide to these cultivars based on their susceptibility classification to BBR would be more difficult. Furthermore, it is interesting to note that future climatic conditions in the Bordeaux region could be relatively similar to the current climatic conditions characterizing the Chilean region considered in the present study (Pañitrur-De la Fuente et al. 2016). Under this context of climate change, strategies may be orientated by adapting the cultivar choice to future possible climatic scenarios, considering both the potential disease development and the associated cultivar susceptibility.

Further investigation should be conducted to better understand the relationships between the classification of cultivars according to their susceptibility to *B. cinerea* and other variables (e.g., clone, vigour, and rootstock) to develop management and integrated pest management strategies.

Conclusions

The results of this study demonstrated that the classification of different wine cultivars according to their susceptibility to *B. cinerea* was generally similar in both countries, despite the contrasting climatic conditions and management practices. Sauvignon Blanc and Gewürztraminer were the most-susceptible cultivars, whereas Petit Verdot, Cabernet Sauvignon, Mourvèdre and Syrah were rather resistant or highly resistant. These results are in accordance to previous studies; however, for the other cvs that we evaluated, their ranking differed to some extent compared with data from the literature. This difference is presumably caused by variations in the agronomic and/or environmental conditions under which the field experiments were performed. The interfering effects of various factors, such as clone, rootstock, and cluster compactness related to flower abortion are discussed in detail and should be considered in further studies aiming to compare cultivar susceptibility to the pathogen.

The maturity of cultivars seems to be a major determining factor in the susceptibility to *B. cinerea*. In our study, the relationship between fruit maturity and susceptibility to the pathogen was positive and exponential, indicating that the most mature grapevine cultivars were the most susceptible. This could be explained by the increasing sugar concentrations in ripening berries, which promote fungal colonization, and by the longer time during which later grapevine cultivars are exposed to favourable conditions for disease development.

The cultivar is a principal and permanent factor affecting the susceptibility to *B. cinerea*, which could be modified by climate and agronomic management, which are considered as variable factors. Thus, the cultivar remains a key parameter in decision support systems, and the fruit maturity could be used to support this. Further investigation should be conducted to better understand the relationship between susceptibility to *B. cinerea* and other variables (e.g., clone, vigour, and rootstock) to develop management and integrated pest management strategies.

CHAPTER 2

Key early risk indicators of Botrytis Bunch Rot development: Berry skin tannin content and grapevine vigor.

(To be sent to: Phytopathology).

Abstract

Substantial economic losses have been estimated in vineyards worldwide due to Botrytis bunch rot (BBR). The control of this disease is still largely based on the use of periodic synthetic fungicide spraying and thereby, control strategies must be optimized to limit fungicide residues in viticulture. In order to evaluate early plant features related to the potential susceptibility to *B. cinerea* to be used as disease risk indicators, a study was carried out between 2010 and 2016 in France and Chile. In this study, grapevine features related with the susceptibility to BBR, notably pectin and tannin content in berry skin and the vegetative growth, were evaluated early in the season and correlated with the disease development at harvest. Furthermore, regression models including these grapevine features and climatic conditions before harvest were performed to better understand the disease development. The results showed that the tannin content in berry skin and grapevine vegetative growth were significantly correlated with both BBR incidence and severity at harvest, whereas the pectins only showed significant correlations with BBR severity. Lastly, all regression models that explain BBR development were highly significant, suggesting that they could be used as tools to control BBR.

Keywords: *Botrytis cinerea*, Integrated Pest Management (IPM), Decision Support System (DSS), Susceptibility, *Vitis vinifera*.

Introduction

Botrytis cinerea is a polyphagous fungus that infects more than 1400 species of cultivated plants, including grapevines (Elad et al. 2016). This necrotrophic pathogen, responsible for Botrytis bunch rot disease (BBR), is able to infect all the organs of the plant, but mainly damages ripening berries. It can penetrate the tissues either through wounds or directly by the cell wall (Elad and Evensen, 1995). During spring, the dispersal spores of this fungus can infect floral tissues and/or fruit pedicel during the flowering-setting period, considered as a major epidemiological step. After this period, the pathogen rests in a latency state until veraison, at which time the susceptibility of grape clusters to *B. cinerea* increases (Elmer and Michailides 2004). Both vine yield and quality of wine can be reduced due to this fungus (Ky et al. 2012) and therefore, substantial economic losses (~2 billion $US per annum) have been reported in vineyards worldwide (Elmer and Michailides 2004).

The control of BBR is still largely based on the use of periodic synthetic fungicide spraying. However, the restriction of fungicides is becoming more and more necessary in order to reduce the negative effects on human health, the environment (Damalas and Eleftherohorinos 2011; Fenner et al. 2013) and limit residues on harvest (Verger and Boobis 2013). Therefore, strategies to control *B. cinerea* must be optimized notably by considering the main principles of Integrated Pest Management (IPM), according to which disease risk must be assessed before direct control measures are used (IOBC 2007). Disease risk assessment should consider factors that favor the disease development such as climate, crop susceptibility and key grapevine phenological stages.

The climate and microclimate are considered as the main factors involved in *B. cinerea* development (Latorre et al., 2015, Pieri and Fermaud 2005). Specifically, the temperature and relative humidity within the cluster zone are key conducive factors to BBR infections. These factors contribute to the presence of free water at the fruit surface, which is essential for conidial germination and berry infection. Rainfall also contributes to the presence of free water on berries, which is crucial especially during the ripening period, i.e. post-veraison, to facilitate secondary infections via sporulating conidia. (Thomas et al., 1988; Broome et al., 1995; Coertze and Holz, 2002; Latorre et al., 2002; Steel et al., 2011; Ciliberti et al., 2015 a, b; Ciliberti 2016). Thus, considering the prime importance of climatic and microclimatic

conditions for BBR development, different models have been proposed to predict or explain disease infections (Broome et al., 1995; Ciliberti et al., 2015 a, b; Ciliberti 2016).

On the other hand, the grapevine susceptibility to BBR depends on various genetic and phenotypic traits, such as cluster compactness and morphological, anatomical, and chemical features of the berry skin (Latorre et al., 2015, Pieri and Fermaud 2005). The cell walls are among the first plant tissue structures that *B. cinerea* encounters when infecting and colonizing the berry skin and it can contribute to susceptibility as well as resistance to the pathogen (Blanco-Ulate et al. 2016). An important component of berry skin cell walls are pectins, particularly abundant in the middle lamella and the corners of cells (Mohnen 2008). Since pectins are a potential source of nutrients for the pathogen, they appear to be one of the main cell wall targets for the fungus during infection (Blanco-Ulate et al 2014). Then, the growth of *B. cinerea* may be favored greatly by the sugars released from hydrolyzed pectins (Zhang et al. 2013).

In contrast to pectins, other components, including tannins, are deposited in the berry skin cell walls, which provide a protective barrier to the fungus (Amrani Joutei et al., 1994; Lecas and Brillouet, 1994; Schlosser et al., 2008, Deytieux et al. 2009). The tannins are produced at variable concentrations and they are able to inhibit the cell wall-degrading fungal enzymes e.g. lacasse, giving partial resistance to berries against the pathogen (Bachmann and Blaich, 1979; Goetz et al., 1999; Tabacchi, 1994). Taking into account the two berry features mentioned above, the Agricultural Research Institute (INRA) in Bordeaux has calculated a disease risk index by dividing pectin contents by tannin compounds in berry skin to provide information to grapevine growers about the potential risk of BBR development.

Another feature of prime importance is grapevine vigor since high canopy and/or foliar density also favors BBR development (Valdés-Gómez et al. 2008, Latorre et al. 2015). Dense grape canopies are associated with longer periods of wetness within the cluster zone, which predisposes berries to be infected by the fungus, increasing their susceptibility to the disease (Steel 2001). Therefore, prophylactic cultural methods for controlling BBR disease include crop canopy management. In vineyards, for example, an effective practice to reduce BBR development is leaf removal (Gubler et al 1987, Percival et al 1994, Elmer and michailides 2004, Zoecklein et al 1992). This practice aims to limit the vegetative growth around the

cluster zone to improve air circulation and expose fruit to light, reducing the disease infections. Thus, in New Zealand, leaf removal carried out between late flowering and berry pea-size is considered as the most effective cultural management for controlling BBR (Elmer and Wood 2016).

Regarding critical periods of disease development, key stages in disease control in grapevines are at flowering and at bunch closure and, presumably also, in between (Ciliberti 2015a, Zoffoli et al., 2009). Although *B. cinerea* appears and develops mostly late in the growing season, early infections play a key role in disease development (Ciliberti 2015a, Elmer and Michailides, 2004). Then, fungicide applications are used during these periods in order to reduce the early fungal inoculum (Dubos 2002). Despite the importance of these critical stages, few studies have investigated the relationships between the disease development at harvest and grapevine features evaluated at early vine phenological stages (e.g. berry pea-size and pre bunch-closure). Consequently, no forecasting tools and/or risk assessment indices are available to evaluate early grapevine susceptibility to the fungus.

We hypothesized that some specific grapevine features evaluated at early phenological stages, notably pectin and tannin contents in berry skin and vegetative growth, may account for part of the BBR incidence and severity at harvest. Thus, the main objective of this work was to evaluate early plant features related to the potential susceptibility to *B. cinerea* to be used as disease risk indicators. For that, pectin and tannin contents in berry skin as well as the vegetative growth were measured at berry pea-size stage and related with the disease attack at harvest. Lastly, the climatic conditions before harvest were also taken into account in this study in order to better understand the part played by these early disease risk indicators and grapevine features in BBR development during the season.

Materials and methods

Experimental sites.

Experiments were carried out in two experimental vineyards of the cultivar Merlot. The first one was located in the Aquitaine Region (France) in the site “Grande Ferrade” (Villenave d’Ornon 44°47′15.4″N, 0°34′37.43″W, 22 m.a.s.l).The other one was situated in the Maule

Region (Chile) in the site "Panguilemo" (Panguilemo, 35°22.24' S, 71°35.62' W, 125 m.a.s.l), which also included the cultivar Sauvignon Blanc. The general characteristics of both experimental vineyards are summarized in Table 1. The experiments were carried out during seven seasons in France (2010 to 2016) and two seasons in Chile (2014-15, 2015-16).

Table 1. Field characteristics of the experimental fields.

