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ORIGINAL PAPER

Foliar application of urea to "Sauvignon Blanc" and "Merlot" vines: doses and time of application

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Abstract A careful control of the N nutritional status of grapevines can have a determining effect on wine characteristics; therefore a suitable management of N fertilization might allow some wine parameters to be modified, thereby improving product quality. The aim of this study was to determine the effect of foliar application of urea at different doses and different times of the growing season on the parameters of Sauvignon Blanc and Merlot grape juice. The research described herein involved Sauvignon Blanc and Merlot grapevines (*V. vinifera* L.) at a commercial vineyard and was conducted over 2 years. In the first year, N treatment involved a foliar application at a dose of 10 kg N ha^{-1} during veraison, whereas in the second year it involved a foliar urea application at two doses (10 and 50 kg N ha^{-1}) and at three different times—3 weeks before veraison, during veraison and 3 weeks after veraison. In this second year, the urea applied at a dose of 10 kg N ha^{-1} was isotopically labelled with 1% ^{15}N. Chemical parameters, yeast assimilable N, amino acid content, amino acid profile and N isotopic composition were determined for all treatments. Grape and grape-juice parameters for Merlot were found to be more affected by N fertilization than for Sauvignon Blanc and were also more affected during the second year than during the first year, thus indicating that the climatic characteristics of each campaign could affect these parameters. The yeast assimilable N in grape juice was found to be higher for late applications of foliar urea, with application of the higher dose of urea during veraison increasing the amino acid and proline contents in both varieties. The isotopic analysis data showed that the urea applied to leaves was transferred to the berries, with the maximum translocation in Sauvignon Blanc occurring for the post-veraison treatment and in Merlot for the veraison treatment. We can therefore conclude that foliar application of urea could modify grape juice quality and could therefore be used as a tool for obtaining quality wines.

Keywords *Vitis vinifera* · Isotopic composition · Nitrogen · Amino acids · N-transport

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Introduction

Although soil is generally the main source of nutrients for plants, foliar fertilization may rapidly and efficiently satisfy nutritional needs during a critical growth stage (Mengel 2002) and ultimately modify the ratio between vegetative and reproductive growth. Thus, it has been found that foliar fertilization can improve final grape quality and ensure good yields (Policarpo et al. 2006). A high penetration rate is one of the pre-requisites for efficient foliar nutrition. Urea, due to its intrinsic characteristics such as small molecular size, non-ionic nature and high solubility, is usually taken up rapidly through the leaf cuticle. Urea can be supplied to plants through the foliage, facilitating optimal nitrogen management, which minimizes nitrogen losses to the environment (Witte et al.

2002). Fertilization is known to be one of the viticultural techniques that most affects both vineyard yield and grape-juice and wine quality. However, due to variations in cultivar, climate and soil, the response of grapevines to N fertilization varies considerably. The supply of fertilizer usually results in an increased yield, although excessive or unbalanced applications have a negative effect on fruit quality. Nitrogen stimulates plant growth and the attainment of a sufficient leaf area, and moderate nitrogen supplies before flowering favour the synthesis of polyphenols in the grape (Keller and Hrazdina 1998). However, an increase in the productive capacity of the plant is often associated with an increase in berry size and therefore also in the pulp/skin ratio, thus resulting in a dilution of the anthocyanins and tannins in the grape juice (Spayd et al. 1994). Excessive nitrogen levels also delay ripening (Keller et al. 1999) and result in poorly colored fruit (Kliewer 1977), and also produce more vegetative growth, which competes with sugar translocation and pigment accumulation in the grape. These effects also interfere with the metabolic pathways responsible for the synthesis of those compounds responsible for berry aroma and flavour, as reviewed by Bravdo and Hepner (1987). However, a low level of N in the fruit is also associated with sluggish or stuck fermentation. Yeast assimilable nitrogen (YAN) compounds are required by yeast for growth and metabolism during the fermentation process (Bisson 1991), therefore imbalances and deficiencies in the supply of YAN are the most common cause of fermentation problems (Jiranek et al. 1995). The main sources of YAN are amino acids and ammonium, although free amino acids are reported to represent 50–90% of the YAN in grapes (Kliewer 1968). Many researchers have reported an increased concentration of YAN in the fruit in response to increased N fertilization in the vineyard (Spayd et al. 1994; Kliewer and Cook 1974). Indeed, both the concentration and type of nitrogenous compounds present in the fruit can affect the rate of fermentation, and these depend on grapevine variety and growing conditions (Spayd et al. 1994).

