Response of ‘Italian Riesling’ Leaf Nitrogen Status and Fruit Composition (*Vitis vinifera* L.) to Foliar Nitrogen Fertilization

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Additional index words. *Vitis vinifera*, foliar nitrogen fertilization, leaf nitrogen status, fruit composition, free amino nitrogen, ammonia, yeast assimilable nitrogen

Abstract. Two-year study was conducted on Italian Riesling cultivar with the aim to compare the effect of foliar sprays with different nitrogen forms on grapevine leaf N status, yield, and nitrogen compounds in grape juice. Treatments included no fertilization (control), soil NPK treatment, and three foliar treatments [amino acids, urea, ammonia (NH₄⁺)/nitrate] applied four times during season, also treated with soil NPK. The application of 1% w/v urea significantly increased leaf total leaf N content in the second year of study. However, there were no effects on N compounds in grape juice, since changes in free amino nitrogen (FAN), NH₄⁺, and consequently yeast assimilable nitrogen (YAN) were not consistent among the treatments and experimental years. Although increase of vine leaf N status was achieved by 1% w/v urea, additional modifying of application time (by moving it closer to veraison) is needed, with the aim to increase N compounds in grape juice as well.

Nitrogen is a macronutrient with major role in plant growth as it is a constitutive part of nucleic acids, chlorophyll, amino acids, and therefore essential for the cell proliferation during intensive vegetative growth (Jackson, 2000). Moreover, nitrogen is required during alcoholic fermentation of must since yeasts use it for their cell growth (Keller, 2010). Its deficiency can cause stuck or sluggish fermentation (Jiranek et al., 1995). Content of nitrogen compounds in must also affects formation of volatile compounds during alcoholic fermentation (Barbosa et al., 2009, 2012).

Nitrogen fertilization is a regular viticultural practice proved to be affecting vine yield (Bell and Henschke, 2005; Smart and Robinson, 1991; Spayd et al., 1993), grape ripening and fruit composition (Christensen et al., 1994; Linsenmeier et al., 2008; Spayd et al., 1994), must nitrogen compounds (Linsenmeier et al., 2008; Neilsen et al., 2010; Schreiner et al., 2014) as well as grape

and wine aromatic profile (Barbosa et al., 2009; Garde-Cerdan et al., 2014; Giorgessi et al., 2001). Addition of nitrogen to must to provide sufficient YAN is a common procedure in winemaking practice since it stimulates sugar utilization and improves fermentation rate (Arias-Gil et al., 2007).

Common viticultural practice includes nitrogen soil application in the form of mineral fertilizers. It was subject of interest by various authors who considered different ways to improve nitrogen soil availability, utilization, grapevine supply and hence alcoholic fermentation (Bell and Robson, 1999; Christensen et al., 1994; Conradie, 1992; Holzapfel and Treeby, 2007; Schreiber et al., 2002).

In recent years, foliar nitrogen application occurred as additional way of improvement of nitrogen fertilization since it is less dependent on weather conditions (Jreij et al., 2009) and less harmful to environment, considering nitrate soil leaching (Dong et al., 2005; Schreiber et al., 2002). Several experiments of foliar use of different N forms have been reported, including comparison of urea, nitrate, and NH₄⁺ (Porro et al., 2010). Moreover, foliarly applied proline, phenylalanine, and urea were compared with commercial nitrogen fertilizers,

considering effect on grape amino acid content (Garde-Cerdan et al., 2014).

Foliar N application can also be considered as a tool for improvement of wine sensory characteristics since positive effect on grape volatile composition (Garde-Cerdan et al., 2014) and enhancement of grape and wine phenolic content (Portu et al., 2015a, 2015b) have been determined.

However, comparison of foliarly applied different N forms did not include the study of their effect on vine N supply, in addition to their effect on grape amino acid content.

Also, as previously reported, foliar nitrogen application is usually performed around veraison (Cheng et al., 2009; Jreij et al., 2009; Lacroux et al., 2008), which proved to be an effective way to improve berry YAN content since nitrogen translocation in that period is directed mainly to berries. However, in vineyards with general nitrogen deficiency caused by both soil and grapevine low N status that can occur as a problem since general improvement of nitrogen vine supply is required.

