Effects of Vine or Bunch Shading on the Glycosylated Flavor Precursors in Grapes of *Vitis vinifera* L. Cv. Syrah

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Effects of the modification of vine or bunch environment on glycoconjugates were studied in Syrah berries over two years. Vines were shaded from berry set to maturity, with black polyethylene nets of different mesh size to obtain 30 and 50% of the direct sunlight. Bunches were naturally shaded by the leaves or artificially with 90% shade bags. Sun-exposed berries were chosen as control berries. A quantitative decrease in levels of glycoconjugates was observed in shaded bunches, particularly for phenolic and C_{13}-norisoprenoidic glycosides. In the same way, vine shading caused a decrease in the contents of glycosides of terpenols, phenols, and C_{13}-norisoprenoids in berries, but the grape environment (microclimate) affected the berry composition more than the vine environment. A cluster thinning experiment confirmed the independence of grapes with regard to the plant for the biosynthesis of the C_{13}-norisoprenoid glycosides.

Keywords: *Vitis vinifera; shade; sun exposure; glycoconjugate; aroma*

INTRODUCTION

In Syrah berries, the aroma potential is characterized by an abundant bound fraction as compared to the free one (Abbott et al., 1990). Among bound compounds, C_{13}-norisoprenoids and volatile phenols are predominant (Abbott et al., 1990; Sefton et al., 1993).

Not only the region and the region-related factors but also the bunch microclimate were shown to modify bound monoterpene and C_{13}-norisoprenoid levels in grapes (Reynolds and Wardle, 1989; Marais et al., 1992; Macaulay and Morris, 1993).

C_{13}-norisoprenoids display interesting olfactory properties (Ohloff, 1978). Consequentely, the glycoconjugated C_{13}-norisoprenoids identified in grapes (Strauss et al., 1987; Sefton et al., 1989; Winterhalter et al., 1990; Baumes et al., 1994) represent an important aroma source in wines.

Carotenoids are generally considered to be precursors of C_{13}-norisoprenoids (Isoe et al., 1972; Enzell, 1985; Williams et al., 1992; Winterhalter, 1993), although the enzymatic systems involved in higher plants have not yet been discovered. The effects of the modifications of vine and bunch environment by a shade cloth on the carotenoid composition in Syrah grapes were previously reported (Bureau et al., 1998). The aim of this study was to investigate the composition of C_{13}-norisoprenoid glycosides and carotenoid degradation products, as well as other glycoconjugates such as glycosylated C6 compounds, alcohols, terpenols and volatile phenols, in grapes subjected to the same environment modifications.

In addition, the effects of the modification of the grape environment on the biosynthesis of these secondary metabolites were compared to those of the bunch environment to assess the independence of the grape. To support this hypothesis of the grape independence, a thinning experiment was also carried out.

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MATERIALS AND METHODS

1. Plant Materials and Treatments. *1.1. 1995 Experiment. a. Plant Materials.* The experiment was conducted in a vineyard of *Vitis vinifera* L. cv. Syrah in the INRA experimental station, Domaine de Pech-Rouge (Aude), France. Vine spacing was 1.1 m in west-northwest/east-southeast oriented rows, with 2.2 m between rows. Vines were trained to bilateral cordon (0.5 m above ground), and the foliage was supported by two wires.

b. Treatments. The imposed shading treatments were put in place when berries reached ∼2−4 mm in diameter (June 20). Artificial bunch and vine shadings were provided with black polyethylene shade nets of different mesh sizes (Diatex S.A., Lyon, France), holding, respectively, 50 and 90% of the direct radiance of sun.

Bunches chosen randomly (one per vine) under the foliage were put in 90% shading nets. The polyethylene net was supported by a wire so that there was no contact between the cluster and the shade net.

Vines were shaded with rectangular shading cages (5 m × 1 m × 2 m), and three adjacent vines were treated per cage.

Three shading treatments were compared to a sunny control: Su, berries of external sides of the bunches exposed to direct sunlight (control); B90, 90% shaded bunches; V50, 50% shaded vines; V90, 90% shaded vines.

c. Grape Sampling. For each traitment, ∼4 kg of grapes was picked at random 40 days after the beginning of veraison (September 4).

1.2. 1996 Experiment. a. Plant Materials. The experiment was conducted in a vineyard of *V. vinifera* L. cv. Syrah in the INRA experimental station, Domaine du Chapitre (Hérault), France. Vine spacing was 1.2 m in northeast/southwest oriented rows, with 2.5 m between rows. Vines were trained to bilateral cordon (0.6 m above ground), and the foliage was supported by two wires.

b. Grapevine Treatments. The imposed shading treatments were put in place when berries reached ∼5 mm in diameter (June 21). The same shading treatments were carried out, except for the 90% vine shading, which was replaced by a 70%

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vine shading. In addition, a sample of natural shaded grapes was also analyzed. Shading treatments included the following: Su, berries of external sides of the bunches exposed to direct sunlight (control); Sh, shaded grapes under foliage; B90, 90% shaded bunches; V50, 50% shaded vines; V70, 70% shaded vines.

c. Grape Sampling. As previously described, grapes were picked at random, 43 days after the beginning of veraison (September 5) for Su and Sh, 47 days after the beginning of veraison (September 9) for V50 and B90, and 50 days after the beginning of veraison (September 12) for V70.

1.3. Thinning Experiment, Modification of Bunch Number per Vine. The exposed leaf area/crop weight ratio was modified by decreasing the bunch number per vine. This experiment was carried out in the above-mentioned homogeneous vineyard (Domaine du Chapitre) in 1996.

a. Grapevine Treatments. The treatments were applied just after the berry set (June 25), and 10 vines were chosen at random for each treatment: V2**,** unthinned vines (control); V1**,** vines with 50% of the bunches removed; V1/2**,** vines with 50% of the bunches removed and 50% of the berries removed from the sides and the tail of each remaining bunch.

b. Sampling. Grapes were picked at maturity (September 6).

1.4. Light Absorption and Temperature Measurements. Light was measured with a photoelectric cell sensitive to visible radiations (400−700 nm). This cell was connected to a quantum radiometer/photometer (LI-COR 190 SA quantum sensor). All measurements were performed in August, on sunny days, at 1:00 p.m. The values of light passing through the shade fabric or inside the canopy were compared to the value of the direct sunlight and were expressed as a percentage of this last value.

The ambient temperature was measured with a thermometer, sheltered in a box placed at the cluster height on the row side opposite the sun. The berry temperature was measured with a radiothermometer.

