IMPACT OF VARYING DEGREES OF POWDERY MILDEW INFECTION (*UNCINULA NECATOR* (SCHWEIN.) BURRILL) ON THE VOLATILE COMPOUNDS OF CHARDONNAY GRAPES, MUST AND WINE

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Abstract

Aims: The aims of this study are (i) to trace the major components responsible for the unpleasant mushroom, earthy and geranium odour in the transformation from grapes to wine in response to powdery mildew infection intensities, (ii) to determine the impact of an aggressive infection on volatile compounds in grapes and wine, and (iii) to determine their effect on the olfactory-taste evaluation of the resulting Chardonnay wine.

Methods and results: A GC-MS technique was used to investigate the impacts of different degrees of oidium infections (0, 10, 50 and 100 % of infected grapes) on the presence of volatiles in grapes, musts and wines. The number of volatiles was higher in oidium-infected grapes than in non-infected grapes, especially for terpenes, aldehydes and alcohols, with 1-octen-3-ol, 2-octen-1-ol, 2-heptanol and 2-octenal, the volatiles reminiscent of mushroom-, geranium-, and earthy-like odours, being detected in oidium-infected grapes only. After overnight clarification, 1-octen-3-ol and 2-heptanol but also methyl-2-furoate were the only mushroom reminiscent volatiles detected in the musts. The infection also caused higher amounts of acetic acid, furfural and some of its derivates (2,5-furandicarboxaldehyde, 5-hydroxymethyl furfural, furaneol), most ethyl esters (except ethyl caprylate), 1-hexanol, maltol and 1-decanol in musts. After alcoholic fermentation the number of esters in young wines increased, whereas that of aldehydes decreased, especially in the wines produced from severely infected grapes. Though it was not detected by sensory analysis, we assume that the intensification of the odours reminiscent of pineapple, banana and coconut in the aged wines produced from infected grapes was likely a consequence of the increased amount of esters.

Conclusion: Powdery mildew infection statistically decreased the freshness but enhanced the malty, sweet mouthfeel of Chardonnay wine, and neither the mushroom odour nor taste were perceived, which contradict some previously published findings.

Significance and impact of the study: This study gives an answer to the significant problem of wine production from oidium-infected grapes. It reports for the first time the traceability of volatiles highly related to mushroom, earthy and geranium odours from the Chardonnay grapes to the wines. The overnight must clarification decreased the impacts of unpleasant volatiles on must and wine odour. This suggested that the oidium infection of the grapes does not necessarily hinder the production of wine, as no unpleasant odour could be sensory evaluated, not even in the wines made from highly infected grapes.

Key words: aroma, Chardonnay, *Uncinula necator*, powdery mildew (oidium), secondary metabolites

Abbreviations:
HS-SPME Headspace solid-phase microextraction;
GC-MS Gas chromatography coupled to mass spectrometry;
DVB/CAR/PDMS Divinylbenzene/Carboxen/Polydimethylsiloxane fibres;
PCA Principal component analysis;
PCR Principal component regression;
PLS Partial least squares regression

Résumé

Objectifs: Les objectifs de cette étude sont de (i) diagnostiquer les principaux éléments responsables de l'odeur désagréable de champignons, de terre/terreuse et de géranium dans la transformation du raisin en vin en réponse à l'intensité de l'infection par l'oïdium, (ii) déterminer l'impact d'une infection sévère sur les composés volatils contenus dans le raisin et le vin, et (iii) déterminer leurs effets sur l'odorat et le goût lors de l'évaluation des vins Chardonnay produits.

Méthode et résultats: La technique GC-MS a été utilisée pour déterminer l'impact de différents degrés de l'infection appelée oïdium (0, 10, 50 et 100 % de raisins infectés) sur la présence de composés volatils dans les raisins, les moûts et les vins. Le nombre de composés volatils identifiés était plus élevé dans les raisins infectés que dans ceux non-infectés, spécialement pour les terpènes, aldéhydes et alcools, dont 1-octen-3-ol, 2-octen-1-ol, 2-heptanol et 2-octenal, des composés volatils associés à l'odeur de champignons, de géranium et de terre, ont été identifiés uniquement dans les raisins infectés. Après une nuit de clarification du moût, le 1-octen-3-ol et le 2-heptanol, mais aussi le methyl-2-furoate ont été les seuls composés volatils de champignons identifiés dans les moûts. L'infection a aussi induit une très grande quantité d'acide acétique, de furfural et certains de ses dérivés (2,5-furandicarboxaldehyde, 5-hydroxyméthyl furfural, furaneol), de la plupart des esters d'éthyle (sauf le caprylate d'éthyle), du 1-hexanol, du maltol et du 1-decanol dans le moût. Après la fermentation alcoolique, le nombre d'esters identifiés dans les vins jeunes a augmenté, alors que celui des aldéhydes a diminué, et plus particulièrement dans les vins produits à base de grappes sévèrement infectées. Bien que cela n'a pas été perçu lors de la dégustation, nous supposons que l'intensification des odeurs évocatrices d'ananas, de banane et de noix de coco dans les vins vieillis produits avec des grappes infectées est due à l'abondance d'esters identifiés dans ces vins.

Conclusion: L'infection appelée oïdium diminue statistiquement la fraîcheur du vin, mais met en valeur des tonalités de malt et le côté sucré du Chardonnay. De plus, ni l'odeur de champignon ni son goût n'ont été détectés, ce qui contredit certains articles publiés à ce sujet.

Signification et impact de l'étude: Cette étude donne une réponse à l'important problème lié à la production de vin issu de raisins infectés par l'oïdium. Elle montre le suivi de substances volatiles associées aux notes de champignon, de géranium et de terre depuis les raisins aux vins de Chardonnay. Un débourbage d'une nuit a permis de diminuer les impacts possibles des composés volatils responsables des nuances aromatiques désagréables dans les moûts et les vins. Nos travaux suggèrent que l'infection par l'oïdium de la vigne ne doit pas nécessairement entraver la production de vin, parce que l'odeur fongique désagréable n'est pas perçue sensoriellement dans la plupart des vins issus de raisins infectés par l´oïdium.

Mots clés: arôme, Chardonnay, *Uncinula necator*, oïdium, métabolites secondaires

manuscript received 6th April 2011 - revised manuscript received 15th August 2012

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J. Int. Sci. Vigne Vin, 2012, **46**, n°4, 305-320

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INTRODUCTION

Powdery mildew of vine crops, a fungal disease caused by *Uncinula necator*, first appeared in European vineyards in the middle of the 19th century. Nowadays, in seasons with oidium-favourable weather conditions, it still represents one of the most frequent and serious diseases in susceptible grapevine (*Vitis vinifera* L.) varieties, affecting both leaves and berries. In severe infections, the epidermal cells of the berry skin become necrotic, progressively drying out the skin and usually causing it to split, which represents favourable conditions for the development of other diseases like grey rot. Powdery mildew infection also affects vine growth and yield by reducing the pruning weight (Pool *et al.*, 1984 ; Calonnec *et al.*, 2004) and leads to a delay in ripening by producing lower total soluble solids content and higher titratable acidity (Stummer *et al.*, 2003 ; Calonnec *et al.*, 2004). Previous studies have shown that the presence of the fungus frequently leads to the development of organoleptic and olfactory defects in grape and wine (Darriet *et al.*, 2002 ; Calonnec *et al.*, 2004) ; it is the exact same organoleptic and olfactory attributes that are considered crucial for the consumers' acceptance of the wine (Yegge and Noble, 2001).

During the winemaking process, the primary aromatic compounds, particularly monoterpenes, are usually transformed into less odorant ones after skin damage (berry crushing), which are later intensified during fermentation (Rusjan *et al.*, 2009 and 2012). During must fermentation, ethanol and many other substances are produced, among which aliphatic alcohols, esters, acids, aldehydes and thiols prevail. The esterases hydrolyze the ethyl esters of fatty acids, which mostly contribute to the secondary aroma of the wine. The subsequent processes occur during wine maturation and aging and are associated with the formation of the tertiary aroma, which, in particular, is related to a decrease in the amount of acetic esters of aliphatic alcohols and an increase in the amount of ethyl esters of mono and dicarboxylic acids and aliphatic aldehydes (Ribéreau-Gayon *et al.*, 2000).

Chardonnay (*Vitis vinifera* L.) is the most widely cultivated French white grapevine variety in the world but also the most susceptible to powdery mildew. Around 140 aroma compounds have been identified in Chardonnay wines (Lee and Noble, 2003 ; Li *et al.*, 2007 ; Jaffré *et al.*, 2010), among which esters represent the most important group of odorants (Li *et al.*, 2007 ; Jaffré *et al.*, 2010). Among 81 odour-active compounds detected in Chardonnay wines (Lee and Noble, 2003), only 11 have been identified as contributing to the distinctiveness of the wine aroma (Acree *et al.*, 1984), among which isoamyl acetate, 2-phenylethyl acetate and linalool responsible for the fruity and floral notes (Lee and Noble, 2003).