The aim of this work was therefore to evaluate the effect and the efficiency of urea foliar application on fruit composition.

Materials and methods

This work was carried out at a trellised vineyard containing Sauvignon Blanc and Merlot varieties belonging to Bodegas Inurrieta in Falces (Navarra) over a two-year period. Climatic data were collected from May to September at a weather station placed in the experimental plot. The average maximum temperature for the two-year study period was 25.6°C, the average minimum temperature was 13.7°C for the first year and 14.5°C for the second year, and the accumulated rainfall was 74 mm in the first year and 255 mm in the second year. This year the precipitation had irregular time distribution, so that 74% of the total rainfall occurred in the month of May when the vine water requirements were lower. According to Soil Taxonomy the soil is classified as a Fluventic Haploxerept with silty clay texture. Following the cultural practices of the area, only Sauvignon Blanc variety was irrigated by drip irrigation. Irrigations were performed from May to August according to the recommendations of Allen et al. (1998) (total irrigation cycles: 8; average quantity per cycle: 4.5 L m^{-2} in the first year and total irrigation cycles: 9; average quantity per cycle: 8.8 L m^{-2} in the second year).

Randomly selected vines from the two test varieties were treated as follows. In the first year, a single foliar urea application at a dose of 10 kg N ha^{-1}, applied during veraison, was compared with control treatment (without applied N), whereas in the second year the urea was applied in two doses (10 and 50 kg N ha^{-1}) at three times of plant growth, namely 3 weeks before veraison (time I), during veraison (time II), and 3 weeks after veraison (time III) and the results compared with the control treatment (without applied N). For urea application, the leaves were sprayed with a solution containing the amount of urea corresponding to each treatment at 200 mL per plant and with the grape bunches covered to prevent contamination. In the second year, the urea applied at a dose of 10 kg N ha^{-1} contained 1% of the tracer element ^{15}N. The test plot comprised five plants, with four repetitions for each treatment.

At harvest time, bunches were gathered and the grape juice extracted for each type of treatment. Fully developed, undeformed berries from various different bunches were chosen for the extraction to make the sample as significant as possible. These berries were then placed in a vegetable mill and crushed with a wooden pestle. The grape juice from each sample was centrifuged at 5,000 rpm for 15 min, after which the supernatant was collected and frozen for later analysis. The grape juice was subjected to the following measurements: weight, pH, total acidity by potentiometric assessment, degrees Baumé by refractometry, sugars (fructose + glucose) by enzymatic methods and probable alcohol content by alcoholometry. The easily assimilable nitrogen in the grape juice was determined by the method described by Aerny (1996), the amino acid profile by capillary electrophoresis using a Beckman-Coulter PA-800 apparatus for laser-induced fluorescence detection, and the total amino acid content corresponded to the sum of the individual values for every amino acid except proline. Once the berries from which to extract the different grape juices had been selected, the weight of 100 grapes was calculated and 15 berries were taken from each sample and placed in appropriately marked paper bags. These samples were then dried in an oven for about 10 days at 75°C. After drying, samples of the second year, control and with applied urea at a dose of 10 kg N ha^{-1} contained 1% of the ^{15}N tracer element, were taken and were ground in liquid nitrogen to homogenize. The resulting material was then further ground with a tissue homogenizer in stainless steel tanks. Subsamples of this material (1 mg of dry weight) were used to determine the $\delta^{15}N$ isotope content using a mass spectrometer (Delta Plus, Thermoquest, Finnigan) coupled to an NC 2500 elemental analyser (CE Instruments, Milan). The percentage of incorporated N in berries of N applied in leaves was calculated using the natural abundance calculated with $\delta^{15}N$, as described previously by Ariz et al. (2011). This value was then applied to the biomass produced and expressed as the amount of N added.

The data obtained were analyzed using a one- or two-way analysis of variance (ANOVA) depending on the parameter, with statistical significance being established at 95% for both analyses. The SPSS statistics program was used to perform all calculations. The one-way ANOVA was used for parameters that compare the time of application of urea or urea dose used for each variety separately; the least significant difference (LSD) test was applied and different letters were assigned for significant inter-treatment differences detected using the F-Fisher test. The two-way ANOVA was used for parameters that compare the effect of urea dose applied (D) and timing of urea (T), and the interaction of both variables ($D \times T$) for each variety separately and significant differences are expressed by asterisks.

