Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis vinifera* L cv Sauvignon blanc in field conditions

Catherine Peyrot des Gachons,¹ Cornelis Van Leeuwen,^{1,2*} Takatoshi Tominaga,¹ Jean-Pierre Soyer,³ Jean-Pierre Gaudillère³ and Denis Dubourdieu¹

¹*Faculté d'Œnologie de Bordeaux, 351 Cours de la Libération, F-33405 Talence Cedex, France* ²*ENITA de Bordeaux, 1 Cours du Général de Gaulle, BP 201, F-33175 Gradignan Cedex, France*

³INRA-ECAV, BP 81, F-33883 Villenave d'Ornon, France

Abstract: S-Cysteine conjugate precursors of three volatile thiols were monitored in Vitis vinifera L cv Sauvignon blanc grapes during fruit ripening to assess the influence of vine water and nitrogen status on the grape aroma potential in field conditions. Four dry farmed plots were studied in the Pessac-Léognan and Graves appellations (Bordeaux area) in 1998, which was a very dry vintage, and in 1999, when regular summer rainfall occurred. Soil water-holding capacity ranged from very low to high. Soil total nitrogen content was related to soil organic matter content, which was highly variable on the four plots. Vine vigour was enhanced by both high water and nitrogen status. Major compounds in grapes depended mainly on vine water status. Water deficit-stressed vines produced small berries with low sugar and low total acidity. Grape aroma potential was highest in vines under mild water deficit and moderate nitrogen supply. Severe water deficit stress seemed to limit aroma potential, as did nitrogen deficiency. Consequences for site selection and irrigation management for Sauvignon blanc are discussed. © 2004 Society of Chemical Industry

Keywords: vine; *Vitis vinifera*; Sauvignon blanc; water deficit; leaf water potential; carbon isotope composition; nitrogen status; soil; terroir; shoot growth; leaf area; grape composition; grape aroma; aroma precursors; volatile thiols; 4MMP; 4MMPOH; 3MH

INTRODUCTION

Vine development and fruit composition are highly dependent on environmental conditions and particularly on vine water status and vine nitrogen status.¹ Many papers report on the influence of water deficits on vine development and yield²⁻⁵ and on fruit composition.^{4,6–10} Vine nitrogen status also greatly influences growth and yield parameters¹¹⁻¹³ as well as fruit ripening.^{11,14-19} Most of these studies were carried out on red grape varieties. Some show a clear positive effect of water deficit on berry phenolic compound concentration and quality potential for red wine making.^{4,7–10} Moderate nitrogen deficiency can also enhance phenolic compound synthesis.^{10,16,18,19} As red wine quality greatly depends upon phenolic compound concentration in grapes, it seems clear that moderate environmental stress, and particularly low water and nitrogen availability, enhances quality potential in red grapes.

Very few data have been published on the influence of environmental conditions on quality potential

in white grapes. Unlike in red grapes, phenolic compounds do not play a positive role in white grape quality. High phenolic content in white grape juice is responsible for unstable colour and bitterness.²⁰ White grape quality mainly depends upon its aroma potential. For some white wine aromas, eg terpenols, one part is directly extracted from the grapes during vinification, without transformation,²¹ and another part is glycosidally bound.²²⁻²⁵ However, very little of this last fraction is transformed into aroma during vinification, because glycosidase enzymes have very low activity at grape juice and wine pH.26,27 Aromatic varieties such as Muscats produce white wines with a terpenol-dominated aroma. Their aromatic potential can easily be assessed during grape ripening either by direct gas chromatography of the free terpenols or by gas chromatography after hydrolysis of the bound terpenols.²⁸ The grapes of other varieties such as Vitis vinifera L cv Sauvignon blanc contain mainly nonvolatile, bound aromas. Some of these aromas are liberated during the fermentation processes, making

E-mail: k-van-leeuwen@enitab.fr

^{*} Correspondence to: Cornelis Van Leeuwen, ENITA de Bordeaux, 1 Cours du Général de Gaulle, BP 201, F-33175 Gradignan Cedex, France

Contract/grant sponsor: Comité Interprofessionnel des Vins de Bordeaux

⁽Received 9 June 2003; revised version received 24 May 2004; accepted 22 June 2004) Published online 24 September 2004

^{© 2004} Society of Chemical Industry. J Sci Food Agric 0022-5142/2004/\$30.00

the wine odorous. The evolution of the aroma potential of these grape varieties during the ripening process is much more difficult to assess.

Recently, three major aroma compounds of the wine produced from Sauvignon blanc grapes were identified: 4-mercapto-4-methylpentan-2-one (4MMP), 4-mercapto-4-methylpentan-2-ol (4MMPOH) and 3-mercaptohexan-1-ol (3MH).^{29,30} Their analysis by gas chromatography coupled with mass spectrometry makes it possible to quantify aroma intensity in Sauvignon blanc wines. These aromas exist in Sauvignon blanc grapes as non-volatile S-cysteine conjugate precursors.³¹ Peyrot des Gachons et al³² developed a method for quantifying these precursors. Although this method is delicate and time-consuming, it allows the quantification of Sauvignon blanc aroma precursors directly in grape juice. Thus aroma potential in Sauvignon blanc grapes can be assessed during grape ripening.

The objective of this research was to study the influence of vine water and nitrogen status on vine development and fruit quality of Sauvignon blanc grapes in field conditions. During grape ripening, not only major compounds such as sugar and organic acids were analysed, but also precursors of three major volatile compounds of the Sauvignon blanc aroma: p-4MMP, p-4MMPOH and p-3MH. Aroma potential of Sauvignon blanc grapes varied widely in relation to vine water and nitrogen status. The possible use of these observations in site selection for Sauvignon blanc grapes and in optimising irrigation and fertilisation practices for this variety is discussed.

