Assessment of grey mould (*Botrytis cinerea*) impact on phenolic and sensory quality of Bordeaux grapes, musts and wines for two consecutive vintages

I. KY[1]*, B. LORRAIN[1]*, M. JOURDES[1], G. PASQUIER[1], M. FERMAUD[2], L. GÉNY[1], P. REY[2], B. DONECHE[1] and P.-L. TEISSEDRE[1]

[1]Unité de recherche Œnologie EA 4577, USC 1219 INRA, Institut des Sciences de la Vigne et du Vin, Université Bordeaux Segalen, 210, chemin de Leysotte, 33882 Villenave d'Ornon Cedex, France

[2]UMR SAVE Santé & Agroécologie du Vignoble 1065, INRA, ISVV, 71 ave Edouard Bourlaux, BP 81, 33883 Villenave d'Ornon Cedex, France

* These authors contributed equally to the work.

Corresponding author: Professor Pierre-Louis Teissedre, fax +33 557575813, email p.teissedre@u-bordeaux2.fr

Abstract

Background and Aims: The impact of grey mould (*Botrytis cinerea* (*B. cinerea*)) was quantified on chemical, phenolic and sensory qualities of grapes, derived musts and wines.

Methods and Results: Analyses were carried out by using naturally or artificially infected grape berries at ripeness or overripeness. In grape seeds, chemical analyses revealed no major differences between healthy and rotten grapes. In grape skins of Botrytis-affected berries, concentrations of all the phenolic compounds (anthocyanins and proanthocyanidin monomers, dimers and trimer) decreased drastically. Mean degree of polymerization of the proanthocyanidin polymeric fraction was also affected in skins. Chemical analyses of musts and wines made with different percentages of rotten berries showed a moderate impact of the pathogen on their phenolic composition. Nevertheless, sensory analyses underlined a loss of wine sensory quality perceptible from a threshold as low as 5% of Botrytis-affected grapes onwards.

Conclusion: Phenolic variations and the associated negative impact in grapes, derived musts and wines may be related to oxidation phenomena from *B. cinerea*. The main effects of severity/age of grey mould and the level of berry maturity are also discussed.

Significance of the Study: *B. cinerea* drastically affects the phenolic and organoleptic properties of grape skins and derived wines. Therefore, prophylactic actions early in the vineyard, evaluation of the sanitary status of the harvested grapes and berry sorting are primordial even under low disease pressure.

Keywords: *anthocyanin*, Botrytis cinerea, *disease incidence*, *disease severity*, *grape*, *grey mould*, *must*, *proanthocyanidin*, *sensory analysis*, *total phenolic*, *wine*

Introduction

Botrytis cinerea (*B. cinerea*) is a ubiquitous, filamentous and necrotrophic fungus attacking over 200 different plant species (Jarvis 1977). This is one of the principal causes of quantitative and qualitative degradation in many crops. In many vineyards around the world, this pathogen is responsible for one of the most serious diseases affecting grapevine (*Vitis vinifera* (*V. vinifera*) L.), namely Botrytis bunch rot and/or grey mould.

Grey mould attacks are usually very heterogeneous in space and time. At flowering and after veraison, grape bunches can be infected and, partly or totally, destroyed by *B. cinerea*. Various key factors have been shown to play an important part in the epidemiology of the disease in the vineyard, i.e. climatic conditions, grape variety susceptibility, latent infections at or just after flowering, vine vegetative and reproductive vigour, genetic structure of pathogen populations as well as morphological and biochemical features of grape bunches and berries (Martinez et al. 2005, 2008, Valdes-Gomeza et al. 2008, Deytieux-Belleau et al. 2009).

Chemical control remains the main way to reduce both the incidence and severity of grey mould by spraying aerial parts of vines (fructiferous zone) with specific anti-Botrytis fungicides. However, the chemical control of Botrytis diseases is impeded by the development of resistant strains to many botryticides (Elad and Evensen 1995, Leroux 2004) and the negative public perception regarding the safety of pesticides (Gullino and Kuijpers 1994). In many countries, environmental policies advise the increase of organic farming zones and/or restrict the use of chemicals. New management techniques need to be developed, and alternative products such as biofungicides, mineral oil and plant hormones are currently being studied (Jacometti et al. 2010). Nevertheless, until effective solutions are found, Botrytis bunch rot still constitutes one of the major concerns of vine growers.

From both economical and oenological qualitative points of view, *B. cinerea* can have dramatic consequences for vine growers and wine producers. First, considering quantitative aspects, the disease is known to drastically reduce yield at harvest. Technical reports indicate that in the Alsace region (France), harvest losses because of grey mould were evaluated at 27% from 1976 to 1980 (Dubos 1999). As for wine volumes, it may be considered that 35% of rotten berries at harvest

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reduces the volume of red wine from 700 hL to 500 hL (Dubos 1999). Second, concerning the oenological quality impact, *B. cinerea* is known to affect grape chemical composition and, in particular, to damage the major qualitative compounds such as sugars, organic acids, varietal aromas and phenolic compounds (Ribéreau Gayon et al. 1980). In addition to wine browning, off-flavours and aromatic flaws may appear, resulting in further organoleptic degradations of wines. Another oenological issue from *B. cinerea* infection is clarification difficulties of wines produced from infected grapes (Dubourdieu 1978).

In response to a pathogen attack, plants, and especially grapevine, are generally able to mount a spectrum of defence responses. In addition to mechanical barriers in the skin and epidermic cell layers, grape skin contains preformed and/or induced fungal inhibitors, mainly phenolic compounds (Jarvis 1980). In grapes, the main classes of phenolic compounds are stilbenes, anthocyanins and condensed tannins, also called proanthocyanidins. Among the *trans*-isomer stilbene compounds, resveratrol (3,5,4-trihydroxystilbene) is produced by vines in response to a fungal infection. Anthocyanins are the pigmented compounds responsible for red wine colour and are essentially located in grape skins. Proanthocyanidins include a large range of phenolic compounds constituted of flavan-3-ol monomer subunits. Their structures vary according to the nature of their constitutive subunits, the mean degree of polymerization (mDP) and linkage position (Prieur et al. 1994, Cheynier et al. 2006). In response to grey mould infection, the grape skin is capable of synthesizing stilbenic phytoalexins (predominance of *trans*-resveratrol) having fungicidal properties (Jeandet et al. 1991). Moreover, many studies agree in suggesting that plant proanthocyanidins maintain *B. cinerea* in a quiescent stage, leading to delayed development of symptoms (Van Baarlen et al. 2007). Proanthocyanidins may also act as competitive inhibitors of *B. cinerea* laccase, in particular stilbene oxidase, thereby preventing detoxification of the stilbenic phytoalexin (Goetz et al. 1999, Van Baarlen et al. 2007).

If phenolic compounds are proved to be of great importance for grape constitutive defence against *B. cinerea* at the 'plant level', their significance is also crucial from an oenological point of view, because of the organoleptic qualitative properties they will confer to their derived wines.

During the red winemaking process, proanthocyanidins and anthocyanins are extracted from seeds and skins. Proanthocyanidins are of great importance to sensory red wine quality because of their astringent and bitter properties (Gawel 1998, Peleg et al. 1999). They also play a role of prime importance in the long-term colour stability *via* the chemical reactions with anthocyanins (copigmentation and/or condensation) (Somers 1971, Vivar-Quintana et al. 1999). Molecular size of proanthocyanidins (mDP) affect their relative bitterness and astringency (Gawel 1998, Peleg et al. 1999, Cheynier et al. 2006). Monomers are more bitter than astringent, whereas the reverse is true for derivates characterized by a large molecular weight.