Results and discussion

The characteristic parameters for the grapes and grape juice obtained from the Sauvignon Blanc and Merlot varieties subjected to different N treatments in this two-year study can be found in Table 1. Foliar urea application to Sauvignon Blanc vines in veraison during the first year did not significantly affect the grape juice or grape parameters, whereas it produced a significant decrease in total acidity and an increase in the degrees Baumé, sugar content and probable alcohol content in the Merlot variety. In the second year, the grape juice pH increased in those plants treated with urea with respect to the control treatment for both varieties. The total acidity was found to be significantly higher for late urea applications in both varieties, although this parameter was not affected by the dose of urea applied. None of the treatments applied had a significant effect on the degrees Baumé or sugar content in Sauvignon Blanc, whereas the probable alcohol content decreased with an increase in nitrogen dose, although only when the urea was applied during stage III. For Merlot variety in the second year, the degrees Baumé, sugar content and probable alcohol content increased significantly with foliar urea application, although this increase was greater for the lower dose of urea. Berry size showed no clear relationship to foliar fertilization or the stage at which it was applied for either variety (Table 1). The different behaviours observed for the two varieties studied as regards the variation in characteristic grape and grape-juice parameters with foliar urea application could be explained by an interaction of these N treatments with meteorological aspects. Water conditions have long been recognized to be an important factor affecting grape quality and therefore the sensory attributes of the resulting wine. Indeed, many papers have reported extensively on the effects of water deficit on the accumulation of various grape metabolites such as sugars and tartaric acid (Koundouras et al. 2006). In our study, whereas the Sauvignon Blanc vines were irrigated by drip irrigation to control their water status, the Merlot vines were not irrigated and therefore probably suffered greater water stress due to the high temperatures and low and irregular rainfall recorded in the study area.

The supply of nitrogen stimulates vegetative growth, thereby producing new foliar tissue. Consequently, the source-sink competition may be reversed after application, to the detriment of the berries, as the sugars migrate towards the new tips undergoing growth. Indeed, Xia and Cheng (2004) found that foliar urea application decreased the starch, glucose and fructose content but that the sucrose concentration remained unaffected in vine plants one-year-old. These authors concluded that a high percentage of non-structural carbon is incorporated into proteins and free amino acids upon foliar urea application.

The yeast assimilable N and total amino acid content were found to be higher in Sauvignon Blanc than in Merlot, whereas the proline content was higher in the Merlot variety (Table 2). The yeast assimilable N and total amino acid content in grape juice did not present significant differences between the control and urea treatment at veraison stage in the first year for either variety. In the second year, however, the yeast assimilable N in grape juice increased significantly with foliar urea application, with this increase being greater the later the application for both varieties. There is no clear trend that might explain how the urea dose affects this parameter. A low yeast assimilable N content in grape juice leads to low yeast populations and poor fermentation vigour, an increased risk of slow fermentations, an increased production of undesirable thiols (e.g. hydrogen sulfide) and higher alcohols, and a decreased production of esters and long chain volatile fatty acids. A high yeast assimilable N level in grape juice leads to increased biomass and higher maximum heat output due

Table 1 Characteristic parameters for the grapes and grape juice of Sauvignon Blanc and Merlot varieties in the 2 years of study treated with the different N treatments

	pH	Total acidity (g Tartaric acid L^{-1})	Baumé degrees	Sugars (fructose + glucose) (g L^{-1})	Probable alcohol content (alcoholic degrees)	100 grapes' weight (g)
1st year						
Sauvignon Blanc						
Control	3.16 a	10.8 a	12.8 a	225 a	13.2 a	162 a
Urea II (10 kg N ha^{-1})	3.13 a	11.0 a	11.7 a	201 a	11.8 a	166 a
Merlot						
Control	3.40 a	7.8 a	13.6 a	239 a	13.6 a	138 a
Urea II (10 kg N ha^{-1})	3.45 a	6.8 b	14.2 b	257 b	14.7 b	136 a
2nd year						
Sauvignon Blanc						
Control	3.26	8.64	11.1	186	11.1	142
Urea I (10 kg N ha^{-1})	3.27	8.22	11.0	186	11.0	117
Urea I (50 kg N ha^{-1})	3.35	9.47	11.0	185	11.0	163
Urea II (10 kg N ha^{-1})	3.40	9.03	11.7	197	11.7	147
Urea II (50 kg N ha^{-1})	3.42	7.67	11.8	201	11.9	142
Urea III (10 kg N ha^{-1})	3.50	9.28	12.1	205	12.2	144
Urea III (50 kg N ha^{-1})	3.28	10.32	9.9	165	9.8	154
Merlot						
Control	3.51	5.59	14.3	245	14.5	134
Urea I (10 kg N ha^{-1})	3.49	4.51	15.4	266	15.8	127
Urea I (50 kg N ha^{-1})	3.61	5.35	14.5	246	14.6	127
Urea II (10 kg N ha^{-1})	3.71	5.44	15.2	256	15.2	131
Urea II (50 kg N ha^{-1})	3.63	5.43	14.9	252	15.0	132
Urea III (10 kg N ha^{-1})	3.80	5.43	15.2	256	15.2	122
Urea III (50 kg N ha^{-1})	3.61	6.09	14.7	248	14.8	139
Sauvignon Blanc						
D × T	*	N.s.	N.s.	N.s.	*	*
D	N.s.	N.s.	N.s.	N.s.	*	*
T	N.s.	*	N.s.	N.s.	N.s.	N.s.
Merlot						
D × T	*	N.s.	N.s.	N.s.	N.s.	*
D	N.s.	N.s.	*	*	*	N.s.
T	*	*	*	*	*	N.s.

