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ORIGINAL RESEARCH ARTICLE

Carryover effects of crop thinning and foliar N fertilisation on grape amino N composition

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ABSTRACT

Nitrogen (N) is an essential element for vine development and yield; it is also involved in the winemaking process and significantly affects wine composition. It is therefore essential to control and optimise plant N use to ensure an adequate N composition of the grapes at harvest. An improved understanding of the impact of cultivation practices on plant N metabolism would allow a better orientation of technical choices with the objective of quality and sustainability (i.e., fewer inputs, more efficiency).

Our trial focused on the impacts of fertilisation and crop thinning on grape N composition. A wide crop load gradient was set up in a homogeneous plot of Chasselas (*Vitis vinifera* L.) in an experimental vineyard in Switzerland. Foliar urea was applied at veraison in order to compare it with an unfertilised control. Vine development and grape composition were evaluated over two years, with particular attention to the carryover effects of both fertilisation and crop thinning.

Foliar N fertilisation effectively increased the amount of N in grapes at harvest in the same year, but had no impact on grape ripeness or carryover effect on year n + 1. Conversely, crop thinning improved grape maturity by reducing fruit N and C demand. Interestingly, amino N proportions could be distinguished according to crop load, while the global grape N concentration at harvest remained unchanged. Some amino acids were more affected by crop thinning than others. The concentrations of alanine, γ-aminobutyric acid (GABA), serine and threonine were reduced by crop thinning. Crop thinning had a strong carryover effect on year n + 1. The carryover impact of crop thinning on grapes in terms of both maturation index and N composition could be observed at the onset of grape ripening on year n + 1.

This experiment highlighted the influence of the previous year's agricultural practices on grape C and N accumulation before and during the ripening phase. Consequently, the modulation of grape composition at harvest should be considered over two consecutive years. These results will contribute to the improvement of predictive models and sustainable agronomic practices in perennial crops.

KEYWORDS: crop regulation, green harvest, nitrogen use efficiency, yeast assimilable nitrogen, aroma

INTRODUCTION

Nitrogen (N) represents approximately 1.5 % of plant dry weight and is an essential element for vegetative and reproductive development. It plays a major role in plant metabolism and is required in larger amounts than any other nutrient applied to crops. Considering that only 30–40 % of the applied N is actually utilised by crops, correct management of N metabolism is necessary for improving N use efficiency and achieving sustainable production of grapes with a high-quality potential (Kant *et al.*, 2011).

N excess and N deficiency are both highly detrimental to the sustainable production of quality grapes. On the one hand, N excess exacerbates plant vigour, inducing excessive leaf area and increasing sensitivity to grey rot. On the other hand, N deficiency results in weak vegetative growth, low berry set and altered fruit composition at harvest. Considering that N restriction in year n affects yield formation in year n + 1, it consequently reduces the long-term production potential, in terms of both quantity and quality (Guilpart *et al.*, 2014).

N restriction also affects the winemaking process. Free amino acids (FAAs) represent 60–80 % of total N in grape must (Aerny, 1996). FAAs with a primary amine ($-NH_2$), together with ammonium (NH_4^+, 5–20 %), are the major N forms assimilable by yeasts during the winemaking process; thus, they are named yeast assimilable N (YAN). YAN concentration is often suboptimal in musts, which consequently reduces yeast growth and alcoholic fermentation kinetics. Below 200 mg YAN/L, fermentation duration is negatively correlated to YAN concentration for a clarified must under medium concentration of total soluble sugars (TSS). Below 140 mg/L, there is a major risk of stuck fermentation (Bell and Henschke, 2005). Moreover, FAAs are precursors of many secondary metabolites in grapes and wines, particularly volatile compounds responsible for wine aromas, such as terpenes, thiols and esters (Verdenal *et al.*, 2021a). Under YAN restriction, the production of these secondary metabolites is affected, modifying the wine sensory profile, even when corrective practices during winemaking are applied (Ugliano and Henschke, 2009). Consequently, proper vineyard N management should be a prerequisite to producing grapes naturally balanced in FAA compounds, thus offering the winemaker an improved potential for producing good quality wines.

Grapevine N status may vary considerably, not only in relation to environmental conditions (i.e., climate and soil), but also depending on the agronomic practices. Agronomic practices often combine tradition and innovation, with the aim of matching production targets in terms of yield and grape composition. Vine growers constantly adjust their agronomic choices concerning plant material, soil management, vine balance and vineyard inputs.

Vine balance is a term used to express the balance between vegetative growth and reproductive development in plants. A balanced vine can produce fully ripe grapes while building up nutrient reserves for the following year (Howell, 2001). Conversely, an excessive fruit load can alter grape ripening in terms of carbon accumulation (Kliewer and Dokoozlian, 2005). Additionally, for any other parameter held constant, an excessive leaf area can alter N accumulation in grapes, particularly the concentration of YAN (Spring *et al.*, 2012). There are two ways to increase the leaf-to-fruit ratio: either by increasing canopy size or by limiting crop load. The consequences of these two actions on the total N amount in the whole plant and on the YAN concentration in the grapes are different under the cool-climate conditions of Switzerland (Figure 1; Verdenal *et al.*, 2021b). A leaf-to-fruit ratio in the range of 1.0–1.2 m^2 of exposed leaves per kg of fruits is usually recommended for the cultivar Chasselas under Switzerland's cool climate conditions (Murisier and Zufferey, 1997; Verdenal *et al.*, 2016).

