

# COMBINED EFFECTS OF SOIL-APPLIED AND FOLIAR-APPLIED NITROGEN ON THE NITROGEN COMPOSITION AND DISTRIBUTION IN WATER STRESSED *VITIS VINIFERA* L. CV SAUVIGNON BLANC GRAPES

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## Abstract

**Aims:** The aim of this work is to test the effects of soil-applied nitrogen (N) at budbreak and subsequent foliar-applied N at veraison on the N composition and partitioning in berries of water stressed *Vitis vinifera* L. cv. Sauvignon blanc vines.

**Methods and results:** N fertilizer was applied to the soil at budbreak at doses of 30 or 60 kg N/ha, while the control did not receive any treatment. This did not increase N content of leaves and the vines showed symptoms of N deficiency from the beginning of the season. In order to overcome this deficiency, N foliar sprayings were applied at veraison at doses of 2.5 or 5 kg N/ha to vines having received 30 or 60 kg N/ha of soil-applied N, respectively. Total N of berry flesh responded to N foliar fertilization more than that any other berry part, whereas amino acids in skins were the more affected by N foliar fertilization than those of other berry parts. Only the 60 soil/5 foliar N treatment produced a measurable increase in the total, assimilable and amino N in berry juices at maturity. Assimilable N was a better indicator for N summer uptake by the vine than total N. Of all amino acids, arginine showed the highest increases following N fertilization and could be considered among the better indicators to distinguish between N summer fertilization treatments.

**Conclusion:** In conditions of severe water deficit and N deficiency, fertilization at a dose of 60 kg/ha soil-applied N combined with 5 kg/ha foliar-applied N improved fruit fermentability. Results support the use of foliar fertilization at veraison as a tool for enhancing grape quality and to a certain extent the style of wine.

**Significance and impact of study:** This work helps to provide insight into the effect of N soil fertilization along with foliar fertilization on water-stressed vines. This may be useful in fertilization programs in the Mediterranean area and may help to choose the type and the rate of the N fertilization in case of severe vine water deficit. Also, we provide information of utmost importance on the distribution of summer foliar-applied N in grape tissues.

**Key words:** Grapevine, nitrogen fertilization, water stress, nitrogen content

## Résumé

**Objectif :** La présente étude a été conçue pour tester l'effet de la fertilisation azotée conventionnelle au débourrement, suivi d'une fertilisation foliaire à veraison, sur la composition azotée et la distribution de l'azote dans les baies de *Vitis vinifera* L. cv. Sauvignon blanc issues d'une parcelle ayant subi une contrainte hydrique sévère.

**Méthodes et résultats :** Une fumure azotée de 30 kg N/ha ou 60 kg N/ha a été appliquée au sol au débourrement, et ses effets comparés à un témoin sans fumure azotée. Ces amendements n'ont pas augmenté la teneur des feuilles en N et des symptômes de carence ont été décelés tôt en saison. Pour ces raisons, des apports foliaires de 2,5 kg N/ha ou 5 kg N/ha ont été effectués chez les vignes ayant reçu respectivement 30 kg N/ha ou 60 kg N/ha auparavant. Les résultats indiquent que l'azote total de la pulpe est le plus augmenté par la fertilisation azotée. Seul l'apport de fertilisant le plus important a produit un effet significatif sur la quantité d'azote total, assimilable et aminé des moûts à maturité. L'azote assimilable est un indicateur plus pertinent que l'azote total pour différencier le niveau de l'alimentation en azote de la vigne en fonction de la fertilisation foliaire effectuée en été. L'arginine est l'acide aminé libre majoritaire dans les baies des vignes ayant reçu l'amendement le plus fort ; ce composé azoté augmente le plus, suite aux fertilisations azotées foliaires et se révèle donc l'indicateur le plus pertinent pour les différencier.

**Conclusion :** Dans des conditions de contrainte hydrique sévère et de carence azotée des baies, une fertilisation de 60 Kg N/ha au sol, suivie d'une autre de 5 Kg N/ha au feuillage a amélioré la fermentescibilité des moûts.

**Signification et impact de l'étude :** Ce travail fournit des informations sur les effets des fumures azotées appliquées au sol et par voie foliaire. Il peut servir aux programmes d'amélioration de la fertilisation azotée dans les conditions de contrainte hydrique en zone méditerranéenne et contribue à mieux en choisir le type et le taux. L'étude montre également les variations induites par la fumure azotée sur les formes d'azote dans les baies, et en particulier la répartition des acides aminés selon le stade phénologique et le taux de l'apport azoté.