Property	**France**	**Chile**
Experimental Period	2010 to 2016	2014-15 and 2015-16
Vineyard planting year	1991	2006
Rootstock	101-14	Own-rooted
Location (WGS84)	44°47′N, 0°34′ W	35°22' S, 71°36' W
Spacing (m x m)	1.8 x 1.0	2.0 x 1.0
Trellis System	Vertical Shoot Positioning	
Pruning System	Double Guyot	Two-bilateral spur cordon
Irrigation system	Non-irrigated	Drip irrigation (one dropper per plant with a flow rate of 4 L / h)

Experimental design and cropping conditions

In France, six or eight (according to the year) replications were distributed in a randomized design in the grapevine field, each replication consisted in a total of 5 consecutive vines. In Chile, the cultivars (Merlot and Sauvignon blanc) were replicated four times in a randomized block design in order to minimize the effect of soil slope. Each block consisted in a total of 15 adjacent vines. In order to evaluate the grapevine development to *B. cinerea* at harvest, no specific fungicide with known activity against this pathogen was applied in any site nor season. The vineyards were protected against European Grapevine Moth and sulphur sprays were applied to avoid Powdery Mildew. Furthermore, Downy Mildew was controlled only in France with four fungicide applications per season. In Chile, due to the unfavourable conditions for grapevine Downy Mildew, no sprays were applied in any season nor site.

Climatic characterization

Climatic conditions for each studied season were characterized at each site by an automatic weather station (AWS) (Adcon Telemetric, A730, Klosterneuburg, Austria in Chile and Cimel Electronique S.A.S, CimAGRO, Paris in France) installed 50 m from the trial fields. AWS provided data at 15 min intervals on air temperature, relative humidity and

precipitation. Two climate indices were calculated using the pluviometry data in order to estimate favorable conditions for disease development after veraison and to account for differences in BBR development between both countries. For that, the cumulative amounts of rain (mm) from 15 (PL15) and 35 (PL35) days before harvest were calculated in each season and site.

BBR incidence and severity

In all growing seasons BBR incidence and severity were visually evaluated at harvest (about 25° Brix) in approximately 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 on a per replicate basis. 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 tannin compounds were performed to relate berry skin susceptibility to *B. cinerea*. For this, 20 clusters per experimental plot were randomly collected at berry pea-size stage (Eichhorn and Lorenz's growth stage 31) (Eichhorn and Lorenz, 1977) and immediately stored at -20°C. The clusters closest to the trunk and located in the shade in the afternoon were collected from representative standard plants, e.g. normal in vigor, without visible diseases or disorders. Once in the laboratory, berries were peeled to obtain 30 g of skin from which 15 g were used to determine pectin concentration and 15 g for the tannin assessment. To prevent oxidation of the skins during the peeling, we processed the fruit at temperatures below 0 ° C using ice and liquid nitrogen.

Pectin content in berry skin:

No-alcohol-soluble compounds (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. The solid material component was re-suspended in ethanol 95% and re-centrifuged similarly, usually 3 times until the liquid supernatant was totally decolorized. The resulting NAS fraction was dried overnight at 60°C and ground to a fine powder (<100 um). Then, 0.1 g of NAS fraction

was diluted in 20 ml of distilled water and 0.2 ml of ethanol 95% and shaken horizontally for 16 h at room temperature (~24°C). The Water-soluble pectins (WSP) were extracted from the NAS fraction by centrifugation (10,000 g) for 20 min at 0°C. The supernatants were then diluted at 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). Thus, 1.5 ml of sulfuric acid 95% was added to 300 µl of the previously prepared solution. Immediately after mixing, the tubes containing the solutions were placed in a water-ice bath for 3 min. They were then heated in a boiling water bath for 6 min and immediately cooled again in a water-ice bath. Thirty µl of m-hydroxydiphenyl reagent was added to each tube. After mixing, the tubes were left in the dark for 20 min before reading the absorbance at 520 nm.

Tannin content in berry skin:

The tannin content (TAN) was extracted 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 (~24°C). Berry skins were stirred at 150 rpm with 5 ml of methanol containing 0.1% of 12 N HCl (Gagné et al. 2006), and filtered using a Flacon filter (100 µm). The tannin content was determined by spectrophotometry and expressed as mg g^{-1} skin using an adaptation of the methodology proposed by Ribéreau-Gayon and Stonestreet (1966). To the 0.2 ml of the previously prepared solution, 2.8 ml of distillated water and 3 ml of HCl 12 N were added. The resulting solution was divided in two (Sample 1, Sample 2) and one of them (Sample 2) was heated in a boiling water bath for 30 min and immediately cooled in a water-ice bath. After that, 0.25 ml of ethanol 95% was added to each sample and, after mixing, they were left in the dark before reading the absorbance at 550 nm. Finally, the tannin concentration was determined as shown in the equation below. All the experiments were performed in triplicate.

$$\text{Tannins} = \frac{(\text{absorbance sample 2} - \text{absorbance sample 1}) \text{ x } 76.35}{2 \text{ x skin berry mass (g)}} \quad (1)$$

Vegetative growth

Grapevine vegetative growth was measured at 300 growing degree-days (GDD) accumulated from flowering, roughly equivalent to berry pea-size stage (Eichhorn and Lorenz's growth

stage 31). The GDD accumulated were calculated on a basis of daily average temperature and a base temperature for grapevine of 10°C. The normalized difference vegetation index (NDVI) was obtained from measurements performed as described by Drissi et al (2009) using a Greenseeker® (*N-Tech* Industries, Ukiah, CA and Oklahoma State Univ., Stillwater). The results were expressed as a numerical index ranging from 0 to 1, with the 1 value representing the maximum vigor and leaf density.

Statistical analyses

On the one hand, the relationships between BBR incidence and severity with the explanatory variables pectins (WSP) and tannins (TAN) were explored by using correlations and linear regressions. To determine if a correlation was significant, the Pearson's correlation coefficient was calculated based on the P-value = 0.05. On the other hand, the relationship between BBR (incidence and severity) and the vegetative growth (NDVI) was plotted and modeled by using a nonlinear model based on the equation $BBRinc/sev = a*(NDVI)^{b}$. Furthermore, multiple linear regressions were performed by including as explanatory variables: the biochemical (WSP, TAN), vegetative (NDVI) and climate indices (PL15 and PL35). In this last analysis, the climate indices were also considered in order to better understand the part played by the early disease risk indices and grapevine features in BBR development during the season. BBR incidence and severity values were previously transformed by using Arcsinus function to improve variances homogeneousness. Variance inflation factors (VIFs) were calculated in order to detect multicollinearity with the set of explanatory variables. Finally, variables were selected by using stepwise analysis with a P = 0.05 significance level to keep in the model. All data analyses were performed using the SAS University Edition software.

Results

Climatic conditions

On the one hand, in France, all vine growing seasons were characterized by humid and temperate conditions, which favored *B. cinerea* development (Figure 1a). The average temperature between budbreak to harvest was similar in all seasons in France, fluctuating

between 18°C and 19°C (data not shown). Pluviometry differed between seasons as shown from April to October, i.e. during the spring and summer period; for example, the least amount of rainfall was recorded in 2011 (275 mm) versus the highest value that was registered in 2013 (593 mm).

On the other hand, Chilean conditions were characterized, in all studied seasons, by dry and temperate spring and summer periods, which were not conducive to disease development (Figure 1b). From budbreak to harvest, the average air temperature was similar in whatever the season, fluctuating between 18°C and 19°C (data not shown) as in France. Nevertheless, the total rainfall from October to April, i.e. during Chilean spring and summer, was much lower than in France. Only 36 mm and 115 mm were recorded in 2014-15 and 2015-16, respectively. Furthermore, rain periods were mostly observed before veraison, leading to unfavorable conditions for BBR development before and at harvest.

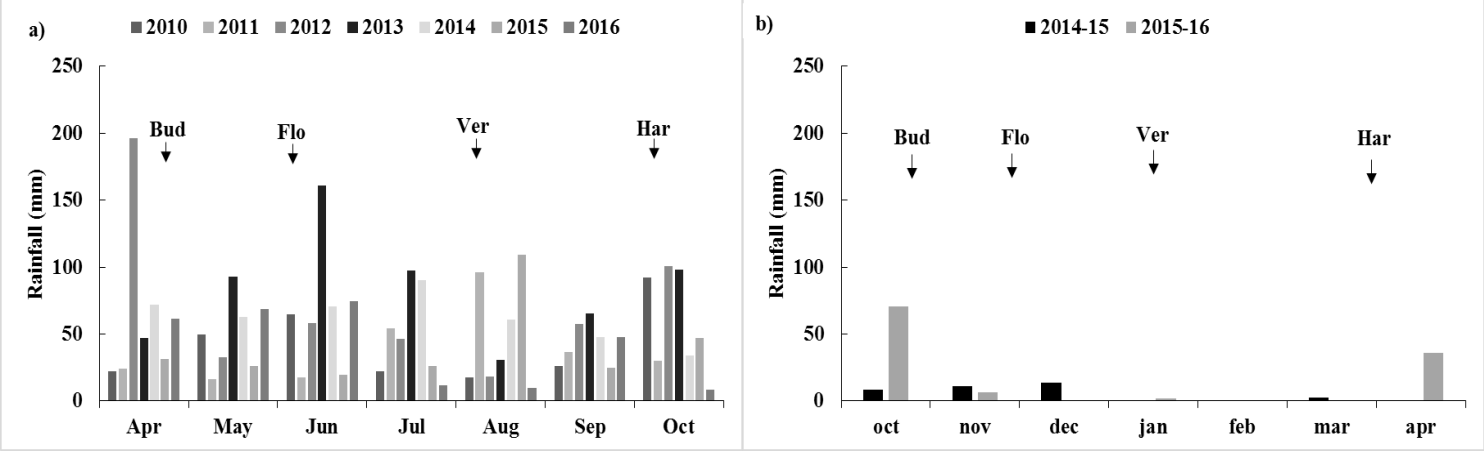

Figure 1: Monthly cumulated rainfall (mm) in France (a) and Chile (b). Bud= Budbreak; Flo= Flowering; Ver= Veraison; Har= Harvest.

