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EFFECTS OF FLUSILAZOLE, A STEROL BIOSYNTHESIS INHIBITOR FUNGICIDE, ON THE FREE AND BOUND AROMA FRACTION OF MUSCAT OF ALEXANDRIA WINES

INFLUENCE DU FLUSILAZOLE, UN FONGICIDE INHIBITEUR DE LA BIOSYNTHÈSE DES STÉROLS, SUR LES FRACTIONS LIBRES ET LIÉES DE L'ARÔME DES VINS DE MUSCAT D'ALEXANDRIE

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Abstract : Vines from Muscat of Alexandria cultivar were subjected to flusilazole treatments, a sterol biosynthesis inhibitor fungicide (SBI), over 2 years. Effects of these treatments on the corresponding wines were assessed by analyzing the free and bound aroma fractions using selective retention on Amberlite XAD-2 and gas chromatography-mass spectrometry. Quantitative changes in the composition of the free and bound fractions of the treated wines were observed. Levels of varietal, prefermentative and fermentative compounds were modified and a regulatory mechanism for sterol synthesis in yeast involving the monoterpenes could be hypothesized.

Résumé : Des vignes de Muscat d'Alexandrie ont subi des traitements de flusilazole, un fongicide inhibiteur de la biosynthèse des stérol (IBS), pendant 2 ans. Systémique, très efficace contre l'Oïdium et le Black-rot, ce fongicide est couramment utilisé sur vigne. Son mode d'action primaire est de bloquer les enzymes à cytochrome P450 impliquées dans la voie de biosynthèse de l'ergostérol chez les champignons parasites (la C14α lanostérol déméthylase, la Δ^{22} désaturase). Toutefois, il peut également inhiber les voies de biosynthèse des phytostérols et des gibbérellines ce qui peut arrêter la croissance des plantes traitées. Afin de vérifier les effets de tels traitements sur la composante aromatique des vins correspondants, les composés libres et liés des vins traités ont été extraits et séparés sur Amberlite XAD-2 et analysés par chromatographie en phase gazeuse couplée à la spectrométrie de masse. Des modifications dans la composition des fractions libres et liées ont été observées. Les concentrations en arômes variétaux, fermentaires et préfermentaires ont été modifiées et un mécanisme de régulation pour la synthèse des stérols dans la levure mettant en jeu les monoterpènes peut être envisagé.

Key words : wine, aroma, flusilazole, fungicide, SBI
Mots clés : vin, arôme, flusilazole, fongicide, IBS

INTRODUCTION

Triazole derivative flusilazole, [bis(4-fluorophenyl)methyl(1H-1,2,4-triazol-1-ylmethyl)silane], belonging to the azole class of sterol biosynthesis inhibitors (SBI), is commonly used on vines to combat fungal diseases, e.g. rust and powdery mildew. SBI exert their fungitoxic effects primarily by inhibiting the C 14 α-lanosterol demethylase as well as Δ^{22} desaturase which are cytochrome P450 dependent enzymes involved in sterol biosynthesis (LEROUX, 1981, 1987a and 1987b ; SCHEINPFLUG and KUCK, 1987). In fungi treated there is a marked accumulation of 14 α-methylsterol, such as obtusifoliol, and a decline of the usual sterols, e.g. ergosterol. The effect is postulated to destabilize the membrane function and leads to cell death (BUCHENAUER, 1987). However, triazole derivatives can also inhibit the biosynthesis of phytosterols and gibberelines, which can stop the growth of treated plants (BUCHENAUER and ROHNER 1981 ; NES *et al.*, 1982 ; KOLLER, 1987).

In a previously work, we have reported the effects of flusilazole and triforine treatments on the aroma of *Vitis vinifera* L. grape, cv. Muscat of Alexandria,

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(AUBERT *et al.*, 1997). In this present study, we only reported the influence of flusilazole on the aroma of wines derived from treated grapes.

MATERIALS AND METHODS

I — REAGENTS AND CHEMICALS

Analytical grade solvents were further purified by distillation before use. Amberlite XAD-2 resin from Fluka was purified before use (GÜNATA *et al.*, 1985). Enzymatic preparations, Pektolase 3PA and Hemicellulase REG2, were purchased from Grinsted and Gist Brocades respectively. Flusilazole [bis(4-fluorophenyl)methyl(1H-1,2,4-triazol-1-ylmethyl) silane] was provided by DuPont de Nemours : the 100 g/l commercial formulation was used (Olymp 10 EW).

II — PLANT MATERIALS AND TREATMENTS

Grapes of the *Vitis vinifera* L., cultivar Muscat of Alexandria, were harvested, in September 1995 and 1996, from the vineyard of Villeroy-Listel, Sète, France. Flusilazole treatments were as previously described (AUBERT *et al.*, 1997).

III — WINE PROCESSING

Wines were made by standard wine processing in inert tanks using the following techniques : De-stemming and crushing ; addition of SO_2 (5 g/hl) and skin contact during 4 hours at room temperature ; pressing the grapes, addition of 20 g/hl dry yeast (*Saccharomyces cerevisae*, K1, ICV-INRA, Montpellier, France), fermentation at 24-25°C for 8 days. The wines were stored for one month (18-20°C) in glass until analysis.

