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Effect of Cladosporium rot on the composition and aromatic compounds of red wine

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Abstract

High incidences of Cladosporium rot frequently occur (over 50% infected berries per clusters) in delayed harvests in Cabernet Sauvignon and other red cultivars of *Vitis vinifera* in Chile. The objective of this study was to determine the effect of Cladosporium rot (*Cladosporium cladosporioides* and *C. herbarum*) on composition and aroma of red wine. Cabernet Sauvignon and Carménère wines made with 50% of Cladosporium rot infected grapes (CRG) were characterized and compared with wines made identically with apparently healthy grapes (AHG). Wine composition, color, and aroma were determined and the wines were subjected to sensory evaluation. Differences in volatile acidity, residual sugar, anthocyanins, color hue, and tannins between CRG wines and AHG wines of both Cabernet Sauvignon and Carménère were significant ($p<0.05$). In Cabernet Sauvignon, differences in the aromatic profiles were obtained between CRG wines and AHG wines. Panelists negatively differentiated the aroma and astringency of the CRG wines. Cladosporium rot reduced color, aroma, and flavor in Cabernet Sauvignon and Carménère wines. To our knowledge, this is the first report demonstrating the effect of Cladosporium rot, a common late fall disease of grapevine in Chile, on red wines, and the results suggest the need to prevent it for obtaining high quality wines.

Additional key words: aromas, *Cladosporium* spp., color, delayed harvest, fungal disease.

Resumen

Efecto de la cladosporiosis en la composición y compuestos aromáticos de vinos tintos

En Chile, en cosechas retrasadas de Cabernet Sauvignon y otros cultivares tintos de *Vitis vinifera* ocurre con frecuencia una alta incidencia de cladosporiosis (> 50% de las bayas afectadas por racimo). El objetivo de este estudio fue determinar el efecto de la cladosporiosis (*Cladosporium cladosporioides* y *C. herbarum*) sobre la composición y aroma del vino tinto. Se elaboraron vinos Cabernet Sauvignon y Carménère con 50% de bayas infectadas con cladosporiosis (CRG), se caracterizaron y se compararon con vinos preparados de forma idéntica pero con bayas aparentemente sanas (AHG). Se determinó la composición, color y aroma de los vinos y se evaluaron sensorialmente. Se encontraron diferencias significativas ($p<0,05$), entre vinos CRG y AHG, en acidez volátil, azúcares residuales, antocianinas, matiz de color y taninos, tanto en Cabernet Sauvignon como en Carménère. En vinos Cabernet Sauvignon se encontraron diferencias en el perfil aromático entre vinos CRG y AHG. Los panelistas diferenciaron negativamente el aroma y astringencia de los vinos CRG. La cladosporiosis redujo el color, aroma y sabor en vinos Cabernet Sauvignon y Carménère. Según la bibliografía consultada, éste es el primer reporte que demuestra el efecto de la cladosporiosis, una patología común en los viñedos chilenos de uvas tintas a fines de otoño y que indica la necesidad de prevenirla para obtener vinos de alta calidad.

Palabras clave adicionales: aromas, *Cladosporium* spp., color, cosecha retrasada, enfermedad fungosa.

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Abbreviations used: AHG (apparently healthy grapes), CRG (Cladosporium rotten grapes), MLF (malolactic fermentation), OAV (Odor activity value), PVC (polyvinyl chloride), TA (titratable acidity), TSS (total soluble solid content).

Introduction

Cladosporium rot occurs frequently in wine grapes (*Vitis vinifera* L.) in the Central Valley of Chile. It is particularly severe (over 50% infected berries per clusters) when growers delay harvest considerably in order to optimize phenolic composition to assure the best possible wine quality (Pszczólkowski *et al*., 2001; Briceño and Latorre, 2007). Therefore, it mainly occurs in overripe berries of red cultivars, e.g. Cabernet Sauvignon, Carménère, and it is characterized by an olive green mold that invades the grape skin superficially (Briceño and Latorre, 2007, 2008). In Chile, it has been associated to *Cladosporium herbarum* (Pers.:Fr.) Link and *C. cladosporioides* (Fresen.) De Vries (Mujica and Vergara, 1980).

The quality of the wines is highly dependent on the grape composition at harvest (Ribéreau-Gayon and Peynaud, 1960). Therefore, the ripeness of the pulp, optimal ratio between the total soluble solid content (TSS) and the titratable acidity (TA) (Barceló, 1997), is recognized as a critical factor for quality wines. Consequently, grapes for winemaking have been harvested in accordance with these parameters (TSS > 22.5% and TA=6-7 g L^{-1} of tartaric acid) in Chile (Pszczólkowski *et al*., 2001). However, this harvest criterion does not necessarily coincide with "phenolic ripeness" of seeds and skins, needed for obtaining quality red wines (Saint-Cricq *et al.*, 1998). Therefore, growers postpone harvest waiting for a softening of the tannins from the seeds and skins. This has resulted in an excessive delay in harvest dates, with a negative impact on the quality and phytosanitary condition of the grapes. A slow or incomplete fermentation frequently occurs in musts made with late harvest grapes, and it has been postulated that a considerably loss of color, flavor, aroma, and other sensorial attributes occur associated to Cladosporium rot (Pszczólkowski *et al*., 2001). Therefore, the objective of this study was to determine the effect of Cladosporium rot on the physicochemical and volatile composition of these red wines.