BBR incidence and severity

In France, the Merlot cultivar showed average values of disease incidence and severity of 57.1% and 8.6% for the seven studied years, respectively. Nonetheless, the disease level varied greatly between years depending mainly on climatic conditions (Figure 2). For example, 2013 was the most conducive to BBR development, with mean incidence and severity values of 98.7% and 20.5%, respectively. In 2010, on the contrary, disease pressure was the lowest among all evaluated years, with an incidence of 28.7 % and a severity value

of 4.7 %. The Merlot cultivar evaluated in Chile did not show any disease development in all studied seasons. As for the Sauvignon blanc cultivar under the Chilean conditions, it showed, in both seasons, a mean disease incidence and severity of 8.4 % and 1.2%, respectively. Thus, this cultivar showed lower disease incidence and severity values than the Merlot cultivar evaluated in France in all study seasons (Figure 2).

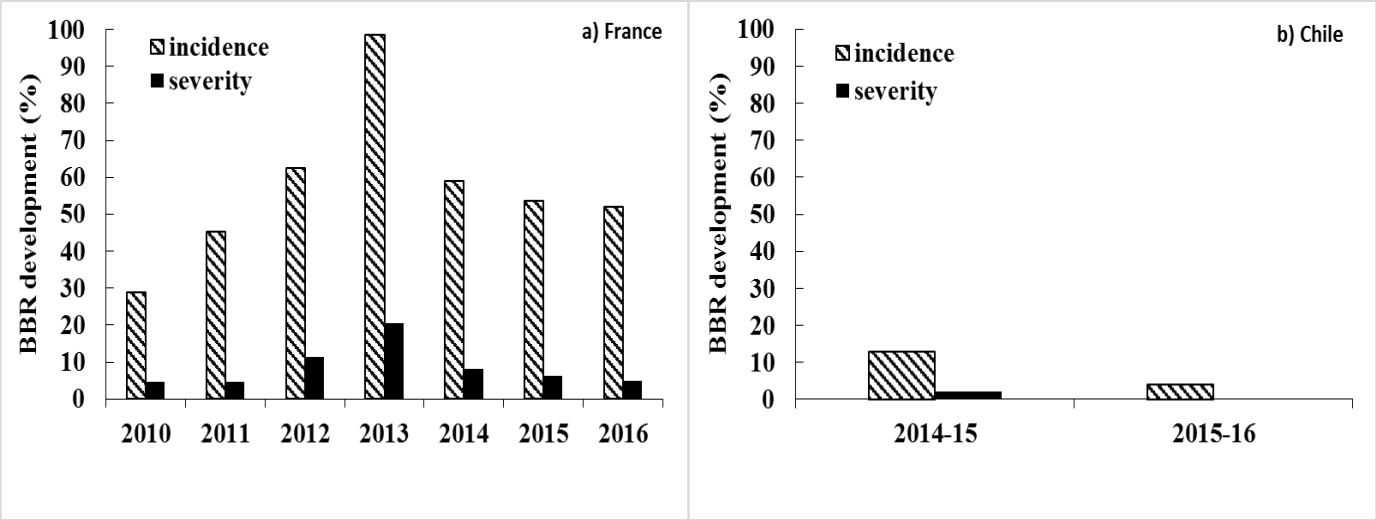

Figure 2: Mean BBR Incidence and Severity values (%) according to the season under field conditions for Merlot cultivar in France (a) and Sauvignon Blanc cultivar in Chile (b). Merlot in Chile did not present BBR development.

Relationships between berry skin components and BBR intensity at harvest

Positive relationships were observed between the pectin contents in the berry skin and BBR incidence and severity at harvest (Figure 3a, 3c). In addition, negative relationships were observed between tannin compounds and disease incidence and severity at harvest (Figure 3b, 3d). All correlations were significant except for the relationship between pectins and BBR incidence (Table 2). The Merlot cultivar evaluated in Chile showed low pectin and high tannin concentrations in berry skins, which was not favorable to the pathogen. Likewise, under Chilean growing conditions, the Sauvignon blanc cultivar showed greater pectin values and similar tannin values as Merlot in both seasons. Accordingly, in Chile, disease development was slightly higher in Sauvignon blanc compared with Merlot in both seasons.

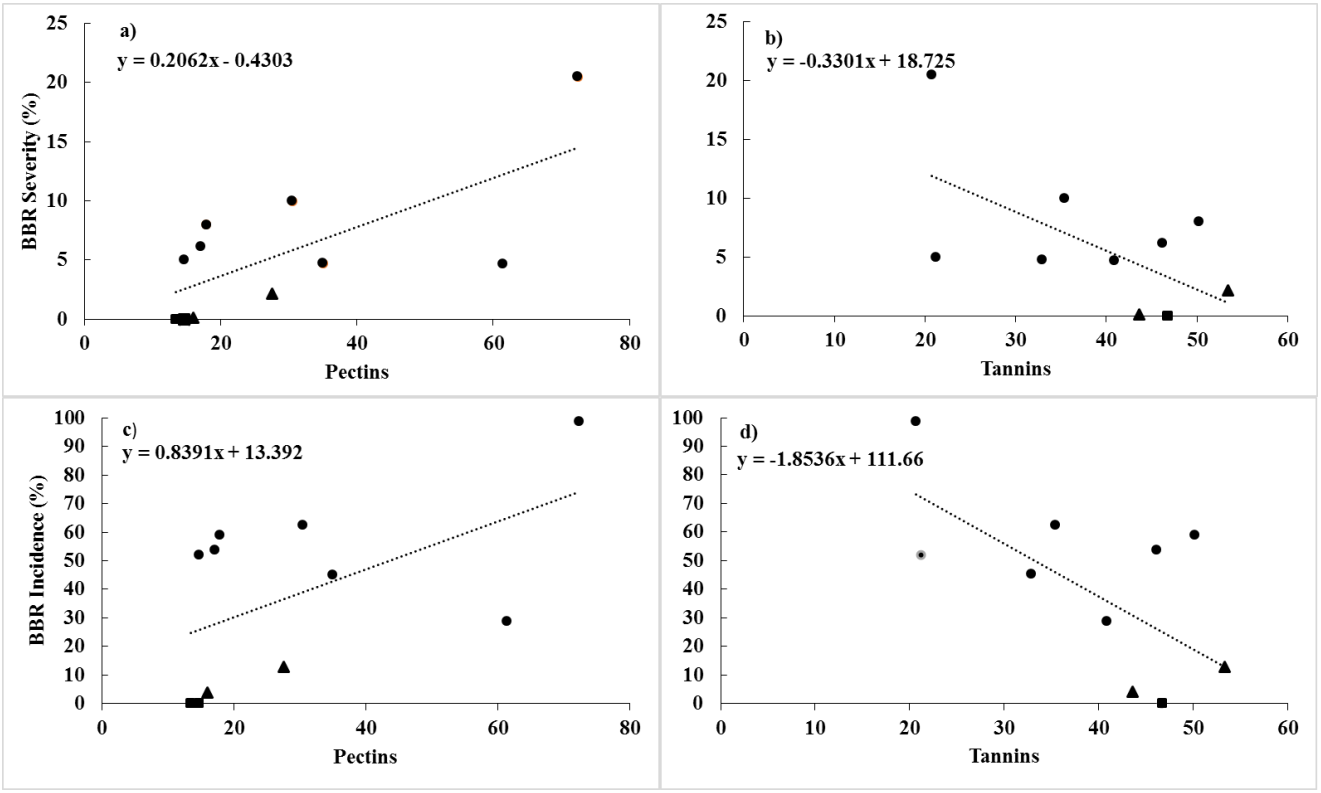

Figure 3: Relationships between BBR Severity (%) with Pectins (a) and Tannins (b); and BBR Incidence (%) with Pectins (c) and Tannins (d). Merlot in France (●), Merlot in Chile (■), Sauvignon Blanc in Chile (▲). Pectins expressed as mg galacturonic acid g^{-1} NAS and Tannins as mg tannins g^{-1} skin.

Table 2: Statistical significance and associated overall coefficients for relationships between BBR Incidence and Severity (%) and Pectins and Tannins.

	Pectins				Tannins			
	dF	**R^2**	**r**	**P- value**	**dF**	**R^2**	**r**	**P- value**
BBR Incidence	10	0.29	0.54 ns	0.09	10	0.42	-0.65 *	0.03
BBR Severity	10	0.49	0.70 *	0.02	10	0.38	-0.62 *	0.04

Where: dF = Degrees of freedom; R^2 = Coefficient of determination; R = Pearson's correlation coefficient, significant at P = 0.05 *. Pectins expressed as mg galacturonic acid g^{-1} NAS and Tannins as mg tannins g^{-1} skin.

Relationships between vegetative growth and BBR intensity at harvest

Exponential relationships were observed between the NDVI values evaluated at 300 GDD from flowering and the BBR incidence and severity at harvest (Figure 4). The relationships between these variables were positive, showing that plants with higher vegetative growth in

an early phenological stage were more susceptible to *B. cinerea* at harvest. For BBR incidence the equation was $y = 183.5 * x^{3.8}$ (Figure 4a), whereas for BBR severity it was $y = 107.7 * x^{9.2}$ (Figure 4b), with "y" representing the % of BBR incidence or severity and "x" the NDVI value. The pattern was similar for both disease incidence and severity, but with a steeper curve for the relation between NDVI and disease severity. A trend is noticeable, showing a change in BBR incidence and severity occurring around NDVI values of 0.5 and 0.6, respectively. According to these equations, for NDVI = 0.7, the incidence and severity reached approximately 50% and 5%, respectively (Figure 4 a, b).