VI — ANALYSIS OF WINES

1) Chemical analysis

Each wine was analyzed for density, sugar, pH and ethanol using standard procedures (table I).

2) Flusilazole residue analysis of musts and wines

50 ml of samples were transferred into a 500 ml separatory funnel, 250 ml of distilled water and 50 ml of a saturated solution of sodium chloride were added, and the medium was extracted with 3x50 ml of hexane/diethyl ether (50:50 v/v). The collected organic phases were dried over anhydrous sodium sulfate and evaporated to dryness under vacuum. The residue was dissolved in 2 ml of ethyl acetate and 10 µl of tebuconazole (200 µg/ml) was added as internal standard. Analysis was carried out by GC and all experiments were performed in triplicate (table 1).

3) Fractionation of the free and bound fractions of the aroma

Fractionation of the free and bound fractions of the aroma of wines was carried out on Amberlite XAD-2 resin according to the method of GÜNATA *et al.* (1985), modified by VOIRIN *et al.* (1992). 10 µl of 4-nonanol (3.2 mg/ml in ethanol) was added as inter-

TABLE I

Chemical and flusilazole residue analysis of musts and wines[a]

Tableau I — Analyses chimiques et de résidus de flusilazole dans les moûts et les vins

	1995			1996		
	control	flusi-3	flusi-6	control	flusi-3	flusi-6
Musts						
Density (20°C)	1,079	1,076	1,079	1,071	1,076	1,068
Sugar ($g.l^{-1}$)	205	200	194	168	162	156
pH (20°C)	3,91	3,94	3,85	3,59	3,55	3,63
Flusilazole residue (ppb)	—	< 0,005	0,018	—	nd[b]	0,01
Wines						
Ethanol (%)	12,30	11,98	11,22	10,00	9,58	9,15
Ethanol (%) calculated[c]		12,00	11,64		9,65	9,29
Sugar ($g.l^{-1}$)	< 2	< 2	< 2	< 2	< 2	< 2
pH (20°C)	3,66	3,64	3,59	3,34	3,55	3,33
Flusilazole residue (ppb)		nd	nd		nd	nd

[a]flusi-3 : three applications each 4 weeks ; flusi-6 : six applications each 2 weeks - [b]not detected
[c]calculated from the sugar concentration of each must and reduced to the control
[a]flusi-3 : trois applications toutes les 4 semaines ; flusi-6 : six applications toutes les 2 semaines - [b]non détecté
[c]calculé à partir de la concentration en sucre de chaque moût et ramené au témoin

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nal standard to 50 ml of wine samples before fractionation (each wine was diluted 5 times in distilled water). These samples were passed through a XAD-2 column at a flow-rate of about 1-1.5 ml/min. The column was rinsed by 50 ml of distilled water. The free fraction was eluted with 50 ml of pentane-dichloromethane (2:1 v/v). The pentane-dichloromethane extract was dried over anhydrous sodium sulfate and concentrated at 40°C using a Vigreux column to a final volume of about 400 µl. The organic extract was stored at -20°C before GC and GC-MS analyses.

The bound fraction was eluted with 50 ml of ethyl acetate. The ethyl acetate extract was dried over anhydrous sodium sulfate and concentrated to 1 ml under vacuum at 40°C. The extract was then transferred to a small vial and concentrated to dryness (50-55°C) under a stream of nitrogen.

4) Enzymatic hydrolysis of bound fraction

100 µl citrate-phosphate buffer (0.1 M, pH 5.0) was added to the above glycosidic extract. The mixture was washed 5 times using 100 µl pentane-dichloromethane (2:1 v/v), then 100 µl of the enzymatic solution (12 mg Pektolase 3PA and 12 mg Hemicellulase REG2 in 1 ml citrate-phosphate buffer , 0.1 M, pH 5.0) was added to the extract. After stirring, the vial was sealed and placed in a water-bath at 40°C for 16 h. The mixture was then extracted 5 times with 100 µl pentane-dichloromethane (2:1 v/v). After addition of 10 µl 4-nonanol (3.2 mg/ml in ethanol), as internal standard, the medium was concentrated to a final volume of 200 µl by a Vigreux column (40°C). The organic extract was stored at -20°C before GC and GC-MS analyses.

All experiments were performed in triplicate.

5) Gas Chromatography and Gas Chromatography-Mass Spectrometry analysis

Chromatographic conditions for the analysis of the free and bound fractions were as previously described (AUBERT *et al.*, 1997).

Chromatographic conditions for the analysis of residues were as followed : a Hewlett-Packard 5890 Series II gas chromatograph fitted with a DB-1 fused silica capillary column (J&W SCIENTIFIC ; 30 m x 0.32 mm i.d. ; 0.5 µm film thickness) and a NPD detector was used. The operating conditions were as followed : the injector temperature program was set at 105°C to 250°C at 150°C/min, then isothermal for 5 min. The oven temperature program was set at 100°C to 250°C at 20°C/min, then isothermal for 5 min. The detector temperature was held at 300°C. The nitrogen carrier gas flow rate was 1.5 ml/min and 1 µl injections were performed on-column.