MATERIALS AND METHODS Experimental plots

The most renowned white Bordeaux wines are produced in the Pessac-Léognan and Graves appellations south of the town of Bordeaux. Four plots, located

in commercial vineyards in these appellations and planted with *Vitis vinifera* L cv Sauvignon blanc, were studied. Plots were medium- to high-density plantings. Vines were guyot pruned and the training system was a trellised vertical shoot positioning. Other characteristics of the plots are listed in Table 1. Plots were dry farmed and no mineral nitrogen was added during the trial.

Soils

Among these plots, two are located on Quaternary alluvium and two are located on Tertiary limestone. The soils on Quaternary alluvium are acidic and contain a high amount of gravel and sand; the soils on Tertiary limestone have a high pH and their texture is sandy clay.

The plot SG is located on a sandy/gravelly soil. Gravel content is around 40% in the topsoil (0–50 cm) and 20% in the subsoil. The subsoil is very compact and most roots are located in the layer 0–50 cm. Water-holding capacity is low (67 mm over 1 m in depth, calculated according to Rawls *et al*³³) because of shallow rooting and soil gravel content. The pH is very low in the subsoil (4.0) but close to neutral in the topsoil owing to fertilisation practices. This plot was planted after deforestation, which explains the very high organic matter content of the topsoil (over 5%). Consequently, soil total nitrogen content is very high in spite of an organic matter C/N ratio of 26.

The plot GS is located on a gravelly/sandy soil. Although GS is developed on the same geological deposit as SG, it contains a higher amount of gravel (over 50% in the topsoil and over 60% in the subsoil). Although rooting is very deep (over 2 m), soil waterholding capacity is low because of the high amount of gravel (39 mm in the first 80 cm of the soil). Soil organic matter content is average (1.2%). The pH is low in the subsoil but close to neutral in the topsoil.

Characteristic	SG	GS	LSB	LHB
Soil type	Sandy/gravelly soil	Gravelly/sandy soil	Sandy clay on soft limestone bedrock	Sandy clay on hard limestone bedrock
Organic matter content (%)	5.31	1.20	0.81	1.14
Organic matter C/N ratio	26	15	12	12
Soil pH (0–50 cm)	5.7	6.4	7.7	8.0
Geological origin	Quaternary alluvium	Quaternary alluvium	Tertiary limestone (Oligocene)	Tertiary limestone (Oligocene)
Gravel content	Medium	High	Low	Low
Soil depth	Shallow	Deep	Deep	Shallow
Appellation of origin	Pessac-Léognan	Graves	Pessac-Léognan	Graves
Grape variety	Sauvignon blanc	Sauvignon blanc	Sauvignon blanc	Sauvignon blanc
Rootstock	101-14MG	3309C	41B	3309C
Vine density (vines ha^{-1})	7143	5556	8547	5556
Canopy management	Vertical shoot positioning	Vertical shoot positioning	Vertical shoot positioning	Vertical shoot positioning
Pruning system	Cane pruned (Guyot)	Cane pruned (Guyot)	Cane pruned (Guyot)	Cane pruned (Guyot)
Year of planting	1988	1985	1988	1985
Irrigation	Dry farmed	Dry farmed	Dry farmed	Dry farmed

The plot LSB (limestone soft bedrock) is located on a calcareous soil developed on a soft Tertiary limestone bedrock. The soil has a sandy clay texture. From 90 cm in depth the soil contains a significant amount of lime. The pH ranges from 7.5 to 8.0 between 0 and 90 cm in depth and is around 8.5 over 90 cm in depth. Soil water-holding capacity is high (93 mm) owing to the sandy clay texture and the deep rooting (over 140 cm). Soil organic matter and soil total nitrogen content are very low.

The plot LHB (limestone hard bedrock) is located on a calcareous, sandy clay soil. A hard Tertiary limestone bedrock is present at 60 cm in depth. Although vine rooting is limited to 60 cm in depth, this type of bedrock can provide a significant amount of water to the vines. Using neutron moisture probes on a similar soil, Duteau³⁴ showed that about 50% of the water consumed by the vines in a dry vintage was supplied by the bedrock. Consequently, even in dry vintages, vines never face severe water deficit stress on this soil type. The pH of the soil is close to 8.0. Soil organic matter content is average (1.1%).

Climatic conditions

In 1998, temperatures were above average for the Bordeaux area from April to September $(18.3 \,^{\circ}C;$ Figs 1a and 1c). Rainfall during the growing season was low, especially in May (22 mm) and August (14 mm). This vintage can be characterised as warm and dry. The year 1999 was even warmer. The average temperature from April to September was 19.0 $^{\circ}C$ (Fig 1b). Growing season rainfall was slightly above average for the Bordeaux area, with more than 60 mm of rain in every month. No significant climatic variations among the plots were recorded during these two vintages (data not shown).



Figure 1. (a) Rainfall, sunshine hours and temperatures in 1998 in Graves and Pessac-Léognan region (data: Bordeaux Mérignac). (b) Rainfall, sunshine hours and temperatures in 1999 in Graves and Pessac-Léognan region (data: Bordeaux Mérignac). (c) Long-term means of rainfall, sunshine hours and temperatures in Graves and Pessac-Léognan region (data: Bordeaux Mérignac).

Variables measured

Vine water status

Changes in vine water status during the season were determined by five predawn leaf water potential measurements³⁵ carried out between the end of June and early September. Each value is the average of eight replicates. Water uptake conditions during the ripening period were also determined by measuring ${}^{13}C/{}^{12}C$ carbon isotope discrimination in grape sugars at ripeness ($\delta^{13}C$).^{36,37}

Vine nitrogen status

Grape juice nitrogen content is a sensitive indicator for assessing vine summer nitrogen uptake in field conditions.^{17,38} Grape juice total nitrogen and grape juice NH_4^+ contents were measured four times during grape ripening (12 replicates per plot).

Vine development and vigour

On 33 vines per plot the length of one shoot per vine was measured every 10 days until growth cessation. To prevent the cutting of these shoots by the hedging machines, they were trained horizontally on the lowest wire of the trellising system. Shoot growth rate $(cm day^{-1})$ was calculated for each period between two measurements. Leaf area was determined immediately after harvest by measuring leaf dry weight on 10 vines per plot according to Sepúlveda and Kliewer.³⁹ Pruning weight was measured in December 1998 and December 1999 on 12 plots of six vines.

