EFFECT OF FOLIAR NITROGEN AND SULPHUR APPLICATION ON AROMATIC EXPRESSION OF *VITIS VINIFERA* L. cv. SAUVIGNON BLANC

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Abstract

Aims: It is well known that vine nitrogen deficiency can negatively impact on aroma in white wines. Soil nitrogen fertilization enhances aroma expression, but it also increases vine vigour and susceptibility to grey rot. The aim of this work was to investigate the impact of foliar nitrogen as well as foliar nitrogen and sulphur applications on aromatic expression, vigour and susceptibility to grey rot of *Vitis vinifera* L. cv. Sauvignon blanc.

Methods and results: The impact of foliar nitrogen (N) and sulphur (S) application on aromatic expression of *Vitis vinifera* L. cv. Sauvignon blanc has been investigated. On a plot where vine nitrogen status is naturally low and water availability not limiting, foliar nitrogen and sulphur fertilization (10 kg/ha of N and 5 kg/ha of S) increased yeast available nitrogen content. Vine vigour and maturity level of grapes were not modified compared to the control. The wines produced from N+S vines contained more volatile thiols and glutathione. These results were confirmed by a tasting of wines produced with grapes from the experimental plots. Foliar nitrogen fertilization alone (10 kg/ha of N) also increased Sauvignon blanc aromatic expression, but less so than the N + S treatment.

Conclusions: Foliar N and foliar N + S applications can enhance aromatic expression in *Vitis vinifera* L. cv. Sauvignon blanc wines without increasing vine vigour and infestation by grey rot.

Significance and impact of the study: Vine nitrogen deficiency can negatively impact on grape aroma potential. Soil nitrogen application can increase vine nitrogen status, but it has several drawbacks: it increases vigour and enhances *Botrytis* susceptibility. This study shows that foliar N and foliar N + S applications can improve vine nitrogen status and enhance aroma expression in Sauvignon blanc wines without the negative impact on vigour and *Botrytis* susceptibility. Although this study was carried out on Sauvignon blanc vines, it is likely that foliar N or foliar N + S applications will have similar effects on other grapevine varieties containing volatile thiols (Colombard, Riesling, Petit Manseng and Sémillon).

Key words: nitrogen, sulphur, foliar fertilization, vine, Sauvignon blanc, N-tester, volatile thiols, glutathione

Résumé

Objectif : Il est admis qu'une carence en azote de la vigne déprécie l'arôme du vin blanc. Une fertilisation azotée au sol augmente l'expression aromatique du vin, mais aussi la vigueur de la vigne et la sensibilité des raisins à la pourriture grise. L'objectif de ce travail a été d'évaluer l'effet d'une fertilisation foliaire en azote seule ou associée à du soufre sur l'expression aromatique du vin blanc de *Vitis vinifera* L. cv. Sauvignon blanc, sur la vigueur et la sensibilité des grappes à la pourriture grise.

Méthodes et résultats : L'effet de la pulvérisation foliaire d'azote et de soufre sur l'expression aromatique de *Vitis vinifera* L. cv. Sauvignon blanc a été évalué. Sur la parcelle d'étude, la vigne était légèrement carencée en azote et l'alimentation en eau était non limitante. L'apport foliaire d'azote (N) et de soufre (S) juste avant véraison (10 kg/ha N et 5 kg/ha S) a permis d'augmenter la teneur en azote du moût sans modifier l'expression végétative et le niveau de maturité des raisins. Les vins issus de la modalité N + S contenaient plus de thiols volatils et plus de glutathion. Ce résultat est confirmé par une dégustation de vins obtenus par micro-vinification. Un apport d'azote foliaire seul avant véraison (10 kg/ha N) a également amélioré le potentiel aromatique du vin, mais à un niveau moindre.

Conclusion : Une pulvérisation foliaire de N ou de N + S peut améliorer l'expression aromatique de vins produits avec des raisins de *Vitis vinifera* L. cv. Sauvignon blanc sans augmenter la vigueur de la vigne et l'intensité de la pourriture grise.