Many studies showed that Botrytis bunch rot significantly affects grape and wine quality, mainly through chemical composition changes (Ribereau Gayon et al. 1979, Ribéreau Gayon et al. 1980, Pallotta et al. 1998). However, most of these studies do not take into account recent advances in analytical chemistry tools, and their results are mainly descriptive and qualitative (in terms of global parameters and/or trends). Furthermore, even for quantitative aspects, little information is available especially concerning relationships between oenological features (chemical and sensorial composition of botrytized wines) and epidemiological data (disease incidence/severity rates, age of grey mould development). In this perspective, this study was aimed at defining an acceptable grey mould infection level or threshold below which phenolic and sensory quality of Bordeaux red wines remains unaffected. The first objective was to quantitatively assess the Botrytis effect on the phenolic composition of Bordeaux Merlot grapes with a focus on proanthocyanidin and anthocyanin fruit composition. After the winemaking process, and for different percentages of grey mould-affected grapes, the Botrytis impact was investigated on the chemical, phenolic and sensorial quality of derived musts and wines.

Materials and methods

Experimental materials

Chemicals. Deionized water was purified with a Milli-Q water system (Millipore, Bedford, Massachusetts, USA). Acetonitrile, ethyl acetate, chloroform, methanol, ethanol and acetone were of high-performance liquid chromatography (HPLC) grade and purchased from Scharlau (Sentmenat, Barcelona, Spain). Ammonia (25%), o-phosphoric acid (85%) and ammonium dihydrogen phosphate were from VWR-Prolabo (Fontenay sous Bois, France).

All other chemicals were purchased from Sigma Aldrich (Saint Louis, MO, USA). The Laboratory of Organic Chemistry and Organometallic (Université Bordeaux 1) synthesized B3 [(+)-catechin-(4α-8)-(+)-catechin] and B4 [(+)-catechin-(4α-8)-(-)-epicatechin] dimers, trimer (T) [(+)-catechin-(4β-8)-(+)-catechin-(4β-8)-(-)-epicatechin] (Tarascou et al. 2006).

Fruit sampling, inoculation by *B. cinerea* and extraction. Grapes (*V. vinifera*, cv. Merlot Noir) were collected from an INRA experimental vineyard located near Bordeaux (Villenave d'Ornon). The vineyard was planted in 1991 on a gravely soil and was grafted onto '101-14' rootstock with a planting density of approximately 5350 vines/ha, a row by vine spacing of 1.7 × 1.10 m and a north-south orientation. The experimental plot was not treated with anti-Botrytis fungicides during either the 2009 or 2010 growing seasons. In 2009, following climatic conditions not conducive to a natural development of *B. cinerea*, visually healthy grape bunches were sampled randomly at maturity on the 23rd of September ('Mat' modality). The bunches were then inoculated by spraying a spore suspension (1×10^6 conidia/mL; conidia issued from 8-day-old Potato Dextrose Agar plates) until the complete fruit surface was covered. The *B. cinerea* single-spore isolate 213 was selected from the collection of UMR SAVE, Bordeaux, because it had been characterized as virulent and belonging to the II-*transposa* type, in the group-II clade (Martinez et al. 2003, 2005). Grape bunches were incubated for 8 days at room temperature within a moist chamber (i.e. a plastic box containing absorbent paper in the base soaked with sterile water). A visual sorting of berries was then performed to remove undesirable rotten berries following a fungal development (blue-green colour) because of *Penicillium spp., Alternaria spp*. or *Clostridium spp*. as well as development of acetic bacteria (red-pink colour). Moreover, in order to use berries naturally infected by *B. cinerea* for this 2009 vintage, overmature grapes ('Overmat') were also harvested (21st Oct.) either visually healthy or expressing typical symptoms of grey mould. In 2010, climatic conditions conducive to the pathogen allowed us to get sufficient natural development of grey mould and to randomly collect 100 kg of healthy and rotten berries on the 3rd of Oct. During berry ripening, S and acidity of the grapes were periodically determined by classical analyses (OIV 2011). In parallel, berries were analysed for their phenolic richness and for their extractable and total anthocyanin contents (Glories and Augustin 1993). Grey mould inci-

dence (frequency percentage of rotten bunches) and severity (frequency percentage of rotten berries) were estimated in the experimental vine plot by rating 200 bunches randomly selected every week. The percentage of rotten berries was assessed visually for every sampled bunch.

Botrytized berries were separated from healthy ones, and part of the berries (healthy and affected) was frozen until chemical analyses. The other part was used for small-scale winemaking.

Grape tannins extraction. Extracts from seeds and skins were prepared in duplicate according to our previous study (Lorrain et al. 2012). Briefly, crude tannin extracts were first prepared. In a second step, they were purified to obtain two distinctive fractions: a low molecular-weight procyanidin fraction (monomeric/oligomeric tannins) and a high-weight procyanidin fraction (polymeric tannins).

Grape anthocyanins extraction. Anthocyanins were extracted from skins according to our previous study (Lorrain et al. 2012).

Small-scale winemaking. Different batches of microvinification were carried out in duplicate. In 2009, 0 (control), 5 and 15% (w/w) of botrytized Merlot grapes ('Mat' modality) were added to healthy grapes to contain 10 kg per tank. For this vintage, the same procedure was achieved with 0 (control), 5 and 20% (w/w) of botrytized grapes ('Overmat' modality). In 2010, this process was applied with 0 (control), 5, 10 and 15% (w/w) of 'naturally' botrytized Merlot grapes. Each batch of 10 kg grapes was mechanically crushed-destemmed and collected in a 10 L aluminum tank. Crusher pressure was adjusted in order to obtain the same juice volume in all the tanks (constant ratio pomace/juice). Before addition of a 6 g/hL dose of aqueous bisulfite solution 18% (Laffort, Bordeaux, France) in each tank, a 60 mL sample of must was collected from each batch and frozen until chemical analyses.

Saccharomyces cerevisiae yeasts (Zymaflore F15®, Laffort) were prepared by rehydration in a warm aqueous mixture of sugar, nutrients (Thiazote®, Laffort) and an alcoholic fermentation activator (Superstar®, Laffort) for 15 min. Twenty millilitres of this leaven was applied in each tank (after a must maceration of 24 h) in order to reach concentrations of 15 g/hL for the activator, 20 g/hL for the nutrients and 20 g/hL for the yeasts. Fermentations were achieved at a control temperature of 20°C and monitored each day by temperature and density measurements. After 14 days, densities were stable for each batch and concentrations of reducing sugars were lower than 2 g/L, indicating that alcoholic fermentations were complete. Wines were separated from the pomace by moderate manual pressing and poured into 1.5 L glass bottles. Malolactic fermentation was carried out by inoculation of a commercial lactic acid bacterium, *Oenococcus oeni* (Lactoenos 450 Preac®, Laffort) at 2 g/hL in mixture with its activator (Energizer®, Laffort) at 5 g/hL. Malic acid concentration was followed by enzymatic kit (R-Biopharm, Saint Didier au Mont d'Or, France), and when lower than 0.2 g/L, finished wines were racked into 1.5 L bottles, and 6 g/hL of aqueous bisulfite solution 18% was added. Wine bottles were completely full (for minimum air content) and sealed with cork stoppers and stored in the dark at 20°C until analysis.

We obtained a total of six musts and six wines in duplicate ('Mat': 0, 5, 15% and 'Overmat': 0, 5 and 20% of botrytized berries) for the 2009 vintage and four musts and four wines in duplicate for the 2010 vintage ('control' or 0, 5, 10 and 15% of botrytized berries).

Chemical analyses

Classical oenological analyses (musts and wines). In musts, total acidity (TA), assimilable nitrogen, malic acid concentrations, reducing sugars and consecutive probable alcohol were determined by the international methods of OIV (OIV 2011). Concerning wines, reducing sugars, TA, malic acid, alcohol (% vol) and pH were achieved by an infrared technique using Foss WineScan 79000 (Foss, Nanterre, France).

Global phenolic analyses (grape crude extracts, musts and wines). Preparation of samples. Total polyphenol, tannin and anthocyanin contents were determined from grape skin and seed crude extracts, from musts and from wines according to our previous study (Lorrain et al. 2012).