The development of the powdery mildew fungus on the berry surface leads to organoleptic defects (mushroom-like, mouldy, earthy and geranium-leaf odours) in the must (Darriet *et al.*, 2002 ; Guerche *et al.*, 2006), and in some cases in the wines, however the latter are most commonly contaminated by the cork (Ullrich and Grosch, 1987). The compounds responsible for some of these unpleasant odours have already been detected : 1-octen-3-one, 1-octen-3-ol, 1,5-octadien-3-ol, 3-octanone and 1,5-octadien-3-one (Swoboda and Peers, 1977 ; Assaf *et al.*, 1997 ; Darriet *et al.*, 2002). Most of the ketones listed are degradation products from unsaturated fatty acids owing to a variety of chemical oxidation reactions (Ullrich and Grosch, 1987). Wines made from ≥ 3 % of infected berries have been given significantly lower quality scores and described as having a mildew-like odour as well as a greater perceived bitterness (Pool *et al.*, 1984 ; Ewart *et al.*, 1992) – but this was not the case in a later work done by Calonnec *et al.* (2004).

The traceability of volatile compounds from grapes with different degrees of powdery mildew infection to wines is still uncertain. In particular, the mechanism by which the disease impairs other typical volatile aroma compounds in Chardonnay wine (Lee and Noble, 2003) has been poorly investigated, although it has been cited in literature that in partially diseased grapes these compounds are transformed during the vinification process (Darriet *et al.*, 2002 ; Guerche *et al.*, 2006). The aims of this study, therefore, are (i) to trace the major components responsible for the unpleasant mushroom, earthy and geranium odour in the transformation from grapes to wine according to different infection intensities, (ii) to determine the impact of an aggressive infection on volatile compounds in grapes and wine, and (iii) to determine their effect on the olfactory-taste evaluation of the resulting Chardonnay wine.

The HS-SPME analyses were identical for all samples. The amount of individual volatile compounds, expressed as a peak area, gave enough information on the composition and profile of the volatiles present in grapes, must and wines. In conclusion, our study was conducted to stress out the main volatiles responsible for the unpleasant odour.

MATERIALS AND METHODS

1. Grape and wine samples

The Chardonnay (*Vitis vinifera* L.) grapes were produced in 2009 in the vineyard of the Biotechnical Faculty, University of Ljubljana (Slovenia). Two hundred plants were selected and treated with different concentrations of phytochemicals against *Uncinula necator* (Schwein.) Burrill. At the optimal technological ripeness stage (10th September), the grapes were harvested

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manually and separated in four different boxes according to the degree of infection: control (healthy, disease-free bunches), 10 % (app. 10 % of berries per bunch were infected), 50 % (half of berries per bunch were infected), and 100 % (all berries per bunch were infected). Grape infections at harvest were determined according to the EPPO standards (1999). Each "infection group" was subject to four separate vinifications under equal conditions: after the bunches were destemmed, crushed and pressed, the obtained musts were decanted into 50-L glass vessels, immediately treated with sulphur dioxide (50 mg $K_2S_2O_5$ per L of must) to prevent oxidation and then left for overnight clarification (static sedimentation; cooling of musts to 10 °C; without any other oenological agent). Equal clarification was conducted on all must samples to standardise any possible influence on volatile compounds (Moio *et al.*, 2004; Kechagia *et al.*, 2008). Afterwards, 0.25 g of rehydrated yeast (*Saccharomyces cerevisiae*, Uvaferm 43, Lallemand) per litre of must were added to all vessels. Must fermentation continued at 17 °C without any agitation. Young wines were decanted a week after the end of fermentation, bottled in 0.75-L bottles and corked. The bottled wines were stored in the cellar facilities (15 °C) for 4 months before analyses.

2. WineScan FT120 analysis of must and wine

The must and wine quality parameters were analysed in four replications per treatment with WineScan FT120 (Foss, Denmark). The measured frequencies were processed through a series of mathematical procedures (which include Fourier transformation) to an absorbance spectrum. A calibration process involving multivariate statistical procedures, such as principal component analysis (PCA), principal component regression (PCR) and partial least squares (PLS) regression was used (Nieuwoudt *et al.*, 2004).

3. Volatile compounds extraction and HS-SPME-GC-MS analysis

For the research, an HS-SPME (headspace solid-phase microextraction) was applied. Whilst a powerful tool in volatile analyses, it has one main drawback: the quantitative analysis of the mixture of volatile compounds present in the vapour phase can only be expressed as a relative value and not as an absolute one. By selecting the mixed polarity phase (Divinylbenzene/Carboxen/Polydimethylsiloxane) (DVB/CAR/PDMS) it was possible to determine a larger number of volatiles than with a PDMS and/or polyacrylate (PA) fibre. Although there were some differences among the fibres used (PA, PDMS or PDMS/DVB, PDMS/CAR, PDMS/DVB/CAR), extracting the matrix in comparison to its vapour phase turned out to be an even greater differences occurred extracting the matrix in comparison to its vapour phase (in case of HS-SPME) (Pawliszyn, 1999), because of the various distribution constants, which are specific to each analyte. If only one analyte had been the target compound, a standard addition method could have been employed for quantification. In our study, the volatile profile was investigated, focusing on the identification of compounds that, to our knowledge, are the most important compounds defining grape, must and wine odour. It is impossible to quantify them individually because they express different distribution constants and adsorption capacities and have to be analysed and quantified separately in order not to exceed the retention capacity of the fibre (Castro *et al.*, 2008).

The extraction of aroma compounds followed a previously described procedure (Prosen *et al.*, 2007). Briefly, 10-mL batches of fresh grape, must and wine were placed in 20-mL vials and corked with a septa stopper, and then each vial was thermostated for 15 min at 50 °C. A SPME (DVB/CAR/PDMS, 50/30 µm, Sigma Aldrich) fibre was inserted in the headspace; the volatile compounds were sampled for 35 min. The selected fibre, which has already been reported as being considerably sorptive (Kataoka *et al.*, 2000), was subsequently inserted into the injector port of a gas chromatograph and desorbed for 10 min. A GC-MS (gas chromatography coupled to mass spectrometry) (Agilent 6890 Series GC System with Agilent 5973 Mass Selective Detector) bound to an Rtx-20 column (60 m, 0.25 mm ID, 1 µm df, Restek, USA) was used for the analyses of volatile compounds in samples.

The oven temperature program was as follows: initial temperature was 50 °C (2 min), then ramped at 10 °C min^{-1} to 210 °C (40 minutes). The injector temperature was 250 °C and the detector temperature was 280 °C. In the mass spectrometer, electron impact (EI) ionization was applied, while chromatograms were recorded in the total ion current (TIC) mode. Compounds were tentatively identified on the basis of their retention times and spectra using the searchable EI-MS spectra library (NIST02).

4. Sensory evaluation of wines

Sensory analyses of the Chardonnay wines were conducted in March and April 2010 at the University of Ljubljana (Biotechnical Faculty) and at the Chamber of Agriculture and Forestry of Slovenia, Nova Gorica.

The four wines of each of the four "infection group" were first compared for between-replicate and between-treatment differences by 20 officially recognized wine tasters using the method of duo-trio difference testing by Amerine *et al.* (1965). All wine samples (25-mL aliquots) were served in covered ISO standard wine glasses (ISO, 1977) at room temperature (22°C). The tasting-room was

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equipped as advised for sensory tastings at international wine competition (OIV, 1994).

The tasters assessed and rated aroma (with smelling) and flavour (with tasting) perceptions in triplicate, following four training sessions (Table 1). Prior to tasting, each taster was provided with information about the study and was instructed on how to complete the wine sensory evaluation sheet. At each session, each assessor evaluated the same four samples, with one example of each treatment in each session. Tasters were asked to rate the intensity of each aroma and "mouthfeel" attribute previously mentioned for Chardonnay wine (Darriet *et al.*, 2002 ; Lee and Noble, 2003 ; Jaffré *et al.*, 2010) according to a descriptive 10-point-scale (0 - without any perception, 2 - poor perception, 4 - average perception, 6 - above average perception, 8 - high perception, 10 - exceptional). Data acquisition was carried out using Statgraphics 4.00 software (Manugistics, Germany).

5. Statistical analysis

Data for volatile compounds are given as an average peak area (x10^6) ± standard errors (SE), while those for quality parameters are given as an average content ± SE. Statgraphics 4.00 software was used for the analyses of the olfactory-taste evaluation of wines according to each treatment. The effects of powdery mildew on the sensory evaluation of wines were analysed using the analysis of variance (ANOVA) and the differences were evaluated using the Duncan test at $P \leq 0.05$ (*), with $P \leq 0.01$ (**) considered as statistically significant.