Signification et impact de l'étude : Une carence en azote déprécie l'arôme du vin blanc. La fertilisation azotée au sol peut augmenter le statut azoté de la vigne, mais a pour inconvénient d'augmenter la vigueur et la sensibilité de la vigne à *Botrytis cinerea*. Cette étude montre que l'application de N ou de N + S peut améliorer l'expression aromatique de vins de Sauvignon blanc, sans avoir les effets négatifs d'augmentation de la vigueur de la vigne et de la sensibilité à la pourriture grise. Cette étude a été réalisée sur Sauvignon blanc, mais il est probable que la pulvérisation foliaire de N ou de N + S aura des effets similaires sur d'autres cépages qui contiennent des thiols volatils (Colombard, Riesling, Petit Manseng et Sémillon).

Mots clés : azote, soufre, fertilisation foliaire, vigne, Sauvignon blanc, N-tester, thiols volatils, glutathion

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INTRODUCTION

Nitrogen has a major impact on vine development and grape composition. High nitrogen status increases vine vigour, yield and sensitivity to fungi, particularly *Botrytis cinerea* (KLIEWER and COOK, 1974 ; DELAS *et al.*, 1991). Low vine nitrogen status increases berry sugar content and total phenolics (DELAS *et al.*, 1991 ; SOYER *et al.*, 1995 ; CHONÉ *et al.*, 2001a, TREGOAT *et al.*, 2002). Hence, moderate to low nitrogen uptake by vines improve grape quality potential in red wine production. Must nitrogen status also interferes with vinification. Low nitrogen content reduces the ability of the must to ferment and might lead to sluggish or stuck fermentations (MASNEUF *et al.*, 2000; SPRING, 1999). Low nitrogen status in *Vitis vinifera* L. cv. Sauvignon blanc limits cysteinylated aroma precursor and glutathione synthesis and increases phenolic compounds in grapes (CHONÉ, 2001; PEYROT DES GACHONS *et al.*, 2005; CHONÉ *et al.*, 2006). Consequently, nitrogen deficiency reduces aromatic quality and ageing potential of wines produced from Sauvignon blanc.

Nitrogen and sulphur leaf applications are easily assimilated in wheat. Simultaneous applications of nitrogen and sulphur facilitate their assimilation in grains (TÉA *et al.*, 2003 ; TÉA *et al.*, 2007). This synergy between nitrogen and sulphur tends to show interactions in their respective pathways. In vigourous grapevines (*Vitis vinifera* L. cv. Colombard) foliar nitrogen was more easily assimilated if sprayed together with sulphur. Wines produced from vines treated with foliar nitrogen and sulphur contained more volatile thiols and showed improved aromatic profile (DUFOURCQ, 2006 and 2007).

Volatile thiols are responsible for the varietal aroma of Sauvignon blanc wines (DARRIET *et al.*, 1995; TOMINAGA *et al.*, 1998a):

- 3-mercaptohexan-1-ol (3MH) (grapefruit),
- 3-mercaptohexyl acetate (A3MH) (passion fruit),
- 4-mercapto-4-methylpentan-2-one (4MMP) (box tree, broom),
- 4-mercapto-4-methylpentan-2-ol (4MMPOH) (citrus zest).

These compounds also participate in the aroma of other grapevine varieties such as *Vitis vinifera* L. cv. Colombard, Sémillon, Manseng and Riesling (TOMINAGA *et al.*, 2000). The volatile thiols are not present in grape berries but are released during fermentation by the action of yeast (MURAT *et al.*, 2001) from odourless precursors whose structure has been identified as being S-cysteine conjugates (TOMINAGA *et al.*, 1998a).

The aromatic potential of grapes also involves glutathione and phenolic compounds. During the pre fermentation processing of grapes, phenolic compounds can give way to quinones, particularly so when exposed to oxygen. These are very reactive molecules that can dramatically reduce precursors of volatile thiols in must and wines. Low phenolic compound content in Sauvignon blanc grapes limits the risk of aromatic compound losses and so does protection against oxidation. Glutathione in musts and wines, which is very reactive to quinones, plays an important role in protecting varietal volatile thiols from oxidation (DUBOURDIEU *et al.*, 2001). High glutathione content in grapes is an important factor in aroma protection in Sauvignon blanc grapes.

