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Impact of *Plasmopara viticola* infection of Merlot and Cabernet Sauvignon grapes on wine composition and flavor

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ABSTRACT

This work reports the identification of volatile compounds involved in the particular and atypical flavor detected in *Vitis vinifera* red Merlot and Cabernet Sauvignon wines made with grapes infected and wilted by brown rot (*Plasmopara viticola*). Must made from withered grapes had green aromas while red wines were marked by intense odor reminiscent of green, herbaceous notes but also figs and cooked fruit. Thanks to GC-O and GC–MS analysis, cooked fruit notes were identified as 3-methyl-2,4-nonanedione, γ-nonalactone and γ-decalactone, whereas herbaceous and green aromas were identified as (*Z*)-1,5-octadien-3-one and 3-isobutyl-2-methoxypyrazine. We show that the organoleptic impact of *P. viticola* is more pronounced in Merlot wines compared to Cabernet Sauvignon ones. The highest levels of 3-methyl-2,4-nonanedione (75.3 ng/L) were found in old Merlot wines made with 20% infected berries, suggesting the incidence of berry quality on the ability of a wine to age.

1. Introduction

Nowadays, regardless of the geographical location of the vineyards in the world, it is not possible to produce quality wines without taking into account the health status of the grape at harvest. Vineyards enjoying an oceanic climate with relatively high annual rainfall such as the Bordeaux vineyard with *V. vinifera* grapes are mainly concerned by *Plasmopara viticola* disease, also called downy mildew. This pathogen was first introduced into European vineyards from North America in the 1870s (Millardet, 1881) before spreading to all major grape-producing regions in the world. It infects all green parts of the plant causing extensive losses in grape yield in favorable weather conditions (Dubos, 2002). The damage caused by the pathogen can lead to quantitative losses by infecting inflorescences and bunches and to qualitative losses by causing early defoliation of the plant and changes in the chemical composition of berries (Jermini, Blaise, & Gessler, 2010a, 2010b). While the control of downy mildew requires regular application of fungicides (Butault et al., 2010), the intensive use of chemicals is becoming more and more restrictive owing to the human health risk and the negative environmental impact.

Before flowering, the inflorescences contaminated by *P. viticola* dry up and fall off. The attacks, lasting from flowering to the beginning of fruit set, produce grey rot characterized by the development of greyish fructifications. Later in the season, attacks on still green berries, and then their colonization, cause the formation of brown lines or mottles that appear and cause the browning of the tissues, i.e. necrosis called brown rot (Fig. 1, supplementary data).

The attacked berries wither but do not soften. Thus, these "green" grape berries that are withered as a result of the pathogen can be found in tanks during winemaking when the grapes are harvested mechanically without sorting.

In 1893, Viala first described the impact of the vinification of these "diseased" berries on the quality of the wines and observed the "disappearance of the bouquet in high-quality wines" (Viala, 1893). Recent preliminary work carried out in the laboratory has made it possible to highlight the presence of nuances associated with a mixture of odors reminiscent of cooked fruits (prune, cooked peach, fig) and both herbaceous odors (ivy, geranium) in red wines from Merlot grape varieties. These nuances were perceived as detrimental to the organoleptic quality of the wines.

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Other observations showed that these shades seemed to be absent from white Chardonnay wines made from grapes withered under the action of the same pathogen. These observations seemed surprising in the light of previous work dealing with the incidence of other well-known cryptogamic diseases, such as powdery mildew (*Erysiphe necator*) or grey mold (*Botrytis cinerea*) on the quality of red and white grapes and wines where moldy, earthy descriptors characterize their odors (Calonnec, Cartolaro, Poupot, Dubourdieu, & Darriet, 2004).

The specificity of these sensorial descriptors probably suggests a different origin of the chemical marker of this off-flavor in red wines. Indeed, in the aforementioned settings, it has been established that the fungus plays a predominant role in the formation of the odorous compounds characteristic of the defects of the earthy-fungal type perceptible directly in must and also detected in wines. The compounds responsible for these odors are now known: 1-octen-3-one, (-)-geosmin and in some cases 1-nonen-3-one (La Guerche, Dauphin, Pons, Blancard, & Darriet, 2006; Pons et al., 2011).

Also, it is likely that other odorous compounds associated with specific mechanisms are involved in the formation of the herbaceous-cooked fruit nuances of the wines resulting from the vinification of grapes infected by brown rot.

For this reason, this study reports new data concerning the identification of compounds associated with herbaceous/cooked fruit character of wines made with grapes infected by *P. viticola*. Experiments in the laboratory and in the field were also performed to assess the impact of increasing concentrations of withered Merlot and Cabernet Sauvignon berries infected by the pathogen on the herbaceous/cooked fruit odor intensity and their associated molecular markers in wines.

2. Materials and methods

2.1. Chemicals

Dichloromethane (99.99%) was supplied by Fisher Scientific (Illkirch, France) and absolute ethanol (99.9%) by Merck (Semoy, France). All the reference compounds were provided by Sigma-Aldrich (Saint-Quentin-Fallavier, France): 3-octanol (99%), γ-lactones and δ-decalactone (99%), ammonium sulfate (greater than99%), 3-isobutyl-2-methoxypyrazine (99%), alkanes (C_7-C_{30}). 3-Methyl-2,4-nonanedione (greater than 99%) was purchased from Chemos GmbH (Germany); (*Z*)-1,5-octadien-3-one sample (97%, 1 g/L in hexane) was a gift from Nestlé® (Lausanne, Switzerland).

2.2. Grape selection

In 2009 and 2012, two vineyards in Bordeaux area were selected as experimental plots. The first experiment concerned Merlot grapes (2009 vintage), the second Cabernet Sauvignon grapes (2012 vintage). For each plot, four rows (80 vines) divided in two were selected. Vines were trained on a vertical shoot trellising system and cane-pruned. The first part of the row received chemical treatments and the second received none. For the non-fungicide modality, no fungicide was applied at any time during the growing season. For the fungicide modality, fungicides were applied according to the specific grape diseases in each vineyard. In the 2009 and 2012 experiments, fungicides were sprayed on average 10 times to control the main diseases, such as downy mildew, powdery mildew and black rot.