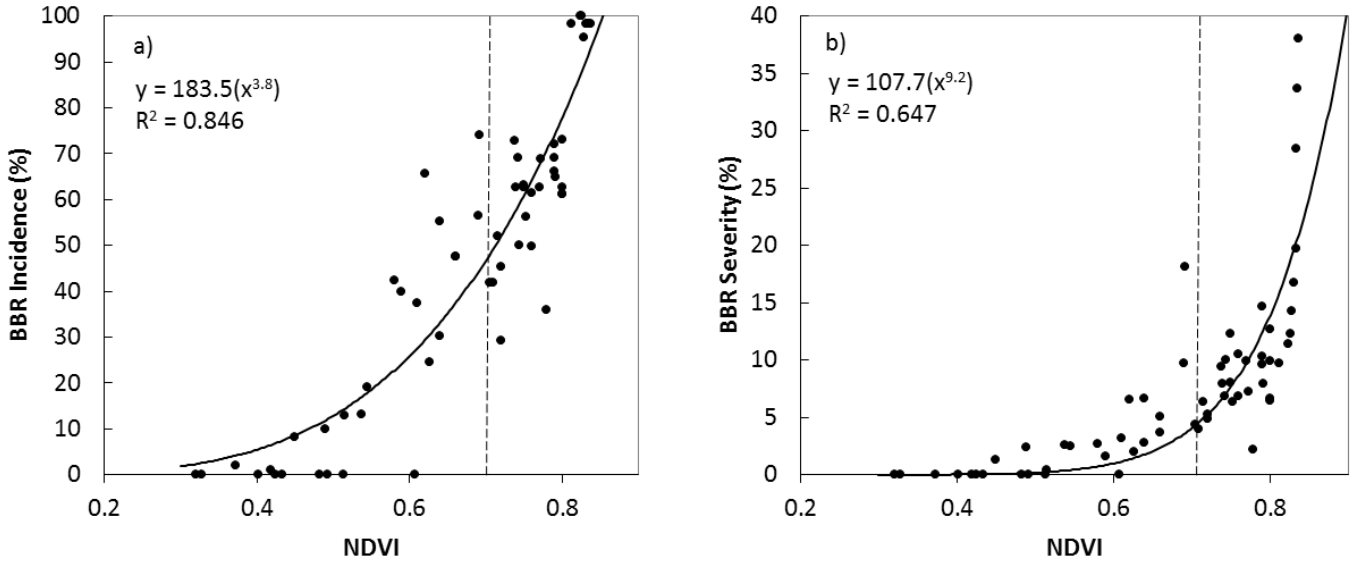

Figure 4: Relationship between and BBR Incidence (%) with NDVI (a) and; BBR Severity (%) with NDVI (b) in France and Chile.

BBR intensity at harvest and its relationship with different explanatory variables: berry skin components, vegetative growth and climate.

Multiple linear regression analyses were performed in order to study the relationships between the following explanatory variables: pectins, tannins, vegetative growth and climate with the response variables: BBR incidence (Table 3) and BBR severity (Table 4). Variance inflation factors (VIFs) were calculated in order to detect multicollinearity with the set of explanatory variables. All VIF values were low (6.3 or less), thus multicollinearity was unlikely to exist. Therefore, all the predictor variables were used for the following regression

analysis. First, the best model for explaining BBR incidence included three variables: i) the pluviometry cumulated 35 days before harvest (PL35) as the predominant explanatory variable (R^2 = 0.79), ii) the NDVI and; iii) the tannin content in berry skins. These two last variables added 0.11 and 0.04 to the model's overall R^2, respectively. The overall R^2 was very high, reaching 0.94 and highly significant (P = 0.0002). Second, according to R^2 value, the best model for estimating BBR severity included two variables: i) the pluviometry cumulated 15 days before harvest (PL15), which was the predominant explanatory variable (R^2 = 0.65) and; ii) the vegetative growth (NDVI) (Table 4). This second variable added 0.17 to the model's overall R2, which was very high, reaching 0.82 and highly significant (P = 0.0012).

Table 3: Summary of the multiple regression model for BBR Incidence at harvest.

Independent variables	VIF	Regression function	Model variables	R-Square	P-value
PL35	6.30	Inc = -96.33 + 0.59 (PL35) + 111.97 (NDVI) + 0.99 (TAN)	PL35	0.79	0.0050
WSP	2.45		NDVI	0.11	0.0071
TAN	2.97		TAN	0.04	0.0818
NDVI	2.55		Model	0.94	0.0002
PL15	4.12	Inc = -28.50 + 84.06 (NDVI) + 0.393 (PL15)	NDVI	0.67	0.0170
WSP	3.33		PL15	0.13	0.0563
TAN	2.15				
NDVI	2.06		Model	0.80	0.0017

Where Inc = BBR Incidence at harvest (%); PL35 = Pluviometry cumulated 35 days before harvest (mm); PL15 = Pluviometry cumulated 15 days before harvest (mm); WSP= Pectins (mg galacturonic acid g^{-1} NAS); TAN = Tannins (mg tannins g^{-1} skin); NDVI = Normalized Difference Vegetation Index (dimensionless); VIF = Variance Inflation Factor.

Table 4: Summary of the multiple regression model for BBR Severity at harvest.

Independent variables	VIF	Regression function	Model variables	R-Square	P-value
PL35	6.30	Sev = 4.12 + 0.19 (PL35)	PL35	0.75	0.0005
WSP	2.45				
TAN	2.97				
NDVI	2.55		Model	0.75	0.0005
PL15	4.12	Sev = -7.68+ 0.16 (PL15) + 24.02 (NDVI)	PL15	0.65	0.0215
WSP	3.33		NDVI	0.17	0.0266
TAN	2.15				
NDVI	2.06		Model	0.82	0.0012

Where Sev = BBR Severity at harvest (%); PL35 = Pluviometry cumulated 35 days before harvest (mm); PL15 = Pluviometry cumulated 15 days before harvest (mm); WSP= Pectins (mg galacturonic acid g-1 NAS); TAN = Tannins (mg tannins g-1 skin); NDVI = Normalized Difference Vegetation Index (dimensionless); VIF = Variance Inflation Factor.

Discussion

Berry skin features as potential early indicators of BBR development.

Berry skin features were measured early in the season, specifically at berry pea-size grapevine stage, at which time *B. cinerea* could be resting inside the berry in a latent state. As an expected result of our scientific rationale, the correlation between berry skin pectins and BBR severity at harvest was significant and positive because sugars issued from pectins are important substrates for the pathogen growth (Blanco-Ulate et al 2014; Zhang et al. 2013). This may be accounted for by grape berry skins which contain high levels of pectins, becoming then potentially more prone to infection and colonization by *B. cinerea*. Although pectins were significantly correlated with BBR severity, this berry feature was not significantly correlated with BBR incidence. This may be explained because the pectins favor the growth and colonization of the fungus following its penetration process within the fruit. However, the infection may occur independently and be governed by other key features such as the water activity (Aw) at the berry surface (Deytieux et al. 2009, Fermaud et al. 2011) and the presence of micro-cracks or pores in berry skins (Mlikota-Gabler et al 2003). Furthermore, pectins are not the only polysaccharide substances in cell walls degraded by the fungus. Since *B. cinerea* is well known as a necrotrophic and pectinolytic fungus (Ten Have et al., 2002), there are other types of polysaccharides in the primary cell wall, e.g. cellulose and hemi-cellulose, which are also possible growth substrates for the pathogen (Kars and van Kan 2004). Therefore, this skin pectin content should be considered as one possible susceptibility indicator but not potentially the only one to predict the final BBR attack on grapes.

On the other hand, a key result in this study is to demonstrate that tannins were significantly and negatively correlated with both BBR severity and incidence. These relationships were expected due to the rationale that tannins are constitutive antifungal compounds in berry skins, playing a potential important role in resistance to *B. cinerea* (Goezt et al. 1999, Pezet et al., 2004). Different studies agree that tannins delay the development of the disease symptoms maintaining *B. cinerea* in a quiescent stage (Hills et al., 1981; Jersch et al., 1989; Hebert et al., 2002). This fungal quiescence may be due to the inhibition of fungal enzyme activity, such as polygalacturonases, cellullases and laccases (Porter and Scwartz, 1962;

Bachmann and Blaich, 1979; Hills et al., 1981; Jersch et al., 1989; Tabacchi, 1994; Goetz et al. 1999; Pezet et al. 2004). Furthermore, it has been demonstrated that more tolerant grape cultivars show higher quantities of tannins in berries and its inhibitory effect on enzyme activity remained until harvest (Pezet et al. 2003). Other studies have also shown that the high resistance in immature strawberry fruits against *B. cinerea* may be attributed to proanthocyanidin, a condensed tannin compound (Schlösser, 1985). Moreover, field treatments of grapevines with a plant activator, i.e. benzothiadiazole, induced resistance against BBR, which also has been associated with an increase of proanthocyanidins in berry skins. Thus, the total tannin content in berry skins has been considered as a major factor affecting both growth and berry colonization by the fungus and, therefore, a feature of prime importance, presumably accounting for grapevine berry ontogenic resistance to the pathogen (Deytieux et al. 2009).

Although different works also have shown significant correlations between grapevine susceptibility to BBR and phenolic contents in the berry skin (Padgett and Morrison 1990; Sarig et al. 1998; Dubos and Roudet 2003; Pezet et al 2004; Deytieux et al. 2009), all of them have been carried out in laboratories and they did not consider the effect of such a relationship under natural conditions. Therefore, the present work is the first study that relates the tannin content in berry skin to BBR development under field conditions. This results suggests that tannin content in berry skin measured at an early phenological stage, might be used as an indicator to estimate the potential susceptibility of grape berries to the pathogen. Nevertheless, it should remain as a trend indicator as there are other important epidemiological factors, such as microclimatic conditions, grapevine vegetative growth, fruit maturity at harvest and interactions with microorganisms that may also affect the BBR development (Fermaud 1989; Broome et al., 1995; Valdés-Gómez et al. 2008, Pañitrur-De la Fuente et al. 2017). Then, the interpretation of this indicator for practical use should always consider environmental conditions such as the climate at the end of the season, the fruit maturity and/or the vegetative growth, considered the two major factors affecting *B. cinerea* development (Valdés-Gómez et al. 2008, Latorre et al. 2015).

Even though tannins are constitutive elements on grape skins, there are some environmental factors that could modify this content and, therefore, also affect berry susceptibility to BBR. Different studies demonstrated that water stress conditions may induce changes in phenolic

composition by increasing the concentration of tannins in grape skins (Kennedy 2002; Roby et al 2004; Casassa et al 2015; Cáceres-Mella 2016). This is of great interest because a controlled water deficit is a common practice in several wine regions, e.g. central valley of Chile, with the objective to improve the organoleptic wine quality (Kennedy et al. 2002, Acevedo-Opazo et al. 2010). Therefore, the higher skin tannin contents observed in Chile in this study could be explained by a low rainfall in the vine growing period and a regulated deficit irrigation in our experimental site. Similarly, the low tannin values observed in some years in France, could be caused by very low precipitations, mainly after flowering (no irrigation in this vineyard). Thus, tannins content could be modulate by different water conditions of each season (due to rain and irrigation), which could affect the biosynthesis of the phenolic compounds (Casassa et al 2015).

Lastly, in the present study, we also calculated the same index as this one calculated by the INRA in Bordeaux, corresponding to the ratio of pectin contents divided by the tannin contents. In our conditions, the index values ranged from 0.3 to 0.5 in Chile and 0.4 to 1.5 in France. Nevertheless, the relationship between the index and the BBR development was not significant (data not shown), suggesting that the use of pectins and tannins, as separate indicators, shows better results.