In a research programme conducted in 2017–2018 at Agroscope, Switzerland, vines were shown to be able to maintain a constant concentration of YAN in the must at harvest, despite variations in crop load (Verdenal *et al.*, 2020). Moreover, the grapevine adjusted N uptake and root mobilisation to its crop load, which had carryover effects on plant N content in the year n + 1 (Verdenal *et al.*, 2021b). The experiment was conducted over two seasons, and the present manuscript addresses the formation of the FAA pool in grape must as a function of crop thinning and fertilisation and their carryover effects in the following year. For this purpose, the composition of the musts and their FAA contents were assessed both at the onset and the end of grape ripening (i.e., veraison and harvest) over two consecutive years.

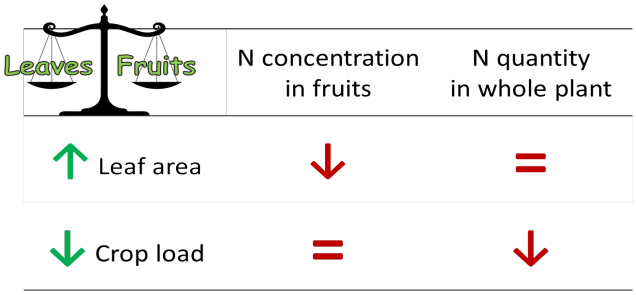

FIGURE 1. Variations in fruit N concentration and whole-plant total N as a function of the leaf-to-fruit ratio.

To increase the leaf-to-fruit ratio, one can either increase leaf area or decrease crop load (adapted from Verdenal *et al.*, 2021b).

MATERIALS AND METHODS

The materials and methods for the entire project are detailed in Verdenal *et al.* (2021b). Only the materials and methods regarding the specific results described in this manuscript are presented.

1. Experimental site and plant material

The trial was conducted over two years (2017–2018) at the Agroscope experimental station in Pully, Switzerland (46°30'45.8"N, 6°40'05.7"E). The local climate is temperate.

During the first vine-growing season (April–October 2017), total precipitation was 562 mm, and daily mean temperature was 16.6 °C. The climatic conditions in 2018 were drier and warmer than in 2017, with 412 mm of total precipitation and an average daily mean temperature of 17.8 °C from April through October (data from the Swiss meteorological station in Pully). The low-calcareous colluvial soil of the site was composed of 47 wt.% sand, 38 wt.% silt and 15 wt.% clay. The soil contained 1.75 wt.% of organic matter, 0.10 wt.% of total N and 4.3 wt.% of carbonates (eq. $CaCO_3$), and the pH was 7.9. Phosphorus (8.2 mg/kg), potassium (25.2 mg/kg) and magnesium (11.4 mg/kg) were not restrictive for vine growing.

Vitis vinifera L. cv. Chasselas grafted onto rootstock 3309 C was planted in 2013 in 90 L pots filled with the local soil described. The pots were then buried in the soil at a density of 8,330 vines per ha (1.5 × 0.8 m). Soil water-holding capacity was estimated at 11 L per pot, according to Saxton *et al.* (1986). To prevent possible water restriction, midday stem water potential was monitored several times during the two summers using a pressure chamber (Model 600; PMS Instruments, Albany, NY, USA) (Scholander *et al.*, 1965). Vines were rain-fed with a back-up drip-irrigation, which was used twice in July in each season (i.e., a total 12 L water per plant and per year) to maintain a midday stem water potential above 0.8 MPa (no water deficit). Vines were trained to a single Guyot trellis system, with 60 cm trunk height and seven shoots per cane. The canopy was trimmed at 120 cm above the trunk three times per season: on the day of year (DOY) 164, 191 and 215 in 2017 and on DOY 162, 183 and 218 in 2018. The dates of the main phenological stages were similar in 2017 and 2018: 50 % bud break (phenological scale BBCH 05) (Lancashire *et al.*, 1991) occurred on DOY 94 and 99 respectively; 50 % flowering (BBCH 65) occurred on DOY 164 and 161; 50 % veraison (BBCH 85) occurred on DOY 214 both years; and harvest was performed on DOY 257 and 269 respectively. At the end of 2017, winter pruning wood were removed from the experimental plot. The plants were organised into eight homogeneous groups of 12 plants each and separated by buffer plants to minimise cross-contamination from fertilisation. Despite homogeneity in terms of plant material and growing conditions, seven out of the 96 vines were identified as outliers (i.e., low vigour, low photosynthetic activity, low fruitfulness, low berry set and incomplete winter cold hardening) and were discarded to optimise the homogeneous conditions of the trial.

2. Experimental treatments and sampling

Two factors of variation were set in this trial: fertilisation and crop load. Three fertilisation levels were established: a non-fertilised control treatment (CT), a treatment with one fertilisation in 2017 only (F17), and a treatment with fertilisation in both 2017 and 2018 (F17+18). In 2017, the groups of 12 plants corresponding to the F17 and F17+18 treatments each received 2.4 g N per plant (20 kg N/ha) in the form of urea, applied on the leaves around veraison, split into four applications (DOY 199, 208, 214 and 226). In 2018, only the plants from the treatment F17+18 received 2.4 g of urea again in the same conditions (DOY 198, 204, 211 and 219). The foliar urea was carefully applied plant by plant on both sides of the canopy (dilution 3.44 % w/v) with hand sprayers (Spray-matic 1.25; Birshmeier, Stetten, Switzerland). No other fertilisation was applied during the trial.