2. Extraction and Determination of Glycoconjugates. *2.1. Preparation of Samples.* Immediately after harvest, grapes were washed, dried, frozen at −20 °C, and stored prior to analysis. Two hundred grams of berries was deseeded and ground under liquid nitrogen using a Dangoumau ball grinder.

Fifty grams of the powder obtained was suspended in 100 mL of pure water containing 0.5 g of d-gluconic acid lactone (Sigma) to inhibit grape β-D-glucosidase (Razungles et al., 1993). After 15 min of stirring at 4 °C, the mixture was centrifuged (9000*g*, 20 min, 3 °C). The supernatant was filtered through glass wool.

The juice was stirred in the presence of 1 g of polyvinylpolypyrrolidone (Sigma), previously prepared, to eliminate the high levels of phenolic compounds capable of inhibiting the glycosidase activities. The mixture was filtered again through glass wool.

2.2. Fractionation of Free and Bound Fractions of Aroma. The free and bound fractions were separated by adsorption/desorption on Amberlite XAD-2 resin (copolymeric polystyrene and divinylbenzene, 50−80 mesh) (Fluka), according to the method of Günata et al. (1985a) as modified by Razungles et al. (1993).

The clear juice (100 mL) was passed through the XAD-2 column at a flow rate of 1 mL·min^{-1}. The column was rinsed with 100 mL of pure water to eliminate sugars, acids, and other low molecular weight polar compounds.

The free fraction was eluted with 50 mL of pentane/dichloromethane (2:1, v/v). The free fraction was not treated in this work because of its lack of interest.

The bound fraction was eluted with 50 mL of methanol. The methanol extract was concentrated to 1 mL under vacuum (rotavapor) at 35 °C. The extract was then transferred into a small tube and concentrated to dryness at 50 °C under a stream of nitrogen.

2.3. Enzymatic Hydrolysis of the Bound Fraction. The dried glycosidic extract was dissolved in 100 μL of citrate−phosphate buffer (0.2 M, pH 5). The mixture was washed five times with 200 μL of pentane/dichloromethane (2:1, v/v) to eliminate possible traces of free volatiles. Two hundred microliters of enzymatic solution [70 mg of Pektolase 3PA (Grinsted) with glycosidase activities: β-D-apiofuranosidase (18.8 nkat), α-L-rhamnopyranosidase (2.3 nkat), α-L-arabinofuranosidase (212.9 nkat), and β-D-glucopyranosidase (83.4 nkat) in 1 mL of citrate−phosphate buffer (0.2 M, pH 5)]. After stirring, the tube was sealed and placed in a water bath at 40 °C for 16 h. The mixture was then extracted five times with 200 μL of dichloromethane. After the addition of 5 μL of 4-nonanol (3.2 g·L^{-1}) as internal standard, the extract was concentrated to a final volume of 400 μL using a Vigreux column at 47 °C.

2.4. Gas Chromatography (GC) and Gas Chromatography−Mass Spectrometry (GC-MS) Analysis. a. GC Analysis. A Varian 6500 gas chromatograph equipped with a DB-Wax fused silica capillary column (J&W Scientific; 30 m × 0.32 mm i.d.; 0.5 μm film thickness) and an FID detector was used. Operating conditions were as follows: the injector temperature program was set to rise from 20 to 245 °C at 180 °C·min^{-1} and then was isothermal for 80 min. The oven temperature program was set to rise from 60 °C (3 min isothermal) to 245 °C at 3 °C·min^{-1} and then was isothermal for 20 min. The detector temperature was held at 250 °C.

Hydrogen carrier gas flow rate was 1.2 mL·min^{-1}. One microliter was injected.

b. GC-MS Analysis. A gas chromatograph (Hewlett-Packard 5890 series II) was fitted with the above-mentioned column. Temperature programs of the injector and oven were as described above. Helium N60 carrier gas flow rate was 1.3 mL·min^{-1}. A Hewlett-Packard 5889 A mass spectrometer equipped with a quadrupole detector was used for electron impact (EI) mode spectra. The transfer line from GC to MS was heated to 250 °C. The source temperature was kept at 250 °C. EI was recorded at 70 eV in the mass range of *m/e* 29−350 at 1 s intervals. Identifications were carried out by linear retention index, by EI mass spectra with published data, or with data from authentic compounds. One microliter was injected.

c. Statistical Analysis. The analyses of the bound compounds were performed in triplicate with an internal standard (50 g of powder each from the same 200 g of berry powder, see section 2.1). The means of the three concentrations and the standard deviations are reported in the tables. For each compound, the variance analyses were performed between the control berries and each shaded treatment: the values in italic type were significantly different from the control at $p < 0.05$.

RESULTS AND DISCUSSION

1. Effect of Vine or Bunch Shading on Berry Growth and Ripening. In 1995, the 90% vine shading (V90) delayed dramatically both ripening and growth of the berries (Table 1). It was not treated in this work and was replaced with the 70% vine shading in 1996. These important physiological modifications were not observed for the berries of the 90% bunch shading (B90).

The effects of the other vine or bunch shadings on the berry weights and the berry sugar levels are given (Table 1). Except for V90, the shading treatments applied from berry set to maturity did not affect berry growth. However, they generally caused a delay of ripening. Indeed, the berries of the shaded bunches (B90 and Sh) and the berries of the 70% shaded vines (V70) had lower maturity indices than the sunny berries (Su) and the berries of the 50% shaded vines (V50) (Table 1). These observations were already discussed in a previous paper (Bureau et al., 1998). The delay of ripening could explain some differences in bound aroma levels insofar as their levels were shown to increase or decrease during the grape ripening (Cordonnier and Bayonove, 1981; Wilson et al., 1984; Günata et al., 1985b).