In 2012, healthy Merlot grapes and dried berries infected by *P. viticola* were collected at the vineyard and kept at −20 °C before use in laboratory experiments.

2.3. Supplementation experiments at lab scale

2.3.1. Experimental design

Seven modalities corresponding to increasing concentrations of berries infected with brown rot (Merlot, 2012 vintage) were selected: 0, 1, 2, 5, 8, 10, 15 g/75 mL. The berries were added to the must just before adding commercial dry active yeast (20 g/hL, *S. cerevisiae*, FX10 strain; Biolaffort, Bordeaux, France), previously rehydrated according to the manufacturer's recommendations. The bottles (100 mL) were stored in a controlled temperature room at 24 °C. Fermentation kinetics was monitored thanks to daily measurement of carbon dioxide loss. After alcoholic fermentation, lees were removed by centrifugation (5 min, 6000 rpm), samples were sulfited (50 mg/L) and stored at 4 °C before SPME-GC–MS and sensorial analysis experiments. Experiments were performed in triplicate.

2.3.2. Must composition

The must selected for microvinification in the laboratory was a red Merlot must from 2012 vintage (DUCOURT vineyards, Bordeaux) whose standard analytical characteristics (analyses carried out by Sarco Laboratory, Floirac) were as follows: reducing sugars: 209 g/L, tartaric acid (TA) 4.33 g/L (H_2SO_4), pH 3.59, total sulfur dioxide SO_2T 36 mg/L, l-malic acid at 3.79 g/L, yeast assimilable nitrogen (YAN) 124 mg/L. The must was slightly deficient in nitrogen. To avoid sluggish alcoholic fermentation, the yeast assimilable nitrogen (YAN) concentration was adjusted to 200 mg/L before each trial by a 0.3 g/L Thiazote solution (i.e. 36.9 mg/L).

2.3.3. Synthetic must composition

This was as described by Marullo (2005). Briefly, the medium was buffered to a pH of 3.3 and contained (g/L): glucose (100); fructose (100), l-tartaric acid (3), citric acid (0.3), l-malic acid (0.3), mineral salts, vitamins, amino acids, anaerobic growth factors and a nitrogen source: 190 mg/L YAN provided by $(NH_4)_2SO_4$. This medium is routinely used on the day of its preparation.

2.4. Study of origin of dried fruit-herbaceous character: A sensorial approach

A synthetic must (75 mL) was supplemented or not with 9 g of Merlot berries (2012 vintage) wilted by the action of brown rot per four treatments. 1. Control treatment, must fermented without berries (AF−) 2. Maceration in must, 5 days at 24 °C, without alcoholic fermentation (M+AF−). 3. Maceration for 5 days at 15 °C, then berries removed before rehydrated yeast addition and alcoholic fermentation (M+B− AF+). 4. Fermentation with berries (B+ AF+); berries and rehydrated yeast were supplemented to the must. After maceration and alcoholic fermentation, samples were centrifuged (5 min, 6000 rpm) and intensity of nuances was evaluated.

2.5. Winemaking conditions

Red wines were made with grapes naturally infected by *P. viticola* and increasingly supplemented with control grapes (grapes from fungicide treatment modality). During the experiments, incorporation was carried out in % of withered berries per cluster for the six modalities in 2009 (0, 2, 5, 10, 15, 20%) and five modalities in 2012 (0, 2, 5, 10, 20%) per the following protocol.

Harvesting was carried out manually by removing possible foci of grey rot (*B. cinerea*) from the plot. Each modality contained 40 kg of grapes vinified in 50-L stainless steel tanks.

Standard winemaking procedures were followed including alcoholic and malolactic fermentation thanks to commercial dry active yeast (20 g/hL, *S. cerevisiae*, Actiflore cerevisiae strain; Biolaffort, France) and bacterial strains (1 g/hL, Vitilactic F; Martin Vialatte

Oenologie, Epernay, France). At the end of malolactic fermentation, red wine was sulfited at 50 mg/L (100 g/L solution in water; Biolaffort, France). Before bottling the wine was fined and filtered. Wines were filled into 750-mL glass Bordeaux bottles and closed using standard commercial practices. Sulfur dioxide was adjusted at 30 mg/L before bottling and sealed with a synthetic stopper. Bottles were kept in a dark and temperature-controlled chamber (16 °C) until required for analysis.

2.6. Sample preparation

2.6.1. Liquid/liquid extraction

Wine samples (100 mL) were spiked with 100 μL 3-octanol (100 mg/L EtOH) as internal standard (IS). The wines were extracted three times with 10, 5, and 5 mL dichloromethane by magnetic stirring (10, 5, 5 min; 750 rpm). The three organic phases obtained after each extraction were blended, dried over anhydrous sodium sulfate, and concentrated to 0.5 mL under a nitrogen stream.

2.6.2. HS-SPME extraction

The methodology was adapted from different works reporting the assay of lactones (Langen, Wang, Slabizki, Wall, & Schmarr, 2013) and 3-isobutyl-2-methoxypyrazine (Kotseridis et al., 2008) in different matrices. One milliliter of wine and 9 mL of mQ water were placed in a 20-mL vial containing 5 g ammonium sulfate and 10 μL IS1 (3-octanol, 100 mg/L, EtOH) and 10 μL IS2 (2-octanol, 100 mg/L, EtOH). The vial was sealed with a PTFE-lined cap (Chromoptic, Villejust, France). Volatiles were isolated from the headspace using a 65 μm polydimethylsiloxane-divinylbenzene fiber (PDMS/DVB; Supelco, Lyon, France). Headspace-SPME parameters were set as follows: extraction temperature 50 °C, incubation time 5 min, extraction time 25 min, agitation speed 500 rpm. Desorption of volatile compounds was performed in the PTV injector of the GC.