The crop load treatment was set for each group of 12 plants. A large crop load gradient was built by crop thinning in each group of 12 plants at bunch closure (phenological stage BBCH 77; DOY 193 in 2017 and DOY 179 in 2018), keeping two to ten bunches per plant. Crop thinning in 2018 was based on the yield at harvest 2017 in order to maintain each plant under the same yield conditions over the two consecutive seasons and promote cumulative responses. For statistical analyses, the plants from each group were split into two sub-groups: low-yield conditions (LYC) and high-yield conditions (HYC). The threshold for splitting the groups of plants sampled in 2017 was 7.0 tons/ha at veraison (1 group, CT) and 13.0 tons/ha at harvest 2017 (2 groups, CT and F17), based on the median crop load by the time of sampling. Due to a higher yield potential in 2018, the thresholds in the groups of plants sampled at veraison 2018 (2 groups) and at harvest 2018 (3 groups: CT, F17 and F17+18) were 12.5 tons/ha and 21.0 tons/ha respectively. Each plant was considered a replicate.

The groups of vines were harvested separately on one of the four following sampling dates: veraison 2017, harvest 2017, veraison 2018 or harvest 2018. For each sampling date, the number of plants sampled (i.e., 12, 24 or 36) was related to the fertilisation levels at that date (i.e., one, two or three): only one group of 12 vines at veraison 2017 (CT); two groups at harvest 2017 and veraison 2018 (CT and F17); and three groups at harvest 2018 (CT, F17 and F17+18).

3. Field measurements, fruit analyses and data treatment

The field measurements and sample preparations were conducted as described by Verdenal *et al.* (2021b). Vine fruitfulness was determined before crop thinning and expressed as the number of bunches per shoot. The total leaf area (TLA) per vine was assessed with the non-destructive method of Mabrouk and Carbonneau (1996), based on the strong correlation between the length of a shoot and its TLA. To determine this equation in our context, 15 shoots from 15 different buffer plants were collected on DOY 206 in 2017. The total shoot length (TSL, main shoot + laterals) was measured, and the TLA was determined with a leaf area metre (LI-3100 C; Li-Cor Biosciences, Lincoln, NE, USA). As a result, Equation (1) allowed the transformation of measured TSL into estimated TLA for both seasons ($r = 0.98$):

$$TLA = 14.4 \times TSL + 161.5 \quad (1)$$

Fruit composition was analysed in centrifuged fresh must aliquots collected from each harvested plant. Total N content in must was measured by elemental analysis/isotope ratio mass spectrometry. A Carlo Erba 1108 elemental analyser (Fisons Instruments, Milan, Italy) was coupled with a Conflo III interface to a Delta V Plus isotope ratio mass

TABLE 1. Field measurements and must compositions at harvest 2018 as a function of 2017 N fertilisation and crop load.

Variable	N fertilisation 2017			Crop load			Interaction fertilisation × crop load
	0 kg/ha	20 kg/ha	*p*-value	LYC	HYC	*p*-value	
Bud fruitfulness (bunches per shoot)	2.2	2.1	n.s.	2.0	2.2	n.s.	n.s.
Bunches per vines	5.5	5.7	n.s.	2.8	8.1	***	n.s.
Total leaf area (m^2/plant)	1.86	1.92	n.s.	1.82	1.96	n.s.	n.s.
Crop load (g/plant)	2.80	2.57	n.s.	1.47	3.79	***	n.s.
Leaf-to-fruit ratio	0.94	0.83	n.s.	1.32	0.49	***	n.s.
Bunch weight (g)	549	482	n.s.	538	492	n.s.	n.s.
Yield (kg/m^2)	2.3	2.1	n.s.	1.2	3.2	***	n.s.
TSS (Brix)	19.5	20.2	n.s.	20.6	19.2	**	n.s.
Maturity index (TSS/TA)	4.2	4.4	n.s.	4.8	3.9	**	n.s.
pH	3.45	3.56	*	3.57	3.45	*	n.s.
TA (g tatrate/L)	4.8	4.7	n.s.	4.4	5.0	**	n.s.
Tartaric acid (g/L)	5.3	5.2	n.s.	5.0	5.4	**	n.s.
Malic acid (g/L)	2.0	2.3	n.s.	2.0	2.3	n.s.	n.s.
K (mg/L)	1629	1808	**	1788	1662	*	n.s.
NH_4^+ (mg/L)	16	12	n.s.	11	17	n.s.	n.s.
Primary amino N (mg N/L)	81	82	n.s.	84	78	n.s.	n.s.
YAN (mg N/L)	94	92	n.s.	94	92	n.s.	n.s.
Aromatic precursor N (mg N/L)	16.7	16.6	n.s.	17.2	16.1	n.s.	n.s.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s., not significant. Data from harvest 2018, cultivar Chasselas, Switzerland. HYC, high-yield conditions; LYC, low-yield conditions. N fertilisation was applied once at veraison in 2017. Crop thinning was realised at bunch closure in both 2017 and 2018.