Table 1. Effects of Vine or Bunch Shading on Syrah Berry Weights and Maturity

	treatments 1995							treatments 1996									
	Su[a]	B90		V50		V90		Su	Sh	B90		V50		V70			
photosynthetic active radiations (% of direct sunlight value)	100	9.3 AC[b]	2.2 IC[b]	53.3 AC	5.9 IC	9.3 AC	2.3 IC	100	5.1	9.2 AC	1.9 IC	52.1 AC	4.0 IC	25.3 AC	2.4 IC		
berry wt (g per berry)	1.65	1.70		1.69		0.84		2.12	2.17	1.98		2.13		2.07			
pH (20 °C)	3.65	3.50		3.40		3.20		3.60	3.48	3.70		3.71		3.60			
sugar ($g \cdot L^{-1}$)	216	169		212		124		199	176	192		194		174			
total acidity ($mequiv \cdot L^{-1}$)	52	60		56		95		72	92	74		101		97			
maturity index[c]	4.15	2.82		3.26		1.30		2.76	1.91	2.59		1.92		1.79			

[a] Su, control berries exposed to direct sunlight; Sh, berries from the shaded bunches under the foliage; B90, berries from the 90% shaded bunches; V50, V70, V90, berries from the 50, 70, and 90% shaded vines, respectively. [b] AC, sensor placed above the canopy; IC, sensor placed inside the canopy. [c] Maturity index = sugar ($g \cdot L^{-1}$)/total acidity ($mequiv \cdot L^{-1}$) values corresponding to samples of 100 berries.

2. Effect of Bunch Shading on Glycoconjugated Contents. In 1995, the bunch shading (B90) decreased C6 compound glycosides, although in 1996 this decrease was not observed (Figure 1; Table 2). In the same way, the contents of alcohol glycosides were shown to depend on year. Thus, B90 displayed lower levels in 1995 and higher levels in 1996 than the control. The B90 bunch shading decreased the total level of terpenyl glycosides in both years. However, in 1996, the difference with the control was significant for the artificial shading but not demonstrated for the natural one (Sh). Moreover, a vintage effect was particularly remarkable on monoterpenyl glycosides, which increased by ~50% in 1996 with respect to 1995.

The total amount of bound volatile phenols was clearly lower in the berries of shaded bunches (B90 and Sh) than in the sun-exposed berries (Su) (Table 2). In the same way, Zoecklein et al. (1998) reported that leaf removal increased the concentration of total phenol glycosides in Riesling and Chardonnay berries.

The volatile phenols are formed via the shikimic pathway, like other well-studied phenolic compounds. Many works reported the effects of the cluster exposure on the berry compositions of the soluble phenolic compounds. For instance, some anthocyanin and flavonol contents decreased in grape berries grown in natural or artificial shade (Kliewer, 1977; Morrison and Noble, 1990; Gao and Cahoon, 1994; Price et al., 1995). Moreover, some enzymes of the shikimic pathway were light-stimulated. That was the case of the shikimate kinase and the phenylalanine ammonia-lyase (Roubelakis-Angelakis and Kliewer, 1986; Richter, 1993). Accordingly, glycosides of methyl vanillate, zingerone, vanillol, guaiacyl ethanol, and methyl syringoate were significantly less abundant in the shaded berries (B90 and Sh) than in the sun-exposed berries (Su). However, the bunch shading did not modify the levels of methyl salicylate, eugenol, 4-vinylphenol, 3,4-dimethoxyphenol, and benzyl salicylate glycosides. Moreover, other important shikimate derivatives such as benzyl alcohol and 2-phenylethanol were not affected.

The total amounts of the bound C_{13}-norisoprenoids were much higher in the sun-exposed berries (Su) than in the shaded bunches (B90 and Sh) (Table 2), in agreement with the findings of Marais et al. (1992). The effect of light on the increase of β-damascenone precursor concentration in the cell culture of concord grapes (Shure and Acree, 1994) and of 3-hydroxy-β-damascone concentration in hypocotyls of *Phaseolus vulgaris* (Kato-Noguchi, 1996) was reported, but this fact was not confirmed by Marais et al. (1992). C_{13}-norisoprenoids would come from carotenoid degradation (Isoe et al., 1972; Enzell, 1985; Kanasawud and Crouzet, 1990; Lutz and Winterhalter, 1992), which increases with light (Isoe et al., 1972; Pesek and Warthesen, 1990). In grape berries, the total carotenoid content decreases during veraison and ripening (Razungles et al., 1988), yet their content decrease was more important in the sun-exposed berries (Su) than in the shaded berries (B90 and Sh) (Bureau et al., 1998). This could explain why the C_{13}-norisoprenoid contents were higher in the sun-exposed berries (Su) than in the shaded berries (B90 and Sh). The same trend was observed for most bound C_{13}-norisoprenoids (Table 2). According to Enzell (1985), Williams et al. (1992), and Winterhalter (1993), grape C_{13}-norisoprenoids would be the degradation products of lutein, neoxanthin, violaxanthin, and 5,6-epoxylutein. This was consistent with the previous observation that the level decrease of lutein, flavoxanthin, and a neochrome−neoxanthin mixture during grape ripening was light-stimulated (Bureau et al., 1998).

The changes in volatile phenol and C_{13}-norisoprenoid levels in the Syrah berries due to natural shading (Sh) were similar to those in artificial shading (B90). This result was consistent with the similarity in the sunlight transmissions by the foliage and the 90% shading bags. However, the natural shading produced a cooler ambient temperature (5 °C lower) and the artificial shading produced a warmer ambient temperature (2 °C higher) compared to the sun-exposed treatment. Thus, glycoconjugates could be more influenced by light environment than temperature, but this statement should be confirmed by further experiments.

3. Effects of Vine Shading on Glycoconjugate Contents. The total amounts of glycosylated C6 compounds and alcohols were slightly modified by vine shading (V50 and V70). However, regarding the major alcohols, vine shading caused a decrease in the phenylethanol levels but not in the benzyl alcohol levels (Table 2).

The berries of the shaded vines (V50 and V70) tended to have lower bound terpenol levels than the sun-exposed berries (Su), in particular for geraniol, (*E*)- and (*Z*)-8-hydroxylinalool, geraniol hydrate, and geranic acid (Table 2).

Vine shading caused a lowering in the bound volatile phenol contents in the berries. This decrease was higher in 1996 (V50 and V70) than in 1995 (V50) (Table 2). It was previously reported that vine shading decreased the total soluble phenol fraction in Cabernet Sauvignon grapes (Morrison and Noble, 1990).

Contrary to bound 3,4-dimethoxyphenol, the levels of bound ethyl homovanillate, zingerone, and vanillol decreased in the berries of the shaded vines (Table 2).

