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ABSTRACT

Botrytis bunch rot (BBR) is a major disease occurring in vineyards worldwide. Its control is still largely based on the use of synthetic fungicide sprayings at predetermined intervals, which often produces negative residues in grapes and wines that may affect the environment and/or human health. To rationalize BBR management, disease risk indicators were developed and evaluated in a set of field experiments carried out between 2010 and 2019 in France and Chile. Key indicators include early grapevine vegetative growth, i.e. ground-based normalized difference vegetation index (NDVI), and the potential berry susceptibility to *Botrytis cinerea*, which is driven by tannin content in the skin. Under these contrasting weather and cropping conditions, regression analyses, including weather information, showed a highly significant positive correlation between the early NDVI, measured at berry pea size stage, and BBR incidence or severity at harvest, whereas the opposite was demonstrated for tannin content in the berry skin measured at an early herbaceous fruit stage. The exponential relationship between the final disease severity and the early NDVI allowed us to identify a possible threshold NDVI value, i.e. between 0.5 and 0.6, under which the BBR severity should be lower or close to 5% at harvest (BBR tolerance threshold for wine quality). Accordingly, in a leaf-removal vineyard experiment in France, the NDVI level at berry pea size stage was strictly controlled to correspond to three different increasing index values: 0.45, 0.60, and 0.78. Following this increase in NDVI, a significant increase in final BBR severity was noticeable, i.e. 2.4%, 6.6% and 9.9%, respectively. Very interestingly, the NDVI increase was also related to a significant decrease in the tannin content in the skin of herbaceous berries at veraison, from 47 to 22 mg tannin/g skin. All regression analyses explaining BBR development were highly significant, showing the weather conditions before harvest as the primary factor. They also show that some of the disease risk indicators chosen, especially early NDVI, could be used in the future as tools in decision support systems for deciding to spray and/or scheduling optimized fungicide applications during the season.

KEYWORDS

Botrytis cinerea, vegetative growth, integrated pest management (IPM), susceptibility, skin tannin content, skin pectin content, *Vitis vinifera*

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/2954

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), can infect all the organs of the plant but mainly damages ripening berries. BBR can reduce vine yield and compromise the wine quality, since a vintage contaminated by the pathogen at harvest at 5% severity will show irreversible consequences on the organoleptic features in a qualitative red wine (Ky *et al.*, 2012). *Botrytis cinerea* can penetrate vine tissues either through wounds or directly via the cell wall. During springtime, airborne spores of *B. cinerea* can infect floral tissues and/or fruit pedicel and the pathogen remains in a latency state until veraison, the point when the susceptibility of grape berries to *B. cinerea* begins to increase (Pezet *et al.*, 2003; Pezet *et al.*, 2004; Elmer and Michailides, 2004; Deytieux-Belleau *et al.*, 2009). Furthermore, although flowering is a key BBR epidemiological stage (Gonzalez-Domínguez *et al.*, 2019), latent infections may also establish at later stages throughout the season (Hill *et al.*, 2014) and direct infections may play an important epidemiological role during berry maturation, when the ontogenic resistance of berries decreases (Elmer and Michailides, 2004; Deytieux-Belleau *et al.*, 2009; Hill *et al.*, 2014).

The control of BBR still largely relies on the use of synthetic fungicide spraying at predetermined intervals. However, restrictions on the use of fungicides are increasing to reduce the negative effects of these pesticides in terms of fungal resistance, human health, and the environment (Fenner *et al.*, 2013). Therefore, strategies to control *B. cinerea* must be optimized, notably by considering the main principles of integrated pest management (IPM), according to which the risk to disease must be assessed before direct control measures are used (IOBC, 2007). Disease risk assessment should consider factors that favor disease development, including weather conditions, crop susceptibility and key grapevine phenological stages.

The weather conditions and canopy microclimate are considered the main factors involved in *B. cinerea* development (Pieri and Fermaud, 2005; Latorre *et al.*, 2015; Hill *et al.*, 2019). 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 berry surface, which is essential for conidial germination and berry infection. Rainfall and fog periods also contribute to the presence of free water on berries that is crucial during flowering, by increasing both direct and/or latent infections, and during the ripening post-veraison period by facilitating secondary infections via asexual conidia from sporulating host tissues (Broome *et al.*, 1995; Elmer and Michailides, 2004). Accordingly, different weather-based models have used this information to predict or explain *B. cinerea* infections and disease progress (Broome *et al.*, 1995; Ciliberti *et al.*, 2015a; Ciliberti *et al.*, 2015b, Ciliberti *et al.*, 2016; Hill *et al.*, 2019).

Furthermore, grapevine susceptibility and/or resistance to BBR depends on various genetic and phenotypic traits, such as morphological, anatomical, and chemical features of the berry skin (Deytieux-Belleau *et al.*, 2009; Latorre *et al.*, 2015; Blanco-Ulate *et al.*, 2016). The skin tissue is acting as a barrier by both physical mechanisms, including cuticle integrity, skin anatomy, microcracks, and biochemical mechanisms based on induced and/or pre-formed defense compounds. The cell walls are among the first plant tissue structures that *B. cinerea* encounters when infecting and colonizing the berry skin, and therefore can contribute to susceptibility to the pathogen (Blanco-Ulate *et al.*, 2016). An important component of berry skin cell walls consists of pectins, polysaccharidic polymers that are abundant in the middle lamella and the cell corners and are the main cell wall targets during infection by *B. cinerea* (Blanco-Ulate *et al.*, 2016). Then, the growth of *B. cinerea* may be favored by the sugar nutrients released from hydrolyzed pectins. In contrast to pectins, other components, including tannins, are deposited in the berry skin cell walls, which provide a protective barrier against the fungus (Lecas and Brillouet, 1994; Schlosser *et al.*, 2008; Deytieux-Belleau *et al.*, 2009). Tannins are produced at variable concentrations and inhibit the fungal enzymes (such as laccases) degrading the cell wall, giving partial resistance against the pathogen (Goetz *et al.*, 1999).

Another key feature involved in BBR development is grapevine vigor favoring *B. cinerea* infection as the foliar density increases (Valdés-Gómez *et al.*, 2008; Latorre *et al.*, 2015). Dense grapevine canopies have been associated with increased periods of wetness within the cluster zone, following rainfall, dew or fog, which results in more conducive environmental conditions to the pathogen (Fermaud *et al.*, 2001; Elmer and Michailides, 2004; Pieri and Fermaud, 2005; Hill *et al.*, 2019). Therefore, important prophylactic cultural methods for controlling BBR include grapevine canopy management practices that reduce canopy vegetative expression, growth, and/or foliar density around the clusters, for improving air circulation and exposing fruit to light. First, vine training and pruning systems may be manipulated (Elmer and Michailides, 2004; Elad, 2016). Second, a very effective practice to reduce BBR development is leaf-removal in the fruit zone (Percival *et al.*, 1994; Elmer and Michailides, 2004). In New Zealand, as in other grapevine-growing countries such as France, leaf-removal is implemented between late flowering and berry pea size stages, and is considered the most effective cultural management for controlling BBR (Elmer and Wood, 2016). Leaf-removal before flowering has also been shown to be effective to reduce BBR, by interfering with bunch compactness of Merlot grapes (Sivilotti *et al.*, 2016).

