NITROGEN PARTITIONING AMONG VINE ORGANS AS A CONSEQUENCE OF CLUSTER THINNING

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Abstract:
Context and purpose of the study - Agroscope is investigating the impact of yield on nitrogen (N) partitioning in grapevine and on must composition. The mechanism of N assimilation, partitioning and mobilization from the reserves is studied through foliar application of ^{15}N isotope-labelled urea over a two-year period. The final scope is to optimize fertilizer use efficiency and grape composition. Here are summarized the results from the first year of experimentation.
Material and methods - Two blocs (control and test) of 12 homogeneous potted grapevines each (*Vitis vinifera* L. Chasselas) were grown under field conditions. During summer 2017, cluster thinning allowed to create a large yield gradient (from 0.5 to 2.5 kg/m^2 of soil). Vegetative development—canopy weight, leaf area, photosynthesis activity—and yield parameters —bud fruitfulness, bunch and berry weights, number of bunches and total yield per vine— were measured. All the vines were excavated at harvestand the organs were separated (roots, trunk, canopy, pomace and must), with the aim of monitoring N partitioning in the plant. The test bloc received 20 kg/ha of foliar-applied ^{15}N labelled urea at veraison. Total organic carbon and nitrogen and their stable isotope compositions were determined in each organ, using EA-IRMS. The musts were analysed for their content of soluble sugars, acids, NH_4^+ and amino acids.
Results - Grapevine compensated higher N demand from the grapes by assimilating more N through leaves and roots and mobilizing more N from reserves. The foliar supply of urea limited N mobilization from the roots, preserving the reserves for the following year. Must amino-acid profiles varied significantly with the yield. Yield had no impact neither on vegetative development nor on grape maturation. With increasing yield, N concentration remained constant in the canopy and grapes at harvest, to the detriment of the N content in roots. Urea assimilation was positively correlated with the yield (r = 0.68, P = 0.029). Urea supply had a positive impact on yeast assimilable nitrogen concentration in the must only under higher yield conditions.
Keywords: Nitrogen, partitioning, yield, foliar urea, isotope labelling, amino acids.

1. Introduction

Yeast assimilable nitrogen content (YAN) in the must—composed of ammonium (NH_4^+) and free amino acids (AA)—is a relevant parameter which influences fermentation kinetics and wine bouquet (Bell and Henschke 2005; Hannam et al. 2016). Below 200 mg N/L in the must, fermentation duration is negatively correlated to YAN concentration; below 140 mg N/L, it seriously increases the risk of stuck fermentation and organoleptic deviations, particularly in white wine (Bell and Henschke 2005; Torrea et al. 2011). To limit those risks, N completion in the must at the beginning of fermentation—mainly in a form of diammonium phosphate—has become a widespread practice in wine making. However, a correlation between the chemical changes induced by nitrogen supplementation and the consequent sensory modifications in the wine, if any, has never been clearly established (Torrea et al. 2011). Therefore, wine sensory profile mainly depends on the initial grape composition at harvest, which has to be managed directly in the vineyard (Gutiérrez-Gamboa et al. 2019).
The accumulation of aromas in the grapes appears to differ from the accumulation of other compounds normally associated with berry ripening (González-Barreiro et al. 2015). It has been established that

over-cropping can delay grape ripening and reduce fruit and wine quality (Petrie et al. 2006; Rutan et al. 2018). Consequently, cluster thinning has become a common practice to regulate yield. Several studies reported on the influence of fruit load on total organic carbon partitioning (Morinaga et al. 2003; Dai et al. 2010; Dayer et al. 2016), but it is still unclear how fruit load influences accumulation of N compounds in grapes. The correlation of YAN concentration in the must with the canopy size was shown in a previous study, but no correlations with yield was observed (Verdenal et al. 2016). As stated by González-Barreiro et al. (2015), knowledge of how cultivation methods influence the formation of aroma compounds would assists grape growers in fine-tuning their long-term practices, like pruning, cluster thinning, irrigation and fertilization. This paper focuses on the impacts of cluster thinning and foliar-N fertilization i) on N mobilization and partitioning in the vine and ii) on the grape composition at harvest, by using a ^{15}N-labelling approach.