RESULTS AND DISCUSSION

1. Determination of wine quality parameters

The quality parameters of the wines produced from grapes with various degrees of powdery mildew infection were measured with WineScan FT120 (Table 2). The results showed that powdery mildew infection affected some of the wine characteristics : whilst the alcohol, malic acid and SO_2 (both free and molecular) content increased with increasing degree of infection, that of total extract, reducing sugars, and tartaric and citric acids decreased (Table 2), which was unexpected and partly contrary to the data reported by Stummer *et al.* (2003) and Calonnec *et al.* (2004).

Table 1 – Preparation of the reference standards for the sensory evaluation of Chardonnay wine vintage 2009.

Attribute (aroma)	Standard mixture - references*
	Odour (smelling)
Pineapple	7 mL canned pineapple juice (Fructal)
Coconut	0.5 mL of essence oil of coconut
Pear	3 drops of ethyl acetate in 50 mL pear juice (Fructal)
Floral	Geranium - fresh rubbed leaves of geranium
	Iris – 0.1 mg irone
	Lilia – 2 mg hydroxyl citronellal
	Rose – 2 mg citronellol
Honey	15% v/v honey in dd water
Yeast	1.0 g of leaven in 50 mL of dd water
Mushroom	10 µL of a 1 mg mL^{-1} 1-octen-3-ol solution
Earthy	62 µg L^{-1} geosmin
Butter	2 g of butter in 50 mL of dd water
	Taste (tasting)
Sweetness	15 g L^{-1} sucrose
Acidity	1 g L^{-1} tartaric acid
Freshness	-

*Standards were diluted/mixed to a final volume of 100 mL in a neutral Chardonnay wine - vintage 2009. The liquid standards were presented in a covered tasting wine glass (app. 30-mL aliquots).

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Table 2 - General composition (mean ± SE; n=4) of Chardonnay wines according to the degree of grape infection by powdery mildew.

Wine compositions	Degree of grape infection			
	0%	10%	50%	100%
Alcohol (vol.%)	14.2 ± 0.1 a	14.7 ± 0.1 b	14.8 ± 0.1 b	15.2 ± 0.2 b
Free SO_2 (mg/L)	33.0 ± 1.5 a	40.0 ± 3.0 b	49.0 ± 0.6 c	48.3 ± 1.7 c
Molecular SO_2 (mg/L)	0.50 ± 0.01 a	0.67 ± 0.03 b	0.70 ± 0.01 b	0.57 ± 0.03 a
Density (20 °C)	1.00 ± 0.01	0.99 ± 0.01	0.99 ± 0.01	0.99 ± 0.01
Total extract (g/L)	46.7 ± 0.3 b	35.3 ± 0.9 b	23.3 ± 0.3 a	24.7 ± 0.3 a
Reducing sugars (g/L)	2.3 ± 0.2 c	1.4 ± 0.6 a	1.6 ± 0.3 a	1.2 ± 0.5 a
Total acidity (g/L)	5.80 ± 0.01 b	6.07 ± 0.03 c	5.63 ± 0.07 a	5.83 ± 0.03 b
pH value	3.65 ± 0.01 b	3.59 ± 0.01 a	3.68 ± 0.01 c	3.77 ± 0.01 d
Malic acid (g/L)	2.63 ± 0.03 a	2.77 ± 0.03 b	2.77 ± 0.03 b	3.17 ± 0.03 c
Tartaric acid (g/L)	1.80 ± 0.01 c	1.50 ±0.01 b	0.90 ± 0.10 a	0.80 ± 0.01 a
Citric acid (g/L)	1.14 ± 0.02 c	0.92 ± 0.01 b	0.64 ± 0.02 a	0.67 ± 0.01 a
Lactic acid (g/L)	0.30 ± 0.01 c	0.27 ± 0.03 bc	0.20 ± 0.01 ab	0.17 ± 0.03 a
Volatile acidity (g/L)*	0.35 ± 0.01	0.33 ± 0.01	0.35 ± 0.01	0.37 ± 0.01

*expressed as acetic acid
**values within rows followed by different letters indicate significant differences at $P \leq 0.05$ (Duncan test)

2. Analysis of volatile compounds in grapes infected by powdery mildew

The crude extract obtained from the various "infection groups" of Chardonnay grapes (1000 g) was analysed using GC-MS (Table 3). Generally speaking, the number and abundance of volatile compounds detected in grapes showed a tendency to increase with increasing degree of infection, especially the terpenic compounds, aldehydes and alcohols.

With respect to terpenic compounds, citronellol and geraniol were only detected in the most severely (100 %) infected grapes (Table 3), which is in accordance with the affirmations of Pattnaik *et al.* (1997) about the antifungal activity of terpenes. Furthermore, the oxygenation of citronellol usually leads to the formation of rose oxide (2H-pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)) (Alsters *et al.*, 2010), and therefore the presence of rose oxide in the severely infected grapes was expected (Table 3). Alsters *et al.* (2010) also noted that the presence of rose oxide, which has already been detected in other fruits such as lychee and 'Gewürztraminer' grapes, may contribute to an odour reminiscent of roses.

The most abundant group of volatile compounds in grapes were the aldehydes (Table 3), which, according to Gómez *et al.* (1995), are also the most aromatic ones. The number of aldehydes showed a tendency to increase with increasing degree of grape infection (Table 3); when predominant, these compounds may lead to an unpleasant, stuffy and pungent odour (Li *et al.*, 2007; Rusjan, 2010). According to Li *et al.* (2007) the unpleasant waxy, buckwheat, green, herbaceous and fungal odours found in infected grapes could largely be ascribed to the presence of decanal, 2-decanal, *n*-nonanal, 2-octenal and heptanal. On the other hand, the grape infection decreased the presence of hexanal and 2-hexenal (Table 3), which may contribute to odours reminiscent of apple and freshly cut grass ("refreshing of grape") (Li *et al.*, 2007). Hexanal and 2-hexenal are products of the oxidation of linoleic and linolenic acids catalysed by the lipoxygenase enzyme (E.C. 1-13-11-12), followed by peroxidase activity and of the isomerisation of 3-hexenal, respectively (Drawert *et al.*, 1966). Drawert *et al.* (1966) also showed that peroxidase activity abbreviated the late ripening period, which suggested acceleration in the ripening process of the infected grapes. Furfural and 5-hydroxymethyl furfural, which were identified only in the most infected grapes (Table 3), both result from sugar degradation and cyclization (Hoydonckx *et al.*, 2007). Grosch (1993) and Bastos *et al.* (2006) reported that furfural and 5-hydroxymethyl furfural may contribute to traces of an almond, baked-bread, honey, buckwheat, or mate tea-like odour, which is undesirable in the context of grape quality.

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Table 3 - Volatile compounds (Peak Area, x10^6) assayed in Chardonnay grapes according to the degree of grape infection by powdery mildew (*Uncinula necator*).

