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Effects of copper fungicide spraying on volatile thiols of the varietal aroma of Sauvignon blanc, Cabernet Sauvignon and Merlot wines

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Summary

In a three-year experiment, the effect of pre-veraison copper sprayings of vines on must composition and some volatile thiols contributing to the varietal aroma of Sauvignon blanc, Cabernet Sauvignon and Merlot wines was studied in comparison with folpet sprayings in the Bordeaux winegrowing region. The reactivity of copper residues with thiols, mainly during the alcoholic fermentation, had a dramatic effect on the concentration of 4-mercapto-4-methylpentan-2-one and 3-mercaptohexanol in wines. However, copper sprayings, preferentially on the foliage, did not significantly increase copper residues in must; thiol concentrations in wines were very close to those found in wines obtained from vines sprayed with folpet. Therefore, this mode of spraying can be used to avoid the effects of copper treatment at veraison on the volatile thiols of the wines varietal aroma.

K e y w o r d s : fungicide, copper, folpet, thiol, Sauvignon blanc, Cabernet Sauvignon, Merlot, grape maturation.

Introduction

Since the second half of the 19th century in most vineyards in the world, it is necessary to control parasitic fungi by application of fungicides. For many decades, sulphur and copper were the only agents effective for downy mildew (*Plasmopara viticola*) and powdery mildew (*Uncinula necator*) control. Starting in the 1950s, the range of traditional fungicides widened to include products of organic synthesis; at present many of these agents are available to protect vineyards. Observation of undesired oenological effects caused by these fungicides has stimulated research on fungicides in must and wine (CABRAS and ANGIONI 1987; CABRAS *et al.* 2000; LEMPERLE 1988; NAVARRO *et al.* 1999), on the way these residues affect alcoholic fermentation (SAPIS-DOMERCQ *et al.* 1976, 1978; MINARIK 1979; HATZIDIMITRIOU *et al.* 1997) and on organoleptic defects of wines (RAUHUT 1989; MAUJEAN *et al.* 1993). All these factors are now taken into consideration when these substances undergo certification. Some research teams have looked at the possible consequences of applying certain phytosanitary products on the composition of must and wine (DOIGNON *et al.* 1992; HATZIDIMITRIOU *et al.* 1996; AUBERT *et al.* 1997; OLIVA *et al.* 1999).

In recent years in our laboratory the chemical characterisation of compounds of the variety specific aroma of Sauvignon blanc, Cabernet Sauvignon and Merlot wines have demonstrated the presence of volatile thiols at trace levels (DARRIET *et al.* 1995; TOMINAGA *et al.* 1996, 1998 a, 2000; BOUCHILLOUX *et al.* 1998 a, b) and special methods to determine these compounds have been developed (BOUCHILLOUX *et al.* 1996; TOMINAGA *et al.* 1998 b, 2000).

The reactivity of volatile thiols with certain metals, in particular copper, led us to determine the effect of copper residues on thiols responsible for the varietal aroma of wines. In our initial studies conducted mainly with gas phase chromatography coupled with an olfactometric detection method, we demonstrated that the amount of 4-mercapto-4-methylpentan-2-one decreased after application of copper sulphate to vines (HATZIDIMITRIOU *et al.* 1996). For three years, we have studied the effect of anticryptogamic treatments with folpet and copper on the volatile thiols of the variety specific aromas of Sauvignon blanc, Merlot and Cabernet Sauvignon wines.

Material and Methods

E x p e r i m e n t a l f i e l d : From 1996 to 1998, experiments were performed in different places of the Bordeaux area with cvs Sauvignon blanc, Cabernet Sauvignon and Merlot (Tab. 1). A random-block scheme (Fischer blocks) was used with three repetitions for each fungicide spray, each of these was divided in sub-plots. The number of vines of each plot was variable. Within each sub-plot, generally in the central row, a zone was kept free for sampling grapes to determine the stage of berry development and to harvest for vinification.