In this context, it is important to quantify the vegetative expression of the vineyards and associate it with BBR development. In grapevine, the vegetative expression can be quantified by direct methods (leaf area and/or pruning mass) or indirect methods, such as the normalized difference vegetation index (NDVI). The NDVI was originally used to determine the density of green parts on a patch of land, using the difference between spectral bands (near-infrared and red light) recorded by multispectral cameras mounted on satellites (Rouse *et al.*, 1974). The NDVI is the ratio of the difference between the reflectance in the near-infrared and the red regions of the spectrum to the sum of these two values (Rouse *et al.*, 1974). The NDVI exploits the spectral properties of green plant tissues due to two complementary grounds. First, light reflected from leaves is enriched in the far-red region of the spectrum. Second, chlorophyll makes leaves strong absorbers of photons in the red region of the spectrum. Thus, in viticulture, when considering vertical shoot positioned vines during the growing season, the NDVI has the potential to represent, quantitatively, the leaf area index in the center of the row, taking also into account the gap fraction of the canopy (Drissi *et al.*, 2009). Furthermore, the use of NDVI may have many applications, for example, used as a proxy for tree biophysical features (van Dijke *et al.*, 2019) or for studying cultivated plant biomass and the nitrogen status (Cao *et al.*, 2013; Yao *et al.*, 2014; Zhang *et al.*, 2017). In viticulture, it has been also used near harvest for predicting yield (Sun *et al.*, 2017; Di Gennaro *et al.*, 2019) and characte-rizing the mature grape composition (Ferrer *et al.*, 2019). The veraison stage has been proposed as the best phenological stage to measure and correlate the NDVI value with harvest yield and/or grape composition (Sun *et al.*, 2017; Di Gennaro *et al.*, 2019; Ferrer *et al.*, 2019). However, under Mediterranean conditions the spatial NDVI pattern (airborne image) acquired at 15 to 20 days before veraison showed a significant correlation with an image acquired at harvest (Kazmierski *et al.*, 2011).

Furthermore, different studies have been published that show the relationship between grapevine vegetative growth and BBR development (Table 1). Unlike the other studies based on one cultivar in one site for –two or three seasons, only one study by Valdés-Gómez *et al.* (2008) proposed a vegetative growth threshold by integrating different cultivars and

TABLE 1. Scientific reports evidencing the relationship between grapevine vegetative growth and BBR development.

Vegetative growth indicator	Index threshold value	Phenological stage	Reference
Leaf area	NA	Pea size stage	Percival *et al.* (1994)
Pruning mass	NA	Winter	Reynolds and Wardle (1994)
Pruning mass	NA	Winter	Intrieri *et al.* (2001)
Pruning mass	0.5 to 0.6 kg/m	Winter	Valdés-Gómez *et al.* (2008)
NDVI	NA	Veraison	Gatti *et al.* (2017)
NDVI	NA	Veraison	Ferrer *et al.* (2019)

NA, not available; NDVI, normalized difference vegetation index.

management conditions worldwide. Furthermore, in all the studies (Table 1), the relationship between grapevine vegetative growth and BBR development was always based on a late vigor evaluation during the season, i.e. from veraison onwards including winter. Such late-assessed management indicators are then of very limited practical use for scheduling of phytosanitary applications and/or adjustment of the management prophylactic practices that must be implemented as early as possible during the season.

Key stages in grapevine BBR control are flowering and bunch closure, and presumably stages between these phases (Zoffoli *et al.*, 2009). Although *B. cinerea* develops mostly late in the growing season, early infections play a key role in disease development and fungicide applications are used during these periods to reduce the early fungal inoculum and/or infection (Ciliberti, 2015a; Elmer and Michailides, 2004; Hill *et al.*, 2014; Pezet *et al.*, 2003; Pezet *et al.*, 2004). Despite the importance of these critical stages, only a few studies have investigated the relationships between disease development at harvest and grapevine features evaluated at early vine phenological stages (e.g., berry pea size and pre bunch closure) (Deytieux-Belleau *et al.*, 2009; Intrieri *et al.*, 2001; Percival *et al.*, 1994; Valdés-Gómez *et al.*, 2008). 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 (berry pea size and pre bunch closure), including pectin and tannin contents in berry skin and the grapevine vegetative growth, may account for the BBR incidence and severity at harvest. Thus, the objective of this work was to develop and evaluate early BBR risk indicators related either to the potential favorable microclimatic effect of the vine vigor on *B. cinerea* or to the grapevine fruit susceptibility to the pathogen. A more focused and key aim was to establish and characterize the relationships between the early NDVI, considered as a crucial factor influencing microclimate in the bunch zone, and the BBR incidence and severity at harvest.

MATERIALS AND METHODS

1. Experimental sites

Experiments were carried out in two vineyards with the Merlot cultivar: the first was in the Aquitaine Region (France) at the site ‘Grande Ferrade’ (Villenave d’Ornon 44°47′15.4′′N, 0°34′37.43′′W, 22 m.a.s.l); the second was in the Maule Region of Chile (Panguilemo, 35°22′14.4′′ S, 71°35′37.2′′ W, 125 m.a.s.l), which also included a trial with Sauvignon blanc. This last highly susceptible cultivar to *B. cinerea* was included in order to express BBR symptoms under the Chilean climatic conditions, which are not very favorable to BBR development (Pañitrur-De la Fuente *et al.*, 2018). Furthermore, analyses using pooled data, from two cultivars differing in susceptibility, could give more generic results than those based on one cultivar only. The suitable general characteristics of the two experimental vineyards are summarized in Table 2. The experiments were carried out during eight seasons in France (2010 to 2016 and 2019) and three seasons in Chile (2014–15, 2015–16, 2016–17).

TABLE 2. Vineyard characteristics of the experimental fields.

Field characteristics	France	Chile
Experimental period	2010 to 2016	2014–15, 2015–16 and 2016–17
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 × m)	1.8 × 1.0	2.0 × 1.0
Trellis system	Vertical shoot positioning	Vertical shoot positioning
Pruning system	Double guyot	Two-bilateral spur cordon
Toping and trimming	Four times per season	Once per season
Soil management	Chemical weed control over the whole soil surface	Chemical weed control over the whole soil surface
Drip irrigation system	Non-irrigated	One dropper per plant, flow rate of 4 L/h
Irrigation frequency	None	Twice per week, 3000–4000 m^3/ha/season

2. Assessment of vegetative growth

Grapevine vegetative growth was measured at 300 growing degree days (GDD) accumulated from flowering '50% caps off = full bloom' (code 23), roughly equivalent to the berry pea size stage (code 31). Cumulated GDDs were calculated based on the daily average temperature measured at each experimental field and a base temperature for grapevines of 10°C. The NDVI was obtained from measurements performed using a hand-held GreenSeeker® RT-100 (Trimble Agriculture division, Sunnyvale, California, USA) as described by Drissi *et al.*, (2009). This last study was used as our experimental rationale because it corresponded to similar vineyard conditions, row architecture and trellising system (Table 2). This hand-held unit optical sensor uses high-intensity light emitting diodes (LEDs) at 660 nm (*R*) and 770 nm (NIR), pulsed at high frequency. The magnitude of the light reflected off the target is measured by a photodiode detector and electronic filters remove all background illumination. The temperature-stable sensor scans a 61 × 1 cm area. Unlike standard remote sensing and standard use of the GreenSeeker (vertical toward the ground), it was oriented horizontally toward the canopy at a 1 m distance, i.e. the GreenSeeker® was pointed sideways at the vertical shoot positioned vines. Thus, a grass cover below the vines could not interfere with the NDVI measurements. The use of a screen placed behind the canopy (on the other side of the vine row where measurements were executed) was necessary to differentiate canopy from background interference. According to Drissi *et al.* (2009), a white screen with a low NDVI value (~0.08) was used for all NDVI measurements. The results were expressed as a numerical index ranging from 0 to 1, with the 1 value representing the maximum vigor, corresponding to maximum LAI (and leaf density) in the center of the rows (NDVI values not scaled to the center of row values). In Chile, four replications per season were performed and between six and eleven replications in France, according to the season.

