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PLANT NITROGEN ASSIMILATION AND PARTITIONING AS A FUNCTION OF CROP LOAD

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Abstract

Aims: The optimization of nitrogen use efficiency (NUE, i.e. uptake, assimilation and partitioning) is a solution towards the sustainable production of premium wines, while reducing fertilization and environmental impact. The influence of crop load on the accumulation of N compounds in fruits is still poorly understood. The present study assesses the impacts of bunch thinning on NUE and the consequences on the free amino N (FAN) profile in fruits.

Methods and Results: A large crop load gradient was imposed by bunch thinning (0.5 to 2.5 kg m^{-2}) in a homogeneous plot of 225 vines. Isotope-labelled foliar urea (10 atom % ^{15}N) was applied on the canopy of the fertilized treatment at veraison. The plants were excavated at four phenological stages over the two seasons (bud burst, flowering, veraison and harvest) and were individually split into five plant parts (roots, trunk, canopy, pomace and must). Total nitrogen and its stable isotope composition were determined in each part, with the aim of monitoring NUE as a function of crop load and fertilization.
The N concentration in fruits either at veraison or at harvest was not related to crop load variation. N concentration was maintained in the must to the detriment of N content in the roots. The root dry weight was 15 % lower and the root N quantity 27 % lower under high yielding conditions (HYC, compared to low yielding conditions LYC). The fertilizer N uptake was 41 % higher under HYC than under LYC. Consequently, urea supply had a positive impact on the yeast assimilable N concentration in the must (+55 mg L^{-1}) only under HYC. However, the must FAN profile was significantly affected by the crop load, suggesting a possible modification of the aroma potential, independently from fertilization and grape maturation.
Conclusion: Using a ^{15}N-labeling method, we demonstrate that grapevine has a strong ability to regulate nitrogen uptake and reserve mobilization to maintain a constant fruit N concentration despite changes in crop load. Foliar-urea fertilization at veraison was more efficient under HYC and helped to fulfill grape N demand, while limiting the mobilization of N reserves. However, the crop load affected the must FAN profile, inducing a possible modification of the fruit aroma.

Significance and Impact of the Study: These findings highlight the great capacity of plants to adapt their N metabolism to constraints, e.g. bunch thinning in this case. These results are important to improve perennial fruit crop production through higher fertilization efficiency and lower environmental impact. Without fertilization, plant nutrition can be enhanced through the optimization of agricultural practices. The root activity appears to be key for understanding the mechanisms that balance N nutrition in plants.

Keywords: Nitrogen partitioning, crop load, isotope labelling, amino acids, vines

Introduction

Plant growth is often limited in the natural environment by nitrogen (N) availability. Despite the low requirement of grapevines, N restriction reduces the yield, affects N accumulation in fruits and has consequences on the winemaking process in terms of fermentation kinetics and wine aroma development (Schreiner *et al.* 2014). Fifty to seventy percent of N provided to crops via fertilization is generally lost by leaching and gaseous emission (Masclaux-Daubresse *et al.*, 2010). Therefore, improving N use efficiency (NUE) through the adaptation of agricultural practices is critical to enhance productivity and to preserve the environment. In viticulture, the optimum yield is generally not the maximum allowed by the conditions of the vineyard. Bunch thinning—i.e., crop load limitation by removing a proportion of fruits early in the season—is a common practice to manipulate the source-to-sink ratio and enhance fruit maturation, i.e. sugar accumulation (Petrie and Clingeleffer, 2006). In viticulture, the leaf-to-fruit ratio (light-exposed leaf area per kg of fruit) is an indicator whether a plant is in balance (Howell, 2001). Vine balance is implicitly understood based on the principle of carbon balance, although N balance is equally of importance. As an example, independently from the plant vigour, an oversized canopy (i.e. due to an excessive trimming height) affects N partitioning in the plant and potentially induces fruit N deficiency, despite available N resources to the plant (Spring *et al.,* 2012). This trial was set up to observe the impact of crop load on the N partitioning in the plant and on the grape composition. It used a ^{15}N-labelling method to monitor N uptake and partitioning through the plant over two years.