**Mots clés :** vigne, fertilisation azotée, stress hydrique, concentration en azote

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## INTRODUCTION

Vine nitrogen (N) nutrition is an important factor for wine quality and plays an essential role in the vineyard and in the winery. Vine N status can affect vine vigor and fruit yield (EWART and KLIEWER, 1977; SPAYD *et al.*, 1993; BELL and ROBSON, 1999). Furthermore, N is required for yeast growth and completion of alcoholic fermentation in grape juice (BISSON, 1991); concentrations of 130 to 160 mg/L of yeast assimilable nitrogen (primary amino acids and  $\text{NH}_4^+$ ) are required for complete fermentation (AGENBACH, 1977; BELY *et al.*, 1990; SPAYD *et al.*, 1995; FERRARI, 2002). Moreover, CHONÉ *et al.* (2006) showed that in the case of *Vitis vinifera* L. cv. Sauvignon blanc vines, an unlimited N supply to the vine is an important factor for optimum varietal aroma expression.

It is known that N fertilization can increase the berry content of N compounds (KLIEWER, 1971; BELL *et al.*, 1979; BERTRAND *et al.*, 1991; SPAYD *et al.*, 1991, 1994 and 1995; CHONÉ *et al.*, 2006). However, the effect of N supply may vary depending upon other factors, in particular soil water status, and N uptake can be in limited supply to the clusters due to drought conditions. This is because plant-available N is dissolved in the soil solution, and its uptake by grapevines depends on the water flow through the soil-root-shoot pathway (KELLER, 2005).

Summer drought in the Mediterranean area has become increasingly frequent in recent years, most likely as a consequence of the global warming process. (LAGET *et al.*, 2008). In these conditions, soil fertilization of N is not the best solution to enhance low N levels in musts, and N foliar fertilization may boost the N content of berries of water-stressed vines since foliar absorption is independent of the soil water status. Therefore, the advantage of foliar application is to guarantee N assimilation by the plant as well as the rapidity of this process. On the other hand, foliar supply is only effective in plants approaching N deficiency since leaves are not the preferential organs of N absorption (DELAS, 2000). For these reasons, foliar spraying of fertilizers is of practical use in the vineyard to overcome temporary deficiencies. Foliar applications of N are often used as a supplement to an appropriate seasonal soil fertilizer program; emergency foliar applications for suddenly recognized deficiencies may be warranted, but only soil applications should be considered for a durable supply (CHRISTENSEN, 2005).

This study focuses on the efficiency of N soil application at budbreak coupled with N foliar application at veraison in the case of Sauvignon blanc berries in a vineyard in a situation severe water deficit. The objective was to test the effect of the combined actions of these two fertilization techniques on the evolution of the N

composition and its distribution in berries of water-stressed vines. We identify threshold values of N soil and foliar fertilization required to optimize the fermentability of musts obtained from water-stressed vines.

## MATERIALS AND METHODS

### 1. Location, vine material and experimental set up

This study was conducted in 2005 on a parcel of the « Rieucoulon » estate located on the outskirts of Montpellier, France. Non-irrigated, 18 year old *Vitis vinifera* L. cv. Sauvignon blanc vines grafted on 110 Richter were trained in vertical shoot positioning trellising system with Royat cordon pruning. The parcel, of 3333 vines/ha density was selected for its low vigour, symptoms of N deficiency (yellow green foliage) and the low N content of its musts during the preceding years. The soil was uniform, dominated by clay with a low proportion of rocks and a presence of limestone between 60 cm and 90 cm depth.

First, two levels of N were applied via soil fertilization at budbreak (day 117) and then N was applied around veraison (day 210) by foliar pulverization. Two levels of nitrogen fertilization were studied:

- 30 S/2.5 F: soil application of 30 kg N/ha one week after budbreak (day 117) using ammonium nitrate (50% ammonia, 50% nitrate – 33.5% N), and foliar application of 2.5 kg N/ha around veraison (day 210) using urea (46% N).

- 60 S/5 F: soil application of 60 kg N/ha after budbreak and foliar application of 5 kg N/ha around veraison.

Each treatment was replicated three times, each replicate comprising 40 vine stocks. Note that no rainfall occurred within the week following foliar fertilization, which reduced the possibility of the applied N being washed off the leaves.

### 2. Vine water status

Seasonal water status was determined by means of the pre-dawn leaf water potential ( $\psi_{pd}$ ) (CARBONNEAU, 1998) measured using the Scholander pressure chamber (SCHOLANDER *et al.*, 1965). Six healthy adult leaves, taken from the middle third of the major branches, were collected during the season from each treatment replicate and used for  $\psi_{pd}$  measurements. Hence, values of  $\psi_{pd}$  of the treatments were taken as the mean value after 6 x 3 measurements.