NDVI as potential early indicator of BBR susceptibility.

Different studies in the literature have investigated the relationship between grapevine vegetative growth and BBR development (Percival et al. 1994; Reynolds and Wardle, 1994; Smithyman et al. 1997; Intrieri et al. 2001; Morlat and Bodin 2006; Valdés-Gómez et al. 2008). Accordingly in our present study, positive relationships between NDVI (indicator of vine growth) and BBR development (disease incidence and severity) were observed. Dense grape wine canopies are associated with increased duration periods of wetness after rainfall resulting in increased susceptibility to *B. cinerea* (Steel 2001). Therefore, prophylactic cultural methods for controlling *Botrytis* disease include managing of the crop canopy. In the vineyard, for example, an important cultural method is removal of leaves. Furthermore techniques associated with vine training and pruning systems may also reduce significantly BBR (Elmer and Michailides 2004; Elad 2016). Leaf removal from the fruit zone has been

adopted as an effective practice in vineyards to reduce significantly *Botrytis* epidemics in European, Californian and Australian vineyards (Gubler et al. 1987; Zoecklein et al 1992; Percival et al. 1994; Elmer and Michailides 2004). In New Zealand this practice, performed between late flowering and berry pea sized is considered as the most effective cultural tool for managing BBR (Elmer 2016). Such practices, above all, tend to limit the vegetative growth around the clusters for improving air circulation in the bunch zone, exposing fruit to light and thereby reducing BBR infections.

Considering the importance of the vegetative growth in *Botrytis* epidemics, it was necessary to develop an indicator of vigor that allows growers to estimate the potential susceptibility of grapevine to the pathogen. Our results suggest that the NDVI measured early in the season could meet this goal. Ky et al. (2012) indicate a threshold value of 5% BBR severity on grape berries at harvest above which negative organoleptic consequences are perceived in the wine. Then, regarding relationship between NDVI and BBR, a NDVI value close to 0.7 (Figure 4) early in the season, i.e. at berry pea-size stage, could be proposed as an adapted threshold to be used for disease management between bunch closure and harvest. Under this value, the BBR severity should be in general lower than 5% as it was observed in the contrasting climatic and cropping conditions from our study.

BBR development and climate effect.

The results of the present study allowed us to identify the tannins and NDVI as potential early indicators of *Botrytis* development in the vineyard. In some years, although the amount of skin components and the vegetative index values may have been similar in the two countries, BBR development was always much higher in France than in Chile. These high differences in disease incidence and severity between the two countries were mainly due to the climatic conditions before harvest. Under our experimental conditions, the means temperatures were similar in both countries, nevertheless the rainfall was much greater in France than in Chile during all seasons, enhancing to a great extent the relative humidity within the grape canopy. Different climatic factors including temperature, relative humidity and rainfall after veraison were tested and correlated with BBR. Among them, rainfall resulted to be the most relevant factor involved in *Botrytis* development (data not shown).

Environmental conditions conducive to *B. cinerea* infection have been extensively studied and it is widely accepted that microclimatic conditions, specifically temperature and relative humidity within the cluster zone, are key factors for disease development (Broome et al. 1995; English et al. 1989; Nair and Allen 1993; Steel et al. 2011; Thomas et al. 1988; Valdés-Gómez et al., 2008). Thus, Ciliberti et al. (2016) have recently demonstrated that optimal conditions for *B. cinerea* sporulation are temperatures between 15 and 20°C and relative humidity higher than 65%. In addition to temperature and relative humidity, rainfall is also crucial to facilitate infections via sporulating conidia, especially during the ripening period. (Thomas et al., 1988; Broome et al., 1995; Coertze and Holz, 2002; Latorre et al., 2002; Steel et al., 2011; Ciliberti et al., 2015 a, b; Ciliberti 2016). All these factors contribute to the evaporative potential within the bunch zone and therefore the presence of free water at the fruit surface, which is essential for conidial germination and berry infection (Thomas et al. 1988; Broome et al. 1995; Nair and Allen 1993; Coertze and Holz 2002; Latorre and Rioja, 2002; Steel et al. 2011; Ciliberti et al., 2015a, b;Ciliberti et al. 2016).

***Regression model of BBR severity and incidence*.**

Linear regression models developed in the present study, using the early indicators of berry susceptibility (pectins, tannins and NDVI) as well as climate indicators at the end of berry maturity, allowed us to determine and select major explanatory variables in BBR development. All our optimized models, both for incidence and severity, included climatic indices, which corroborates the prime importance of this factor in BBR epidemiology as above stated. The best models that explained BBR incidence and severity included the pluviometry recorded during 35 and 15 days before harvest, respectively. This could be due to the fact that incidence is related with the epidemic onset and first infections, whereas the severity corresponded to the spread of these infections. Thus, the earlier favorable climatic conditions are needed for the onset of disease infections (incidence), while the later weather conditions are required for the disease spread (severity).

A second very important result of this analysis was to show the vegetative growth as the other major factor in determining *Botrytis* development because it was selected in both BBR severity and incidence models. This result confirms previous studies which showed that

grapevine vegetative growth is a key factor affecting *Botrytis* development (Valdés-Goméz et al., 2008). Regarding the berry skin features, only the tannins were included in the BBR incidence model, corroborating that they should be considered as a better indicator of berry susceptibility to the pathogen than pectins. Interestingly, previous studies have investigated the relationship between the amount of skin tannins at harvest with the vegetative growth of vines between budbreak and veraison, observing that less vigorous vines tend to have greater amounts of these phenolic compounds (Cortell et al. 2005). Finally, it is interesting to highlight that all the regression models that explain BBR incidence and severity were highly significant and thereby they could be used as tools for study disease development under different growing conditions. Likewise, they could be used in control strategies, for example in specific DSS to limit pesticide fungicides, such as in the case of Powdery mildew (Delière et al. 2015; Valdés-Gómez et al. 2017)

During the recent decades, restriction in fungicide application have been increasing to reduce their negative impact on the environment and to limit pesticide residues at harvest (Verger and Boobis 2013, Fenner et al. 2013). Nonetheless, control of *B. cinerea* still largely depends on the use of chemical specific fungicides and then protection strategies require to be optimized. In this context, cultural management practices are key in a BBR control strategy aiming to reduce fungicide spraying. In order to help growers decide which or when a cultural management practice should be applied, it is necessary to have information about the crop susceptibility to *B. cinerea*. Our results point out two early risk disease indices, e.g. NDVI and tannins, which could be used to characterize the grapevine susceptibility to the pathogen. Therefore, the vegetative index (NDVI) may be used in IPM strategies as a management indicator to decide, for example, to implement cultural practices such as leaf removal and heading and/or shoot removal. On the other hand, the amounts of tannins in berry skins could be used as a disease risk assessment to help the decision of spraying a fungicide. Is important to highlight that these two risk indices would be available early in the season, allowing to orient phytosanitary strategies at early phenological stages, e.g. at prebunch-closure or at veraison, which are two critical moments for controlling the disease.

In addition to these indicators, is important to consider as a potential management indicator, as well as in a future DSS, the cultivar susceptibility, which is a key parameter in BBR susceptibility (Pañitrur-De la Fuente et al. 2017). In the present study we have only presented

the Merlot cultivar in both countries and Sauvignon Blanc only in Chile. Nevertheless, the indices of susceptibility to BBR may be adapted according to the specific cultivar. Further investigations may include, in addition to the test of various other grapevine cultivars, the level of the pathogen inoculum to better understand the relationships between *B. cinerea* inoculum in an early phenological stage and the BBR attack at harvest as it was investigated by Calvo-Garrido et al. (2015).

Conclusions

The regression models developed in this study significantly explained the BBR development at harvest. These models included two early variables: grapevine vegetative growth (NDVI) and tannin content in berry skins, as well as two late variables: cumulated rainfall at 15 and 35 days before harvest. The two early grapevine features, i.e. the vegetative growth (NDVI) and the amount of tannins in berry skins, were significantly correlated with the susceptibility of grapevine to *B. cinerea*. Both NDVI and tannin content, evaluated at berry pea-size stage (approx. 100 days before harvest), are of great interest to be used in IPM strategies as disease risk indicators before implementing direct control measures. Since they are potential early indicators, they could be used to orient phytosanitary managements at critical moments for controlling BBR, such as prebunch-closure and/or at veraison. Furthermore, the models proposed in this work could be used in control strategies against BBR, for example in specific DSS to limit pesticide fungicide residues in viticulture.

In the present paper, we confirmed the climatic conditions before harvest, specifically the accumulated rainfall, as the main factor explaining BBR infections. Then, the pluviometry was the principal factor that explained the great differences in BBR intensity at harvest between Chile and France. Secondly, the grapevine vigor was demonstrated as the other important factor influencing the disease incidence and severity. New investigations should consider other early epidemiological important factors, i.e. cluster compactness and pathogen inoculum, in order to better understand their relationships with BBR at harvest and to improve management and control methods in IPM strategies.

GENERAL CONCLUSIONS

Traditional control of Botrytis Bunch Rot (BBR) depends heavily on routine application of fungicides, whether or not it is necessary, leading to negative effects on both human health and the environment. Restriction in fungicide applications has become necessary and alternative strategies for rational suppression of the pathogen are urgently needed. A large amount of research projects around the world have been dedicated to studying various aspects of the biology, epidemiology and control of *B. cinerea*, however, few of them have dealt with developing and implementing rational approaches for disease control. Therefore, this doctoral thesis aimed to study different factors related with BBR development to be used as disease risk indicators in a specific rational disease strategy.

First of all, the cultivar's susceptibility to *B. cinerea* was studied under two contrasting climatic and cropping conditions (Bordeaux Region in France and the Maule Region in Chile), and the effect of fruit maturity on such susceptibility was also investigated. The results of this study showed a similar cultivar classification according to their susceptibility in both contrasting conditions. Sauvignon Blanc and Gewürztraminer resulted to be the most susceptible cultivars, whereas Petit Verdot, Cabernet Sauvignon, Mourvèdre and Syrah were rather resistant or highly resistant. This study points out that the cultivar is a permanent factor affecting *B. cinerea* epidemics and therefore, it should be considered as a key parameter in decision support systems. Although the classification proposed in this research is of great interest to be used in rational disease managements, it is important to highlight that in climatic and cropping conditions very different from those of Bordeaux and Maule Valley, some cultivars could present a different susceptibility from the one proposed here. This situation occurred when results of this study were compared with some research from the literature. This difference is presumably caused by variations in agronomic factors, e.g. rootstock, clone, and vine training system, which also can affect the crop susceptibility to the pathogen. For example, all cultivars evaluated in this work were trained to vertical shoot positioning. However, their susceptibility may be modified if they are trained to another system, e.g. pergola system. Further studies should be carried out to better understand the relationship between agronomic factors and the crop's susceptibility to the pathogen to improve integrated disease management strategies. Otherwise, a positive and exponential relationship between fruit maturity and susceptibility to the pathogen was observed in this study,

indicating that most mature cultivars were the most susceptible. Therefore, the maturity of cultivars seemed to be a major factor determining the susceptibility to *B. cinerea*, which could support the cultivar classification.