Global analyses procedures. These procedures are described in our previous study (Lorrain et al. 2012). Briefly, the total phenol content (TPC) was determined by Folin–Ciocalteu test, and gallic acid was used as a standard in order to express the results as mg of gallic acid equivalents (GAEs). The total tannin content was measured by acidic hydrolysis of proanthocyanidin, resulting in carbocation formation partially converted into red cyanidin (Ribéreau Gayon and Stonestreet 1966). Anthocyanin content was determined by the SO_2 bleaching procedure (Ribéreau Gayon and Stonestreet 1965).

Proanthocyanidins and anthocyanins HPLC analyses (grapes purified extracts and wines). Proanthocyanidin monomers and oligomers analyses. Monomeric/oligomeric tannin extracts were solubilized in a methanol/water solution (50:50, v/v) at concentrations of 1 g/L for seed extracts and 6 g/L for skin extracts. Wines were filtered (0.45 µm) and directly injected for HPLC analyses.

The equipment used for HPLC analysis consisted of a Thermo-Finnigan UV-vis detector (UV-vis 200, Thermo Scientific, West Palm Beach, FL, USA), a Thermo-Finnigan autosampler and a Thermo-Finnigan ternary pump coupled to an Xcalibur data treatment system. Analytical conditions (column, gradient, mobile phase, peaks monitoring) are detailed in our previous study (Lorrain et al. 2012). The results were converted in mg of dried skin or seed weights and in mg/L of wine.

Determination of mDP. The proanthocyanidin mDP were determined for seed and skin extracts both in monomeric/oligomeric tannin fraction and in polymeric tannins fractions as well as in wines by the means of phloroglucinolysis (Drinkine et al. 2007). Analytical procedures were the same as used in our previous study (Lorrain et al. 2012).

Anthocyanins analyses. Before injection, skin anthocyanin extracts were dissolved in water/methanol solution (50:50, v/v) at concentration of 10 g/L. Wines were filtered (0.45 µm) and directly injected for HPLC analyses. HPLC-ultraviolet analyses were performed according to our previous study (Lorrain et al. 2012).

Sensory analyses (wines). Sensory analyses were carried out 3 months after the wines were bottled. The wines were evaluated by 20 judges, from the Oenology department of the University of Bordeaux. They were all selected on the basis of interest and availability as well as their experience in red wine sensory analysis. All analyses were performed in a specific room

at 20°C with isolated booths. Three different triangle tests (IS0 4120:2007) were first set up (each winemaking duplicate was assessed in a single replicate), in order to determine any significant difference between the control wine sample (0% of affected grapes) and wines containing different percentages of affected grapes (5, 15 (*in vitro* inoculation), 5 and 20% (overmature) in 2009 and 5, 10 and 15% in 2010). Twenty-millilitre samples were presented in dark ISO-approved wine glasses labelled with three-digit random codes with a randomized presentation across panellists. In following sessions, panellists were asked to describe the aromatic profile of each wine. Four different 20-mL wine samples were presented per session in the same conditions as for triangle tests. After smelling and tasting the wines, judges marked the intensity of each chosen attribute on a 0 to 7 scale. The attributes were chosen from a list made with those normally used for wine sensory description (fruitiness, reduced, oxidized, acidity, bitterness, astringency). The panellists rinsed their mouth with water, and they were forced to have a rest of 30 s before being allowed to assess the next sample. A factorial analysis of variance (ANOVA) was performed to test the effects of variation factors (samples, judges and the interaction 'samples × judges') on each sensory attribute. A significant effect of judges was observed, while the interaction 'samples × judges' remained insignificant.

Statistics

All measurements were performed in duplicate. Results are expressed as means ± standard deviation. One-way ANOVA was performed to test the effects of variation factors (different samples) on each variable (TPC, total tannin, anthocyanin, phenol concentrations, mDP, etc . . .). If significant effects were found at a 95% confidence interval, ANOVA was followed by a Tukey's honestly significant difference (HSD) and Duncan's post-hoc test to identify differences among groups. Statistical analyses (ANOVA, Tukey's HSD and Duncan's post-hoc tests) were performed using the ANOVA of Statistica V.7 Software (Statsoft Inc., Tulsa, Oklahoma, USA).

Results

Grey mould development and features

This study was conducted in both seasons 2009 and 2010, with each exhibiting different climatic conditions. In August and September 2009, dry and stable anticyclonic weather conditions resulted in a low intensity of grey mould in the experimental vineyard and a quasi-absence of disease risk (Table 1). When technical and phenolic maturities were achieved (harvest: 23rd of September), disease incidence and severity reached 7.1 and 0.2%, respectively. Therefore, the quasi-absence of *B. cinerea* led us to artificially inoculate visually healthy mature berries (modality 'Mat'). These artificially rotten berries were introduced into a micro-scale vinification process after 8 days of incubation post-inoculation. Twenty-eight days after harvest (21st Oct), the overmaturated berries naturally infected by the pathogen (modality 'Overmat') were characterized by a mean age of diseased berries of 9 days and a high disease incidence reaching 78.2% (Table 1). In 2010, more conducive climatic conditions resulted in sufficient natural development of grey mould characterized by a mean disease age on infected berries of 4 days and a natural mean disease incidence of medium intensity, i.e. 23.4% (Table 1).

Grape maturity level at harvest

The maturity level of berries from Merlot variety was characterized at harvest on healthy berries in 2009 ('Mat' modality) and 2010 (Table 1). In 2009, harvest occurred on the 24th of September. The berries reached a good level of technical maturity with high sugar content (S) predicting an alcohol content of 12.8% (v/v) in derived wines, a moderate TA

Table 1. Characterization of grey mould development in 2009 and 2010 and the associated maturity level of healthy grapes.

Grey mould characteristics	2009		2010
	2nd Oct (Mat)	21st Oct (Overmat)	3rd Oct
	Artificial inoculation grape harvest: 23rd Sept	Natural rot development	Natural rot development
Disease mean age (day)	8	9	4
Disease incidence (%)	7.1	78.2	23.4
Disease severity (%)	0.2	18.9	3.8
Healthy berry maturity	24th Sept	—	30th Sept
100-berry sample weight (g)	188 ± 12	na	158 ± 7
Sugars (g/L)	218	na	245
pH	3.94	na	3.37
Total acidity (g H_2SO_4/L)	4.26	na	4.1
RPT (A_{280} × dilution)	na	na	52.4
Mp%	na	na	27.7
EA%	na	na	56.2

All measurements were performed in duplicate. Results are expressed as means ± standard deviation. — RPT, total phenolic richness; A_{280}, absorbance at 280 nm; Disease incidence, frequency % of rotten bunches. Disease severity, frequency % of rotten berries. EA%, anthocyanin extractability corresponding to the cellular maturity index; Mat, artificially inoculate visually healthy mature berries; Mp%, seed maturity index; na, not assessed; Overmat, overmaturated berries naturally infected by the pathogen; RPT, total phenolic richness.

corresponding to a maturity index or S/TA ratio of 51.2. In 2010, technical and phenolic maturities were achieved on the 23rd of September (harvest), but it was necessary to wait 10 extra days (3rd Oct) to get sufficient natural development of *B. cinerea*. This 10-day delay accounted for an important sugar accumulation in berries, predicting a wine alcohol content higher than 14% (v/v). Acidity was moderate, quite similar to 2009, and the S/TA ratio amounted to 59.8. Lastly, both seeds and skins exhibited a good phenolic maturity level (Table 1).

Berry phenolic composition in 2010

In 2010 only, the berry phenolic composition was compared between healthy and rotten berries following the natural development of Botrytis in mature grape berries. Because of the quantitative lack of harvested grapes in 2009, healthy and Botrytis-affected grapes ('Mat' or 'Overmat' berries) were preferentially kept for winemaking. In order to discriminate between healthy and botrytized grapes, some global analyses were first conducted by using procedures usually employed for wine analysis: TPC, total tannin and total anthocyanin. Both kinds of crude extracts, seeds and skins, were analysed after solubilization at appropriate concentrations in model solutions

Botrytis impact on seed phenolic composition

A significant TPC decrease was observed in seeds between healthy and affected grapes (51.1 vs 42.6 mg GAE/g dw, respectively), while no significant variation was detected for total tannin (Table 2).