### 3. Vine N status

a) Leaf, must, skin and seed samples

Thirty healthy adult leaves were picked during the season - from fruit set to maturity - from the middle third of the major branches of each of the treatment replicates.

Two hundred berries were randomly hand-picked during the season (from fruit set until harvest) from the designated vines, according to a published sampling method (CARBONNEAU *et al.*, 1991), in which bunch fragments are randomly picked from the midsection of the cane or the cordon of the vines except those from the first row of the parcel. Sampled berries were then washed and wiped dry. Skins and seeds were manually separated from the flesh and the juices were obtained by manually pressing the berries.

In order to extract the amino fraction, the skins were freeze-dried (-85 °C, under vacuum, 24 hours) to obtain dry skins which were mashed to obtain a dry powder. A 100 mg aliquot of this powder was then dissolved in 4 ml of 5% perchloric acid and subjected to a 12 hour continuous agitation.

All samples and extracts were stored at -20 °C until analysis.

#### b) Analytical methods

Total N was quantified by the Kjeldahl method (KJELDAHL, 1883) modified as follows: either 10 g of whole berries, 1 g of skins, 2.5 g of seeds or 10 ml of berry juice are first digested in 20 ml of 96% sulfuric acid in the presence of a catalyst, heated and then distilled. The released ammonia is trapped in 4% boric acid and the conjugate base thus produced titrated with a 0.1 M HCl solution. Proteins were quantified by the Bradford method (BRADFORD, 1976) optimized according to MARCHAL and SEGUIN (2004). Assimilable N consisting of ammonium ions and the amino acids except proline were determined by the formol titration method of Sørensen (SØRENSEN, 1907) as follows: A volume of 50 ml of must titrated to pH 8, was added to 25 ml formal (also adjusted beforehand to pH 8). The acidification caused by the reaction between the formal and primary amino nitrogen was then titrated to pH 8 with 0.1 M NaOH solution. The «NOPA» method (DUKES and BUTZKE, 1998) was used to quantify global primary amino N.

These methods were statistically validated and were found to be suitable for berry analyses (coefficient of variation, CV < 5%). Individual amino acids in juices and skins were quantified by a liquid chromatography method, as described by PRIPIS-NICOLAU *et al.* (2001), with fluorescence detection by using pre-column derivatization with orthophthaldialdehyde and iodoacetic acid. The latter is used to render the method applicable to sulphur-containing amino acids, in particular cysteine. The

resulting isoindole fluorescent derivatives were separated on a reverse phase chromatography column (125 x 4 mm) Lichospher® RP-18 (5 µm) protected by a guard column (4 mm x 4 mm dp 5 µm).

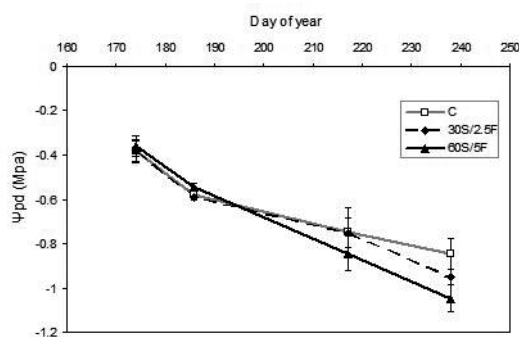
## 4. Statistical analysis

All statistical analyses were performed by ANOVA (*p*-value < 0.05) followed by Tukey range test, in order to determine which means were significantly different from one another. These analyses were accomplished by the RGui software, version 2.7.1.

## RESULTS AND DISCUSSION

### I. Grapevine water status

Pre-dawn leaf water potential ( $\psi_{pd}$ ) decreased from the end of June (day 174) until the end of August (day 238) (figure 1). According to the CARBONNEAU (1998) classification, these values correspond to mild water deficit (values < -0.6 MPa) from fruit set (day 174) until bunch closure (day 187), and to severe water deficit from bunch closure until the end of the season (day 238). Moreover,  $\psi_{pd}$  did not vary in the three treatments until bunch closure. On August 5th (day 217),  $\psi_{pd}$  of the «60S/5F» treatment was significantly lower than the control (Test of Tukey, *p* < 0.05), whereas  $\psi_{pd}$  of «30S/2.5F» did not differ from the control. Furthermore, on August 26th (day 238), the entire vineyard was under severe water deficit conditions (< -0.8 MPa) and all the fertilized vines were under significantly greater water deficit than the control (figure 1). This result may be related to the fact that N fertilization can increase the vine susceptibility to drought (KELLER, 2005).