Compound	Degree of grape infection**				Statistically differences	P.I.
	0%	10%	50%	100%		
Terpenic compounds						
Linalool	n.d.	1.88 ± 0.04	7.03 ± 3.41	3.74 ± 1.34	*	↑
Citronellol	n.d.	n.d.	n.d.	7.11 ± 0.02	*	↑
Geraniol	n.d.	n.d.	n.d.	2.83 ± 0.04	*	↑
Rose oxide	n.d.	n.d.	4.10 ± 0.05	5.37 ± 0.41	*	↑
Acids						
Acetic acid	n.d.	n.d.	n.d.	18.7 ± 0.91	*	↑
Hexanoic acid	n.d	n.d	2.89 ± 0.04	8.22 ± 0.64	*	↑
Aldehydes						
Hexanal	165.0 ± 2.4	122.0 ± 9.1	104.0 ± 10.8	31.7 ± 2.4	*	↓
2-Hexenal, (*E*)-	385.0 ± 10.0	252.9 ± 47.9	258.1 ± 29.9	114.9 ± 11.8	*	↓
Furfural	n.d.	n.d.	n.d.	9.94 ± 0.04	*	↑
5-Hydroxymethyl furfural	n.d.	n.d.	n.d.	46.2 ± 0.0	*	↑
Heptanal	n.d.	1.61 ± 0.03	4.37 ± 0.79	3.25 ± 0.84	*	↑
2,4-Hexadienal, (*E*)-	4.63 ± 0.05	5.57 ± 0.04	4.67 ± 0.88	2.59 ± 0.02		
Sorbaldehyde	n.d.	4.90 ± 0.03	1.52 ± 0.16	4.79 ± 0.06	*	↑
2-Heptenal, (*Z*)-	n.d.	3.50 ± 0.05	6.55 ± 0.31	5.11 ± 0.83	*	↑
Octanal	n.d.	4.90 ± 0.03	1.51 ± 0.16	4.79 ± 0.06	*	↑
2-Octenal, (*E*)-	n.d.	2.22 ± 0.02	1.96 ± 0.54	2.67 ± 0.26	*	↑
n-Nonanal	2.10 ± 0.04	6.75 ± 0.02	3.21 ± 0.03	8.94 ± 0.11	*	↑
Decanal	1.37 ± 0.24	1.67 ± 0.22	4.3 ± 1.7	4.57 ± 0.84		
2-Decenal, (*Z*)-	n.d.	n.d.	n.d.	7.22 ± 3.11	*	↑
Benzaldehyde	14.1 ± 0.1	11.1 ± 0.1	n.d.	n.d.	*	↓
Esters						
Ethyl acetate	n.d.	n.d.	41.9 ± 0.0	81.5 ± 2.3	*	↑
Alcohols						
3-Methyl-1-butanol	n.d.	5.04 ± 0.03	4.52 ± 0.01	11.8 ± 1.6	*	↑
Ethanol	n.d.	n.d.	15.5 ± 0.8	42.6 ± 0.3	*	↑
1-Hexanol	38.3 ± 0.8	41.4 ± 1.4	124.0 ± 10.2	159.0 ± 13.0	*	↑
2-Hexen-1-ol, (*E*)-	72.2 ± 9.1	70.9 ± 2.2	104.4 ± 2.3	96.2 ± 6.1		
2-Heptanol	n.d.	n.d.	2.36 ± 0.49	5.21 ± 2.11	*	↑
1-Octanol	n.d.	3.54 ± 0.00	5.26 ± 0.06	7.46 ± 1.08	*	↑
1-Octen-3-ol	n.d.	2.83 ± 0.04	5.50 ± 0.07	11.4 ± 0.02	*	↑
2-Octen-1-ol, (*Z*)-	n.d.	n.d.	3.55 ± 0.16	4.12 ± 0.44	*	↑
1-Nonanol	n.d.	1.89 ± 0.20	4.12 ± 0.02	5.31 ± 0.06	*	↑
Phenylethyl alcohol	n.d.	2.39 ± 0.23	3.48 ± 0.05	6.76 ± 1.45	*	↑
Other compounds (Carbonyl compounds)						
Methoxy-phenyl oxime	2.60 ± 0.03	3.88 ± 0.42	4.10 ± 0.36	5.71 ± 0.34	*	↑
6-Methyl-5-heptene-2-one	1.61 ± 0.03	0.96 ± 0.04	3.04 ± 0.57	4.33 ± 0.05		
Dihydromaltol	n.d.	n.d.	n.d.	11.8 ± 0.2	*	↑

n.d. : not detected; P.I. : possible impacts of the infection on the amount of volatile compounds (↑ - increase, ↓ - decrease)
*Statistically significant differences between fungi-infected and -free grapes (Duncan test; $P \leq 0.05$)
**Data are means ± standard errors obtained from four analyses per treatment

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The second most abundant volatile compounds detected in grapes were alcohols (Table 3), which are mostly derived from the fermentation process by microorganisms (yeasts, bacteria) (Gómez *et al.*, 1995). Most of the alcohol compounds were determined in the most severely infected grapes (50 % and 100 %), except for 1-hexanol and 2-hexen-1-ol, which may contribute to an odour reminiscent of freshly cut grass (Li *et al.*, 2003 ; Prosen *et al.*, 2007). Furthermore, except for 2-hexen-1-ol the peak areas of alcohols generally increased with increasing degree of infection, particularly ethanol, 1-hexanol, 3-methyl-1-butanol, 1-octen-3-ol, 1-octanol and phenylethyl alcohol (Table 3). Volatile compounds responsible for the mushroom-, geranium-, green- and earthy-like odours such as 1-octen-3-ol, 2-octen-1-ol, 2-heptanol and 2-octenal (Assaf *et al.*, 1997 ; Darriet *et al.*, 2002 ; Guerche *et al.*, 2006) were also detected in our study, especially in infected grapes (Table 3). Tressl *et al.* (1982) pointed out that the accumulation of 1-octen-3-ol in grape could also be linked to the presence of ascomycetes and basidiomycetes, in which case 1-octen-3-ol is a degradation product of unsaturated lipid. Furthermore, Zawirska-Wojtasiak (2004) and Husson *et al.* (2005) asserted that 2-octen-1-ol is one of the main characteristic fungal odours produced by moulds like *Aspergillus* and *Penicillium*, with an intense olfactory perception (Amerine *et al.*, 1965). Up to the present day 2-octen-1-ol has been detected by Tressl *et al.* (1982) in white musts only, which was confirmed by our results as well (Table 3). 2-Heptanol was evidenced by Morales *et al.* (2005) as an off-flavour in olive oils, usually providing an earthy odour. Husson *et al.* (2005) found that 2-heptanol is a metabolite of *Penicillium camemberti* and *Penicillium roqueforti*, whilst Guerche *et al.* (2006) pointed out its possible contribution to a fungal odour in the context of red grapes only. Our study proved the presence of 2-octen-1-ol and 2-heptanol in powdery mildew-infected Chardonnay grapes as well. In the most severely infected grapes, dihydromaltol (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one) was also detected, which may contribute to a caramel odour (Preininger *et al.*, 2009). In a previous study (Shaw *et al.*, 1971), dihydromaltol is mentioned as a major product in all systems containing hexoses and was found to be converted into maltol under specific reaction conditions (Maillard reaction). In our study, it was also identified in all musts produced from infected grapes (Table 4).

Here the presence of acetic acid in infected grapes could be explained with the statement of Shaw *et al.* (1971), citing that acetic acid bacteria (*Acetobacter* spp.) colonize damaged berries and cause the emergence of acetic acid. Moreover, according to Shaw *et al.* (1971), these organisms are present in all stages of winemaking, from the mature grapes and the vinification process to the bottled wines. The higher amounts of acetic acid and ethanol in the highly infected grapes may have also yielded higher amounts of ethyl acetate through esterification (Table 3), as already reported by Ribéreau-Gayon *et al.* (2000). The higher abundance of acetic acid in infected grapes in combination with ethyl acetate may contribute to an unpleasant odour reminiscent of glue, acetone and vinegar (Rusjan, 2010).

3. Analysis of volatile compounds in musts produced from grapes infected by powdery mildew

Volatile compounds in Chardonnay musts were assayed after overnight must clarification (Table 4). Compared to the volatiles detected in the grapes (see Table 3), the clarified musts contained a higher number of carboxylic acids, alcohols and esters but fewer C_6 aldehydes and terpenic compounds, which can be partly explained by an early start of the fermentation (Ribéreau-Gayon *et al.*, 2000).

Esters are usually derived from inorganic or organic acids, most commonly carboxylic acids, and alcohols (Ribéreau-Gayon *et al.*, 2000). Therefore, in the presence of both substrates a higher amount of esters in musts, compared to grape, was expected and proven. The presence of methyl-2-furoate, ethyl decanoate and hexyl acetate was only detected in musts produced from infected grapes (Table 4). Further, the peak area of ethyl acetate and ethyl caproate increased with increasing degree of infection. Mauriello *et al.* (2001) has already pointed out the contribution of methyl-2-furoate (methyl pyromucate) to a fungal, mushroom odour, which is a product of lactic acid bacteria (*Lactobacillus*) linked to excessive glucose and limited oxygen conditions. Li *et al.* (2007) reported that greater peak areas of ethyl caproate, ethyl acetate and hexyl acetate in musts produced from infected grape may intensify the odour reminiscent of pineapple, banana and pear odour.

Additionally, the powdery mildew infection affected the presence of many alcohol compounds in musts as well (Table 4). The most abundant assayed alcohols in musts were 1-octanol, 1-hexanol, ethanol and 1-nonanol (Table 4). The infection increased the peak areas of many alcohols, except of 2-methyl-1-butanol, 2-heptanol, 1-octanol and 1-nonanol. After the overnight must clarification, the present volatile alcoholic compounds, which according to Darriet *et al.* (2002) significantly contribute to the mushroom odour, decreased, where only 1-octen-3-ol remained in musts (Table 4). Considering the reports by Joyeux *et al.* (1984) on the presence of methyl-1-butanols as natural products in fruits and their synthesis by lactic acid bacteria (*Streptococcus*, *Leuconostoc*), we cannot determine whether the infection affected the abundance of these compounds in musts (Table 4). According to Joyeux *et al.* (1984) and Li *et al.*

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Table 4 - Volatile compounds (Peak Area, x10^6) assayed in Chardonnay must according to the degree of grape infection by powdery mildew (*Uncinula necator*).