MATERIALS AND METHODS

1. Location, vine material and experimental set up

This research was conducted in 2007 in a Bordeaux vineyard (Château La Louvière, Appellation of Controlled Origin Pessac-Léognan). The study plot was planted in 1988 with Sauvignon blanc vines, grafted on 41B rootstock. Plant density is 8500 vines/ha. Training system is a double guyot pruned vertical shoot positioned trellis. Soil type is a medium deep lime holding loamy clay soil developed on a Tertiairy Miocene deposit (CALCOSOL following the « RÉFÉRENTIEL PÉDOLOGIQUE FRANÇAIS », BAIZE et GIRARD, 1995). This plot was chosen because it exhibited a slight nitrogen deficiency induced by a low soil organic matter content (10 g/kg).

On this plot, four treatments were compared:

- CONTROL: control without fertilization,
- SOILN: 30 kg/ha of nitrogen (ammonium nitrate) applied to the soil just after flowering,
- LEAFN: 10 kg/ha of nitrogen (urea) in two applications prior to veraison,
- LEAFNS: 10 kg/ha of nitrogen (urea) and 5 kg of sulphur (micronized sulphur) in two applications prior to veraison.

Each treatment was conducted with four randomnized replicates of 20 vines each.

During the season, no potassic fertilisation and copper treatments were implemented on the study plot.

2. Vine water status

Vine water status was assessed at veraison by means of stem water potential measurements (CHONÉ *et al.*, 2001b), carried out with a pressure chamber (SCHOLANDER *et al.*, 1965). Each value is the means of four replicated measurements.

3. Nitrogen status

Vine nitrogen status was assessed by means of two indicators:

a- « N-Tester » readings

Leaf blade coloration was measured with an « N-Tester » device (YARA, Oslo, Norway). After measurements realized on 30 leaf blades, « N-Tester » indicates a value, without units, that represents chlorophyll concentration, in relation to plant nitrogen status. High values indicate high nitrogen status (SPRING, 1999). Two measurements per replicate are carried out from fruit set through harvest at monthly intervals.

b- Yeast available nitrogen

Yeast available nitrogen (YAN) was assessed in grape juice at harvest. Grape juice YAN content is an accurate indicator of vine nitrogen status (VAN LEEUWEN *et al.*, 2000). YAN was analyzed with the modified Sörensen method (AERNY, 1996).

4. Vine vigour and production

Four vines per replicate were harvested. Average number of grapes per vine was calculated and so was average weight of one grape. Average yield per vine was determined. In December 2007, 4 vines per replicate were pruned. Annual pruning wood from primary and secondary shoots were weighed separately.

5. Botrytis infestation

Grape infestation with gray rot (*Botrytis cinerea*) was determined at harvest. 16 measurements per replicate were carried out and averaged.

6. Major compounds in grape juice at harvest

200 berries were sampled randomly the day prior to the harvest of the plot by the estate. Berry weight was assessed and berries were pressed in a small laboratory press. Grape sugar was measured with a hand hold refractometer and total acidity by titration with NaOH 0.1N. Juice malic acid was measured by an enzymatic kit (Isitec-Lab, France) and juice tartaric acid by colorimetry after reaction with vanadic acid.

7. Small scale vinification

5 kg of grapes were harvested on four vines per replicate on 28 August 2007. Replicates were assembled two by two. Hence, two small scale vinifications were carried out for each treatment. Grapes were pressed so as to obtain 700 mL of juice for 1 kg of grapes. 50 mg/L of SO_2 was added to the must. Must was protected by CO_2 against oxidation. Pectinase enzymes were added (10 mg/L, Lafazym CL, Laffort Oenology, Bordeaux) and juice was cleared by cold settlement to 200 NTU turbidity. Must was inoculated with Zymaflore®VL3 yeast at 300 mg/L (Laffort Oenologie, Bordeaux, France) and adjusted to 200 mg/L yeast available nitrogen with ammonium sulphate. The temperature of fermentation was around 17-18 °C. Sugar transformation in ethanol was monitored daily by must density measurements and analysis of residual sugar when density was under 1.000. When residual sugar was under 2 g/L, 50 mg/L of SO_2 was added.

8. Major compounds in wine

After the end of the fermentation, wine volumic alcohol content, total acidity, malic and tartaric acid and pH were measured with classical analytical methods.