RESULTS AND DISCUSSION

Flusilazole, a sterol biosynthesis inhibitor fungicide, was used on grapes of Muscat of Alexandria, well known for its intense floral aroma and its ability to give such wines. Two treatment levels were used for flusilazole in 1995 and 1996. The first involved one application every 4 weeks for 12 weeks (3 applications). The second involved one application every 2 weeks for 12 weeks (6 applications). The wines were made from sound grapes, harvested from the treated vines 2 weeks after the last spraying and from the control vines (AUBERT *et al.*, 1997). The effects of these applications were assessed by analyzing the free and bound aroma constituents of the wines in triplicate with internal standard, and the relative data were considered : the levels and the standard deviations obtained are reported in tables II and III. Variance analysis were performed between the control and each treated sample : the values underlined were significantly different to that of the control ($p<0.05$).

As previously described, no significant effect on the berry weight was observed with flusilazole treatments, however a slight decrease in sugar content was observed in the treated samples (AUBERT *et al.*, 1997). Accordingly, chemical analysis of the wines displayed a decrease in ethanol content in the samples treated (table I), in agreement with the results previously reported by DOIGNON and COUTY (1992). However these decreases in ethanol content were higher for 6 applications of flusilazole, than what would be expected from the sugar content of the grapes analyzed. As in all the samples the residual sugar content were lower than 2 g/l, this was indicative of an effect of SBI on the sugar metabolism of yeast during fermentation.

In every experiment, flusilazole residues in musts were either not detected or lower than 0.02 ppm (table I). No delay time in fermentation were observed in our experiments which was consistent with the results previously reported by DOIGNON (1991), who found no delay time in the fermentation of a synthetic medium below 0.05 ppm of flusilazole. In each experiment, no flusilazole residue was found in wines (table I).

I — INFLUENCE OF FLUSILAZOLE ON THE FREE FRACTION OF MUSCAT OF ALEXANDRIA WINES (Table II)

The flusilazole treatments led to a decrease in the total amount of free C6 compounds in wines. The decrease was most marked on the samples subjected to highest application rates. Among the C6 compounds, the highest decrease occurred for hexanol. DOIGNON and COUTY (1992) had already observed a decrease of hexanol in wines made from Semillon grapes trea-

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TABLE II

Influence of flusilazole on the free aromatic fraction of Muscat of Alexandria wines. Treatments[a] 1995-1996 (amounts in µg/l wine)[b]

Tableau II — Influence du flusilazole sur la fraction libre de l'arôme des vins de Muscat d'Alexandrie. Traitements[a] 1995-1996 (concentrations en µg/l de vin[b])