As for proanthocyanidin composition, the flavan-3-ol monomers (C, EC, ECG) and oligomers, i.e. B1, B2, B3, B4 dimers and the trimer T, were identified and quantified in both seeds and skins (Table 2). Significant differences between healthy and botrytized berries in seed phenolics were only observed for C, B3 and trimer concentrations which decreased

Table 2. Concentrations and structural characteristics of phenolic compounds in seeds and skins of healthy grapes and botrytized grapes (vintage 2010).

	2010			
	Seeds		Skins	
	Healthy	Botrytized	Healthy	Botrytized
TPC	51.1 ± 1.3[b]	42.6 ± 0.6[a]	24.6 ± 0.9[b]	4.0 ± 0.2[a]
Total tannin	137.6 ± 5.5[a]	120.8 ± 2.6[a]	73.0 ± 3.9[b]	21.9 ± 0.3[a]
Total anthocyanin	nd	nd	27.8 ± 0.5[b]	5.0 ± 0.0[a]
C	2.156 ± 0.030[b]	1.609 ± 0.089[a]	0.027 ± 0.001[b]	0.013 ± 0.001[a]
EC	2.583 ± 0.008[a]	2.256 ± 0.103[a]	0.013 ± 0.000[b]	0.004 ± 0.001[a]
ECG	0.263 ± 0.001[a]	0.223 ± 0.028[a]	nd	nd
B1	0.158 ± 0.008[a]	0.127 ± 0.009[a]	0.040 ± 0.000[b]	0.006 ± 0.000[a]
B2	0.639 ± 0.017[a]	0.564 ± 0.007[a]	0.013 ± 0.001[b]	0.003 ± 0.000[a]
B3	0.312 ± 0.008[b]	0.243 ± 0.003[a]	0.008 ± 0.000[b]	0.003 ± 0.000[a]
B4	0.304 ± 0.009[a]	0.260 ± 0.014[a]	nd	nd
T	0.185 ± 0.002[b]	0.142 ± 0.003[a]	0.031 ± 0.001[b]	0.007 ± 0.000[a]
Monomeric/oligomeric fraction				
mDP	2.1 ± 0.0[a]	2.1 ± 0.0[a]	3.8 ± 0.4[a]	5.6 ± 0.4[a]
%G	20.4 ± 1.1[b]	17.3 ± 1.0[a]	33.2 ± 3.7[a]	31.8 ± 4.3[a]
%P	nd	nd	26.1 ± 0.0[a]	42.5 ± 1.1[b]
Polymeric fraction				
mDP	9.4 ± 0.2[a]	9.2 ± 0.3[a]	13.1 ± 0.8[b]	7.8 ± 0.8[a]
%G	34.6 ± 1.6[b]	30.8 ± 1.0[a]	6.9 ± 1.9[a]	10.0 ± 1.1[a]
%P	nd	nd	57.6 ± 3.9[a]	69.8 ± 2.0[a]
Dp	nd	nd	2.333 ± 0.218[b]	0.315 ± 0.066[a]
Cy	nd	nd	0.603 ± 0.100[b]	0.167 ± 0.114[a]
Pt	nd	nd	1.942 ± 0.138[b]	0.301 ± 0.069[a]
Pn	nd	nd	1.346 ± 0.006[b]	0.315 ± 0.040[a]
Mv	nd	nd	7.346 ± 0.745[b]	1.300 ± 0.213[a]
Ac	nd	nd	0.587 ± 0.037[b]	0.000 ± 0.000[a]
Coum	nd	nd	1.469 ± 0.124[b]	0.503 ± 0.154[a]

Analysis of variance to compare data: for each vintage, values with different letters within each row are significantly different (Duncan's test, $P < 0.05$). Healthy and botrytized berries were harvested on the 3rd Oct 2010. Data are means of duplicate determinations. Results are expressed in mg/g dw seed or skin ± standard deviation over the two replications in one grape sample. %G, percentage of galloylation; %P, percentage of prodelphinidins; Ac, sum of peonidin-3-*O*-acetylglucoside and malvidin-3-*O*-acetylglucoside; coum, sum of peonidin-3-*O*-(coumaroyl)glucoside and malvidin-3-*O*-(coumaroyl)glucoside; Cy, cyanidin-3-*O*-glucoside; Dp, delphinidin-3-*O*-glucoside; mDP, mean degree of polymerization; Mv, malvidin-3-*O*-glucoside; nd, not detected; Pn, peonidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; TPC, total phenol content.

(from 25, 22 and 23%, respectively) following fungal attack. Proanthocyanidin features, including mDP, percentage of galloylation (%G) and percentage of prodelphinidins (%P) were also investigated for two kinds of fractions: (i) a monomeric/oligomeric and (ii) a polymeric tannin fraction (Table 2). Seed tannin mDP was not significantly influenced by the pathogen in both fractions. The %G decreased significantly in the infected berries in both fractions. However, these changes in %G (20.4 vs 17.3 and 34.6 vs 30.8) were low, indicating a low impact of Botrytis on seed phenolic composition. Because of the more direct contact between skins and the grey mould pathogen, more effects and variations were expected in the case of the skin phenolic composition.

Botrytis impact on skin phenolic composition

Skin phenolic composition was highly significantly affected by *B. cinerea* infection. Major content variations between healthy and rotten grapes were detected for all phenolic compounds as shown by TPC, total tannin and anthocyanin contents which decreased markedly by 84, 70 and 82%, respectively (Table 2).

Proanthocyanidin composition analysis revealed significant reductions of all monomer, dimer and trimer compounds assessed in this study. The concentration decrease in Botrytis-affected grapes varied from 52% for catechin to 85% for B1 dimer (Table 2). Skin mDPs were also assessed in the two kinds of proanthocyanidin fractions. In the monomeric/oligomeric fraction, mDP values were not statistically differentiated (3.8 vs 5.6), in spite of an apparent increase following pathogen infection (Table 2). Conversely, in the polymeric fraction, healthy grapes exhibited an mDP of 13.1, while the mDP was considerably reduced (7.8) in rotten grapes.

Similarly, skin anthocyanin composition revealed a marked and significant decrease because of the pathogen (globally of 80%) which was noticeable for all the investigated anthocyanins: delphinidin-, cyanidin-, petunidin-, peonidin- and malvidin-3-*O*-glucosides as well as 3-*O*-acetylglucosides and 3-*O*-coumaroylmonoglucosides (Table 2).

Must analysis

In 2009, no significant variations were recorded between musts containing 0 (control), 5, 15 ('Mat') or 20% ('Overmat') of botrytized grapes for all the analysed classical parameters: reducing sugars, pH, assimilable nitrogen, malic acid, TA (data not shown). Similarly, in 2010, the same result was obtained when considering batches of must including 0 (control), 5, 10 and 15% of Botrytized grapes (data not shown).

For the 2009 vintage, the global phenol analyses showed no significant variations in TPC ('Mat' and 'Overmat'), while must total tannin contents were not assessed in 2009. However, a 46% significant decrease in total anthocyanin content was noticeable between control must and must supplemented with 15% of rotten berries ('Mat') (Figure 1d). However, no impact of Botrytis was noticed for the second trial using overmature berries ('Overmat') (Figure 1d). In 2010, a steady and significant decrease in TPC was recorded between control and the 15% must batch (Figure 1a). However, no significant variation was observed in total tannin content (Figure 1b). Lastly, as in 2009 ('Mat'), an important and progressive decline of anthocyanin content was because of an increasing percentage of botrytized grapes in musts (Figure 1c). There was a 57% anthocyanin drop in the must containing 15% of rotten berries (Figure 1c).

Thus, these results mainly reflected the differences in grape phenolic composition, particularly in skins, between healthy and botrytized grapes as previously assessed. The decrease in anthocyanin content was the most significant and reproducible in both consecutive vintages (2009 'Mat' and 2010).