The first year compared a control treatment without foliar urea application and a treatment with foliar urea application at a dose of 10 kg N ha^{-1} for each variety, the second year compared two doses of urea (10 and 50 kg N ha^{-1}) and three times of foliar application of urea, I pre-veraison, II veraison, III post-veraison, for each variety. Statistical analysis of the first year is a one-way Anova which compares the dose of urea applied to each variety separately, the second year data are analyzed with a two-way Anova which analyzes the effect of the dose of urea (D) and the application time of urea (T) and the interaction of both variables (D × T) for each variety separately, asterisk, significant differences at 95%; N.s., no significant differences, (n = 5)

to greater fermentation vigour, as well as increased formation of ethyl acetate, acetic acid and volatile acidity (Bell and Henschke 2005). There are, however, discrepancies as regards the minimum amount of assimilable nitrogen necessary in grape juice to ensure problem-free alcohol production. Some authors, for instance, have proposed that a concentration of 200 mg L^{-1} of assimilable nitrogen is acceptable (Trioli and Paronetto 1992). All the treatments in our study, including the control, exceeded this concentration.

As far as proline is concerned, the content of this amino acid was significantly higher in the Merlot variety than in the Sauvignon Blanc variety. As mentioned above, the Sauvignon Blanc vines were irrigated whereas the Merlot vines were not, thus meaning that the latter were exposed to water deficit. In this respect, it is well documented that

Table 2 The yeast assimilable N, total aminoacids and proline content in grape juice of Sauvignon Blanc and Merlot varieties in the two years of study treated with the different N treatments

	Yeast assimilable N in grape juice (mg L^{-1})	Total amino acids in grape juice (mg L^{-1})	Proline in grape juice (mgl pro L^{-1})
1st year			
Sauvignon Blanc			
Control	292 a	2783 a	380 a
Urea II (10 kg N ha^{-1})	280 a	3095 a	276 b
Merlot			
Control	156 a	1182 a	1990 a
Urea II (10 kg N ha^{-1})	194 a	1144 a	2139 a
2nd year			
Sauvignon Blanc			
Control	353	2226	264
Urea I (10 kg N ha^{-1})	371	3795	471
Urea I (50 kg N ha^{-1})	465	1567	161
Urea II (10 kg N ha^{-1})	492	2133	264
Urea II (50 kg N ha^{-1})	491	7187	414
Urea III (10 kg N ha^{-1})	634	5967	437
Urea III (50 kg N ha^{-1})	514	2847	218
Merlot			
Control	187	628	1242
Urea I (10 kg N ha^{-1})	126	661	1345
Urea I (50 kg N ha^{-1})	242	797	1380
Urea II (10 kg N ha^{-1})	298	968	1886
Urea II (50 kg N ha^{-1})	232	2757	2369
Urea III (10 kg N ha^{-1})	428	875	1506
Urea III (50 kg N ha^{-1})	318	1070	1656
Sauvignon Blanc			
D × T	*	*	*
D	N.s.	N.s.	N.s.
T	*	*	*
Merlot			
D × T	*	*	N.s.
D	N.s.	*	*
T	*	*	*