**Figure 1 - Evolution of predawn leaf water potential of the three N treatments during the season.**

C: control with no N fertilization treatment; 30 S/2.5 F: fertilization of 30 Kg/ha soil-applied N at budbreak (117) and 2.5 Kg/ha foliar-applied N at veraison (day 210); 60 S/5 F: fertilization of 60 Kg/ha soil-applied N at budbreak and 5 Kg/ha foliar-applied N at veraison. Error bars represent standard deviations.

## 2. Grapevine N status

Vine N status was evaluated by the mean N content of leaf tissues and berries - the N content of leaves and of musts are two complementary indicators of vine N status as shown by GAUDILLÈRE *et al.* (2003). The N content of leaves comes from vine N reserves at the beginning of the season and from N absorption from bloom onwards, while N in berries is mainly the result of the N uptake during the summer period.

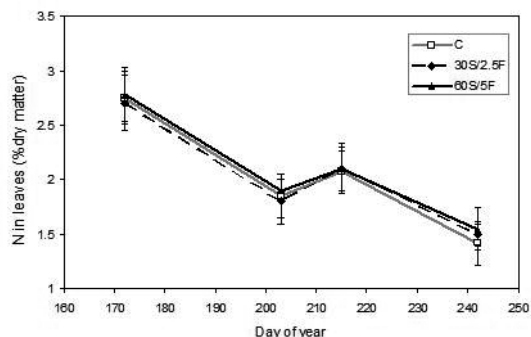
### a) Total N content of leaf tissues

N in leaves responds to the vine N uptake during the first part of the growth cycle when leaves are strong sinks (CONRADIE, 1986 and 1991). However, it may be observed in figure 2, that the N content of leaf tissues did not respond to soil N fertilization. This may be attributed to i) limiting soil moisture which reduces N availability; ii) significant vine water deficit that was a limiting factor in N uptake by the roots, which has been also demonstrated for the principal vine cations (Etchebarne *et al.*, 2009).

### b) Total N content of whole berries

It is important to present the results of N analysis both in terms of mg/berry to provide information about the absorption of N and the synthesis of N compounds, and in terms of mg/L or mg/Kg to provide information about the concentration related to the volume of the fruit. The volume of the berries of « 60S/5F » treatment was 11% greater than that control berries and « 30S/2.5F » treatment.

At fruit set, for the control and both treatments, the total N content per berry averaged about 0.88 mg. It remained almost constant before veraison (day 206) but started to increase significantly from veraison in all three cases (figure 3). This increase is certainly related to significant N translocation to the berries which serve as important sinks during ripening (CONRADIE, 1991). N foliar fertilization was applied on day 210 and from this



**Figure 2 - Evolution of total N in leaves (% dry matter) of the three N treatments during the season.** Error bars represent standard deviations.

day onwards, the N content of « 60S/5F » berries was greater than that control berries, whereas no difference was found between the control and the « 30S/2.5F » treatment. At maturity (day 232), the « 60S/5F » treatment increased the total N content per berry by 23% as compared to the control.

Figure 4 shows that the evolution of N concentrations per kg of whole berries varied throughout the season and it would appear that this evolution is affected by two principal factors, i.e., N absorption and translocation to berries and berry dilution during growth and development. N concentration decreased significantly between fruit set (day 171) and bunch closure (day 187) for the control and both treatments. It may thus be assumed that the dilution process was dominant between fruit set and bunch closure. On the other hand, the time trends shown in figure 4 between bunch closure (day 187) and veraison (day 210) were not significant. N concentration increased significantly from veraison onwards only in the case of the « 60S/5F » treatment, which indicates that N absorption and translocation to the berries was dominant after N foliar fertilization (day 210) in the berries of « 60S/5F ». The latter N treatment increased the berry total N content at maturity by 21% as compared to the control.

### c) Total N distribution in the different berry parts

**Table 1 - Total N content (mg/berry) and percent N partitioning in different berry parts (flesh, skin, seeds), for the three treatments, during ripening.**

		Ripening (Day 213) (3 days after foliar fertilization)		
Total N content		C	30S/2.5F	60S/5F
of berry flesh	in mg/flesh x berry	0.24 ± 0.03 a	0.32 ± 0.06 ab	0.40 ± 0.04 b
	in % of N content of whole berry	24	31	34
of berry skin	in mg/skin x berry	0.20 ± 0.01 a	0.20 ± 0.01 a	0.22 ± 0.03 a
	in % of N content of whole berry	19	19	19
of berry seed	in mg/seeds x berry	0.60 ± 0.04 a	0.52 ± 0.06 a	0.56 ± 0.05 a
	in % of N content of whole berry	57	50	47

Values followed by the same letter in the same line did not differ significantly (test of Tuckey,  $p < 0.05$ ).