F u n g i c i d e s p r a y i n g s : Against downy mildew we sprayed folpet (commercial formulation FOLPAN 80 WDG, 80 % of active ingredient (a.i.), Makteshim Agan, Israel, 1500 g of a.i. per ha) and copper (Bordeaux mixture RSR, copper sulphate, 20 % of active ingredient, Elf-Atochem, France, 3000 g of a.i. per ha). The concentrations correspond to

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Table 1

Characteristics of the experimental sites

Variety Site	Sauvignon blanc Entre Deux Mers	Cabernet Sauvignon Margaux	Merlot Pomerol
Year of plantation	1989	1989	1937
Vines per ha	3300	5900	5900
Sub-plot	3 rows of 50 vines	8 rows of 30 vines	8 rows of 38 vines
Sampling zone	40 vines	80 vines	80 vines

those used in the certification process in viticulture by the French Ministry of Agriculture. Treatments were carried out with pneumatic pulverizers generally used in vineyards. The fungicide preparation (100 l ha^{-1}) was sprayed on the entire foliage of the vines, except for treatments with direct spraying on the leaves. Folpet was used as a reference fungicide according to a previous work (HATZIDIMITRIOU *et al.* 1996) and applied between stage E (BAGGIOLINI 1952) and 30 ± 5 d before harvesting every 10 d. This corresponds to 9 or 10 treatments during the growing season. Bordeaux mixture was sprayed from "cluster closure" stage L to the onset of veraison instead of folpet. In case of one copper spraying, this was done at the onset of veraison. If there were two, spraying was also done at the end of "cluster closure". In the case of three copper treatments, the first was done at the onset of "cluster closure", the following two at 10 and 20 d later. Moreover, complementary phytosanitary treatments were performed against powdery mildew (*Uncinula necator*) and against grey rot (*Botrytis cinerea*) in all plots. Against powdery mildew, wettable sulphur was sprayed (10.0 kg ha^{-1} of a.i. every 10 d) up to flowering then with a sterol biosynthesis inhibitor fungicide (tebuconazole, 100 g ha^{-1} of a.i., Corail, Bayer S.A.) applied once after flowering and then every 14 d. Against *Botrytis cinerea*, vines were sprayed around flowering with a mixture of diethofencarbe and carbendazime (Sumico, 2 l ha^{-1}, Sopra) and again at mid-veraison with iprodione (1.5 kg ha^{-1}, Rovral, Aventis).

To protect grapes from copper residues, bunches were hermetically sealed by small bags (polypropylene, Lembal, Libourne, France) before spraying; they were removed 24 h later. For specific spraying of foliage, the pulverising jet was narrowed to the leaves accordingly. Treatments were similar but the active ingredient was two-fold less (1500 g ha^{-1}).

Must analysis: Approximately 40-45 d after mid-veraison, grapes were harvested in all sub-plots (approximately 15-20 kg of grapes per sub-plot) which had been exposed to the same spraying procedure in order to mix them and to constitute 30 kg of Sauvignon blanc grapes and 40 kg of Cabernet Sauvignon and Merlot grapes respectively, for vinification. Analyses were performed on juices obtained from crushed berries corresponding to mixed grapes originating from sub-plots having undergone the same spraying modality, except in 1997 where grapes from each sub-plot were analysed (Tab. 2). A must sample was taken after crushing of grapes for classical analyses (sugar content was determined by Fehling analysis and titratable acidity by 0.1 M NaOH).

Copper content in grapes and must: Copper content in grapes and must was determined by Atomic Emission Spectrometry (AES) using the Liberty 220 system (Varian, USA) in the automatic mode after constitution of calibration curves with copper sulphate concentrations ranging from 0.1 to 5 mg l^{-1} in model solution (50 g l^{-1} of glucose, 250 mg l^{-1} of KCl). Each sample was analysed 4 times and the repeatability was close to 1-3 %.