2. Material and methods

Vineyard site and experimental setup.The experiment was conducted in 2017 at Agroscope in Pully, Switzerland. Two homogeneous blocks (control and test) of twelve vines each—*Vitis vinifera* L. cv. Chasselas grafted onto rootstock 3309C—were planted in 2013 in 90 L underground pots and trained in a single Guyot training system. In each block, three subgroups of vines were created by cluster thinning at the phenological stage of bunch closure on 07/12—i.e. 2/5/8 clusters/vine—with the aim of creating a large yield gradient. The local natural soil was the only source of nutrients for the vines before the nitrogen (N) fertilisation was implemented in this study. 20 kg N/ha of ^{15}N-labelled urea (10 atom% ^{15}N, Sigma-Aldrich®) was applied to the test block around veraison period in four applications (07/18, 07/27, 08/02 and 08/14). The control block was identical to the test block but received no N fertilization.

Field measurements and plant sampling.During the season, vegetative development was monitored through the following measurements: chlorophyll index (N-tester®), light-exposed and total leaf areas, bud fruitfulness, cluster and berry weights, yield, leaf-to-fruit ratio (light-exposed leaf area divided by yield). At harvest time, all the vines were excavated and then partitioned into five organs: roots, trunk (including cane), canopy (including trimming collected throughout the season), pomace and must. Each vine was treated as a separate replicate. The clusters were pressed manually in a strainer to separate the must from the pomace. The five plant organs were fresh weighed. An aliquot (100 mL) of the fresh must was separated for chemical analysis. A second aliquot (25 g) of the must was freeze-dried, while the four other plant organs were dried at 60°C until constant weight. The dried plant parts were weighted and then ground to a fine powder (<1300 µm). After freeze-drying the must was in form of spangles.

Analyses.pH, total soluble solids (TSS), total acidity (g tartaric acid /L), tartaric and malic acids and YAN using an infrared spectrophotometer (FOSS WineScan) were determined on fresh must samples. Free amino acids (AA) were derivatized and quantified by HPLC. The ammonium was quantified by enzyme assay (Methods of Biochemical Analysis and Food Analysis, Boehringer Mannheim GmbH, 1997). The carbon and nitrogen stable isotope compositions (δ^{13}C and δ^{15}N) of organs and musts were determined by elemental analysis - isotope ratio mass spectrometry (EA/IRMS). The total organic carbon (TOC) and organic nitrogen (TON) contents were determined from peak areas of the major isotopes.

Calculation and data treatment. The N quantity (*QN*) in each organ was calculated as $QN_{organ} = DW_{organ} \times TON$, where *DW* is the organ dry weight (g) and *TON* is the organ N dry weight proportion (*%DW*). The abundance of ^{15}N (*A%*) or the proportion of heavy isotope per 100 atoms was calculated as: $A\%=R/(R+1)\times100$, where R is the ratio of the heavy to light isotopes (^{13}C/^{12}C, ^{15}N/^{14}N). The relative specific abundance (*RSA*)—proportion of newly incorporated N atoms relative to total N atoms in each organ—was calculated as follow (Deléens et al. 1997): $RSA = (A\%_{sample} - A\%_{control}) / (A\%_{nutrient} - A\%_{control})$. The new N pool for each organ was calculated as follow: $(new\ N\ pool)_{organ} = RSA_{organ} \times QN_{organ}$. The new N pool for the whole vine is the sum of the new N pools of the five organs. Thus, the percent partitioning (*%P*, also called distribution) of the new N in the organs was calculated as: $\%P_{organ}=(new\ N\ pool)_{organ} / (new\ N\ pool)_{vine} \times 100$. Two 6-vine groups were created in each block: low yield and high yield, 1.3 kg/m^2 at harvest being the threshold. Three vines were eliminated due to abnormally low berry set rate, low vigour or broken cane. The statistical analysis was performed using ©XLSTAT 2018.1.50011. The significance of differences between treatments was evaluated with analysis of variance (ANOVA, *P* values < 0.05) followed by multiple comparison Newman-Keuls test. Principal component analysis was used to study the AA composition.