Material and methods

Winemaking. Three and four grape lots, each of 15 kg per cultivar, were harvested in May when Cladosporium rot incidence was above 50% (CRG) in a commercial Cabernet Sauvignon and Carménère vineyards in 2006 and 2007, respectively. An equal number of lots of apparently healthy grapes (AHG) were harvested as controls in the same vineyards. Each grape lot was made into wine in separate 20 L polyvinyl chloride (PVC) containers. The Cladosporium rotted grapes used in this study exhibited an olive green superficial mold caused by *C. herbarum* and *C. cladosporioides*.

Cabernet Sauvignon and Carménère bunches were destemmed, manually crushed, and fermented in the presence of skins and seeds. The nitrogen content of the musts was corrected with 300 mg L^{-1} ammonium phosphate and then treated with 20 mg L^{-1} pectolytic enzymes (Rapidase® ex color, Gist-brocades, Beverages Ingredients Group, France) and 30 mg L^{-1} sulphur dioxide (SO_2). The musts were homogenized, and the temperature and density were determined before the addition of 200 mg L^{-1} of *Saccharomyces cerevisiae* (Anchor WE 372, Anchor Bio-technologies, Eppindust, South Africa), which was previously hydrated at 37ºC for 30 min.

The containers were sealed with water valves to maintain semi-anaerobic conditions, and fermentation took place in an isothermal fermentation chamber at 28-30ºC. Skins were pushed down daily and the temperature and density of each wine were determined. At the end of fermentation, the free run wine was transferred into 5 L glass bottles that were kept in the isothermal chamber (20-24ºC) until the end of malolactic fermentation (MLF), which was determined by paper chromatography (Ribéreau-Gayon and Peynaud, 1962). The wines obtained were decanted into clean containers and treated with 35 mg L^{-1} SO_2. The wines were then stored at 0ºC for 30 days and then the free SO_2 was corrected to 35 mg L^{-1} (Buechsenstein and Ough, 1978).

Basic analysis of must and wine. The TSS content, pH, and TA of musts were determined. Analytical determinations of alcohol, TA, pH, volatile acidity and reducing sugars were assessed in the wines (Bordeu and Scarpa, 1998).

Color and phenolic composition of wine. The content of anthocyanins, total polyphenols (A_{280}), color hue (A_{420}/A_{520}), and color intensity ($A_{420+520+620}$) were determined spectrophotometrically (Spectronic Genesys 2, Milton Roy, USA) (Bordeu and Scarpa, 1998). The tannin content was determined by acid butanolysis (Vivas *et al*., 2004).

Analysis of the volatile fraction. The volatile fraction of Cabernet Sauvignon wines was determined and quantified using a gas chromatograph (Hewlett-

Packard 6890 Series, USA) coupled with a Hewlett-Packard 5972 mass detector (GC-MS). Samples (100 mL) of bottled wines were subjected to a double liquid-liquid extraction with 25 mL of dichloromethane. They were mixed for 30 min at 0°C in a nitrogen atmosphere and centrifuged between each extraction (Sorvall®RC 285, Dupont, USA) at 5000 rpm for 20 min to separate the organic phase, which was dried over anhydrous Na_2SO_4. Prior to GC-MS analysis, the extracts obtained were concentrated to 350 µL in a Dufton column in a thermo-regulated bath (Julabo Labortechnik, GMBH, Germany) at 45ºC. As an internal standard, 4-nonanol (100 µL of a 3.5 mg L^{-1} solution) was used as a response factor equal to 1. A DB-WAX ETR column (J&W Scientific, Folson, CA, USA) of 60 m x 0.25 mm x 0.25 µm film thickness was used. The temperature of the injector was 180°C and the GC oven was initially regulated at 40°C for 5 min, increasing 3°C per minute and then 25 min at 240°C. Samples (2 µL) were injected to the chromatograph in splitless mode with an helium flux of 1.9 mL min^{-1}. The compounds obtained in each chromatogram were compared with the information from the NIST-EPA-NIH mass spectral database (Gaithersburg, MD, USA), which is composed of 130,000 spectra. Each wine sample was processed in triplicate.