Wine analysis

After micro-scale winemaking, we obtained, in 2009, six different wines containing 0, 5 and 15% as well as 0, 5 and 20% of botrytized grapes for 'Mat' and 'Overmat' types of berries, respectively. In 2010, four different wines contained 0, 5, 10 and 15% of botrytized grapes. The classical wine analyses, first performed by means of infrared technique were similar in 2009 ('Mat') and 2010 (Table 3). In both cases, only significant differences between the different batches were revealed in total and volatile acidities. The wines issued from 15% rotten batches showed significant increases in TA of 4 and 6% and in volatile acidity of 112 and 10% in 2009 and 2010, respectively. Resulting from the grape highest content in sugar, alcohol volume was higher in 2010 (14.9 vs 13.5 vol%). In 2009, results with overmature fruits ('Overmat') showed similar variations in total and volatile acidities, although they were not statistically significant for TA. Moreover, for these wines, an increase of 6% in alcohol content was noticed in the 20% batch, reflecting the high S concentration in the rotten and overripened berries.

Global phenolic wine analyses, including TPC, total tannin and anthocyanin, underlined only significant and consistent variations in total anthocyanin content consecutively to addition of rotten berries during both vinifications in 2009 ('Mat') and 2010 (Figure 2a,b). The concentration decrease was of 17 and 15% between the control wine and the wine issued from 15% rotten berries in 2009 and 2010, respectively. In 2009, the results from the trial using overmature berries ('Overmat') differed from the others because there was only a significant variation in TPC which decreased from 1776 (GAE) to 1599 mg/L (GAE) between control and 20% wine (Figure 2a).

Looking into the detail of individual proanthocyanidin concentrations, 2009 and 2010 data ('Mat' and 'Overmat') underlined a global trend to increased concentrations of proanthocyanins in wines containing the highest percentages of Botrytis-affected grapes (Figure 3a,b). In 2009, the increase in proanthocyanidin concentration was significant ($P < 0.05$) for the catechin monomer (+18%), B2 (+40%) and B3 (+64%) dimers and the trimers (+20%) for the trial at maturity ('Mat'). At overmaturity ('Overmat'), the increase was significant for catechin (+19%), epicatechin (+22%), B2 (+35%), B3 (+19%) and B4 (+20%) dimers. In 2010, these increases in proanthocyanidin concentration following addition of botrytized berries were less obvious, mostly as a trend, but confirmed significantly for both the catechin monomer (+5%) and the B3 (+8%) dimer.

Proanthocyanidin polymeric characteristics such as mDP, %G, %P in the different wines exhibited no significant variations in 2009 ('Mat' and 'Overmat') (Table 4). Conversely, in 2010, slight significant decreases in mDP, from 4.7 to 4.1, and in %G, from 33.1 to 26.5, were observed in wines containing the highest percentages of rotten berries. By contrast, %P increased with the percentage of botrytized grapes. Although not significant, the increase in %P was also noticeable in 2009, as a trend, in both 'Mat' and 'Overmat' trials.

In both vintages 2009 and 2010, most of the individual anthocyanin concentrations decreased significantly as the percentage of rotten grapes increased in wines (Figure 3c,d). This was observed for the majority of anthocyanins in keeping with the wine total anthocyanin content and the skin phenolic composition. In 2009 ('Mat'), rates of depletion were estimated at −0.44, −3.98, −1.66 and −0.59 mg/L/% of botrytized berries for the peonidin-, malvidin-3-*O*-glucosides and the acetyl- and

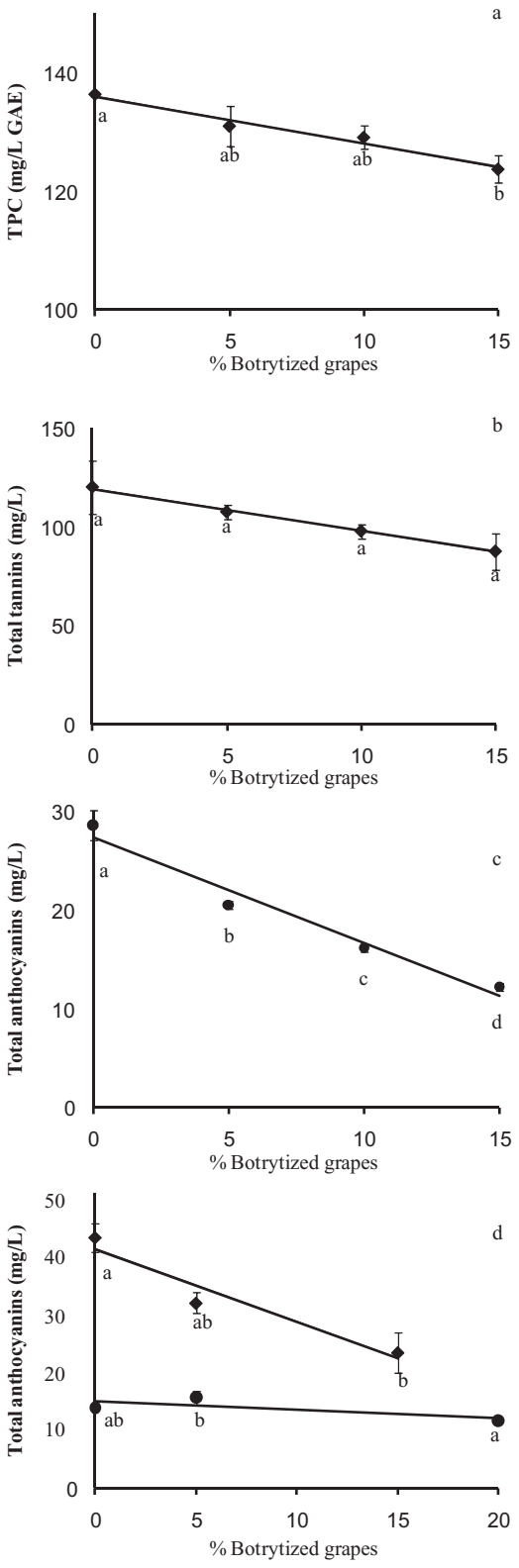

Figure 1. Global phenolic analyses of musts containing different percentages of botrytized grapes. Total phenol content (TPC) in gallic acid equivalent (GAE) (a), total tannin content (b), total anthocyanin content (c) for 2010 vintage and total anthocyanin contents for 2009 vintage (Diamond (◆): artificially inoculate visually healthy mature berries (Mat), circle (●): overmaturated berries naturally infected by the pathogen (Overmat) cf Material and methods) (d). Results are expressed as means ± standard deviation (SD). Error bars represent SD. Analysis of variance to compare data: for each parameter, values with different letters are significantly different (Duncan's test, $P < 0.05$).

Table 3. Classical oenological parameters of wines containing different percentages of Botrytis-affected grapes for vintages 2009 (Mat and Overmat†) and 2010.

		2009					2010				
		Ethanol (% v/v)	pH	Total acidity (g/L‡)	Volatile acidity (g/L‡)	Tartaric acid (g/L)	Ethanol (% v/v)	pH	Total acidity (g/L‡)	Volatile acidity (g/L‡)	Tartaric acid (g/L)
Mat	0%	13.58 ± 0.05^{a}	3.83 ± 0.01^{b}	3.37 ± 0.03^{a}	0.25 ± 0.01^{a}	1.59 ± 0.00^{a}	14.95 ± 0.12^{a}	3.76 ± 0.01^{a}	3.36 ± 0.02^{a}	0.30 ± 0.00^{a}	2.72 ± 0.13^{a}
	5%	13.47 ± 0.04^{a}	3.78 ± 0.00^{a}	3.54 ± 0.03^{ab}	0.31 ± 0.01^{a}	1.62 ± 0.04^{a}	14.88 ± 0.28^{a}	3.69 ± 0.04^{a}	3.66 ± 0.02^{d}	0.32 ± 0.01^{b}	2.65 ± 0.18^{a}
	10%	nd	nd	nd	nd	nd	14.87 ± 0.15^{a}	3.76 ± 0.02^{a}	3.44 ± 0.01^{b}	0.33 ± 0.01^{bc}	2.44 ± 0.02^{a}
	15%	13.48 ± 0.05^{a}	3.81 ± 0.01^{ab}	3.49 ± 0.08^{b}	0.53 ± 0.07^{b}	1.71 ± 0.08^{a}	14.95 ± 0.06^{a}	3.74 ± 0.01^{a}	3.55 ± 0.02^{c}	0.33 ± 0.00^{c}	2.43 ± 0.05^{a}
Overmat	0%	14.65 ± 0.00^{a}	3.86 ± 0.00^{b}	3.66 ± 0.05^{a}	0.21 ± 0.00^{a}	1.82 ± 0.01^{b}	nd	nd	nd	nd	nd
	5%	14.81 ± 0.07^{a}	3.79 ± 0.01^{a}	3.84 ± 0.07^{a}	0.23 ± 0.01^{a}	1.77 ± 0.03^{ab}	nd	nd	nd	nd	nd
	20%	15.52 ± 0.22^{b}	3.89 ± 0.01^{b}	3.80 ± 0.01^{a}	0.28 ± 0.01^{b}	1.70 ± 0.01^{a}	nd	nd	nd	nd	nd