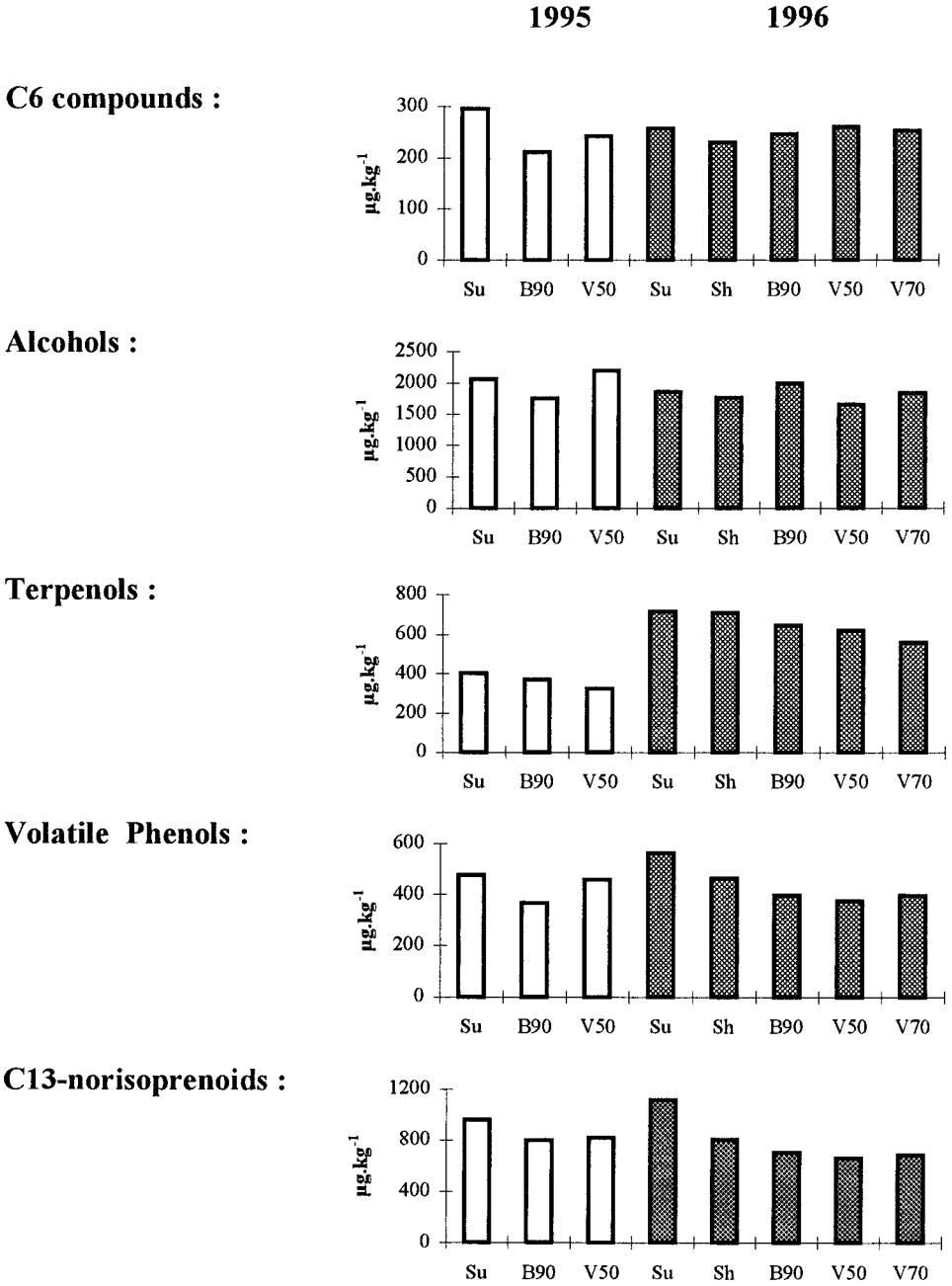

Figure 1. Influence of bunch or vine shading on the glycoconjugated aroma contents of Syrah grapes: treatments in 1995 and 1996 (amounts in micrograms per kilogram of berries). Su, control berries exposed to direct sunlight; Sh, berries from the shaded bunches under the foliage; B90, berries from the 90% shaded bunches; V50 and V70, berries from the 50 and 70% shaded vines, respectively. For 1996, values shown are means of three replications of the same sample.

Some of these compounds seemed to be more sensitive than others.

The total levels of bound C_{13}-norisoprenoids were lower in the berries of the shaded vines (V50 and V70) than in the sunny berries (Su) (Table 2). This was observed for every compound, with the exception of the 4-oxo-7,8-dihydro-β-ionol and 3-hydroxy-β-ionone mixture and 4,5-dihydrovomifoliol in 1995. Moreover, the carotenoid level decrease was lower in grapes of the shaded vines than in the sunny berries between veraison and maturity (Bureau et al., 1998), resulting in lower C_{13}-norisoprenoid accumulation.

Fifty and 70% vine shading caused changes in the glycosylated flavor composition of the Syrah berries. These changes could be due to the foliage shading and/or the grape shading. However, berry composition was less affected by a 50 or 70% vine shading than by a 90% bunch shading (Figure 1). It should be kept in mind that a 90% vine shading dramatically affected both ripening and growth of the berries.

4. Effect of Cluster Thinning on Glycoconjugate Contents. If there was no migration of glycosylated compounds from leaves to grape berries (Gholami et al., 1995), an artificial decrease of fruit amount per vine should not increase the accumulation of these compounds in the remaining clusters. To study the effect of foliage on the berry composition, a cluster thinning experiment was carried out in 1996. The exposed leaf area/crop weight ratio was modified by decreasing the bunch number per vine.

In general, reducing crop level raise berry weight (Reynolds et al., 1994), although, in our conditions, the

Table 2. Influence of Bunch or Vine Shading on the Glycosylated Aroma Contents of Syrah Grapes: Treatments[a] in 1995 and 1996 (Amounts in Micrograms per Kilogram of Berries)[b]