2.7. Capillary gas chromatography – olfactometry (GC-O)

The analysis was carried out alternately by two operators on a Hewlett-Packard HP5890 series II (Agilent Technologies, Palo-Alto, United States) coupled with olfactory detection using ODO-1 installation (Scientific Glass Engineering (SGE), Ringwood, Australia). A 2-μL sample of the extract was introduced onto a polar BP20 capillary column (SGE, 100% polyethylene glycol, 50 m, 0.25 mm i.d., 0.25 μm film thickness) or a BPX5-type fused silica non-polar capillary column (SGE, 5% methylpolysiloxane, 30 m, 0.25 mm i.d., 1 μm). The carrier gas was He (Linde gas, Bordeaux), 5.3 grade, with a flow rate of 1 mL/min for all the analyses. The injector in splitless mode (purge time: 1 min; purge flow 50 mL/min) was set at 230 °C. Oven temperature was initially set at 45 °C for 1 min, then raised to 240 °C at 3 °C/min and held at that temperature for 20 min. Linear retention indices (LRI) were obtained by simultaneous injection of samples and a series of alkanes (C_7-C_{30}, 0.1 g/L pentane).

2.8. Quantification of 3-isobutyl-2-methoxypyrazine (IBMP), lactones and 3-methyl-2,4-nonanedione (MND)

2.8.1. Apparatus and chromatographic conditions

Apparatus and chromatographic conditions were described previously (Pons, Lavigne, Darriet, & Dubourdieu, 2011). Basically, a GC 3400 CX equipped with a PTV injector was coupled with a 4000 MS mass spectrometer; both from Agilent Technology (France), with a polar BP20 column (60 m, 0.22 mm i.d., 0.5 μm film thickness, 100% polyethylene glycol, SGE). Oven temperature began at 45 °C (1 min hold) and increased to 170 °C at 3 °C/min, then increased to 250 °C at 4 °C/min (15 min hold). The inlet temperature was held constant at 240 °C with a constant flow of 1 mL/min helium.

Mass spectrometric conditions were as follows for quantification experiments. The transfer line and manifold were maintained at 230 °C and 70 °C, respectively. Trap temperature was maintained at 150 °C. Full scan EI spectra were acquired with mass range m/z 50–200, scan time 1 s/scan, emission current 60 μA (at 70 eV electron energy), and automatic gain control (AGC) target set at 20,000. CI spectra (μSIS mode) were acquired with methanol as reactant gas. The acquisition was divided into four segments: first segment IS1 (CI, 29–31 min, m/z 71, second segment IS2 (EI, 31–33 min, m/z 59), third segment 3-isobutyl-2-methoxypyrazine (IBMP) (CI, 33–36 min, m/z 167), fourth segment γ-lactones and δ-lactones (EI, 46–60 min, with m/z 85 and 99 respectively), for quantification purpose.

2.8.2. Calibration curves

A young red Merlot wine and a synthetic model wine (12% vol. AT 5 g/L, pH 3.5) were used for the calibration curves. The wine was spiked with IBMP and 3-methyl-2,4-nonanedione (MND) to give concentrations in the range of 4–40 ng/L and 4–100 ng/L, respectively. For γ and δ lactones, 8 concentrations were in the range of 1–150 μg/L. The samples were prepared, extracted and analyzed in triplicate. Data were analyzed and compared using means and relative standard deviation. 3-octanol (IS1) served as internal standard for IBMP and MND whereas 2-octanol (IS2) served as internal standard for lactone quantification.

2.9. Enological parameters

All wines were analyzed by Fourier transform infrared spectrometry (FTIR) with WineScan FT120 (Foss, Hillerød, Denmark) equipment. The following enological parameters were determined: ethanol content (% *v/v*), pH, volatile acidity, residual sugar. Sulfur dioxide was determined by iodometry.

2.10. Sensory analysis

Sensory analyses were conducted in the tasting room of the enology research unit (ISVV, France) including ten individual booths. The installation is designed to limit external factors liable to disturb sensory analysis. In addition, all the tastings were carried out in black glasses filled with 20 mL of wine. For all experiments, glasses were labeled with three-digit random codes and presented to panelists in random order. The panel consisted of 25 judges from the enology research unit (ISVV, France). There were 15 women and 10 men between 20 to 50, all wine-tasting specialists or winemakers. Sensory analysis consisted of ranking tests. For each series, the glasses corresponding to the different modalities were presented in randomized positions relative to the assessors, who were asked to classify the samples on the basis of the herbaceous/cooked fruit descriptor intensity (less to more intense).

2.11. Statistical processing of results

Results obtained from sensory tests were statistically interpreted according to the norms published by the International Organization for Standardization. The agreement of a single group of judges with an a priori ordering of treatment was tested. The sum of ranks R_1, $R_2 \ldots R_p$, for the p samples, corresponding to different concentration levels of berries infected by *P. viticola* was calculated; we then obtained the L value of the norm ISO 8587 corresponding to the L statistic of Page (1963).

All analyses were carried out in triplicate. Standard deviations (SD) were calculated for each data series as an indicator of dataset scatter. A 2-way analysis of variance (ANOVA) was used. The significance of the differences between the means was determined with Tukey's multiple range test with 5% error probability. To assess the relationship between the concentration of withered berries and changes in compound concentration, a Pearson correlation analysis was used. A p-value of <0.05 was taken to be significant.

Principal component analysis (PCA) was applied to discriminate Merlot and Cabernet Sauvignon wines according to different concentrations of infected berries. Related samples corresponding to different treatments tend to be grouped in the same cluster and the degree of difference between the groups and the degree of difference between the clusters was stated according to Ward criterion (Ward, 1963). PCA was applied first to mean values of the analytical and sensorial results obtained during the experiments conducted in the vineyard, followed by hierarchical cluster analysis by means of RECIP application of Spad 8.0 (Coheris, Suresnes, France).