As suggested by Neilsen et al. (2010) and Tozzini et al. (2013), earlier N application could affect both leaf and berry N accumulation. Research on late spring–applied (Conradie, 1990) and early summer–applied (Conradie, 1991) nitrogen showed that N translocation was mainly directed to bunches. Additionally, fall nitrogen fertilization also proved to be efficient in improving petiole N status (Peacock et al., 1991) or berry YAN supply (Hannam et al., 2014).

For this trial, vineyard with a history of low nitrogen status causing difficulties during alcoholic fermentation was selected. Therefore, multiple foliar nitrogen application was investigated, to generally improve grapevine nitrogen supply as well as berry N content.

The objective of this study was to compare effectiveness of different nitrogen forms (amino acids, urea, NH₄⁺/nitrate) in improving vine N supply and berry nitrogen compounds when foliarly applied several times during season. Due to increasing use of some commercial fertilizers, one commercial product containing amino acids was included in this study.

Materials and Methods

Vineyard site and plant material. Two-year study (2012 through 2013) was conducted on Italian Riesling cultivar at Jazbina experimental field (Faculty of Agriculture, University of Zagreb, long. 45°51' N, lat. 16°0' E), which is characterized by moderately warm and rainy continental climate. Experimental vines were planted in 1997 with row spacing of 1.2 between vines × 2 m between rows (4167 vines/ha), oriented east-west. Soil type was anthropogenic pseudogley with clay texture.

Meteorological data were collected at Zagreb Maksimir station, 5 km from experimental site.

Growing season 2012 had total amount of 1857 growing degree day (GDD), with average

Received for publication 4 Dec. 2015. Accepted for publication 26 Jan. 2016.

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growing season temperature of 18.5 °C. Growing season precipitation was 532.5 mm unevenly spread through the ripening season, which was especially notable in August when only 9.8 mm of precipitation was measured. Growing season 2013 was quite cooler, having total of 1718 GDD with 17.8 °C average growing season temperature. Growing season precipitation was 518.4 mm, which in addition to lower temperatures lead to later harvest (25 Sept.) compared with 2012 (18 Sept.).

'Italian Riesling' vines (clone ISV-1) were grafted on SO4 rootstock. Vines were trained to double Guyot, leaving 24 buds per vine. Fruit-bearing wire was set to 80 cm aboveground, with addition of two sets of catch wires at 40 cm intervals from the fruit-bearing wire.

Vineyard was not irrigated and no fertilization was applied 1 year before experiment.

Soil analysis performed during winter period before first experimental year showed that it was very acid with a surface pH (in KCl) of 4.1 (0 to 30 cm deep). The soil was very poor in organic matter, ranging from 0.9% (0 to 30 cm deep) to 1.6% (30 to 60 cm deep). The lower horizon of soil was richer in organic matter due to the trenching of soil up to 60 cm deep, performed before the planting of vines. Available P was lower than 4 mg of P₂O₅/100 mg soil. Available K did not exceeded 12 mg of K₂O/100 mg soil. The soil was moderately supplied with nitrogen, ranging from 0.06% (0 to 30 cm deep) to 0.1% (30 to 60 cm deep). Following soil analysis, mineral fertilizer 7N–8.7P–24.9K was applied to entire experimental plot except control vines.

Viticultural practice during season, usual for this viticultural area, was performed. Application of glyphosate was used in aim to keep the soil beneath the vine rows weed-free. Shoots exceeding the height of the trellis were hedged to 20 cm above the last wire, 4 weeks before veraison. Vines were not subjected to irrigation treatment.

This vineyard site was selected for experiment because of repeating low must YAN content in years before experiment, which indicated low vine N supply. Study with different levels of urea soil application was performed (Karoglan, 2009); however, satisfactory YAN level was not achieved.

Treatments and experimental design. This single-factor experiment consisted of five fertilization levels, designed as latin square. Each repetition included 18 continuous vines. Experiment had a total of 25 units (plots) distributed in five rows, with nontreated rows between experimental ones.