		treatments 1995			treatments 1996				
compound	LRI[c]	Su	B90	V50	Su	Sh	B90	V50	V70
C6 compounds									
1-hexanol	1356	157.6 ± 1.8	*95.7* ± 4.8	*113.0* ± 3.1	144.7 ± 7.8	*110.1* ± 9.4	134.0 ± 2.7	125.3 ± 11.1	*120.8* ± 4.0
Z 3-hexen-1-ol	1386	75.6 ± 0.7	*57.0* ± 1.8	*59.6* ± 1.1	75.3 ± 2.4	84.0 ± 7.1	76.1 ± 1.9	*92.7* ± 7.7	*102.8* ± 3.5
E 2-hexen-1-ol	1408	63.0 ± 0.6	*58.2* ± 1.8	*69.5* ± 1.6	36.9 ± 1.2	35.8 ± 3.0	36.4 ± 0.8	41.4 ± 3.3	*29.2* ± 0.8
total		**296.2 ± 2.6**	***210.9* ± 8.3**	***242.1* ± 5.7**	**256.9 ± 11.4**	**230.0 ± 19.5**	**246.6 ± 5.2**	**259.5 ± 22.1**	**252.9 ± 8.3**
Alcohols									
2- and 3-methylbutanol	1210	65.9 ± 0.8	*45.0* ± 2.7	*51.4* ± 2.5	71.7 ± 6.0	*37.0* ± 4.8	*60.1* ± 2.3	*46.9* ± 1.1	*42.8* ± 2.4
3-methyl-3-buten-1-ol + pentanol	1250	108.8 ± 1.2	*92.2* ± 5.3	*85.9* ± 2.8	97.0 ± 7.6	78.3 ± 9.7	98.5 ± 4.2	83.6 ± 4.9	94.6 ± 5.6
2-methyl-2-buten-1-ol	1322	61.4 ± 1.0	*52.4* ± 2.6	*56.5* ± 1.5	67.5 ± 3.4	*53.2* ± 4.9	73.9 ± 2.3	*56.1* ± 1.5	*55.6* ± 2.4
benzyl alcohol	1870	1521.4 ± 33.4	*1361.3* ± 16.7	*1723.0* ± 29.6	1275.8 ± 23.5	1281.9 ± 15.6	*1475.4* ± 2.1	*1205.0* ± 11.9	*1354.8* ± 16.4
2-phenylethanol	1905	300.7 ± 4.8	*200.1* ± 7.3	*279.2* ± 2.1	337.8 ± 15.5	310.1 ± 8.9	*280.5* ± 2.8	*258.2* ± 10.3	*277.6* ± 5.8
total		**2058.2 ± 40.0**	***1751.0* ± 26.7**	***2196.1* ± 29.8**	**1849.7 ± 19.0**	***1760.6* ± 8.0**	***1988.5* ± 6.5**	***1649.8* ± 12.2**	**1825.3 ± 13.1**
Terpenols									
cis-furan-linalool oxide	1472	4.0 ± 0.1	*5.4* ± 0.1	3.8 ± 0.1	5.6 ± 0.9	6.9 ± 0.5	6.7 ± 0.3	6.9 ± 0.3	6.2 ± 0.1
trans-pyran-linalool oxide	1739	11.1 ± 0.4	11.0 ± 0.2	10.6 ± 0.1	26.0 ± 1.7	25.0 ± 1.5	23.9 ± 0.0	*21.8* ± 1.3	*21.1* ± 0.7
cis-pyran-linalool oxide	1763	11.3 ± 0.4	*12.5* ± 0.4	*9.2* ± 0.1	16.5 ± 1.6	17.5 ± 0.9	16.6 ± 0.8	15.3 ± 0.4	*13.0* ± 0.6
nerol	1800	7.9 ± 0.4	*4.9* ± 0.2	*5.7* ± 0.6	9.7 ± 1.1	8.0 ± 0.4	8.6 ± 1.1	*5.9* ± 0.2	*5.4* ± 0.2
geraniol	1848	44.4 ± 1.7	*31.9* ± 1.0	*28.5* ± 1.4	76.6 ± 3.7	*65.8* ± 2.9	80.7 ± 4.3	*62.4* ± 4.0	*62.0* ± 2.0
3,7-dimethyl-1,5-octadien-3,7-diol	1949	7.5 ± 0.0	*5.8* ± 0.2	7.2 ± 0.3	39.9 ± 2.8	40.9 ± 4.7	*34.0* ± 1.1	35.1 ± 3.4	*32.1* ± 1.1
(*E*)-8-hydroxylinalool	2270	78.3 ± 0.6	*91.9* ± 2.2	*68.5* ± 1.5	143.4 ± 8.9	149.6 ± 11.6	132.0 ± 0.5	124.6 ± 11.0	*120.1* ± 4.9
geraniol hydrate + (*Z*)-8-hydroxy-linalool	2310	185.9 ± 3.7	*163.5* ± 4.3	*154.5* ± 3.3	338.2 ± 24.5	346.4 ± 29.2	*294.4* ± 3.2	305.0 ± 29.6	*252.7* ± 12.5
geranic acid	2329	33.1 ± 22.8	28.0 ± 28.4	*23.6* ± 31.2	49.0 ± 1.5	*42.3* ± 3.8	*36.6* ± 0.9	*35.7* ± 3.7	*35.6* ± 1.4
p-1-menthen-7,8-diol	2517	16.6 ± 14.8	15.0 ± 15.5	*14.0* ± 20.2	8.6 ± 0.6	*6.5* ± 0.6	8.4 ± 0.2	*5.7* ± 0.2	*6.2* ± 0.2
total		**399.9 ± 6.9**	***370.0* ± 7.9**	***325.6* ± 4.7**	**713.4 ± 31.2**	**709.0 ± 55.6**	***641.9* ± 7.7**	**618.6 ± 50.6**	***554.5* ± 20.1**
Phenols									
methyl salicylate	1765	49.2 ± 1.0	48.8 ± 2.5	*81.8* ± 2.1	28.3 ± 1.1	27.3 ± 2.0	29.5 ± 2.2	26.7 ± 0.1	*41.9* ± 1.6
guaiacol	1850	14.4 ± 0.2	*9.9* ± 0.6	*16.9* ± 0.5	15.2 ± 0.7	16.3 ± 0.5	15.8 ± 1.6	14.1 ± 0.8	15.5 ± 1.1
phenol + *o*-cresol	2000	10.7 ± 1.0	*8.7* ± 0.6	9.1 ± 0.5	24.7 ± 1.0	*22.4* ± 0.7	*22.0* ± 0.2	*19.5* ± 0.9	*21.8* ± 0.3
eugenol	2154	3.5 ± 0.2	3.0 ± 0.3	*2.3* ± 0.3	10.7 ± 0.7	*14.2* ± 0.5	*13.4* ± 0.5	10.7 ± 0.8	10.3 ± 0.1
4-vinylguaiacol	2180	24.7 ± 0.6	*14.3* ± 1.1	*10.2* ± 0.2	28.6 ± 1.6	*23.2* ± 0.5	28.6 ± 0.5	*22.9* ± 0.7	28.5 ± 1.2
4-vinylphenol	2377	6.8 ± 0.4	*8.7* ± 1.0	*4.9* ± 0.7	18.9 ± 2.4	14.9 ± 1.0	18.6 ± 1.9	*13.1* ± 0.9	15.7 ± 0.6
methyl vanillate	2586	24.3 ± 0.8	*15.0* ± 0.8	*16.1* ± 1.4	41.5 ± 1.1	*30.9* ± 1.4	*16.6* ± 0.3	*19.6* ± 1.2	*17.4* ± 0.9