Berry composition (major compounds)

Berry samples were taken at four times during grape ripening on 12 blocks per plot. Samples from each block were analysed separately and constitute replicates. Each block contained six vines. Ten berries were sampled on each vine. Berry weight was determined. Then for each block the 60 berries were manually pressed and 30 ml of grape juice was centrifuged. The following analyses were carried out on each replicate of fresh grape juice.

- Sugar content was calculated after determination of total soluble solids with a manual temperaturecompensated refractometer (°Brix, RF233 model, Merck Eurolab, Fontenay sous Bois, France).
- pH was determined using an automated coupled pH meter (Cogetude, Vendôme, France).
- Titratable acidity was determined by titration with 0.05 N NaOH to an end point of pH 7.0 (expressed in g tartaric acid 1^{-1}).
- Organic acids were analysed using a continuous flow analytical system (Traacs 800, Bran and Luebbe, Plaisir, France) and expressed in g1⁻¹. Malic acid was determined by an enzymatic method (Bohringer, Mannheim, Germany), and tartaric acid by colorimetry after reaction with vanadic acid.
- K, Ca and Mg were determined with an inductively coupled plasma atomic emission spectrometer (ICP-AES, Varian Vista, Mulgrave, Australia) after

1:20 (v/v) dilution with 5% HNO₃ (K expressed in gl^{-1} ; Mg and Ca expressed in mgl^{-1}).

- NH₄⁺ and mineral phosphorus were analysed using the Traacs 800 continuous flow analytical system according to the Berthelot method for N and the Duval method for P.
- Total nitrogen was digested with sulphuric acid/ H_2O_2 according to a modified Kjeldahl method, followed by the analysis of NH_4^+ as mentioned previously.⁴⁰

Berry composition (aroma precursors)

One lot of 25 bunches was sampled per plot. Berries were crushed under neutral gas. SO2 was added $(50 \text{ mg} \text{l}^{-1})$. Skins were macerated in grape juice for 18h at 18°C. After maceration, skins were pressed at 0.5 MPa in a pneumatic micro-press (Bellot, Gradignan, France), SO₂ was added again (50 mg l^{-1}) and the grape juice was filtered and stored at -20 °C until analyses. S-Cysteine conjugate precursors of volatile thiols were analysed following the method in Ref 32. In both vintages the concentrations of precursors of 4-mercapto-4-methylpentan-2-one (p-4MMP), 4mercapto-4-methylpentan-2-ol (p-4MMPOH) and 3mercaptohexan-1-ol (p-3MH) were determined at ripeness (ie harvest date of the plot, which was decided by the technical staff of the estate managing the plot). In 1998, five to seven samples were taken from the plots between veraison and harvest to show the evolution of these precursors during fruit ripening.

Data analysis

Data were analysed by means of linear regression (determination coefficient), analysis of variance and Newman-Keuls (NK) comparison of averages. The statistical analysis was carried out using Microsoft Excel and Statbox software.

RESULTS

Vine water uptake conditions in 1998 and 1999

On 9 July 1998 (Julian day 190), predawn leaf water potentials were close to zero on the four plots, showing no limitation in vine water uptake (Fig 2a). At the end of July 1998 (Julian day 208), predawn leaf water potentials were significantly more negative on SG and GS compared with LSB and LHB. Vine water deficit stress continued to increase during August on SG and GS. On 1998 August 20 (Julian day 232), water deficit stress was severe on GS (predawn leaf water potential -0.62 MPa) and very severe on SG (predawn leaf water potential -1.0 MPa). On SG the water deficit stress caused severe defoliation of the vines. Vines did not face water deficit stress on LSB and LHB. In early September, predawn leaf water potential values just showed a slight water deficit on these plots. δ^{13} C values, measured in grape sugars at ripeness, confirmed the water stress on SG and GS (Table 2). Moreover, the values indicated that water



Figure 2. (a) Predawn leaf water potential values measured in 1998 (error bars indicate SD). (b) Predawn leaf water potential values measured in 1999 (error bars indicate SD).

Table 2. Carbon isotope composition of grape sugar (${}^{13}C/{}^{12}C$) at harvest ($\delta^{13}C$, p 1000)

Year	SG	GS	LSB	LHB
1998	-20.9	-22.5	-25.0	-25.9
1999	-24.5	-26.0	-26.1	-26.9

Interpretation: <-25.0, unlimited water uptake conditions during fruit ripening; -25.0 to -24.0, mild water deficit during fruit ripening; -24.0 to -22.0, moderate water stress during fruit ripening; >-22.0, severe water stress during fruit ripening. One replicate per plot for each year.

deficit during grape ripening was slightly more intense on LSB than on LHB.

In 1999, vines did not face severe water deficit stress because of higher rainfall during the season (Fig 2b). Predawn leaf water potential values did not indicate any vine water deficit on LSB and LHB. On GS a slight water deficit occurred at the end of July 1999 (predawn leaf water potential -0.22 MPa, Julian day 208), but the plants quickly recovered owing to rain in August. On SG, soil water-holding capacity is very low because of shallow vine rooting. On this plot, moderate water deficit stress occurred at the end of July, disappeared after rain in the middle of August and reappeared in early September. δ^{13} C values, measured in grape sugars at ripeness, indicated mild water deficit on SG and no water deficit on the other plots (Table 2).

Vine nitrogen status in 1998 and 1999

In 1998, grape juice nitrogen content was very high on SG, high on GS, moderate on LHB and low on LSB (Fig 3a).

In 1999, grape juice nitrogen content was lower on most plots compared with 1998 (Fig 3b). Vine



Figure 3. (a) Total nitrogen and NH_4^+ nitrogen in grape juice at harvest in 1998 (error bars indicate SD). (b) Total nitrogen and NH_4^+ nitrogen in grape juice at harvest in 1999 (error bars indicate SD).

nitrogen status was high again on SG, low on LSB and medium on GS and LHB. Values were not significantly different on GS and LHB for both grape juice total nitrogen and grape juice NH_4^+ .