3. Experimental designs and cropping conditions

3.1. Long-term experiments in France and Chile (2010–2017)

In the non-irrigated French vineyard, between six and eleven replications, according to the years 2010 to 2016, were distributed in a randomized design across the field. Each replication consisted of a total of five adjacent vines. In the drip-irrigated vineyard in Chile, four replicated blocks per vineyard (Merlot and Sauvignon blanc) were distributed in a randomized block design to minimize the effect of soil slope. Each replicated block consisted of a total of 15 adjacent vines. To evaluate the development of *B. cinerea* at harvest, no fungicide with known activity against *B. cinerea* was applied at either site or season. The fungicides used to control other grapevine diseases (powdery and downy mildew) were specific to these target diseases, and not recommended or not registered against *B. cinerea.* Therefore, no side efficacy, or a negligible one, should be expected. The vineyards were protected against European Grapevine Moth, and sulfur sprays were applied in both countries to avoid powdery mildew. Additionally, one application of quinoxyfen (Legend, Dow AgroSciences, Frenchs Forest, NSW, Australia), one of tebuconazol (Corail, Bayer, Lyon, France) and one of trifloxystrobin (Natchez, Bayer, Lyon, France) were used to control powdery mildew in France, whereas one application of flusilazol (Nustar, DuPont de Nemours SA, Cernay, France) and one of penconazol (Topas, Syngenta, Fulbourn, England) were applied in Chile. Furthermore, downy mildew was controlled only in France with four fungicide applications per season, corresponding to two applications of cymoxanil (Option, DuPont) and two copper (Heliocuivre, ActionPin, Castets, France) applications. In Chile, due to the unfavorable conditions for this disease, no sprays were applied.

3.2. One-year controlled leaf-removal (LR) experiment in France (2019)

In France, in the vineyard plot (Merlot) an experiment with controlled LR was carried out in 2019. The aim was to show, experimentally in the vineyard, the effect of three different early NDVI levels on i) the incidence and severity of BBR at harvest, and ii) the skin tannins content in grapevine berries at veraison. According to our results obtained in previous years (Figure 4), three very different early NDVI levels were selected, within the range of possible already observed values (between 0.3 and 0.85), to cause potentially three very different BBR incidences

(Figure 4a): less than approximately 10% incidence when NDVI < 0.45; less than approximately 30% BBR incidence when NDVI < 0.6; and around 60–80% BBR incidence for a NDVI value near 0.8 (near saturation).

Thus, the three experimental controlled LR intensities (treatments) were as follows: severe LR level (NDVI=0.45); intermediate LR level (NDVI=0.60); and control treatment without LR (NDVI=0.78).

The NDVI was obtained from measurements using a hand-held GreenSeeker® RT-100 as described above. Removal of leaves causes NDVI to decrease because the measurement estimates, quantitatively, the vertical leaf area index in the center of the row, taking into account the gap fraction of the canopy (Drissi *et al.*, 2009). Thus, to reach intermediate and severe LR levels, leaves were removed stepwise and the NDVI was measured accordingly and regularly. At pea size stage, the whole experiment was conducted during one day, 3 July, corresponding to 319 GDD accumulated from flowering. The leaves near the clusters were removed by hand on the east side of the row for the severe and intermediate LR treatments, until reaching the targeted NDVI values. For some 14-vines-unit-plots, in the severe LR treatment only, secondary lateral growing shoots within the canopy were also removed. The trial was distributed in a Latin Square (main factor with the three LR treatments) with three replicated blocks, with one block consisting of one entire row of 42 vines (each replication consisted of a total of 14 adjacent vines). In every experimental unit, the skin tannins content was assessed. For this, two clusters, closest to the trunk and located in the shade in the afternoon, were collected at the veraison stage on 5 August, and kept at -20°C until the biochemical analysis described in the 'Tannin content in berry skin' section below. Vineyard phytosanitary management, i.e. pest and disease control, was similar to that described above for this French vineyard plot.

4. Weather data collection and phenology

Weather conditions were monitored at each site with an automatic weather station (AWS) installed 50 m from the trial fields (Adcon Telemetric, A730, Klosterneuburg, Austria in Chile and Cimel Electronique S.A.S, CimAGRO, Paris in France). Air temperature, relative humidity and precipitation were recorded in 15 min intervals. To estimate favorable conditions for disease development after veraison and to account for differences in BBR development between both countries, two climate indices were calculated using pluviometry data as follows: PL15, cumulative amounts of rain (mm) from 15 days before harvest to harvest; PL35, cumulative amounts of rain (mm) from 35 days before harvest to harvest. Both climate indices were calculated in each season and site. The main phenological stages were observed in each season and site using the Eichhorn and Lorenz scale, modified by Coombe (1995), assigning a code number from 1 to 47 to each stage. Phenological observations were performed weekly, by recording at budbreak (first leaf tissue visible, code 4), flowering 'full bloom' (50% caps off, code 23), berry pea size (berries 7 mm diameter, code 31), beginning of bunch closure (code 32), veraison (berries begin to color and enlarge, code 35) and harvest (berries harvest-ripe, code 38).

5. BBR incidence and severity

In the long-term experiments, BBR incidence and severity in all growing seasons were evaluated at harvest (total soluble solids ~25° Brix) in approximately 250 and 300 grape clusters randomly chosen per experimental unit in France and Chile, respectively. In the one-year LR experiment, at harvest (7 October 2019), BBR incidence and severity were measured by sampling randomly a minimum of 100 grape clusters, chosen from the east side of the row, in every experimental unit. In every grapevine cluster sampled, BBR severity was assessed by evaluating, visually, the percentage of the area typically rotted and/or sporulating, by looking at the outer surface of all observable berries. 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 rotted and/or sporulating area. Both incidence and severity were expressed as percentages.

6. Biochemical berry skin assessment

Analyses of pectin and tannins were performed to relate berry skin susceptibility to *B. cinerea*. For this, 20 clusters per experimental plot were collected at berry pea size (code 31) 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 vines, e.g., normal in vigor, and without visible diseases or disorders. Once in the laboratory, berries were peeled to obtain 30 g of skin from which 15 g was used to determine pectin concentration and 15 g was used for the tannin assessment. To prevent oxidation and loss of experimental material during peeling, the fruit was processed at temperatures below 0°C using ice and liquid nitrogen.

6.1. Pectin content in berry skin

Non-alcohol-soluble compounds (NAS fraction) were separated by a fractional process as proposed by Chénet (1997). Skins (5 g) were boiled for 10 min in 250 mL of 95% ethanol, ground 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 95% ethanol and re-centrifuged, usually three 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 µm). Then, 0.1 g of the NAS fraction was diluted in 20 mL of distilled water and 0.2 mL of 95% ethanol and shaken horizontally for 16 h at room temperature (~24°C). 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 1/10, and the concentration of galacturonic acid, expressed as mg/g NAS, was measured in three replicates using an adaptation of the colorimetric method described by Robertson (1979). In brief, 1.5 mL of 95% sulfuric acid was added to 300 µL of the previously prepared solution. Immediately after mixing, the tubes containing the solutions were placed in an iced water bath for 3 min. They were then heated in a boiling water bath for 6 min and immediately cooled again in an iced water bath. Finally, 30 µL of m-hydroxydiphenyl reagent was added to each tube, mixed, and stored in the dark for 20 min before reading the absorbance at 520 nm.