3. Results and discussion

3.1. Origins of cooked fruit/herbaceous nuances: Sensorial approach

First, the origin of these herbaceous/cooked fruit nuances was investigated. To do this, several experiments were carried out in a model medium having a composition close to that of must. The occurrence of these nuances during maceration and alcoholic fermentation and in the presence or not of grapes infected and withered by brown rot was studied (Table 1). The herbaceous nuance was detected in the media after 5 days of maceration (M+ AF−) whereas the cooked fruit nuance was not apparent at this stage (Table 1). The macerate of withered berries evoked green-herbaceous nuances. These were also found in the wine resulting from the fermentation of a macerate of withered berries from which the berries were removed at the beginning of alcoholic fermentation (M+/B− AF+). Thus, it appears that the green/herbaceous nuance results from the solubilization of odorous compounds present in the film of wilted grapes.

The cooked fruit nuances were found only in the fermented modality in the presence of withered berries (B+ AF+) that also presented an intense herbaceous odor. Thus, yeast metabolism seems to play an important role in the formation of the cooked fruit nuances of wines made with grapes infected by brown rot. Thus, these aromatic antagonistic nuances of herbaceous/cooked fruit cannot be dissociated in these red wines.

On the other hand, sensory analysis of wines made with increasing levels of withered berries (Table 1, supplementary data) showed that a panel of tasters was able to classify them according to the intensity of the herbaceous/cooked fruit nuance ($L > L_{0.05}$) and that their intensity was strongly linked to the content of the berries supplemented with must.

Table 1
Effect of maceration (M) and alcoholic fermentation (AF) of media supplemented or not with withered berries infected by *P. viticola* on the formation of cooked fruit and herbaceous nuances.

Treatments[a]	Descriptors[b]	
	Cooked fruit	Herbaceous
AF-	–	–
M+/AF−	–	+
M+/B− AF+	–	+
B+ AF+	+++	+++

[a] (AF) synthetic must after alcoholic fermentation; (M+/AF−) synthetic wine supplemented with withered berries (maceration 5 d, 24 °C); (M+/B− AF+) synthetic must supplemented with withered berries (maceration 5 d, 15 °C) and removed before inoculating the media with yeast; (B+ AF+) alcoholic fermentation with withered berries.
[b] Intensity of aromas perceived by two assessors: - no intensity, + weak, ++ medium, +++ strong intensity.

3.2. Identification of molecular markers of cooked fruit/herbaceous nuances in wine

The most important descriptors related to the aroma of these wines were identified by a trained panel. The terms selected were: "prunes", "figs", "cooked peaches" and "bell pepper".

GC-O analysis of the dichloromethane extract of the Merlot wine made with 20% of withered berries (2009 vintage) and marked by theses odors revealed six odor zones (OZ) corresponding to the aroma of wines (Table 2). OZ1 and OZ2 possessed a strong geranium odor. OZ3 was reminiscent of bell pepper and ivy, whereas OZ4, OZ5 and OZ6 resembled dried/cooked fruit. These OZ seemed to be specific to the wines made with these diseased grapes.

By using GC–MS with chemical standards, it was possible to identify the molecules corresponding to the following retention indices: OZ3, 3-isobutyl-2-methoxypyrazine (IBMP) (RI_{polar}: 1841, $RI_{nonpolar}$: 1369), OZ4 3-methyl-2,4-nonanedione (RI_{polar}: 1741), OZ5 γ-nonalactone, (RI_{polar}: 2058, $RI_{nonpolar}$: 1325) and OZ6, γ-decalactone, (RI_{polar}: 2170, $RI_{nonpolar}$: 1420).

γ-Nonalactone and γ-decalactone reminiscent of coconut and cooked peach were first identified by Schreier in wines (Schreier & Drawert, 1974). In red wines, γ-nonalactone concentrations range from some μg/L to 40 μg/L (Durif wines) (Cooke, Capone, Van Leeuwen, Elsey, & Seeton, 2009) and from traces to 4 μg/L for γ-decalactone in Merlot wines. The origin of γ-nonalactone in grapes and wines is unclear. However, its biosynthesis in fruits might be due to the oxidation of unsaturated fatty acids such as linoleic acid (Tressl, Apetz, Arrieta, & Grunewald, 1978). Moreover,

Table 2
Main odorant zones detected by GC-O during analysis of red Merlot wine made or not with berries infected by *P.viticola.*

Odorant zone	Descriptors	LRI		Compounds	Wine[c]	
		BPX5	BP20		Control	Control + brot[d]
OZ1	Geranium	880	1260	(*Z*)-1,5-heptadien-3-one[a]	–	++
OZ2	Geranium	950	1376	(*Z*)-1,5-octadien-3-one[b]	–	+++
OZ3	Green, bell pepper	1144	1552	3-isobutyl-2-methoxypyrazine[b]	+	+++
OZ4	Fruit pit, minty	/	1741	3-methyl-2,4-nonanedione[b]	–	++
OZ5	Cooked peach, coconut	1325	2058	γ-nonalactone[b]	–	+++
OZ6	Cooked peach	1420	2170	γ-decalactone[b]	–	++

[a] tentatively identified on the basis of odor similarity and IRL found in literature: IRL_{polar} 1278 (Lorber et al., 2014).
[b] Identified by comparison with IRL found in literature and co-injection of pure compound.
[c] odor intensity: – not detected, + weak, ++ medium, +++ high intensity.
[d] berries infected by *P. viticola*.

the metabolism of *S. cerevisiae* has been shown by deuterium labeling to produce γ-nonalactone from linoleic acid (Garbe, Landrock, Hübke, & Tressl, 2005). γ-Decalactone is widely produced by many microorganisms thanks to β-oxidation of hydroxylated fatty acid such as ricinoleic acid (Serra, Fuganti, & Brenna, 2005) or 10-hydroxystearic acid (An & Oh, 2013).