To assay copper on the surface and in the berries during maturation, a sample of 450 berries corresponding to a random sample of 150 berries, cut with their pedicels, in each sub-plot was taken for each type of treatment. Berries were weighed and sonicated for 15 min in 800 ml of a tartaric acid solution at 40 g l^{-1}. The solution was kept at -4 °C until analysis by AES. Then, the berries were sonicated again for 2 min in another tartaric acid solution. This solution, which was also analysed by AES, was considered as a control to verify whether all copper at the surface of the berries was dissolved in the first solution. Then the berries were dried and ground mechanically. For determination of copper in the berries, the ground grapes were mineralised by adding 5 ml of nitric acid (65 %) to 5 g of ground berries in a polyethylene tube. After 12 h at room temperature, the tubes were kept for 8 h in a thermostated bath at 50 °C. Finally, the solution was transferred to a 25 ml flask filled with ultrapure water and kept for analysis.

Wine-making: Sauvignon blanc must was fermented according to the protocol described by HATZIDIMITRIOU *et al.* (1996). Briefly, grapes were destemmed and slightly crushed in the presence of liquid CO_2 (10 g kg^{-1}), then sulphur dioxide (30 mg kg^{-1}) was added and crushed grapes were left to macerate in hermetically sealed stainless steel vats (12 l) under a CO_2 atmosphere (10 °C, 18 h). After maceration, unclarified free run must was collected in the presence of liquid CO_2 and sulphur dioxide was added. When the turbidity level was measured at 150-200 NTU (Nephelometric Turbidity Unit) with a nephelometer, the must was racked in 5-l-flasks, then inoculated with active dry yeast (*Saccharomyces cerevisiae*, strain VL3c, Laffort Cie, Bordeaux) at 100 mg l^{-1} (approximately 5 x 10^6 cells per ml). At the end of the alcoholic fermentation, 60 mg l^{-1} of sulphur dioxide were added and the wines were analysed three months later. For vinification of Cabernet Sauvignon and Merlot, grapes were destemmed and slightly crushed in the presence of liquid CO_2 (10 g kg^{-1}), then placed in 40-l-stainless steel tanks and supplemented with sulphur dioxide (60 mg kg^{-1}). Must was inoculated with active dry yeast

T a b l e 2

Sugar, titratable acidity, pH and copper residues of grape juices in relation with copper sprayings

Spraying variants	Copper (a.i., g ha^{-1})	Sugar (g l^{-1})			Titratable acidity (g l^{-1} tartaric acid)			pH			Copper (mg l^{-1})		
		1996	1997[a]	1998	1996	1997[a]	1998	1996	1997[a]	1998	1996	1997	1998
Sauvignon blanc													
Sauv (1)	none[b]	227	176 ± 11.8	-	6.8	8.3 ± 1.2	-	3.1	3.4 ± 0.1	-	0.8 (0.3)[e]	1.7 (1.0)	-
Sauv (2)	2 x 3000[c]	227	162 ± 9.6	-	6.8	9.3 ± 0.8	-	3.1	3.2 ± 0.2	-	8.4 (1.0)	14.3 (1.8)	-
Sauv (3)	3000	223	164 ± 5.8	-	6.9	9.0 ± 0.8	-	3.1	3.3 ± 0.2	-	3.8 (0.5)	7.3 (1.7)	-
Sauv (4)	3000 (spraying leaves only)[d]	-	180 ± 14.8	-	-	8.1 ± 0.8	-	-	3.3 ± 0.2	-	-	3.5 (0.9)	-
Cabernet Sauvignon													
CS (1)	none[b]	204	186 ± 9.4	194	7.7	5.7 ± 0.5	6.6	3.2	3.3 ± 0.2	3.5	0.7	1.4	2.4
CS (2)	3 x 3000[c]	-	206 ± 12.6	185	-	5.4 ± 0.2	6.8	-	3.2 ± 0.2	3.5	-	37.6	28.7
CS (3)	2 x 3000	202	183 ± 3.6	189	7.1	5.6 ± 0.3	6.2	3.2	3.2 ± 0.1	3.5	17.9	25.1	20.1
CS (4)	3000	202	188 ± 5.9	192	8.0	5.6 ± 0.3	6.2	3.2	3.2 ± 0.1	3.5	11.0	13.6	11.6
CS (5)	3000 (grapes protected)	-	188	192	-	5.5	6.2	-	3.2	3.5	-	2.4	1.4
Merlot													
M (1)	none[b]	218	190 ± 3.6	219	7.8	5.7 ± 0.3	5.1	3.3	3.5 ± 0.1	3.4	1.1	1.3	1.0
M (2)	2 x 3000[c]	209	189 ± 0.9	216	8.1	5.7 ± 0.5	5.1	3.2	3.5 ± 0.2	3.4	9.0	23.3	11.4
M (3)	3000	216	203 ± 4.4	219	8.1	5.7 ± 0.2	5.1	3.2	3.5 ± 0.1	3.4	4.5	16.7	6.4
M (4)	3000 (spraying leaves only)[d]	-	193 ± 6.9	219	-	5.7 ± 0.3	4.7	-	3.5 ± 0.1	3.1	-	3.8	2.3