9. Wine glutathione

Glutathione in its reduced form was assayed by capillary electrophoresis (HP ^{3D} Capillary Electrophoresis System) on a silica capillary (65 cm; 75 µm) with a phosphate buffer (pH=7.5; 0.05M) and at 25KV voltage. Detection was performed by fluorescence (λ excitation 365 nm; λ emission 465 nm) with a method adapted from that of NOCTOR and FOYER (1998) using specific derivatization of SH functions by monobrobimane (MBB) (LAVIGNE *et al.*, 2007). Samples were prepared in the following conditions. To 200 µL of pre-filtered wine were added 10µL dithiotreitol (DTT; 10 mM), 145 µL CHES buffer (0,5 mM, pH 9,3; preparation: 2,58 g 2-(N-cyclohexylamino) ethanesulfonic acid (CHES), in 25 mL distilled water; pH is adjusted to 9,3 with NaOH). After 15 min reaction in the dark, the sample was injected in the hydrodynamic mode (5 s, 50 mbar).

10. Wine volatile thiols

Volatile thiols in wine were concentrated and purified prior to analysis by gaz chromatography coupled with mass spectrometry (GC-MS). Details of the method are described by TOMINAGA *et al.* (1998b).

11. Tasting session

Sensory attributes of wines were assayed on 17 November 2007 by a panel of 23 experts familiar with Sauvignon blanc wines. Typical Sauvignon blanc aroma intensity was rated on a scale from 0 to 5. Each tasted wine was a blend of the two small scale vinifications carried out for each treatment.

12. Data analysis

Statistical data analysis (analysis of variance, means comparison with Newman Keuls test) was carried out with Grimmersoft Statbox and Microsoft Excel software (Redmond, WA, USA).

RESULTS

1. Climatic conditions

Temperatures were close to average during the 2007 growing season (figure 1). Spring was warm and summer relatively cool. 2007 was a rainy vintage, which induced high disease pressure and delayed shoot growth cessation.

2. Vine water status

High summer rainfall (90 mm in August) induced unlimited vine water uptake conditions. Stem water potential values measured at veraison were comprised between 0 and -0.7 MPa, which indicates no water deficit stress (CHONÉ *et al.*, 2001b ; VAN LEEUWEN *et al.*, 2007). PEYROT DES GACHONS *et al.*, 2005 have shown that both water deficit stress and vine nitrogen deficiency can negatively impact aroma potential in Sauvignon blanc grapes. Hence, to study the effect of nitrogen fertilization on aroma compounds in Sauvignon blanc wines it is important to verify the absence of water deficit stress.

3. Vine nitrogen status

a- N-tester values

N-tester values were measured five times during the season, from June through the end of August (figure 2). At veraison (early August) and harvest (end of August) N-tester values are significantly higher in both soil and foliaire nitrogen supplemented vines. However, N-tester values are not significantly different among SOILN, LEAFN and LEAFNS treatments.

b- Yeast available nitrogen in grape juice at harvest

Yeast available nitrogen (YAN) values are low in all treatments, showing that the vines on the plot are nitrogen deficient (table 1). Moreover, their values under 180 mg/L justify must nitrogen supplementation to ensure rapid fermentation. On treatments with leaf nitrogen applications (LEAFN and LEAFNS) YAN is increased by 60 %, showing the ability of the vines to redistribute nitrogen absorbed by the leaves prior to veraison to the grapes. Suprisingly so, the SOILN treatment is not significantly different from the control. This might be related either to late application of soil nitrogen, or limited absorption in this clayey soil.

4. Vine vigour and yield components

Number of bunches per vine, bunch weight, yield and pruning weight were not significantly different among treatments (tabke 2). Neither leaf nor soil applications of nitrogen have impacted on yield components or vine vigour in this study, despite the fact that the control vines were subjected to nitrogen deficiency. This result can be explained by the late application of nitrogen in the season (June for soil application and late July and early August for foliar applications). High vigour is not looked after, because it negatively impacts on grape quality and because it increases susceptibility to *Botrytis*.

5. Botrytis infestation

Botrytis infestation was not significantly different among treatments (table 3). High nitrogen fertilization is reputed to increase grape susceptibility to *Botrytis*. Due to late application, this negative impact of nitrogen fertilization seems to be avoided in this experiment.