6.2. Tannin content in berry skin

The tannin (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 at room temperature (~24°C). Berry skins were stirred at 150 rpm with 5 mL of methanol containing 0.1% 12 N HCl (Gagné *et al.*, 2006) and filtered using a Falcon filter (100 µm). The tannin content was determined by spectrophotometry and expressed as mg/g skin using an adaptation of the methodology proposed by Ribéreau-Gayon and Stonestreet (1966). Next, 2.8 mL of distilled water and 3 mL of HCl 12 N were added to 0.2 mL of the previously prepared solution. The resulting solution was divided into sample 1 and sample 2; then, sample 2 was heated in a boiling water bath for 30 min and immediately cooled in an iced water bath. Then, 0.25 mL of 95% ethanol was added to each sample, and after mixing they were stored in the dark before reading the absorbance at 550 nm. Finally, the tannin concentration was determined as shown in the equation below. All experiments were performed in triplicate.

$$\text{Tannins} = 76.35 \times (\text{absorbance sample2 - absorbance sample1}) / [2 \times (\text{skin berry mass in g})] \quad (1)$$

7. Statistical analyses

The relationships between pectin (WSP) or tannin (TAN) contents in berry skin and BBR incidence and severity were explored using correlations and linear regressions. These analyses were carried out using a mean value of WSP, TAN and BBR infection for each study season. For the biochemical berry skin assessments, WSP and TAN, the mean in every year was calculated by using the three replicate measured values. As for both BBR incidence and severity in each year (and for each cultivar), the mean was based on between six and eleven replicate values in France and four in Chile. To determine if a correlation was significant, Pearson's correlation coefficient was calculated and tested based on $p = 0.05$. The relationship between BBR (incidence and severity) and vegetative growth (NDVI) was plotted and modeled using a nonlinear model. Furthermore, multiple linear regressions were performed by including the biochemical (WSP and TAN), vegetative (NDVI), and climate indices (PL15 and PL35) as explanatory variables. The climate indices (PL15 and PL35) were included to account for the important and well-known major climatic effect during fruit maturation in the BBR epidemiology. BBR incidence and severity values were previously transformed using an arcsinus function to improve variances homogeneousness. Variance inflation factors (VIFs) were calculated to detect multi-collinearity with the set of explanatory variables.

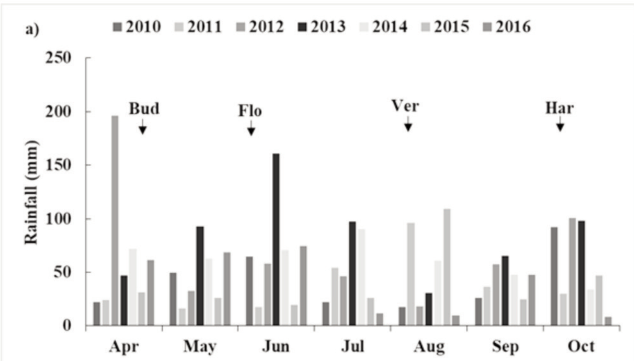
Finally, the number of significant variables to retain in the model was selected by using stepwise analysis with $p = 0.05$. All data analyses were performed using SAS University Edition software.

RESULTS

1. Climatic conditions

In France, all growing seasons evaluated were characterized by humid and temperate conditions, which favored *B. cinerea* development. The average temperature between budbreak and harvest was similar in all seasons, fluctuating between 18°C and 19°C (data not shown), i.e., during the spring and summer period (April to October). Pluviometry differed between seasons, with the lowest rainfall amount in 2011 (275 mm) and the highest value in 2013 (593 mm) (Figure 1a).

The climate conditions in Chile were characterized by dry and temperate spring and summer periods, which were not conducive to BBR development. From budbreak to harvest, the average air temperature was similar in all seasons, fluctuating between 18°C and 19°C, as in France (data not shown). However, the total rainfall during the spring and summer period (October to April) was much lower than in France (Figure 1b). Only 36 mm, 115 mm, and 96 mm were recorded in 2014–15, 2015–16 and 2016–17, respectively. Furthermore, rain periods were mostly distributed before bunch closure, leading to unfavorable conditions, during fruit maturation, for BBR development and disease symptom expression.



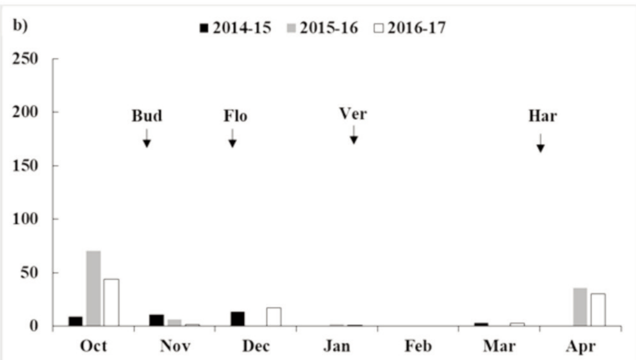


FIGURE 1. Monthly cumulated rainfall (mm) in (a) France and (b) Chile.
Bud= Budbreak; Flo= Flowering; Ver= Veraison; Har= Harvest

2. BBR incidence and severity

In France, for all study seasons, the Merlot cultivar showed average values of disease incidence and severity of 57.1% and 8.6%, respectively. Nonetheless, the disease level varied greatly between years (Figure 2a), depending mainly on weather conditions. For example, 2013 was the most conducive year to BBR development (rainfall of 593 mm in the spring-summer period from 1 April to 31 Oct.), with mean incidence and severity values of 98.7% and 20.5%, respectively. In contrast, disease pressure in 2010 was the lowest among all years (rainfall of 295 mm in the spring-summer period), with an incidence of 28.7% and severity of 4.7% (Figure 2a). In Chile, under less conductive environmental conditions to BBR, the Merlot cultivar evaluated did not show any disease development in all studied seasons. The Sauvignon blanc vineyard, as expected, showed a mean disease incidence and severity of 16.3% and 2.7%, respectively. Thus, Sauvignon blanc in Chile always showed lower disease incidence and severity than the Merlot cultivar in France in all studied seasons (Figure 2).