The first year compared a control treatment without foliar urea application and a treatment with foliar urea application at a dose of 10 kg N ha^{-1} for each variety, the second year compared two doses of urea (10 and 50 kg N ha^{-1}) and three times of foliar application of urea, I pre-veraison, II veraison, III post-veraison, for each variety. Statistical analysis of the first year is a one-way Anova which compares the dose of urea applied to each variety separately, the second year data are analyzed with a two-way anova which analyzes the effect of the dose of urea (D) and the application time of urea (T) and the interaction of both variables (D × T) for each variety separately, asterisk, significant differences at 95%; N.s., no significant differences (n = 5)

such an abiotic stress results in the accumulation of proline, which acts as an osmoregulator in the tissues of many plants, including vines (Ramteke et al. 2001). The proline content in the grape juice obtained from Sauvignon Blanc vines treated with urea was lower than in the control treatment; the proline content for the Merlot variety was not affected by urea application in the first year. The total amino acid and proline contents for the Sauvignon Blanc grape juice obtained in the second year were greater when the urea was supplied at a dose of 10 kg N ha^{-1} at times I and III and a dose of 50 kg N ha^{-1} at stage II. Likewise, the total amino acid and proline contents in Merlot grape juice were higher when the urea was applied during veraison than when applied either before or after. Application

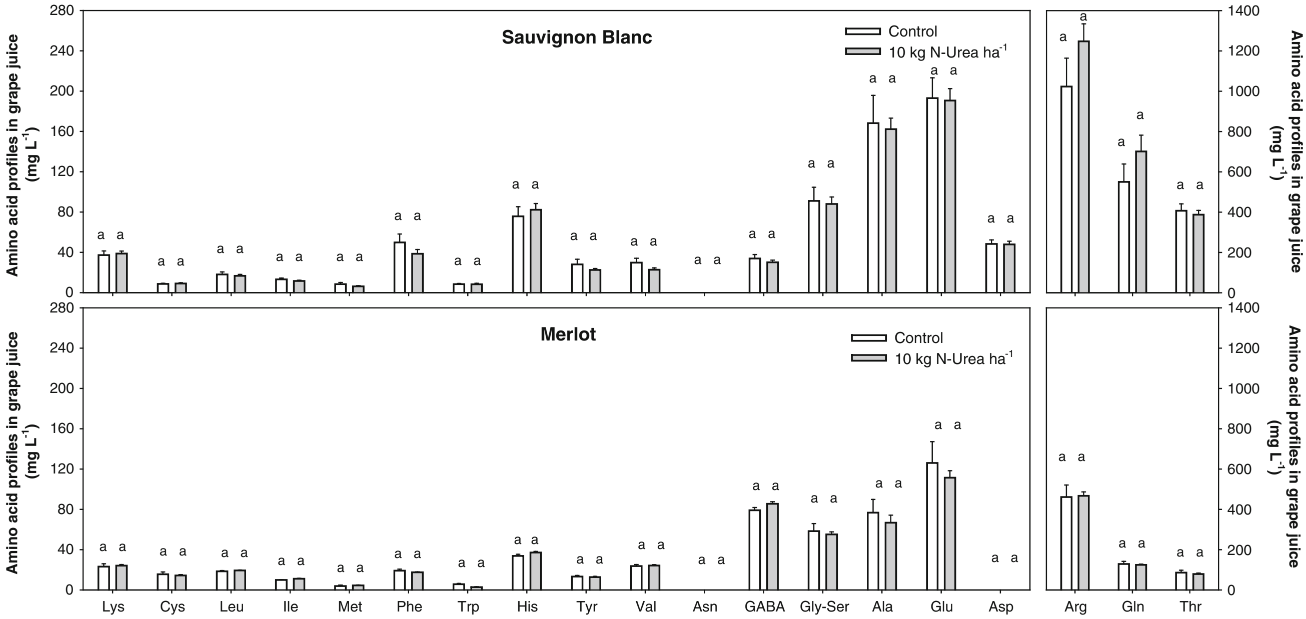

Fig. 1 Amino acid profile in grape juice of N- treatments applied in the first year for Sauvignon Blanc and Merlot variety. Statistical analysis is a one-way Anova which compares the dose of urea applied to each variety separately, (n = 5)