Vegetative development

On LHB, shoot growth rate was high and growth cessation occurred late in the season (Figs 4a and 4b). This resulted in long shoots at the end of the season (over 300 cm in 1998 and 1999, Table 3). Leaf area, pruning weight and yield were also high (Table 3). This plot, where neither vine water status nor vine nitrogen status was severely limited, can be characterised as vigorous.

On LSB, shoot growth rate was low throughout both growing seasons (Figs 4a and 4b). Although growth cessation did not occur very early, the slow growth rate resulted in short shoots at the end of the growing season in 1998 (138 cm) and in medium shoots in 1999 (246 cm, Table 3). Yield was medium in 1998 and high in 1999, while pruning weight was low in both vintages (160 g per vine in 1998 and 250 g per vine in 1999, Table 3), showing low vegetative vigour.

In 1998, shoot growth rate was high on SG at the beginning of the season but dropped dramatically when water stress occurred during July (Fig 4a). Although the shoots stopped growing earlier on this plot than on any other plot in 1998, SG produced the second longest shoots (202 cm, Table 3). Leaf area and yield were small because of the severe water stress, but pruning weight was high. In 1999, shoot growth rate varied within the season depending on vine water status. It was high in June, decreased in July when available water became limiting and increased again in August owing to significant rainfall. Growth stopped in early September (Fig 4b). Yield was medium and pruning weight was high.

Shoot growth rate, shoot length, leaf area and pruning weight were average on GS in both vintages (Figs 4a and 4b, Table 3). Yield was low in 1999 and medium in 1998.

Pruning weight was correlated with berry NH₄⁺ content ($R^2 = 0.55$; p = 0.05; n = 8) but not with vine water status. Secondary leaf area was correlated with vine water status (correlation δ^{13} C-secondary leaf area: $R^2 = 0.85$; p = 0.01; n = 8), as was total leaf area (correlation δ^{13} C-total leaf area: $R^2 = 0.75$; p = 0.01; n = 8). Yield and final shoot length were not correlated with vine water or vine nitrogen status.

Berry composition at ripeness (major compounds)

Harvest date was determined by the technical staff of each estate and mainly based on grape sugar/acid ratio (Table 4). Correlations were established with data collected on the four plots during two vintages (n = 8). Berry weight at ripeness was highly correlated with vine water status (correlation δ^{13} C-berry weight: $R^2 = 0.81$; p = 0.01; n = 8). Water deficit-stressed



Figure 4. (a) Shoot growth rate in 1998. (b) Shoot growth rate in 1999.

1998	SG		GS		LSB		LHB	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Final shoot length (cm)	202	87	159	75	138	62	306	103
n = 33	b		bc		С		а	
Primary leaf area at harvest ($m^2 ha^{-1}$)	7600	1500	6200	1900	5500	1800	8400	2100
<i>n</i> = 10	ab		ab		b		а	
Secondary leaf area at harvest (m ² ha ⁻¹)	3600	1700	6300	2500	7500	2200	9100	3300
<i>n</i> = 10	b		ab		а		а	
Total leaf area at harvest (m ² ha ^{-1})	11 200	2500	12500	5100	13000	4000	17 500	5200
<i>n</i> = 10	b		ab		ab		а	
Pruning weight (tha ⁻¹)	3.5	0.32	2.6	0.33	1.4	0.17	3.5	0.40
n = 12	а		b		С		а	
Yield (t ha ^{-1})	8.5	2.1	12.8	3.0	12.0	3.2	19.5	3.1
n = 12	С		b		b		а	
1999	SG		GS		LSB		LHB	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Final shoot length (cm)	246	106	278	122	246	110	372	138
n = 33	b		b		b		а	
Primary leaf area at harvest ($m^2 ha^{-1}$)	8600	1500	7100	1900	8000	1800	9800	2100
<i>n</i> = 10	ns		ns		ns		ns	
Secondary leaf area at harvest (m ² ha ⁻¹)	8800	2300	11900	3700	9200	2300	12300	3400
<i>n</i> = 10	ns		ns		ns		ns	
Total leaf area at harvest (m ² ha ^{-1})	17 400	2700	19000	5300	17 200	4000	22 100	5300
<i>n</i> = 10	ns		ns		ns		ns	
Pruning weight (t ha^{-1})	3.6	0.47	3.0	0.34	2.1	0.45	4.0	0.42
n = 12	а		b		С		а	
Yield (t ha ⁻¹)	11.2	2.3	8.5	2.6	15.7	5.6	18.8	2.6
n = 12	b		b		а		а	

Table 3. Vine development and vine vigour parameters

Letters a, b, c indicate differences at p = 0.05 level (NK test); n, number of replicates; SD, standard deviation.

vines produced small berries, particularly on SG in 1998. Vine nitrogen status did not have an effect on berry size in this study. Vine water deficit stress negatively affected berry sugar content at harvest (correlation δ^{13} C-berry sugar content at ripeness: $R^2 = 0.59; p = 0.05; n = 8$). SG produced berries with very low sugar content in 1998 when vines were severely water stressed. The same plot produced berries with the highest sugar content in 1999 when water deficit was only mild. Vine nitrogen status did not affect berry sugar content at ripeness. Titratable acidity was determined by berry malic acid content $(R^2 = 0.72; p = 0.01; n = 8)$ instead of by berry tartaric acid content ($R^2 = 0.22$; ns (not significant)) or berry potassium content ($R^2 = 0.02$; ns). Grape juice pH was correlated with titratable acidity $(R^2 = 0.71; p = 0.01; n = 8)$ as well as with malic acid content ($R^2 = 0.71$; p = 0.01; n = 8). Titratable acidity depended on vine water status (correlation δ^{13} C-titratable acidity: $R^2 = 0.74$; p = 0.01; n = 8) but not on vine nitrogen status (correlation grape juice total nitrogen-titratable acidity: $R^2 = 0.40$; ns). The water-stressed plots SG and GS in 1998 produced berries with low titratable acidity and malic acid content and high pH. Differences in acidity among plots were much smaller in 1999 when vine water uptake conditions were similar. Water deficit-stressed vines had a higher total nitrogen content (correlation δ^{13} C-grape juice total nitrogen: $R^2 = 0.50$; p = 0.05; n = 8).

