

Nitrogen and carbohydrate reserves in the grapevine (*Vitis vinifera* L. 'Chasselas'): the influence of the leaf to fruit ratio

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Summary

Seasonal patterns of total organic nitrogen (N) and total non-structural carbohydrate (TNC) concentrations in relation to the leaf-fruit ratio (source-sink) were measured over three years at different grapevine phenological stages in one- and two-year-old canes, trunks and roots of the cultivar 'Chasselas' (*Vitis vinifera* L.). The highest N and TNC concentrations were observed during the period from dormancy until budbreak. A decrease in the N and TNC reserves was measured in the different organs (canes, trunks and roots) from budbreak, reaching minimum values around flowering, except for the N concentration in the roots, which was lowest during the period between bunch closure and veraison. N storage was highest in the roots and occurred from veraison until leaf fall. The N concentration in the trunks and canes represented approximately half of that measured in the roots. TNCs accumulated preferentially in the roots and also in the trunks and canes during the growing season. The leaf area per vine (or canopy height) and yield both influenced the N concentration in the roots. High yield and low leaf area per vine decreased the N concentration in the roots. The leaf-fruit ratio, expressed as the "light-exposed leaf area per kg fruit", substantially influenced the N and TNC concentrations in the roots at harvest. The highest N and TNC concentrations in the roots were obtained when the leaf-fruit ratio approached 2.0 m² of light-exposed leaf area per kg fruit.

Key words: nitrogen reserves; total non-structural carbohydrates; source-sink ratio; roots; wood.

Introduction

Carbon and nitrogen reserves in roots and wood fractions play a major role in vine longevity and grape quality potential (KELLER 2010). N reserves in the form of amino acids, either soluble (mainly arginine) or incorporated into proteins (KLIOWER 1967, SCHALLER *et al.* 1989, XIA and CHENG 2004), as well as carbohydrates in the form of starch

and soluble sugars (WINKLER and WILLIAMS 1945, EIFERT *et al.* 1961, ZAPATA *et al.* 2004, SMITH and HOLZAPFEL 2009) contribute to vegetative and root growth in spring (CHENG *et al.* 2004).

According to KELLER (2010), up to half of the N demand during canopy development can be provided by these reserves. Due to important vegetative growth in spring, nutrient demand is greatest at this time, and N and TNC concentrations in reserve organs are generally lowest around flowering, even though most soil N uptake occurs only after flowering (CONRADIE 1986, PEACOCK *et al.* 1989). Nevertheless, various authors (KELLER and KOBLET 1995, KELLER *et al.* 2001) noted that insufficient availability of N reserves, due to excessive yield or inadequate N replenishment in the previous season, could affect shoot elongation, leaf area development and fruit set. N reserve mobilization is the major process involved in N allocation to the growing tissues, at least until flowering (ZAPATA *et al.* 2004). The significant accumulation of soluble sugars and amino acids in the berries at veraison is dependent on import of sucrose from photosynthesizing leaves and woody storage organs (KELLER 2010). N and TNC storage may begin in the main reserve organs (trunks and roots) only once the plant requirements for growth and fruit production have been satisfied. In fact, N and TNC storage occurs when the resource supply exceeds demand. In general, the roots represent the most efficient sink organs to store N and C compounds (starch, soluble sugars) compared with other organs such as trunks, canes and shoots (LOESCHER *et al.* 1990, ZAPATA *et al.* 2004).

N and TNC utilization and storage during the season also depend on environmental conditions such as temperature (cool or hot climate) (WAMPLE *et al.* 1993), water supply (PELLEGRINO *et al.* 2014), light level (SCHREINER *et al.* 2012) and plant factors (photosynthetic conditions in the late season, and leaf N recycling during senescence, BATES *et al.* 2002). Moreover, WEYAND and SCHULTZ (2006) showed that the pruning system can influence N and TNC storage in the woody parts of a grapevine with respect to the leaf area developed by the training system. Under a cool climate, a sufficient leaf-fruit (source-sink) ratio is required to ensure adequate grape ripening (MURISIER and ZUFFEREY 1997, KLIOWER and DOKOOZLIAN 2005) and

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reserve recovery, e.g. for carbon (ZUFFEREY *et al.* 2012). This study presents the seasonal dynamics (2000-2002) of N and TNC reserves in different wood fractions (one- to two-year-old canes and trunks) and roots of field-grown vines. The impact of leaf area and yield per vine on the N concentration of the wood and roots was assessed, primarily through the leaf-fruit ratio (light-exposed leaf area per kg fruit).