Compounds	RI	Treatments 1995 control		Treatments 1995 flusi-3		Treatments 1995 flusi-6		Treatments 1996 control		Treatments 1996 flusi-3		Treatments 1996 flusi-6	
C6 compounds													
hexanol	*1353*	1169,3 ±	62,6	1047,0 ±	55,0	1038,5 ±	27,7	892,1 ±	44,7	825,9 ±	121,2	746,4 ±	16,9
Z 3-hexen-1-ol	*1374*	880,6 ±	169,2	761,4 ±	9,4	738,8 ±	17,6	494,4 ±	24,5	559,6 ±	30,0	476,9 ±	13,9
E 3-hexen-1-ol	*1414*	53,5 ±	0,9	42,2 ±	0,4	46,1 ±	3,4	35,3 ±	2,2	41,1 ±	2,1	34,6 ±	1,2
sum of C6 compounds		**2103,4 ±**	**211,2**	**1850,7 ±**	**53,1**	**1823,4 ±**	**44,2**	**1421,8 ±**	**69,1**	**1426,5 ±**	**143,6**	**1257,9 ±**	**31,9**
terpenols													
cis furan-linalool oxide	*1445*	19,8 ±	1,8	15,4 ±	0,8	24,9 ±	2,6	17,1 ±	1,1	13,3 ±	0,3	14,3 ±	0,2
trans furan-linalool oxide	*1472*	44,4 ±	0,8	52,0 ±	5,2	46,8 ±	3,6	41,8 ±	3,3	22,5 ±	3,2	29,1 ±	1,3
linalool	*1602*	1043,0 ±	35,8	782,3 ±	13,6	945,6 ±	5,0	785,2 ±	4,3	682,4 ±	13,1	599,1 ±	8,9
ho-trienol	*1612*	97,8 ±	1,5	93,5 ±	3,0	121,0 ±	2,8	127,3 ±	0,9	114,8 ±	2,0	90,6 ±	1,7
α-terpineol	*1698*	260,3 ±	16,3	197,7 ±	7,5	234,5 ±	13,7	257,0 ±	1,0	249,6 ±	3,2	221,1 ±	4,4
trans pyran-linalool oxide	*1723*	289,8 ±	4,9	224,0 ±	1,2	260,6 ±	12,8	151,7 ±	1,7	148,2 ±	6,6	110,5 ±	1,4
cis pyran-linalool oxide	*1767*	75,5 ±	10,3	47,4 ±	5,1	55,0 ±	0,8	31,3 ±	0,9	28,3 ±	0,6	22,8 ±	0,4
citronellol	*1773*	73,2 ±	0,8	78,8 ±	2,2	68,1 ±	2,6	75,6 ±	1,4	53,4 ±	0,8	62,1 ±	1,6
nerol	*1804*	47,2 ±	1,7	32,2 ±	0,3	34,7 ±	0,5	61,2 ±	8,6	54,9 ±	1,5	46,2 ±	11,8
geraniol	*1832*	137,4 ±	5,0	104,4 ±	2,4	86,6 ±	0,6	164,0 ±	1,5	100,1 ±	1,1	88,6 ±	1,7
3,7-dimethyl-1,5-octadien-3,7-diol	*1957*	453,9 ±	18,6	389,9 ±	29,8	402,4 ±	3,2	69,5 ±	14,2	48,6 ±	27,0	81,5 ±	13,6
3,7-dimethyl-1,7-octadien-3,6-diol	*2135*	56,3 ±	1,9	35,5 ±	4,6	48,7 ±	0,6	9,8 ±	1,4	7,5 ±	1,9	12,0 ±	1,5
sum of terpenols		**2598,6 ±**	**85,4**	**2053,2 ±**	**18,9**	**2328,8 ±**	**30,8**	**1791,9 ±**	**30,9**	**1523,8 ±**	**15,6**	**1377,9 ±**	**16,3**
alcohols													
2-methylpropanol	*1108*	2379,9 ±	281,4	3207,4 ±	185,5	3357,3 ±	185,6	3883,2 ±	399,0	3545,9 ±	977,6	3653,1 ±	691,1
2- and 3-methylbutanol	*1215*	177332,9 ±	796,3	242825,4 ±	13583,0	191728,4 ±	1762,5	102349,1 ±	5986,6	119323,1 ±	14930,5	138760,5 ±	8951,1
1-pentanol	*1257*	29,7 ±	7,4	16,0 ±	2,6	22,6 ±	0,5	26,3 ±	1,8	26,0 ±	2,3	18,1 ±	2,2
benzyl alcohol	*1871*	48,7 ±	1,8	46,2 ±	0,9	52,8 ±	1,3	85,9 ±	5,2	34,5 ±	2,5	70,2 ±	5,5
2-phenylethanol	*1904*	74574,9 ±	3975,6	98284,8 ±	3291,8	71820,9 ±	2966,4	32567,0 ±	1287,8	37974,4 ±	1370,4	41705,3 ±	981,9
sum of alcohols		**254366,2 ±**	**3117,8**	**344379,8 ±**	**15203,3**	**266982,0 ±**	**2585,3**	**138911,5 ±**	**7304,8**	**160903,9 ±**	**17156,4**	**184207,3 ±**	**10372,8**
esters													
2- and 3-methylbutyl acetate	*1143*	686,2 ±	22,0	373,0 ±	62,9	576,7 ±	84,2	136,6 ±	31,5	393,2 ±	108,3	227,7 ±	27,3
ethyl hexanoate	*1227*	61,4 ±	2,7	40,1 ±	5,2	59,7 ±	3,8	39,8 ±	9,4	43,8 ±	13,4	29,9 ±	6,9
ethyl octanoate	*1436*	55,3 ±	1,2	76,9 ±	15,3	80,6 ±	1,9	66,5 ±	19,5	57,7 ±	15,6	44,7 ±	10,8
diethyl succinate	*1678*	65,8 ±	6,1	49,9 ±	4,8	82,1 ±	8,5	89,6 ±	17,2	68,4 ±	18,4	65,3 ±	10,5
2-phenylethyl acetate	*1808*	64,1 ±	0,4	52,8 ±	17,8	38,6 ±	10,0	30,6 ±	4,3	27,5 ±	0,8	23,1 ±	5,9
sum of esters		**932,9 ±**	**21,9**	**592,8 ±**	**89,3**	**837,7 ±**	**90,3**	**327,4 ±**	**60,0**	**560,3 ±**	**141,0**	**369,0 ±**	**51,6**

[a]flusi-3 : three applications each 4 weeks ; flusi-6 : six applications each 2 weeks. [b]mean of three experiments ; underlined values were significantly different from the control ($p<0.05$) ;
[c]linear Retention Index calculated on DB-WAX capillary column using n-hydrocarbons standards (VAN DER DOOL and KRATZ, 1963)

[a]flusi-3 : trois applications toutes les 4 semaines ; flusi-6 : six applications toutes les 2 semaines ; [b]moyenne de trois répétitions ; les valeurs soulignées sont significativement différentes par rapport au témoin ($p<0,05$) ; [c]Index de Rétention linéaire obtenu sur colonne capillaire DB-WAX à partir d'hydrocarbures standards (VAN DER DOOL and KRATZ, 1963)

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ted with flusilazole. The only discrepancy was seen in 1996 with (Z and E) 3-hexen-1-ol after 3 applications. In an earlier study, we had also reported that the use of SBI on vine decreased the levels of the C6 compounds formed when crushing the grapes treated, and postulated that SBI treatments could affect the enzyme involved at some stage of the lipid degradation leading to C6-compounds (AUBERT *et al.*, 1997). As the only effect of yeast on the formation of these C6 compounds is to reduce the ones at a higher oxidation state than hexanol in hexanol and/or hexenols, it is consistent to find the similar result in wines (JOSLIN and OUGH, 1978).

