LEAF-TO-FRUIT RATIO AFFECTS THE IMPACT OF FOLIAR-APPLIED NITROGEN ON N ACCUMULATION IN THE GRAPE MUST

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Abstract

Aims: Agroscope investigated the impact of the leaf-to fruit ratio on nitrogen (N) partitioning in grapevine following a foliar urea application with the aim of increasing the yeast assimilable nitrogen (YAN) concentration in the must.

Methods and results: Foliar urea was applied to field-grown *Vitis vinifera* L. cv. Chasselas grapevines as part of a split-plot trial with two variable parameters: canopy height (90 or 150 cm) and fruit load (5 or 10 clusters per vine). Foliar application of 20 kg/ha of ^{15}N-labelled urea (10 atom % ^{15}N) was performed at veraison. The isotope labelling method allowed to observe foliar-N partitioning in the plant at harvest. The leaf-to-fruit ratio varied between 0.4 and 1.6 m^2/kg, and strongly impacted the N partitioning in the grapevines. Total N and foliar-N partitioning was mainly affected by the variation of canopy height. The YAN concentration varied from 143 to 230 mg/L (+60 %) depending on the leaf area. An oversized canopy (+31 % DW) induced a decrease in the total N concentration of all organs (-17 %), and a decrease in YAN quantity in the must in particular (-53 %). A negative correlation between the N concentration and the carbon isotope discrimination (CID) could be pointed out in a condition of no water restriction (e.g., $R^2 = 0.65$ in the must).

Conclusion: An excessive leaf area can induce YAN deficiency in the must. Thus, a balanced leaf-to-fruit ratio – between 1 and 1.2 m^2/kg – should be maintained to guarantee grape maturity, YAN accumulation in the must and N recovery in the reserve organs.

Significance and impact of the study: The results of this study encourage further research to understand the role of other physiological parameters that affect N partitioning in the grapevine – YAN accumulation in the must in particular – and add new perspectives for N management practices in the vineyard.

Key words: grapevine, foliar urea, ^{15}N-labelling, yeast assimilable nitrogen

Résumé

Objectifs: Agroscope a étudié l'impact du rapport feuille-fruit sur la répartition de l'azote (N) dans la vigne après un apport d'urée foliaire, avec l'objectif d'améliorer son efficacité sur l'accumulation de l'azote assimilable par les levures dans le moût.

Méthodes et résultats: De l'urée foliaire a été appliquée sur *Vitis vinifera* L. Chasselas dans le cadre d'un essai en split-plot comprenant deux variables: hauteur de feuillage (90 ou 150 cm) et charge en raisin (5 ou 10 grappes par cep). Toutes les variantes ont reçu 20 kg/ha d'urée marquée (10 atom % ^{15}N) à la véraison. La technique du marquage isotopique a permis d'observer la distribution de l'azote foliaire dans la plante au moment de la vendange. Le rapport feuille-fruit a varié de 0.4 à 1.6 m^2/kg et a fortement influencé la répartition de N dans la vigne. La distribution du N total et du N foliaire ont été principalement affectés par la hauteur de feuillage. La concentration d'azote assimilable par les levure (YAN) dans le moût a varié de 143 à 230 mg/L (+60 %) en fonction de la surface foliaire. Un feuillage surdimentionné (+31 % matière sèche) a entraîné une baisse de concentration du N total dans toute la plante (-17 %), et plus particulièrement une baisse de la quantité de YAN dans le moût (-53 %).

Conclusion: Une surface foliaire excessive peut entraîner une carence en YAN dans le moût. Un rapport feuille-fruit équilibré – entre 1.0 et 1.2 m^2/kg – doit être maintenu de façon à garantir la pleine maturation des raisins, l'accumulation du YAN dans le moût et le stockage de N dans les organes de réserves.

Signification et impact de l'étude: Cette étude encourage le développement de la recherche afin de comprendre le rôle d'autres facteurs physiologiques dans la distribution de N dans la vigne, et dans l'accumulation du YAN dans le moût en particulier. Ces résultats apportent de nouvelles perspectives à la pratique et à la gestion de la fertilisation azotée au vignoble.