3. Relationships between berry skin components and BBR intensity at harvest

Positive relationships were observed between the pectin content in the berry skin and BBR incidence and severity at harvest when considering all study seasons (Figure 3a,c), whereas negative relationships were observed between the tannin content and disease incidence and severity at harvest (Figure 3b,d). All correlations, obtained by analyzing pooled data from France and Chile, were significant at $p = 0.05$, except for the relationship between the pectin and BBR incidence (Table 3). However, it is important to highlight that the R^2 of all these

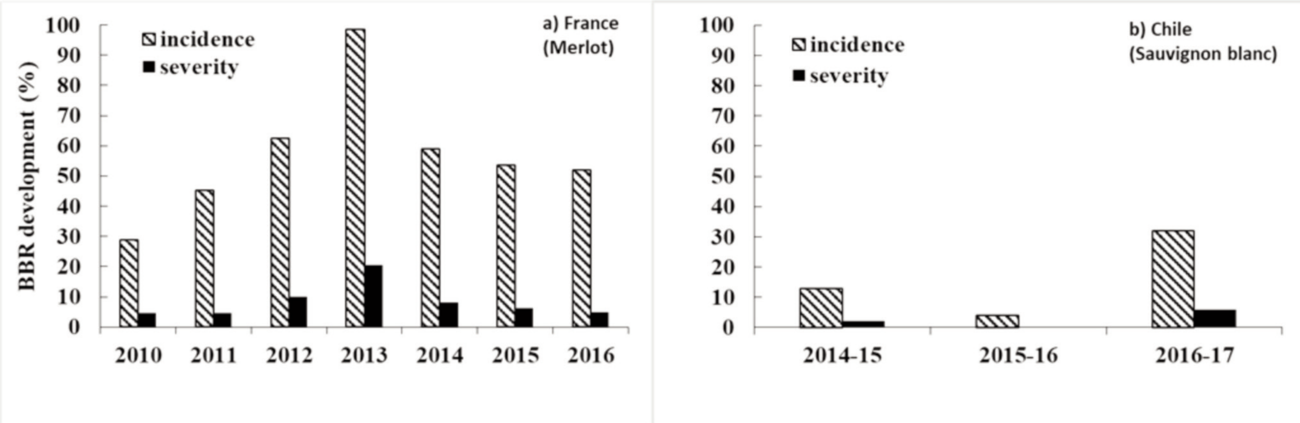


FIGURE 2. Mean BBR incidence and severity (%) according to the season under field conditions for (a) the Merlot cultivar in France and (b) the Sauvignon blanc cultivar in Chile.
Merlot in Chile did not show any BBR development and symptoms.

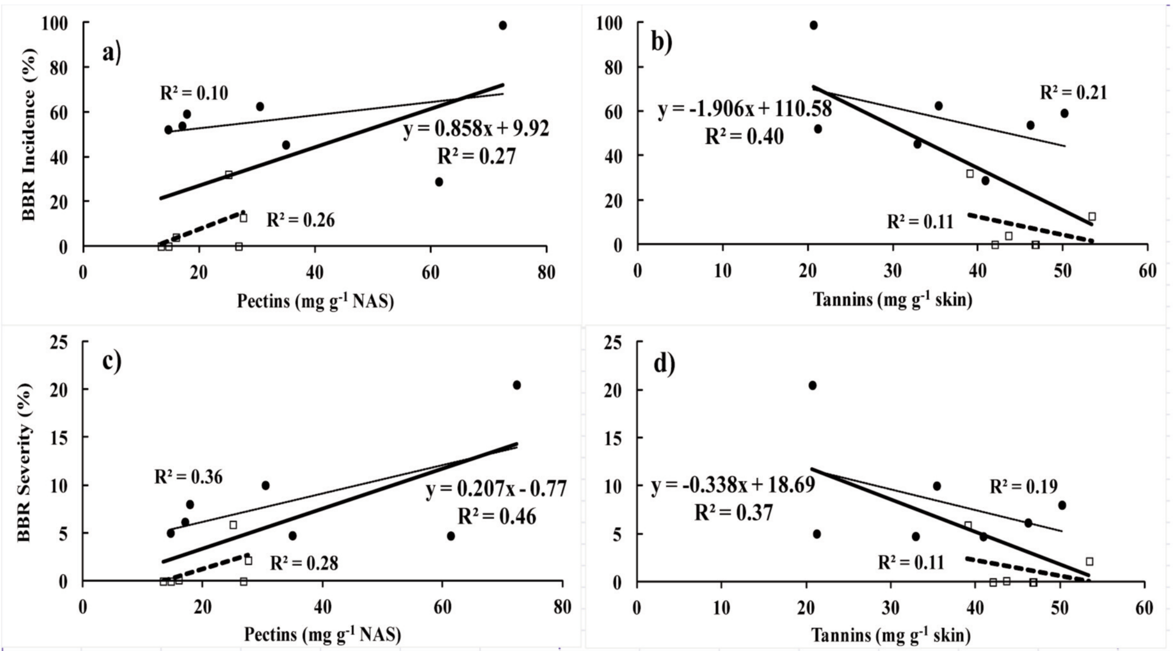


FIGURE 3. Relationships between BBR incidence (%) with the concentration in the young berry skin of (a) pectins and (b) tannins. Relationships between BBR severity (%) with the concentration in the young berry skin of (c) pectins (d) and tannins. In France (·) using cv. Merlot noir; in Chile (□) using cv. Merlot noir or Sauvignon Blanc. Each point corresponds to a study season. Statistical significances are indicated in Table 3.

overall relationships were relatively low (<0.5) (Table 3).

4. Relationships between vegetative growth and BBR intensity at harvest

Exponential relationships were established between the NDVI values evaluated at 300 GDD after flowering and BBR incidence and severity at harvest (Figure 4). The relationship between BBR (incidence and severity) and vegetative growth (NDVI) was plotted and modeled using a nonlinear model based on the equation BBRinc/sev = a*(NDVI)^b, which was the best model according to the R^2 value. The relationships between these variables were positive, showing that vines with higher vegetative growth in an early phenological stage

TABLE 3. Statistical significance with corresponding overall coefficients for relationships between BBR incidence (%) and severity (%), and pectin and tannin concentration in the berry skin.

	Pectins				Tannins			
	dF	R^2	r	P- value	dF	R^2	r	P- value
BBR incidence	12	0.27	0.52 ns	0.07	12	0.40	-0,63	*0,02
BBR severity	12	0,46	0,68	*0,01	12	0,37	-0,61	*0,03

dF = residual degrees of freedom; R^2 = coefficient of determination; R = Pearson's correlation coefficient, significant a p = 0.05 (*)

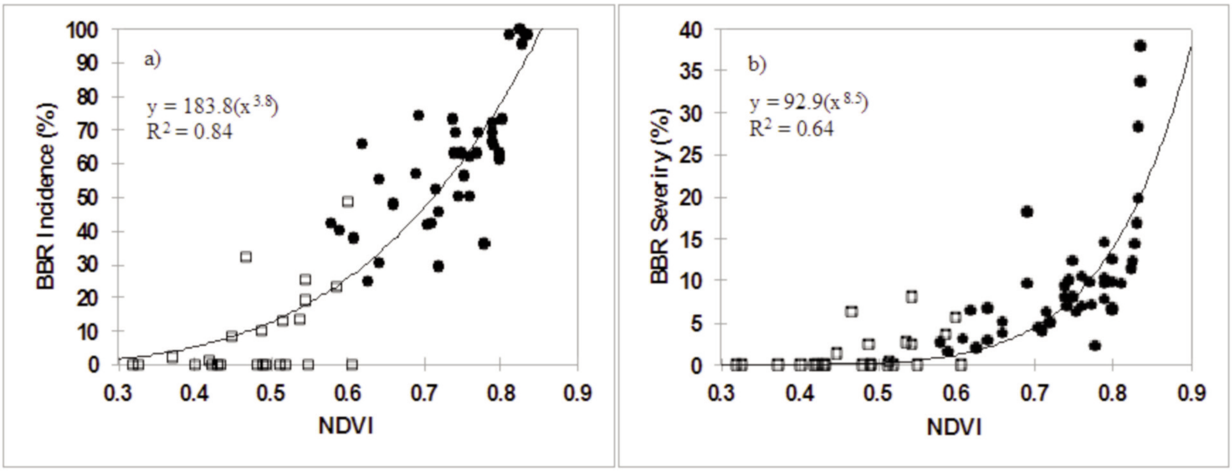


FIGURE 4. Relationships in France (●) and Chile (□) between (a) BBR incidence (%) with early NDVI evaluation and (b) BBR severity (%) with NDVI.

were more likely to be attacked by *B. cinerea* during the season. The pattern was similar for both disease variables, but with a steeper curve between NDVI and disease severity (Figure 4b). A trend was noticeable, showing a change in BBR incidence and severity when NDVI values reached approximately 0.5 and 0.6, respectively.