†'Mat', artificially inoculate visually healthy mature berries; 'Overmat', overmaturated berries naturally infected by the pathogen. ‡Equivalent sulfuric acid for total acidity, equivalent sulfuric acid for volatile acidity. Data are means of duplicate determination, ±standard deviation over the two replications. Analysis of variance to compare data: for each parameter, values with different letters within each column are significantly different (Duncan's test, $P < 0.05$). nd, not detected.

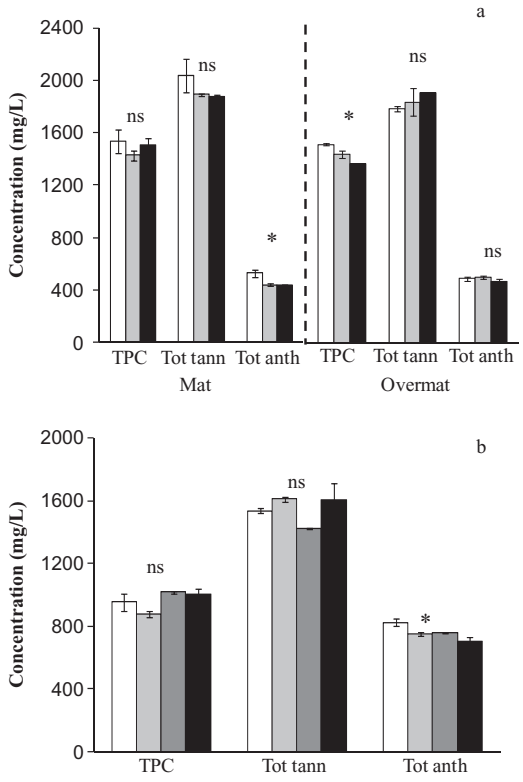

Figure 2. Total phenol content (TPC) in gallic acid equivalent, total tannin (Tot tann) content and total anthocyanin (Tot anth) content of wines containing different percentages of botrytized grapes for 2009 vintage (a) and for 2010 vintage (grey colour gradations from blank, control or 0% to black, 15 (artificially inoculate visually healthy mature berries (Mat)) or 20% (overmaturated berries naturally infected by the pathogen (Overmat)) with 5% intermediates for 2009 vintage. Grey colour gradations from blank, control or 0% to black, 15% with 5 and 10% intermediates for 2010 vintage). Results are expressed as means ± standard deviation (SD). Error bars represent SD. Analysis of variance to compare data (Duncan's test, $P < 0.05$): for each compound, 'ns' means none significant difference between the different percentages, while '*' indicates significant differences at minimum between the two extreme percentages (0 vs 15 or 20% for 1009, 0 vs 15% for 2010).

coumaroyl-glucosides, respectively. In 2010, these rates were slightly lower, reaching –0.32, –1.66, –1.19 and –0.55 mg/L/% of botrytized berries. Finally, for the 'Overmat' trial in 2009, the depletion rates were the lowest, i.e. –0.22, –1.14, –0.54 mg/L/% of botrytized berries for the peonidin-, malvidin-3-*O*-glucosides and the acetylglucosides, respectively.

Further investigation of sensory properties of botrytized wines firstly showed, by a triangle test, that judges easily distinguished between a control wine made from healthy grapes and wines obtained from 15 or 20 and 5% of rotten berries in 2009 ('Mat' and 'Overmat') (Table 5). In 2010, the results were less obvious. Astonishingly, judges found it easier to recognize the difference between control and low percentage of rotten berries (5%) wines than between control and higher percentage of rotten berry (15%) wines. Nevertheless, these tests confirmed that only 5% of rotten grapes in the winemaking process could be enough to discriminate between these botrytized wines and the control wines (Table 5). Wine characterization in 2009 based on three criteria, i.e. fruit (nose and mouth), astringency and bitterness, the ANOVA analysis revealed no significant distinction between control and botrytized wines for these parameters (data not shown). On the other hand, during triangle tests,

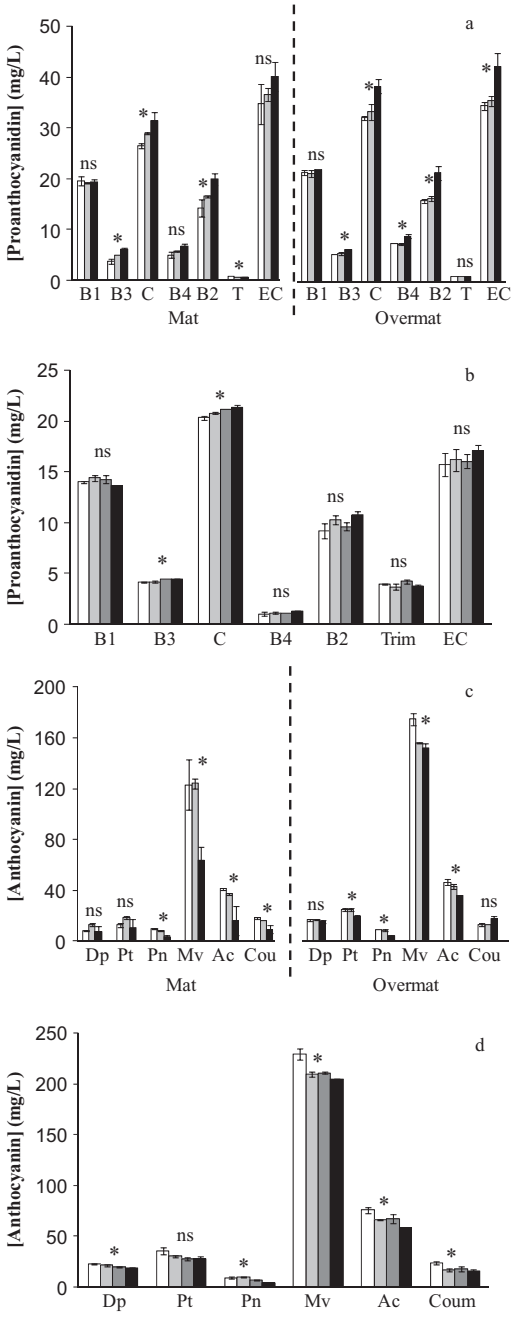

Figure 3. Individual proanthocyanidin concentrations (a: 2009 vintage; b: 2010 vintage) and individual anthocyanin concentrations (c: 2009 vintage; d: 2010 vintage) of wines containing different percentages of Botrytis-affected grapes (grey colour gradations from blank, control or 0% to black, 15 (artificially inoculate visually healthy mature berries (Mat)) or 20% (overmaturated berries naturally infected by the pathogen (Overmat)) with 5% intermediates for 2009 vintage. Grey colour gradations from blank, control or 0% to black, 15% with 5 and 10% intermediates for 2010 vintage). Results are expressed as means ± standard deviation (SD). Error bars represent SD. Analysis of variance to compare data (Duncan's test, $P < 0.05$): for each compound, 'ns' means none significant difference between the different percentages, while '*' indicates significant differences at minimum between the two extreme percentages (0 vs 15 or 20% for 1009, 0 vs 15% for 2010). Ac, sum of peonidin-3-*O*-acetylglucoside and malvidin-3-*O*-acetylglucoside; Coum, sum of peonidin-3-*O*-(coumaroyl)glucoside and malvidin-3-*O*-(coumaroyl)glucoside; Cy, cyanidin-3-*O*-glucoside; Dp, delphinidin-3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside.