Materials and Methods

Experimental Setup and Sampling

The experiment was conducted in 2017 and 2018 at Agroscope (Pully, Switzerland). 225 cultivars of *Vitis vinifera* L. Chasselas (grafted onto 3309 C) were planted in 2013 in 90 L underground pots, filled with low-calcareous colluvial soil (clay 15wt %, silt 38wt %, sand 47wt % and carbonates 4.3wt % equivalent $CaCO_3$). The vines were trained in a single Guyot trellis system, with 60 cm trunk height and 7 shoots per cane. The canopy was trimmed at 120 cm above the top of trunk, three times per season. The plot was divided into 14 homogeneous blocks (12 plants each) separated by buffer plants and corresponding to the three fertilization treatments: control treatment (C); treatment with one fertilization in 2017 (F17); and treatment with a fertilization in both 2017 and 2018 (F17+18). The fertilization consisted of spraying 2.4 g N per plant (20 kg N ha^{-1}) on the canopy at veraison in a form of ^{15}N-labelled urea (10 atom % ^{15}N, Sigma-Aldrich). Each block was excavated at one of the four major phenological stages (i.e. bud burst, flowering, veraison and harvest) over the two seasons. Consequently, a block of treatment C was excavated at each stage from bud burst 2017 to harvest 2018 (total 8 blocks); a block of treatment F17 was excavated at each stage starting from harvest 2017 (i.e. after 2017 urea application) to harvest 2018 (5 blocks); and a block of treatment F17+18 was excavated only at harvest 2018 (1 blocks). In each block, a large crop load gradient was built by bunch thinning at bunch closure. The field measurements and sample preparations were conducted as described in Verdenal *et al.* (2020). By the time of excavation, each vine was unearthed separately, split into five parts (i.e. roots, trunk, canopy, pomace and must), fresh (FW) and dry (DW) weight recorded and then powdered for further analyses.

Analyses

The stable C and N isotope compositions were determined by isotope ratio mass spectrometry, as detailed in Spangenberg and Zufferey (2018). The δ values were reported in milliurey (mUr) in conformity with the International System of Units. The total organic C (TOC) and total N (TN) contents were determined from peak areas of the major isotopes. Fruit composition was analyzed in centrifuged fresh must aliquots collected from the vines excavated at veraison and harvest. An infrared spectrophotometer (WineScan, FOSS NIR Systems) was used to determine the pH, total soluble solids (TSS), titratable acidity (TA), potassium (K) and tartaric and malic acids. The ammonium (NH_4^+) was quantified using an enzymatic test kit (Boehringer Mannheim GmbH). Free amino acids (FAA) were separately quantified in the must by ultrahigh-performance liquid chromatography-mass spectrometry, following the methods detailed in Verdenal *et al.* (2020).

Data Treatment

For the interpretation, the data from each block were split in two groups of vines, i.e. low-yielding conditions (LYC) versus high-yielding conditions (HYC). The data treatment was realized based on the method detailed in Verdenal *et al.* (2020). The N quantity (NQ) in each organ was calculated as NQ = DW × TN. The abundance of ^{15}N (A%), i.e. the proportion of heavy isotope per 100 atoms, was calculated as: $A\%=R/(R+1)\times 100$, 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—was calculated as follows (Cliquet *et al.* 1990): RSA = ($A\%_{sample}$ -

A%$_{control}$) / (A%$_{nutrient}$ - A%$_{control}$). The new N pool (NNP) for each plant part was calculated as follows: NNP = RSA × QN. The new N pool for the whole plant is the sum of the NNPs of the five plant parts. The partitioning (%P, also called distribution) of the NNP in the plant parts was calculated as:

$\%P_{plant\ part} = NNP_{plant\ part} / NNP_{whole\ vine} \times 100$.

Results and Discussion

A large gradient of crop load was set for two years (e.g. 0.8 to 4.4 kg m^{-2} at harvest in 2018). N concentration in the must remained uniform in all plants despite the crop load variations (94 ± 17 mg L^{-1} in C treatment at harvest in 2018). Two physiological processes could be observed. First, root N mobilization was higher under HYC in answer to the higher fruit N demand (Figure 1).