[a] Values corresponding to the average and standard deviations of determinations on each sub-plot.
[b] Sprayings were realised with folpet (1500 g a.i. ha^{-1}) with a frequency of 10 d.
[c] Sprayings before and after copper sprayings with folpet.
[d] Copper sprayings were realized only on the upper part of the foliage without spraying grapes.
[e] Values in brackets correspond to copper concentration in must after clarification.
- Not determined.

(*S. cerevisiae*, strain F10, Laffort Cie) at 100 mg l^{-1} and fermented at 25 °C in a thermostated room. Then, at the end of alcoholic fermentation, the crushed fermented and settled marc was left to macerate for 15 d under CO_2. Wines were then run off in 5-l-flasks and inoculated with lyophilised lactic bacteria for malolactic fermentation (Chr. Hansen, Horsholm, Denmark). At the end of malolactic fermentation, wines were sulphited (50 mg l^{-1}), bottled with cork stoppers and stored at 10 °C before analysis three months later.

Gas chromatography-mass spectrometry: Thiol compounds were analysed by GC-MS (method of Tominaga *et al.* 1998 b). The reproducibility for volatile thiol in wines was close to 5 %.

Sensorial analysis: Fruitiness and global complexity of the varietal aroma of wines was estimated 4-6 months after the end of vinification by 8-10 judges. Tastings were done randomly using glasses corresponding to AFNOR standards (Association Française des Normes).

Statistical data processing: The significance of the differences for sugar concentration, titratable acidity, 3-mercaptohexanol concentration in wines and wine tasting data were studied by a Newman-Keuls mean comparison test ($\alpha = 0.05$; Statbox software ©).

Results and Discussion

Grape composition at harvest: For the three experimental years with Bordeaux mixture (3000 g ha^{-1} applied one, two or three times before and at the onset of veraison, the sugar content in must was quite similar to that determined in must from the same vines but treated with folpet (maximum difference 9 g l^{-1}, Tab. 2). Moreover, statistical analysis of sugar concentration in grapes during maturation after sampling in sub-plots in Margaux and Pomerol vineyards did not evidence any significant effect of copper sprayings on sugar content in grapes in comparison with folpet (data not shown). Titratable acidity and pH were also quite similar (Tab. 2). Compared to 1996, Sauvignon showed a lower sugar content in 1997 with copper sprayings. As previously observed (Hatzidimitriou *et al.* 1996), copper sprayings can sometimes affect sugar accumulation during maturation of Sauvignon blanc. These results could be related either to a direct effect of copper on sugar metabolism in grapes or to an indirect effect due to the consequences of copper sprayings on vine physiology, these effects being modulated by climate, canopy and yield.