With the aim to compare foliar sprays of different nitrogen forms, using fertilizers frequently used in local viticultural practice, following sprays were applied: amino acids (Drin®, Green Has Italia, Canale d'Alba, Italy), urea (urea), and NH₄⁺/nitrate (ammonium nitrate) containing 6.3%, 46%, and 17.5% nitrogen, respectively. Treatments were five levels of fertilization as follows: 1) control without fertilization (C), 2) early-season soil application of 250 kg/ha 7N–8.7P–24.9K (NPK) 3) NPK treatment with addition of

foliar amino acids applications (0.25% w/v Drin), (DR), (4) NPK treatment with addition of foliar urea applications (1% w/v urea) (UR), and (5) NPK treatment with addition of ammonium/nitrate applications (0.25% w/v ammonium nitrate) (AN). Application of commercial products with different N content, applied in concentration following manufacturers' recommendation led to different N quantities. However, intention was to directly evaluate practical effectiveness of applied fertilizers, based on the results of experiment.

Foliar applications were repeated at four growth stages: young shoot with eight leaves separated, before flowering, berries pea size, and after harvest, which represent stage 15, 18, 31, and 41 according to modified Eichorn and Lorenz system (Coombe, 1995). Fertilizers were applied using backpack sprayer until runoff. Sprays containing N were applied in 926 L·ha⁻¹ of water. The rates of N were 17.04 kg N/ha/year for UR treatment, 1.64 kg N/ha/year for AN treatment, and 0.60 kg N/ha/year for DR treatment. In both years, foliar sprays were applied early in the day (before 12:00 AM), so temperatures never exceeded 25 °C during treatment application. There were no leaf damages observed after treatment application.

Leaf nitrogen measurements. Before every foliar application leaf samples were collected, consisting 10 visually healthy opposite basal cluster leaves per each repetition, positioned between nodes 2 and 4. Leaves were collected according to standard method for leaf diagnosis that recommends sampling of blades opposite the basal clusters, established by the Organisation Internationale de la Vigne et du Vin (OIV, 1996). The leaves were dried at 105 °C according to Wermelinger and Koblet (1990). The total leaf nitrogen was determined according to Kjeldahl method (AOAC, 1995).

Yield components and juice analysis. Grapes were harvested at their full maturity when the total soluble solids (%SS) of 100 randomized collected berries remained constant for a few days, which was on 18 Sept. 2012 [261 day of the year (DOY)] and 25 Sept. 2013 (268 DOY).

Number of clusters and yield per vine were determined. Cluster weight, yield per vine, and yield per hectare were calculated based on collected data.

Clusters were separately destemmed and crushed for each experimental plot and submitted to juice analysis. %SS were measured using handheld refractometer (MASTER-OE; Atago, Tokyo, Japan). Titratable acidity (TA, g·L⁻¹) was measured by titration with 0.1 M NaOH according to OIV (2013) method. Additionally, samples were taken for each repetition and frozen at –18 °C for following juice analysis of nitrogen compounds.

Juice nitrogen compounds. Frozen juice samples were thawed at room temperature (21 °C) to determine content of must nitrogen compounds. FAN content (mg·L⁻¹) was determined using ultraviolet spectrophotometer (SPECORD 400; Analytik Jena AG, Jena,

Germany), by nitrogen by o-phthaldialdehyde assay (NOPA) procedure according to Dukes and Butzke (1998). NH₄⁺ content (mg·L⁻¹) was measured with Megazyme Ammonia Assay Kit and procedure (Megazyme, Chicago, IL), according to the method of Bergmeyer and Beutler (1990), using ultraviolet spectrophotometer (SPECORD 400). YAN (mg·L⁻¹) was calculated as a sum of FAN and NH₄⁺ content.

Statistical analysis. All variables were analyzed by one-way analysis of variance, using foliar spray treatments as factors. Data were examined separately by year. Treatment means were compared using Fisher's least significant difference test to establish whether there were significant differences among treatments ($P \leq 0.05$). All statistical analysis were conducted using Statistica software (Version 5.0; Statsoft Inc., Tulsa, OK).