Odor activity values (OAVs) were calculated by dividing the mean concentration of each aromatic compound by its odor threshold value, and it was used to estimate the sensory contribution of the aromatic compounds to the overall wine flavor. The OAVs in Cabernet Sauvignon wines were calculated on the basis of odor threshold values reported previously (Guth, 1997; Ferreira *et al*., 2000, 2002; López *et al*., 2002; Culleré *et al*., 2004; Peinado *et al*., 2004; Selli *et al*., 2004; Gómez-Míguez *et al*., 2007).

Sensory evaluation. The sensory evaluation was performed in one session in individual booths. Each replicate was presented as pairs of wines with both treatments in random order for each of the cultivars. The samples were served at room temperature (18-20ºC) in glasses identified with a random 3-digit number and covered with a polyethylene cover. The sensorial tasting panel was composed of 11 panelists (oenologists) with no previous training, 4 women and 7 men, between 25 and 60 year old, and a set of parameters describing appearance, aroma and mouth-feel was evaluated on a graphical scale of 10 cm length, where 0 and 10 represented low and high perception of each parameter respectively.

Statistical design and analysis. The effect of vintage year and Cladosporium rot on the musts and wines, phenols and the volatile fraction of the wine were studied by two-way analysis of variance (ANOVA) with four replicates, considering vintage year as main factor and health grape status (with and without visible Cladosporium rot symptoms) as sub-factor. The SigmaStat® 3.1 software (Systat Software Inc., San José, CA, USA) was used.

The results of the sensory evaluation were analyzed separately for each of the parameters using a two way analysis where the type of wine (made with or without Cladosporium-rotted grapes) was the main factor and panelists the sub-factor. Data were analyzed with the aid of SigmaStat® 3.1.

Results

Basic composition of must and wine. Cladosporium rotted berries were significantly ($p=0.05$) lighter than apparently healthy berries with a mean weight reduction of 14.3% in Cabernet Sauvignon and 12.5 to 25% in Carménère. Significant differences in TA, and pH were obtained among must prepared with diseased berries and apparently healthy berries, but differences in TSS content were only significant in Carménère. In Carménère must, the interaction between vintage year and berry weight, TSS and pH were significant ($p<0.041$), while in Cabernet Sauvignon must, only the interaction between vintage year and TA was significant ($p<0.026$), meaning that differences in the above mentioned parameters were significant only one season (Table 1).

In both cultivars, Cabernet Sauvignon and Carménère, the volatile acidity and reducing sugars were significantly higher in CRG wines than AHG wines. Moreover, ethanol content was significantly different in Carménère wines but not in Cabernet Sauvignon. The significant interaction in residual sugars again reflects differences only one season. Wine acidity and pH results were not clear.

Color and phenolic composition of wine. Color hue and total tannins were always higher in CRG wines than in AHG wines. Total phenols were significantly higher in CRG wines than AHG wines prepared with grapes cv. Carménère. Except for Carménère in 2007, the CRG wines were characterized by significantly lower anthocyanin content than AHG wines (Table 2).

Analysis of the volatile fraction. A total of 93 and 130 volatile compounds including acids, alcohols, alde-

Table 1. Effect of the vintage year and Cladosporium rot on berry weight and must composition of *Vitis vinifera* cvs. Cabernet Sauvignon and Carménère

Parameters	Vintage years [1]				*P* values [2]		
	2006		2007		Year (Y)	Cladosporium rot (C)	Y × C interaction
	CRG [3]	AHG [4]	CRG	AHG			
Cabernet Sauvignon							
Berry weight (g) [5]	nd [6]	nd	1.2	1.4	nd	0.011	nd
Total soluble solids (%)	24.9	23.9	24.1	23.4	< 0.001	0.685	0.026
pH	3.5	3.4	3.9	3.7	< 0.001	0.005	0.980
Titratable acidity (g L^{-1}) [7]	4.1	4.9	4.1	4.7	< 0.001	0.003	0.084
Carménère							
Berry weight (g) [5]	1.5	2.0	1.4	1.6	< 0.001	< 0.001	< 0.001
Total soluble solids (%)	26.3	25.7	26.1	24.7	0.007	< 0.001	0.041
pH	4.2	4.0	4.1	3.9	< 0.001	< 0.001	0.020
Titratable acidity (g L^{-1}) [7]	3.7	4.2	3.6	4.1	0.166	< 0.001	0.978

[1] Each value is the mean of three and four infield replicates in 2006 and 2007, respectively. [2] A two-way analysis of variance probability values. [3] CRG = Cladosporium rotted grapes with 50% incidence caused mainly by *C. cladosporioides* and *C. herbarum*. [4] AHG = apparently healthy grapes. [5] Mean of 50 berries; [6] nd, not determined; [7] Expressed as tartaric acid

hydes and ketones, esters, furans, lactones, norisoprenoids, phenols, sulphur derivatives, and vanillin derivatives were identified in wine samples in 2006 and 2007, respectively (Table 3).