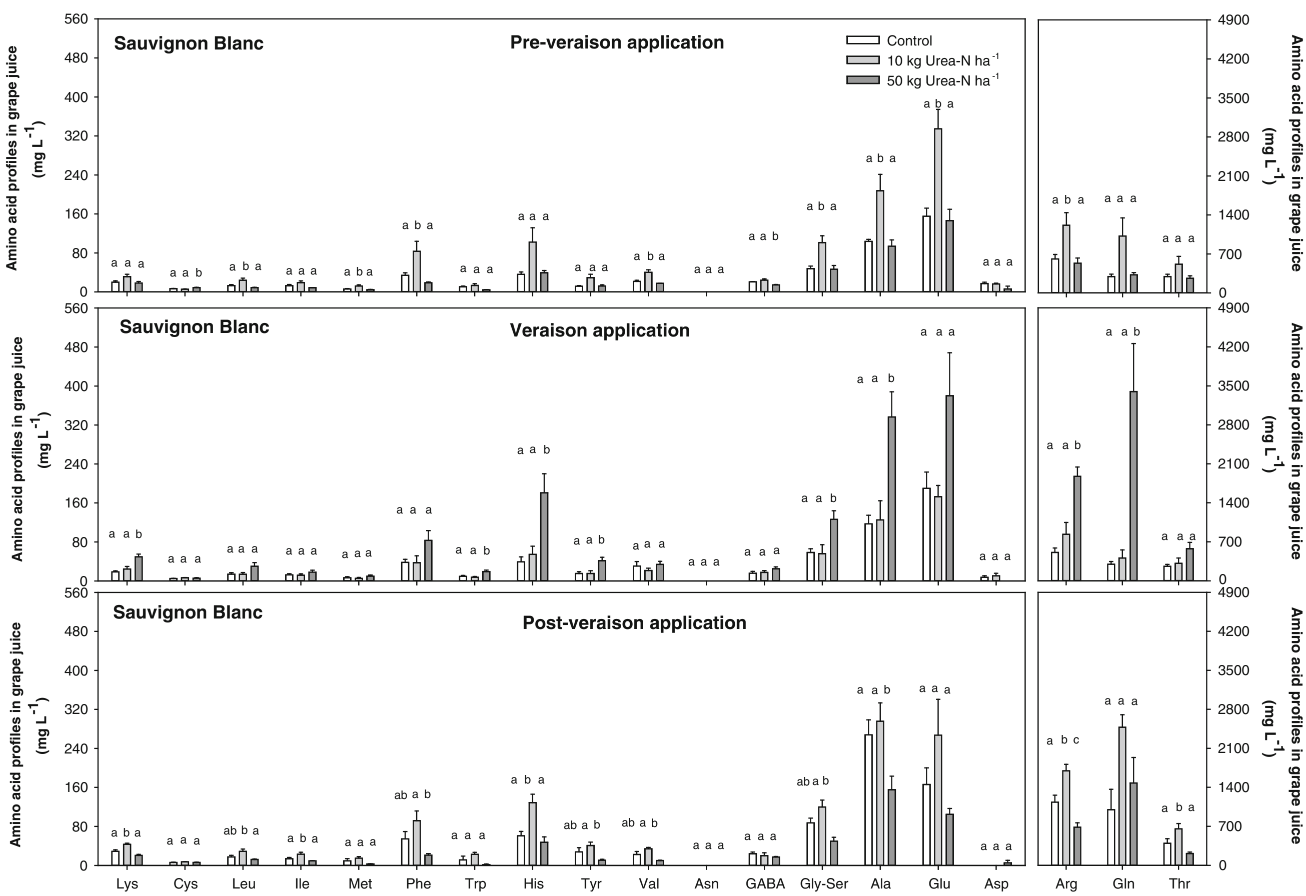

Fig. 2 Amino acid profile in grape juice of N- treatments applied in the second year for Sauvignon Blanc variety. Statistical analysis is a one-way Anova which compares the dose of urea applied at each time of application, (n = 5)