In 1995 and 1996, the total amount of free alcohols were significantly higher in wines derived from treated grapes. This was mainly due to the levels of the most abundant ones, 2-phenylethanol and 2- and 3-methylbutanols. These results were consistent with those previously reported in wines made from grapes treated with flusilazole (Semillon and Sauvignon cultivars) (DOIGNON and COUTY, 1992). However, some discrepancies were observed for 2-phenylethanol for 6 applications in 1995 and for 2-methylpropanol for both applications in 1996.

The fusel alcohols, 2-methylpropanol, 2- and 3-methylbutanol and 2-phenylethanol, are mainly produced during fermentation by yeast through the catabolism and the anabolism (from sugar) of the corresponding amino acids (ETIEVANT, 1991 ; NYKANEN and SUOMALAINEN, 1983). Thus, contrary to the other minor alcohols (1-pentanol, benzyl alcohol), their concentration increased greatly from grapes to the corresponding wines. Since the same results had been observed in the fermentation of a synthetic medium added with flusilazole (DOIGNON, 1991), one can conclude that SBI treatments influence the formation of alcohols during fermentation essentially by a direct action on the metabolism pathways of yeast. Furthermore, as SBI treatments of vines modify the composition of grape, *i.e.* the fermentation medium (AUBERT *et al.*, 1997, HOUTMAN *et al.*, 1980), an indirect action can also be hypothesized. However, no coherent effect of flusilazole on the levels of the esters formed by yeast, except for the free 2-phenylethyl acetate, was observed in our experiments, contrary to their decrease previously reported by DOIGNON (1991) (cv. Ugni-blanc, Sauvignon and Sémillon).

In both experiments, the total amount of free monoterpenols decreased significantly in wines made from treated grapes. Similar results have been obtained on the wines of non-monoterpenol dependent cultivars (DOIGNON and COUTY, 1992). However the data given here demonstrated more clearly the effects of flusilazole treatments on monoterpenols, due to the abundance of these compounds in Muscat wines. These effects were more important in 1996 than in 1995, but some discrepancies with this global behavior were observed. The typical compounds of muscat wine flavor, linalol, geraniol and nerol (BAYONOVE and CORDONNIER, 1971 ; CORDONNIER, 1973, BOIDRON and TORRES, 1979) were significantly lower in the assays than in the controls. Nevertheless, for both experiments, the decrease of the level of geraniol was generally more important (about 40 p. cent at the highest application rate) than those of the levels of linalol or nerol. We reported previously (AUBERT *et al.*, 1997) that such treatments on vines (cv. Muscat of Alexandria) decreased the levels of free monoterpenols in grape berries, but these effects were sharper in wines.

II — INFLUENCE OF FLUSILAZOLE ON THE BOUND FRACTION OF MUSCAT OF ALEXANDRIA WINES (Table III)

In 1995 and 1996, the levels of the bound C6-alcohols decreased in wines made from treated grapes. Regarding the relatively low levels of these bound compounds in wines compared to the free ones, these variations seemed negligible. Nevertheless, they were the reverse to what had been observed in grapes treated with flusilazole (AUBERT *et al.*, 1997).

In both experiments, the total amount of bound monoterpenols decreased significantly with increased applications of flusilazole. The decrease of these compounds could not be explained by their hydrolysis, as the corresponding free monoterpenols showed the same behavior. These results contrasted with those we reported on grapes in which such treatment increased the levels of bound monoterpenols (AUBERT *et al.*, 1997). Particularly, the typical compounds of Muscat aroma, geraniol, linalool and nerol, were significantly lower in the wines made from treated grapes than in the control, while the sum of their levels was about 1.2-1.4 times higher in treated grapes than in the control grapes (figure 1) (AUBERT *et al.*, 1997). A similar trend was observed for benzyl alcohol and 2-phenylethanol. Thus the decreasing effects observed during wine making of the treated grapes were more important than shown when comparing only the treated and the control wines. To our best knowledge, it is the first time that such results have been observed.

Finally, the only coherent effect of flusilazole on the levels of bound phenol and C13-norisoprenoids was a decrease of the levels of the last ones in 1996 (significant with 6 applications), but some discrepancies with this effect were observed in 1995.