Compound	Degree of grape infection**				Statistically differences	P.I.
	0%	10%	50%	100%		
Terpenic compounds						
Rose oxide	n.d.	n.d.	n.d.	4.47 ± 0.13	*	↑
Acids						
Acetic acid	1.39 ± 0.51	3.35 ± 1.10	7.73 ± 1.32	7.93 ± 0.98	*	↑
Oxalic acid	38.8 ± 1.1	27.9 ± 2.4	15.8 ± 0.03	9.97 ± 0.31	*	↓
Formic acid	42.0 ± 0.0	4.29 ± 0.04	n.d.	n.d.	*	↓
Pyruvic acid	n.d.	n.d.	12.5 ± 0.7	18.8 ± 0.0	*	↑
Hexanoic acid	n.d.	4.53 ± 0.05	3.00 ± 0.03	2.84 ± 0.91	*	↓
Decanoic acid	n.d.	n.d.	17.3 ± 4.5	13.6 ± 0.2	*	↑
Aldehydes						
2-Methyl butanal	3.45 ± 0.04	1.28 ± 0.08	n.d.	n.d.	*	↓
Methylglyoxal	n.d.	n.d.	n.d.	3.29 ± 0.04	*	↑
Hexanal	13.6 ± 0.0	9.27 ± 0.63	8.22 ± 1.90	7.58 ± 0.71	*	↓
2-Hexenal	20.9 ± 0.6	16.0 ± 0.05	18.4 ± 0.57	9.32 ± 0.56	*	↓
Furfural	34.9 ± 0.3	129.0 ± 16.5	153.0 ± 25.0	237.0 ± 65.8	*	↑
n-Nonanal	5.94 ± 0.16	5.33 ± 0.16	3.08 ± 0.12	1.99 ± 0.05	*	↓
Benzeneacetaldehyde	9.84 ± 0.08	11.6 ± 0.21	11.6 ± 0.79	13.9 ± 0.10	*	↑
2,5-Furandicarboxaldehyde	8.72 ± 0.01	11.2 ± 2.20	30.9 ± 3.71	25.0 ± 1.90	*	↑
5-Acetoxymethyl-2-furaldehyde	31.7 ± 4.3	14.3 ± 0.07	4.58 ± 1.94	2.60 ± 0.39	*	↓
5-Hydroxymethyl furfural	31.7 ± 4.3	75.1 ± 5.5	151.0 ± 56.7	328.5 ± 6.1	*	↑
Esters						
Methyl 2-furoate	n.d.	n.d.	n.d.	0.49 ± 0.05	*	↑
Ethyl acetate	29.1 ± 1.0	37.0 ± 1.0	63.8 ± 1.2	73.0 ± 7.1	*	↑
Ethyl caproate	2.99 ± 0.26	6.39 ± 2.17	7.82 ± 0.31	7.66 ± 0.12	*	↑
Ethyl caprylate	7.39 ± 0.24	n.d.	n.d.	n.d.		
Ethyl decanoate	n.d.	n.d.	14.0 ± 2.1	22.0 ± 0.3	*	↑
Hexyl acetate	n.d.	2.20 ± 0.07	5.51 ± 0.23	6.50 ± 0.24	*	↑
Alcohols						
Ethanol	34.9 ± 3.3	46.4 ± 1.3	67.3 ± 0.6	53.2± 1.5	*	↑
2-Methyl-1-butanol	13.5 ± 0.4	6.72 ± 0.12	3.15 ±0.38	4.46 ± 0.71	*	↓
3-Methyl-1-butanol	7.64 ± 0.35	6.37 ± 0.13	7.0 ± 0.41	8.33 ± 0.16		
3-Hexen-1-ol	6.98 ± 0.10	14.6 ± 1.4	16.1 ± 0.1	19.2 ± 3.9	*	↑
1-Hexanol	304.0 ± 26.2	363.0 ± 24.9	445.0 ± 13.2	486.0 ± 17.5	*	↑
2-Heptanol	15.8 ± 0.3	14.0 ± 0.4	3.77 ±0.87	2.20 ± 0.05	*	↓
1-Octen-3-ol	2.04 ± 0.34	2.90 ± 0.23	7.71 ± 2.09	8.66 ± 0.05	*	↑
6-Methyl-1-heptanol	19.5 ± 0.2	22.3 ± 0.7	13.2 ± 0.05	7.35 ± 0.81		
1-Octanol	999.0 ± 67.6	883.5 ± 27.9	840.0 ± 59.1	826.5 ± 12.6	*	↓
Maltol	n.d.	9.07 ± 0.07	19.6 ± 3.9	38.4 ± 1.8	*	↑
1-Nonanol	38.6 ± 0.8	29.8 ±0.24	20.8 ± 3.3	18.5 ± 1.1	*	↓
Phenylethyl alcohol	4.33 ± 0.09	13.1 ± 0.3	13.2 ± 3.6	16.9 ± 0.3	*	↑
1-Decanol	n.d.	n.d.	6.22 ± 0.61	14.6 ± 1.07	*	↑
Other compounds (Carbonyl compounds)						
Acetol	2.06 ± 0.04	2.02 ± 0.03	4.17 ± 0.04	14.2 ± 0.81		
2-Cyclopentene-1,4-dione	18.6 ± 3.60	4.82 ± 0.10	10.6 ± 0.05	15.7 ± 0.24		
1-Hydroxy-2-propanone	5.57 ± 0.01	19.3 ± 3.6	20.6 ± 0.0	23.4 ± 0.0	*	↑
Dihydromaltol	5.56 ± 0.04	19.3 ± 0.4	20.6 ± 0.0	23.4 ± 0.1	*	↑
Furaneol	7.34 ± 0.29	8.15 ± 1.6	33.2 ± 2.0	60.5 ± 2.12	*	↑
5-Hydroxymaltol	264.0 ± 33.6	1.71 ± 0.15	12.3 ± 0.04	24.1 ± 2.0	*	↓

n.d. – not detected; P.I. – possible impacts of the infection on the amount of volatile compounds (↑ - increase, ↓ - decrease)
*Statistically significant differences between fungi-infected and -free grapes (Duncan test; $P \leq 0.05$)
**Data are means ± standard errors obtained from four analyses per treatment

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(2007) the isomers of methyl-1-butanol might contribute to fused-oil-, whisky-, and onion-like but also fermented, yeasty, and bread-like odours. The presence of greater peak areas of 1-nonanol and 1-octanol in musts produced from disease-free grapes may contribute to a pleasant, fresh odour, reminiscent of freshly cut grass, citrus and coconut (Li *et al.*, 2007). We have to note the presence of maltol and 1-decanol in musts produced from infected grapes, whose peak areas increased with increasing degree of infection (Table 4).

The peak areas of the aldehydes benzeneacetaldehyde, 5-hydroxymethyl furfural, furfural, 2,5-furandicarboxaldehyde and methylglyoxal were larger in musts produced from infected grapes (Table 4), which can result in a sweet, smoky odour with traces of almond, honey and caramel (Nieuwoudt *et al.*, 2004; Li *et al.*, 2007). Inoue and Kimura (1995) and later Ko *et al.* (2005) noted that methylglyoxal (pyruvaldehyde) is an aldehyde form of pyruvic acid, an offshoot of glycolysis found in some prokaryotes, which converts glucose into methylglyoxal and then into pyruvate. In the same metabolic pathways pyruvic acid and 1-hydroxy-2-propanone are also involved (Ko *et al.*, 2005); both were detected here as well, namely in high peaks in musts from infected grapes (Table 4).

The set of assayed acids in musts showed a tendency to increase with increasing degree of infection, except for the oxalic, formic and hexanoic acid (Table 4). The dynamic of acetic acid in our study is in accordance with the findings of Joyeux *et al.* (1984), who reported the frequent accumulation of acetic acid during spontaneous bacteria fermentation. Li *et al.* (2007) noted that the presence of acetic acid may contribute to vinegar- or acetic-like odours, which, according to Ribéreau-Gayon *et al.* (2000), is likely to decrease must quality.

According to Preininger *et al.* (2009), the presence of maltol, dihydromaltol, furaneol and their decomposition products at higher temperatures, such as a 2-hydroxy-2-cyclopentene-1-one, may lead to a caramel odour in musts produced from infected grapes (Table 4). Some of the carbonyl compounds detected here have already been detected in oak barrel-aged wines (Baltes *et al.*, 1989). After must clarification, the peak area of rose oxide also diminished and was detected only in the must produced from 100 % infected grape (Table 4). Alsters *et al.* (2010) asserted that the presence of rose oxide can contribute to odours reminiscent of roses and other flowers.

