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

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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.

Key words: *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.*, 2014 b; 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.*, 2014 b; 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.

Material 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): $y = [a \times Teqb \times (1 - Teq)]c / [1 + \exp(d - e \times RH/100)]$.

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 (pre-véraison applications) or 300 l/ha (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	Agrisnergie 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	De Sangosse (Pont-du-Casse, France)	NODU Vert Biocontrol list (France)
Fungicover	Fatty acid emulsion	15 g/l	BioDurcal (Granada, Spain)	Registered in Spain
M3AEY	Terpenes	4 l/ha	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).

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.

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.

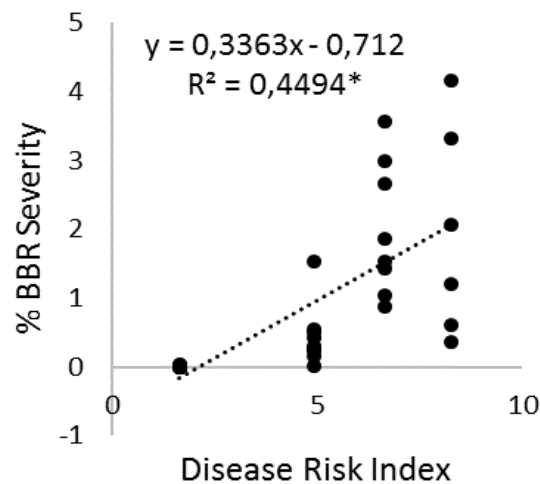


Figure 1. Correlation between percentage of *Botrytis* bunch rot severity and Disease Risk Index (cumulated daily values) based on temperature and relative humidity. Data from six replicate plots in an experimental vineyard near Bordeaux (2012 to 2015). BBR assessment was carried out approximately 30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

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	
	Σ Wind (m/s)	Σ ¹ PP (mm)	Σ ² DRI	% <i>B. cinerea</i> infection on calyptras	Σ Wind (m/s)	Σ PP (mm)	Σ 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

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

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*.

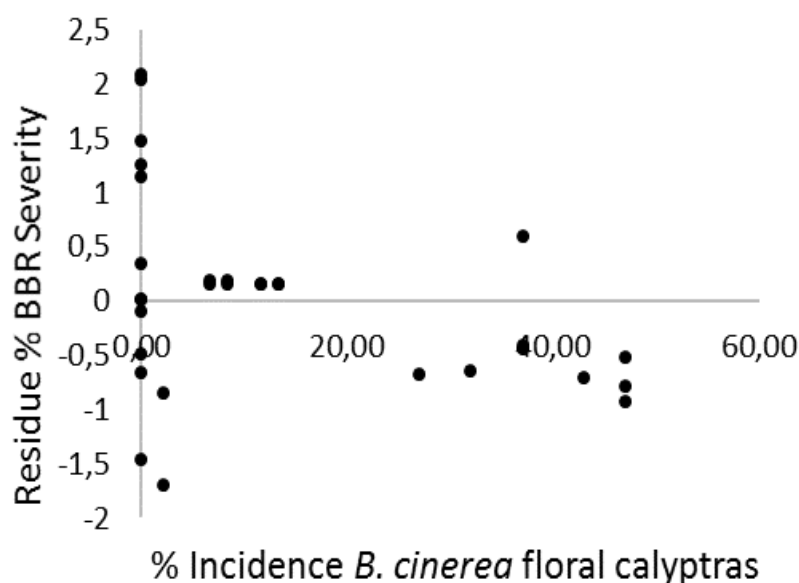


Figure 2. Correlation between residues issued from the previous regression analysis and *B. cinerea* incidence on floral calyptras (%). Data from six replicate plots in an experimental vineyard near Bordeaux (2012 to 2015). *B. cinerea* incidence on floral calyptras was assessed at the end of flowering. 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.

Flowering treatments have demonstrated to be the most effective in a variety of fungicide timing experiments (Calvo-Garrido *et al.*, 2014 b; 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.*, 2014 b). 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 3 a), 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 3 b), 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.*, 2014 a; 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.

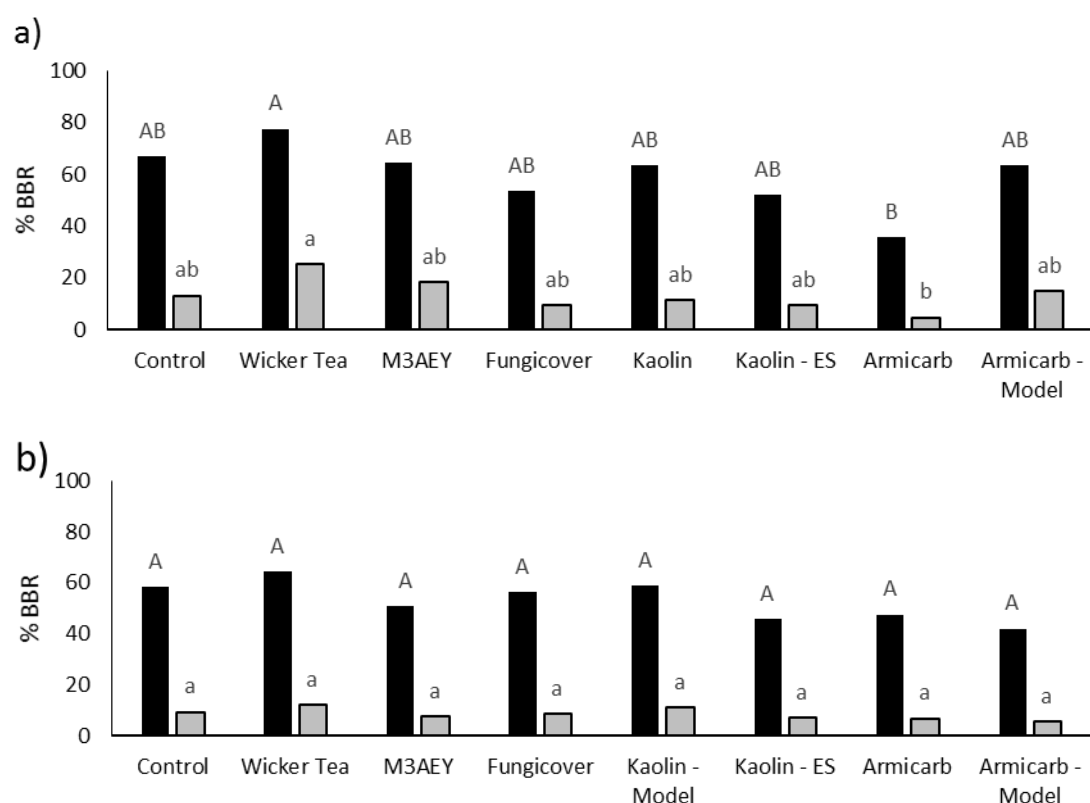


Figure 3. Efficacy of natural products applied to control *Botrytis* bunch rot in two organic vineyards near Bordeaux in 2015. Incidence (black bars) and severity (grey bars) were assessed at commercial harvest in St. Yzan (a) and Montagne (b) field sites. Treatments consisted on 5 or 6 spray applications (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.

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.

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