IBMP, which is reminiscent of bell pepper, has a perception threshold of 15 ng/L (Roujou de Boubee, Van Leeuwen, & Dubourdieu, 2000). It is the main known compound to date responsible for the green pepper aromas of wines made from many grape varieties, including Cabernet Sauvignon, Cabernet Franc and Sauvignon Blanc (Augustyn, Rapp, & Van Wyk, 1982). It is also found in lower concentrations in other grape varieties (Merlot in particular). It is naturally present in the grape and particularly in the skin (Roujou de Boubee et al., 2000) and its concentration drops sharply during maturation, especially under the influence of temperature and light. Therefore, a significant content in grapes at harvest is associated with a lack of maturity and affects the aromatic quality of a wine.

3-Methyl-2,4-nonanedione (MND), reminiscent of anise and fruit pit, is a β-diketone identified in prematurely aged red wines marked with an intense prune flavor (Pons, Lavigne, Frérot, Darriet, & Dubourdieu, 2008). MND content was low (some ng/L) in non-oxidized red wines and higher in oxidized red wines (340 ng/L max), always exceeding its perception threshold (16 ng/L). The role of oxidation in its formation in wines has also been demonstrated. Oxidation of furanoid fatty acids (FFA) is thought to result in this dione in several matrices (Guth & Grosch, 1991), but the origin of MND is still unclear in wines.

Fine GC-O-MS analysis of the samples in which OZ1 and OZ2 were detected did not allow us to obtain a chromatographic peak, probably owing to the very low levels of these compounds in the samples.

In wines, geranium odors are not so common. However, three compounds have already been described in wines which have corresponding odors: (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (Janusz et al., 2003), 2-ethoxy-3,5-hexadiene (Rapp, 1992) and (*Z*)-1,5-octadien-3-one (Darriet et al., 2002). The LRI values of the latter compound reported by several studies were 1369 (Steinhaus, 2015), 1372 (Lorber, Schieberle, & Buettner, 2014) on polar and 980 (Lorber et al., 2014) on non-polar column, respectively, and were similar to those obtained for OZ2 (LRI_{pol}: 1376, LRI_{apol}: 960). The injection of the pure product allowed us to confirm its identification by GC-O. The odor of the pure product was clearly reminiscent of that of geranium leaf. Its perception threshold in must is 9 ng/L (Allamy, Darriet, & Pons, 2017). It has already been detected by GC-O in grapes infected by bunch rot complexes implicating *Botrytis cinerea* (Vacher et al., 2008). The metabolism of *S. cerevisiae* can reduce it to the slightly odorous (*Z*)-5-octen-3-one (Darriet et al., 2002). Linolenic acid and its *n*-3 homologues are thought to be precursors during its chemical oxidative formation in butter (Rychlik, Schieberle, & Grosch, 1998). Moreover, its enzymatic formation and that of its corresponding alcohols by lipoxygenase-catalyzed conversion of linoleic acid and linoleic acid were previously demonstrated in mushrooms (Tressl, Bahri, & Engel, 1982).

The compound reminiscent of geranium associated with OZ1 had an LRI similar to (*Z*)-1,5-heptadien-3-one (IRL_{pol}: 1278 IRL_{nonpol}: 861). According to Lorber (Lorber et al., 2014), it has a mix of odors reminiscent of metallic and geranium odors, *i.e.* descriptors similar to those associated with OZ1.

3.3. Chemical analysis of volatile compounds

We analyzed the volatiles in red wines made with different concentrations of withered berries. The analysis of γ-nonalactone and γ-decalactone was extended to other γ and δ-lactones (Table 3) already described in red wines and presenting a similar aroma reminiscent of fresh coconut and fruity nuances. To correlate the quantitative data with the aroma of the wine itself, the odor activity values (OAV) of the compounds were calculated on the basis of odor thresholds determined in model wine solution.

Detected at trace level in must and at a low level in the control wine, γ-nonalactone concentrations increased almost linearly ($r = 0.975$) according to the amount of berries added to reach 133.5 μg/L (OAV = 4.9) in the 10 g modality. In this last modality, γ-nonalactone contributes directly to the flavor of the wine sample. Its detection threshold was reached at 1 g of withered berry.

As for γ-nonalactone, the addition of berries infected by brown rot induced the formation of other lactones to a lesser extent, except for γ-dodecalactone, which remained stable whatever the quantity of berries added (Table 3).

For the 10 g treatments the incidence of alcoholic fermentation on the formation of these lactones was investigated under our experimental conditions. For the 10 g treatment the concentration of lactones released during maceration of the whole berries in a hydroalcoholic solution was evaluated. After 5 days at 24 °C, the lactones were detectable in non-negligible quantities in the hydroalcoholic solution (Table 3), although the levels were much lower than those found in the wine after alcoholic fermentation.

Interestingly, γ-nonalactone has also been reported at higher levels in wines made with late-harvested shriveled berries of Shiraz grapevine (Šuklje et al., 2016). This suggests the potential similarity between early shriveling due to the pathogen and late shriveling due to abiotic stress (sunburn, late-season fruit dehydration).

Table 3
Incidence of increasing concentrations of withered berries infected by *P. viticola* on volatile compounds concentration in Merlot wines. ($n = 3$).