Mots clés: vigne, urée foliaire, marquage ^{15}N, azote assimilable

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INTRODUCTION

Nitrogen (N) represents 1 to 4 % of the dry weight of the vine and plays a key role in the plant development, as a component of proteins, DNA and chlorophyll. The optimization of N fertilisation practices in viticulture is required for quality and sustainability of the production while preserving the environment (Champagnol, 1984). In particular, the concentration of yeast assimilable nitrogen (YAN) in the must at harvest is a relevant parameter used to determine wine quality because both its concentration and composition affect the alcoholic fermentation and the formation of aroma compounds (Rapp and Versini, 1991; Bell and Henschke, 2005). Extreme YAN deficiency can even induce atypical ageing off-flavours in wine (Linsenmeier *et al.*, 2007; Reynard *et al.*, 2011). The YAN concentration is usually enhanced by the addition of diammonium phosphate to the must to improve the fermentation kinetics, but it does not appear to have any beneficial effect on wine aroma (Lorenzini and Vuichard, 2012). Indeed, the main source of aroma precursors in the must are free amino-acids (AA), which represent approximately 80 wt. % of the YAN (Ribéreau-Gayon *et al.*, 2004). Hence, it is necessary to correct the YAN concentration, including the AA concentrations, early in the season through the foliar application of urea to grapevine canopies (Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; Hannam *et al.*, 2013; Nisbet *et al.*, 2014). However, Spring *et al.* (2011) observed cases of N-deficient musts produced from vigorous grapevines, despite a high level of N in the soil and an absence of water restriction. Knowing that N supply impacts the development of biomass and N allocation in the vine (Metay *et al.*, 2014), the uptake and subsequent translocation of N are key processes in the development of good wine after the application of foliar fertiliser (Porro *et al.*, 2010). As a consequence, the technical and physiological parameters that can improve the efficiency of foliar urea fertilisation, such as application timing (Conradie, 2005; Lasa *et al.*, 2012), canopy height and yield (Murisier and Zufferey, 1997; Spring *et al.*, 2011), must be optimized to increase the YAN concentration in the must at harvest. The YAN concentration in must was found to be higher when urea was applied to the vine at veraison (Verdenal *et al.*, 2015). Though, very few studies have determined the impact of the leaf-to-fruit ratio on N partitioning with a focus on the YAN accumulation in the must (Kliewer and Ough, 1970; Schreiber *et al.*, 2002; Peyrot des Gachons *et al.*, 2005). The leaf-to-fruit ratio, i.e., the light-exposed leaf area per fruit quantity (m^2/kg), is known as an essential parameter in grape growing: on one hand, the leaf area, as a source of nutrients, affects the leaf gas exchanges and the quantity of carbohydrates available through photosynthesis for vegetative growth and grape maturation; on the other hand, the grapes, as a sink of nutrients, affect the quantity of C and N required for their maturation (Murisier and Zufferey, 1997; Morinaga *et al.*, 2003; Kliewer and Dokoozlian, 2005; Etchebarne *et al.*, 2010). A balanced grapevine is one that can produce mature grapes while building its reserves for the next year (Champagnol, 1984; Zufferey *et al.*, 2012).

The aim of this study was to gain a better understanding of N partitioning in the vine at harvest as a function of canopy height and fruit load after foliar urea application at veraison.

MATERIALS AND METHODS

1. Vineyard site

The experiment was conducted in 2013 at Agroscope (Pully, CH) on field-grown *Vitis vinifera* L. cv. Chasselas (clone 800) grafted onto rootstock 3309C. During the vine growing season (April-October), the local average temperature was 15.7 °C and the total precipitation was 930 mm (2013 data, Pully meteorological station, www.agrometeo.ch). July was the warmest month, with an average temperature of 21.8 °C, and August was the driest month, with only 50 mm of precipitation. The vineyard soil was a noncalcareous colluvial soil containing 17 wt. % clay, 46 wt. % sand and 4 wt. % total carbonate as $CaCO_3$. The soil organic matter content was 1.7 wt. %. In May, 30 kg N/ha were applied to the soil and were the only fertilisation before foliar urea was applied in this study. There were no visual symptoms of deficiencies of essential elements such as P, K, Mg or B during the whole season of the experiment. The water-holding capacity was high and non-limiting (> 250 mm). The vines were planted in 2007 at a density of 5880 vines/ha (2 x 0.85 m). Similar to regional practices, the vines were pruned using a single Guyot training system (vertical shoot-positioning) with 7 shoots/plant, and lateral shoots were removed from the bunch area.

2. Experimental design

Five treatments (A, B, C, D, E) consisting of five vines each – each vine being a replicate – were established as presented in Table 1, with the aim of obtaining a large range of leaf-to-fruit ratios. Two factors of variation were chosen, namely, 1) canopy height: during vegetation development, the canopy was trimmed at two different heights (150 cm in treatments A, B and C; 90 cm in treatments D and E), and 2) yield: two yield restrictions were applied (5 bunches per vine in treatments A, B and D; 10 bunches per vine in treatments C and E). Bunch thinning was performed in July before bunch closure. N (5 kg/ha) was applied once a week for four weeks (total 20 kg/ha) in the form of ^{15}N-labelled urea (10 atom % ^{15}N, $CO(^{15}NH_2)_2$, Sigma-Aldrich, St-Louis, MO, USA). The period of N application covered the period of veraison. N was applied to the canopy (treatments B, C, D and E) at a dilution rate of 3.3 % (w/v). The method of isotopic labelling allowed the tracking of urea N in the plant and the description of its partitioning in the grapevine organs depending on the leaf-to-fruit ratio. The urea application dates were 08/14, 08/21, 08/26 and 09/02. The control treatment (A) did not receive nitrogen fertilisation.

3. Field measurements and plant sampling

For each replicate, bud fruitfulness was estimated and expressed as number of bunches per shoot. Shoot trimming was conducted three times during the season (four times for treatments D and E, which had a lower canopy height). The shoots were weighed fresh (g/plant), and the data were

Table 1 - Description of the five treatments of the experiment. 5 kg of urea per hectare were supplied to the canopy once weekly for four weeks at veraison. The concentration of urea used was 3.3 %w/v. The control treatment A received no nitrogen fertilization (cv. Chasselas, Pully, 2013).

Treatment	Canopy height (cm)	Fruit load (bunches/plant)	Nitrogen supply
A (control)	150	5	0 N
B	150	5	20 kg N/ha
C	150	10	20 kg N/ha
D	90	5	20 kg N/ha
E	90	10	20 kg N/ha

pooled per vine at the end of the season. The light-exposed leaf area (m^2/m^2 of ground area) was estimated using Carbonneau's method (1995) only once per treatment, since the percentage of holes could not be estimated for each vine separately. The yield was determined for each vine (kg/plant).

At harvest time, each of the 25 vines were dug up, with maximum recovery of roots, and then partitioned into five parts : roots, trunk (including cane), vegetative parts (shoots, leaves, total trimmings), pomace and must. Only the trimmings were collected throughout the season as explained above. For each replicate, the bunches were pressed in a Speidel-20 press to separate the must from the pomace. An aliquot (100 mL per vine) of the must was separated immediately after pressing for chemical analysis (see below).