In a second part of this work, early grapevine features related with the final disease development were investigated and proposed as risk indicators. Specifically, the tannin content in berry skins and the vegetative growth, expressed as NDVI, were significantly correlated with both BBR incidence and severity at harvest. Therefore, these two features could be used as disease risk indicators in IPM strategies. Since both indicators were evaluated early in the season (berry pea-size stage), they may be useful to decide if it is necessary or not to apply fungicides at critical moments like cluster closure and/or veraison. Moreover, the vegetative index (NDVI) might also be used as a management indicator to implement cultural practices such as leaf removal. A NDVI value of 0.7 was identified as a possible threshold above which the BBR severity should be lower than 5% and therefore, not affect the quality of wines. Pectin contents in berry skins were only correlated with BBR severity and thereby, they could be considered as one possible susceptibility indicator, but not as the only one. Lastly, climatic conditions before harvest were also investigated and regression models based on climatic indices, i.e. cumulated rainfall 15 and 35 days before harvest, and the early grapevine features were proposed to evaluate the potential susceptibility to *B. cinerea* at harvest. All optimized models included climatic indices, which corroborates the prime importance of this factor in BBR epidemiology. Moreover, the best models that explained the BBR development at harvest also contained the NDVI and tannin contents in berry skins, confirming that they could be used as risk indicators in a decision support system to orient control measures.

Finally, floral calyptra infection rate was studied as another potential early disease risk indicator (annex 1). This work demonstrated no significant correlation between this indicator and BBR development in mature berries (after veraison). Therefore, according to these results, the percentage of *B. cinerea* infection of floral calyptras might not be a reliable early indicator of the BBR epidemic. New experiments under different vineyard conditions may be conducted to find a relationship between floral infection rate and BBR intensity in ripening berries.

PERSPECTIVES

This doctoral study makes a significant contribution to a rational strategy for controlling *B. cinerea* in grapevines. In this work, factors related with BBR development were investigated and proposed as disease risk indicators. Specifically, it was possible to set up permanent (cultivar susceptibility) and variable (NDVI and berry skin features) indicators that may be used in a decision support system to control the disease. Furthermore, potential thresholds for some of these indicators were identified. Among the variable indicators, NDVI was the easiest index to asses and so it may be a good field management indicator. Concerning pectin contents in berry skins, its assessment was more time-consuming and expensive in terms of costs, which may complicate its use in a practical way. On the other hand, tannin skin compounds were easier and faster to asses than pectins and thereby, this parameter should remain a potential field risk indicator. Moreover, new technologies, e.g. hyperspectral imagery, could facilitate their determination in a near future, making this berry feature a valuable disease risk indicator.

The knowledge achieved in this work could be used to improve the Decision Support Rule (DSR) proposed by French pathologists and consequently, a "new DSR" could be built by considering the indicators suggested in this study. This new DSR is presented in Figure 1. In this DSR, cultivar susceptibility to BBR is used early in the season (at flowering) to decide the first fungicide spraying. After this, at bunch closure, tannin and pectin content in berry skins are proposed instead of potential receptivity of berries (index calculated by dividing pectin by phenolic compounds) used in the original DSR. Also, at this phenological stage the NDVI indicator was added, which incorporates the vegetative influence in host susceptibility to BBR. At veraison, cluster compactness was maintained as a key indicator. Literature information and complementary data collected in the Merlot cultivar (not shown in this work) were used to identify a potential threshold for this variable (compactness index = 3). Nevertheless, it may be important to asses this parameter in other grapevine cultivars as they present different cluster architectures, which affects BBR disease. Finally, at veraison-harvest period, literature information was used to propose a potential climatic indicator, since this type of indicator was not investigated in the present work. For example, Ciliberti et al. (2015, 2016) have proposed climatic equations that predict *B. cinerea* infections during berry

ripening and thereby, they could be useful as climatic risk indicators to control disease after veraison. Nonetheless, these models should be validated under different field conditions before its implementation.

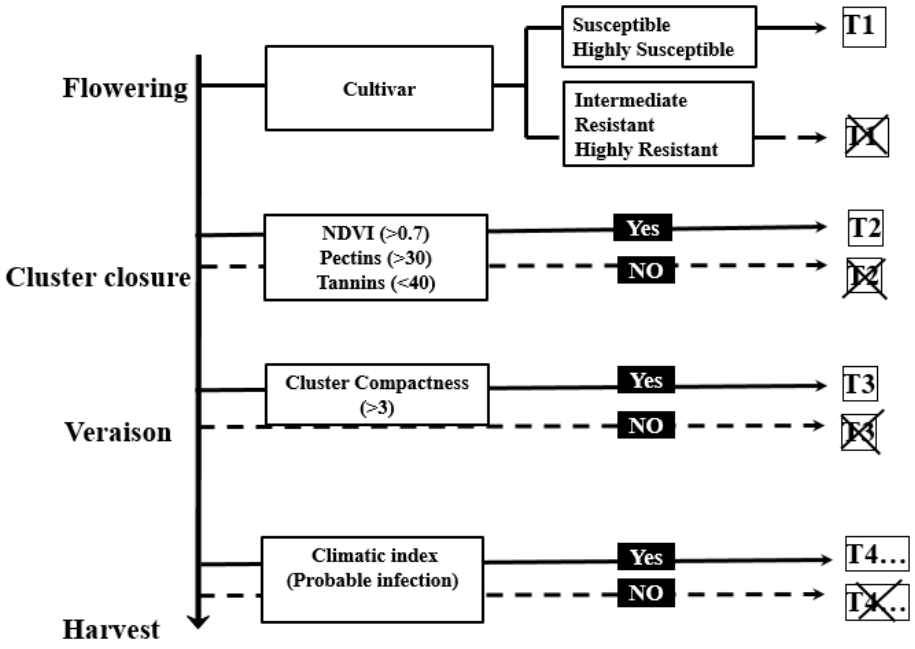

Figure 1: Decision Support Rule proposed to control *B. cinerea* in vineyards. Where NDVI and Cluster Compactness are expressed as a dimensionless index, Pectins as mg galacturonic acid g-1 NAS, and Tannins as mg tannins g^{-1} skin.

The new DSR should be tested under different vineyard conditions before its implementation at the commercial level. Additional questions or gaps may be solved to improve the strategy. For example, in this work factors related with the plant and the environment were proposed as risk indicators, nonetheless, nothing was proposed considering the pathogen (e.g. genetic features and inoculum). Furthermore, and considering cultivar as a key parameter, future research should evaluate risk indicators in different cultivars to know if it is necessary to propose thresholds cultivar-dependant. Lastly, it may be interesting to test and/or adapt disease risk indicators for controlling BBR using biological fungicides. The integration of all this knowledge would allow managing *B. cinerea* in grapevines in a more rational manner in a few years, controlling the disease primarily by non-chemical measures and using fungicides as a complementary measure, only when needed.

ANNEX 1

Epidemiology of Botrytis bunch rot in Bordeaux vineyards and alternative control strategies

(Presented in: IOBC-WPRS Meeting of the Working Group on "Integrated Protection and Production in Viticulture" 2015).

Abstract: *Botrytis* bunch rot (BBR) is a major fungal disease of grapevine worldwide caused by *Botrytis cinerea*. The pathogen presents a complex life cycle in the vineyard with a great genetic variability, multiple biological forms and various infection pathways highly dependent on meteorological conditions. Losses at harvest can be very important quantitatively as well as qualitatively by modifying wine quality from 5% of rotted berries upwards.

Extensive research on BBR epidemiology has been carried out at INRA Bordeaux-Aquitaine evaluating and developing disease risk indicators. An interesting case of study is the *B. cinerea* floral calyptras infection rate as a potential early indicator of disease development and losses at harvest. From 2011 to 2015, *B. cinerea* infection of calyptras from an experimental Bordeaux vineyard (cv. Merlot) was evaluated at the end of flowering. The potential relationships between the infection on calyptras and the climatic conditions are analysed and discussed. However, no significant correlation was observed between the indicator and BBR disease incidence or severity.

Additionally, alternative strategies to chemical fungicides have been evaluated in different Bordeaux organic vineyards in 2015. Natural products, already commercialized for their use in organic viticulture, were applied at key phenological stages or following a disease risk index. Results indicated the reduced interest of a wicker tea product, whereas potassium bicarbonate, kaolin and a fatty acid products showed BBR reduction and may be good candidates as alternative strategies for BBR control.

Keywords: *Vitis vinifera*, latent infection, kaolin, saprophytic, bunch trash, abiotic factors

Introduction

Botrytis Bunch Rot (BBR), caused by the fungus *Botrytis cinerea*, is one of the most challenging diseases of grapevine. This necrotrophic pathogen may drastically reduce both yield and wine quality (Bezier *et al.*, 2002, Lipsa *et al.*, 2012), especially sensory qualities (Jacometti *et al.*, 2010), which are perceived in the wine from a threshold of 5% of diseased berries at harvest (Ky *et al.*, 2012). The epidemic development in vineyards is initiated by primary infections of young vegetative parts by airborne conidial inoculum, following winter conservation by saprophytic colonization of necrotic debris and/or pathogen sclerotia (Elmer & Michailides, 2004). In spring, infections may develop in floral tissues, followed by a period of latency until véraison (Pezet *et al.*, 2003). Main infection pathways for ripening berries are airborne conidial inoculum, latent infections and infections coming from saprophytic mycelium (Elmer & Michailides, 2004). Latent infections initiated in floral tissues, as well as saprophytic colonisation of necrotic tissues, have been sometimes associated with final disease severity in berries (Calvo-Garrido *et al.*, 2014b, Nair *et al.*, 1995, Sanzani *et al.*, 2012, Wolf *et al.*, 1997). However, the importance of flower infection in the BBR epidemiology is not generally recognized (Nair & Allen, 1993) and the quantitative relationship between floral infection and final disease expression in mature berries has not been established clearly (Calvo-Garrido, et al., 2014b, Elmer & Michailides, 2004, Holz *et al.*, 2004). Therefore, more research is needed to further investigate such a relationship. In addition, early season indicators of disease risk may be very useful for vineyard managers and, due to this potential relationship, the incidence of *B. cinerea* in floral tissues could represent an early and very helpful indicator of secondary inoculum level and, hence, of BBR risk.