4. Analysis of volatile compounds in young wines produced from grapes infected by powdery mildew

The assayed volatile compounds in young wines produced from grapes with various degrees of powdery mildew infection are given in Table 5. Most of the analysed volatile compounds in typical Chardonnay wines, regarding the impacts of various factors (yeasts, vinification practices, spraying, aging, etc.) have already been reported by Lee and Noble (2003), Li *et al.* (2007) and Jaffré *et al.* (2010). In the present study, 15 esters, 11 alcohols, 7 acids, 4 other carbonyl compounds and 3 aldehydes were assayed in young Chardonnay wines (Table 5).

The most abundant esters were ethyl esters, particularly ethyl decanoate, ethyl caprylate and ethyl laurate, followed by ethyl hexadecanoate and ethyl myristate (Table 5), which partially coincides with reports by Li *et al.* (2007) and Jaffré *et al.* (2010) for Chardonnay wine. Except for ethyl hexadecanoate, their peak areas generally decreased in young wines produced from infected grapes, compared to those from disease-free grapes. On the other hand, the content of ethyl acetate, ethyl caproate, isoamyl octanoate, isoamyl decanoate, diethyl succinate and ethyl nonanoate increased with increasing powdery mildew infection (Table 5), which, according to Li *et al.* (2007), may contribute to a sweet odour reminiscent of pineapple and banana.

Considering the peak areas of esters and their dynamics with respect to the degree of infection (Table 5), and according to the findings of Li *et al.* (2007) and Jaffré *et al.* (2010), it can be claimed that young wines produced from infected grapes may possess an odour reminiscent of fruits, pineapple and banana, whilst those from disease-free grapes may have a more floral, grape- or iris-like odour.

During alcoholic fermentation, the peak areas of aldehydes in all treatments was decreased considerably, where only furfural, methyl-5-furfural and 5-hydroxymethyl furfural were assayed (Table 5). Increasing powdery mildew infection further decreased the abundance of assayed aldehydes in young wines. Furfural and 5-hydroxymethyl furfural have already been assayed in wines (Bastos *et al.*, 2006) and may contribute to an odour reminiscent of caramel and mate tea and to a smoky flavour.

At the beginning of fermentation nearly all C_6 aldehydes was converted into their respective alcohols, which, as reported by Ramey *et al.* (1986), are less aromatic. The number of assayed alcohols was lower in young wines than in musts (Table 5), which was unexpected based on results by Ribéreau-Gayon *et al.* (2000). The most abundant assayed alcohols in young wines were ethanol, glycerol and 3-methyl-1-butanol, followed by 2-methyl-1-butanol and phenylethyl alcohol (Table 5). However, higher ethanol contents were determined in young wines produced from 50 % and 100 % infected bunches, whilst glycerol responded quite proportionally to the degree of infection. Ribéreau-Gayon

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Table 5 - Volatile compounds (Peak Area, x10^6) assayed in young Chardonnay wine according to the degree of grape infection by powdery mildew (*Uncinula necator*).

Compound	Degree of grape infection**				Statistically differences	P.I.
	0%	10%	50%	100%		
Acids						
Acetic acid	12.5 ± 0.0	18.9 ± 2.0	54.0 ± 8.0	54.0 ± 9.7	*	↑
Formic acid	6.20 ± 0.34	n.d.	n.d.	n.d.	*	↓
Pyruvic acid	3.21 ± 0.02	3.29 ± 0.02	4.34 ± 0.04	11.3 ± 0.05	*	↑
Hexanoic acid	5.19 ± 0.42	8.38 ± 4.8	8.50 ± 1.03	13.9 ± 4.8	*	↑
3-Hydroxybutanoic acid	8.67 ± 2.28	12.20 ± 0.91	16.0 ± 0.05	7.83 ± 0.16		
Octanoic acid	48.3 ± 2.3	52.4 ± 1.7	44.7 ± 0.2	38.7 ± 0.8		
Decanoic acid	28.5 ± 0.5	39.9 ± 1.0	40.9 ± 1.1	63.7 ± 1.7	*	↑
Aldehydes						
Furfural	43.3 ± 1.6	29.8 ± 0.02	7.83 ± 0.4	3.12 ± 0.03	*	↓
Methyl-5-furfural	124.2 ± 0.2	22.2 ± 0.0	17.4 ± 0.0	11.0 ± 3.0	*	↓
5-Hydroxymethyl furfural	124.0 ± 12.7	91.8 ±10.7	23.8± 3.0	8.65 ± 0.29	*	↓
Esters						
Ethyl acetate	14.8 ± 0.3	17.1 ± 0.6	21.9 ± 0.6	37.7 ± 0.9	*	↑
Ethyl butyrate	7.04 ± 0.18	3.86 ± 0.18	1.69 ± 0.33	1.89 ± 0.28	*	↓
Isoamyl acetate	54.8 ± 0.1	24.3 ± 4.3	25.3 ± 0.3	16.1 ± 1.8	*	↓
Ethyl caproate	3.42 ± 0.03	20.9 ± 2.8	90.9 ± 1.1	122.0 ± 10.1	*	↑
Ethyl myristate	47.3 ±1.9	41.5 ± 4.8	27.1 ± 0.9	25.6 ±0.1	*	↓
Ethyl caprylate	4996 ± 128	3427 ± 258	2988 ± 85	2286 ± 208	*	↓
Diethyl succinate	2.12 ± 0.01	4.99 ± 0.20	6.93 ± 0.74	6.54 ± 1.42	*	↑
Isopentyl hexanoate	6.77 ± 0.15	6.93 ± 0.32	6.33 ± 0.32	2.62 ± 0.1		
Ethyl nonanoate	16.4 ± 0.2	16.6 ± 1.2	32.6 ± 5.6	34.8 ± 0.1	*	↑
2-Phenylethyl acetate	25.5 ± 0.0	28.2 ± 0.8	35.4 ± 0.2	37.7 ± 0.5	*	↓
Ethyl decanoate	7989 ± 260	7590 ± 56	6788 ± 69	6744 ± 133	*	↓
Isoamyl octanoate	27.8 ± 0.1	28.9 ± 0.5	36.2 ± 0.4	41.8 ± 1.4	*	↑
Ethyl laurate	2419 ± 46	2154 ± 127	1334 ± 22	1154 ± 66	*	↓
Isoamyl decanoate	41.8 ± 1.1	48.4 ± 0.2	49.7 ± 0.4	53.9 ± 0.01	*	↑
Ethyl hexadecanoate	70.1 ± 0.0	66.0 ± 8.6	93.9 ± 1.2	82.7 ± 0.3		
Alcohols						
Ethanol	1917 ± 36	2598 ± 400	3024 ± 229	3679 ± 139	*	↑
Isobutanol	11.20 ± 0.02	9.82 ± 0.71	4.46 ± 0.01	4.06 ± 1.44	*	↓
2-Methyl-1-butanol	113.5 ± 0.9	44.1 ± 3.1	36.2 ± 1.5	35.0 ± 2.4	*	↓
3-Methyl-1-butanol	150.2 ± 3.8	90.2 ± 3.9	91.3 ± 1.0	82.8 ± 3.9	*	↓
2,3-Butanediol	20.8 ± 0.2	41.2 ± 7.0	67.0 ± 2.0	125.2± 2.7	*	↑
Propane-1,2-diol	2.57 ± 0.01	2.63 ± 0.03	2.86 ± 0.03	4.32 ± 0.03	*	↑
1-Hexanol	4.34 ± 0.11	4.58 ± 0.02	4.61 ± 0.16	5.30 ± 0.35	*	↑
3-Methyl-2-hexanol	n.d.	4.51 ± 0.04	16.4 ± 0.01	19.6 ± 1.7	*	↑
Glycerol	506.2 ± 42.4	511.0 ± 34.9	802.0 ± 20	1350.0 ± 42	*	↑
1-Octanol	39.2 ± 3.8	41.4 ± 3.8	28.9 ± 0.2	32.6 ± 1.6		
Phenylethyl alcohol	99.8 ± 0.0	31.8 ± 1.9	137.7 ± 2.8	122.0 ± 10		
Other compounds (Carbonyl compounds)						
Acetol	6.46 ± 0.22	4.63 ± 0.18	3.26 ± 0.32	2.64 ± 0.65	*	↓
Dihydro-2,5-furandione	3.48 ± 0.33	4.97 ± 1.22	6.27 ± 0.63	11.5 ± 0.55	*	↑
Dihydromaltol	1.76 ± 0.31	5.67 ± 0.14	96.5 ± 4.3	145.3 ± 5.4	*	↑
5-Hydroxymaltol	3.31 ± 0.33	10.57 ± 0.51	11.32 ± 0.13	19.11 ± 0.14	*	↑

n.d. – not detected; P.I. – possible impacts of the infection on the amount of volatile compounds (↑ - increase, ↓ - decrease)
*Statistically significant differences between fungi-infected and -free grapes (Duncan test; $P \leq 0.05$)
**Data are means ± standard errors obtained from four analyses per treatment

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et al. (2000) reported the importance of glycerol, the major fermentation by-product of *Saccharomyces cerevisiae*, as an indirect contributor to the sweetness, body and fullness of wines' sensory character. The infection also decreased the amount of 2- and 3-methyl-1-butanol and isobutanol, which, according to the findings of Li *et al.* (2007), may give a more pungent, oily, bread-like, yeasty, and greenish odour to the young wines, and increased that of 2,3-butanediol and 3-methyl-2-hexanol, which likely contributed to a sweeter, musty and corny odour. According to the study of Darriet *et al.* (2002), the optimal function of *Saccharomyces cerevisiae* and possible activity of enone reductases during fermentation could have caused the total disappearance of compounds reminiscent of fungal aroma (Table 5).

