THE IMPACT OF VINE NITROGEN STATUS ON AROMA POTENTIAL EXPRESSION IN *VITIS VINIFERA* L. CV. SAUVIGNON BLANC

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Abstract

In interaction with climate and genetic or human factors, the soil is a major component of the viticulture terroir. The mineral composition of the soil influences vine performance and wine sensory attributes. Among the elements that vines take from the soil, nitrogen is the one that has the strongest impact on vine physiology, vigor and grape composition. In addition to its major effect on primary metabolites in berries, nitrogen plays also a decisive role in the secondary metabolism, especially in the production of key compounds for berries quality, like volatile thiols, methoxypyrazines and glutathione (GSH).

To study the effect of nitrogen on these target metabolites, an experiment on Sauvignon blanc vines was performed in Bordeaux and Sancerre areas (France). Four nitrogen treatments were applied: control, soil application of 50kg N/ha, soil application of 100kg N/ha and foliar application of 15kg N/ha. Secondary metabolites were measured in grape berries and in wines produced through small scale vinifications.

Yeast Assimilable Nitrogen and N-tester measurements showed a significant difference in vine nitrogen status among the four treatments. The analysis of volatile compounds showed an increase in the content of 3-sulfanylhexan-1-ol precursors (P-3SH) and GSH in berries from vines with high N status. Similar effect of nitrogen was observed on the concentration of 3SH and GSH in wine.

This study will allow better management of vine nitrogen status in vineyards allowing a quantitative and qualitative control of grape berries.

Keywords: terroir, nitrogen, Sauvignon blanc, berry, wine, volatile thiols, methoxypyrazines, glutathione.

INTRODUCTION

A viticultural terroir is an ecosystem, in which the vine is in interaction with factors of the natural environment, such as climate and soil (van Leeuwen and Seguin 2006). It can be described as the combination of pedoclimatic conditions (soil type, climate), plant material (cultivar, clone and rootstock), as well as viticultural techniques (pruning system, canopy management, etc.). Soil is considered as an important factor in terroir expression (van Leeuwen et al. 2004). Soil influences vine behavior and wine quality through the temperature in the root zone, and through water and mineral supplies. Among soil minerals, nitrogen is obviously the one that most influences plant development and berry composition. Many studies deal with the influence of various levels of nitrogen fertilization on these parameters. Concerning plant development, nitrogen has a major impact on vine vigor, yield and sensitivity to fungi, particularly *Botrytis cinerea* (Bell et al. 1979). Regarding berry composition, vine nitrogen status influences berry sugar content, total phenolics and aroma compounds such as volatile thiols precursors (Choné et al. 2006; Lacroux et al. 2008).

Volatile thiols contribute to wine flavor, particularly in the varietal aroma of Sauvignon blanc wines and other white and red varieties. One of the most important thiols in Sauvignon blanc aroma is the 3-sulfanylhexan-1-ol (3SH) with a grapefruit flavor. This volatile thiol is not present in grape berries but is released during fermentation by the action of yeast (Murat et al. 2001) from odorless precursors whose structure has been identified as being *S*-conjugates to glutathione or cysteine (Pgsh-3SH and Pcys-3SH) (Tominaga et al. 1998). Glutathione in must and wine plays an important role in protecting varietal volatile thiols from oxidation

(Lavigne et al. 2007). Hence, high glutathione content in grapes is an important factor in aroma protection in wine production from Sauvignon blanc grapes.

The present study demonstrates the effect of nitrogen fertilization on 3SH and its precursors and on glutathione in grape berries, must and wines in the absence of water deficit. Vigor was controlled to avoid indirect effects of nitrogen through modification of microclimate in the bunch zone.