Copper concentrations in must from grapes sprayed with Bordeaux mixture were up to 37.6 mg·l^{-1} for three successive treatments in the pre-veraison period (Tab. 2). Overall, copper concentrations were proportional to the number of sprayings, as noted elsewhere (Hatzidimitriou *et al.* 1996). However, before alcoholic fermentation, concentrations were higher in the must of red varieties than in must of white varieties (Tab. 2) because copper concentration decreased during skin contact and pressing, probably by adsorption on solids of the grapes. For all folpet sprayings, small amounts of copper were always found (up to 2 mg l^{-1}), probably due to naturally occurring copper in vines.

In 1997, during maturation of Cabernet Sauvignon, we noticed that copper concentration on the surface of berries decreased regularly (Tab. 3). At the same time, copper concentrations in the berries increased in proportion to the amounts of fungicide sprayed around veraison (Tab. 3). The proportion of copper in grapes was as high as 3.2 mg kg^{-1} (three copper sprayings) compared to a maximum of 1.1 mg kg^{-1} (folpet). This underlines that copper may migrate into grapes, as previously noted by Bernard and Dallas (1979). If the Bordeaux mixture was sprayed on vines with clusters being hermetically protected, copper concentrations at the surface of berries were much lower compared to unprotected clusters; copper concentrations on protected clusters were close to those found after spraying of folpet (Tab. 3). Copper concentrations in the berries were somewhat higher compared to berries from vines sprayed with folpet, suggesting a migration of copper from the leaves into the grape.

Volatile thiols in wine: The main effect of copper sprayings on vine concerns the varietal aroma of young Sauvignon blanc, Cabernet Sauvignon and Merlot wines, to which some volatile thiols clearly contribute (Bouchilloux *et al.* 1998 a, b; Tominaga *et al.* 1998 a b; Blanchard *et al.* 1999). For each vintage between 1996 and 1998 and for all plots studied, wines from grapes sprayed with copper had thiol concentrations which were much lower than those in wines originating from the same plots but sprayed with folpet (Tab. 4). The difference in thiol concentration was particularly striking for 3-mercaptohexanol in Sauvignon blanc, Merlot and Cabernet Sauvignon wines

Table 3

Copper concentration at the surface and in the berries (in brackets) of Cabernet Sauvignon (CS) grapes during maturation in relation to copper sprayings (Margaux 1997)

	Copper concentration (mg kg^{-1})				
Date	CS(1)	CS(2)	CS(3)	CS(4)	CS(5)
H-42[a]	0.40 (1.0)	26.8 (2.3)	20.2 (1.8)	13.2 (1.7)	0.28 (0.9)
H-21	0.42 (0.9)	24.2 (2.6)	13.9 (1.9)	11.0 (1.7)	0.24 (1.4)
H-14	0.34 (1.0)	22.2 (3.2)	12.8 (2.2)	8.6 (2.0)	0.72 (1.8)
H-7	0.36 (1.0)	21.2 (2.1)	13.2 (2.0)	8.2 (1.8)	0.78 (1.7)
H	0.36 (1.1)	16.4 (2.0)	11.6 (1.9)	8.6 (1.3)	0.68 (1.7)

[a] Last spraying was realised 42 d before harvest (H-42).

Table 4

Volatile thiols concentrations in Sauvignon blanc, Cabernet Sauvignon and Merlot wines in relation to different sprayings

Spraying variants	3-mercaptohexanol (ng l^{-1}) 1996	3-mercaptohexanol (ng l^{-1}) 1997	Newman-Keuls[b] (significant difference 5 %)	4-mercapto-4-methylpentan-2-one (ng l^{-1}) 1996	4-mercapto-4-methylpentan-2-one (ng l^{-1}) 1997
Sauvignon blanc					
Sauv (1)	151 (100)[a]	1876 (100)	-	14 (100)	8.0 (100)
Sauv (2)	9 (6)	194 (10)	-	0	4.0 (50)
Sauv (3)	17 (11)	1249 (67)	-	4 (29)	6.0 (75)
Sauv (4)	- 2062	(110) -	-	-	15.0