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TABLE III

Influence of flusilazole on the bound aromatic fraction of Muscat of Alexandria wines. Treatments[a] 1995-1996 (amounts in µg/l wine)[b]

Tableau III — Influence du flusilazole sur la fraction liée de l'arôme des vins de Muscat d'Alexandrie. Traitements[a] 1995-1996 (concentrations en µg/l de vin[b])

Compounds	RI	Treatments 1995 control	Treatments 1995 flusi-3	Treatments 1995 flusi-6	Treatments 1996 control	Treatments 1996 flusi-3	Treatments 1996 flusi-6
C6 compounds							
hexanol	*1353*	12.3 ± 1.0	9.8 ± 1.3	10.5 ± 0.5	12.7 ± 0.1	11.3 ± 0.4	9.0 ± 0.9
Z 3-hexen-1-ol	*1374*	11.1 ± 2.1	9.4 ± 2.0	10.4 ± 1.3	11.6 ± 0.8	11.2 ± 0.9	11.0 ± 1.1
E 2-hexen-1-ol	*1414*	6.2 ± 1.6	4.8 ± 0.3	5.7 ± 0.5	7.2 ± 0.4	5.4 ± 0.2	4.6 ± 0.5
sum of C6 compounds		**29,7 ± 4,7**	**24,0 ± 3,5**	**26,6 ± 1,0**	**31,5 ± 1,4**	**27,9 ± 0,7**	**24,6 ± 2,5**
terpenols							
trans furan-linalool oxyde	*1445*	80.5 ± 9.2	43.8 ± 6.2	53.7 ± 2.3	44.4 ± 0.3	22.4 ± 3.6	34.7 ± 4.4
cis furan-linalool oxyde	*1472*	3.2 ± 0.4	2.5 ± 0.3	4.2 ± 0.1	5.5 ± 0.1	5.9 ± 0.9	5.7 ± 1.3
linalool	*1555*	373.3 ± 22.2	255.8 ± 26.4	229.9 ± 19.9	103.8 ± 10.9	72.6 ± 10.6	71.6 ± 2.5
ho-trienol	*1612*	4.2 ± 0.5	4.2 ± 0.6	4.3 ± 0.2	4.3 ± 0.1	4.3 ± 0.1	5.4 ± 0.8
α-terpineol	*1698*	11.3 ± 1.0	10.2 ± 1.1	9.7 ± 0.6	5.4 ± 0.3	5.3 ± 1.0	7.8 ± 2.4
trans pyran-linalool oxyde	*1739*	49.6 ± 0.9	40.0 ± 4.9	34.6 ± 3.4	20.2 ± 0.6	12.0 ± 1.4	14.0 ± 1.0
cis pyran-linalool oxide	*1767*	4.3 ± 0.4	3.7 ± 1.3	3.4 ± 1.2	5.9 ± 0.2	2.4 ± 0.8	4.6 ± 2.1
citronellol	*1773*	4.5 ± 1.2	5.2 ± 2.2	3.2 ± 0.6	4.2 ± 0.6	4.0 ± 0.7	4.2 ± 0.1
nerol	*1804*	83.4 ± 5.1	64.8 ± 6.4	55.2 ± 3.9	46.6 ± 12.7	37.0 ± 10.9	30.2 ± 0.1
geraniol	*1854*	206.6 ± 25.1	180.7 ± 0.4	144.3 ± 24.7	137.8 ± 22.5	141.9 ± 28.4	98.8 ± 1.8
3,7-dimethyl-1,5-octadien-3,7-diol	*1957*	166.7 ± 24.0	123.5 ± 10.9	47.4 ± 23.8	196.5 ± 34.6	194.7 ± 1.2	87.0 ± 6.0
linalool hydrate	*1988*	16.1 ± 2.6	17.8 ± 1.9	11.2 ± 2.1	29.7 ± 2.8	31.5 ± 2.1	18.9 ± 0.4
3,7-dimethyl-1,7-octadien-3,6-diol	*2135*	35.8 ± 1.7	55.7 ± 3.0	52.1 ± 3.8	22.1 ± 2.8	24.6 ± 6.1	24.9 ± 5.5