All plant parts were weighed fresh before drying at 60 °C until a constant weight. The musts were frozen while agitated in liquid nitrogen to ensure homogeneity and then dried in a lyophilizer. The dry weight (DW, g) and the percentage of dry mass (%DW) were determined for each plant part. The dried organs were then ground into a fine powder (< 1300 µm) for organic carbon and nitrogen stable isotope analysis, except for the dried musts, which remained hard and sticky.

4. Must analyses

The fresh must samples were analysed at the Institute for Food Sciences (IDA, Agroscope) to determine general parameters, e.g., total soluble solids (TSS, °Brix), titratable acidity (TA, g/L as tartaric acid), tartaric and malic acids (g/L), pH, and YAN (mg/L) using an infrared spectrophotometer (FOSS WineScan™).

Primary free AA were quantified using an Agilent 1200 HPLC System equipped with a Zorbax Eclipse AAA column. The OPA-AA derivatives were detected using UV absorbance and expressed in mg N/L considering all the N atoms of each AA (as detailed in Verdenal *et al.*, 2015). The ammonium concentration (NH_4^+, mg/L) was quantified using an enzymatic method (Boehringer Mannheim, 1997).

5. C and N analyses

The total organic C and N (TOC and TON values, respectively, expressed as % DW) and the stable C and N isotope composition ($\delta^{13}C$ and $\delta^{15}N$ values, respectively) of the plant parts were determined at the Stable Isotopes Laboratory of the University of Lausanne (UNIL) using elemental analysis-isotope ratio mass spectrometry (EA-IRMS). On one hand, the measurement of $^{13}C/^{12}C$ ratio at natural isotopic abundance in the must at harvest is an integrated index of the water restriction experienced by the vine during grape maturation (Van Leeuwen *et al.*, 2001). On the other hand, the measurement of $^{15}N/^{14}N$ ratio after the labelled urea application allowed to describe N distribution in the grapevine. The stable C and N isotopic compositions are reported as $\delta^{13}C$ and $\delta^{15}N$ values, respectively, as the per mil (‰) deviations of the isotope ratio relative to known standards :

$$\delta = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$$

where R is the ratio of the heavy to light isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$). All the isotopic analyses were performed in duplicate (as detailed in Verdenal *et al.*, 2015).

6. Calculations

- A maturity index was calculated as follows :

$$Maturity\ index = \frac{°Brix \times 100}{TA}$$

In the different plant parts of each vine, the following parameters were calculated as detailed in Verdenal *et al.* (2015) :

- The total N quantity (QN, g) :

$$QN_{organ} = DW_{organ} \times \%N_{organ}$$

- The abundance (A%) or proportion of heavy isotope ^{15}N per 100 N atoms :

$$A\% = \frac{R}{R+1} \times 100$$

- The relative specific abundance (RSA) or proportion of newly incorporated N atoms (from urea) relative to total N atoms in each organ. In other words, the RSA represents the importance of the organ sink strength, which is independent of its size (Deléens *et al.*, 1997) :

$$RSA = \frac{A\%_{sample}\ enrichment}{A\%_{nutrient}\ enrichment} = \frac{A\%_{sample} - A\%_{control}}{A\%_{nutrient} - A\%_{control}}$$

- The new N pool (g). The new N pool of the whole vine is the sum of the new N pools of each plant part:

$$\text{new N pool}_{\text{organ}} = \text{RSA}_{\text{organ}} \times \text{QN}_{\text{organ}}$$

$$\text{new N pool}_{\text{whole vine}} = \sum_{\text{organ}=1}^{5} \text{new N pool}_{\text{organ}}$$

- The partitioning (%P) or distribution of the new N in the different plant parts. The sum of all the %P of a vine equals 100 %.

$$\%\text{P}_{\text{organ}} = \frac{\text{new N pool}_{\text{organ}}}{\text{new N pool}_{\text{vine}}} \times 100$$

7. Statistical analyses

The differences between the control A and the other treatments were statistically evaluated using analysis of variance (ANOVA, p values < 0.05), and then a multiple comparison using the Newman-Keuls test was conducted using ©XLSTAT 2014.2.02. The control A was used as a reference without N supply, then ignored to focus on a second experimental design (split-plot) considering only the four other treatments (B, C, D and E). The split-plot design, which considered the canopy height and fruit load as main and sub-factors, respectively, allowed the separate determination of the impact of each factor and then their possible interaction.

RESULTS

1. Phenology and bud fruitfulness

The 2013 vintage induced a late phenological development: cv. Chasselas flowering occurred on the 4th of July, whereas the 1993-2012 average flowering date was the 15th of June, and veraison occurred on the 23rd of August. There was no difference in phenology between the treatments. The grapes were harvested on the 9th of October, just before full maturity to preserve grape integrity and prevent rot. The effects of canopy height and fruit load on the vine parameters, yield components and must composition at harvest are presented in Table 2. The average bud fruitfulness was 1.8 ± 0.2 bunches per shoot (average ± 1 SD), with no difference between treatments.

2. Leaf-to-fruit ratio

The fruit loads were 2.0 ± 0.5 and 3.8 ± 0.4 kg/plant in the treatments with 5 and 10 bunches per plant, respectively (Table 2). The light-exposed leaf areas were 0.9 ± 0.0 and 1.4 ± 0.1 m²/m² in the treatments with canopy heights of 90 and 150 cm, respectively. As a consequence of the variability of these two factors of variation, the leaf-to-fruit ratio varied between 0.4 and 1.6 m²/kg and had a considerable influence on grape maturity.