	3-mercaptohexanol (ng l^{-1}) 1996	3-mercaptohexanol (ng l^{-1}) 1997	3-mercaptohexanol (ng l^{-1}) 1998		3-mercapto-2-methylpropanol (µg l^{-1}) 1997[c]	3-mercapto-2-methylpropanol (µg l^{-1}) 1998
Cabernet Sauvignon						
CS(1)	519 (100)	930 (100)	2137 (100)	A	25.0 (100)	80.7 (100)
CS(2)	-	340 (37)	136 (6)	-	14.4 (58)	29.5 (37)
CS(3)	30 (6)	340 (37)	152 (7)	B	11.6 (46)	24.6 (31)
CS(4)	206 (40)	590 (63)	467 (22)	B	21.9 (88)	52.0 (64)
CS(5)	-	1087 (117)	1770 (82)	-	24.4 (98)	62.3 (77)
Merlot						
M(1)	293 (100)	4550 (100)	357 (100)	A	38.3 (100)	50.3 (100)
M(2)	120 (41)	980 (22)	117 (33)	B	17.0 (44)	36.7 (73)
M(3)	397 (135)	1870 (41)	173 (49)	AB	43.6 (113)	53.4 (101)
M(4)	-	5370 (118)	286 (80)	-	50.5 (132)	46.6 (92)

[a] Values in brackets indicate the percentage of thiol concentration in the wine in relation to the concentration in the reference wine.
[b] Newman-Keuls statistical analysis to determine the level of difference of 3-mercaptohexanol concentrations (expressed in %) to the folpet and copper sprayings in experiments with Cabernet Sauvignon [Comparison of CS (1), (3), (4)] and Merlot [Comparison of M (1), (2), (3)]. The letters A, B or AB mean that the values are statistically different.
[c] 3-mercapto-2-methylpropanol was not quantified in Cabernet Sauvignon and Merlot wines from 1996 vintage.
- Not determined.

and for 4-mercapto-4-methylpentan-2-one concentrations in Sauvignon blanc. A comparison of 3-mercaptohexanol concentrations in wines made from folpet-sprayed vines with those in wines from copper-sprayed vines showed decreases between 89 and 37 % (one copper treatment at 3000 g ha^{-1}) and a decrease between 94 and 63 % (two sprayings with Bordeaux mixture) (Tab. 4). The effect of copper sprayings on 3-mercaptohexanol concentrations (%) in Merlot and Cabernet Sauvignon wines in comparison with wines obtained from folpet-sprayed vines was statistically significant for the three years (Tab. 4). 4-mercapto-4-methylpentan-2-one concentrations in Sauvignon blanc wines were distinctly lower compared to those in wines from folpet plots (at least a 50 % difference of 4-mercapto-4-methylpentan-2-one with two copper sprayings and at least a 25 % difference with one copper spraying). These differences were less pronounced for 3-mercapto-2-methylpropanol, another more abundant thiol originating possibly from fermentation (Bouchilloux *et al.* 2000) (decrease between 12 and 70 % in comparison with wine made from folpet-treated vines) (Tab. 4). Moreover, if grapes were protected from copper during the last spraying at veraison (CS 5), the 3-mercaptohexanol content in Cabernet Sauvignon wines was very close to that obtained from folpet-treated vines. This result was closely correlated with the low concentrations of copper residues in must before alcoholic fermentation (Tab. 2).

A significant statistical difference was also established for the sensorial analysis of the wines with reference to aroma complexity and fruitiness (Tab. 5). When applying the low olfactory perception threshold of 3-mercaptohexanol or 4-mercapto-4-methylpentan-2-one in the model solution (60 ng l^{-1} and 0.8 ng l^{-1}, respectively; Tominaga *et al.* 1998 b, Darriet *et al.* 1995) and the ratio of the concentration of these compounds on the olfactory perception threshold in reference wines and wines obtained from copper-sprayed vines, these data were very coherent with sensorial analysis.