Cladosporium rot significant reduced the concentration of decanoic acid in CRG wines in both vintage years. Among alcohols, isoamyl alcohol and 2-phenylethanol had the highest concentrations but the effect of Cladosporium was not significant. Other alcohols were significantly ($p<0.001$) altered in CRG (Table 3). Among ten ester compounds, eight were significantly altered in both vintage years. For instance, isoamyl acetate, ethyl 3-hidroxybutirate and phenyl acetate were significantly higher in AHG wines than CRG wines. Higher concentration of furan compounds were found in CRG wines than AHG wines, but significant effect of Cladoporium rot was only determined for furfural in 2006. Norisoprenoid compounds were only detected in 2007 and their concentration significantly increases in CRG wines. Phenol compounds were always higher in CRG wines than AHG wines but, only phenol, and 3,4,5-trimethoxy-phenol were consistently altered in both vintage years. Benzaldehyde, ethyl vanillate and γ-butyrolactone were significantly higher in CRG wines than AHG wines in both vintages years (Table 3).

OAVs were calculated for 49 of the 167 compounds, but only seven compounds had OAVs higher than one and significant differences between AHG wines and CRG wines in both vintage years (Table 4). With the exception of isoamyl acetate and 2,3-butanedione, the effect of CRG on the other compounds was dependant significantly ($p<0.009$) on the vintage year. The interaction between vintage year and Cladosporium rot was only significant ($p=0.048$) in relation to γ-butyrolactone (Table 4).

Sensory evaluation. In Cabernet Sauvignon wine, all parameters were significantly affected by Cladosporium rot ($p<0.045$), except for acidity (Table 5). In Carménère wine, the effect of Cladosporium rot was significant only in relation to aroma and tannins. In both cases a significant interaction with panellist was found in several parameters, showing some discrepancy in the panel.

In Cabernet Sauvignon, color, aroma and acceptability were found to be considerably lower in CRG wines than AHG wines, while sweetness, alcohol, acidity and astringency were higher in CRG wines than AHG wines. In Carménère aroma was lower in CRG than AHG wines and astringency was higher in CRG wines than AHG wines (data not shown).

Discussion

On the basis of the results obtained in this study, the delayed harvest of Cabernet Sauvignon and Carménère,

Table 2. Effect of vintage year and Cladosporium rot on the composition of Cabernet Sauvignon and Carménère wines in 2006 and 2007 vintages

Parameters	Vintage years[1]				*P* values[2]		
	2006		2007		Year (Y)	Cladosporium rot (C)	Y × C interaction
	CRG[3]	AHG[4]	CRG	AHG			
Cabernet Sauvignon							
Ethanol (%, v/v)	15.3	14.1	13.0	13.0	< 0.001	0.083	0.107
Titratable acidity (g L^{-1})[5]	6.3	6.2	4.2	4.8	< 0.001	0.010	0.002
pH	3.75	3.67	4.34	4.18	< 0.001	0.001	0.156
Volatile acidity (g L^{-1})[6]	0.78	0.72	0.92	0.80	< 0.001	0.002	0.241
Reducing sugars (g L^{-1})	3.27	2.34	1.75	1.49	< 0.001	0.002	0.011
Total phenols (A_{280})	67	61	51	54	< 0.001	0.339	0.020
Total tannins (g L^{-1})	5.0	4.4	3.4	3.0	< 0.001	0.020	0.582
Anthocyanins (mg L^{-1})	255	402	305	380	0.318	< 0.001	0.024
Color intensity ($A_{420+520+620}$)	11.4	9.1	9.0	10.8	0.570	0.647	0.003
Color hue (A_{420} /A_{520})	0.72	0.59	0.91	0.84	< 0.001	< 0.001	0.047
Carménère							
Ethanol (%, v/v)	16.0	14.9	14.6	13.7	< 0.001	0.001	0.511
Titratable acidity (g L^{-1})[5]	4.6	4.2	3.9	3.9	< 0.001	0.063	0.036
pH	4.33	4.42	4.39	4.33	0.621	0.641	0.025
Volatile acidity (g L^{-1})[6]	0.83	0.67	0.80	0.71	0.844	0.008	0.368
Reducing sugars (g L^{-1})	3.72	2.30	1.99	1.51	< 0.001	< 0.001	0.005
Total phenols (A_{280})	78	69	77	69	0.762	0.001	0.880
Total tannins (g L^{-1})	3.4	1.7	2.8	1.5	0.097	< 0.001	0.375
Anthocyanins (mg L^{-1})	672	903	843	781	0.379	0.010	<0.001
Color intensity ($A_{420+520+620}$)	21.7	20.6	21.4	19.1	0.263	0.054	0.440
Color hue (A_{420} /A_{520})	0.79	0.70	0.82	0.79	< 0.001	< 0.001	0.003

[1] Each value is the mean of three and four infield replicate in 2006 and 2007 respectively. [2] A two-way analysis of variance probability values. [3] CRG = Cladosporium rotted grapes with 50% incidence caused mainly by *C. cladosporioides and C. herbarum*. [4] AHG = apparently healthy grapes. [5] Expressed as tartaric acid. [6] Expressed as acetic acid.

which is a common practice for obtaining the phenolic maturity of red wine grapes in Chile resulting in a severe development of Cladosporium rot, considerably affected the physicochemical profile of Cabernet Sauvignon and Carménère wines. In agreement with previous work both *C. cladosporioides* and *C. herbarum* were identified on disease samples (Pszczólkowski *et al*., 2001; Briceño and Latorre, 2007).