MATERIALS AND METHODS

Location, vine material and experimental set up

This research was conducted in 2013 in French vineyards (Château Couhins, Pessac-Léognan, Bordeaux and Château Fontaine-Audon, Sancerre). The study plot was planted with Sauvignon blanc vines. These plots were chosen because nitrogen status was low in the previous year (Yeast Assimilable Nitrogen (YAN) < 100 mg/l). Hence, though modulated nitrogen supply a gradient of treatments with increasing vine nitrogen status can be obtained. On each plot, four treatments were compared: control without fertilization, Soil N50: 50 kg/ha of nitrogen applied to the soil in two applications, Soil N100: 100 kg/ha of nitrogen applied to the soil in two applications and Foliar N15: 15 kg/ha of nitrogen (urea) in three applications. Each treatment was conducted with four randomized replicates of 10 vines each. Vines were irrigated during all the season and similar cultivation practices were adopted by the two vineyards during the grape-growing period. One hundred fresh berries were randomly sampled from each bloc at five different stages: bunch closure, mid-veraison, veraison, mid-maturity and maturity. All plant materials were frozen immediately in liquid nitrogen, grounded and stored at -80°C until implementation of analyses. Analyses were also carried out on grape must and wines after small scale vinifications.

Vine water status

Vine water status was assessed several times during the season by means of stem water potential measurements as described by Choné et al. 2001.

Nitrogen status

Vine nitrogen status was assessed by means of two indicators: i) the N-tester indicator that measure chlorophyll concentration which is in relation with plant nitrogen status and ii) the Yeast Assimilable Nitrogen (YAN) in grape juice at harvest (van Leeuwen et al. 2000). YAN was analyzed with a WineScan (FOSS®).

Vine vigor

Vine vigor was determined by measuring primary and secondary leaf area as described by (Mabrouk and Carbonneau 1996) and by weighing pruning mass.

***S*-conjugate precursors of 3SH and glutathione (GSH)**

In grape berries and in musts of Sauvignon blanc, *S*-conjugate precursors of 3SH was purified on C18 columns and then analyzed by liquid chromatography coupled to a mass spectrometry. Glutathione in its reduced form was assayed by the method described by Noctor and Foyer 1998 and Lavigne et al. 2007.

Wine volatile thiols

Volatile thiols in wine were concentrated and purified prior to analysis by gas chromatography coupled with mass spectrometry (GC-MS).

RESULTS AND DISCUSSION

Vine water status

To study the effect of nitrogen fertilization on aroma compounds in Sauvignon blanc variety, it is important to verify the absence of water deficit because it can influence aroma potential in Sauvignon blanc grapes (Peyrot des Gachons et al. 2005). Vines were irrigated and stem water potential values, between 0 and -0.75 MPa, indicated that they did not face any water deficit in this experiment (Choné et al. 2001).

Vine nitrogen status

N-tester values were measured several times during the season, from June through the end of August. N-tester values are significantly higher in both soil and foliar nitrogen supplemented vines compared to the control. At harvest, YAN content was higher in fertilized vines; this difference was highly significant. Hence, this demonstrated that applied nitrogen was well assimilated.

Vine vigor

Neither soil nor leaf applications of nitrogen impacted on primary or secondary leaf area in this study. However pruning mass per vine increased significantly with fertilization. Nitrogen was applied in several times, and relatively late in the season, which explains that vigor was only slightly increased by nitrogen. Hence, nitrogen application did not modify (or only marginally) microclimate in the bunch zone. In the absence of water deficit, any modification of aroma compounds in this study would thus be the result of a direct effect of nitrogen status.

***S*-conjugate precursors of 3SH**

In both vineyards and for all treatments, glutathionylated precursor of 3SH (Pgsh-3SH) content increased during ripening. Moreover its content is significantly related to nitrogen status: Pgsh-3SH content is higher in both soil and foliar treatments compared to control, in particular during mid-maturity and maturity stages (figure 1).

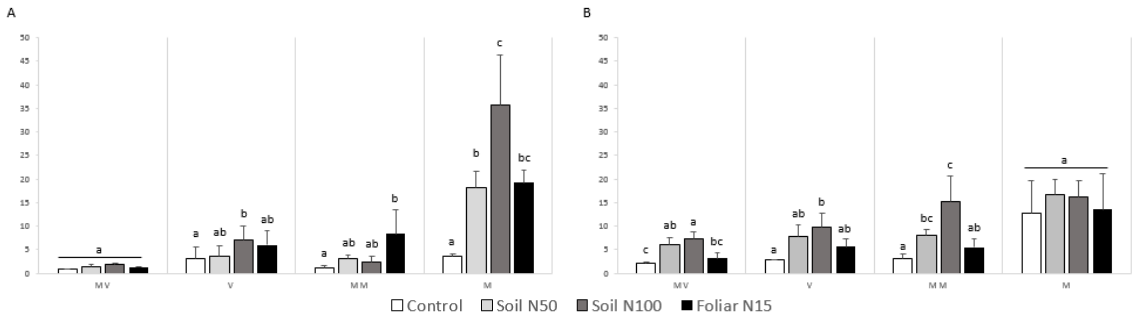

Figure 1: Pgsh-3SH content (µg/l) during ripening in (A) Bordeaux and (B) Sancerre vineyards.
The results represent the mean ± standard error of four biological replicates ($p<0.05$).