**Table 2 - Total N content (mg/berry) and percent N partitioning in different berry parts (flesh, skin, seeds), for the three treatments at ripeness.**

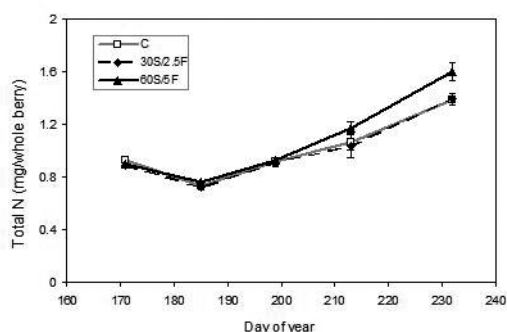
Total N content		Ripeness (Day 232)		
		C	30S/2.5F	60S/5F
of berry flesh	in mg/flesh x berry	0.34 ± 0.05 a	0.31 ± 0.02 a	0.52 ± 0.09 b
	in % of N content of whole berry	24	22	30
of berry skin	in mg/skin x berry	0.26 ± 0.01 a	0.28 ± 0.01 a	0.35 ± 0.04 a
	in % of N content of whole berry	19	21	21
of berry seed	in mg/seeds x berry	0.78 ± 0.03 a	0.80 ± 0.07 a	0.83 ± 0.10 a
	in % of N content of whole berry	57	57	49

Values followed by the same letter in the same line did not differ significantly (test of Tuckey,  $p < 0.05$ ).

**Table 3 - Total and assimilable N concentrations in musts (mg/L) and the ratio of assimilable N/total N of the three treatments during ripening and at ripeness.**

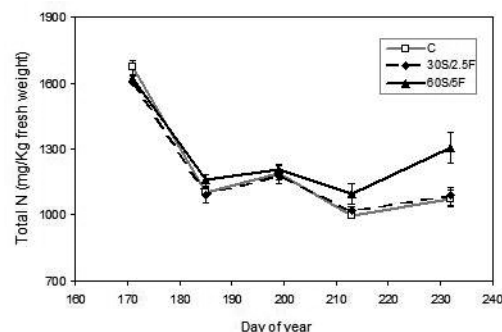
	During ripening (Day 213)			Ripeness (Day 232)		
	C	30S/2.5F	60S/5F	C	30S/2.5F	60S 5F
Total N (mg/L)	275 ± 26 a	371 ± 38 ab	441 ± 21 b	328 ± 50 a	292 ± 27 a	490 ± 80 a
Assimilable N (mg/L)	54 ± 4 a	74 ± 3 b	131 ± 7 c	70 ± 10 a	71 ± 14 a	136 ± 30 b
Assimilable N in % of total N	20	20	30	21	24	28

Values followed by the same letter in the same line did not differ significantly (test of Tukey,  $p < 0.05$ ).



**Figure 3 - Evolution of berries total N content (in mg/whole berry) of the three N treatments from fruit set up to ripeness.**

Error bars represent standard deviations.



**Figure 4 - Evolution of the concentrations of total N in whole berries (in mg/Kg of fresh weight) of the three N treatments from fruit set up to ripeness.**

Error bars represent standard deviations.

Total N content per each berry part (flesh, skin, seeds) is presented in terms of mg/berry in order to make a comparison between the N content of the different berry parts. The first finding was that the total N of berries was found primarily in the seeds, followed by the flesh then by the skins (table 1). During ripening (day 213), the N content of berry flesh of « 30S/2.5F » and « 60S/5F » vines was higher than that of the control by 33% and 66%, respectively (table 1). However, only the increase in the case of « 60S/5F » treatment was statistically significant ( $p < 0.05$ ). Moreover, the N content of skin and seeds per

berry were not significantly affected by fertilization at this date.

Table 2 shows that the N treatments had little effect on the N content of skins and seeds at maturity. In addition, only the effect of the « 60S/5F » treatment was significant for berry flesh: the N content of « 60S/5F » berry flesh was higher by 53% than that of the control. The N content of berry flesh as a percentage of the total N of whole berries accounted the highest percentage in the case of the « 60S/5F » treatment (table 1). These results confirm

that berry flesh is the compartment most affected by N fertilization and seeds were the least affected by N treatments. The « 60S/5F » treatment increased the total amount of N per berry by about 0.32 mg as compared to the control: 0.18 mg in the flesh; 0.09 and 0.05 mg in the skin and in the seeds, respectively. Thus, it may be concluded that N absorbed during the summer was distributed in berries at maturity in the ratio of 56% in berry flesh, 28% in berry skin and 16% in seeds.