spectrometer (Thermo Fisher Scientific, Bremen, Germany) operated under continuous helium flow, as detailed in Spangenberg and Zufferey (2018). The repeatability was better than 0.2 wt.%. An infrared spectrometer (WineScan; FOSS NIR Systems, Hilleroed, Denmark) was used to determine the pH, TSS, titratable acidity (TA), potassium (K^+) and contents of tartaric and malic acid content. The ammonium (NH_4^+) was quantified using an enzymatic test kit (Boehringer Mannheim GmbH, Mannheim, Germany). The primary amino N (PAN) concentration – excluding proline and hydroxyproline, which are not assimilable by yeast in the fermentation conditions – was determined with the o-phthalaldehyde (OPA) method using the Primary Amino Nitrogen kit (Bio Systems, Barcelona, Spain). The must YAN concentration was computed by summing the content of NH_4^+ and PAN, both expressed in mg N/L (Bell and Henschke, 2005).

To determine the FAA profiles (in %) of the grape musts, the FAAs were separately quantified in the must aliquots by ultrahigh-performance liquid chromatography-mass spectrometry using an Infinity 1290 HPLC system connected with an electrospray interface (ESI) to a 6460C Triple Quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Liquid chromatography was performed with an Intrada amino acid column (50 x 3 mm; Imtakt, Portland, OR, USA), following the methods detailed in Verdenal *et al.* (2020). Detection was achieved by multiple reaction monitoring. The calibration was done with standards for each FAA separately, according to their abundance. The repeatability of the values was better than 5 % and 10 % for high and low abundances respectively. FAA concentrations were reported in mg N/L. Aromatic precursor N was calculated by summing up the concentrations of asparagine, isoleucine, leucine, phenylalanine, threonine, tyrosine and valine (Valdés *et al.*, 2019).

Data treatment was realised using XLSTAT version 2020.5.1 software (Addinsoft, Paris, France). Each sampling date was subjected to a separate statistical analysis. For each sampling date, the determination of the effects of crop load, fertilisation and their interaction was determined using analysis of variation (ANOVA). Principal component analysis was used to discriminate the must FAA profiles at each sampling date.

RESULTS

1. Carryover effects of fertilisation and two-year crop thinning at harvest n + 1

2017 fertilisation had a negligible impact on the grape composition at harvest in 2018. Only the pH was increased by 0.1 and K^+ concentration was increased by 11 %, in

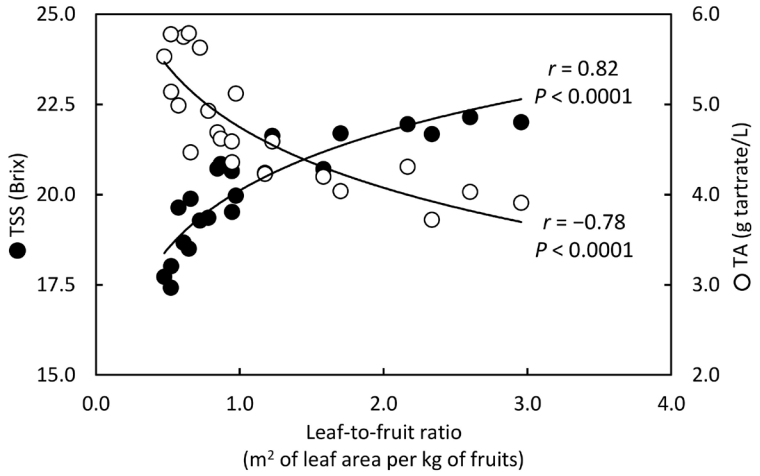

FIGURE 2. Variation of total soluble sugars (TSS) and titratable acidity (TA) in the must at harvest as a function of the leaf-to-fruit ratio.

Data from harvest 2018, cultivar Chasselas, Switzerland. Crop thinning was implemented at bunch closure in both 2017 and 2018.

TABLE 2. Must amino acid profiles (% of total FAA) at harvest 2018 as a function of 2017 N fertilisation and crop load.