Cysteinylated precursor content of 3SH (Pcys-3SH) in berries from Bordeaux vineyard is stable during early stages of berry development. It increased with foliar application at mid-maturity and then decreased for all treatments at maturity. However, this decrease is lower in fertilized treatments compared to control (figure 2A).

In berries from Sancerre vineyards, Pcys-3SH concentration increased during ripening and it is higher in fertilized vines compared to the control (figure 2B).

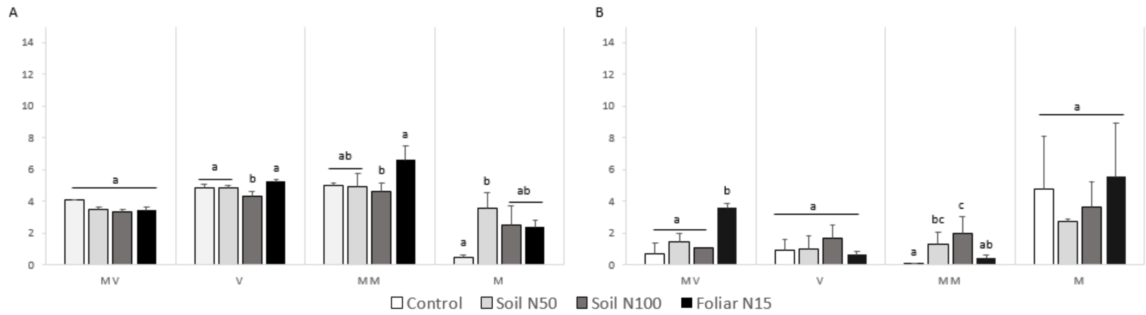

Figure 2: Pcys-3SH content (µg/l) during ripening in (A) Bordeaux and (B) Sancerre vineyards.
The results represent the mean ± standard error of four biological replicates ($p<0.05$).

In Sauvignon blanc musts obtained from the Bordeaux area, nitrogen fertilizations led to a significant increase in the concentration of both precursors (Pgsh-3SH and Pcys-3SH) compared to the control (figure 3). Furthermore and compared to Pgsh-3SH, the levels of Pcys-3SH in the must were lower in all treatments.

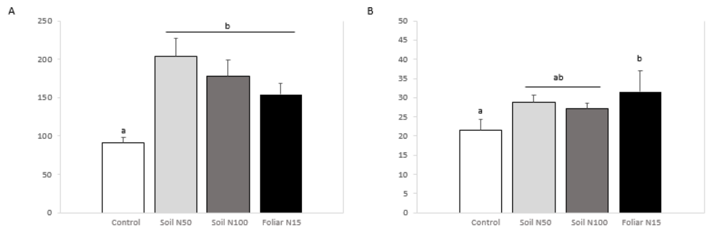

Figure 3: Pgsh3SH (A) and Pcys-3SH (B) content (µg/l) in must obtained from the Bordeaux area.
The results represent the mean ± standard error of four biological replicates ($p<0.05$).

These results showed that higher vine nitrogen status increased cysteine and glutathione precursor synthesis in Sauvignon blanc grapes and musts.

Wine volatile thiols

In wine obtained by small scale vinification, 3SH content is significantly different between the soil and foliar treatments and the control (figure 4): 3SH levels are higher in fertilized modalities compared to the control. These results indicate that wines, produced from vines with higher nitrogen status, contained more 3SH.