Berry aroma potential

In 1998, p-4MMP content at ripeness was high on LHB, average on LSB and GS and low on SG (Fig 5a). In 1999, p-4MMP content was medium to high on SG and GS and low on LSB and LHB (Fig 5b). p-4MMPOH content and p-3MH content showed a similar distribution among the soils and vintages (Figs 5a and 5b). Higher levels were recorded in 1998 from vines grown in the soils on the Tertiary deposits (LSB and LHB) and in 1999 on the Quaternary alluvium (SG and GS). The concentration of p-4MMPOH was particularly high in 1999 on SG and the concentration of p-3MH peaked in 1998 on LHB. In 1999 the highest aroma potential was reached on SG and in 1998 on LSB.

Precursors of volatile thiols were analysed in 1998 on the experimental plots weekly from veraison (Figs 6a-6c) and continued until 1 or 2 weeks after harvest on 25 non-harvested vines. Concentrations of p-4MMP increased until a maximum and then decreased (Fig 6a). On SG and GS the maximum value occurred before harvest and was considerably lower compared with LSB and LHB. On LSB and

C Peyrot des Gachons et al

Table 4. Berry compositio	n at harvest date	(major compounds)
---------------------------	-------------------	-------------------

1998 Sampling date (calendar day) Sampling date (Julian day)	SG 14 Sept 257		G 7 S 25	GS 7 Sept 250		LSB 4 Sept 247		LHB 15 Sept 258	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Berry weight (g)	1.19	0.13	1.71	0.25	1.75	0.17	2.21	0.16	
n = 12	С		b		b		а		
Sugar (g I ⁻¹)	163	7	182	6	209	11	188	7	
n = 12	С		b		а		b		
Titratable acidity (g tartaric acid I^{-1})	5.6	0.47	5.0	0.62	7.0	0.45	6.8	0.42	
<i>n</i> = 12	b		b		а		а		
рН	3.42	0.06	3.40	0.09	3.20	0.04	3.07	0.06	
n = 12	а		а		b		С		
Malic acid (g I^{-1})	1.34	0.31	1.61	0.38	2.41	0.33	2.21	0.25	
n = 12	С		b		а		а		
Tartaric acid (g I^{-1})	6.8	0.43	5.5	0.44	6.6	0.37	6.0	0.27	
n = 12	а		С		а		b		
K (g I ⁻¹)	1.489	0.105	1.394	0.075	1.525	0.103	1.060	0.095	
n = 12	а		b		а		С		
Mg (mg l ⁻¹)	62.3	3.0	54.8	6.0	63.1	10.4	45.9	3.4	
n = 12	а		b		а		С		
Ca (mgl ⁻)	37.8	5.3	35.6	5.3	52.8	7.8	42.9	5.5	
n = 12	bc		С		a		b		
Mineral phosphorus (mg l ⁻)	77.7	8.9	57.1	5.7	70.5	8.2	52.2	5.8	
n = 12	а		С		b		С		
1000	S	G	GS		LSB		LHB		
1999	2 Sept		2 Sept		6 Sept		13 5	13 Sept	
Sampling date (calendar day)	245		245		249		256		
	<u> </u>								
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Berry weight (g)	1.78	0.11	1.87	0.10	2.05	0.21	2.07	0.11	
n = 12	b		b		а		а		
Sugar (g I^{-1})	214	11	197	7	205	14	211	7	
n = 12	а		b		ab		а		
Titratable acidity (g tartaric acid I^{-1})	5.9	0.65	7.4	0.65	7.6	0.44	7.7	0.39	
n = 12	b		а		а		а		
pH	3.31	0.04	3.13	0.03	3.23	0.04	3.16	0.04	
n = 12	а		С		b		С		
Malic acid (g I ⁻⁺)	1.81	0.48	2.48	0.44	2.41	0.41	2.08	0.24	
n = 12	b		а		а		ab		
Tartaric acid (gl ⁻)	5.6	0.34	6.1	0.34	6.6	0.36	6.5	0.23	
n = 12	C	0.000	d Loot	0.400	a	0.077	a	0.000	
K (g1 ')	1.320	0.060	1.361	0.108	1.491	0.077	1.95	0.063	
n = 12	b		b	0.0	a		b 10 5	0.0	
Mg (mgl ⁻)	56.5	2.9	53.9	6.3	49.8	8.6	49.5	3.0	
$\begin{array}{l} 11 = 12 \\ 0 = (m \pi 1^{-1}) \end{array}$	а	0.0	a	07	a	7 4	a 10 0	FO	
Ca (IIIGI)	20.5	2.3	27.0	2.1	37.2	7.1	40.6	5.2	
11 = 12	0		~		-		~		
Minoral phoophorus (mart-1)		4.0	D GC O	7.0	a 71 0	7.0	a 50 4	60	
Mineral phosphorus (mg l ⁻¹)	62.5	4.8	р 66.9	7.0	a 71.6	7.0	52.4	6.2	

Letters a, b, c indicate differences at p = 0.05 level (NK test); n, number of replicates; SD, standard deviation.

LHB, berry p-4MMP content continued to increase respectively 1 and 2 weeks after harvest before starting to decrease. There was a considerable time span between the maximum on GS (2 September, Julian day 245) and the maximum on LHB (23 September, Julian day 266). Concentrations of p-4MMPOH increased slowly from veraison to harvest (Fig 6b). The increase was most pronounced on LHB, where it tripled during fruit ripening. Concentrations of p-3MH had a more chaotic development and, globally, remained relatively stable during fruit ripening (Fig 6c). Grape p-3MH content was highest on LHB at veraison and remained so until harvest.