Li *et al.* (2007) affirmed that saturated fatty acids contribute to an unpleasant odour reminiscent of rancid butter and cheese. The most abundant assayed acids in young wines were octanoic and decanoic acid, followed by acetic and 3-hydroxybutanoic acid (Table 5). The peak areas of hexanoic, decanoic, acetic and pyruvic acids increased with the powdery mildew infection, whilst for the other assayed acids no impact of infection was observed (Table 5).

The abundance of other analysed carbonyl compounds generally increased with the severity of grape infection, with the exception of acetol (Table 5). Yaylayan and Mandeville (1994) and Shinoda *et al.* (2005) reported that dihydromaltol, 5-hydroxymaltol and the previously mentioned 5-hydroxymethyl furfural are secondary metabolites from *Penicillium echinulatum* and the decomposition products of fructose (hexoses) mostly, which causes browning, a non-enzymatic oxidation that reduces wine quality.

5. Analysis of volatile compounds in wines produced from grapes infected by powdery mildew

According to Ribéreau-Gayon *et al.* (2000), the tertiary volatile compounds formed during wine maturation and aging are predominantly alcohols and esters. This was also observed in our study (Table 6). Many of the analysed volatiles have already been reported as typical compounds of Chardonnay wine (Li *et al.*, 2007 ; Jaffré *et al.*, 2010), whilst others are reported here for the first time. In matured Chardonnay wines, ethyl esters, especially ethyl decanoate, ethyl caprylate, ethyl laurate and ethyl caproate predominated, irrespective of infection treatment (Table 6). After bottling, the peak areas of most assayed esters decreased in comparison to those in young wines, especially ethyl caprylate, ethyl laurate, ethyl decanoate, isoamyl decanoate and ethyl hexadecanoate (Table 6). In comparison to the esters from disease-free grapes, larger peak areas were determined for most assayed esters in wines produced from infected grapes, except for ethyl hexadecanoate, ethyl myristate, diethyl succinate, 2-phenylethyl acetate and isoamyl octanoate. The presence of the analysed esters in matured Chardonnay wines produced from disease-free grape may contribute to an odour reminiscent of pineapple and banana (Li *et al.*, 2007), whereas those from infected grape may contribute to an unpleasant odour of pear, similar to that of ripe, sour fruits and iris.

At wine aging, a decrease in alcohol content was observed, irrespective of infection treatment, among which the most abundant were ethanol, 1-octanol, glycerol, phenylethyl alcohol and 3-methyl-1-butanol (Table 6). In matured wines produced from infected grapes, a decrease in the peak areas of ethanol, 1-octanol and 2,3-butanediol but an increase in glycerol was determined. Moreover, propane-1,2-diol and 3-methyl-2-hexanol (isoamyl alcohol) were only analysed in the previously mentioned wines ; in combination with glycerol, they may have contributed to a mild, choking alcohol and sweet odour (Li *et al.*, 2007). The presence of propane-1,2-diol only in mature wines produced from the most severely infected grapes (Table 6) confirmed the affirmations by Hartley (1947) about the presence of propane-1,2-diol in the context of antimicrobial activity.

During bottle aging, the average abundance of most aldehydes decreased, particularly in wines produced from infected grapes, where furfural, methyl-5-furfural and 5-hydroxymethyl furfural were detected (Table 6). Except for 5-hydroxymethyl furfural, their peak areas increased mostly in wines produced from 100 % infected grapes, which, according to the study by Li *et al.* (2007), can intensify the impacts on unpleasant musty, sweet and buttery odour.

Li *et al.* (2007) affirmed that acids, particularly hexanoic, octanoic and decanoic ones significantly contribute to the odour of Chardonnay wine. Wine maturation generally decreased the abundance of organic acids, irrespective of powdery mildew infection (Table 6). Acetic acid was detected in matured wines produced from the most severely infected grapes exclusively (Table 6), which was expected according to affirmations of Shaw *et al.* (1971). Li *et al.* (2007) reported a possible contribution of acetic acid to a vinegar-like, rancid, fatty and unpleasant odour.

Fewer carbonyl compounds were detected in matured wine compared to young wine. This was observed particularly in wines produced from infected grapes, mostly in the abundance of acetol and dihydromaltol. Acetol (1-hydroxy-2-propanone) was detected only in matured wines produced from 100 % infected grapes (Table 6) and according to Ribéreau-Gayon *et al.* (2000)

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Table 6 - Volatile compounds (Peak Area, x10^6) assayed in matured Chardonnay wine according to the degree of grape infection by powdery mildew (*Uncinula necator*).

Compound	Degree of grape infection**				Statistically differences	P.I.
	0%	10%	50%	100%		
Acids						
Acetic acid	n.d.	n.d.	n.d.	12.3 ± 0.0	*	↑
Formic acid	n.d.	n.d.	n.d.	15.0 ± 1.0	*	↑
Pyruvic acid	n.d.	n.d.	n.d.	13.4 ± 0.0	*	↑
Oxalic acid	3.66 ± 0.02	n.d.	n.d.	2.84 ± 0.04		
Hexanoic acid	n.d.	5.08 ± 0.01	6.80 ± 0.04	7.91 ± 0.02	*	↑
3-Hydroxybutanoic acid	22.3 ± 0.0	11.5 ± 0.4	n.d.	n.d.	*	↓
Octanoic acid	22.9 ± 0.0	25.6 ± 0.5	43.8 ± 0.9	50.2 ± 0.9	*	↑
Decanoic acid	23.6 ± 1.2	26.6 ± 1.8	28.8 ± 0.3	32.8 ± 1.0	*	↑
Aldehydes						
Furfural	2.07 ± 0.05	12.6 ± 0.0	21.7 ± 0.3	25.6 ± 0.2	*	↑
Methyl-5-furfural	15.3 ± 1.6	19.5 ± 0.0	27.2 ± 0.4	29.5 ± 0.4	*	↑
5-Hydroxymethyl furfural	6.17 ± 0.46	5.73 ± 0.86	5.62 ± 0.21	3.65 ± 0.51		
Esters						
Ethyl acetate	16.1 ± 1.1	20.7 ± 0.9	21.9 ± 0.1	23.4 ± 0.7	*	↑
Ethyl butyrate	n.d.	2.07 ± 0.11	2.36 ± 0.14	6.02 ± 0.10	*	↑
Isoamyl acetate	21.8 ± 1.7	24.5 ± 0.7	26.7 ± 0.5	30.4 ± 0.2	*	↑
Ethyl caproate	81.2 ± 0.6	101.5 ± 1.2	100.9 ± 3.5	218.5 ± 2.8	*	↑
Ethyl myristate	14.2 ± 0.4	16.4 ± 0.6	10.6 ± 0.5	7.63 ± 0.34		
Ethyl caprylate	1426 ± 94	1660 ± 38	2045 ± 44	3620 ± 89	*	↑
Diethyl succinate	2.53 ± 0.02	2.36 ± 0.22	3.19 ± 1.01	9.41 ± 0.22		
Isopentyl hexanoate	3.22 ± 0.65	3.43 ± 0.27	4.10 ± 0.07	5.75 ± 0.26	*	↑
Ethyl nonanoate	14.7 ± 0.0	16.07 ± 0.2	18.9 ± 1.9	21.2 ± 0.1	*	↑
2-Phenylethyl acetate	26.1 ± 0.2	32.2 ± 1.1	29.8 ± 1.9	23.9 ± 0.1		
Ethyl decanoate	3170 ± 90	3784 ± 119	4796 ± 116	5802 ± 70	*	↑
Isoamyl octanoate	22.3 ± 0.4	24.8 ± 0.5	19.4 ± 0.6	15.6 ± 0.6		
Ethyl laurate	288.9 ± 1.2	508.6 ± 0.8	567.0 ± 30.2	833.6 ± 15.1	*	↑
Isoamyl decanoate	7.82 ± 0.01	15.9 ± 0.1	16.9 ± 0.3	25.7 ± 1.2	*	↑
Ethyl hexadecanoate	6.28 ± 0.14	4.88 ± 0.02	8.72 ± 1.47	13.1 ± 0.82		
Alcohols						
Ethanol	2100 ± 98	2460 ± 64	2510 ± 96	3260 ± 37	*	↓
Isobutanol	4.31 ± 0.30	3.46 ± 0.01	9.15 ± 0.41	15.3 ± 0.46		
2-Methyl-1-butanol	33.6 ± 0.4	36.7 ± 1.6	39.1 ± 0.7	91.7 ± 1.6	*	↑
3-Methyl-1-butanol	77.0 ± 1.2	66.1 ± 1.0	82.9 ± 3.4	110.1 ± 1.8		
2,3-Butanediol	96.1 ± 6.8	10.2 ± 3.4	36.8 ± 3.8	47.8 ± 0.1	*	↓
Propane-1,2-diol	n.d.	n.d.	n.d.	1.79 ± 0.04	*	↑
1-Hexanol	2.23 ± 0.02	4.83 ± 0.05	3.75 ± 0.38	n.d.		
3-Methyl-2-hexanol	n.d.	8.55 ± 0.01	8.96 ± 0.05	21.2 ± 0.3	*	↑
Glycerol	116.6 ± 1.0	452.2 ± 33.2	599.4 ± 28.1	617.2 ± 11.0	*	↑
1-Octanol	1110 ± 21	51.1 ± 0.7	36.3 ± 1.6	33.6 ± 0.1	*	↓
Phenylethyl alcohol	122.3 ± 0.5	157.7 ± 0.0	132.0 ± 8.0	99.7 ± 0.7		
Other compounds (Carbonyl compounds)						
Acetol	n.d.	n.d.	n.d.	7.57 ± 0.3	*	↑
Dihydro-2,5-furandione	12.3 ± 0.5	12.0 ± 0.0	10.4 ± 0.5	10.6 ± 0.3		
Dihydromaltol	26.8 ± 0.2	28.7 ± 0.0	142.0 ± 1.9	165.8 ± 0.0	*	↑
5-Hydroxymaltol	10.0 ± 1.0	3.36 ± 0.1	13.2 ± 6.4	13.1 ± 0.4		