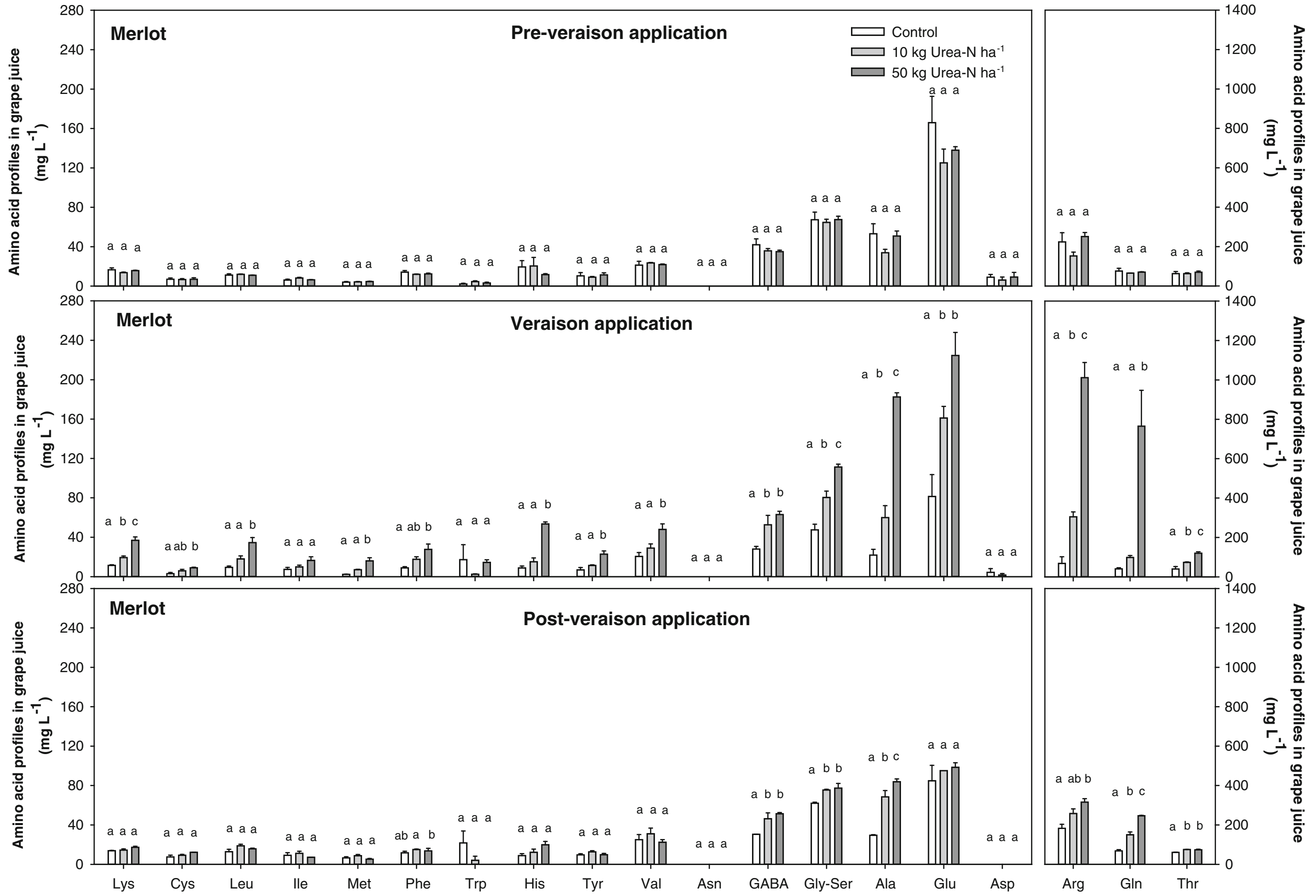

Fig. 3 Amino acid profile in grape juice of N- treatments applied in the second year for Merlot variety. Statistical analysis is a one-way Anova which compares the dose of urea applied at each time of application, (n = 5)

of the higher dose of urea increased the value of these parameters during veraison (Table 2).

Nitrogen compounds in the grape juice are of great interest in wine-making as they are crucial nutrients for yeast. Indeed, grape juices with high yet balanced amino acid contents result in a greater synthesis of ethyl esters, which are considered to be desirable for wine quality due to their fruity aromas (Lacroux et al. 2008).

Figures 1, 2, and 3 show the amino acid profiles in grape juice for the different N treatments during this two-year study. The foliar application of urea during the first year did not change the amino acid profile of grape juice from either of the varieties studied significantly (Fig. 1). In the second year, foliar urea application to Sauvignon Blanc vines during veraison and after veraison resulted in a greater increase in some amino acids, primarily arginine and glutamine. Indeed, this increase was significant for doses of 50 kg N ha^{-1} during veraison and for doses of 10 kg N ha^{-1} post-veraison (Fig. 2). Likewise, the data show that the foliar application of urea before and after veraison barely affected the amino acid profiles in grape juice from the Merlot variety, whereas the application of urea during veraison increased the content of the majority of amino acids, with this increase being greater for the high dose of urea. It is well known in viticulture and oenology that "good-quality" fruit requires a halt to herbaceous growth around veraison so that the berries can become the main sink. The amino acids whose contents increased most were arginine, glutamine and alanine (Fig. 3). Arginine is a good nitrogen source for the alcoholic fermentation of wine since it provides four nitrogen atoms per molecule and yeasts can incorporate it directly into proteins or store it in vacuoles as a nitrogen reserve (Watson 1976). Moreover, a somewhat lower increase in the amino acid threonine, which, together with phenylalanine and aspartic acid, appears to be one of the amino acids with the greatest influence on wine aroma composition,was reported by Hernandez-Orte et al. (2006).