acetovanillone	2620	nq	nq	nq	23.5 ± 3.1	19.1 ± 0.6	*17.1* ± 0.5	17.9 ± 2.5	*14.7* ± 1.0
3,4-dimethoxyphenol	2750	16.8 ± 0.4	*22.2* ± 0.3	17.5 ± 1.2	10.3 ± 0.7	*14.7* ± 0.8	*14.7* ± 0.3	*16.8* ± 1.9	*16.0* ± 0.8
ethyl homovanillate	2759	14.9 ± 1.7	*5.9* ± 0.5	*7.4* ± 1.0	8.8 ± 0.6	7.3 ± 0.7	6.8 ± 1.1	*4.7* ± 0.9	*3.4* ± 0.4
benzyl salicylate	2767	4.2 ± 0.3	*14.9* ± 1.6	*24.9* ± 1.5	10.5 ± 0.4	9.7 ± 1.1	10.5 ± 0.3	*8.3* ± 1.1	*9.6* ± 0.3
zingerone	2779	24.1 ± 2.2	*13.8* ± 0.8	*11.4* ± 1.3	11.1 ± 0.5	*5.7* ± 0.2	*6.6* ± 0.4	*5.0* ± 0.1	*3.3* ± 0.2
vanillol	2787	19.0 ± 2.0	*9.5* ± 0.9	*7.1* ± 0.6	13.4 ± 0.5	13.3 ± 0.8	*7.4* ± 0.6	*6.7* ± 0.4	*5.9* ± 0.1
vanilloyl methyl ketone	2800	8.8 ± 0.5	8.9 ± 0.9	*6.3* ± 0.5	26.2 ± 1.7	*16.2* ± 1.3	*11.6* ± 0.1	*19.6* ± 1.9	*21.5* ± 1.5
tyrosol isomer	2820	31.9 ± 2.3	26.6 ± 2.8	*23.6* ± 3.1	35.5 ± 0.9	*27.5* ± 2.2	*26.9* ± 0.5	*24.0* ± 0.9	*22.7* ± 0.5
guaiacyl ethanol	2830	36.9 ± 2.5	*11.8* ± 1.1	*17.1* ± 1.3	83.5 ± 5.5	*26.8* ± 1.1	*15.0* ± 1.1	*12.1* ± 0.2	*12.6* ± 0.2
syringaldehyde + methyl 4-hydroxy-benzoate	2930	20.6 ± 1.6	18.1 ± 2.1	*27.3* ± 0.5	30.9 ± 0.6	29.9 ± 1.8	*23.6* ± 1.5	*21.7* ± 1.0	*21.7* ± 1.2
methyl syringoate	2957	25.5 ± 1.1	*8.3* ± 0.7	*7.6* ± 1.2	27.6 ± 2.5	*19.8* ± 0.4	*8.1* ± 0.1	*10.4* ± 0.8	*9.8* ± 0.6
guaiacyl propanol	2969	24.4 ± 1.6	29.0 ± 3.2	*56.6* ± 2.3	31.2 ± 5.0	32.2 ± 2.7	38.3 ± 3.2	25.2 ± 3.9	25.7 ± 2.6
methyl 2,6-dihydroxy-benzoate + tyrosol	2985	48.4 ± 3.5	*30.9* ± 1.3	*58.5* ± 3.9	25.2 ± 2.1	*32.0* ± 1.1	*14.5* ± 0.8	24.1 ± 1.3	27.3 ± 1.8
unknown phenol	3042	65.4 ± 3.9	58.4 ± 4.6	*50.2* ± 2.8	56.3 ± 3.2	57.8 ± 4.1	*49.5* ± 2.2	50.8 ± 4.2	50.8 ± 3.0
total		**474.7 ± 15.5**	***366.8* ± 6.3**	***456.9* ± 9.7**	**561.9 ± 21.9**	***461.1* ± 13.0**	***395.2* ± 6.4**	***373.6* ± 19.6**	***396.2* ± 7.7**
C13-norisoprenoids									
3-hydroxy-β-damascone	2531	61.8 ± 1.0	*58.1* ± 1.9	*45.5* ± 1.7	40.3 ± 0.5	38.0 ± 4.8	*33.1* ± 1.0	*32.9* ± 3.8	*31.7* ± 1.6
unknown norisoprenoid (MW = 212)	2571	31.0 ± 1.2	*24.1* ± 1.2	*16.1* ± 1.7	20.8 ± 0.6	*16.1* ± 1.6	*15.4* ± 0.2	*13.8* ± 1.3	*14.1* ± 0.5
3-oxo-α-ionol	2629	165.5 ± 2.4	*157.2* ± 2.4	*153.8* ± 2.8	141.1 ± 2.5	126.7 ± 8.7	*122.0* ± 1.7	*107.4* ± 10.9	*109.4* ± 5.6
3-hydroxy-7,8-dihydro-β-ionol	2659	50.7 ± 1.8	*29.2* ± 1.8	*41.0* ± 1.8	47.5 ± 1.7	*20.5* ± 1.9	*15.8* ± 1.6	*13.7* ± 0.2	*14.9* ± 1.4
4-oxo-7,8-dihydro-β-ionol + 3-hydroxy-β-ionone	2672	13.0 ± 0.4	13.7 ± 0.3	*19.2* ± 0.9	14.1 ± 0.3	*10.3* ± 0.8	*5.9* ± 0.5	*6.1* ± 0.3	*6.8* ± 0.6
3-oxo-3,4-dihydro-actinidiolide	2681	14.8 ± 0.7	*6.4* ± 0.8	13.7 ± 0.9	6.7 ± 0.5	*3.3* ± 0.2	*2.1* ± 0.0	*2.8* ± 0.1	*2.4* ± 0.2
3-oxo-7,8-dihydro-α-ionol	2704	39.1 ± 1.3	*29.3* ± 4.1	*25.3* ± 0.7	52.9 ± 2.2	*33.5* ± 1.4	*32.7* ± 1.5	*24.1* ± 0.8	*25.8* ± 2.2
3-hydroxy-7,8-dehydro-β-ionol	2746	25.6 ± 0.1	23.9 ± 1.2	*20.8* ± 0.6	25.9 ± 1.6	22.8 ± 2.4	*18.9* ± 0.4	*19.0* ± 1.8	*20.2* ± 1.1
4,5-dihydrovomifoliol	3062	61.2 ± 1.0	*83.3* ± 3.9	*69.7* ± 5.0	65.5 ± 1.6	*41.2* ± 2.9	*38.2* ± 2.2	*31.2* ± 1.0	*34.2* ± 2.5
unknown norisoprenoid (MW = 226)	3070	15.7 ± 0.2	*5.2* ± 0.2	*6.4* ± 0.5	25.6 ± 1.1	*10.8* ± 0.8	*13.4* ± 0.7	*11.0* ± 0.5	*9.7* ± 0.1
vomifoliol	3128	461.2 ± 18.5	*355.5* ± 2.8	*394.2* ± 9.2	634.3 ± 19.3	*451.4* ± 34.6	*387.7* ± 11.7	*378.5* ± 18.8	*397.5* ± 18.8
7,8-dihydrovomifoliol	3183	16.1 ± 0.5	*13.1* ± 0.4	*9.6* ± 0.7	42.2 ± 2.5	*27.9* ± 1.5	*20.8* ± 0.8	*17.7* ± 1.4	*19.2* ± 1.1
total		**955.7 ± 23.5**	***799.0* ± 1.2**	***815.3* ± 12.5**	**1116.9 ± 26.5**	***802.4* ± 59.8**	***706.0* ± 15.2**	***658.4* ± 38.5**	***685.9* ± 34.6**