Currently *B. cinerea* is primarily controlled by specific synthetic fungicides. Their intensive use has generated several problems, such as: i) development of resistant strains (Walker *et al.*, 2013), ii) high economic cost, iii) residues in grapes and wines due to late applications and iv) adverse effects on human health and environment (Elmer & Michailides, 2004). Thus, new alternative products to control BBR are necessary. Nonetheless, the supply of biocontrol products is still limited. For example in France, there are only three products registered against *Botrytis* in vineyards (Serenade Max®, Armicarb® and Botector®). Their efficacy may be highly variable depending on specific vineyard conditions and there is a need for growers

to better know the specific efficacy and the factors for a successful application of these products in a particular growing region.

The aims of this study are: 1) to evaluate the infection of floral tissues, to possibly use it as an early indicator of BBR epidemic, by establishing a correlation between percentage of *B. cinerea* incidence on floral calyptras and BBR incidence and severity after véraison; 2) to quantify the efficacy of five natural products already commercialised for controlling BBR in Bordeaux organic vineyards.

Materials and methods

Early disease risk indicator: The case study of floral calyptras

Experimental field site: The relationship between *B. cinerea* infection percentages of calyptras and of BBR incidence and severity on berries was studied from 2012 to 2015 in an INRA experimental vineyard (cv. Merlot) near Bordeaux, France. The vines were planted in 1991 with a density of approx. 5300 vines ha^{-1}. A total of 6 to 9 replicate plots (5 to 6 vines each) were distributed on the field site, depending on the season (n = 31). No phytosanitary products were applied in these plots during the four growing seasons.

Botrytis infection of calyptras: Calyptras were collected at the end of flowering (80-100% calyptras fall). Inflorescences were shaken to collect the calyptras in empty sterile Petri dishes. Calyptras were then stored at -20 ° C. A total of 48 calyptras per plot were deposited randomly at a rate of 6 calyptras per malt agar plate (8 plates per plot). After incubation for 15 to 30 days at 15-18 °C, the number of *B. cinerea* colonies was assessed and the *B. cinerea* infection of calyptras (%) was calculated

BBR development on berries: The incidence (%) and severity (%) of BBR on berries was recorded by assessing visually 30 bunches per replicate plot. The assessment was carried out 30 days after mid-véraison, which represents an early development stage of BBR in maturing berries, when the effect of secondary inoculum sources inside the bunch may influence the first disease symptoms.

Meteorological data and Disease Risk Index calculation (DRI): Hourly data of Temperature (T) and Relative Humidity (RH), collected by an automatic weather station at

the field site, were introduced in the formula for calculating potential infection rate in mature berries as published by Ciliberti *et al.*, (2015): $y = [a \times Teqb \times (1\text{-}Teq)]c/[1 + exp(d\text{-}e \times RH/100)]$

DRI was calculated as the average value per day (0:00 h to 23:59 h).

Field evaluation of alternative strategies to control BBR

In 2015, two organically managed experimental field sites (cv. Merlot) were used, one located at Montagne (St. Emilion area) and the other at St. Yzan (Medoc area). Experimental design included four replicate plots per treatment, with 10 adjacent vines per replicate plot, where first and last were considered as buffer lines. Product application rate was of 200 L ha^{-1} (pre-véraison applications) or 300 L ha^{-1} (véraison to harvest). Applications were carried out with a motorised backpack sprayer (table 1).

Table 1. Natural products applied against BBR on Bordeaux vineyards in 2015.

Commercial name	Active ingredient	Dose	Brand	Registration status
Sokalciarbo Surround	Calcined Kaolin	10 Kg ha^{-1}	Agrisynergie De Sangosse (Pont-du-Casse, France)	NODU Vert Biocontrol list (France)
Wicker tea (*Salix* spp)	Dried plant	10% dilution of concentrated solution (100 g of in 3 L)	Bioservices (France)	Registered in France
Armicarb	Potassium bicarbonate	3 Kg ha^{-1}	De Sangosse (Pont-du-Casse, France)	NODU Vert Biocontrol list (France)
Fungicover	Fatty acid emulsion	15 g L^{-1}	BioDurcal (Granada, Spain)	Registered in Spain
M3AEY	Terpenes	4 L ha^{-1}	Sumi-Agro (Paris, France)	Authorised for research issues (France)

Applications were carried out following: 1) key phenological stages: 10% Flowering, 100% Flowering to Fruit set, Pre-bunch closure, Véraison and Fruit ripening (one and two sprays during fruit ripening in St. Yzan or Montagne sites, respectively). A specific early season treatment with Calcined Kaolin was included, consisting of the three first applications only.

2) Decision rules based on DRI: during post-véraison period, hourly weather forecast data (48h forward) for T and RH were introduced in the DRI formula to obtain a daily forecasted DRI for the following days. Decision rules were applied to these DRI values in order to trigger or not a field application. The practical outcome of this DRI-based strategy was three applications after véraison in 2015. The products applied using DRI were: Calcined Kaolin and K-Bicarbonate products in Montagne, Bicarbonate product in St. Yzan.

Statistical analysis

Percentage data of incidence and severity of BBR was correlated by Simple Linear Regression (SLR) to the percentage of *B. cinerea* incidence on floral calyptras and to cumulated DRI values for each season considered. A residue analysis was performed in order to take into account the effect of climatic conditions on BBR progression, represented by the DRI. Residue was calculated as the distance from each particular BBR Severity value to the regression line between % BBR Severity and % DRI cumulated values. Variability in the residue values may be linked to secondary inoculum presence, since the effect of climatic conditions was the same for all the replicates within one season. These residue values were then correlated by SLR to the *B. cinerea* incidence on floral calyptras (%). Treatment effects in the field efficacy experiment were explored by Analysis Of Variance. Significant treatment differences were determined by Newman–Keuls Test ($p = 0.05$). Every statistic procedures were performed using XLSTAT software (Addinsoft, Paris, France).

Results and discussion

Early disease risk indicator: The case study of floral calyptras

The linear regression analysis showed no significant positive correlation between the *B. cinerea* incidence (%) of floral calyptra and the BBR (%) incidence or severity ($r = -0.44$ and $r = -0.47$, respectively; data not shown). This result confirmed the complexity of the relationship between secondary inoculum built up and BBR intensity in maturing berries. This BBR level is significantly determined by favourable weather conditions for *B. cinerea* development. The influence of weather conditions was evidenced by the significant positive

relationship between cumulated DRI values and BBR severity (%) ($p < 0.01$; Figure 1) or incidence (%) (r = 0.73, $p < 0.01$; data not shown).

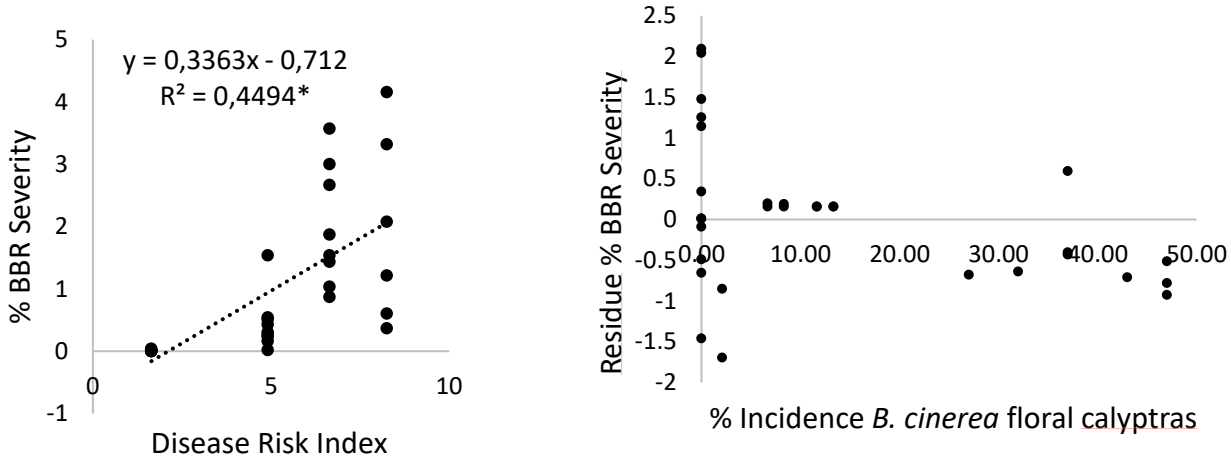

Figure 1: Correlation between percentage of *Botrytis* bunch rot severity and Disease Risk Index (cumulated daily values) based on temperature and relative humidity. Data from six replicate plots in an experimental vineyard near Bordeaux (2012 to 2015). BBR assessment was carried out approximately 30 days after mid-véraison.

Figure 2: Correlation between residues issued from the previous regression analysis and *B. cinerea* incidence on floral calyptras (%). Data from six replicate plots in an experimental vineyard near Bordeaux (2012 to 2015). *B. cinerea* incidence on floral calyptras was assessed at the end of flowering. BBR assessment was carried out 30 days after mid-véraison. Residue was calculated as the difference between each severity value and the severity value predicted by the regression line.

Influence of weather conditions was also evidenced by some trends shown in Table 2. Significant differences were detected between seasons in the three *B. cinerea* and BBR variables. Higher DRI values in the post-véraison period corresponded to significantly higher BBR, whereas this trend was not clear for the *B. cinerea* incidence on floral calyptras. In 2014, the lack of relationship between calyptras infection and pre-flowering rainfall or cumulated DRI values (i.e. 0.0 % incidence after 56.5 mm of rainfall) was not explained by variations in the cumulated wind speed and may depends upon other factors non included in this study, highlighting again the complexity of the floral infection process.

Table 2: Climatic features of two main periods during grapevine phenology (approx. 20 days before calyptras assessment and 30 days after mid-véraison) and quantification of *B. cinerea* infection in necrotic calyptras and maturing berries (cv. Merlot) near Bordeaux.