Table 4. Proanthocyanidin characteristics (mDP, %G, %P) of wines containing different percentages of Botrytis-affected grapes for vintages 2009 (Mat and Overmat†) and 2010.

		mDP		%G		%P	
		2009	2010	2009	2010	2009	2010
Mat	0%	2.3 ± 0.1[a]	4.7 ± 0.1[c]	4.9 ± 1.7[a]	33.1 ± 0.5[b]	17.5 ± 1.0[a]	23.4 ± 3.0[a]
	5%	2.3 ± 0.2[a]	4.6 ± 0.1[bc]	7.6 ± 3.2[a]	27.7 ± 0.1[a]	15.6 ± 3.9[a]	30.6 ± 0.3[b]
	10%	nd	4.4 ± 0.1[ab]	nd	29.2 ± 0.7[ab]	nd	29.0 ± 0.1[ab]
	15%	2.2 ± 0.3[a]	4.1 ± 0.0[a]	7.0 ± 1.4[a]	26.5 ± 1.9[a]	23.8 ± 9.2[a]	30.9 ± 0.5[b]
Overmat	0%	2.1 ± 0.2[a]	nd	4.6 ± 0.3[a]	nd	15.6 ± 4.0[a]	nd
	5%	2.2 ± 0.1[a]	nd	6.1 ± 2.5[a]	nd	14.6 ± 0.7[a]	nd
	20%	2.1 ± 0.2[a]	nd	4.5 ± 1.2[a]	nd	20.6 ± 4.4[a]	nd

†'Mat',artificially inoculate visually healthy mature berries; 'Overmat', overmaturated berries naturally infected by the pathogen. Data are means of duplicate determinations. Results are expressed as means ± standard deviation. Analysis of variance to compare data: for each parameter, values with different letters within each column are significantly different (Duncan's test, $P < 0.05$). %G, percentage of galloylation; %P, percentage of prodelphinidins; mDP, mean degree of polymerization; nd, not detected.

Table 5. Results of the triangle tests performed by 20 panellists for vintages 2009 (Mat and Overmat†) and 2010.

	Batch vs control batch	Number of correct answers		Off-flavour quotations	
		Rep 1	Rep 2	Rep 1	Rep 2
2009					
Mat	5%	19***	18***	15	13
	15%	15***	17***	12	12
Overmat	5%	18***	17***	17	16
	20%	13**	13**	13	11
2010					
	5%	14***	14***	13	14
	10%	13**	12*	12	12
	15%	8	8	7	6

*Significance level 5%; **Significance level 1%; ***Significance level 0.1%. †'Mat', artificially inoculate visually healthy mature berries; 'Overmat', overmaturated berries naturally infected by the pathogen.

more than the half of the judges clearly indicated off-flavours development such as 'damp earth', 'reduced', 'wild mushroom' and 'vegetal' smells (Table 5). In 2010, a significant decrease in astringency was perceived by judges following increased percentages of botrytized grapes in parallel to the appearance of off-flavours.

Discussion

In this study, the impact of grey mould (*B. cinerea*) was investigated on phenolic composition of Merlot grapes and derived musts and wines containing various percentages of rotten berries. The sensory profiles of the derived wines were also assessed. All analyses were attempted in order to define a 'tolerable infection threshold' to be applied to mature berries at harvest.

B. cinerea *impact on berry phenolic composition*

B. cinerea impact was investigated on the chemical composition of both seeds and skins. In the 2010 vintage, *B. cinerea* had little effect on seed phenolic composition (Table 2). Such results could be expected because fungal development is mostly localized within the berry skin, resulting in a limited contact between *B. cinerea* and the seed (Jarvis 1977). On the other hand, in the skin, a significant impact of the pathogen was observed affecting and damaging several phenolic compounds with decreases in concentration of greater than 50% (Table 2).

During *B. cinerea* infection and grape tissue colonization, the mycelium grows at the fruit surface and within the skin tissue, leading to a direct contact between the skin constituents, notably the phenolic compounds and the fungal extracellular enzymes. In numerous studies, laccases have been isolated and purified from *B. cinerea* (Bollag and Leonowicz 1984, Oszmianski et al. 1985, Pezet 1998). These extracellular enzymes catalyze the oxidation of a large variety of phenolic compounds. Oszmianski et al. (1985) investigated the enzymatic oxidation by *B. cinerea* laccase of a grape seed extract containing gallic acid, catechin, epicatechin and four proanthocyanidin dimers. HPLC determination showed considerable depletion because of oxidation of all these phenolic compounds. In our study, a similar oxidation process is assumed to occur with the anthocyanins appearing as most sensitive because their concentrations decreased by more than 80%. The larger molecules, such as polymeric proanthocyanidins, may also have been more affected than the smaller ones (monomers, dimers and trimers) as shown by the significant decrease in mDP revealed in the tannin polymeric fraction. The trimer and dimers (B1, B2, B3) appeared also to be more degraded (77, 75, 62 and 77%, respectively) than the two monomers (C and EC) which decreased by 52 and 66%, respectively. This agrees with previous results showing kinetic variations in oxidation for the different phenolic compounds present in the seed extracts (Oszmianski et al. 1985).

In this study, the dimeric proanthocyanidins were more quickly degraded than catechin and epicatechin. In complex media such as extracts, the lower breakdown of these last two monomers might result from competitive reactions, while a synergistic effect might cause the degradation of other proanthocyanidins that are slightly reactive in pure solution (Oszmianski et al. 1985). This phenomenon may result from coupled chemical oxidations with quinones formed in the early stages of the enzymatic oxidation. On the other hand, it has been shown that enzymatic oxidation of catechin in pure solution leads to the formation of several dimers, i.e. colourless and yellow dimers (Guyot et al. 1995, 1996, Osman et al. 2007). This may

account, in our study, for the brown-yellow colouring noticeable on the skins outer and the increase, although not significant, in mDP of the tannin monomeric/oligomeric fraction (3.8 in healthy vs 5.6 in rotten berries). Conversely, in the polymeric fraction, an important decrease in mDP was recorded following infection by the pathogen in the grape skin. However, no depolymerization enzymatic activities attributed to *B. cinerea* have been previously reported in the literature. Thus, it can be suggested that the oxidation of polymeric proanthocyanidins may cause complex structural rearrangements and/or oxidative breakdown of some interflavane linkages cleavable, and thus, not quantified by phloroglucinolysis any more.

B. cinerea *impact on must phenolic composition*

By chemically analysing different musts containing various percentages of rotten grapes, significant decreases in total anthocyanin were detected in 2009 'Mat' and in 2010 (1.3 and 1.1 mg/L/% of rotten berries, respectively) (Figure 1). They were related to the phenolic composition variations previously measured in skins.

In grape berries, laccase from *B. cinerea* is soluble and in musts, this enzymatic activity remains. Juices from recently contaminated grapes are characterized by a laccase activity reduced by half compared with juices from old rot (Grassin and Dubourdieu 1989) (Fermaud, unpublished data, 2012). Consequently, the age of grey mould development may be considered as a major factor in influencing the level of laccase activity in rotten berries and derived musts. Regarding Botrytis rot age in our study (Table 1), the contaminated fruit sampled in our trials might be characterized by a weaker laccase activity (not measured) in 2010 compared with 2009. This hypothesis would be consistent with the anthocyanin depletion rate being slightly higher in 2009 ('Mat') than in 2010. As for the 2009 'Overmaturity' results, must anthocyanin concentrations appeared low in the control musts from healthy berries resulting naturally from the effect of the fruit overripening process (Fournand et al. 2006, Holt et al. 2010). Under these particular conditions, the effect of *B. cinerea* activity seems to be more difficult to demonstrate significantly as the potential variations between batches are reduced.