[a] Su, control berries exposed to direct sunlight; Sh, berries of the shaded bunches under the foliage; B90, berries of the 90% shaded bunches; V50 and V70, berries of the 50 and 70% shaded vines, respectively. [b] Mean of three replications of the same sample; the values in italic type were significantly different from the control sunny berries Su ($p < 0.05$); nq, not quantified. [c] LRI, linear retention index calculated on DB-Wax capillary column.

Table 3. Effects of Bunch Number per Vine on Syrah Berry Weights and Maturity

	V2[a]	V1	V1/2
crop wt (kg per vine)	2.538	1.388	0.741
berry wt (g per berry)	2.35	2.33	2.35
pH (20 °C)	3.53	3.63	3.61
sugar ($g \cdot L^{-1}$)	174	203	203
total acidity ($mequiv \cdot L^{-1}$)	80	82	82
maturity index[b]	2.17	2.47	2.47

[a] V2, unthinned vines (control); V1, vines with 50% of the bunches removed; V1/2, vines with 50% of the bunches removed and 50% of the berries removed from each remaining bunch. [b] Maturity index = sugar ($g \cdot L^{-1}$)/total acidity ($mequiv \cdot L^{-1}$).

bunch number decrease did not modify the berry growth (Table 3) as mentioned by Iacono et al. (1994). However, it accelerated berry ripening slightly. The sugar levels were higher in the V1 and V1/2 berries than in the V2 berries.

The total amounts of bound C6 compounds were lower in V1 and V1/2 berries than in V2 berries (Table 4) in accordance with their higher ripening, as previously observed on free C6 compounds (Cordonnier and Bayonove, 1981). Indeed, this difference was particularly important for (*Z*)-3-hexen-1-ol, which decreased sharply during maturation. On the contrary, the bound terpenol levels were higher in the V1 and V1/2 berries than in the V2 berries. Their levels rose when the bunch number per vine was reduced. This increase could also result from the higher maturity of the V1 and V1/2

Table 4. Influence of Bunch Number per Vine on the Glycosylated Aroma Contents of Syrah Grapes: Treatments[a] in 1996 (Amounts in Micrograms per Kilogram of Berries)[b]