3. Results and discussion

No impact of yield neither on grape maturation nor on TON concentration in the must at harvest.The grape *DW* was largely increased between low- and high-yielding conditions (+155 % in the control treatment; +117 % in the urea treatment). Despite yield variation, TSS and YAN concentrations in the must remained unchanged, as shown in other studies (Keller et al. 2005; Mawdsley et al. 2018; Wang et al. 2018). This was most probably due to low-limiting leaf-to-fruit ratio (average 0.7 m^2/kg for high yield) on the grape maturation and large carbohydrate reserves at the beginning of the experiment. Additionally, the grapevine appeared to adapt its metabolism through the modulation of combined morphological and physiological mechanisms, as explained hereafter.

Limitation of root growth and smaller root N reserves under high-yielding conditions.The root growth tended to be lower under high-yielding conditions (i.e. -17 %*DW* and -14 %*DW* in the control and urea treatments respectively). There was no difference in terms of *DW* between low and high yields after one season, but the observed tendency may become significant over a longer term. Morinaga et al. (2003) observed that under high-yielding conditions the growth of fine roots and lateral shoots is reduced, while the respiration rate of fine roots is higher. High yielding vines have to draw C and N from the reserves mainly located in trunk and roots, to fill in the maturing fruits (Howell 2001). Under high-yielding conditions, grape maturation (i.e. accumulation of the major part of the photosynthetic carbohydrates) appeared as a priority over root development. Therefore, the TOC and TON contents increased in the grapes, proportionally to the fruit load, while the root growth was limited and consequently the C and N storage capacity. In fact, in the control treatment, the N reserves in the roots of vines were reduced by 27 % on average in high yield conditions. Other studies have shown that accumulations in the roots was restricted by the presence of fruit before and after veraison (Rodriguez-Lovelle and Gaudillere 2002; Rossouw et al. 2017). This suggests that several years of overproduction could potentially induce an important reduction of N reserves, which may reduce vigour, bud fruitfulness and even vine sustainability in the long term.

Higher N uptake under high-yielding conditions.The foliar-N assimilation rate was on average 37 % under high-yielding conditions and only 26 % under low-yielding conditions (Fig. 1). The *RSA*—a measure of the N sink strength, independently from the organ size—was the highest in the must (10 ± 3 % TON). When yield was greatly increased, grape N demand increased consequently, inducing modifications in the N partitioning. This result confirms findings from Verdenal et al. (2016) suggesting that increasing N uptake is a reaction of the vine to yield variations for maintaining grape N concentration. The foliar N assimilation was a function of both plant vigour and yield, which can be assessed on our dataset by the equation: Assimilation rate = 8.97 + 1.22*[canopy weight] + 7.44*[yield] (n = 10; r = 0.90). Additionally, higher-yielding conditions might also have stimulated root assimilation from the soil in contrast to root growth. This may explain why after N-labelling, the *TON* concentration was significantly higher in the roots under high-yielding conditions (+20 %), while the *RSA* of labelled N was lower. This suggests the presence of a non-labelled N source, which could only be the N assimilated by the roots from the soil. This could be confirmed in a longer-term experiment.

Yield effect on AA profile.Despite an unchanged concentration in the must, the *YAN* composition varied significantly in terms of AA profile (Fig. 2). The AA profile was more affected by the yield than by the urea supply. Under high-yielding conditions, the percentage of alanine, serine, threonine and GABA were higher, and those of arginine, histidine, tyrosine, tryptophan and isoleucine were lower. The relation between the AA profile and the wine aroma profile remains unclear. The impact of cluster thinning on the volatile compounds can be either positive or negative; it seems to vary depending on the grapevine variety, the period of bunch thinning and the leaf-to-fruit ratio (Alem et al. 2019). Further research is required to understand the mechanisms involved in the accumulation of aroma compounds in grapes and wines, in relation to AA profiles.

Preservation of root N reserves after foliar urea supply.The efficiency of foliar-urea application depended on the fruit load. Under low-yielding conditions, the impact of urea supply on the *YAN* concentration in the must remained insignificant. However, under high-yielding conditions, the urea assimilation rate was higher and the *YAN* concentration in the must was increased by 34 % (P = 0.021). In these conditions, significantly less labelled N was distributed in the roots (-50 %) and in the trunk (-38 %), while a higher fraction tended to be distributed to the canopy (+14 %) and to the grapes (+35 %) (Fig. 3). In terms of partitioning, only 9 % of labelled N was accumulated in the roots, against 18% under low-yielding conditions. N quantity in the roots did not decrease due to urea supply when the yield

increased, while it did in the non-fertilized vines. The urea supply allowed fulfilling the grape N demand, while preserving the N reserves in the roots.