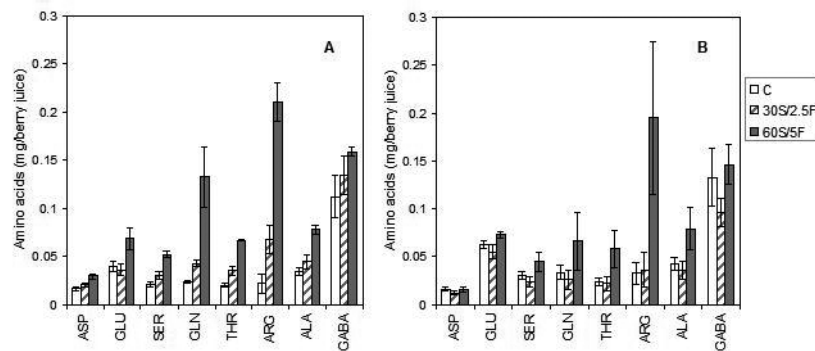
d) Total and assimilable N content of berry juices

Three days after N foliar fertilization (day 213), the « 30S/2.5F » treatment produced an increase in total and assimilable N by 35% and 37%, respectively in comparison to the control (table 3), and the « 60S/5F » treatment increased total and assimilable N by 60% and 143%, respectively, as compared to the control (table 3).

At maturity, only the « 60S/5F »-treated vines retained the increased concentrations of total and assimilable N

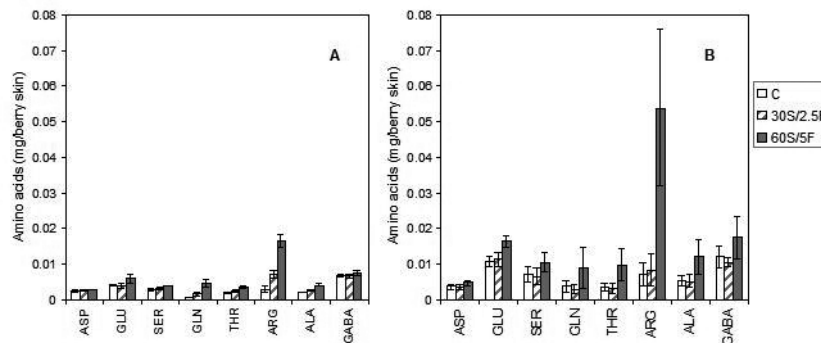
in juice observed on day 213 (49% and 94% respectively; table 3), though only the increase in assimilable N was statistically significant. The concentrations of assimilable N in the « 60S/5F » juice was 136 mg/l at maturity which shows that in the conditions of this experiment, the foliar fertilization dose should be at least 5 kg N/ha in order to correct must N deficiency. It may be observed that the effect of the « 30S/2.5F » treatment was only temporary since the increases obtained in N concentrations of berry juice three days after foliar fertilization were no longer found twelve days later (table 3).

Assimilable N proved to be a better indicator than total N of the efficiency of N fertilization treatments, since it provided a more significant differentiation between treatments (table 3). Moreover, the ratio of assimilable N as a percentage of total N was increased by foliar N fertilization, which applied at veraison, promotes synthesis of simple assimilable forms of N (amino acids and ammonium ions); this concurs with the fact that absorbed



**Figure 5 - Amino acid content of the juice (in mg/berry juice) of the three N treatments at two phenological stages: A: during ripening (day 213) and B: at ripeness (Day 232).**

ASP: Aspartic acid, GLU: Glutamic acid, SER: Serine, GLN: Glutamine, THR: Threonine, ARG: Arginine, ALA: Alanine, GABA:  $\gamma$ -aminobutyric acid. Error bars represent standard deviations.



**Figure 6 - Amino acid content of the skin (in mg/berry skin) of the three N treatments at two phenological stages: A: during ripening (day 213) and B: at ripeness (Day 232).**

ASP: Aspartic acid, GLU: Glutamic acid, SER: Serine, GLN: Glutamine, THR: Threonine, ARG: Arginine, ALA: Alanine, GABA:  $\gamma$ -aminobutyric acid. Error bars represent standard deviations.