3. Must composition

The maturity index varied significantly from 173 to 197 (Table 2). The average TSS content in the must was 17.1 ± 0.9 °Brix with a minimum value of 16.0 °Brix in treatment E and a maximum value of 18.2 °Brix in treatment B. The

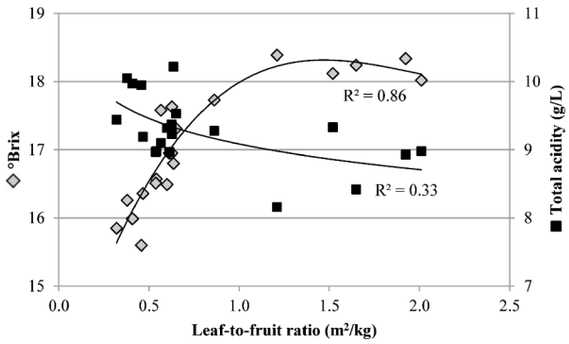

Figure 1 - Impact of leaf-to-fruit ratio variation (m²/kg) on total soluble solid (TTS, °Brix) and titratable acidity (TA, g/L as tartaric acid) (cv. Chasselas, Pully, 2013).

Brix degree was initially positively correlated with the leaf-to-fruit ratio (R^2 = 0.86) but then reached a plateau when the leaf-to-fruit ratio exceeded approximately 1.2 (Figure 1). Meanwhile, TA (average 9.3 ± 0.5 g/L) and YAN (average 186 ± 42) were negatively correlated with canopy height (R^2 = 0.33 and 0.37, respectively). No interaction was observed between the fruit load and canopy height. The control A could only be distinguished from the treatment B by its YAN content in the must, which was significantly lower (93 versus 143 mg/L).

4. Dry organic matter

The effects of the fruit load and canopy height on the DW, TOC, $\delta^{13}C$, TON and $\delta^{15}N$ values are presented in Table 3. The average whole plant DW for all treatments was 1.83 ± 0.28 kg. As expected, the grape and canopy DW values were correlated with the fruit load and canopy height, respectively, while the roots and trunk DW values were not influenced by these factors.

5. TOC and $\delta^{13}C$

The average whole plant TOC was 46.5 ± 0.6 % DW and was only influenced by the fruit load. The TOC values at harvest varied between the different plant parts: the lowest average TOC was found in the must, which was only 39.1 ± 0.9 % DW. When considered individually, the plant parts did not exhibit TOC variations with respect to the leaf-to-fruit ratio (Table 3). No interaction was observed between the two factors of variation. The $\delta^{13}C$ values varied between -29.5 ‰ in the pomace and -27.7 ‰ in the must. The $\delta^{13}C$ values were significantly lower in each vine part (increasing carbon isotopic discrimination, CID) when the canopy height was more important, while fruit load had no effect. For example, $\delta^{13}C$ in the must varied between -28.7 and -28.3 ‰ (-28.5 ± 0.1 ‰) when canopy height was 150 cm and between -28.0 and -27.2 ‰ (-27.7 ± 0.2 ‰) when canopy height was 90 cm. An interaction in terms of $\delta^{13}C$ could be observed in the roots between the two factors of variation.

6. TON, $\delta^{15}N$ and QN

The average whole plant TON was 0.84 ± 0.07 % DW and varied from 0.28 ± 0.08 % DW in the must to 1.69 ±

0.21 % DW in the pomace (Table 3). All the plant part TON values were highly negatively correlated with canopy height and there was no interaction between the two factors of variation. Conversely, the fruit load only influenced the must and root TON values: the must TON increased by 0.04 % DW with an increase in the fruit load, while the root TON decreased by 0.06 % DW. As expected, the ^{15}N abundances in the control A samples were similar in all plant parts and were equivalent to a natural abundance of 0.37 atom % (results not shown). In the ^{15}N-labelled treatments, the $\delta^{15}N$ values showed important variations between plant parts and ranged from 1514 ‰ ($A\%_{must}$ = 0.92 ± 0.25 %) in the roots to 6170 ‰ in the must ($A\%_{must}$ = 2.57 ± 0.25 %) (Table 3). Both the fruit load and canopy height influenced new N abundance in the reproductive organs (grapes) and the reserve organs (trunk and roots) without influencing A% in the canopy, which remained constant at 1.25 ± 0.11 A%. The $\delta^{15}N$ value in the trunk was only influenced by fruit load. An interaction between the two factors in terms of ^{15}N abundance was observed in the grapes and the trunk. The average QN in the whole plant was 15.2 ± 1.8 g and increased with fruit load but was independent of canopy height. The average QN in the canopy (7.6 ± 1.0 g) represented 50 % of the TON in the plant and was correlated with canopy height. Conversely, the average QN in the must (1.0 ± 0.5 g) represented only 6 % of the TON, which was the smallest N pool in the vine. Only the QN in the grapes was correlated with both factors of variation: it was positively correlated with the fruit load and negatively correlated with the canopy height. The interaction between both factors only influenced the QN in the pomace.