n.d. – not detected; P.I. – possible impacts of the infection on the amount of volatile compounds (↑ - increase, ↓ - decrease)
*Statistically significant differences between fungi-infected and -free grapes (Duncan test; $P \leq 0.05$)
**Data are means ± standard errors obtained from four analyses per treatment

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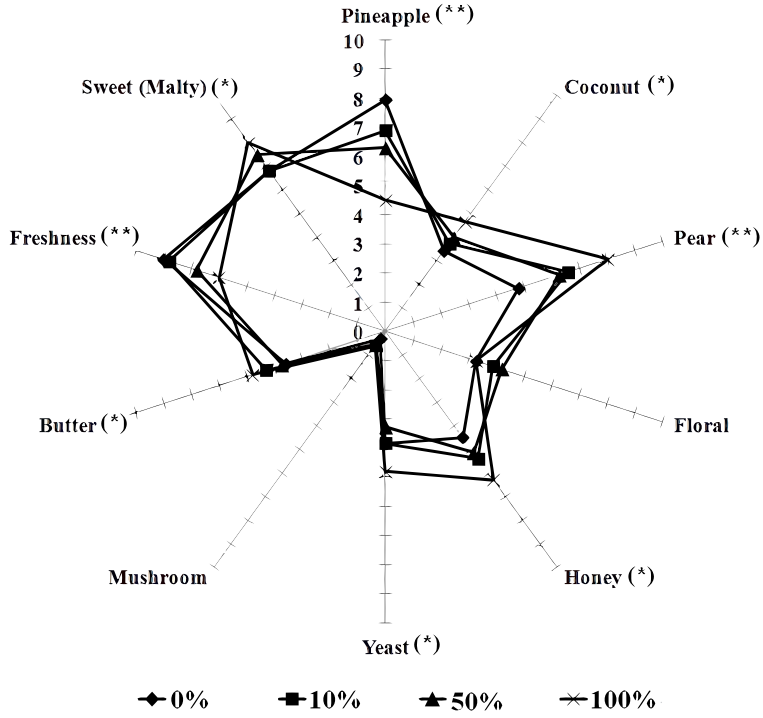


Figure 1 - Polar plot analysis showing olfactory attribute scores of mature Chardonnay wines produced from grapes with different degrees of powdery mildew infection (*Uncinula necator*). Each value is the mean score from four fermentation replicate wines that were presented to 20 tasters in two replicate sessions. The differences were evaluated using Duncan test at P ≤ 0.05 (*); P ≤ 0.01 (**) represents statistically significant differences.

it can be formed by the dehydration of less volatile glycerol. According to reports by Li *et al.* (2007), these volatile carbonyl compounds may contribute to an unpleasant, acrid, pungent, caramel-like odour and may occur with surprising intensity in wines produced from disease-free grapes.

6. Olfactory evaluation of Chardonnay wines produced from grapes infected by powdery mildew

The average scores of the olfactory evaluation of the wines made from grapes with different degrees of powdery mildew are presented as a "spider-web" diagram (Fig. 1). In wines produced from disease-free grapes, a higher intensity of sensory attributes was observed, which was also preferred by the testers in our study.

The wines produced from infected grapes yielded a moderately intense, slightly unpleasant odour. These wines, compared to wines produced from disease-free grapes, statistically differed especially in the reminiscence of pineapple, pear, honey, coconut, yeast and butter (Fig. 1). Statistical significance was also observed for freshness, with wines from infected grapes showing less freshness and a more "closed" nose compared to wines produced from disease-free grapes.

According to the volatile compounds detected by chemical analysis (see Table 6), the freshness of the wines produced from disease-free grapes was unexpected (Fig. 1). The olfactory tests showed the presence of an intense odour and trace flavours of pineapple, pear and floral characteristics, which have already been mentioned for Chardonnay wine by Stummer *et al.* (2003 and 2005) and Guchu *et al.* (2006). But on the other hand, the olfactory evaluation rejected the affirmation of a fungal (mushroom), earthy odour in wines produced from infected grapes, which contradicts previous reports by Stummer *et al.* (2003 and 2005). Furthermore, Calonnec *et al*. (2004) reported that during tasting the differences between Sauvignon blanc wines were clearly discriminated at the 50 % infection level only, whereas in Cabernet-Sauvignon wines these differences were detected when the proportion of diseased berries reached 25 %. The disappearance of the fungal aroma could be explained by the optimal activity of *Saccharomyces cerevisiae* and of enone reductases during alcoholic fermentation (Darriet *et al.*, 2002).

The study has shown the impact of powdery mildew infection on some volatile compounds in wine and consequently on their olfactory evaluation, where odours reminiscent of sweet, honey and malt flavours increased

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and predominated and subsequently decreased the freshness of matured Chardonnay wine.

CONCLUSIONS

The study focused on the investigation of volatile compounds in grapes with different infection levels of powdery mildew (*Uncinula necator*), as well as in the musts and wines produced from these infected grapes, with an HS-SPME-GC-MS analysis, the most powerful tool regarding aroma compounds. The fungal infection increased the amount of volatile compounds, especially in grapes, where the compounds 1-octen-3-ol, 2-octen-1-ol, 2-octenal and 2-heptanol reminiscent of mushroom, geranium and earthy-like odours were detected. Their presence in musts was reduced after overnight must clarification, after which only 1-octen-3-ol and 2-heptanol were determined. Moreover, the odour reminiscent of fungi or mushroom could have been intensified by the presence of methyl-2-furoate (methyl pyromucate), detected only in musts produced from the most severely infected grape. Furthermore, higher amounts of many other volatile compounds were detected in musts produced from infected grapes, especially those that probably contribute to the smoky-sweet odour reminiscent of caramel. After alcoholic fermentation, the amount of esters was increased, whilst that of aldehydes was drastically reduced in young wines. Furthermore, volatile compounds reminiscent of mushroom were not detected. The infection additionally decreased the peak areas of the most abundant esters (ethyl decanoate, ethyl laurate and ethyl caprylate). In matured wines produced from disease-free grapes the volatiles reminiscent of floral, grape- and iris-like odours predominated, although this was not confirmed by the olfactory evaluation of the wines. By infection, the amount of volatiles giving the wine a fruitier, pineapple-, pear- or banana-like odour was enhanced, whereas the fungal odour was not perceived. The olfactory evaluation of the wines confirmed the impact of the powdery mildew disease on wine quality, particularly in decreasing its freshness and increasing its sweet, malty taste.

Acknowledgements: We would like to express our gratitude to the Biotechnical Faculty of the University of Ljubljana and to the Chamber of Agriculture and Forestry of Slovenia for financial support.

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