N is first transformed to assimilable N before being transformed to more complex forms.

e) Amino N content of grape juice and skins

Amino N accounted for 60% to 70% of the assimilable N of juices and followed the same trends as the latter. At maturity, an increase of 69% (compared to the control) of the amino N in juice of the « 60S/5F » treatment was observed. Of the 21 primary amino acids analyzed in juices and skin extracts, the major ones were aspartic and glutamic acids, glutamine, serine, threonine, arginine, alanine, and  $\alpha$ -aminobutyric acid (GABA), representing about 80% of the total amino acid content in juices and in skins, data which concur with those of RIBÉREAU-GAYON *et al.* (1998). GABA was the major amino acid in the juices and the skins of the control and the « 30S/2.5F » berries, whereas arginine was the major amino acid in the juices (23 % of total amino acid content) and the skins (27 % and 31 % of total amino acid content on day 213 and 232, respectively) of the « 60S/5F » berries (figures 5AB, and 6AB, and table 4).

During ripening, the « 30S/2.5F » and « 60S/5F » treatments produced an increase in arginine in the berry juice of 205% and 853%, respectively (figure 5A), and in berry skin by 150% and 480%, respectively (figure 6A) in comparison to the control. The « 30S/2.5F » and « 60S/5F » treatments produced an increase in arginine in the berry juice of 9% and 490% respectively (figure 5B) and in berry skin of 17% and 655%, respectively (figure 6B) at maturity (day 232). Arginine has long been considered as an indicator of vine N status (KLIEWER and COOK, 1974; YU GAO and CAHOON, 1990). In fact, when conditions are favorable for N uptake, excess glutamine is converted to arginine which is the main storage component of N in vineyards (KLIEWER, 1967; SCHALLER *et al.*, 1989). This result thus indicates once more that N uptake was efficient only in the case of the « 60S/5F » treatment and that arginine proved to be the best indicator to discriminate between the N foliar fertilization treatments.

It may be observed that the « 60S/5F » treatment increased the amount of amino acids to a greater extent in juice than in the skins (147% versus 80%) during

**Table 4 - Concentrations of amino acids in juices and skins of the three treatments at ripeness. Values followed by the same letter in the same line did not differ significantly (test of Tukey,  $p < 0.05$ ).**

Amino acids	Amino acids concentrations in musts (mg/L)			Amino acids concentrations in skins (mg/Kg fresh weight)		
	C	30S/2.5F	60S/5F	C	30S/2.5F	60S/5F
Aspartic acid	15.5 ± 2.7 a	11.9 ± 1.6 a	14.9 ± 2.5 a	44.3 ± 9.4 a	39.5 ± 6.6 a	51.8 ± 8.3 a
Glutamic acid	59.7 ± 3.4 a	52.9 ± 8.4 a	69.7 ± 5.3 a	129.0 ± 22.1 a	123.5 ± 17.9 a	179.1 ± 19.0 a
Cysteine	0.2 ± 0.0 a	0.2 ± 0.0 a	0.3 ± 0.1 a	-	-	-
Asparagine	1.2 ± 0.2 a	0.9 ± 0.2 a	1.8 ± 0.6 a	4.6 ± 1.6 a	3.6 ± 0.2 a	5.7 ± 1.8 a
Serine	28.4 ± 4.1 a	23.4 ± 6.1 a	43.2 ± 11.5 a	86.1 ± 31.3 a	71.6 ± 22.6 a	113.9 ± 29.8 a
Histidine	0.5 ± 0.1 a	0.4 ± 0.2 a	1.0 ± 0.4 a	-	-	-
Glutamine	31.7 ± 7.4 a	26.0 ± 10.6 a	64.4 ± 29.6 a	45.9 ± 19.6 a	33.5 ± 13.3 a	97.9 ± 60.0 a
Glycine	13.1 ± 3.2 a	11.2 ± 3.9 a	14.4 ± 3.3 a	53.2 ± 21.4 a	42.7 ± 17.8 a	48.2 ± 11.4 a
Threonine	22.6 ± 3.9 a	21.3 ± 8.5 a	56.1 ± 19.8 a	42.6 ± 15.2 a	34.6 ± 14.6 a	107.5 ± 51.1 a
Arginine	31.4 ± 11.1 a	35.5 ± 18.1 a	188.2 ± 23.0 <b>b</b>	86.6 ± 41.2 a	91.0 ± 46.0 a	591.3 ± 81.0 <b>b</b>
Alanine	40.6 ± 7.1 a	34.5 ± 10.5 a	76.2 ± 22.3 a	64.7 ± 20.2 a	57.6 ± 16.3 a	132.1 ± 53.4 a
GABA	126.9 ± 31.2 a	92.2 ± 17.7 a	139.7 ± 21.4 a	144.9 ± 40.5 a	117.0 ± 12.0 a	191.1 ± 67.7 a
Tyrosine	2.8 ± 0.5 a	2.9 ± 1.2 a	4.9 ± 1.2 a	6.6 ± 2.4 a	6.0 ± 2.9 a	8.3 ± 3.4 a
Valine	16.6 ± 4.0 a	14.4 ± 6.2 a	33.4 ± 13.9 a	38.8 ± 15.1 a	32.8 ± 14.2 a	71.3 ± 33.7 a
Methionine	0.9 ± 0.3 a	0.8 ± 0.4 a	2.9 ± 1.8 a	8.7 ± 1.5 a	10.5 ± 1.3 a	9.2 ± 1.0 a
Tryptophane	9.4 ± 3.0 a	5.7 ± 3.4 a	12.9 ± 7.1 a	20.7 ± 8.2 a	13.5 ± 9.6 a	30.6 ± 20.1 a
Phenylalanine	15.1 ± 4.1 a	10.9 ± 4.8 a	26.2 ± 11.8 a	34.7 ± 13.6 a	26.6 ± 13.7 a	61.7 ± 32.3 a
Isoleucine	8.6 ± 2.6 a	7.1 ± 3.6 a	18.1 ± 9.5 a	19.7 ± 8.6 a	15.0 ± 8.8 a	38.1 ± 22.4 a
Leucine	9.9 ± 3.2 a	8.0 ± 4.5 a	23.7 ± 12.8 a	25.9 ± 9.9 a	22.2 ± 11.6 a	55.4 ± 31.3 a
Ornithine	2.7 ± 0.3 a	2.3 ± 0.5 a	5.4 ± 3.4 a	44.3 ± 26.4 a	38.3 ± 16.0 a	44.5 ± 9.1 a
Lysine	1.6 ± 0.3 a	1.5 ± 0.2 a	3.5 ± 0.9 a	16.1 ± 12.4 a	16.9 ± 4.7 a	16.7 ± 5.6 a