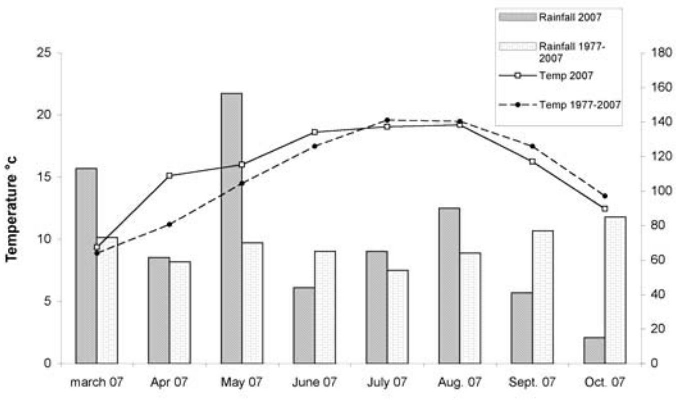

Figure 1 - Monthly temperature and rainfall data from April through September in Pessac-Léognan (near Bordeaux): comparison of 2007 with average values 1977 - 2007

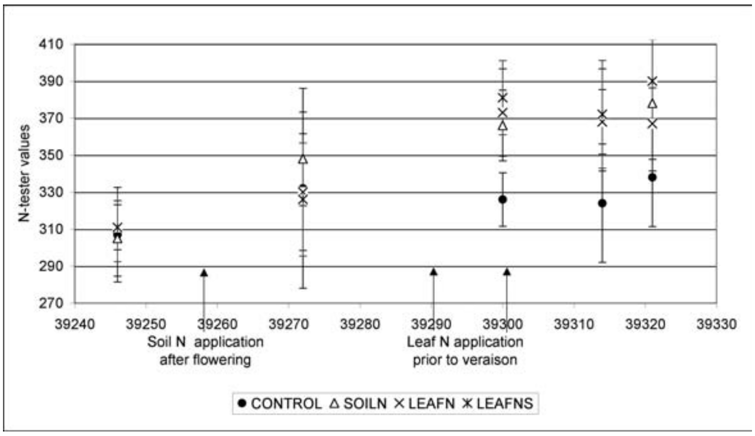

Figure 2 - N-tester values during the season: comparison of leaf N supplemented treatment, leaf N + S supplemented treatment, soil N supplemented treatments and control

Table 1 - Yeast Available Nitrogen content in grape juice at ripeness (27 August 2007) : comparison of leaf N supplemented treatment, leaf N + S supplemented treatment, soil N supplemented treatment and control

	Yeast Available Nitrogen (mg/L)			
	CONTROL	SOILN	LEAFN	LEAFNS
Average	72.7 B	83.7 B	112.3 A	115 A
Standard deviation	6.5	14.7	8.6	1.0
p (F<f)	0.007			

Letters A, B, C indicate differences at p < 5 % (Newman-Keuls test) ; NS = Not Significant;

Table 2 - Vine vigour and yield components: comparison of leaf N supplemented treatment, leaf N + S supplemented treatment, soil N supplemented treatment and control

	CONTROL		SOILN		LEAFN		LEAFNS		
	Average	SD	Average	SD	Average	SD	Average	SD	p(F<f)
Bunches/vine	9.1 NS	2.1	8.8 NS	1.9	9.5 NS	2.5	9.2 NS	0.8	0.85
Bunch weight (g)	175 NS	78	126 NS	22	140 NS	33	105 NS	13	0.1
Yield (T/ha)	13.1 NS	3.1	12.1 NS	2.6	12.9 NS	0.5	10.1 NS	0.9	0.26
Total pruning weight (g)	987 NS	170	992 NS	271	1060 NS	91	1112 NS	240	0.5
Secondary pruning weight / Total pruning weight	0.20 AB	0.01	0.27 A	0.03	0.25 AB	0.06	0.18 B	0.01	0.02

Letters A, B, C indicate differences at p < 5% (Newman-Keuls test) ; NS = Not Significant; SD = standard deviation

6. Major compounds in grape must

No differences in grape composition were observed at harvest date for major grape compounds (table 3). Soil or leaf nitrogen fertilization did not interfere with grape composition, nor did it delay maturity in the conditions of this trial.