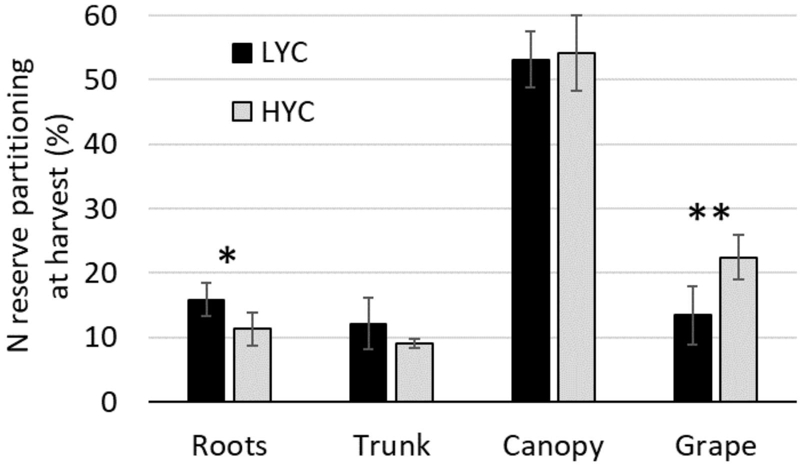

Figure 1: Partitioning of the N reserve at harvest 2018 (residual labelled NQ from 2017 present in roots and trunk at bud burst 2018). Chasselas cv., Switzerland, 2018. * *P*-value < 0.05; ** *P*-value < 0.01. LYC = low yield conditions; HYC = high yield conditions.

Second, HYC strongly stimulated fertilizer N uptake (+41 % in comparison to LYC, average 2017-2018). Fertilization under HYC had a positive impact on the must YAN at harvest (+ 54 mg L^{-1} in 2018; *P-value* = 0.032), while fertilization under LYC had no significant impact. Foliar fertilization at veraison 2017 had no carryover impact on the must YAN content of the following year. Consequently, NQ significantly increased in the grapes under HYC, proportionally to the fruit load, without excessive mobilization and downsizing of the root N reserves (Figure 2).