8-hydroxy-dihydro-6,7-linalool + citronellol hydrate	*2216*	20.9 ± 4.5	28.7 ± 4.4	16.9 ± 3.9	19.2 ± 0.1	17.2 ± 3.1	15.7 ± 2.8
E 8-hydroxylinalool	*2275*	71.9 ± 13.3	86.4 ± 12.7	59.3 ± 13.6	58.7 ± 2.4	41.0 ± 6.9	38.2 ± 4.7
Z 8-hydroxylinalool + geraniol hydrate	*2318*	54.5 ± 12.2	83.5 ± 16.9	51.6 ± 14.3	51.1 ± 2.4	44.2 ± 8.8	34.0 ± 4.8
geranic acid	*2335*	23.3 ± 10.3	27.6 ± 28.7	25.3 ± 21.9	69.5 ± 14.1	47.9 ± 21.1	54.9 ± 19.1
p-1-menthen-7,8-diol	*2526*	4.0 ± 0.6	7.5 ± 2.0	4.8 ± 0.6	5.6 ± 0.6	6.2 ± 0.6	4.6 ± 1.1
Z 8-hydroxygeraniol	*2603*	18.9 ± 5.0	35.5 ± 3.9	15.6 ± 4.9	14.2 ± 0.1	10.9 ± 1.9	8.9 ± 0.4
sum of terpenols		**1244,8 ± 100,3**	**1092,6 ± 88,5**	**838,7 ± 58,4**	**859,6 ± 76,3**	**737,7 ± 111,4**	**570,3 ± 12,1**
alcohols							
benzyl alcohol	*1871*	58.8 ± 3.7	56.4 ± 18.4	48.1 ± 6.4	45.7 ± 7.2	46.0 ± 3.6	35.8 ± 5.4
2-phenylethanol	*1904*	168.3 ± 38.2	131.9 ± 4.1	96.2 ± 15.9	75.2 ± 4.0	70.1 ± 7.4	50.5 ± 1.3
sum of alcohols		**227,1 ± 40,0**	**188,3 ± 14,6**	**144,3 ± 21,4**	**120,9 ± 11,3**	**116,1 ± 10,9**	**86,3 ± 4,1**
phenols							
2-(3-methoxy-4-hydroxyphenyl)-ethanol	*2832*	10.7 ± 3.4	18.0 ± 4.1	11.0 ± 3.5	4.9 ± 0.1	4.9 ± 0.3	4.7 ± 1.9
unknown phenol	*2933*	4.7 ± 1.1	6.6 ± 0.6	2.5 ± 0.8	5.0 ± 0.9	4.8 ± 0.4	4.1 ± 0.1
sum of phenols		**15,4 ± 4,4**	**24,6 ± 4,4**	**13,6 ± 4,3**	**9,9 ± 1,0**	**9,7 ± 0,7**	**8,8 ± 2,0**
C13 norisoprenoïds							
3-hydroxy-β-damascone	*2535*	66.4 ± 2.9	74.1 ± 0.1	64.7 ± 0.2	49.8 ± 3.2	47.8 ± 10.1	40.7 ± 2.7
unknown C13 (I)	*2572*	20.6 ± 2.6	20.9 ± 3.0	18.4 ± 2.1	12.0 ± 0.3	10.1 ± 1.6	9.5 ± 1.4
3-oxo-α-ionol	*2637*	26.3 ± 2.6	24.9 ± 2.9	23.1 ± 2.3	23.5 ± 1.0	22.5 ± 4.2	16.4 ± 1.2
unknown C13 (II)	*2654*	8.3 ± 0.7	10.7 ± 1.3	8.6 ± 1.3	3.9 ± 0.0	3.7 ± 0.5	2.5 ± 0.5
3-hydroxy-7,8-dehydro-β-ionol	*2667*	8.7 ± 1.9	7.4 ± 3.0	7.2 ± 1.8	10.9 ± 1.0	10.0 ± 1.4	10.0 ± 0.5
3-hydroxy-7,8-dihydro-β-ionol	*2757*	9.0 ± 0.6	11.7 ± 0.6	16.5 ± 0.4	8.6 ± 0.3	10.4 ± 2.2	6.7 ± 0.2
vomifoliol	*3131*	24.7 ± 5.5	26.1 ± 5.3	22.0 ± 4.0	13.7 ± 0.2	13.1 ± 3.4	6.0 ± 0.4
sum of C13 norisoprenoïds		**163,9 ± 12,7**	**175,8 ± 10,6**	**160,4 ± 10,4**	**122,4 ± 5,6**	**117,5 ± 23,4**	**91,8 ± 3,1**