DISCUSSION AND CONCLUSION

Vine water status as well as vine nitrogen status were assessed by two techniques, showing consistent results.



Figure 5. (a) Volatile thiol content in berries at harvest in 1998 (error bars indicate coefficient of variation). (b) Volatile thiol content in berries at harvest in 1999 (error bars indicate coefficient of variation).

 δ^{13} C values were highly correlated with minimum predawn leaf water potential ($R^2 = 0.92$; p = 0.001; n = 8). Tregoat *et al*¹⁰ showed that the combined use of these two techniques allows a precise monitoring of vine water status in field studies. Vine nitrogen status was assessed by measuring total nitrogen and NH₄⁺ in grape juice according to Van Leeuwen *et al*.¹⁷ Values of total nitrogen and NH₄⁺ were highly correlated ($R^2 = 0.92$; p = 0.001; n = 48).

Vine vigour depended on both vine water status and vine nitrogen status.¹⁸ Limiting vine nitrogen uptake resulted in low shoot growth rate during shoot development, as was the case on LSB in 1998. The effect of limiting vine water uptake was shown on SG in 1998. Shoot growth rate started at a high level but dropped as soon as vines faced water deficit. Grape juice from water deficit-stressed vines contained more nitrogen (correlation δ^{13} C-grape juice total nitrogen: $R^2 = 0.50; p = 0.05; n = 8$). It is likely that high nitrogen status increases the susceptibility of vines to water stress. High nitrogen uptake promotes shoot growth early in the season and consequently results in high leaf area. High leaf area increases vine water use and favours the emptying out of soil water reserves. When water deficit stress becomes severe, part of the leaves can fall as a reaction by the plant to protect itself against excessive water loss. This scenario clearly took place on SG in 1998. Conversely, low vine nitrogen status limits shoot growth and leaf area. Subsequently, water use by vines is diminished, as was shown by Pieri et al.⁴¹

Berry composition at harvest was more influenced by vine water status than by vine nitrogen status. Severe water deficit stress provoked low berry sugar content and low titratable acidity, because malic acid content was low. This berry composition is not favourable for producing high-quality white wines. In Bordeaux a titratable acidity of 7.5 g tartaric acid 1^{-1} is considered optimum for the production of well-balanced white wines.²⁰ In 1998, a very dry vintage, all the experimental plot titratable acidity values were below 7.5 g tartaric acid l^{-1} . Titratable acidity was particularly low on SG and GS, which faced severe water deficit stress. In 1999, when rainfall occurred more regularly during the summer, titratable acidity was close to 7.5 g tartaric acid l^{-1} on three experimental plots (GS, LSB and LHB). Average berry sugar content was also higher in 1999. Water deficit stress reduced berry size. In this study there was no relationship between berry size and berry aroma precursor content in Sauvignon blanc.

4MMP is responsible for the box tree and broom aromas in Sauvignon blanc wines.²⁹ Its detection threshold in a model solution is very low (0.8 ng l^{-1}) .²⁹ 4MMPOH smells of citrus zest (detection threshold in model solution 55 ng l⁻¹) and 3MH of grapefruit and passion fruit (detection threshold in model solution 60 ng l^{-1}).³⁰ Although the concentration of volatile thiols in wine is directly related to the concentration of their precursors,^{42,43} only a small percentage of the precursors are effectively transformed into aroma during vinification. According to Peyrot des Gachons,⁴³ the average level of transformation is 1.4%



Figure 6. (a) Evolution of berry p-4MMP content during fruit ripening in 1998 (error bars indicate coefficient of variation). (b) Evolution of berry p-4MMPOH content during fruit ripening in 1998 (error bars indicate coefficient of variation). (c) Evolution of berry p-4MH content during fruit ripening in 1998 (error bars indicate coefficient of variation).

for p-4MMP, 3.0% for p-4MMPOH and 4.2% for p-3MH.

In 1998, which was a dry vintage, the highest aroma potential was achieved on the plots with the

greatest water reserves (LSB and LHB). In 1999, which was a wet vintage, the highest aroma potential was achieved on the plots with the lowest water reserves. SG and GS had mild water deficit stress



Figure 7. Correlation between berry p-4MMP content at harvest date and intensity of water deficit stress, assessed by carbon isotope composition of grape sugar.

in 1999. These results seem to indicate that severe water deficit stress limits aroma potential in Sauvignon blanc grapes but that mild water deficit might enhance it. When the four soils and two vintages are plotted together, berry p-4MMP content is highest when vines face mild water stress ($-26 < \delta^{13}C < -24$, Fig 7). This is consistent with the observation that white Bordeaux wines generally lack aroma expression in dry vintages.²⁰ Low vine nitrogen status on LSB might explain the lower concentrations of volatile thiol precursors on this plot in 1998 compared with LHB, while vine water status was similar. Despite a tendency of depressed aroma potential when vine nitrogen status is low, no correlation can be established with the available data between vine nitrogen status and grape aroma potential, because the effect of vine water status interferes with the effect of vine nitrogen status. For example, on SG in 1998, vine nitrogen status is high but aroma potential remains low because of severe water stress. Although these results need to be confirmed by experiments in controlled conditions, they seem to indicate that the highest aroma potential is built up in Sauvignon blanc grapes when vines face mild water deficit stress and when nitrogen status is non-limiting.

Considering perception threshold values of volatile thiols and the percentage of transformation of precursors into odorous thiol, p-4MMP and p-3MH have a higher contribution to the aroma potential of the Sauvignon blanc grapes in this study than p-4MMPOH.43 Because p-3MH content remained relatively stable from veraison to harvest, variations in aroma potential of Sauvignon blanc grapes during fruit ripening depended mainly on the evolution of p-4MMP content. In 1998, grapes were picked after the maximum p-4MMP content on SG and GS and before the maximum on LSB and LHB. Ideally, grapes should be picked when p-4MMP content is the highest. However, it was not possible to pick grapes earlier on SG and GS because of low sugar content. When p-4MMP content was maximum, sugar content was only 160 gl⁻¹ on SG (9 September, Julian day 252) and 174 gl^{-1} on GS (2 September, Julian day 245). Conversely, grapes could have been picked a few days later on LSB and LHB, but not very much so, because otherwise titratable acidity would have been too low.