Amino acids	N fertilisation 2017			Crop load			Interaction fertilisation × crop load
	0 kg/ha	20 kg/ha	*p*-value	LYC	HYC	*p*-value	
Alanine	7.77	7.97	n.s.	7.12	8.56	**	n.s.
Arginine	20.45	19.49	n.s.	18.06	21.68	n.s.	n.s.
Aspartic acid	5.41	5.45	n.s.	4.65	6.15	*	n.s.
Asparagine	0.47	0.45	n.s.	0.45	0.47	n.s.	n.s.
Citrulline	0.48	0.47	n.s.	0.51	0.44	n.s.	n.s.
Cysteine	n.d.	n.d.	–	n.d.	n.d.	–	–
GABA	7.56	8.48	n.s.	6.98	9.02	**	n.s.
Glutamine	1.45	0.92	***	1.05	1.28	n.s.	n.s.
Glutamic acid	11.09	10.26	n.s.	11.76	9.65	n.s.	n.s.
Histidine	1.84	1.69	n.s.	1.66	1.86	n.s.	n.s.
Hydroxyproline	0.45	0.46	n.s.	0.48	0.44	n.s.	n.s.
Isoleucine	1.83	1.70	n.s.	1.79	1.73	n.s.	n.s.
Leucine	1.10	1.05	n.s.	1.09	1.06	n.s.	n.s.
Lysine	0.32	0.29	n.s.	0.30	0.30	n.s.	n.s.
Methionine	0.96	0.91	n.s.	0.96	0.90	n.s.	n.s.
Ornithine	0.41	0.36	n.s.	0.40	0.37	n.s.	n.s.
Phenylalanine	1.27	1.19	n.s.	1.14	1.31	n.s.	n.s.
Proline	18.42	21.52	n.s.	25.11	15.39	***	n.s.
Serine	6.57	6.22	n.s.	5.76	6.96	**	n.s.
Threonine	6.58	5.92	n.s.	5.32	7.07	**	n.s.
Tryptophane	1.31	1.20	n.s.	1.21	1.29	n.s.	n.s.
Tyrosine	0.86	0.81	n.s.	0.83	0.83	n.s.	n.s.
Valine	2.34	2.24	n.s.	2.35	2.24	n.s.	n.s.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s., not significant; n.d., not detectable. Data from harvests 2018, cultivar Chasselas, Switzerland. HYC, high-yield conditions; LYC, low-yield conditions. N fertilisation was applied once at veraison in 2017. Crop thinning was implemented at bunch closure in both 2017 and 2018.

comparison with that of the CT (Table 1). Maturity index (4.3), YAN (93 mg/L) and the aromatic precursor N (16.7 mg/L) remained unchanged.

Crop thinning increased the leaf-to-fruit ratio and consequently enhanced grape maturity at harvest, particularly when the leaf-to-fruit ratio was lower than 1.0 m^2/kg (Figure 2). Under LYC, the maturity index increased by 23 %, with a higher TSS (+7 %) and a lower TA (-12 %), principally due to a lower concentration of tartaric acid (-7 %). K^+ concentration was also increased by crop thinning (+8 %). Crop thinning had no significant impact on grape N content. No significant interactions were observed between the two factors of variation.

In terms of amino N profiles, 2017 fertilisation only affected the proportion of glutamine (-40 %) (Table 2). Conversely, crop thinning reduced highly significantly ($p < 0.01$)

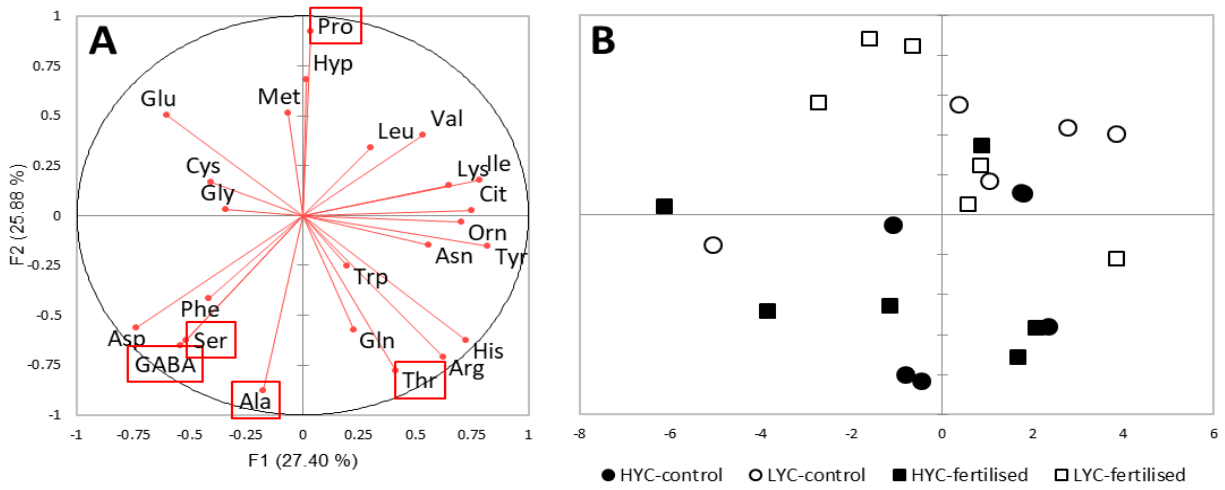

FIGURE 3. Principal component analysis (PCA) of the amino N profiles of the must at harvest.

Data from 2018, cultivar Chasselas, Switzerland. N fertilisation was applied once at veraison in 2017. Crop thinning was implemented at bunch closure in both 2017 and 2018. (A) Variables: correlations among amino acid concentrations. The amino acids, which were highly affected by crop load ($p < 0.01$), are highlighted in red. (B) Observations: shorter distances between observations indicated similar amino N profiles. HYC, high-yield conditions; LYC, low-yield conditions.

TABLE 3. Field measurements and must composition at veraison 2018 as a function of 2017 N fertilisation and crop load.