ripening (day 213), while the reverse was the case (120% - skins versus 73% - juice) at maturity (day 232). This result confirms that skins are particularly affected by N foliar fertilization during late ripening.

The total amount of amino acids in juice decreased between ripening (day 213) and maturity (day 232) in the juice of both the « 30S/2.5F » (24%) and « 60S/5F » (8%) treatments. This decrease may be due to the formation of more complex N forms such as proteins from amino acids. Conversely, the total amount of amino acids of the control juices increased by 29%.

The total amount of amino acids in skins increased between ripening and maturity by 133%, 84% and 185%, respectively in the skins of control, « 30S/2.5F » and « 60S/5F » treatments. This observation supports the hypothesis that skins serve as active sinks of N at the end of ripening, particularly as regards amino acids. Moreover, these results concur with RIBÉREAU-GAYON *et al.* (1998) who reported that amino acids of berry juice may be translocated to the skins during ripening. Furthermore this may also account for the decrease between ripening and maturity of the amino acids content in the juice of « 30S/2.5F » and « 60S/5F » treatments.

## CONCLUSION

The effect of N foliar fertilization with urea at veraison, in combination with soil fertilization at budbreak on total, assimilable and free amino N content of berries of water stressed *Vitis vinifera* L. cv. Sauvignon blanc vines was studied. It was observed firstly that the N content of leaves was unaffected by the N foliar fertilization applied around veraison, a result which confirms that N in leaves is not affected by summer N supply. Secondly, the fertilization of 30 kg/ha of soil-applied N coupled with 2.5 kg/ha of foliar-applied N was not sufficient to significantly increase juice N content of water -stressed vines at maturity. In fact the small effect that was observed with 2.5 kg/ha foliar-applied N dose was temporary, as analysis twelve days after foliar fertilization showed that only berries which received the 5 kg N/ha foliar-applied treatment had a significantly higher N content than the control. Thirdly, assimilable N proved to be a better indicator than total N in distinguishing between the N foliar fertilization treatments applied at veraison. However, the concentration of arginine provided the greatest discrimination between treatments, and its concentration was dramatically increased by fertilization treatments. Fourthly, the total N content of berry flesh was more affected by N foliar fertilization than other berry parts, whereas total N in seeds was the least affected. Amino acids began to accumulate in skins during ripening showing that this organ acts as an active sink of amino acids at this stage, and in fact, the skin amino acid content was more affected

than the berry amino acid content by foliar fertilization. This study also showed that N soil supply failed to increase the total N content of leaves and berries before veraison, whereas N foliar application of 5 kg N/ha lead to increases in total, assimilable and amino N of the berries at a period for which the low soil moisture involves poor root N absorption.

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