7. RSA, new N pool and partitioning

The effects of canopy height and fruit load on the RSA, new N and its partitioning in the plant are presented in Table 4. The average RSA in the whole plant was 12 ± 1 % and was independent of both factors of variation. The average RSA in the different plant parts varied between 6 ± 3 % in the roots and 23 ± 3 % in the must. Grapes (must and pomace) had the strongest sink-effect in all the treatments. The RSA was affected by both factors of variation in the roots and grapes and only by the fruit load in the trunk. The RSA was indeed higher in the grapes when the fruit load and/or canopy height were lower. The RSA in the canopy remained constant at 9 ± 1 % and was independent of both factors of variation. A strong interaction between both factors was observed in the grapes and the trunk, as well as in the whole plant. As a consequence of the RSA and organ size, the new N pool in the grapes and roots also varied with both factors of variation. The new N quantity in the whole plant was reduced by approximately 10 % by the increase in canopy size and tended to be reduced (only significant at $p < 0.10$) by the decrease in fruit load. An interaction between the factors of variation was observed only in the pomace. The variation in the new N pool in the different organs as a function of the leaf-to-fruit ratio is shown in Figure 2. The new N pools in the canopy and trunk were independent of the leaf-to-fruit ratio (R^2 = 0.06 and 0.03 for the canopy and trunk, respectively). When the ratio increased to 1.6, the new N pool in the grapes decreased considerably (from 1.07 to 0.28 g, R^2 = 0.93), while it increased slightly in the roots (from 0.03 to 0.18 g, R^2 = 0.63). With respect to the TON, a similar correlation was observed between new N in the grapes (must + pomace) and reserves (trunk + roots) (R^2 = 0.42), indicating that reserve organs take advantage of new N partitioning under a lower fruit load. In the case of a higher canopy, the new N content in the roots increased by 40 %, while the new N content in grapes was reduced by 33 % (Table 4). New N partitioning was clearly affected by organ size (canopy and/or fruits) and

Table 2 - The effect of canopy height and fruit load on vine parameters, yield components and must composition at harvest. The split-plot analysis allowed the separate determination of the impact of both factors of variation (fruit load and canopy height) and their interaction. For each factor, the average of two treatments is presented. B (5 bunches; 150 cm canopy), C (10; 150), D (5; 90) and E (10; 90). (cv. Chasselas, Pully, 2013).

Variable	Control A	Fruit load per vine			Canopy height			Interaction
		5 bunches (average B-D)	10 bunches (average C-E)	p value	90 cm (average D-E)	150 cm (average B-C)	p value	p value
Fertility (bunches/shoot)	1,9	1,8	1,8	0,723	1,9	1,7	0,060	0,953
Exposed leaf area (m^2/m^2)	1,5	1,2	1,1	-	0,9	1,4	-	-
Total trimmings (g/plant)	452	447	429	0,599	524	351	**0,002**	0,216
Leaf-fruit ratio (m^2/kg)	1,4	1,2	0,5	-	0,6	1,1	-	-
Fruif load (kg/plant)	1,9	2,0	3,8	**<0.0001**	3,1	2,8	0,097	0,055
Brix degree	18	17,7	16,5	**<0.0001**	16,6	17,6	**0,001**	0,652
Total Acidity (g/L)	9	9,1	9,4	0,130	9,6	8,9	**0,005**	0,766
Maturity index	202	195	175	**0,002**	173	197	**0,001**	0,508
Tartaric acid (g/L)	6,4	6,8	6,7	0,336	6,9	6,7	0,202	0,669
Malic acid (g/L)	4,7	5,1	5,4	0,067	5,5	5,0	**0,0001**	0,618
pH	3,1	3,1	3,1	0,400	3,1	3,1	0,488	0,219
YAN (mg/L)	93	178	194	0,197	222	151	**<0.0001**	0,982

Table 3 - The effect of canopy height and fruit load on DW, TOC, $\delta^{13}C$, TON, $\delta^{15}N$ and QN in the different plant parts at harvest. The split-plot analysis allowed the separate determination of the impact of both factors of variation (fruit load and canopy height) and their interaction. For each factor, the average of two treatments is presented. B (5 bunches; 150 cm canopy), C (10; 150), D (5; 90) and E (10; 90). (cv. Chasselas, Pully, 2013).

Variable	Organ	Control A	Fruit load per vine			Canopy height			Interaction
			5 bunches (average B-D)	10 bunches (average C-E)	p value	90 cm (average D-E)	150 cm (average B-C)	p value	p value
DW (g)	Must	232	254	458	**< 0.0001**	374	339	0,140	0,135
	Pomace	110	113	206	**< 0.0001**	162	156	0,635	0,055
	Canopy	734	649	597	0,283	543	703	**0,012**	0,349
	Trunk	436	386	414	0,341	393	408	0,550	0,747
	Roots	258	281	296	0,683	271	305	0,291	0,814
	Whole plant	1770	1684	1971	**0,046**	1742	1912	0,117	0,818
TOC (% DW)	Must	38,8	38,9	39,4	0,232	39,3	39,0	0,450	0,581
	Pomace	47,8	49,3	48,5	0,371	49,7	48,1	0,148	0,265
	Canopy	46,0	47,1	46,0	0,079	46,6	46,6	0,985	0,462
	Trunk	49,5	49,1	48,9	0,643	48,8	49,1	0,396	0,941
	Roots	49,2	49,1	49,6	0,081	49,5	49,2	0,354	0,141
	Whole plant	46,5	46,8	45,9	**0,004**	46,3	46,4	0,713	0,387
$\delta^{13}C$ (‰)	Must	-28,3	-28,1	-28,1	0,577	-27,7	-28,5	**<0.0001**	0,119
	Pomace	-29,3	-29,1	-29,3	0,341	-28,9	-29,5	**0,005**	0,714
	Canopy	-29,3	-28,6	-28,6	0,792	-28,3	-29,0	**0,004**	0,889
	Trunk	-28,5	-28,3	-28,3	0,803	-28,1	-28,5	**0,013**	0,390
	Roots	-28,5	-28,2	-28,3	0,521	-28,1	-28,4	**0,011**	**0,030**
TON (% DW)	Must	0,13	0,26	0,30	**0,05**	0,35	0,21	**<0.0001**	0,684
	Pomace	1,34	1,70	1,67	0,727	1,79	1,58	**0,028**	0,716
	Canopy	1,20	1,19	1,28	0,153	1,31	1,16	**0,013**	0,630
	Trunk	0,39	0,44	0,42	0,052	0,46	0,40	**0,002**	0,538
	Roots	0,70	0,82	0,76	**0,037**	0,89	0,68	**<0.0001**	0,722
	Whole plant	0,79	0,85	0,83	0,474	0,89	0,79	**0,0001**	0,404
$\delta^{15}N$ (‰)	Must	39	6450	5891	**0,002**	6575	5766	**0,007**	**0,001**
	Pomace	19	4805	4430	**0,012**	4822	4413	**0,016**	**0,001**
	Canopy	16	2475	2430	0,792	2458	2447	0,932	0,233
	Trunk	27	2734	2041	**0,0001**	2377	2398	0,878	**0,013**
	Roots	36	2014	1015	**0,0001**	1194	1834	**0,003**	0,367
QN (g)	Must	0,31	0,70	1,35	**<0.0001**	1,33	0,72	**0,0001**	0,660
	Pomace	1,47	1,90	3,38	**<0.0001**	2,87	2,41	**0,006**	**0,009**
	Canopy	8,76	7,63	7,51	0,735	7,08	8,06	**0,034**	0,093
	Trunk	1,69	1,69	1,75	0,681	1,80	1,64	0,137	0,816
	Roots	1,81	2,28	2,29	0,970	2,43	2,14	0,414	0,887
	Whole plant	14,04	14,21	16,27	**0,025**	15,52	14,96	0,433	0,651