Copper reactivity with thiol compounds is a well known phenomenon in oenology, since copper sulphate (up to 1 mg l^{-1}) is an authorized treatment to remove certain aromatic defects due to thiol compounds (EU Regulation, 822/87). Hatzidimitriou *et al.* (1996) first demonstrated that

Table 5

Sensorial analysis of Sauvignon blanc, Cabernet Sauvignon and Merlot wines in relation to copper sprayings

Spraying variants	Tasting marks (10^{-1}) 1996		Tasting marks (10^{-1}) 1997	Average ± standard deviation	Newman-Keuls[a] (significant difference 5 %)
Sauvignon blanc					
Sauv (1)	8.5		6.3	-	-
Sauv (2)	5.2		2.8	-	-
Sauv (3)	5.3		5.2	-	-
Sauv (4)	-		5.8	-	-
	Tasting marks (10^{-1}) 1996	1997	1998		
Cabernet Sauvignon					
CS(1)	8.3	7.0	7.9	7.7 ± 0.7	A
CS(2)	-	4.8	4.1	-	-
CS(3)	4.7	5.0	5.3	5.0 ± 0.3	B
CS(4)	5.6	4.8	5.3	5.2 ± 0.4	B
CS(5)	-	5.6	6.0	-	-
Merlot					
M(1)	6.3	8.0	7.4	7.2 ± 0.9	A
M(2)	3.7	4.4	4.4	4.2 ± 0.4	B
M(3)	4.6	4.4	4.5	4.5 ± 0.1	B
M(4)	-	8.4	5.5	-	-

[a] Newman-Keuls statistical analysis to determine the level of difference of tasting marks between folpet and copper sprayings for Cabernet Sauvignon (CSCu (1), (3), (4)) and Merlot (MCu(1), (2), (3)). A, B: values are statistically different.
- Not determined.

only one pre- and post-veraison copper spraying (3000 g ha^{-1} of a.i.) to Sauvignon blanc vines (and grapes) led to a large decrease in 4-mercapto-4-methylpentan-2-one concentrations in wines. This was correlated with a lack of typicity. The present results obtained with different varieties clearly demonstrate the impact of copper sprayings at veraison on some volatile thiols contributing to the varietal expression of Sauvignon blanc, Merlot and Cabernet Sauvignon wines. The decrease of thiols can be interpreted as a reaction of copper residues with volatile thiols in the early stage of alcoholic fermentation by *Saccharomyces cerevisiae*, when S-cysteine conjugates precursors for 3-mercaptohexanol, 4-mercapto-4-methylpentan-2-one in the must are revealed in volatile thiols (TOMINAGA *et al.* 1998 c). An effect of copper residues at the surface of the grapes on the biosynthesis of the S-cysteine conjugates during grape maturation cannot be ruled out in the case of two or more copper sprayings since copper penetrates berry skin during spraying (Tab. 3). Measurements of S-cysteine conjugates in grapes during maturation would clarify this point (PEYROT DES GACHONS *et al.* 2000). However, the fact that protection of clusters before a spraying can lead to low levels of copper residues in must and to a thiol concentration in wines comparable to that found in wines obtained from grapes of the same site but sprayed with folpet, suggests that only copper residues on grapes are detrimental to thiols in wines during alcoholic fermentation. On the other hand, it appears that spraying copper onto leaves (one copper spraying, 1500 g ha^{-1}), does not impair varietal aroma.

Copper spraying only to the foliage leads to a decrease of copper residues on grapes and consequently in the must at harvest. This mode of treatment was tested on Sauvignon blanc and Merlot vines in order to study its effects on the thiol content in wines. Copper contents were much less abundant in must if only the foliage was sprayed, and both, 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one concentrations were similar to those obtained from vines sprayed with folpet (Tabs 2 and 4). Tasting marks of wines were relatively higher and close to reference wines (Tab. 5). Treating the foliage but not the grapes to protect vines against downy mildew limits copper residues and their effects on volatile thiols of the varietal aroma.

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