7. Major compounds in wine

The small scale vinifications underwent quick fermentation. Hence, differences in wine composition reflect composition in grape juice, in relation with the nitrogen and sulphur treatments. The wines produced from the four treatments show little differences in major compounds (data not shown). The wine produced from grapes harvested on SOILN contains more alcohol. This difference might be the result of a higher yield from sugar into alcohol on this treatment, because must sugar content was not higher for SOILN.

8. Wine glutathione

Glutathione content is higher in LEAFN and LEAFNS treatments compared to CONTROL and SOILN treatments (figure 3). The addition of sulphur with the foliar N sprayings (LEAFNS treatment) did not increase the wine glutathione content compared to foliar N sprayings (LEAFN treatment). This observation is in contradiction with results from TÉA *et al.* (2003), who showed better foliar N absorption in presence of sulphur, and hence higher glutathione content in plant tissues.

9. Wine volatile thiols

Wine volatile thiol content is not significantly different between the SOILN treatment and the CONTROL (figure 4). 4MMP (Box tree) content is higher in the LEAFN treatment compared to the CONTROL. However, 3MH content (grapefruit) and A3MH content (passion fruit) are not. In the LEAFNS treatment, all three volatile thiols are present in higher concentrations compared to the CONTROL. High vine nitrogen status increases volatile thiol precursors in grapes (PEYROT DES GACHONS *et al.*, 2005). TÉA *et al.* (2007) observed a better nitrogen absorption in wheat in presence of sulphur. Although in our experiment indicators for vine nitrogen status do not significantly differ between LEAFN and

LEAFNS treatment, wines produced from LEAFNS contain more volatile thiols.

10. Tasting session

The positive effect of foliar spraying of nitrogen (LEAFN) on Sauvignon blanc aroma is confirmed by the tasting of the wines produced by small scale vinifications (table 4). Moreover, the wine produced from the LEAFNS treatments is preferred over the LEAFN treatment. The SOILN treatment is not differentiated by the tasting panel from the CONTROL. These results are consistent with the analysis of volatile thiols in the wines.

DISCUSSION AND CONCLUSION

Low nitrogen status in Sauvignon blanc vines can negatively impact on wine quality (PEYROT DES GACHONS *et al.*, 2005). Aroma precursors and glutathione in grape must are reduced, and phenolic compounds are increased (CHONÉ *et al.*, 2006). Soil fertilization with nitrogen can increase vine nitrogen status (SPAYD *et al.*, 1994; BELL *et al.*, 1979). However, soil nitrogen fertilization tends to increase yield and vine vigour (KLIEWER and COOK, 1974; DELAS *et al.*, 1991) and susceptibility of grapes to *Botrytis* (DELAS, 2000). These effects can reduce or annihilate the positive impact of an improved vine nitrogen status. This research points out the effects of foliar nitrogen and sulphur spraying prior to veraison in low nitrogen status vines. Through foliar fertilization, vine nitrogen status can be improved without increasing vine vigour and yield. Major compound composition in grapes is not modified and maturity is not delayed. Must yeast available nitrogen is increased. Hence, it can be expected that these applications speed up the fermentation and might avoid sluggish fermentations. Moreover, nitrogen addition to the must can be limited or avoided. A quick and clean fermentation is a quality factor in wine making, and particularly so for Sauvignon blanc wines (LAVIGNE and DUBOURDIEU, 2002). Sauvignon blanc wines obtained from vines that received foliar nitrogen fertilization prior to veraison are richer in volatile thiols (typical aroma compounds in Sauvignon blanc) and preferred in blind tasting by a professional panel. The effect of foliar nitrogen applications is enhanced when sulphur is applied simultaneously. Glutathione is an important compound for aroma protection in Sauvignon blanc wines (DUBOURDIEU *et al.*, 2001). As foliar nitrogen as well as foliar nitrogen and sulphur applications increase glutathione content in wines, it can be expected that these treatments improve ageing potential of the wines. This research was conducted on low nitrogen vines. More research is needed to assess the impact of foliar nitrogen and/or sulphur applications on Sauvignon blanc vines with moderate or high nitrogen status. Precursors of volatile thiols are present in a large range of white grapevine varieties (Colombard, Petit Manseng, Sémillon and Riesling; TOMINAGA *et al.*, 2000). It can be expected that the effect of foliar nitrogen and sulphur applications is comparable in these varieties to what was observed on Sauvignon blanc.