	Detection threshold[a]	Must	Wines							r^d
			Treatments (g/75 mL)							
			0	1	2	5	8	10	10[c]	
γ-octalactone (μg:L)	7	tr[b]	4.11 (1.1)	5.52 (0.5)	6.14 (1.5)	**10.21 (1.9)**	**11.60 (2.2)**	**14.15 (2.5)**	2.1 (0.6)	**0.991**
γ-nonalactone (μg/L)	27	tr	5.82 (0.6)	**30.55 (1.5)**	**52.47 (3.3)**	**96.41 (7.9)**	**111.90 (12.8)**	**133.54 (13.3)**	12.3 (2.2)	**0.975**
γ-decalactone (μg/L)	0.7	tr	**2.70 (0.3)**	**4.34 (0.2)**	**4.92 (0.3)**	**7.11 (0.4)**	**7.71 (0.5)**	**8.56 (0.5)**	2.1 (0.7)	**0.964**
γ-undecalactone (μg/L)	60	tr	tr	0.71 (0.2)	1.41 (0.3)	2.01 (0.3)	2.13 (0.3)	2.55 (0.3)	tr	**0.924**
γ-dodecalactone (μg/L)	7	tr	1.80 (0.5)	2.15 (0.6)	2.12 (0.7)	2.94 (0.3)	2.84 (0.7)	3.21 (0.8)	tr	**0.942**
MND (ng/L)	16	8	16.2 (2.1)	17.5 (1.9)	**18.5 (2.1)**	**35.2 (2.1)**	**44.1 (3.3)**	**61.1 (8.4)**	**32.1 (3.4)**	**0.983**
IBMP (ng/L)	15	5.8	6.11 (0.9)	8.51 (0.8)	**15.24 (0.8)**	**24.70 (3.4)**	**31.65 (6.8)**	**33.29 (5.2)**	**27.1 (3.3)**	**0.930**

[a] (Gemmert, 2003).
[b] Traces.
[c] Concentrations in hydroalcoholic solutions supplemented with withered berries kept for 5 days at 24 °C ($n = 2$).
[d] Pearson correlations in bold were significant for $\alpha = 0.05$. For each compound, concentration in bold corresponds to OAV > 1.

Concerning MND, it was detected at low levels in must and wine. There was a clear linear correlation with the concentration ($r = 0.983$) of infected berries in the media. The highest level was 61.1 ng/L, which is higher than its perception threshold (16 ng/L). Moreover, it was found at high levels not only in infected grapes but also during alcoholic fermentation. Therefore, the concentration of MND at the end of alcoholic fermentation depends on two phenomena: the first is its reduction due to yeast reductase (as already demonstrated for other carbonyls); the second results in its formation from precursors found at higher levels in infected berries.

The lactones and ketones we identified have precursors belonging to the same fatty acid family. As recently demonstrated by Figueiredo et al. (2017) inoculation of *P. viticola* on Trincadeira leaves induced an increase in lipid peroxidation as well as an increase in H_2O_2 level. Our results suggest the high lipoxygenase activity of the grapes that are able to peroxidize polyunsaturated fatty acids in response to pathogen attack (Howe & Schilmiller, 2002), giving rise to volatile aldehydes and ketones and hydroxy-fatty acid. The latter compounds are metabolized into lactones by yeast.

The control Merlot wine contained a non-negligible level of IBMP that was below its perception threshold (OAV < 1). The addition of increasing amounts of berries infected by brown rot increased the IBMP content of the wines in a linear manner ($r = 0.930$). The detection threshold was reached as of 2 g treatment. From 5 g, the medium contained more than 25 ng/L and more than 33 ng/L (OAV = 2.2) for the modality containing the largest amount of infected berries. At these levels it is likely that IBMP contributes significantly to the bell pepper nuance of these wines.

Maceration experiments indicated that berries infected by brown rot contain significant amounts of IBMP, i.e. withering of the berry due to brown rot concentrates the most stable compounds. Owing to its chemical structure, it is likely that IBMP is one of these compounds and that it occurs in large quantities in berries infected by brown rot.

3.4. Analytical and sensorial effect of vinification of berries infected by *P. viticola* on red Merlot and Cabernet Sauvignon wines over time

We thus identified for the first time some volatile compounds involved in the aroma of wines resulting from microvinification of grapes infected by rot brown under laboratory conditions. We were also interested in their organoleptic impact in conditions closer to real winemaking conditions.

3.4.1. Enological parameters

Table 2 (supplementary data) shows the enological parameters of the different wines produced. Control Merlot and Cabernet Sauvignon wines corresponding to grapes harvested in 2009 and 2012, respectively, had a similar composition, suggesting similar levels of grape maturity. For Merlot wines, the parameters systematically influenced by *P. viticola* infection were pH and total acidity. This is probably due to green berry dehydration, which increases the acid concentration in wines. Surprisingly, this was not clear for Cabernet Sauvignon wines since the addition of withered berries had no effect on standard enological parameters. Moreover, the wines produced had similar average ethanol levels.

3.4.2. Sensorial analysis of young and aged red wines

Wines were tasted twice during the first year of their production and after 6 and 3 years of aging for Merlot and Cabernet Sauvignon wines, respectively. Results concerning the ranking of Merlot and Cabernet Sauvignons wines according to the intensity of cooked fruit/herbaceous character are shown in Table 4. The panel was able to rank the wines according to the concentration of withered berries; a greater quantity added to the must resulted in a higher intensity of odors perceived. These odors were stable over time for both Merlot and Cabernet Sauvignon wines.

3.4.3. Assay of volatile compounds in young and aged wines

For each batch, the overall compounds previously identified were quantitated over time. The analysis of red wines samples vinified in the presence of increasing levels of berries infected by brown rot revealed that γ-nonalactone, and to a lesser extent γ-octalactone and γ-decalactone, increased in a linear manner in Merlot wines (Table 5). On the contrary, unlike what we obtained at laboratory scale, γ-undecalactone, γ-dodecalactone and δ-decalactone were not detected in these wines. In Cabernet Sauvignon wines, γ-nonalactone was detected at a lower concentration than in Merlot wines, and it was the only lactone that correlated well with the proportion of infected berries ($r = 0.979$). The level of γ-nonalactone was greater in young Merlot wines produced with 2% infected berries than in the control sample. From 5% infection the concentration reached 38.3 μg/L (OAV = 1.4), thereby exceeding its detection threshold. Similar results were observed for γ-decalactone. A modification in γ-octalactone level was observed with 10% infected berries but its concentration did not reach the level of detection, so its direct contribution to the aroma of wine was not established. Nevertheless, it may contribute, thanks to the phenomenon of additivity with other lactones with similar chemical structures and aroma descriptors (Jarauta, Ferreira, & Cacho, 2006).