5. BBR at harvest related to explanatory variables: berry skin components, vegetative growth and climate

Multiple linear regression analyses were performed to study the relationships between the two response variables of BBR incidence and BBR severity at harvest and the following explanatory variables: i) pectins, ii) tannins, iii) vegetative growth early in the season, and iv) rainfall late in the season using the two indices PL15 and PL35 (Tables 4 and 5). All variance inflation factor (VIF) values were low (5.6 or less), indicating that multicollinearity was unlikely to exist with the set of explanatory variables. Therefore, all the predictor variables were used for the following regression analyses. Despite the contrasting weather conditions in Chile and France, it was possible to identify the main factors explaining the BBR development. The best models, explaining both BBR incidence and severity, included the pluviometry, a few weeks before harvest, and the NDVI evaluated early in the season.

First, the best model explaining BBR incidence, showing the highest R^2 value, included the variables i) pluviometry cumulated 35 days before harvest (PL35) as the predominant explanatory variable (R^2 = 0.74), and ii) NDVI early in the season, which added 0.06 to the model's overall R^2. The overall R^2 was high, reaching a value of 0.80, which was highly significant (p = 0.0003). Figure 5 shows the good relationship (close to the Y = X) between observed BBR incidence and modeled BBR incidence (Table 4, first incidence model) from the long-term experiments in France and Chile (2010–2017). Second, according to the R^2 values, the best regression model for estimating BBR severity was also based on two variables: i) vegetative growth (NDVI), which was the predominant explanatory variable (R^2 = 0.60),

TABLE 4. Summary of the multiple regression models for explaining BBR incidence at harvest.

Independent variables	VIF	Regression function	Model variables	R-Square	P-value
PL35	5.55	BBRinc = -15.96 + 0.38 (PL35) +	PL35	0.74	0.0303
WSP	2.27	55.87 (NDVI)	NDVI	0.06	0.0303
TAN	2.37				
NDVI	2.85		Model	0.80	0.0003
PL15	4.11	BBRinc = -30.69 + 87.69 (NDVI) +	NDVI	0.68	0.0104
WSP	3.22	0.38 (PL15)	PL15	0.10	0.0581
TAN	2.01				
NDVI	2.11		Model	0.78	0.0006

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.

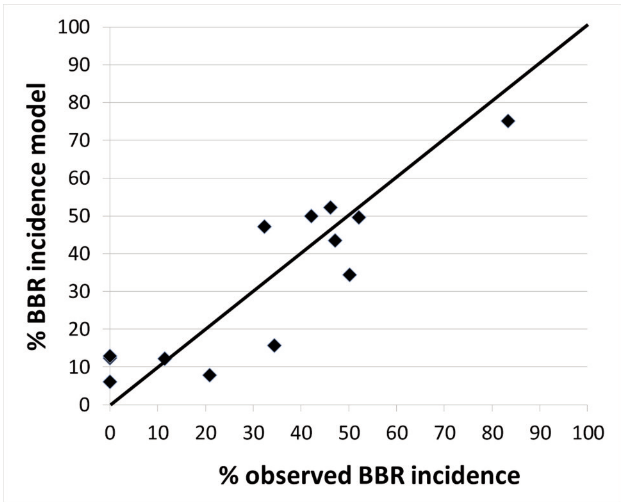


FIGURE 5. Relationship between observed and modeled BBR incidences (Table 4, first incidence model) from the long-term experiments in France and Chile (2010–2017). The Y = X equation is also indicated.

and ii) pluviometry cumulated 15 days before harvest (PL15) (Table 5). This second variable added 0.14 to the model's overall R^2, which reached the high value of 0.74 and was highly significant ($p = 0.0012$).

6. BBR at harvest related to berry skin tannin content and vegetative growth in the controlled leaf-removal (LR) experiment in France (2019)

The three experimental controlled LR-intensity treatments were associated with NDVI levels of 0.45 following severe LR, 0.60 with intermediate LR, and 0.78 in the control treatment without LR. As shown in Figure 6, this led to significant differences in the final BBR severity (ANOVA, Pr > F = 0.028). The severity reached 9.9% of rotted berries at harvest in the control without leaf-removal, whereas the severity was minimal (2.4%) in the severe LR treatment and in between (6.6%) following the intermediate LR treatment. Similarly, although not significantly (ANOVA, Pr > F = 0.095), the same trend was shown for BBR incidence at harvest. Furthermore, very interestingly, these differences in the early NDVI at berry pea size stage and in disease severity at harvest were also associated with significant differences in the tannin content in the fruit skin (Figure 6). The LR treatments caused significant differences in the tannin content measured in herbaceous berries sampled at veraison (ANOVA, Pr > F = 0.001). There was a marked increase from 22 mg/g of fresh skin in the control treatment with the higher canopy density (no LR) to 46.8 mg/g of fresh skin following the severe LR treatment. Thus, the tannin content in the berry skin, measured at the beginning of fruit maturation, increased significantly as the leaf density in the cluster zone decreased and this was also clearly correlated with the final BBR severity.

Skin tannins content was measured in herbaceous berries at veraison, and both BBR incidence and severity were assessed at harvest (7 October) (cv. Merlot noir, Nouvelle-Aquitaine Region in France, 2019). NDVI measurements and leaf-removal were implemented in a combined way by controlling the LR intensity

TABLE 5. Summary of the multiple regression models for explaining BBR severity at harvest.

Independent variables	VIF	Regression function	Model variables	R-Square	P-value
PL35	5.55	BBRsev = 4.78 + 0.18 (PL35)	PL35	0.66	0.0007
WSP	2.27				
TAN	2.37				
NDVI	2.85		Model	0.66	0.0007
PL15	4.11	BBRsev =-7.68+0.15 (PL15) + 24.67	PL15	0.14	0.0411
WSP	3.22	NDVI	NDVI	0.60	0.0334
TAN	2.01				
NDVI	2.11		Model	0.74	0.0012

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.

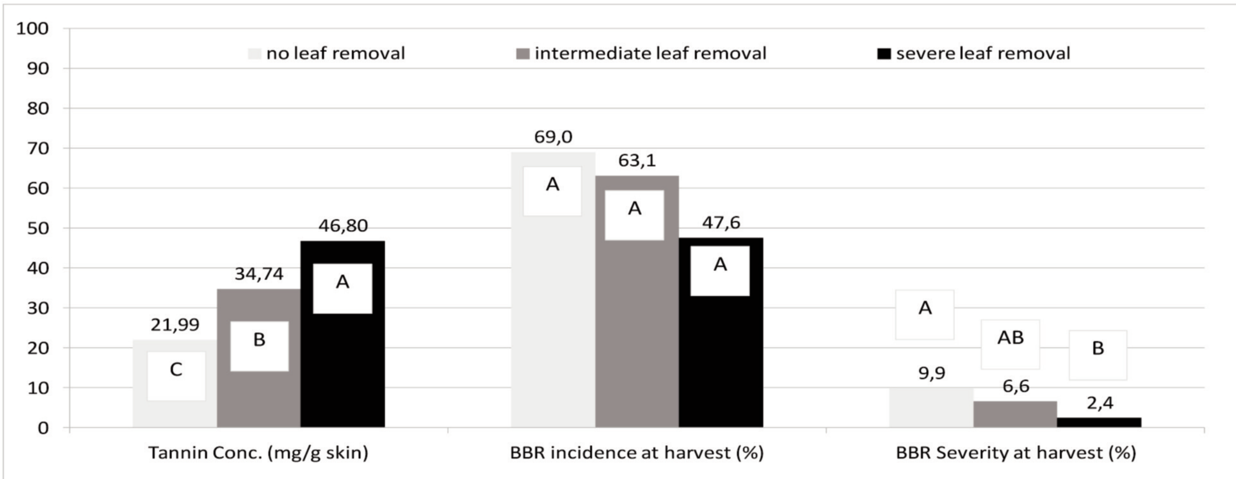


FIGURE 6. Effects on skin tannin concentration and on BBR development of controlled leaf-removal (LR) treatments leading to different NDVI levels of 0.45 (severe LR), 0.60 (intermediate LR) and 0.78 (control without LR).