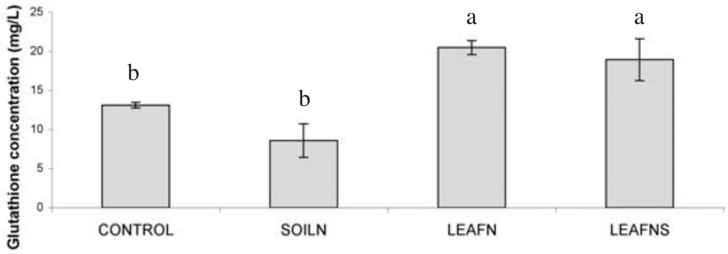

Figure 3 - Glutathione content in wines produced by small scale vinifications: comparison of leaf N supplemented treatment, leaf N + S supplemented treatment, soil N supplemented treatment and control

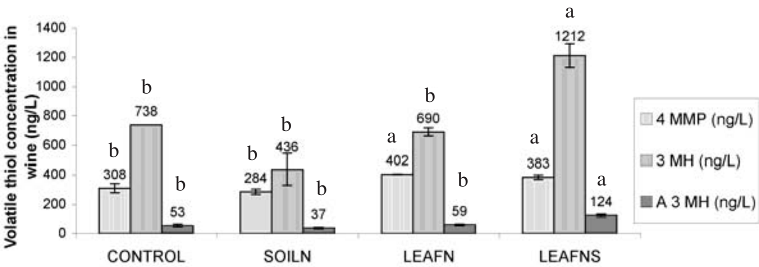

Figure 4 - Volatile thiol concentrations in wines produced by small scale vinification: comparison of leaf N supplemented treatment, leaf N + S supplemented treatment, soil N supplemented treatments and control

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Table 3 - Major compounds and Botrytis level at harvest (27 August 2007) : comparison of leaf N supplemented treatment, leaf N + S supplemented treatment, soil N supplemented treatment and control

	CONTROL		SOILN		LEAFN		LEAFNS		
	Average	**SD**	**Average**	**SD**	**Average**	**SD**	**Average**	**SD**	**p (F<f)**
% vines without Botrytis	85	30.5	67.5	25	62.5	20.6	75	23.8	**0.44**
	NS		**NS**		**NS**		**NS**		
Berry weight (g)	1.68	0.20	1.67	0.11	1.72	0.19	1.80	0.18	**0.8**
	NS		**NS**		**NS**		**NS**		
Sugar (g/L)	204	5	199	6	197	5	199	6	**0.59**
	NS		**NS**		**NS**		**NS**		
Total acidity (g tartaric acid/L)	9.15	0.2	9.3	0.3	9.6	0.3	9.15	0.2	**0.73**
	NS		**NS**		**NS**		**NS**		
Malic acid (g/L)	4.6	0.44	4.9	0.31	5.0	0.38	4.6	0.27	**0.52**
	NS		**NS**		**NS**		**NS**		
Tartaric acid (g/L)	7.4	0.0	7.5	0.1	7.5	0.1	7.4	0.0	**0.1**
	NS		**NS**		**NS**		**NS**		
pH	2.97	0.01	2.97	0.04	2.97	0.03	2.99	0.01	**0.75**
	NS		**NS**		**NS**		**NS**		

Letters A, B, C indicate differences at p < 5 % (Newman-Keuls test) ; NS = not significant; SD = standard deviation

Table 4 - Aroma intensity of the wines obtained by small scale vinifications (scale : 0 - 5, from neutral to very intense); comparison of leaf N supplemented treatment, leaf N + S supplemented treatment, soil N supplemented treatments and control

	Aroma intensity (rated 0 to 5)			
	CONTROL	SOILN	LEAFN	LEAFNS
Average	1.95 C	1.95 C	3.4 B	4.0 A
standard deviation	0.85	0.79	0.84	0.71
p (F<f)	0			

Letters A, B, C indicate differences at p < 5 % (Newman-Keuls test) ; NS = Not significant

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