Material and Methods

Study site and plant material: A field experiment was conducted on *Vitis vinifera* L. 'Chasselas' (clone 14/33-4, rootstock 3309 C, planted in 1986) at the Agroscope research station, in the experimental vineyard of Pully, Switzerland (46°32'N, 7°17'E: 450 m above sea level) between 2000 and 2002. The plot was oriented South with a 10 % to 15 % slope. Vines were pruned in a vertical shoot positioning system (cane pruning), which included six shoots per vine. Two planting densities, *i.e.* 4,900 vines·ha⁻¹ (2.4 x 0.85 m²) and 9,800 vines·ha⁻¹ (1.2 x 0.85 m²), each comprising two canopy heights (0.75 and 1.25 m) with the same trunk height (0.6 m), were compared. The experiment was conducted in a split-plot design with four replicates (blocks) for each treatment (two canopy heights and two planting densities). Each replicate included ten vines. The soil of the Pully vineyard is deep and fertile, with a high water holding capacity estimated to exceed 200 mm over a 2-meter soil depth. The annual and monthly precipitation values were reported in ZUFFEREY *et al.* (2012). The climatic data were collected from a weather station located in the same plot used for this experiment (www.meteosuisse.ch).

Leaf-fruit ratio variation: The leaf-fruit ratio was manipulated in two ways (Table). 1. By varying the canopy height (H): two canopy heights (0.75 and 1.25 m) were maintained throughout the season by successive topplings. The first topping was conducted at the end of flowering on day of year (DOY) 182 at 0.75 m canopy height, and 10 d later at 1.25 m canopy height. The shoots

were re-topped every three weeks to maintain the two distinctive canopy heights. 2. By varying the yield: two yield levels were compared by maintaining 1 or 2 fruit clusters per shoot. Cluster dropping was completed when the grapes were pea-sized (DOY 190-200, depending on the year).

Leaf area measurement: Leaf area (LA) was determined non-destructively several times during the growing season in 2000 by measuring the length of two secondary lateral veins of each leaf lamina. Lengths were converted to areas using allometric equations developed from direct area measurements of previously harvested leaves ($n = 200$; $r^2 = 0.96$). All primary and lateral leaves on two shoots per vine (12 shoots per treatment) were measured to estimate the average leaf area per shoot. The average shoot leaf area was used to estimate total vine leaf area by multiplying the shoot leaf area by the number of shoots per vine. The leaf area exposed to saturating light (PPFD > 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was estimated using Carbonneau's method (1995). This estimation takes into account the height of the effective canopy (H), the canopy width (e), the row spacing (E) and the rate (in percentage) of canopy gaps (T), using the formula: Light-exposed leaf area = $[(2 \times H) + e] / [E \times (1-T)]$. The light-exposed leaf area was estimated annually at veraison (BBCH 81). The leaf-fruit or source-sink ratio was estimated using the ratio of the light-exposed leaf area to yield per vine (light-exposed leaf area per kg fruit).