The results of this research can be used in site selection for non-irrigated Sauvignon blanc vineyards. For maximum aroma expression in Sauvignon blanc grapes, water deficit stress should not be severe and nitrogen status should not be limiting. Shallow and gravelly soils are better suited for high potential red grape production, while deeper soils are better adapted for Sauvignon blanc. However, soils for Sauvignon blanc should not provide an excessive amount of water and nitrogen to the vines. Mild water deficit could possibly have a positive effect on aroma potential in Sauvignon blanc grapes. Excessive nitrogen can enhance susceptibility to Botrytis.20 In irrigated vines, Sauvignon blanc should be watered to achieve and maintain a mild deficit level. Nitrogen deficiency should be avoided, as well as excessively high vine nitrogen levels. The same recommendations can be given for a number of other white grape varieties of Vitis vinifera L (Gewürztraminer, Petit Manseng, Gros Manseng, Sémillon), because volatile thiols also make up part of their aroma.44

Grape sugar and titratable acidity level monitoring is universally used to determine harvest date. In red grapes the analysis of anthocyanin and tannin can provide interesting further information. The assessment of the aroma potential in white grapes could also be helpful and give interesting information to growers.

ACKNOWLEDGEMENTS

We thank the Comité Interprofessionnel des Vins de Bordeaux for financial support, and Christian Molot and Sylvie Milin for assistance with the analyses.

REFERENCES

- Jackson D and Lombard P, Environmental and management practices affecting grape composition and wine quality—a review. Am J Enol Vitic 44:409-430 (1993).
- 2 Hardie W and Considine J, Responses of grapes to water-deficit stress in particular stages of development. Am J Enol Vitic 27:55-61 (1976).

- 3 Matthews M, Anderson M and Schultz H, Phenological and growth responses to early and late season water deficits in Cabernet franc. *Vitis* **26**:147–160 (1987).
- 4 Van Leeuwen C and Seguin G, Incidences de l'alimentation en eau de la vigne, appréciée par l'état hydrique du feuillage, sur le développement de l'appareil végétatif et la maturation du raisin (*Vitis vinifera* variété Cabernet franc, Saint-Emilion, 1990). *J Int Sci Vigne Vin* 28:81–110 (1994).
- 5 Dry P, Loveys B, Düring H and Botting B, Effects of partial root-zone drying on grapevine vigour, yield composition of fruit and use of water, in *Proceedings 9th Australian Wine Industry Technical Conference*, Ed by Stockley C, Sas A, Johnstone R and Lee T. Winetitles, Adelaide, pp 128–131 (1996).
- 6 Seguin G, Alimentation en eau de la vigne et composition chimique des moûts dans les Grands Crus du Médoc. Phénomènes de régulation. *Connaiss Vigne Vin* 9:23-34 (1975).
- 7 Duteau J, Guilloux M and Seguin G, Influence des facteurs naturels sur la maturation du raisin, en 1979, à Pomerol et Saint-Emilion. *Connaiss Vigne Vin* **15**:1–27 (1981).
- 8 Matthews M and Anderson M, Fruit ripening in Vitis vinifera L.: responses to seasonal water deficits. Am J Enol Vitic 39:313-320 (1988).
- 9 Dry P, Loveys B, McCarthy M and Stoll M, Strategic irrigation management in Australian vineyards. *J Int Sci Vigne Vin* 35:129-139 (2001).
- 10 Tregoat O, Van Leeuwen C, Choné X and Gaudillère JP, The assessment of vine water and nitrogen uptake conditions by means of physiological indicators. Influence on vine development and berry potential. *J Int Sci Vigne Vin* 36:133-142 (2002).
- 11 Kliewer W, Effect of nitrogen on growth and composition of fruits from 'Thompson seedless' grapevines. J Am Hort Sci 96:816-819 (1971).
- 12 Conradie W and Saayman D, Effects of long-term nitrogen, phosphorus, and potassium fertilization on Chenin blanc vines. I. Nutrient demand and vine performance. Am J Enol Vitic 40:85–90 (1989).
- 13 Spayd S, Wample R, Stevens R, Evans R and Kawakami A, Nitrogen fertilization of white Riesling grapes in Washington. Effects on petiole nutrients concentration, yield, yield components, and vegetative growth. Am J Enol Vitic 44:378-386 (1993).
- 14 Bell A, Ough C and Kliewer W, Effects on grape-juice and wine composition, rates of fermentation, and wine quality of nitrogen fertilization on *Vitis vinifera* var. Thomson seedless grapevines. *Am J Enol Vitic* **30**:124–129 (1979).
- 15 Spayd S, Wample R, Evans R, Stevens R, Seymour B and Nagel C, Nitrogen fertilization of white Riesling grapes in Washington. Must and wine composition. Am J Enol Vitic 45:23-27 (1994).
- 16 Keller M and Hrazdina G, Interactions of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. *Am J Enol Vitic* 49:341–349 (1998).
- 17 Van Leeuwen C, Friant P, Soyer JP, Molot C, Choné X and Dubourdieu D, Measurement of total nitrogen and assimilable nitrogen in grape juice to assess vine nitrogen status. *J Int Sci Vigne Vin* 34:75–82 (2000).
- 18 Choné X, Van Leeuwen C, Chéry P and Ribéreau-Gayon P, Terroir influence on water status and nitrogen status of non irrigated Cabernet-Sauvignon (*Vitis vinifera*): vegetative development, must and wine composition. S Afr J Enol Vitic 22:8-15 (2001).
- 19 Hilbert G, Soyer JP, Gaudillère JP, Molot C, Giraudon J and Milin S, Nitrogen supply during growth of *Vitis vinifera* L cv Merlot: effect on must quality and anthocyanin accumulation. *Vitis* 42:69–76 (2003).
- 20 Ribéreau-Gayon P, Dubourdieu D, Donèche B and Lonvaud A, *Traité d'Œnologie. Tome 1: Microbiologie du Vin*, *Vinifications.* Dunod, Paris (1998).