Confirming previous results (Pszczólkowski *et al*., 2001; Briceño and Latorre, 2007), the delayed harvest was associated with berry dehydration, 12.5 to 25.0% berry weight losses. The relatively high TSS contents (> 23.4%) increased the risk of delayed must fermentation. In this study, fermentation was delayed between 24 h in Carménère in 2007 and 96 h in Cabernet Sauvignon in 2006 in relation to AHG. These differences in the fermentation period can result from differences in TSS contents but it is possible that the high proportion of Cladosporium rotted berries could affect yeast performance. However, further research is needed to verify this subject. Independently of the grape cultivar, wines produced with 50% Cladosporium rotted grapes (CRG) consistently had higher residual sugars (9.2 to 61.7%) than AHG wines in both vintage years, reflecting incomplete fermentations.

Important changes in phenolic composition occur during grape ripening that improve red wine quality (Kennedy *et al*., 2000, 2006; Harberston *et al*., 2002; Adams, 2006; Kennedy, 2008). On the basis of this knowledge, Chilean growers considerably delay harvest of Cabernet Sauvignon and other red cultivars until late in autumn (April-May) waiting for the best possible phenolic composition of berries. However, this delay often implies that Cladosporium infected

Table 3. Effect of vintage year and Cladosporium rot in aroma profile obtained by gas chromatography- mass spectrometry in Cabernet Sauvignon wines in 2006 and 2007 vintages

Compounds[1]	Concentration in wines (μg L^{-1})				*P* values of variables[2]		
	2006		2007		Year (Y)	Cladosporium rot (C)	Y × C interaction
	CRG[3]	AHG[4]	CRG[3]	AHG[4]			
Acids							
Hexanoic acid	1263	1608	1120	1134	0.013	0.138	0.110
Octanoic acid	1054	1632	721	1058	< 0.001	0.250	< 0.001
Decanoic acid	333	487	242	371	0.114	0.040	0.839
Alcohols							
Isoamyl alcohol	87201	89956	72327	83257	0.004	0.184	0.038
3-Methyl 1-pentanol	84	145	80.1	121.7	0.139	< 0.001	0.278
1,3-Butanediol	1090	504	966	545	0.701	< 0.001	0.449
Benzyl alcohol	686	459	1810	1508	< 0.001	< 0.001	0.497
2-Phenylethanol	37208	38480	38246	44128	0.009	0.052	0.006
Esters							
Ethyl propionate	117	116	80.7	70.8	< 0.001	0.092	0.172
Propyl acetate	34	37	30	22	< 0.001	0.318	0.021
Isobutylacetate	19.6	31.6	26.5	23.8	0.774	0.028	0.002
Isoamyl acetate	331	515	435	541	0.125	0.004	0.340
Ethyl 3-hydroxybutirate	282	285	253	313	0.962	0.008	0.015
Ethyl acetate	229	614	425	626	0.054	< 0.001	0.084
Diethyl di malate	486	161	223	0	< 0.001	< 0.001	0.219
Phenyl ethyl lactate	nd	nd	875	1097	nd	0.010	nd
Mono methyl succinate	nd	nd	83.3	381	nd	0.026	nd
Monoethyl succinate	nd	nd	41059	48831	nd	0.028	nd
Furans							
Furfural	17.3	6.2	nd	nd	nd	0.025	nd
Furfuryl alcohol	32.3	20.1	12.0	0	0.011	0.090	0.988
Furan 2,5-dimethyl	9.9	0	nd	nd	nd	0.111	nd
Norisoprenoides							
3-Hydroxy-β-damascone	nd	nd	331	198	nd	0.050	nd
3-Hydroxy-5,6-epoxy-β-ionone	nd	nd	111	24.9	nd	0.023	nd
Phenols							
p-Cresol	nd	nd	13.3	0	nd	0.033	nd
Phenol	24.5	9.3	15.6	0	< 0.001	< 0.001	0.904
3,4-Dimethoxy phenol	33.8	9.2	71	65.5	< 0.001	0.113	0.295
3,5-Dimethoxy phenol	nd	nd	70.2	0	nd	0.138	nd
3,4,5 –Trimethoxy phenol	55.0	0	176.1	97.4	< 0.001	< 0.001	0.334
Methyl p-hydroxy benzoate	34.3	6.3	nd	nd	nd	< 0.001	nd
Ethyl p-hydroxy benzoate	103.6	11.3	nd	nd	nd	0.003	nd
Others							
γ-Butyrolactone	4973	5262	5992	7749	< 0.001	0.011	0.048
Benzaldehyde	24.3	6.8	130	0	0.047	0.007	0.028
Ethyl vanillate	767	193	691	491	0.112	< 0.001	0.015