Plant samples, nitrogen and carbohydrate analyses: Samples of one-year-old canes, two-year-old canes (fruit canes), trunks and roots were collected from each vine plant during the main developmental stages of the grapevine following the development scale of LORENZ *et al.* (1994): winter dormancy BBCH 0, budbreak BBCH 11, flowering BBCH 65, veraison BBCH 81, harvest BBCH 91 and leaf fall BBCH 97. At each phenological stage, three replicate vines per treatment (a total of 12 vines on the same block) were mechanically excavated, extracting the maximum possible quantity of roots. Approximately 1 kg of roots of all lengths and diameters were collected, immediately washed and frozen using liquid nitrogen. The roots were then stored at -20 °C. Wood samples were also collected destructively using pruning shears. Approximately 400 g of each of the three wood types (one- and two-year-old canes and trunks) were collected from each vine, frozen using liquid nitrogen, and stored at -20 °C for analysis. All root and wood samples were weighed in the field before freezing to determine their fresh weight and then reweighed prior to cryo-dessication (freeze-drying) at Eurolyo laboratory, Chartres (France). Each freeze-dried sample was finely ground to 1.2 mm.

Nitrogen and carbohydrate analyses: The samples collected in 2000, 2001 and 2002 were analyzed for total organic nitrogen (N) using 500 mg of dried and homogenized material following the Kjeldahl method (Ref. 07M084, 2014, Soil-Conseil laboratory ISO 17025, Changins, Switzerland). Soluble sugars (including glucose, fructose and sucrose) and starch were analyzed enzymatically (kit LISA 200C, CETIM, France) and then measured spectrophotometrically at 340 nm using an ELx800UV automated micro-plate reader (Bio-Tek Instruments Inc., Vermont, USA) as described by GOMEZ *et al.* (2007).

Table

Plant density, row spacing, foliage height and cluster number per shoot of 'Chasselas' grapevines in Pully, Switzerland from 2000-2002

Plant density (vines·ha ⁻¹)	Row spacing (m)	Foliage height (m)	Clusters per shoot
9800	1.2	0.75	1
			2
		1.25	1
			2
4900	2.4	0.75	1
			2
		1.25	1
			2

Yield and fruit composition: All grapes from each vine were harvested separately. The effective yield (kg fruit per vine) and berry weight (based on 50 berries per vine) were measured. Grapes from each vine were crushed separately to quantify the soluble solids content, pH and total acidity: the analytical parameters were measured using the WinScan® at the laboratory in Agroscope. The WinScan® is an instrument based on the Fourier transform infrared spectroscopy that allows the analysis of the major grape quality and wine parameters (FOSS NIRSystems, USA).

Statistical analysis: Duncan test was performed to assess significant differences between treatments (One-Way analysis of variance) using SigmaStat 3.1 (Systat Software, Point Richmond, CA). The relationship between variables was analyzed by simple linear regression and Pearson's coefficient of determination (R^2). *, ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

Results

Seasonal dynamics of nitrogen and carbohydrate reserves: The levels of N reserves were highest during dormancy (BBCH 0), budbreak (BBCH 11) and leaf fall (BBCH 97) in the wood fractions (canes and trunks) and roots (Fig. 1 D-F). The roots contained the highest N reserves among all organs, with maximum values reaching 1.2 % (on a dry weight (DW) basis), which was almost double the N concentration in the trunks and canes. The N concentration measured in the canes and trunks tended to decline after budbreak (Fig. 1 D-E), followed some time later by the roots, and reached the lowest values at flowering and bunch closure in the canes and trunks, and at veraison in the roots. We observed a slight increase in the N concentration of the canes and trunks after bunch closure in 2000, which persisted until post-harvest and even until leaf fall in 2001. During the period from leaf fall until budbreak, the root N concentration tended to increase. The seasonal dynamics of the TNC reserves in the roots (Fig. 1 G - I), trunks and canes were generally similar to those of the N reserves. However, the TNC reserves decreased shortly before budbreak until flowering in the roots and trunks; N reserve mobilization appeared only later in May and continued until post-veraison. The recovery of the TNC reserves occurred mainly from post-flowering until leaf fall.