The analysis of wines aged in bottles (Table 5) showed that the γ-lactone concentration tends to increase during bottle aging. This was particularly clear for γ-nonalactone in wines made with withered Merlot and Cabernet sauvignon berries but also for control wine. Concerning Cabernet Sauvignon wines, although

Table 4
Incidence of increasing concentration of withered berries infected by *P. viticola* on herbaceous/cooked fruit odor intensity in young and aged Cabernet Sauvignon and Merlot wines.

Vines		Aging time (years)	Sum of ranks						L[b]	$L_{0.05}$	$L_{0.01}$
			% berries								
			0	2	5	10	15	20			
Merlot	T0	1	19	21	32	51	64	65	1071[c]	928	946
	T1	6	12	30	33	42	39	54	843[d]	777	793
Cabernet-Sauvignon	T0	0.7	25	27	32	42	/	54	613[c]	570	584
	T1	3	20	24	25	37	/	44	511[d]	477	487

[a] $R_1 \ldots R_4$ are sums of ranks for treatments 0 to 20.
[b] L was calculated as described in ISO 8587 (Garde-Cerdán & Ancín-Azpilicueta, 2006) for Page test: $L = \sum_{i=1}^{p} iR_i$. This value was compared to those found in tables in the case of complete block.
[c] $n = 14$.
[d] $n = 10$.

Table 5
Evolution of volatile compounds in Merlot and Cabernet Sauvignon wines during bottle aging according to percentage attack of grapes by *P. viticola*. ($n = 2$).

vine	% brot berries[b]	Volatile compounds[a]									
		γ-octalactone (μg/L)		γ-nonalactone (μg/L)		γ-decalactone (μg/L)		MND (ng/L)		IBMP (ng/L)	
		T0[c]	T1	T0	T1	T0	T1	T0	T1	T0	T1
Merlot	0[c]	0.51 (0.21)	1.90 (0.41)	2.33 (0.50)	10.4 (2.22)	0.61 (0.31)	0.92 (0.21)	Tr	12.11 (1.09)	6.25 (0.59)	4.5 (0.87)
	2	0.48 (0.23)	1.62 (0.42)	14.15 (0.55)	20.9 (3.94)	0.57 (0.62)	1.01 (0.22)	8.10 (2.21)	13.17 (1.51)	7.28 (1.11)	6.2 (1.11)
	5	0.42 (0.28)	2.42 (0.39)	**38.33 (2.21)**	**54.4 (3.16)**	**1.22 (0.26)**	1.13 (0.15)	9.83 (1.13)	12.26 (1.53)	6.22 (1.55)	6.8 (1.05)
	10	2.3 (0.11)	3.45 (0.36)	**49.51 (4.42)**	**68.2(5.95)**	**1.34 (0.25)**	**1.30 (0.23)**	12.74 (1.24)	**47.15 (1.91)**	10.9 (1.98)	7.1 (0.96)
	15	3.2 (0.18)	3.27 (0.45)	**51.80 (3.31)**	**70.2 (7.14)**	**2.11 (0.18)**	**1.84 (0.22)**	12.11 (2.26)	**50.64 (2.51)**	**15,7 (1.97)**	12.2 (1.56)
	20	4.9 (0.21)	6.29 (0.50)	**83.14 (3.9)**	**109.5 (9.13)**	**2.52 (0.5)**	**2.71 (0.18)**	**17.87 (1.30)**	**75.28 (2.92)**	**19,9 (1.24)**	**16.9 (1.89)**
r [c]		***0.972***	***0.915***	***0.960***	***0.958***	***0.982***	***0.949***	***0.889***	***0.965***	***0.973***	***0.951***
Cabernet Sauvignon	0	Nd	1.46 (0.56)	1.26 (0.5)	7.5 (1.11)	Tr	0.92 (0.21)	Tr	4.17 (0.81)	4.72 (1.25)	5.10 (1.23)
	2	0.62 (0.22)	1.42 (0.64)	3.89 (0.29)	10.3 (2.38)	Tr	1.37 (0.21)	Tr	9.76 (1.82)	6.55 (1.02)	5.82 (0.86)
	5	0.55 (0.20)	1.96 (0.57)	4.63 (0.45)	15.1 (2.17)	0.60 (0.33)	1.56 (0.23)	4.17 (1.21)	9.89 (1.83)	7.21 (1.51)	6.1 (1.06)
	10	0.8 (0.29)	1.91 (0.43)	12.2 (1.1)	**32.1 (5.43)**	0.68 (0.22)	**1.83 (0.15)**	14.22 (2.31)	**18.4 (1.22)**	8.80 (1.23)	6.2 (1.09)
	20	0.95 (0.19)	1.98	15.9 (2.8)	**49.3 (4.72)**	**1.55 (0.54)**	**1.90 (0.25)**	**18.91 (2.42)**	**29.9 (1.52)**	9,1 (0.79)	7.9 (1.52)
r[c]		***0.811***	***0.751***	***0.965***	***0.991***	***0.976***	*0.851*	***0.960***	***0.990***	***0.877***	***0.967***

[a] For each compound, concentrations in bold correspond to OAV > 1.
[b] Results concerning wines made with healthy grapes and grapes infected with increasing% of berries infected by brown rot (Brot).
[c] For Merlot (T0 = 1 y. after bottling; T1 = 6 y. after bottling), for Cabernet Sauvignon (T0 = 0.7 y. after bottling; T1 = 3 y. after bottling).
[d] Pearson correlation in bold is significant for $\alpha = 0.05$.