- 21 Ribéreau-Gayon P, Boidron JN and Terrier A, Aroma of Muscat grape varieties. J Agric Food Chem 23:1042-1047 (1975).
- 22 Williams P, Strauss C, Wilson B and Massy-Westropp R, Novel monoterpenes disaccharide glycosides of *Vitis vinifera* grapes and wines. *Phytochemistry* 21:2013–2020 (1982).
- 23 Wilson B, Strauss C and Williams P, The distribution of free and glycosidally-bound monoterpenes among skin, juice and pulp fractions of some white grape varieties. *Am J Enol Vitic* 37:107-111 (1986).
- 24 Strauss C, Wilson B and Williams P, Novel monoterpene diols and dioglycosides in *Vitis vinifera* grapes. J Agric Food Chem 36:569-573 (1988).
- 25 Razungles A, Gunata Z, Pinatel S, Baumes R and Bayonove C, Etude quantitative de composes terpéniques, norisoprénoïdes et de leurs précurseurs dans diverses variétés de grappes. *Sci Alim* 13:59–72 (1993).
- 26 Williams P, Strauss C, Wilson B and Massy-Westropp R, Studies on the hydrolysis of *Vitis vinifera* monoterpene precursor compounds and model monoterpene β-D-glucosides rationalizing the monoterpene composition of grapes. *J Agric Food Chem* **30**:1219–1223 (1982).
- 27 Bayonove C, Gunata Z and Cordonnier R, Mise en évidence de l'intervention des enzymes dans le développement de l'arôme du jus de Muscat avant fermentation: la production des terpénols. *Bull OIV* 57:741–758 (1984).
- 28 Wilson B, Strauss C and Williams P, Changes in free and glycosidally bound monoterpenes in developing Muscat grapes. J Agric Food Chem 32:919–924 (1984).
- 29 Darriet P, Tominaga T, Lavigne V, Boidron JN and Dubourdieu D, Identification of a powerful aromatic component of *Vitis vinifera* L var Sauvignon wines: 4-mercapto-4methylpentan-2-one. *Flav Fragr J* 10:385-392 (1995).
- 30 Tominaga T, Furrer A, Henry R and Dubourdieu D, Identification of new volatile thiols in the aroma of *Vitis vinifera* L var Sauvignon blanc wines. *Flav Fragr J* 13:159–162 (1998).
- 31 Tominaga T, Peyrot des Gachons C and Dubourdieu D, A new type of flavor precursors in *Vitis vinifera* L cv Sauvignon blanc: *S*-cysteine conjugates. *J Agric Food Chem* 46:5215–5219 (1998).
- 32 Peyrot des Gachons C, Tominaga T and Dubourdieu D, Measuring the aromatic potential of *Vitis vinifera* L cv Sauvignon blanc grapes by assaying *S*-cysteine conjugate compounds, precursors of the volatile thiols responsible for the varietal aroma of wines. *J Agric Food Chem* 48:3387-3391 (2000).
- 33 Rawls W, Brakenseik D and Saxton K, Estimation of soil water properties. *Trans ASAE* 25:1316–1320 (1982).
- 34 Duteau J, Contribution des réserves hydriques profondes du calcaire à Astéries compact à l'alimentation en eau de la vigne dans le Bordelais. *Agronomie* 7:859–865 (1987).
- 35 Scholander P, Hammel H, Edda D, Bradstreet E and Hemmingsen E, Sap pressure in vascular plants. *Science* 148:339–346 (1965).
- 36 Van Leeuwen C, Gaudillère JP and Trégoat O, The assessment of vine water uptake conditions by ¹³C/¹²C discrimination in grape sugar. J Int Sci Vigne Vin 35:195–205 (2001).
- 37 Gaudillère JP, Van Leeuwen C and Ollat N, Carbon isotope composition of sugars in grapevine, an integrated indicator of vineyard water status. *J Exp Bot* 53:757–763 (2002).
- 38 Soyer JP, Molot C, Bertrand A, Gazeau O, Rodriguez-Lovelle B and Delas J, Influence de l'enherbement sur l'alimentation azotée de la vigne et sur la composition des moûts et des vins, in 'Œnologie 95', Proceedings of the 5th International Enology Symposium, Ed by Lonvaud-Funel A, et al. Lavoisier Tec et Doc, Paris, pp 81–84 (1996).
- 39 Sepúlveda G and Kliewer W, Estimation of leaf area of two grapevine cultivars (*Vitis vinifera* L) using laminae linear measurements and fresh weight. Am J Enol Vitic 34:221–226 (1983).
- 40 Oliver R, Minéralisation par voie humide en vue de dosages des éléments majeurs dans les plantes. *Analusis* 7:38–41 (1979).

Influence of water and nitrogen deficit on grape aroma potential

- 41 Pieri P, Pradel E, Soyer JP, Valancogne C and Dayau S, Bilans hydriques de la vigne avec et sans enherbement. *Proc 9th GESCO Meet*, pp 175–180 (1996).
- 42 Tominaga T, Recherche sur l'arôme variétal des vins de *Vitis vinifera* L cv Sauvignon blanc et sa genèse à partir de précurseurs inodores du raisin. *PhD Thesis*, Université Victor Ségalen Bordeaux 2 (1998).
- 43 Peyrot des Gachons C, Recherches sur le potentiel aromatique des raisins de *Vitis vinifera* L cv Sauvignon blanc. *PhD Thesis*, Université Victor Ségalen Bordeaux 2 (2000).
- 44 Tominaga T, Baltenweck-Guyot R, Peyrot des Gachons C and Dubourdieu D, Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. *Am J Enol Vitic* 51:178–181 (2000).