4. Conclusions

C and N balance between roots and grapes seems to be essential for long-term vine sustainability and grape composition. The grapevines appeared to be in a constant search for nutrition balance to insure their reproduction. The use of N reserves from the roots and the modification of N assimilation rate allowed the vines to maintain a constant N concentration in the grapes despite variations in yield. Foliar-urea fertilization at veraison was more efficient under high yield conditions and helped fulfilling the grape N demand, while limiting mobilization and downsizing of N reserves. However, the yield significantly affected the AA profiles, suggesting a possible modification of wine aroma profiles despite unaltered grape maturation. Only few studies focused on the impact of cluster thinning—and agronomic practices in general—on volatile organic compounds in grapes. The root development and activity appear to be a key for understanding the mechanisms that balance N nutrition in vines. In the second year of this experiment, the first year residual labelled N in the roots will help to assess the N reserve partitioning during the vegetative growth, while differentiating from root N assimilation.

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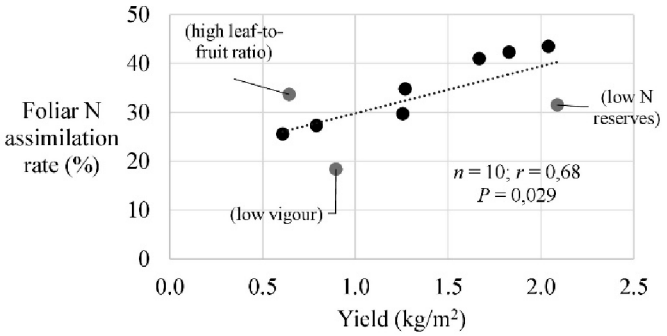

Figure 1: Impact of yield on foliar N assimilation rate
The dots below the line indicate a lower vigour, the dots above the line indicate a higher vigour.

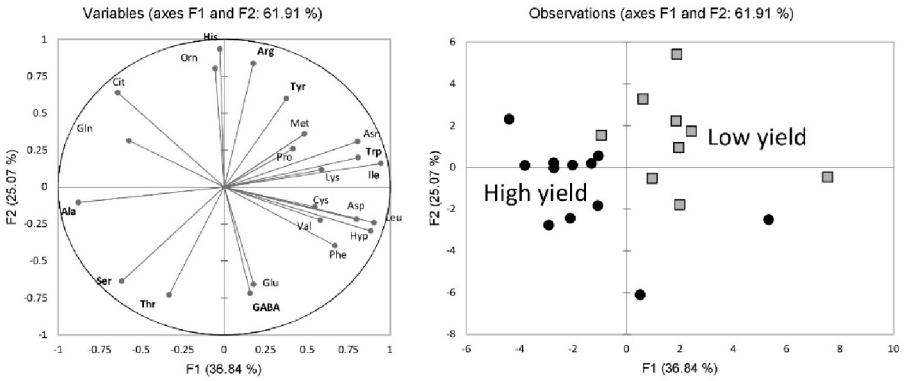

Figure 2: Principal component analysis showing the discrimination between the AA profiles of the musts produced under low- and high-yielding conditions.

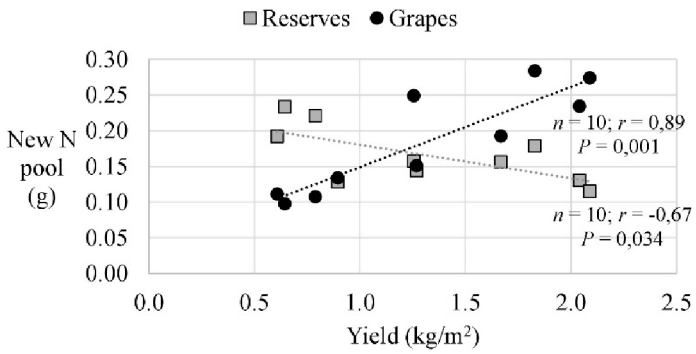

Figure 3: Impact of yield on the new N partitioning in roots and grapes.
Significantly less labelled N was allocated to the roots under high-yielding conditions.