Variable	N fertilisation 2017			Crop load			Interaction fertilisation × crop load
	0 kg/ha	20 kg/ha	*p*-value	LYC	HYC	*p*-value	
Bud fruitfulness (bunches per shoot)	2.0	2.2	n.s.	2.1	2.1	n.s.	n.s.
Bunches per vine	5.8	5.4	n.s.	2.8	8.3	***	n.s.
Crop load (kg/plant)	1.62	1.79	n.s.	0.92	2.49	***	n.s.
Bunch weight (g)	318	360	n.s.	359	319	n.s.	n.s.
Yield (kg/m^2)	1.3	1.5	n.s.	0.8	2.1	***	n.s.
Total soluble sugars (Brix)	11.8	11.7	n.s.	13.0	10.4	***	n.s.
pH	2.98	2.99	n.s.	3.03	2.94	**	n.s.
Titratable acidity (g tartrate/L)	15.0	15.6	n.s.	13.0	17.6	***	n.s.
Tartaric acid (g/L)	7.2	7.1	n.s.	6.7	7.6	***	n.s.
Malic acid (g/L)	10.1	10.9	n.s.	8.6	12.4	***	n.s.
Potassium (mg/L)	1724	1768	n.s.	1653	1839	**	n.s.
Ammonium (mg/L)	70	94	n.s.	52	112	**	n.s.
Primary amino N (mg N/L)	64	75	n.s.	70	68	n.s.	n.s.
Yeast assimilable nitrogen (mg N/L)	121	152	n.s.	113	161	n.s.	n.s.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s., not significant. Data from veraison 2018, cultivar Chasselas, Switzerland. HYC, high-yield conditions; LYC, low-yield conditions. N fertilisation was applied at veraison in the 2017. Crop thinning was implemented at bunch closure in both 2017 and 2018.

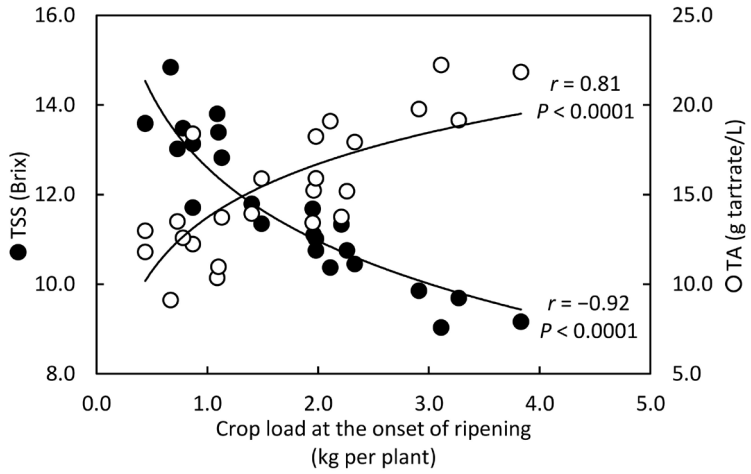

FIGURE 4. Variation of total soluble sugars (TSS) and titratable acidity (TA) in the must at veraison as a function of crop load.

Data from veraison 2018, cultivar Chasselas, Switzerland. Crop thinning was implemented at bunch closure in both 2017 and 2018.

TABLE 4. Must amino acid profiles (% of total FAA) at veraison 2018 as a function of 2017 N fertilisation and crop load.

Amino acids	N fertilisation 2017			Crop load			Interaction fertilisation × crop load
	0 kg/ha	20 kg/ha	*p*-value	LYC	HYC	*p*-value	
Alanine	8.00	9.44	n.s.	9.33	8.11	n.s.	n.s.
Arginine	21.75	22.71	n.s.	20.04	24.42	**	n.s.
Aspartic acid	16.63	15.51	n.s.	15.56	16.59	n.s.	n.s.
Asparagine	0.54	0.57	n.s.	0.50	0.60	**	n.s.
Citrulline	1.61	1.81	n.s.	1.85	1.57	n.s.	n.s.
Cysteine	n.d.	n.d.	–	n.d.	n.d.	–	–
GABA	3.01	2.81	n.s.	2.27	3.54	**	n.s.
Glutamine	4.00	4.57	n.s.	3.91	4.66	n.s.	n.s.
Glutamic acid	8.18	9.46	*	9.35	8.29	n.s.	n.s.
Glycine	2.99	2.12	*	3.01	2.10	*	n.s.
Histidine	2.62	2.51	n.s.	2.47	2.65	n.s.	*
Hydroxyproline	0.09	0.06	*	0.09	0.06	*	n.s.
Isoleucine	1.23	1.16	n.s.	1.29	1.09	***	*
Leucine	0.94	0.88	n.s.	0.96	0.87	*	**
Lysine	0.53	0.41	**	0.50	0.44	n.s.	*
Methionine	0.86	1.09	n.s.	0.99	0.96	n.s.	n.s.
Ornithine	1.31	1.09	*	1.37	1.03	**	n.s.
Phenylalaline	0.85	0.80	n.s.	0.83	0.82	n.s.	**
Proline	1.41	1.92	n.s.	2.18	1.14	**	n.s.
Serine	10.41	9.14	*	10.58	8.97	**	n.s.
Threonine	9.36	8.36	*	9.04	8.67	n.s.	n.s.
Tryptophane	0.77	0.77	n.s.	0.80	0.74	n.s.	n.s.
Tyrosine	1.29	1.29	n.s.	1.31	1.26	n.s.	*
Valine	1.61	1.55	n.s.	1.74	1.41	***	n.s.