B. cinerea *impact on wine chemical and phenolic composition*

Among all classical oenological parameters assessed in this study, the impact of grey mould at maturity was only detected on total and volatile acidities in both 2009 and 2010 wines (Table 3). The synthesis of citric and acetic acids by certain strains of *B. cinerea* has been previously reported (Ribereau Gayon et al. 1979, Ribéreau Gayon et al. 2004, Tosi et al. 2005) which could explain these variations. Nevertheless, the 112% increase in 2009 'Mat' results might also be connected with sour rot deviations appearing with Botrytis inoculation on grapes in spite of the sorting performed after *B. cinerea* inoculation. As for wines from overmature berries in 2010, the increase in %vol alcohol because of a higher S in rotten berries was in good agreement with other studies, notably, those concerning the effect of noble rot also because of *B. cinerea* (Ribéreau Gayon et al. 1980).

Total phenolic measurements in the different wines underlined significant decrease in total anthocyanin contents in 2009 ('Mat') and 2010 (–17 and –15% between control wine and 15%-rot wine, respectively) (Figure 2). In comparison with the decrease of total anthocyanin content in the skins (–82% for 2010), a decline was also expected in the wines. The recorded variations could first appear low but reflect the dilution of botrytized grapes among healthy grapes (15%, w/w). Indeed, relating to 15% of botrytized grapes in the winemaking process and considering a quasi-complete anthocyanin extraction, a simple calculation yields a predicable decline of 12% (15% × 82% = 12%) in wines, which is in good accordance with our measurements. The resulting depletion rates corresponded to 6.1 mg/L/% of rotten berries and 8.1 mg/L/% of rotten berries in 2009 and 2010, respectively. In musts, the total anthocyanin content before maceration (earlier mentioned musts results) decreased by 46% in 2009 and 57% in 2010 in the 15% rot batch, confirming that anthocyanins were very well extracted during the maceration and the winemaking process. Similarly, a TPC decrease of 16 and 84% in seeds and skins was shown for affected grapes, respectively (vintage 2010). In musts before maceration, a significant decrease of 9% between the two extreme batches (0 vs 15%) was only observed in 2010, whereas after winemaking, no variation appeared between the different wines. A similar pattern was noted for the variations in total tannin contents. Thus, good extraction of all phenolic compounds was achieved in the maceration from seeds as well as from skins.

During *B. cinerea* infection, Kamoen (1992) reported that the skin tissues are progressively degraded by fungal extracellular enzymatic activities such as cutinase, polygalacturonase, pectin lyase, pectin-methyl esterase, acid proteinase, glucanase, cellulase, phospholipidase and lipase. These activities should enhance phenolic release, notably from the grape skin, leading to good extraction through vinification.

For both investigated vintages, several individual proanthocyanidin (notably, catechin monomer and B3 dimer) concentrations in wines tended to increase as the percentage of rotten grapes increased (Figure 3). However, the grape berry analyses performed in 2010 showed important decreases in skin proanthocyanidin concentrations following infection by the pathogen. In this context, it should be pointed out that the total tannin content in the skin tissue has been considered as contributing to a large extent to the preformed resistance of the berry to the pathogen (Deytieux-Belleau et al. 2009). During maceration and the winemaking process, a better extraction of phenol compounds is achieved in wines containing rotten grapes because of disruption of the skin cell contents induced by the fungal extracellular enzymatic activities previously mentioned. Another explanation could consider that an oxidation activity still occurred in wines leading to the release of monomers and/or dimers from higher polymerized molecules. Furthermore, comparing rot ages in 2009 (8–9 days) and in 2010 (4 days) affecting *B. cinerea* activity, the more intense increase in monomeric and dimeric proanthcoyanidin concentrations in 2009 compared with 2010 confirm the hypothesis of a persistent laccase activity in the finished wines. This hypothesis could also be supported by the mDP decrease (4.7 to 4.1) recorded in 2010 botrytized wines, but unfortunately this trend was not obvious for the 2009 vintage. These results were also interesting because in grapes, variations related to mDP concerned essentially polymeric skin proanthocyanidin mDP. This observation suggests that low mDP tannins were in majority extracted during maceration.

Anthocyanins still proved to be the most sensitive molecules to enzymatic oxidation because their concentrations remained reduced in wines containing the highest percentages of affected grapes. Comparing the 2009 'Mat' and 2010 data, the decrease was noticeable mainly for the same molecules (peonidin-, malvidin-3-*O*-glucosides, acetyl- and coumaroyl-glucosides). The decrease was more marked in 2009 than in 2010, displaying once more the positive link between the presumed laccase activity intensity and grey mould age.

B. cinerea *impact on wines sensory quality*

The sensory analyses showed that quality of wines containing rotten grapes was negatively affected from the threshold of 5% of botrytized berries upward. This depreciation was mainly attributed to the appearance of off-flavours such as 'damp earth', 'vegetal/herbal like', 'mushroom'. Several studies have shown that grape rot frequently leads to the development of organoleptic deviations in grapes and wines. Organoleptic defects such as mushroom, mouldy and camphoric odours have been reported in musts made from rotten grapes and sometimes also in wines (La Guerche et al. 2006, La Guerche et al. 2007). Geosmin and 1-octen-3-one have been identified in rotten grapes of various varieties as well as in musts made from rotten grapes (Pallotta et al. 1998, Darriet et al. 2000, La Guerche et al. 2007). With relatively low perception thresholds, such compounds persist after alcoholic fermentation and may be responsible for major defects in wine (La Guerche et al. 2006). In 2010, a significant decrease in astringency was perceived in botrytized wines. These results were in good agreement with the mDP decrease that we noticed in the same wines because several authors indicate that molecular size of proanthocyanidins affect their bitterness and astringency (Gawel 1998, Cheynier et al. 2006).

Conclusions

This research provided further evidence that grey mould (*B. cinerea*) is detrimental to grape skin phenolic composition, must and wine composition and sensory properties. Exoenzymes secreted by the fungus and laccase, in particular, are speculated to be the main causal agent resulting in oxidation phenomena and quality depreciation. The strong consequences of *B. cinerea* noticed on phenolic contents as well as on mDP were nevertheless attenuated in the derived wines because of a dilution effect but also a better extractability of phenols from botrytized grapes. A persistent *B. cinerea* activity in derived musts and wines may also explain some monomeric and dimeric proanthocyanidin concentration increases in the wines containing the highest percentages of botrytized grapes. The *B. cinerea* activity was in good agreement with the grey mould age as it was previously known for laccase activity. Nevertheless, laccase activity remains to be measured to confirm its implication in phenol depletion.

This study was performed on two vintages with distinctive climatic conditions leading to different modes of inoculation. In 2009, two kinds of grey mould inoculation were chosen, an artificial inoculation on healthy mature berries and a natural *B. cinerea* inoculation on overmature berries, while in 2010, natural fungus development was achieved because of favourable weather conditions. The data collected from 2009 'Mat' and 2010 followed the same pattern and variations, while those of 2009 'Overmat' differed. This observation underlines different mechanisms concerning *B. cinerea* development during overmaturity and maturity In the case of a *B. cinerea* development on grapes at maturity, germination tube penetration in berries is only possible by exogenous wounds (caused by insects, hail) through stomatal ostriols or peristomatal breaks. On the other hand, in the case of an overmaturity inoculation, the direct penetration of mould germination tubes through the skin complex of berries is easier. Then, the 'Overmat' inoculation is probably closer to the noble rot infection process with some desiccations, reduced berries sizes and consequent concentrations phenomena. In any case, we showed that *B. cinerea* oenological consequences were serious and dramatic from 5% (w/w) of botrytized berries in wines (phenols oxidation, colour changes, off-flavour appearance). From that point of view, ageing of such wines will also be difficult to anticipate with probable underripe phenomena. Then, prophylactic actions as well as the measurement of the vine sanitary state and intensive sorting therefore present here an undeniable interest.

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