Results

Vine nitrogen supply. Vine leaf nitrogen supply affected by foliar-applied different nitrogen forms is indicated by leaf total N (%) (Table 1). Before first foliar application, average leaf total N level in experimental vineyard was very high, 3.43%. The results considering vine nitrogen supply as affected by fertilization treatments were not consistent, especially throughout first year of experiment. Additionally, vine nitrogen supply showed different dynamics through vegetative period of first and second experimental year. In 2012, highest average leaf total N value was at the beginning of the season and afterward was decreasing toward the end of vegetation period. Contrary, in 2013, it peaked at "berries pea size" stage and decreased afterward toward lowest average value at "after harvest" growth stage.

In 2012, foliar treatments did not significantly affect vine nitrogen supply although some significant differences regarding leaf total N occurred at prebloom and after harvest growth stages.

No significant difference was recorded at "berries pea size" stage although leaf total N was increased by UR and AN treatments. However, that increase was nonsignificant neither it repeated at other recorded growth stages.

In 2013, average leaf total N in foliar treatments was higher than in nonfoliar treatments in all growth stages except at the beginning of season ("eight leaves separated" stage).

Also, leaf total N was significantly increased by UR treatment at all observed stages except "eight leaves separated" when slightly higher value was obtained by DR, compared with other treatments. At "eight leaves separated" growth stage, slightly increased leaf total N value by C treatment indicates there was no effect due to foliar treatments from first experimental year.

Yield components and juice quality. None of the fertilization treatments had clear and consistent influence on yield parameters (Table 2). However, during first experimental year, slightly higher values occurred within

Table 1. Effect of nitrogen foliar treatments on vine leaf nitrogen status indicated by leaf total N (%).

Treatments ^c	8 Leaves separated	Prebloom	Berries pea size	After harvest
Year 2012				
C	3.43	3.04 a	2.27	1.78 a
NPK		3.09 a	2.33	1.75 a
DR		3.05 a	2.33	1.65 b
UR		3.01 ab	2.39	1.66 b
AN		2.89 b	2.38	1.75 a
Significance ^y		**	NS	***
Year 2013				
C	3.58	2.92 b	3.62 c	1.50 b
NPK	3.50	2.92 b	3.84 bc	1.48 b
DR	3.60	3.03 ab	3.82 bc	1.52 b
UR	3.50	3.11 a	4.11 a	1.61 a
AN	3.52	2.99 ab	4.01 ab	1.54 ab
Significance	NS	*	***	**

^cNPK, DR, UR, and AN indicate treatments of soil application of 250 kg/ha of NPK fertilizer, NPK treatment with addition of foliar amino acids applications (0.25% w/v Drin), NPK treatment with addition of foliar urea applications (1% w/v urea), and NPK treatment with addition of ammonium/nitrate applications (0.25% w/v ammonium nitrate), respectively. C indicates control treatment without fertilization.

^yResults of analysis of variance: NS, *, **, ***Nonsignificant or significant difference at $P \leq 0.05$, 0.01, and 0.001, respectively.

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ by least significant difference test.

Table 2. Effect of nitrogen foliar treatments on yield components and juice quality.

Treatments ^c	Clusters/vine	Cluster wt (g)	Yield (kg/vine)	Yield (t/ha)	SS (%)	TA (g/L)
Year 2012						
Control	36.7	77.4	2.82	11.8	22.7 b	5.5 a
NPK	36.5	81.7	3.00	12.5	24.0 ab	5.4 ab
DR	39.9	84.3	3.38	14.1	24.3 a	5.0 b
UR	40.1	79.7	3.26	13.6	23.5 ab	5.5 a
AN	45.0	74.3	3.36	14.0	23.4 ab	5.4 ab
Significance ^y	NS	NS	NS	NS	*	**
Year 2013						
Control	26.0	112.6	2.88	12.0	23.2	6.9
NPK	28.7	98.1	2.78	11.6	24.2	6.5
DR	25.7	102.8	2.66	11.1	25.4	7.2
UR	25.9	106.0	2.79	11.6	24.0	6.7
AN	25.0	98.7	2.47	10.3	24.4	6.5
Significance	NS	NS	NS	NS	NS	NS

^cNPK, DR, UR, and AN indicate treatments of soil application of 250 kg/ha of NPK fertilizer, NPK treatment with addition of foliar amino acids applications (0.25% w/v Drin), NPK treatment with addition of foliar urea applications (1% w/v urea), and NPK treatment with addition of ammonium/nitrate applications (0.25% w/v ammonium nitrate), respectively. C indicates control treatment without fertilization.