new N partitioning was balanced accordingly. The trunk was only affected by the fruit load. The new N content in the must varied from 6.1 ± 0.5 % in treatment B (leaf-to-fruit ratio = 1.6) to 20.9 ± 2.3 % in treatment E (leaf-to-fruit ratio = 0.4).

8. Soluble N in the must

The effects of canopy height and fruit load on AA and NH_4^+ concentrations are presented in Table 5. The control treatment A had a significantly lower concentration of total AA and NH_4^+ in the must, i.e., 154 mg N/L versus an average of 313 mg N/L in the four other treatments that received foliar-applied urea. The increase in the fruit load had no significant impact on the total AA concentration but positively affected the NH_4^+ concentration. However, canopy height strongly affected both the total AA and NH_4^+ concentrations: the higher the canopy, the lower the YAN concentration in the must. Most of the AAs were highly negatively correlated with canopy height ($p < 0.001$). No interaction was noted between canopy height and fruit load.

DISCUSSION

1. The foliar urea increased the YAN concentration

The results of this study indicated that there were no side-effects of urea application at veraison on vine physiology.

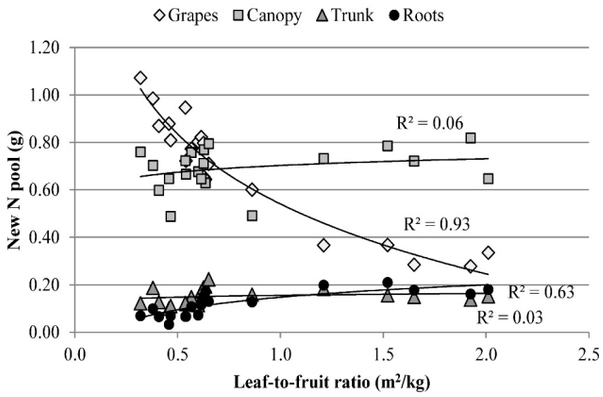

Figure 2 - Impact of leaf-to-fruit ratio variation (m^2/kg) on total soluble solid (TTS, °Brix) and titratable acidity (TA, g/L as tartaric acid) (cv. Chasselas, Pully, 2013).

Besides the increased YAN concentration in the must (+50 mg/L), there was no difference between the control treatment A and treatment B in terms of vigour (leaf area, pruning weight), yield per vine or must composition. This confirms the results obtained by Neilsen *et al.* (2013), who showed that foliar urea fertilisation enhanced the soluble N content in the must without increasing plant vigour.

2. The leaf-to-fruit ratio impacted the must composition

The leaf-to-fruit ratio had a strong impact on the maturity index, which is in line with the results reported in other studies (Murisier and Zufferey, 1997 ; Kliewer and Dokoozlian, 2005). Insufficient canopy height affected grape maturation in terms of TSS accumulation, malic acid degradation and YAN accumulation, while high fruit load only affected TSS accumulation in the must. This would explain the lower correlation of TA with the leaf-to-fruit ratio (R^2 = 0.33 ; Figure 1). These results contrast with those reported by Kliewer and Ough (1970), who found a negative correlation between YAN and fruit load. This divergence can most likely be explained by the wide range of numbers of bunches per vine they studied (i.e., up to 48) or by the different climatic conditions (Köppen-Geiger climate classification subtypes Csa-Csb for the warm to hot summer Mediterranean-like climate in California and Cfb for the marine West coast climate in Pully ; Peel *et al.*, 2007).

3. The leaf-to-fruit ratio did not impact the leaf N-uptake

It is likely that N absorption is determined by favourable atmospheric relative humidity and vintage climatic conditions (Porro *et al.*, 2010 ; Eichert, 2013). Knowing the quantity of urea applied and the average new N quantity in the vine at harvest, ^{15}N-labelling indicated that 48 ± 6 mass % of urea was absorbed through the leaves into the vines. This is a fairly good absorption rate in comparison to the results of other studies, which showed that only 30 to 40 mass % of the applied urea was assimilated by the plant (Jakovljevic *et al.*, 1995 ; Verdenal *et al.*, 2015). N absorption rate was not significantly related to fruit load nor leaf area. However, it tended to be higher when the leaf area was smaller (51 %) and when the fruit load was higher (51 %).