γ-nonalactone was below its detection threshold in young wines, it contributed directly to the aroma of wines after a 3-year aging period and with 10% and 20% treatments.

We also evaluated the incidence of the vinification of berries infected by *P. viticola* on the level of MND, a marker of oxidation in red wine. In young control wines, it was detected at trace level and increased slowly over time. On the contrary, there were significant levels in young red wine made with infected berries and its level was higher than its detection threshold with the 20% modality. In the latter case, its level increased over time and especially in Merlot wines made with at least 10% infected berries. After 6 years of bottle aging, its concentration reached 75.2 ng/L (OAV = 4.7) in the 20% modality, whereas in the control wine its concentration was only 12.1 ng/L (OAV < 1). A similar albeit less intense phenomenon was observed in Cabernet Sauvignon wines. The composition of the berries infected by the pathogen tended to contain higher amounts of precursors of MND, which were slowly oxidized in MND during bottle aging.

IBMP levels increased according to the percentage of infected berries in the media. The control sample had the lowest level, whereas the 20% modality contained the highest level (19.9 ng/L), which was higher than its detection threshold. While its concentration was quite stable over time, its organoleptic impact at intermediate percentages was not so clear, owing to the low level detected, so we were unable to explain the clear herbaceous flavor detected by the panel in these wines. Similar results were observed for Cabernet Sauvignon wines in which levels were systematically below its detection threshold. We thus hypothesized that other volatiles reminiscent of "green" aromas such as (*Z*)-1,5-octadien-3-one, eucalyptol or methyl salicylate, which was recently reported in red wine, may contribute to this character (Darriet & Darriet, 2016).

Finally, these results were consistent with results of sensorial analysis in which a higher intensity of the cooked fruit character was found in wines vinified with increasing levels of *P. viticola* infection. Indeed, the stability of this aroma during bottle aging was demonstrated. Moreover, the organoleptic impact of IBMP was established with highly infected batches beyond 15%.

Cluster analysis performed on Merlot and Cabernet Sauvignon wines spiked with increasing concentrations of infected berries

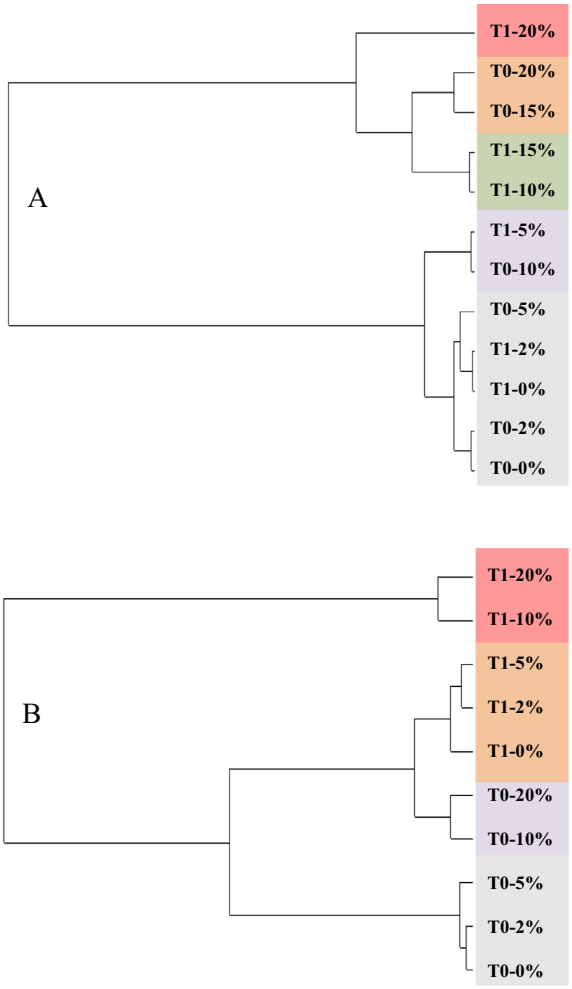

Fig. 1. Cluster dendrogram of treatments according to variety. Samples were distributed by hierarchical cluster analysis into 5 groups for Merlot (A) and 4 groups for Cabernet Sauvignon (B) (different colors) after a PCA applied to the results of analytical and sensorial analysis (Ward's method).

are depicted in Fig. 1. Merlot wine samples were divided into five main clusters whereas Cabernet Sauvignon samples were divided into four main clusters. The effect of aging was more pronounced for Merlot wines since young and old wine samples at T0 and T1 were situated in cluster 1, whereas young and old Cabernet Sauvignon wines were well differentiated according to their age. This means that the kinetic evolution of sensorial and molecular markers in Merlot wines was faster in samples that contained a higher percentage of infection. Moreover, there was a clear separation between samples containing more than 5% infected berries owing to the higher levels of volatile compounds in these samples and a more intense herbaceous/cooked fruit odor.

4. Conclusion

To conclude, this study shows that green berries infected early by *P. viticola* become shriveled and undergo extensive modifications in berry constituents, thereby affecting the chemical and sensory profile of red wines. The intensity of the dried fruit/herbaceous off-flavor associated with the vinification of these grapevines depends on the variety. A clear increase in intensity was observed from 5% infection in Merlot grapes whereas the threshold was higher in Cabernet Sauvignon grapes. Indeed, the similar chemical composition of the control wines suggests that the organoleptic impact of *P. viticola* infection on Cabernet Sauvignon grapes is less pronounced than in Merlot grapes.

The lactones, ketones and methoxypyrazines found in this study were associated with grape metabolism and also produced during alcoholic fermentation with lactones and ketones. The intrinsic quality of the grape and its fine chemical composition therefore has an impact on the aroma of young wines but can affect the ability of a wine to age well.

This study opens up new perspectives for studying the etiology of this phenomenon as well as throwing light on the mechanisms of berry shriveling under the action of *P. viticola*. Further studies are now needed to extend these preliminary findings and to provide a better analytical definition of what grape maturity involves.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.06.087.

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