compound	LRI[c]	V2	V1	V1/2
C6 compounds				
1-hexanol	1356	138.6 ± 5.3	128.7 ± 10.0	127.9 ± 7.3
Z 3-hexen-1-ol	1386	118.2 ± 4.6	*87.8* ± 6.4	*83.1* ± 4.7
E 2-hexen-1-ol	1408	41.1 ± 1.8	36.6 ± 2.3	37.3 ± 2.2
total		**297.9 ± 11.6**	***253.1*** **± 18.7**	***248.3*** **± 14.0**
Alcohols				
2- and 3-methylbutanol	1210	52.4 ± 2.6	54.5 ± 2.1	56.9 ± 4.9
3-methyl-3-buten-1-ol + pentanol	1250	78.7 ± 4.5	83.9 ± 1.8	83.3 ± 6.1
2-methyl-2-buten-1-ol	1322	52.4 ± 2.3	58.0 ± 5.7	*73.5* ± 4.8
benzyl alcohol	1870	1043.5 ± 33.3	*1141.4* ± 31.3	1063.5 ± 13.8
2-phenylethanol	1905	277.2 ± 8.1	*304.1* ± 16.2	*312.6* ± 6.5
total		**1504.3 ± 49.7**	***1641.9*** ± 39.1	**1589.8 ± 22.0**
Terpenols				
cis-furan-linalool oxide	1472	7.5 ± 0.2	7.2 ± 0.3	6.1 ± 0.8
trans-pyran-linalool oxide	1739	17.7 ± 0.6	*20.0* ± 1.2	*19.4* ± 0.7
cis-pyran-linalool oxide	1763	11.4 ± 0.8	13.1 ± 1.1	11.8 ± 0.7
nerol	1800	8.4 ± 0.3	7.8 ± 0.5	*10.7* ± 0.4
geraniol	1848	55.2 ± 0.9	*67.6* ± 2.8	64.8 ± 6.5
3,7-dimethyl-1,5-octadien-3,7-diol	1949	18.7 ± 0.8	*24.4* ± 1.5	*25.4* ± 0.4
(*E*)-8-hydroxylinalool	2270	98.3 ± 2.9	*118.6* ± 9.4	*113.8* ± 3.6
geraniol hydrate + (*Z*)-8-hydroxylinalool	2310	221.4 ± 7.3	*310.9* ± 28.6	*361.0* ± 11.1
geranic acid	2329	9.4 ± 0.4	10.7 ± 1.1	*11.3* ± 0.2
p-1-menthen-7,8-diol	2517	4.7 ± 0.1	*5.2* ± 0.2	5.5 ± 0.6
total		**452.6** ± 12.4	***585.4*** ± 45.3	***629.9*** ± 19.1
Phenols				
methyl salicylate	1765	19.0 ± 1.1	18.7 ± 1.9	18.2 ± 1.4
guaiacol	1850	15.0 ± 0.7	15.5 ± 0.4	14.9 ± 0.8
phenol + *o*-cresol	2000	10.6 ± 0.2	10.7 ± 0.6	*11.4* ± 0.4
eugenol	2154	3.5 ± 0.0	3.2 ± 0.4	*1.5* ± 0.1
4-vinylguaiacol	2180	14.1 ± 0.3	14.9 ± 1.1	14.2 ± 1.6
4-vinylphenol	2377	6.2 ± 0.6	*7.8* ± 0.7	6.6 ± 0.2
methyl vanillate	2586	28.7 ± 0.8	31.2 ± 2.9	*31.3* ± 0.5
3,4-dimethoxyphenol	2750	13.2 ± 0.6	13.3 ± 1.5	13.3 ± 0.5
ethyl homovanillate	2759	10.8 ± 1.1	*8.4* ± 1.0	12.2 ± 1.5
benzyl salicylate	2767	9.9 ± 0.9	*7.8* ± 0.3	*7.7* ± 0.4
zingerone	2779	5.0 ± 0.3	*8.3* ± 0.4	*6.1* ± 0.3
vanillol	2787	14.2 ± 0.8	14.0 ± 1.4	*16.0* ± 0.2
vanilloyl methyl ketone	2800	4.1 ± 0.1	*5.6* ± 0.4	*6.2* ± 0.9
tyrosol isomer	2820	21.1 ± 0.5	21.9 ± 2.0	*25.1* ± 0.5
guaiacyl ethanol	2830	23.9 ± 0.9	*32.0* ± 1.9	*46.8* ± 1.9
syringaldehyde + methyl 4-hydroxybenzoate	2930	7.4 ± 1.5	*4.5* ± 0.5	5.1 ± 0.2
methyl syringoate	2957	11.7 ± 0.9	11.8 ± 1.4	*15.6* ± 1.9
guaiacyl propanol	2969	28.9 ± 2.4	*21.0* ± 2.9	*17.6* ± 1.8
methyl 2,6-dihydroxybenzoate + tyrosol	2985	20.1 ± 2.4	*26.9* ± 0.6	23.2 ± 0.7
unknown phenol	3042	76.6 ± 0.4	83.4 ± 5.6	79.5 ± 3.8
total		**343.8** ± 5.3	**361.1** ± 22.2	***372.6*** ± 8.3
C13-norisoprenoids				
3-hydroxy-β-damascone	2531	33.3 ± 0.9	33.7 ± 2.6	34.3 ± 0.9
unknown norisoprenoid (MW = 212)	2571	10.4 ± 0.3	*14.9* ± 0.2	*15.6* ± 0.3
3-oxo-α-ionol	2629	105.4 ± 2.1	112.4 ± 8.9	107.3 ± 2.5
3-hydroxy-7,8-dihydro-β-ionol	2659	12.4 ± 0.6	*15.5* ± 1.3	*16.6* ± 0.6
4-oxo-7,8-dihydro-β-ionol + 3-hydroxy-β-ionol	2672	9.3 ± 0.3	9.7 ± 0.2	10.5 ± 1.0
3-oxo-7,8-dihydro-α-ionol	2704	28.6 ± 2.5	34.2 ± 3.7	*39.6* ± 3.2
3-hydroxy-7,8-dehydro-β-ionol	2746	18.9 ± 1.3	18.7 ± 1.9	18.4 ± 1.3
4,5-dihydrovomifoliol	3062	32.7 ± 2.2	34.3 ± 2.0	34.9 ± 1.4
vomifoliol	3128	295.2 ± 15.5	305.0 ± 29.1	321.2 ± 27.1
7,8-dihydrovomifoliol	3183	9.0 ± 1.5	*14.5* ± 1.1	10.1 ± 1.6
total		**555.0** ± 23.7	**592.8** ± 48.8	**608.4** ± 29.3

[a] V2, vines with two bunches per shoot; V1, vines with one bunch per shoot; V1/2, vines with a half of the bunch per shoot. [b] Mean of three replications of the same sample; the values in italic type were significantly different from the control berries V2 ($p < 0.05$). [c] LRI, linear retention index calculated on DB-Wax capillary column.

berries, as it was reported previously that total bound terpenols increased with maturation (Wilson et al., 1984; Günata et al., 1985b). Most compounds had the same behavior with the exception of *cis*-furan-linalool oxide and *cis*-pyran-linalool oxide (Table 4).

Several volatile phenols levels were higher in the berries from the thinning treatments, particularly vanilloyl methyl ketone and guaiacyl ethanol, although only the V1/2 total level was significantly higher than in the control. This observation was consistent with the higher accumulation of soluble phenols such as anthocyanins in mature berries from cluster thinning treatments (Reynolds et al., 1994; Dokoozlian and Hirschfelt, 1995), but Iacono et al. (1994) reported that these compounds were not significantly affected by cluster thinning.

Conversely, the total levels of bound C_{13}-norisoprenoids were not modified by thinning. This was consistent with the localization of the potential precursors of bound C_{13}-norisoprenoids, carotenoids, which are biosynthesized in the plastids of the berry pulp and skin (Goodwin, 1980; Razungles et al., 1988). These different results seemed to confirm the hypothesis of grape autonomy with regard to glycoconjugates. Other papers reported the independence of grape berries that could produce glycoconjugates (Bravdo et al., 1990; Gholami et al., 1995), but other thinning experiments are necessary to confirm these previous observations.

5. Conclusions. Bunch environment influenced berry composition. Berries of naturally and artificially shaded bunches had lower glycoconjugate contents than sun-exposed berries. The berry microclimate appeared to be very important. Vineyard management practices leading to an improved sunlight penetration to clusters increased the aroma potential of Syrah berries.

Moreover, vine environment influenced berry composition. Berries of sun-exposed vines had higher phenol, terpenol, and C_{13}-norisoprenoid glycoside contents than berries of 50 and 70% shaded vines. However, bunch environment appeared to have more effect on the berry glycoconjugate composition than vine environment. Bunch exposure raised the glycosylated aroma levels in Syrah berries, which could consequently increase the flavor qualities in future wines.

Finally, cluster thinning stimulated ripening and affected the levels of some bound volatile compounds dependent on maturation. On the other hand, this experiment confirmed the independent behavior of grapes in the biosynthesis of the C_{13}-norisoprenoids.

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