^yResults of analysis of variance: NS, *, **, ***Nonsignificant or significant difference at $P \leq 0.05$, 0.01, and 0.001, respectively.

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ by least significant difference test.

foliar nitrogen treatments compared with nonfoliar ones, in all measured parameters except cluster weight. In 2013, regardless of treatment, yield parameters obtained lower values than in 2012, although average cluster weight was higher.

Also, contrary to 2012, in second experimental year, C treatment, compared with foliar treatments, obtained slightly higher values in all measured parameters.

Juice quality was changed under the influence of some fertilization treatments, although not always reflecting results concerning yield components (Table 2).

The highest %SS value, indicating earlier ripening, in both experimental years was obtained in DR treatment, contrary to C treatment, which obtained the lowest %SS value. However, the difference between those treatments was significant only in the first experimental year. This could also be addressed to meteorological data, since 2012 year was

significantly warmer than 2013. In accordance to %SS and ripening dynamics, TA was significantly lowered by DR treatment in year 2012, a result that did not repeat in following experimental year. In 2013, the lowest TA values were observed within NPK and AN treatments, which is in accordance to their somewhat higher %SS values.

Must nitrogen compounds. Significant differences occurred among treatments regarding must N compounds in the first experimental year. However, treatment with the highest yeast assimilable N content was C, regardless of no fertilization (Table 3). This treatment significantly increased YAN content compared with all other treatments except UR.

Since YAN is a sum of individual nitrogen components (FAN and NH_4^+), its content in must reflected the content of individual nitrogen compounds. However, FAN and NH_4^+ were not equally represented in YAN.

In year 2012, average FAN and NH_4^+ content in YAN was 63% and 37%, respectively. Individual nitrogen compounds were, as well as YAN, the most abundant in the C treatment, which significantly increased FAN content compared with all other treatments except DR. On the other hand, regarding NH_4^+ content, UR treatment was the only one not significantly lower than C. Year 2013 was specific for extremely low values of all measured must nitrogen compounds. YAN content in must was 2-fold or even lower than values measured in 2012. Likewise in first experimental year, average FAN content in YAN was higher (71%) than NH_4^+ content (29%). Contrary to 2012 results, highest FAN and YAN content in 2013 were obtained by AN treatment (Table 3), whereas NH_4^+ content was slightly higher within UR treatment. Nevertheless, all nitrogen compounds obtained very similar values among treatments and consequently no significant differences occurred due to fertilization in 2013.

Discussion

Average leaf nitrogen level in experimental grapevines at the beginning of experiment exceeded expectations, especially considering low soil N supply and low must yeast available amino-N in years preceding this experiment (Karoglan, 2009). It also exceeds average vine leaf N level, which ranges from 1% to 2% (Coombe and Dry, 1992).

Soluble solids showed some changes in response to foliar fertilization, especially to DR treatment, which affected the highest % SS value in both years. The observed increase is not irrelevant when compared with C, since DR grapes show potential to yield wines with more than 1.0 vol% higher alcohols than C grapes.

The most notable effects of foliar nitrogen application were slightly increased values of yield parameters in the first experimental year and improved vine N supply (indicated by leaf total N) in the second year. Improved vine leaf nitrogen supply was to the largest extent due to UR treatment. Impact of UR on leaf total N increase is probably due to the greatest content of N applied with UR treatments (1% w/v).