Figure 4 shows the data for the δ^{15}N content of the berries of both varieties treated with foliar doses of 10 kg N ha^{-1} at the three application times (second year). Berries from the Sauvignon Blanc variety had significantly higher δ^{15}N content than the control berries for all foliar applications with N-labelled urea. A trend whereby the

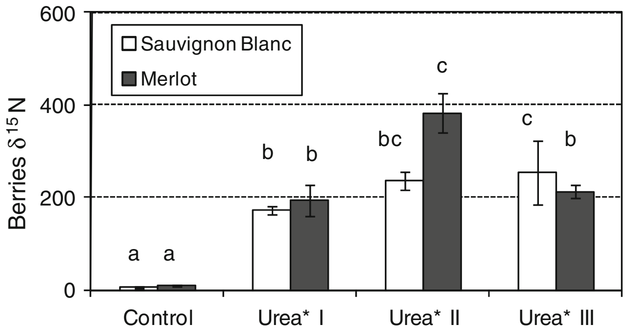

Fig. 4 Berries δ^{15}N of N- treatments applied in the second year. Urea* is 10 kg urea-N ha^{-1} labelled with ^{15}N isotope (1%) and I, II, III is the time of urea foliar application, I pre-veraison, II veraison, III post-veraison. Statistical analysis corresponds to a one-way Anova which compares the application time for each variety separately, (n = 5)

Table 3 Percentage of incorporated N in berries of N applied in leaves in the second year

	Incorporated N in berries of N applied in leaves (%)
Sauvignon Blanc	
Urea I (10 kg N ha^{-1})	35 a
Urea II (10 kg N ha^{-1})	17 a
Urea III (10 kg N ha^{-1})	57 a
Merlot	
Urea I (10 kg N ha^{-1})	28 a
Urea II (10 kg N ha^{-1})	80 b
Urea III (10 kg N ha^{-1})	61 ab

Nitrogen is applied as urea at a dose of 10 kg urea-N ha^{-1} labelled with ^{15}N isotope (1%) in I, II, III time of foliar urea application, I preveraison, II veraison, III post-veraison. Statistical analysis corresponds to a one-way Anova which compares the application time for each variety separately, (n = 5)

δ^{15}N was higher after the later urea application was also observed. In a similar manner to the Sauvignon Blanc variety, all berries from the Merlot variety had significantly higher δ^{15}N content than the control treatment after the foliar application of labelled urea, although application during veraison produced a significant increase in δ^{15}N content (order of 90 top %) with respect to applications prior to and subsequent to this stage. According to our data and depending on the variety and timing of urea, between 17% and 80% of the nitrogen applied to the leaves as urea was absorbed by the berries (Table 3). Schreiber et al. (2002) found that 30% of the nitrogen absorbed by grapevine leaves ended up in the berries, whereas only 2% of root-absorbed nitrogen ended up in the berries. The δ^{15}N parameter represents the incorporation and migration of the urea nitrogen applied to the leaves towards the berries as the nitrogen contained in this the urea included 1% ^{15}N as tracer. The enrichment of a tissue with this isotope produces an increase in the δ^{15}N parameter, which can be explained by incorporation of the urea applied and transported from the leaves to the fruit.

The percentage of N applied to the leaves that was subsequently transported to the berries did not differ significantly between the foliar urea application times for the Sauvignon Blanc variety, whereas this value was significantly higher when the urea was provided post-veraison or during veraison than pre-veraison for Merlot berries (Table 3), thereby appearing to confirm an increased transfer of nutrients from the vegetative part to the berry during veraison in the Merlot variety.

As general conclusions, our study clearly shows that foliar application of urea to *Vitis vinifera L.* has the following consequences for the analytical parameters of grape juice:

(i) It significantly increases the synthesis and accumulation of amino acids such as Arg, Gln and Thr that are rapidly assimilated by the fermenting yeasts, as well as Ala, which is assimilated more slowly, thereby resulting in better fermentation kinetics and higher production of the ethyl esters essential for quality aromatic wine.
(ii) Late application of urea (15 days after veraison) increases the acidity of the resulting wine.
(iii) Late, high-dose application of urea limits the accumulation of sugars in the berry whilst maintaining optimal quality values.
(iv) The berry size is not influenced by either dose or time of application; therefore there is no dilution of any of the other compounds that influence wine quality.

Finally, we note that the contribution of foliar urea to ripening modulates wine parameters of interest such as the nitrogen composition of the must, acidity and sugar accumulation.

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