[1] Representative compounds with significant differences between wines made with and without Cladosporium rotted grapes. [2] A two way analysis of variance probability values are showed. [3] CRG = Cladosporium rotted grapes with 50% incidence caused mainly by *C. cladosporioides* and *C. herbarum*; [4] AHG = apparently healthy grapes. Each value is the mean of three and four infield replicate in 2006 and 2007 respectively; nd = not detected.

Table 4. Aroma descriptor, odour threshold and effect of vintage year and Cladosporium rot on odour activity values (OAV) of compounds in Cabernet Sauvignon wines in 2006 and 2007 vintages. Mean of four infield replicates

Compound	Descriptor [1]	Odour threshold ($\mu g\ L^{-1}$) [1]	OAV [2]				*P* values of variables [3]		
			Year 2006		Year 2007		Year (Y)	Cladosporium rot (C)	Y × C interaction
			CRG [4]	AHG [5]	CRG [4]	AHG [5]			
Ethyl hexanoate	Anise, fruity, floral [c,d]	14 [b]	22.40	25.50	16.34	19.23	< 0.001	0.044	0.941
Isoamyl acetate	Banana, fruity [c,d]	30 [a]	11.03	17.17	14.48	18.04	0.125	0.004	0.340
Isoamyl alcohol	Red fruit, framboise [e], cheese [1a]	30000 [a]	2.91	3.00	2.41	2.78	0.004	0.038	0.181
2-Phenylethanol	Floral, rose, lavander [c,e]	14000 [b]	2.66	2.75	2.73	3.15	0.009	0.006	0.052
2,3-Butanedione	Strawberry, buttery, sweet [c,f]	100 [d]	4.13	7.73	3.58	4.58	0.065	0.028	0.177
γ-Butyrolactone	Floral, caramel, sweet [d]	400 [f]	12.43	13.15	16.38	19.37	< 0.001	0.011	0.048
Octanoic acid	Cheese, fatty, rancid [c,d]	500 [b]	2.10	3.27	1.44	2.12	< 0.001	< 0.001	0.247

[1] Obtained from [a] Guth (1997) (10% water/ethanol solution), [b] Ferreira *et al.* (2000) (11% ethanol/water, 7 g L-1 glycerin, 5 g L-1 tartaric acid at pH 3.4), [c] Ferreira *et al.* (2002) (10% hydro-alcoholic solution at pH 3.2), [d] Peinado *et al.* (2004), [e] Selli *et al.* (2004), [f] Gómez-Míguez *et al.* (2007). [2] OAV = ratio of the compound concentration and odour threshold value. [3] OAVs higher than 1 and significant according to a two way analysis of variance for Cladosporium rot were included. [4] CRG = Cladosporium rotted grapes with 50% incidence caused mainly by *C. cladosporioides* and *C. herbarum.* [5] AHG = apparently healthy grapes.

grape lots are used for winemaking. Total phenols were significantly higher in CRG wines only in Carménère and expected differences between seasons were found in Cabernet Sauvignon (Kennedy, 2008).

Table 5. *P* values showed the effect of panellists and Cladosporium rot on sensory evaluation of wines Cabernet Sauvignon and Carménère.

Parameters	Cabernet Sauvignon			Carménère		
	Panellist (P)	Cladosporium rot (C)	Interaction P × C	Panellist (P)	Cladosporium rot (C)	Interaction P × C
Color	< 0.001	< 0.001	0.002	< 0.001	0.459	0.145
Aroma	0.006	< 0.001	0.002	0.230	0.034	0.015
Sweetness	< 0.001	0.045	0.108	< 0.001	0.951	0.033
Alcohol	< 0.001	0.005	< 0.001	0.205	0.674	0.601
Acidity	0.022	0.170	0.043	0.011	0.884	0.395
Tannins	0.033	< 0.001	< 0.001	0.015	0.040	0.306
Acceptability	< 0.001	< 0.001	< 0.001	0.043	0.363	0.610

Data analysed by two-way analysis of variance.

Berry dehydration and low pulp-seed ratio were possible responsible for the higher tannins and phenol contents found in wines elaborated with grape lots having 50% Cladosporium rotted berries and it may explain the high astringency detected by panelists in the sensory analysis. In spite of this lower pulp-skin ratio, the anthocyanin contents in CRG wines were lower than in AHG wines, confirming previous results on Cabernet Sauvignon elaborated with heavily mold infested grapes (Pszczólkowski *et al*., 2001). This relatively low anthocyanin contents and the high hue values found in the CRG wines, corresponding to an evolution towards a red brick color was also detected by panelists in Cabernet Sauvignon wines.