Significantly higher leaf total N was determined at some stages, which indicates positive impact of application at growth stages: eight leaves separated, prebloom, and berries pea size. Foliar application after harvest intended to increase vine leaf N supply at the beginning of vegetative growth following year ("eight leaves separated" stage) since late-season N application previously occurred as an effective way to improve vine leaf N supply at early stages of the following year (Christensen et al., 1994; Holzapfel and Treeby 2007; Xia et Cheng, 2004). Differently, in some studies it provided only small increase of vine leaf N supply (Brunetto et al., 2005). Our study confirms latter findings since leaf total N was not improved by "after harvest" foliar treatments. No significant differences occurred at this stage, except slightly

Table 3. Effect of nitrogen foliar treatments on must nitrogen compounds.

Treatments ^z	Free primary amino-N (mg·L ⁻¹)	Ammonia (mg·L ⁻¹)	Yeast available amino-N (mg·L ⁻¹)
Year 2012			
Control	40.2 a	28.4 a	68.6 a
NPK	28.3 b	13.6 b	41.9 b
DR	31.2 ab	12.3 b	43.5 b
UR	26.1 b	21.4 ab	47.5 ab
AN	28.7 b	16.5 b	45.2 b
Significance ^y	**	*	**
Year 2013			
Control	14.2	5.0	19.2
NPK	13.6	5.9	19.5
DR	15.2	6.0	21.2
UR	14.0	7.0	21.0
AN	16.7	6.1	22.8
Significance	NS	NS	NS

^zNPK, DR, UR, and AN indicate treatments of soil application of 250 kg/ha of NPK fertilizer, NPK treatment with addition of foliar amino acids applications (0.25% w/v Drin), NPK treatment with addition of foliar urea applications (1% w/v urea), and NPK treatment with addition of ammonium/nitrate applications (0.25% w/v ammonium nitrate), respectively. C indicates control treatment without fertilization.

^yResults of analysis of variance: NS, *, **, ***Nonsignificant or significant difference at $P \leq 0.05$, 0.01, and 0.001, respectively.

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ by least significant difference test.

increased leaf total N for DR, as well as C treatment. Rather high values of N supply in vine leaves from C treatment with no fertilization imply the ongoing effect of previous vine N supply. Therefore, it is not strange that effects of ongoing N fertilization are still to be developed.

The response of experimental vines to foliar treatments was inconsistent across years. Such varying results are in accordance to other findings, where positive effect of N fertilization was not recorded through every year of research (Bell and Robson, 1999; Linsenmeier et al., 2008; Neilsen et al., 2010). Obtained differences, however, are most likely to be related to previous vine nitrogen supply accumulated in years preceding experiment, rather than foliar fertilization since the C and NPK treatments (as well as DR treatment) obtained slightly higher leaf total N. It can also be a consequence of vineyard heterogeneity.

Also, this study has results similar to some other studies where no effect of N treatments (in the form of urea) on vine N status was observed (Hannam et al., 2014; Jreij et al., 2009). However, it is important to point out that, in mentioned studies, foliar application was performed at veraison and, consequently, increased berry N compounds. Such findings point out the need to modify foliar application time to target particular vineyard needs.

No significant differences were observed considering yield parameters. It is in accordance to previous studies (Hannam et al., 2014; Lacroix et al., 2008; Tozzini et al., 2013), where foliar N application had no effect on yield parameters, which is expected since application was performed at veraison when grape composition, rather than yield, is affected.

Although application time in our study was different, lack of significant difference, considering yield parameters, is also not unusual. According to Bell and Henschke

(2005), N fertilization, depending on sufficient or nonsufficient vine N supply, has a positive or negative effect on vegetative growth and yield, respectively. Other authors observed no increase in yield (Lohnertz, 1991) or decrease of yield (Champagnol, 1994) when vine N supply was sufficient.

Despite no significant effect on yield, fertilization seemed to have affected yield parameters in other way. Second year of our study resulted in generally lower yield than 2012, despite higher cluster weight. Higher cluster weight could have been the consequence of very high precipitation in August of 2013, when 145.2 mm of precipitation was measured. This was the trigger for later powdery mildew occurrence, which could lead to lowering yield to some extent. Linsenmeier et al. (2008) explained that abundant N supply leads to decrease of fruit set and therefore to lower yield. Additionally, the berry set period in 2012 had more precipitation, that, compared with 2013, led to higher N soil availability. Therefore, non-sufficient vine N supply, powdery mildew, and weather conditions could have all contributed to generally lower yield in 2013.