according to Greenseeker® NDVI measurements, on one day (3 July 2019), at berry pea size stage, at exactly 319 growing degree days from flowering.

DISCUSSION

The contrasting weather and cropping conditions between Chile and France allowed us to identify robust BBR risk indicators that are potentially useful under conditions from not conducive to very conducive to the disease. The primary risk factor put forward, favoring Botrytis development, is weather conditions, i.e. rainfall near harvest associated with high RH, confirming several previous studies (Pieri and Fermaud, 2005; Valdés-Gómez *et al.*, 2008; Molitor *et al.*, 2016; Ferrer *et al.*, 2017; Hill *et al.*, 2019). BBR development was always much higher in France than in Chile, and this main difference in disease incidence and severity between the two countries was attributed to the weather conditions before harvest. Different climatic factors, including temperature, relative humidity, and rainfall after veraison, were tested for correlation with BBR (data not shown). Among them, rainfall was the most relevant factor involved in *Botrytis* development, in agreement with previous studies (Fermaud *et al.*, 2001; Pieri and Fermaud 2005; Molitor *et al.*, 2016; Ferrer *et al.*, 2017). This may be related to the absolute need for liquid water for conidia to germinate and, then, facilitate conidial infections during the ripening period in increasingly susceptible berries (Broome *et al.*, 1995; Steel *et al.*, 2011; Ciliberti *et al.*, 2015 a,b, 2016). Therefore, it was possible to analyze the relative importance of other proposed risk indicators than the climatic factors. In this context, the development and use of an early risk disease index, such as the early NDVI, was of prime importance.

1. NDVI as a potential early indicator of favorable conditions to final BBR development

In this study, for the first time, based on a large database (two sites, two cultivars and three to seven seasons), an early NDVI measurement was used, at berry pea size, which was positively related to BBR intensity at harvest (both incidence and severity). This early berry pea size stage corresponded, under our trial conditions, to approximately 30 days before veraison (Supplementary Table 1) and this is a very important condition allowing growers to use low-cost NDVI measurements from a practical point of view, as a vineyard management strategy for BBR control. The NDVI also has the advantage of depending on a precise and reproducible evaluation moment during each season, based on the thermal phenological model facilitating the measurement scheduling: 300 growing degree days 'GDD', base temperature = 10°C, accumulated from flowering (50% caps off, code 23). The early NDVI measurement during the season allowed us to avoid the negative effects of NDVI saturation, due to the vineyard canopy closure. The NDVI saturated threshold has been estimated between 0.8 and 0.86 according to different studies (Yang *et al.*, 2008; Zhang *et al.*, 2017). They also showed that at different crop growth stages GreenSeeker-based NDVI account for approximately 90% of biomass variability (NDVI saturation at 0.86). The effects of NDVI saturation in rice crop have also been reported, mainly caused by canopy closure and low transmittance of visible light (Cao *et al.*, 2013; Yao *et al.*, 2014). In our study, the data did not show NDVI values higher than 0.85. This may also be considered very close to the saturation threshold, which is in keeping with previous measurements under similar Bordeaux vineyard conditions (maximum GreenSeeker-based NDVI values recorded just below 0.9) (Drissi *et al.*, 2009).

Because of such a crucial role of early vegetative growth and foliar density in BBR development, the early NDVI indicator tested was also investigated in terms of an associated possible threshold. Under the contrasting climatic and cropping conditions in France and Chile, the relationship between early NDVI and final BBR severity showed that an early NDVI value between 0.5 and 0.6 at berry pea size stage (Figure 4), could be proposed as a potential threshold for subsequent disease management. However, in order to test the robustness of the relationship and the derived threshold, further studies are needed in various climatic zones and grapevine-growing regions worldwide. In the present study, under this NDVI threshold range of 0.5–0.6, the BBR severity at harvest should be lower than 5%, or close to this tolerance value in grape wines (Ky *et al.*, 2012). The last 2019 LR experiment in France confirmed this threshold showing, for an early NDVI value of 0.6 (intermediate LR treatment), a final disease severity of only 6.6%, whereas the control severity without LR reached approximately 10%. Similarly, such a grapevine vigor threshold, for limiting BBR incidence and/or severity, was highlighted in different countries by indicating a critical-threshold value between 0.5 and 0.6 kg/m of pruning mass (Valdés-Gómez *et al.*, 2008 in Table 1).

The importance of LR to reduce BBR risk has also been confirmed and highlighted in our 2019 vineyard experiment in France. The strict control of vegetative density (NDVI level) using LR at berry pea size stage, according to our early NDVI measurement methodology, showed that the three very different increasing NDVI values (0.45, 0.60 and 0.78) resulted in significantly different BBR severities at harvest (2.4%, 6.6% and 9.9%, respectively, Figure 6). To our knowledge, we have demonstrated for the first time that the decrease in the early NDVI level, precisely controlled by LR, caused a significant increase in the tannin content in the skin of herbaceous berries at veraison, from 47 to 22 mg tannin/g skin (Figure 6). Thus, two main reasons may account for the negative effect of reducing vegetative growth and foliar density around the clusters on BBR final severity: i) the well-known microclimate effect, and ii) the increase in fruit defense biochemical mechanisms mediated, notably, by the tannin composition in the berry skin.

Finally, and importantly, the exponential pattern associated with the significant regressions established in the present study (Figure 4) are very similar to the relationship between vine vigor (pruning mass) and BBR infection at harvest based on different cultivars and grapevine-growing regions worldwide (Valdés-Gómez *et al.*, 2008). Thus, all these results further confirm that the disease response to vine vigor may be generalized as an exponential relationship and that at pea size stage the early NDVI gives information on grapevine vigor of great interest similar to the 'pruning mass'. However, the 'pruning mass' indicator is obtained at the end of the season, in winter, that is *a posteriori* and then without showing any forecasting capacity, contrary to the NDVI indicator. The 5% BBR severity threshold has been shown as acceptable because the resulting wines did not evidence any negative organoleptic and/or enological problems (Ky *et al.*, 2012). Finally, this proposed early NDVI threshold should be further investigated in other grapevine-growing regions worldwide and/or confirmed with various cultivars differing in susceptibility to the pathogen.