* $p < 0.05$; *** $p < 0.01$; *** $p < 0.001$; n.s., not significant. Data from veraison 2018, cultivar Chasselas, Switzerland. HYC, high-yield conditions; LYC, low-yield conditions. N fertilisation was applied at veraison in the 2017. Crop thinning was implemented at bunch closure in both 2017 and 2018.

the proportions of few FAAs, that is, alanine (-17 %), GABA (-22 %), serine (-11 %) and threonine (-25 %), while it increased the proportion of proline (+37 %).

Amino N profiles at harvest 2018 were considered as a function of 2017 fertilisation and crop load (Figure 3). Two groups of plants can be distinguished according to yield conditions (i.e., HYC or LYC). No distinction could be made as a function of fertilisation in the previous year. The grape musts under HYC presented higher proportions of alanine, GABA, serine and threonine, while they had a lower concentration of proline.

2. Carryover effect already marked at veraison n + 1

Fertilisation 2017 had no impact at all on the field measurement and grape composition at veraison 2018 (Table 3). Conversely, crop thinning had a highly significant impact on both yield and grape maturity, even at the onset of maturation. Under LYC, TSS was higher (+25 %), and TA was lower (-18 %) due to lower contents of tartaric and malic acids, in comparison with HYC (Figure 4). K^+ and NH_4^+ concentrations were also reduced in a highly significant manner due to crop thinning (-10 % and -54 % respectively), while PAN, YAN and the aromatic precursor N concentrations were not affected.

In terms of amino N profiles in grape must, differences were observed as early as veraison (Table 4). At that phenological stage, the proportion of proline was still below 2 % of total FAAs. In comparison with the non-fertilised treatment, 2017 fertilisation affected the proportions of seven FAAs out of 24, that is, glutamine (+16 %), glycine (-30 %), hydroxyproline (-33 %), lysine (-20 %), ornithine (-15 %), serine (-12 %) and threonine (-11 %). Crop thinning affected the profiles of 11 FAAs, that is, arginine (-18 %), asparagine (-6 %), GABA (-4 %), glycine (+43 %), hydroxyproline (-33 %), isoleusine (+18 %), leucine (+11 %), ornithine (+40 %), proline (+100 %), serine (+18 %) and valine (+21 %).

Amino N profiles of grape musts sampled at veraison were discriminated by crop load and not by fertilisation treatment (Figure 5). At veraison, the grapes under HYC presented lower concentrations of isoleucine, ornithine, proline, serine and valine, while they had higher concentrations of arginine, asparagine and GABA.

DISCUSSION

The environmental conditions of this trial and the initial plant N status were not restrictive (i.e., unlimited water and nutrient supply). The results of this trial should be interpreted for these conditions and not extrapolated to vines under abiotic stress (water deficit and/or N deficiency). Despite the fact that grapevine yield formation occurs over two consecutive years (Guilpart *et al.*, 2014), no change was observed in terms of either bud fertility or bunch weight in the second year of the trial related to either fertilisation or crop load. Despite these unchanged parameters, fertilisation and crop load affected grape composition in different ways.

The year had a major impact on the must parameters. The 2018 maturity index was 39 % higher than that of 2017, with more TSS (+4 %) and less TA (-24 %). Concentrations of both tartaric and malic acids were reduced in 2018 in comparison with 2017, and pH consequently increased (+6 %). The YAN was sharply lower in 2018 (-43 %) due to a reduction in both ammonium (-63 %) and PAN (-38 %). The major differences observed in amino acid profiles between 2017 and 2018 might have been influenced by the drier and warmer conditions of 2018 compared to 2017.

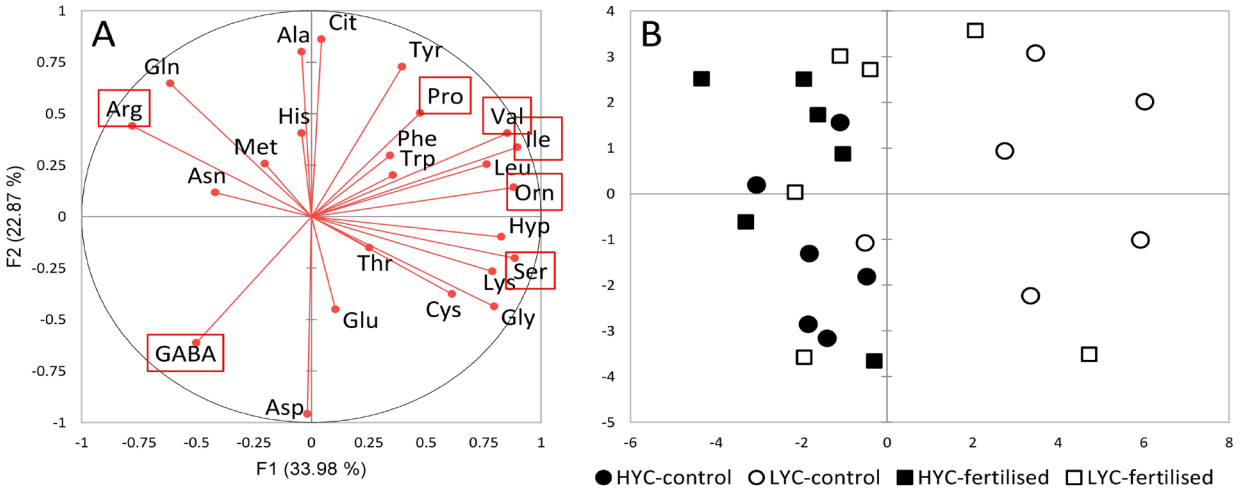

FIGURE 5. Principal component analysis (PCA) of the amino N profiles of the must at veraison.