Average FAN content in total must YAN (63% and 71% in 2012 and 2013, respectively) is in accordance to Kliewer (1969) who established that amino acids make 60% to 90% of total must nitrogen.

However, YAN values in must, regardless of treatment, were below expected values in both experimental years, especially in 2013. This could be explained by lower temperatures recorded in 2013, since some positive effects of foliar sprays on berry N content have been obtained in warmer climate (Lasa et al., 2012). The ripening period of 2013 was also characterized by higher precipitation that may cause leaching loss of N from the soil profile. According to literature (Bell and Henschke, 2005; Jiranek et al., 1995), 140 mg·L⁻¹ is minimum of YAN content in

must, required to avoid fermentation faults (slow, stuck, or sluggish fermentation). Below that threshold, rate or time for completion of fermentation is unsatisfactory (Jiranek et al., 1995). YAN values reported by other authors (Garde-Cerdan et al., 2014; Linsenmeier et al., 2008; Spayd et al., 1994) are usually around 200 mg·L⁻¹ or higher. Another survey (Butzke, 1998) of YAN status in 1523 clarified musts from 968 different cultivars showed that the average was 213 mg·L⁻¹. Although YAN values used by *Saccharomyces cerevisiae* can vary and are dependent on other factors such as yeast strain (Taillandier et al., 2007) and grape juice total SS (%) (Barbosa et al., 2009), in this study unobstructed fermentation could not have been expected. This problem can still be solved by must supplementation with added nitrogen or using yeast strains with a low nitrogen demand.

Previous findings showed that nitrogen fertilization applied either to the soil (Linsenmeier et al., 2008; Spayd et al., 1994) or foliarly (Hannam et al., 2014; Jreij et al., 2009; Tozzini et al., 2013) increases must total N content as well as individual N compounds.

However, N foliar application did not always give consistent and repeated results, regarding juice N compounds. In Lasa et al. (2012) study, increase of juice N compounds (YAN and total amino acid) after foliar urea application depended on season as well as on time of application, with later applications (veraison or 3 weeks after veraison) being more effective in increasing juice N compounds than application 3 weeks before veraison. Mengel (2002) also stated that foliar-applied N may be more effectively absorbed at veraison due to cracks in epicuticular waxes in old leaves that can facilitate absorption. Success of N uptake considering the time of application can also depend on variety (Porro et al., 2006).

In addition to previously mentioned need for modifying the time of foliar N application, we can assume that additional application at veraison is needed to increase berry/must N compounds level, given the particular needs of our experimental vineyard. Possibly, spring foliar fertilization performed in the current study could be reduced to fewer numbers of applications. Increasing the concentrations of nitrogen fertilizers solutions may also solve a problem, keeping in mind that grape leaves are susceptible to burning when concentrations are high.

Finally, the present study as well as many previous results (Garde-Cerdan et al., 2014; Lacroix et al., 2008; Lasa et al., 2012; Portu et al., 2015a, 2015b) lead us to conclusion that nitrogen foliar application does not always show clear and consistent effect on grape composition, since the results tend to vary within the different experiments.

Conclusion

Nitrogen foliar sprays were only partially effective with increasing grapevine leaf N status. Expected increase of juice N

compounds was not achieved. The effects of different N forms via foliar sprays were mostly nonsignificant; however, all N forms foliarly applied somewhat increased leaf total N in most stages of second experimental year. Moreover, urea foliar sprays (1% w/v) significantly increased leaf total N in the second year of study. Improvements in vine N status were not sufficient to increase YAN above recommended 140 mg·L⁻¹. It remains questionable how much of applied N is retained by vine contrary to translocation toward clusters, since grape juice composition changes were not consistent among foliar treatments. Lack of impact on juice N composition requires additional modifying of application time by moving one of application terms closer to veraison, so increase of juice N compounds can be achieved as well.

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