4. The canopy height increased the C isotope discrimination

The TOC concentration per organ was not influenced by the variation of the leaf-to-fruit ratio. However, the CID in all the organs was substantially affected by the canopy height ; the $\delta^{13}C$ values were lower for a larger canopy. Indeed, Farquhar *et al.* (1989) showed that CID is negatively correlated with instantaneous water use efficiency (WUE, estimated as CO_2 assimilation divided by canopy transpiration) and positively correlated with the ratio of intercellular CO_2 concentration to ambient CO_2 concentration (Ci/Ca), which is a balance between stomatal conductance and photosynthetic activity. Therefore, for a given leaf area, CID would depend on either stomatal conductance or photosynthetic activity per leaf (Dubey and Chandra, 2008). In this study, the CID value measured in the must ($\delta^{13}C$ = -28.1 ± 0.4 ‰) did not indicate a water restriction over the period of grape maturation (e.g., Van Leeuwen *et al.*, 2009). As a consequence, CID would have depended mainly on the photosynthetic activity per unit of leaf area and not on stomatal behaviour.

5. The canopy height reduced the N concentration in the whole plant

The impact of canopy height on the N concentration in all the organs suggests that the TON was diluted by the volume of the whole plant : for a constant N quantity (QN = 15.2 ± 1.8 g), the TON in the whole plant was reduced by 12 % DW when exposed leaf area was increased by 55 %. This result confirms the observations of Spring *et al.* (2011).

6. The CID and the N concentration were negatively correlated

These results suggest that an increase in canopy height and therefore leaf area index (LAI, i.e., the total leaf area per m^2 of soil) in the absence of water restriction would have induced a lower photosynthetic activity per unit of leaf area with two major consequences for the whole plant (Figure 3) : (1) a reduction in photosynthetic-N use efficiency (PNUE), which would result in a lower N concentration ; and (2) a reduction in WUE, which would induce a higher CID. This hypothesis can explain the negative correlation between CID and the N content (i.e., R^2 = 0.65 in the must). This relationship was previously observed in *Coffea* by Gutierrez and Meinzer (1994) and in *Arachis* by Nageswara Rao and Wright (1994), but requires further research to confirm the results in grapevine.

7. The fruit load reduced the TON concentration in the roots

On the other hand, the variation in the fruit load resulted in a linear relationship between the grape dry weight (must

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DW + pomace DW) and N quantity (QN) (R^2 = 0.84). A similar trend was shown by González-Real *et al.* (2008) for sweet pepper plants. In other words, the TON concentration in the grapes remained constant at 0.71 ± 0.10 % DW (must DW + pomace DW), and the QN increased accordingly.

However, a negative correlation between the TON values in the must and roots (R^2 = 0.42) suggested that a high fruit load would result in lower N reserves in the roots for the next season. Zapata *et al.* (2004) demonstrated that the N reserves used on the spring flush were precisely those of the roots as the main N storage organ. Crop modellers often consider the roots as an invariant fraction of the whole plant dry mass, without any active role in N partitioning (González-Real *et al.*, 2008). Nevertheless, Morinaga *et al.* (2003) suggested that a larger quantity of bunches (the strongest sink organ) would prevent the development of new fine roots and, as a consequence, would reduce whole root activity and N accumulation. Moreover, lower N reserves in the roots would largely

Table 4 - The effect of canopy height and fruit load on RSA (% TON), new N quantity (g) and its partitioning (%) in the different vine organs at harvest. The split-plot analysis allowed the separate determination of the impact of both factors of variation (fruit load and canopy height) and their interaction. For each factor, the average of two treatments is presented. B (5 bunches; 150 cm canopy), C (10; 150), D (5; 90) and E (10; 90) (cv. Chasselas, Pully, 2013).

Variable	Organ	Fruit load per vine			Canopy height			Interaction
		5 bunches (average B-D)	10 bunches (average C-E)	p value	90 cm (average D-E)	150 cm (average B-C)	p value	p value
RSA (% TON)	Must	24	22	**0,002**	24	21	**0,007**	**0,001**
	Pomace	18	16	**0,012**	18	16	**0,016**	**0,001**
	Canopy	9	9	0,793	9	9	0,934	0,233
	Trunk	10	8	**0,0001**	9	9	0,873	**0,013**
	Roots	7	4	**0,0001**	4	7	**0,003**	0,373
	Whole plant	12	12	0,302	12	11	0,199	**0,001**
New N (g)	Must	0,17	0,30	**0,0001**	0,32	0,15	**0,0001**	0,512
	Pomace	0,35	0,56	**<0.0001**	0,50	0,40	**0,003**	**0,001**
	Canopy	0,70	0,68	0,689	0,65	0,73	**0,025**	0,944
	Trunk	0,17	0,13	**0,030**	0,16	0,15	0,121	0,234
	Roots	0,16	0,08	**0,0001**	0,10	0,14	**0,004**	0,484
	Whole plant	1,55	1,74	0,063	1,73	1,57	**0,028**	0,186
Partitioning (%)	Must	11	17	**<0.0001**	18	9	**<0.0001**	0,291
	Pomace	22	32	**<0.0001**	29	25	**0,002**	**0,005**
	Canopy	46	39	**0,001**	37	47	**<0.0001**	**0,009**
	Trunk	11	8	**<0.0001**	9	9	0,686	0,579
	Roots	11	5	**<0.0001**	6	9	**0,001**	**0,045**