Year	15 days before mid-flowering to floral calyptras assessment			Floral calyptras assessment	Mid-véraison to BBR assessment (approx. 1 month)			[3]BBR development	
	∑Wind (m/s)	∑[1]PP (mm)	∑[2]DRI	% *B. cinerea* infection on calyptras	∑Wind (m/s)	∑PP (mm)	∑DRI	Incidence (%)	Severity (%)
2012	39.0	39.0	2.7	10.0b	46.9	2.5	1.64	0.4b	0.01b
2013	55.7	186.5	6.7	39.3a	43.4	37.5	4.9	11.5b	0.4b
2014	42.0	56.5	5.6	0.0c	51.7	63.5	6.6	36.7a	2.0a
2015	34.6	6.5	4.2	0.7c	48.8	109.5	8.2	29.1a	1.95a

[1]Cumulated rainfall; [2]Disease Risk Index; [3]*Botrytis* Bunch Rot

Since weather conditions are influencing berry infection of maturing berries late in the season, the influence of the secondary inoculum quantity could be better evidenced by removing the effect of meteorology in the statistical analysis. For that purpose, a residue analysis was carried out (see Materials and methods) in order to try to show the effect of differences in secondary inoculum quantity on the variability in BBR incidence and severity.

The correlation between BBR severity residue and the infection of floral calyptras is shown in Figure 2. No significant positive correlation was observed. Therefore, BBR variability observed in the different plots was not related to a lower or higher infestation of floral tissues by *B. cinerea*, although the effect of weather conditions had been partially excluded of the analysis. This result allow us to further discuss the epidemiological role of secondary inoculum build up in grape bunches, the phenomenon of latency and saprophytic colonisation by *B. cinerea*. Flowering treatments have demonstrated to be the most effective in a variety of fungicide timing experiments (Calvo-Garrido, et al., 2014b, Keller *et al.*, 2003, Petit *et al.*, 2010). However, other studies showed early stages in the season to be less important (de Kock & Holz, 1994, Viret *et al.*, 2010). Also, removal of bunch debris at fruit set has shown to reduce, with a certain variability, BBR at harvest (Wolf, et al., 1997). Furthermore, incidence of latent infection and infected fruitlets and calyptras, evaluated at véraison, were correlated to BBR levels at harvest in a Spain field study (Calvo-Garrido, et al., 2014b). In the same study, most of these latent, or saprophytic, infections at véraison had been produced

at flowering. Thus, several studies have shown an important relationship between the early season infestation and the BBR later in the season, even if results are sometimes variable. However, the evaluation of flowering infection in our study has not been a good early risk indicator to predict BBR variability after véraison. Since infections are produced at flowering, as well as secondary inoculum present at véraison seem to be a determining factor, the authors consider that the development and/or disappearance of this floral infection during the early season might be a key point to understand this partially unknown process in the epidemiology of *B. cinerea* in vineyards. Although some recent works are considering this and other topics on BBR epidemiology (Jaspers *et al.*, 2015), more research should be done on the quantification of different inoculum sources and the factors determining their temporal evolution between flowering and fruit ripening.

In any case, the present results confirmed that the *B. cinerea* incidence (%) on floral calyptras might not be a good early disease risk indicator. The easy assessment methodology and the early dates in the season to gain this information made it a good indicator candidate for its adoption by growers and extension services. Nonetheless, the analysis performed in this work did not allow us to show any positive correlation, but new analysis under different vineyard conditions may be also conducted in order to find a relationship between floral infection levels and BBR intensity in maturing fruit.

Field evaluation of new alternative products to control BBR

The percentages of BBR incidence and severity in the control and treated plots are shown in Figure 3. Since treatments were not exactly the same in both sites, results are presented separately. In St Yzan field site (Figure 3a), the untreated control presented 67.0 % incidence and 13.2 % severity. No significant differences were detected between the control and any of the treatments. The treatments showing lower BBR incidence and severity were the Armicarb (35.5 % and 4.7%, respectively), Kaolin-ES (52.0 % and 9.3 %, respectively) and Fungicover (53.5 % and 9.4 %, respectively). Interestingly, two other treatments increased BBR incidence or severity compared with the untreated control: Wicker Tea (77.5 % and 25.2 %, respectively) and M3AEY corresponding to terpenes (64.5 % and 18.2 %,

respectively). The only significant difference was shown between Wicker Tea and Armicarb treatments, which presented the highest and lowest BBR values.

In the Montagne field site (Figure 3b), control presented 58.5 % BBR Incidence and 9.4 % BBR severity. No significant differences were observed among any of the treatments, nonetheless, some of the trends are similar to the results in St. Yzan. For example, the favourable impact of the Wicker Tea product on BBR, as well as the lowest incidence and severity in the Armicarb treatment plots. The terpenes-based product (MA3EY) also presented similar BBR levels than the control, while Fungicover exhibited a relatively lower efficacy in this site, compared with results in St. Yzan site. It is important to mention that Fungicover was applied at the lower rate recommended by the manufacturer and a higher dose could be determinant to achieve higher reductions, as in previous studies (Calvo-Garrido *et al.*, 2014a, Calvo-Garrido *et al.*, 2013).

The Kaolin application showed an intermediate efficacy in both vineyards, especially performing when applications were carried out only before véraison. Although it was not significantly effective in our experiment, this is a common strategy used by organic winegrowers in the region, achieving good results in many cases with a reduced treatment cost. Thus, this early season applications should be also explored in the future, for example, by combining them with a late season treatment using a different product.

Considering the DRI-based applications, focusing on the kaolin product, it did not show a good performance compared to the full season or the early season strategies. As for Armicarb, the application following DRI slightly improved its effect compared to the five-application strategy in Montagne, but it did not in St. Yzan. This different efficacy pattern may be related to a heavy rain episode just after the application in St. Yzan (data not shown), while in Montagne the application was carried out after rainfall.

In conclusion, results showed that the percentage of *B. cinerea* infection of floral calyptras does not represent a reliable early indicator of BBR epidemic risk after véraison. Our study evidenced the complexity of BBR epidemics in vineyards and indicated key possible topics for further research, especially the evolution of floral infections between flowering and véraison. Regarding the alternative strategies tested, our results in two organic vineyards pointed, overall, three natural products as the most interesting for BBR control (Armicarb,

Kaolin and Fungicover) and a product with a very low interest as an alternative control strategy in our conditions (Wicker Tea), whereas they also highlighted the importance of dose and application timing when dealing with new strategies.

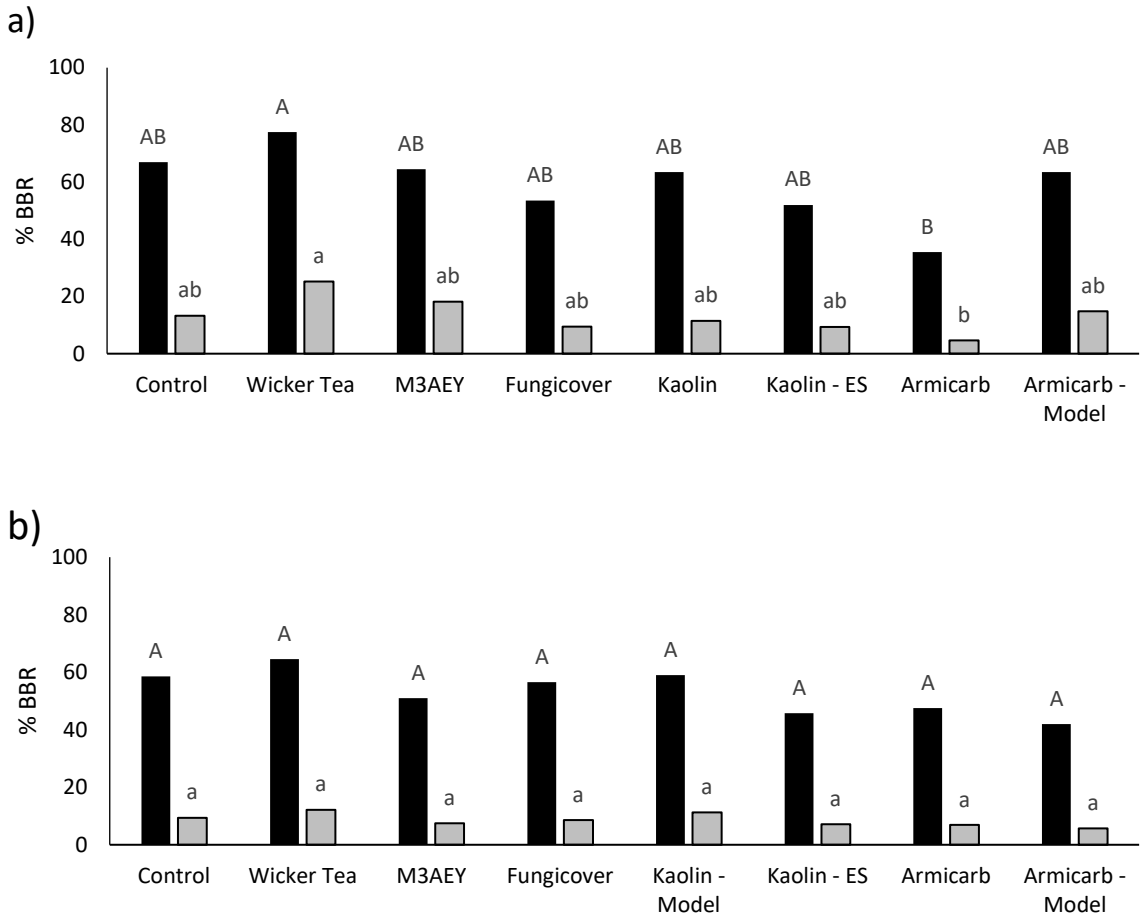

Figure 3. Efficacy of natural products applied to control *Botrytis* bunch rot in two organic vineyards near Bordeaux in 2015. Incidence (black bars) and severity (grey bars) were assessed at commercial harvest in St. Yzan (a) and Montagne (b) field sites. Treatments consisted on 5 or 6 spray applications (St. Yzan and Montagne, respectively) at key phenological stages. ES (Early Season): only three sprays before véraison; Model: only three post véraison sprays, following a decision rule based on a Disease Risk Index. For upper and lower case, values linked by the same letter are not significantly different ($p = 0.05$) according to Newman-Keuls test.

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