[a]flusi-3 : three applications each 4 weeks ; flusi-6 : six applications each 2 weeks. [b]mean of three experiments ; underlined values were significantly different from the control ($p<0.05$) ; [c]linear Retention Index calculated on DB-WAX capillary column using n-hydrocarbons standards (VAN DER DOOL and KRATZ, 1963)

[a]flusi-3 : trois applications toutes les 4 semaines ; flusi-6 : six applications toutes les 2 semaines ; [b]moyenne de trois répétitions ; les valeurs soulignées sont significativement différentes par rapport au témoin ($p<0,05$) ; [c]Index de Rétention linéaire obtenu sur colonne capillaire DB-WAX à partir d'hydrocarbures standards (VAN DER DOOL and KRATZ, 1963)

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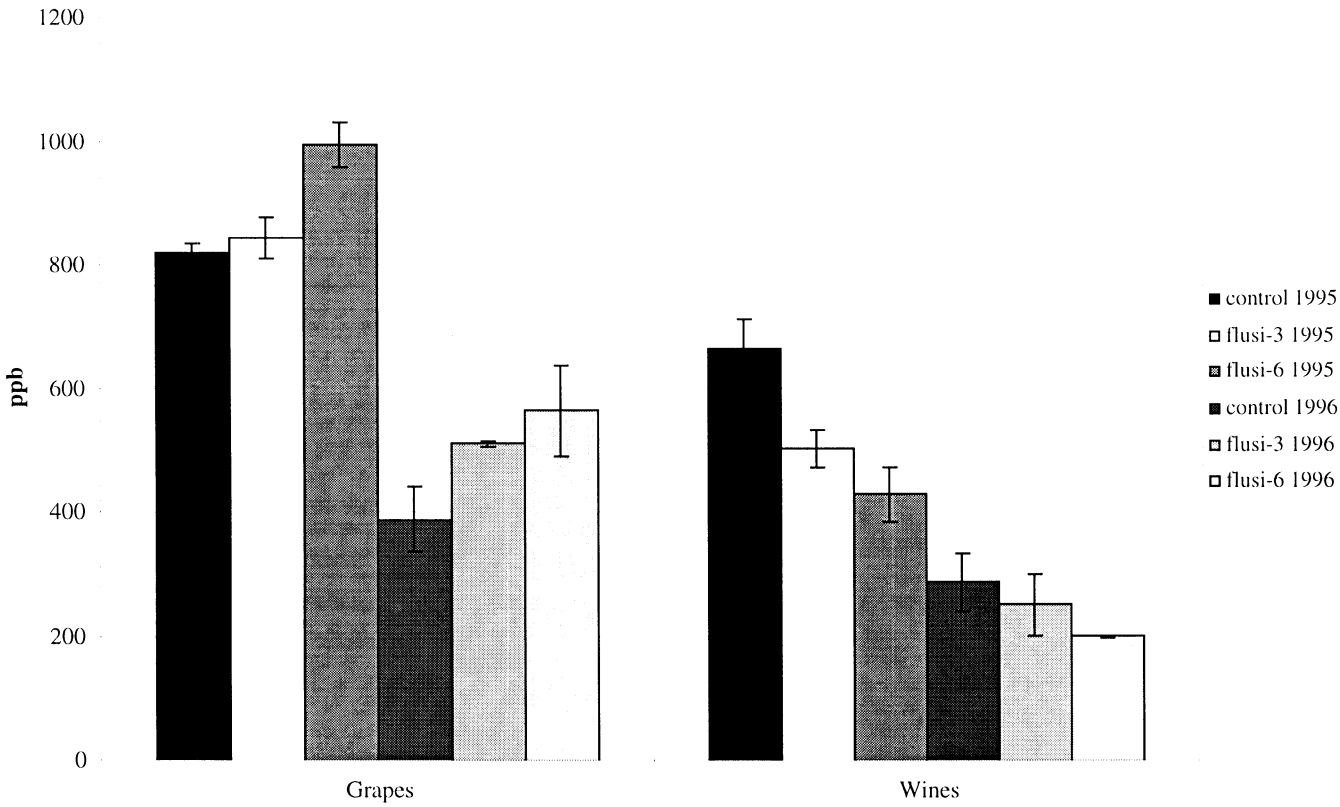


Fig. 1 — Influence of flusilazole on the sum of bound typical compounds of Muscat aroma (linalool + geraniol + nerol) in grapes and wines in 1995 and 1996 experiments

Note : flusi-3 : three applications each 4 weeks ; flusi-6 : six applications each 2 weeks

Fig. 1 — Influence du flusilazole sur la somme des composés liés caractéristiques de l'arôme Muscat (linalol + géraniol + nérol) dans les baies et les vins lors des campagnes 1995 et 1996

Note : flusi-3 : trois applications toutes les 4 semaines ; flusi-6 : six applications toutes les 2 semaines

CONCLUSION

In an earlier study, we had reported that SBI treatments modified the biosynthesis of aroma compounds in grapes (AUBERT *et al.*, 1997). This study clearly showed that flusilazole treatments of vine also decreased the levels of prefermentative C6 compounds and fermentative volatile compounds, such as 2-phenylethanol and 2- and 3-methylbutanol, in wines. A similar decrease of C6 compounds was observed in grapes treated with flusilazole (AUBERT *et al.*, 1997), and these results strongly suggested that flusilazole treatments could affect the enzyme involved at some stage of the lipid degradation leading to C6 compounds. As regards ethanol and fusel alcohols, a direct action of flusilazole treatments on the metabolism pathways of yeast involved in the formation of these alcohols could be hypothesized. Nevertheless, as SBI treatments of vines modified the composition of grape (AUBERT *et al.*, 1997), *i.e.* the fermentation medium, an indirect action could not be discarded. Furthermore, a decrease of the levels of the free and bound monoterpenols was observed in the wines treated suggesting that flusilazole induced some modification of the yeast metabolism involving these free and bound compounds. Indeed, PAISARNRAT (1985) studied the catabolism pathways of 1-^{3}H geraniol in muscat cells cultivated *in vitro*, and concluded that geraniol was partially used in the biosynthesis of sterols. On the other hand, a recent work showed that the ergosterol content of *S. cerevisae* decreased when the fermentation medium contained flusilazole (DOIGNON, 1991). Therefore it seemed plausible that in the presence of this SBI, a regulatory mechanism for sterol synthesis in yeast could involve the free and bound monoterpenes.

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