It is probable that this color change results from the physical and chemical skin damages caused by *Cladosporium* spp. that colonized the skin surface of the grapes. However, additional research is needed to probe this hypothesis. Nevertheless, similar results have been reported for wines made grape lots infected with powdery mildew (*Erysiphe necator*), where the loss of red color and low anthocyanin content was mainly due to a low concentration of delphinidin, cyanidin, petunidin, peonidin, and malvidin (Amati *et al.*, 1996; Piermattei *et al*., 1999).

In both Cabernet Sauvignon and Carménère, the sensory panel detected a considerable loss of aromas in CRG wines. In Cabernet Sauvignon wine, it is possible that relatively low contents of compounds such as 2-phenyl ethanol, isoamylacetate, and ethyl hexanonoate, found in the aroma profile, reduced the floral and/or fruity aromas. Additionally, the CRG wines had a larger number and higher concentration of aromatic phenolic compounds than AHG wines. Some of these phenolic compounds contributed aromas like smoke, phenol, and medicine (e.g. guaiacol, *p*-cresol) that could be considered negative because they may mask favorable aromas in Cabernet Sauvignon wines (Ferreira *et al*., 2000, 2002; López *et al*., 2002).

The concentration of esters like monoethyl succinate, isoamyl acetate and ethyl 3-hydroxybutyrate were in lower concentrations in CRG wines than AHG wines which may affect the fruity aroma. In both vintage seasons, γ-butyrolactone was significantly lower in CRG wines AHG wines. This could be an undesirable factor considering that γ-butyrolactone confers pleasant aroma with floral and sweet attributes (Selli *et al*., 2004). Even if compounds with pleasant aromas like ethyl vanillate, benzaldehyde and furans were relatively high in CRG, these compounds had OAV<1, therefore, they are not important in their olfactory properties.

With the exception of octanoic acid, most compounds with important olfactory properties (OAV>1) and significant differences between CRG wines and AHG wines have positive sensory properties. Therefore, the lost of fruity aroma associated to Cladosporium rotted grapes in Cabernet Sauvignon wines could be primarily due to a loss of esters (e.g. isoamyl acetate), lactones (e.g. γ-butyrolactone), ketones (e.g. 2,3-butanedione), and alcohols (e.g. 2-phenylethanol). Previous studies have demonstrated the loss of varietal aromas in wines elaborated with grapes infected with powdery mildew, however, the compounds affected were not determined (Calonnec *et al*., 2004).

Finally, it was interesting that some volatile phenol compounds (i.e. 3,5 dimethoxy phenol, *p*-cresol) and furan compounds (2,5 dimethyl furan) were found only in wines made with 50% Cladosporium rotted grapes. It is unknown whether these are unique compounds associated to *C. cladoporioides* and/or *C. herbarum*. Additional investigations are needed in order to confirm this and to determine the effect of these compounds on the sensory qualities of red wines.

On the basis of the results obtained, the use of grape lots having 50% Cladosporium rotted grapes, caused by *C. cladosporioides* and *C. herbarum*, negatively affected Cabernet Sauvignon and Carménère wines, reducing color, aroma, and flavor significantly. An important reduction in fruity and floral aromas was obtained in Cabernet Sauvignon wines. Additionally, Cladosporium rot considerably reduced berry weight and so yields of Cabernet Sauvignon and Carménère grapevines.

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References

ADAMS D.O., 2006. Phenolics and ripening in grape berries. Am J Enol Vitic 57, 249-256.

AMATI A., PIVA A., CASTELLARI M., ARFELLI G., 1996. Preliminary studies on the effects of *Oidium tuckeri* on the

phenolic composition of grapes and wines. Vitis 35, 149-150.

BARCELO J.M., 1997. La gestion de la maturation: le premier acte œnologique. Incidence sur les profile de vins de Syrah dans les Côtes du Rhône. Rev Fr Oenol 165, 24-26. [In French].

BORDEU E., SCARPA J., 1998. Análisis químico del vino. Ediciones Universidad Católica de Chile, Santiago, Chile. 253 pp. [In Spanish].

BRICEÑO E.X., LATORRE B.A., 2007. Outbreaks of Cladosporium rot associated with delayed harvest wine grapes in Chile. Plant Dis 91, 1060-1060. doi:10.1094/PDIS-91-8-1060C.

BRICEÑO E.X., LATORRE B.A., 2008. Characterization of Cladosporium rot in grapevines, a problem of growing importance in Chile. Plant Dis 92, 1635-1642. doi:10.1094/PDIS-92-12-1635.

BUECHSENSTEIN J.W., OUGH C.S., 1978. SO_2 determination by aeration-oxidation: a comparison with Ripper. Am J Enol Vitic 29, 161-164.