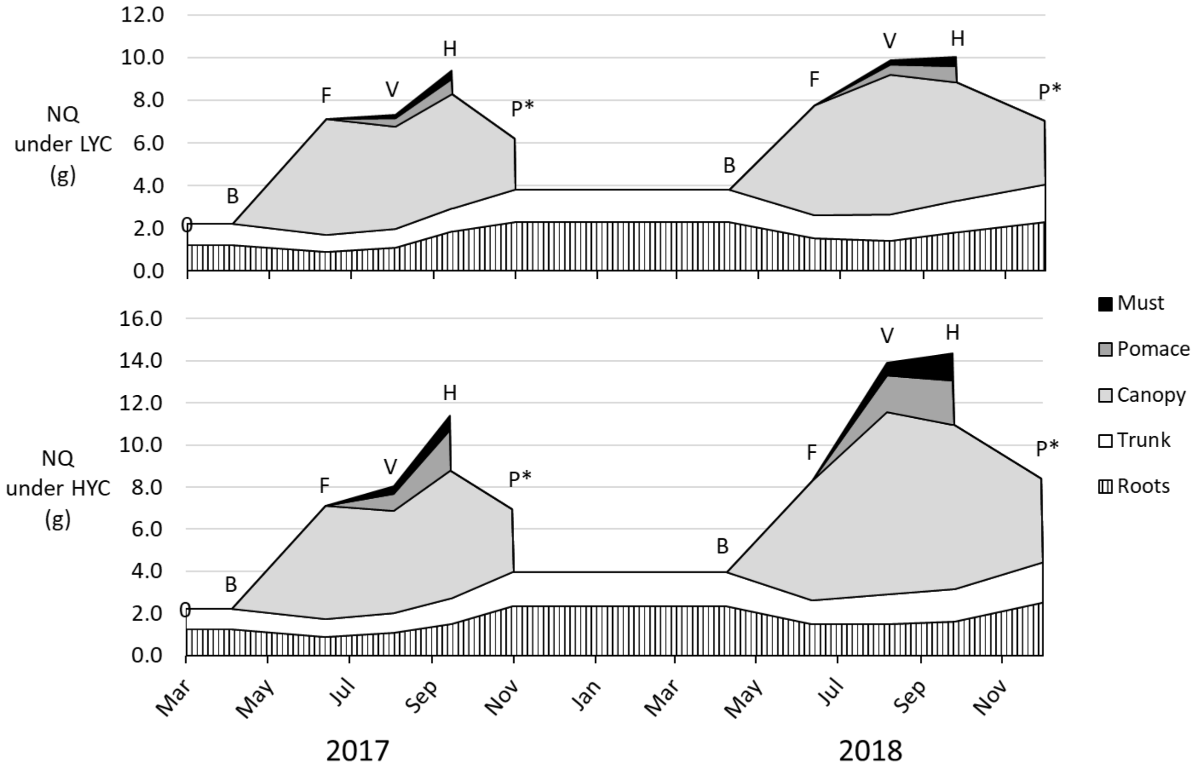

Figure 2: Distribution of the total nitrogen in the vine over two years in response to a change in fruit load. Chasselas cv., Switzerland, 2017 and 2018. NQ = nitrogen quantity; LYC = low yielding condition; HYC = high yielding condition; B = bud burst; F = flowering; V = veraison; H = harvest; P = pruning (*hypothetic values).

Bunch thinning did not improve the must YAN concentration but affected the FAA profile, suggesting a modification of the potential aroma profile. The year highly influenced the FAA. LYC promoted the proportion of Pro, while it reduced Ala, GABA, Gln, Gly, Ser and Thr. It is therefore questionable whether the crop load limitation has a positive impact on the grape composition and ultimately on the wine quality. The discrimination in terms of FAA between LYC and HYC could be observed already at veraison.

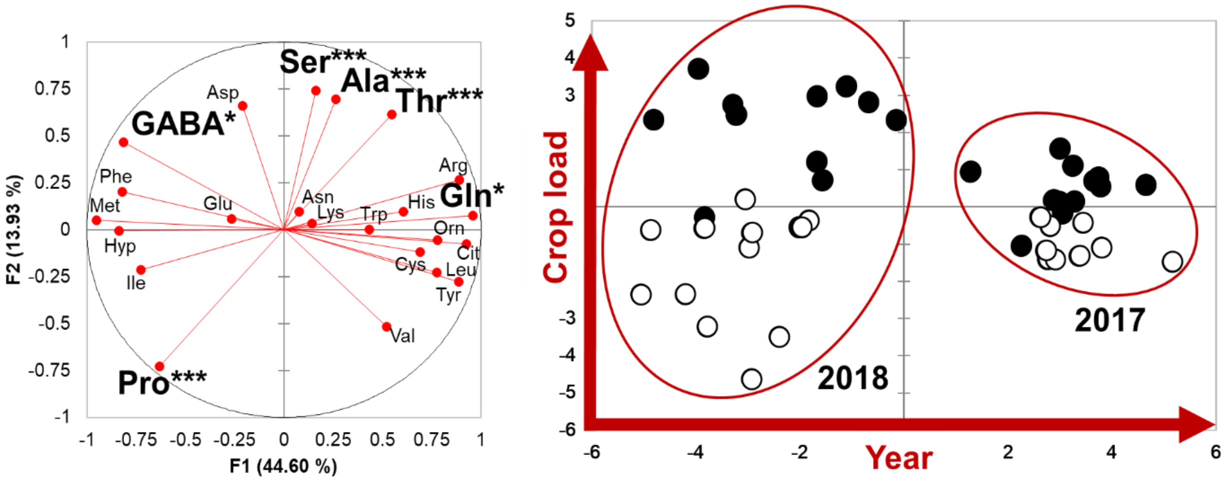

Figure 3: Principal component analysis showing the discrimination between the AA profiles of the musts produced under low- (white dots) and high-yielding conditions (black dots) over two years, 2017 and 2018.

Conclusion

Grapevines modulated both root N reserve mobilization and fertilizer N uptake to maintain a uniform N concentration in the must despite crop load variations. However, crop load affected the FAA composition in the must. This experiment demonstrates the high potential of bunch thinning to control the plant NUE and ultimately the final N composition in fruits at harvest. Root development and activity are both key factors for understanding the mechanisms that balance plant N nutrition. This study encourages further research on the potential of agricultural practices to monitor NUE, with the aim of enhancing crop quality and sustainability.

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