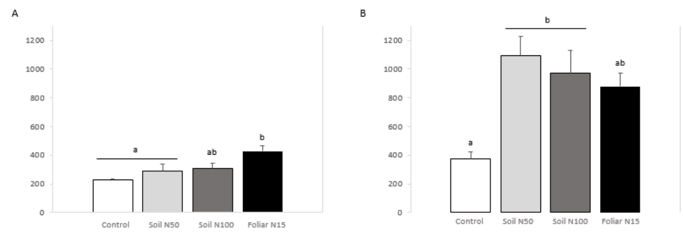

Figure 4: 3SH content (ng/l) in (A) Bordeaux and (B) Sancerre wines.
The results represent the mean ± standard error of four biological replicates ($p<0.05$).

Glutathione

Glutathione is an extremely important metabolite in oenology due to its protective role of volatile thiols from oxidation. Higher glutathione levels will provide a better protection of volatile thiols during grape processing.

In Sauvignon blanc berries of both Bordeaux and Sancerre areas, GSH levels increased during ripening for all treatments and more in fertilized modalities compared to the control, in particular during mid-maturity and maturity stages (figure 5).

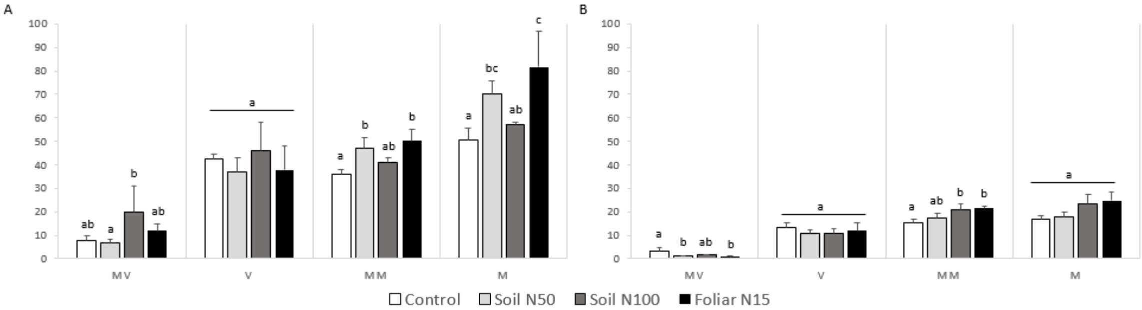

Figure 5: GSH content (mg/l) during ripening in (A) Bordeaux and (B) Sancerre vineyards.
The results represent the mean ± standard error of four biological replicates ($p<0.05$).

In Sauvignon blanc must from Bordeaux vineyards, nitrogen has a positive effect on GSH content (figure 6A). GSH also tended to increase in the corresponding wines (figure 6B), although these results were not significant.

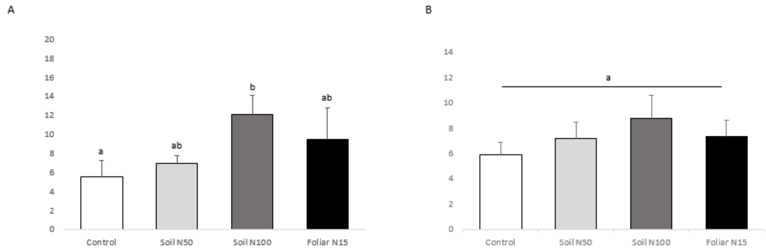

Figure 6: GSH content (mg/l) in Bordeaux (A) must and (B) wines.
The results represent the mean ± standard error of four biological replicates ($p<0.05$).

CONCLUSION

This research points out the effects of vine nitrogen status on aroma compounds in Sauvignon blanc berries, musts and wines. We showed that multiple applications of nitrogen (soil or foliar applications), relatively late in the season, increases vine nitrogen status without increasing vine vigor. In this experiment, we showed that nitrogen applications lead to an increase in levels of precursors of 3SH and to higher glutathione content in grape berries.

Sauvignon blanc musts and wines obtained from vines with higher nitrogen status are richer in 3SH, typical aroma compounds in Sauvignon blanc. As soil nitrogen as well as foliar nitrogen applications increase glutathione content in wines, it can be expected that these treatments improve also ageing potential of the wines.

This research will be completed by a transcriptomic approach to determine which genes control biosynthetic pathways of these compounds and to study their response to nitrogen addition.

This study will allow better management of vine nitrogen status in vineyards allowing a quantitative and qualitative control of grape berry quality potential.

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