2. Berry skin features as potential early indicators of BBR development.

Another important result was to identify the early tannin content of the skins as a candidate BBR risk indicator to estimate the potential susceptibility of berries to the pathogen. Significant correlations between grapevine susceptibility to BBR and phenolic contents in berry skin have been already established, but only in the laboratory (Sarig *et al.*, 1998; Pezet *et al.*, 2004; Deytieux-Belleau *et al.*, 2009). Similarly, in tomato or Arabidopsis, phenolic compounds (lignin or monolignols) could play an important role in plant cell wall fortifications (Blanco-Ulate *et al.*, 2016). However, in table grapes, Mlikota Gabler *et al.* (2003) were not successful to correlate significantly total phenolic content, or catechin content, in the berry skin with the susceptibility level to the pathogen. To our knowledge, our results in grapevine are then the first ones demonstrating such a significant relationship under field conditions. Tannins are constitutive antifungal compounds in berry skins, playing a potentially important role in resistance to the pathogen, in particular berry ontogenic resistance, and more tolerant grape cultivars exhibit higher quantities of tannins in berries (Goetz *et al.*, 1999; Pezet *et al.*, 2003, 2004; Deytieux-Belleau *et al.*, 2009). Condensed tannins (proanthocyanidins), associated with high resistance in immature strawberry fruits, may maintain *B. cinerea* in a quiescent stage and delay symptom development in grapes and strawberries (Jersch *et al.*, 1989; Hebert *et al.*, 2002; Hill *et al.*, 2014). This fungal quiescence may be due to the inhibition of fungal enzyme activity, such as polygalacturonases, cellullases and laccases (Porter and Schwartz, 1962; Hill *et al.*, 1981; Goetz *et al.*, 1999; Pezet *et al.*, 2004). Conversely, the positive correlation shown between berry skin pectins and the BBR final severity could be explained by the sugars issued from pectins promoting pathogen growth (Blanco-Ulate *et al.*, 2016). However, this result should be interpreted with care because the pectin content in berry skin was not significantly correlated with BBR final incidence (R^2 value of 0.27), and low R^2 values are associated with poor predictive power of the regressions.

3. Factors affecting berry skin and grapevine vigor indicators

Environmental factors, management practices (irrigation, fertilization) and some vine features (age, rootstock) can affect grapevine vegetative growth and/or tannin contents in berry skins. This is notably the case of water stress, generated naturally in non-irrigated vineyards or induced by water management in irrigated vineyards (Acevedo-Opazo *et al.*, 2010; Keller *et al.*, 2015). The water management in these experiments in Chile did not promote BBR development because the plant vigor was not increased: irrigation was only implemented to avoid strong water restriction (NDVI values weaker in Chilean vineyards than in the French not-irrigated ones). Furthermore, following drip irrigation, the wetness at the soil surface generally does not reach more than 3% (Ortega-Farías *et al.*, 2007), which should not significantly affect the relative humidity in the cluster zone. Water stress may also induce changes in grapevine phenolic composition (Kennedy *et al.*, 2002; Lorrain *et al.*, 2011; Casassa *et al.*, 2015). A controlled water deficit between flowering and bunch closure is a common irrigation practice to improve the final organoleptic red wine quality in several wine regions worldwide (Kennedy *et al.*, 2002; Acevedo-Opazo *et al.*, 2010). As tannin content in berry skins may be highest at fruit set (Downey *et al.*, 2003), a water restriction before this stage may increase the biosynthesis of tannins, notably because of less vigorous vines (Ristic *et al.*, 2007; Casassa *et al.*, 2015). Less vigorous vines tend to have grapes with greater amounts of skin tannins, with increased polymeric length, accumulating in sun-exposed berries (Keller, 2015). This is clearly confirmed by our one-year controlled LR experiment. Additionally, less vigorous vines favor the air circulation in the cluster zone and are associated with reduced cluster compactness, which are important factors limiting BBR risk (Fermaud *et al.*, 2001; Pieri and Fermaud, 2005; Keller, 2015). Thus, all these mechanisms following water restriction at an early stage during the season, may act together to decrease the infection risk and then BBR development.

In this study, the new NDVI indicator may allow the technician to consider (integrate) the variations in leaf density and/or canopy growth resulting from all agronomical and environmental key factors, such as vine age or the rootstock. Furthermore, other important factors differed between our experimental sites, e.g. soil fertility, which should reinforce the interest for the generic, overall, and significant relationship between the new NDVI indicator and BBR severity shown in this field study.

4. Linear regression models of BBR severity and incidence

All the linear regression models explaining BBR incidence and severity were highly significant. They allowed us to determine and select major explanatory variables in BBR development. All our optimized models, both for incidence and severity, included climatic indices that are then considered as of prime importance in BBR epidemiology. The best models that explained BBR incidence and severity included the pluviometry recorded during 35 and 15 days before harvest, respectively. This difference in the period of time to be considered before harvest could originate from the fact that earlier favorable climatic conditions are needed for new first disease infections (incidence), whereas later favorable weather conditions are required for disease spread out, notably within bunches. In addition to climate, the early vegetative growth indicator (NDVI) was the other major factor of prime importance in relation to *Botrytis* development, because of its significant contribution to BBR development models. This major contribution, especially to BBR severity, could be explained because favorable weather conditions are needed for first disease infections (incidence) and once it occurs, a higher vegetative growth favors the disease spread (severity). This should be due to dense canopies increasing wetness duration within the cluster zone, favoring bunch colonization from one berry to the next, i.e. BBR severity (Valdés-Gómez *et al.*, 2008). Regarding the early berry skin features, none were included in the BBR incidence and severity models, suggesting that they should remain as trend and complementary indicators only. Such berry skin variables, notably pectins, should be considered weak predictors of final BBR intensity in the context of practical BBR management.

During recent decades, restrictions on fungicide applications have been increasing to reduce their negative impact on the environment and to limit pesticide residues at harvest (Fenner *et al.*, 2013). Nonetheless, the control of *B. cinerea* still largely depends on the use of chemical-specific fungicides, and protection strategies require optimization. A reduction of fungicide use could be accomplished by crop cultural prophylactic measures that limit fungal infections and/or spread, and forecast models and DSS that allow growers to better target the timing of fungicide applications. To help growers decide which practice to use 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 to the use of early NDVI values as management indicators in IPM strategies to decide early LR and/or shoot removal at bunch closure. Additionally, the tannin content in berry skins may be used as a complementary key indicator to support a specific fungicide spray decision. Thus, a main prospect is the use of the proposed new early risk indices in integrated vineyard management as key pieces of information to develop IPM control strategies of the disease. In addition to these indicators, it is important to consider, in itself, cultivar susceptibility to the pathogen as another key management indicator (Pañitrur-De la Fuente *et al.*, 2018). Finally, other fungal pathogens of the grape berry could be also concerned by the use of the developed indices, such as *Penicillium expansum*, of prime importance in both table grapes and wine grapes, particularly when associated with *B. cinerea* (Franck *et al.*, 2005; La Guerche *et al.*, 2005; Donoso and Latorre, 2006).

CONCLUSIONS

The management indicators developed in this study significantly explained the BBR development at harvest and therefore may be used as new tools to develop specific DSS to limit botryticide treatments. Main regression models included the cumulated rainfall before harvest, corroborating the importance of this factor in BBR development, and the early grapevine vegetative growth (NDVI), which was the other major factor governing *B. cinerea* infection. Regarding tannin content in berry skin, evaluated at the berry pea size stage, this BBR risk indicator could be used as a trend indicator in IPM strategies. Further, new investigations should consider the cluster compactness and pathogen inoculum throughout the season, to better understand their relationships with BBR at harvest and to optimize more deeply the management and control methods in IPM strategies.

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