Data from veraison 2018, cultivar Chasselas, Switzerland. N fertilisation was applied once at veraison in 2017. Crop thinning was implemented at bunch closure in both 2017 and 2018. (A) Variables: correlations among amino acid concentrations. The amino acids, which were highly affected by crop load ($p < 0.01$), are highlighted in red. (B) Observations: shorter distances between observations indicated similar amino N profiles. HYC, high-yield conditions; LYC, low-yield conditions.

Foliar fertilisation had no carryover effect in year n + 1: it neither exacerbated plant vegetative development nor affected grape composition at harvest. Moreover, fertilisation, in the form of foliar urea applied at veraison, efficiently increased grape N content at harvest exclusively in the same year of its application, particularly the YAN (+27 %, $p = 0.005$, two-year average) and the aromatic precursor N concentration (+27 %, $p = 0.001$, two-year average) (Verdenal *et al.*, 2021b). It improved grape N concentration without affecting grape maturity. Hence, this method is effective in correcting grape N deficiency and preventing vinification issues, as previously investigated by other authors (Lacroux *et al*., 2008; Hannam *et al*., 2016).

Crop thinning enhanced 2018 grape ripening in a highly significant way. Surprisingly, despite variations in both crop load and maturity level, must YAN concentration remained unchanged. Howell (2001) explained that under LYC, the plants required less C and N to meet the demand of the ripening fruits. Vines lower both N uptake from the soil and N mobilisation from the reserves (i.e., mainly in the roots) in response to the lower fruit demand. Under LYC, vines had enhanced root growth and were able to build a larger C and N reserve for the following year (Verdenal *et al.*, 2021b). Consequently, crop thinning induced a strong carryover effect on grape composition in year n + 1, particularly in terms of the maturity level at harvest.

Grape maturity is one of the most determinant variables in grape amino N content, and it has a strong influence on the evolution patterns of all FAAs (Garde-Cerdán *et al.*, 2018). As an example, the accumulation of proline in must usually occurs only at the final stage of grape ripening (Stines *et al.*, 2000). This would explain the higher concentration of proline in 2018 musts, which had a higher maturity level in comparison with those of 2017. Grape ripening in 2018 was impacted by the carryover impact of crop thinning, inducing an extremely high correlation between crop load and the maturity index at harvest 2018 ($r = -0.81$; $p < 0.0001$). Consequently, it was difficult to separate the impact of crop load from the impact of maturity in 2018. However, in 2017, the variation of grape maturity as a function of crop load was not significant ($p = 0.171$), allowing us to distinguish at harvest 2017 the impact of crop load on the FAA profiles from that of grape maturity (Verdenal *et al.*, 2020). Since the two yield conditions (i.e., HYC and LYC) were set only from 2017 (first year of trial), the absence of a relationship between crop load and maturity in that first year was due to the absence of carryover effect.

Four FAAs were highly affected by crop thinning both years, that is, alanine, GABA, serine and threonine, while proline was extremely correlated to the maturity index ($r = 0.94$; $p < 0.0001$) (Verdenal *et al.*, 2021b). Crop thinning reduced the proportions of these four FAAs, thus affecting the subsequent wine composition. The concentration of alanine in grapes increases α-ketopropionic acid and acetaldehyde in wines, often associated with fruity aromas (Verdenal *et al.*, 2021a). The concentration of threonine in grapes is related to 2-ketobutiric acid, propionaldehyde and 1-propanol in wines, and the concentration of serine in must is related to 3-hydroxy-2-ketopropionic acid, glyoxal and glycol content in wine (Garde-Cerdán *et al.*, 2018). These volatile compounds may contribute to wine aromatic complexity. However, the aromatic precursor N, as described by Valdés *et al.* (2019), was not affected by crop load ($p = 0.884$), suggesting no variation in wine aroma potential due to the yield condition. Conversely, the presence of GABA has been suggested as a regulator of plant physiology, which modulates plant growth, development and stress response (Ramesh *et al.*, 2017). The higher GABA content in must at veraison and at harvest in year *n + 1* could therefore be the consequence of the carryover effect of HYC in year n. GABA could potentially be an indicator of high-yield conditions, keeping all other parameters unchanged.

CONCLUSION

This study highlights the strong impact of crop thinning on grape composition at harvest and its carryover effects in year n + 1 in terms of both maturation and N composition. Relative contents in alanine, GABA, serine and threonine vary with crop load. Crop thinning has a strong potential for shortening grape ripening and modulating the amino N profile of the must. These results must be considered in the context of the environmental conditions of the trial (no restrictions for water and N) and the grape variety (*Vitis vinifera* L. Chasselas), as these factors have a dominant impact on the grape N composition. Similar to yield formation, the modulation of grape composition at harvest should be considered over two consecutive years. These results will contribute to the improvement of predictive models for N metabolism in order to promote sustainable agronomic practices in perennial crops.

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