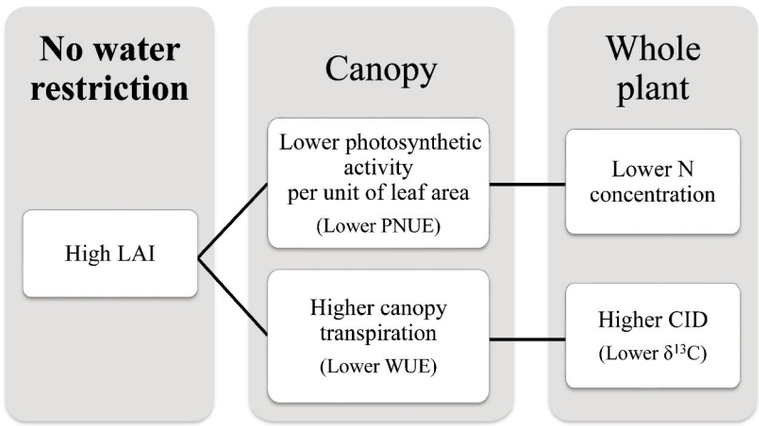

Figure 3 - Relationship between leaf area index (LAI), carbon isotope discrimination (CID) and N concentration in the whole plant under no water restriction (cv. Chasselas, Pully, 2013). PNUE, photosynthetic-N use efficiency; WUE, water use efficiency; $\delta^{13}C$, carbon isotope composition.

Table 5 - The effect of canopy height and fruit load on the concentrations of amino-acids and ammonium in the must. The split-plot analysis allowed the separate determination of the impact of both factors of variation (fruit load and canopy height) and their interaction. For each factor, the average of two treatments is presented. B (5 bunches; 150 cm canopy), C (10; 150), D (5; 90) and E (10; 90). (cv. Chasselas, Pully, 2013).

Amino-acids (mg N/L)	control A	Fruit load per vine			Canopy height			Interaction
		5 bunches (average B-D)	10 bunches (average C-E)	p value	90 cm (average D-E)	150 cm (average B-C)	p value	p value
Arginine	100,6	215,8	229,2	0,335	256,7	188,3	**<0.0001**	0,191
Alanine	7,0	17,0	18,0	0,513	21,9	13,1	**0,001**	0,223
Threonine	6,5	10,5	13,1	**0,005**	14,1	9,5	**0,000**	0,291
Glutamic Ac.	6,0	6,2	5,1	0,077	6,4	4,9	**0,003**	0,483
Aspartic Ac.	5,8	4,8	5,1	0,549	5,5	4,3	**0,000**	0,835
Serine	5,0	9,7	11,1	0,112	13,0	7,8	**0,000**	0,542
Glycine	4,5	4,7	4,6	0,524	4,7	4,6	0,164	0,160
y-aminobutyric acid	3,4	5,7	6,7	**0,009**	6,6	5,8	0,189	0,634
Glutamine	3,3	7,9	12,2	0,107	16,2	3,9	**0,004**	0,488
Histidine	2,6	5,4	5,3	0,750	6,4	4,3	**<0.0001**	0,593
Leucine	2,4	3,2	4,0	0,056	4,8	2,4	**0,001**	0,815
Valine	1,4	2,3	2,5	0,407	2,9	1,9	**0,000**	0,430
Tryptophane	1,2	1,4	1,5	0,095	1,7	1,2	**0,001**	0,065
Phenylalanine	1,1	1,5	1,7	0,033	1,8	1,3	**0,002**	0,057
Isoleucine	0,8	1,0	1,3	0,022	1,5	0,9	**0,001**	0,834
Asparagine	0,7	1,1	1,3	0,279	1,7	0,6	**0,000**	0,346
Tyrosine	0,7	1,4	1,5	0,588	1,6	1,3	**0,000**	0,425
Lysine	0,5	0,8	0,9	**0,001**	1,0	0,8	**<0.0001**	0,145
Methionine	0,5	0,8	1,2	**0,002**	1,4	0,6	**<0.0001**	0,107
Cystine	n.d.	n.d.	n.d.	-	n.d.	n.d.	-	-
Primary AA	154,2	300,6	326,0	0,224	369,5	257,1	**<0.0001**	0,310
NH_4^+ (mg N/L)	19,8	44,0	70,2	**0,005**	80,5	33,7	**<0.0001**	0,177

affect the sustaining growth and the fruiting of grapevine in the following season (Cheng and Xia, 2004).

8. The canopy height highly affected the YAN concentration in the must

With respect to new N, when the leaf-to-fruit ratio was 0.5 m^2/kg or lower, more than half (52 %) of the foliar-applied N went to the grapes (must + pomace) compared with only 23 % when the leaf-to-fruit ratio was 1.5 m^2/kg or higher (Figure 2). In fact, the impact of foliar-N application on YAN and AA concentrations in the must was mostly affected by the excess of leaf area (*p* value < 0.0001). These observations support the results of Spring *et al.* (2011), who showed that YAN-deficient musts could be produced from vigorous vines in spite of high soil N availability, pointing out the key role of canopy management in YAN accumulation in the must.

CONCLUSION

It is a well-established fact that a minimum leaf-to-fruit ratio (approximately 1.0-1.2 m^2/kg in the context of Swiss vineyards) must be maintained to guarantee optimal TSS accumulation in the grapes and N recovery in the reserve organs. However, bearing in mind the major role of YAN in must composition in terms of fermentation kinetics and aroma development, this study demonstrated that excessive leaf area reduces YAN concentration in the must and can potentially induce a YAN deficiency that could impair wine quality. These results add new perspectives regarding foliar-N fertilisation management as a function of the leaf-to-fruit ratio. The development of indicators to manage foliar-N fertilisation efficiency, such as an eventual early YAN concentration monitoring in the grape along with thresholds, would be necessary. Further research is required to better understand the role of the roots in the N partitioning in the vine – e.g., their development, activity and organ size – and to determine other technical and physiological parameters which would optimize N accumulation in the grapes and guarantee the maximum efficiency of foliar urea supply at veraison.

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