CALONNEC A., CARTOLARO P., POUPOT C., DUBOURDIEU D., DARRIET P., 2004. Effects of *Uncinula necator* on the yield and quality of grapes (*Vitis vinifera*) and wine. Plant Pathol 53, 434-445. doi:10.1111/j.0032-0862.2004.01016.x.

CULLERÉ L., ESCUDERO A., CACHO J., FERREIRA V., 2004. Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. J Agric Food Chem 52, 1653-1660. doi:10.1021/jf0350820.

FERREIRA V., LÓPEZ R., CACHO J., 2000. Quantitative determination of the odorants of young red wines from different grape varieties. J Sci Food Agric 80, 1659-1667. doi:10.1002/1097-0010(20000901)80:11<1659::AID-JSFA693>3.0.CO;2-6.

FERREIRA V., ORTÍN N., ESCUDERO A., LÓPEZ R., CACHO J., 2002. Chemical characterization of the aroma of Grenache Rosé wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. J Agric Food Chem 50, 4048-4054. doi:10.1021/jf0115645.

GÓMEZ-MÍGUEZ M.J., CACHO J.F., FERREIRA V., VICARIO I.M., HEREDIA F.J., 2007. Volatile components of Zalema White wines. Food Chem 100, 1464-1473. doi:10.1016/j.foodchem.2005.11.045.

GUTH H., 1997. Quantitation and sensory studies of character impact odorants of different white wine varieties. J Agric Food Chem 45, 3027-3032. doi:10.1021/jf970280a.

HARBERSTON J.F., KENNEDY J.A., ADAMS D.O., 2002. Tannin in skin and seeds of Cabernet Sauvignon, Syrah and Pinot noir berries during ripening. Am J Enol Vitic 53, 54-59.

KENNEDY J.A., 2008. Grape and wine phenolics: Observations and recent findings. Cien Inv Agr 35, 107-120.

KENNEDY J.A., MATTHEWS M.A., WATERHOUSE A.L., 2000. Changes in grape seed polyphenols during fruit ripening. Phytochemistry 55, 77-85. doi:10.1016/S0031-9422(00)00196-5.

KENNEDY J.A., SAUCIER C., GLORIES Y., 2006. Grape and wine phenolics: History and perspective. Am J Enol Vitic 57, 239-248.

LÓPEZ R., AZNAR M., CACHO J., FERREIRA V., 2002. Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. J Chromatogr A 966, 167-177. doi:10.1016/S0021-9673(02)00696-9.

MUJICA F., VERGARA C., 1980. Flora fungosa de Chile. 2nd ed. Ciencias Agrícolas Nº 5. Facultad de Agronomía, Universidad de Chile. Editorial Universitaria, Santiago, Chile. 308 pp. [In Spanish].

PEINADO R.A., MORENO J., BUENO J.E., MORENO J.A., MAURICIO J.C., 2004. Comparative study of aromatic compounds in two Young White wines subjected to pre-fermentative cryomaceration. Food Chem 84, 585-90. doi:10.1016/S0308-8146(03)00282-6.

PIERMATTEI B., PIVA A., CASTELLARI M., ARFELLI G., AMATI A., 1999. The phenolic composition of red grapes and wines as influenced by *Oidium tuckeri* development. Vitis 38, 85-86.

PSZCZÓLKOWSKI P., LATORRE B.A., CEPPI DI LECCO C., 2001. Efecto de los mohos presentes en uvas cosechadas tardíamente sobre la calidad de los mostos y vinos Cabernet Sauvignon. Cien Inv Agr 28, 157-163. [In Spanish].

RIBÉREAU-GAYON J., PEYNAUD E., 1960. Traité d'Œnologie, Tome 1. Maturation du raisin, Fermentation alcoolique, Vinification. Librairie Polytechnique Ch. Béranger, Paris, France. 753 pp. [In French].

RIBÉREAU-GAYON J., PEYNAUD E., 1962. Análisis de vinos. Ediciones Aguilar S.A., Madrid, Spain. [In Spanish].

SAINT-CRICQ N., VIVAS N., GLORIES Y., 1998. Maturité phénolique: définition et contrôle. Rev Fr Oenol 98, 22-25. [In French].

SELLI S., CABAROGLU T., CANCAS A., ERTEN H., NURGEL C., LEPOUTRE J.P., GUNATA Z., 2004. Volatile composition of red wine from cv. Kalecik Karast grown in central Anatolia. Food Chem 85, 207-213. doi:10.1016/j.foodchem.2003.06.008.

VIVAS N., NONIER M-F., VIVAS DE GAULEJAC N., ABSALON C., BERTRAND A., MIRABEL M., 2004. Differenti-

ation of proanthocyanidin tannins from seeds, skins and stems of grapes (*Vitis vinifera*) and heartwood of Quebracho (*Schinopsis balansae*) by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and thioacidolysis/liquid chromatography/electrospray ionization mass spectrometry. Analytica Chimica Acta 513, 247-256.