Influence of the leaf-fruit ratio: The vines with a canopy height maintained at 0.75 m exhibited a leaf area of approximately 2.5 m² from post-flowering to harvest; vines with a canopy height maintained at 1.25 m had a leaf area of 4.5 m² (see results in ZUFFEREY *et al.* 2012). The N concentrations measured in one-year-old (shoots), two-year-old (canes) and older (trunks) wood were similar between the canopy heights (0.75 and 1.25 m) in both seasons (2000 and 2001; Fig. 2 E-F). Moreover, the N reserves in the roots were higher in the vines with a greater leaf area (H = 1.25 m) during the growing season (Fig. 2 G-H). A good correlation ($r^2 = 0.78$ to 0.98) was obtained between the change in leaf area (ΔLA , m² vine⁻¹) and

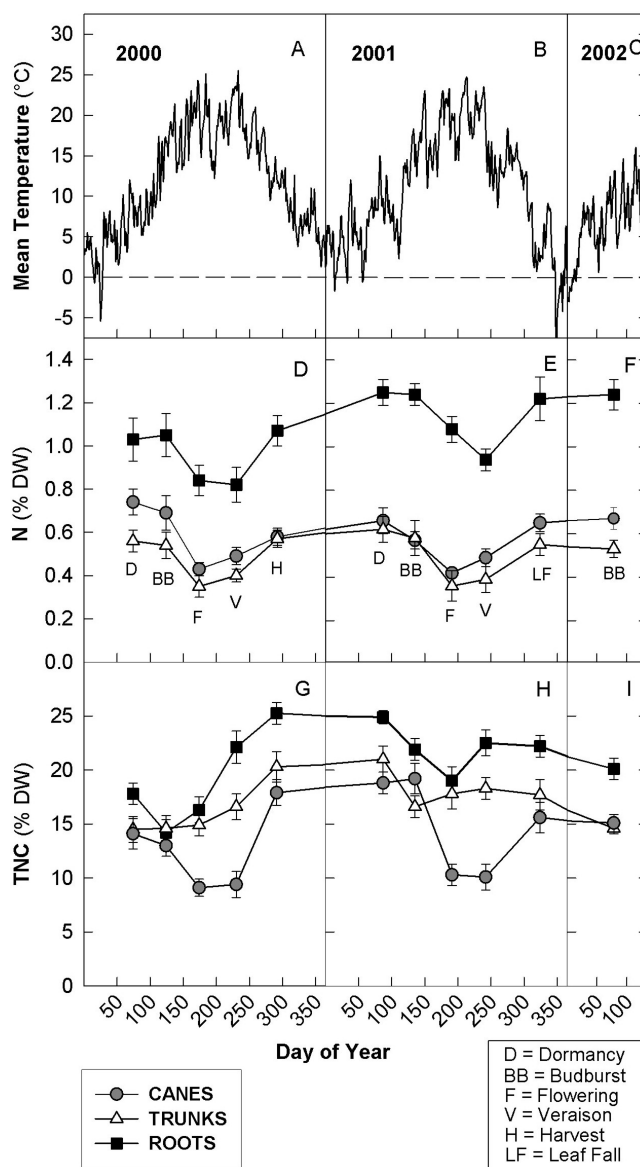


Fig. 1: Mean air temperature (A-C) and seasonal evolution of organic nitrogen (N, D-F) and total non- structural carbohydrate (TNC, G-I) concentrations in the canes (two-year-old canes), trunks and roots of 'Chasselas' grapevines in Pully, Switzerland from 2000 to 2002. The means \pm standard deviation ($n = 12$) are presented. DW: dry weight.

the change in N concentration (ΔN , %) over the time (*i.e.* from budbreak to flowering, flowering to veraison, veraison to leaf fall) in the wood and roots (results not shown) during both years of study. The yield per vine influenced the content of the N reserves measured in the roots at harvest, as shown in Fig. 3. Indeed, the N concentration in the roots tended to decrease with an increase in the yield at harvest. The leaf-fruit ratio (light-exposed leaf area per kg fruit) significantly influenced the N and TNC concentrations measured in the roots at harvest (Fig. 4). An increase in the leaf-fruit ratio resulted in higher soluble solids in the berries, N and TNC concentrations in the roots at the end of the season. The highest N and TNC concentrations were achieved with a leaf-fruit ratio above 2.0 m² per kg fruit. The N and TNC reserves in the roots strongly decreased with a leaf-fruit ratio lower than 1.0 m² per kg fruit.

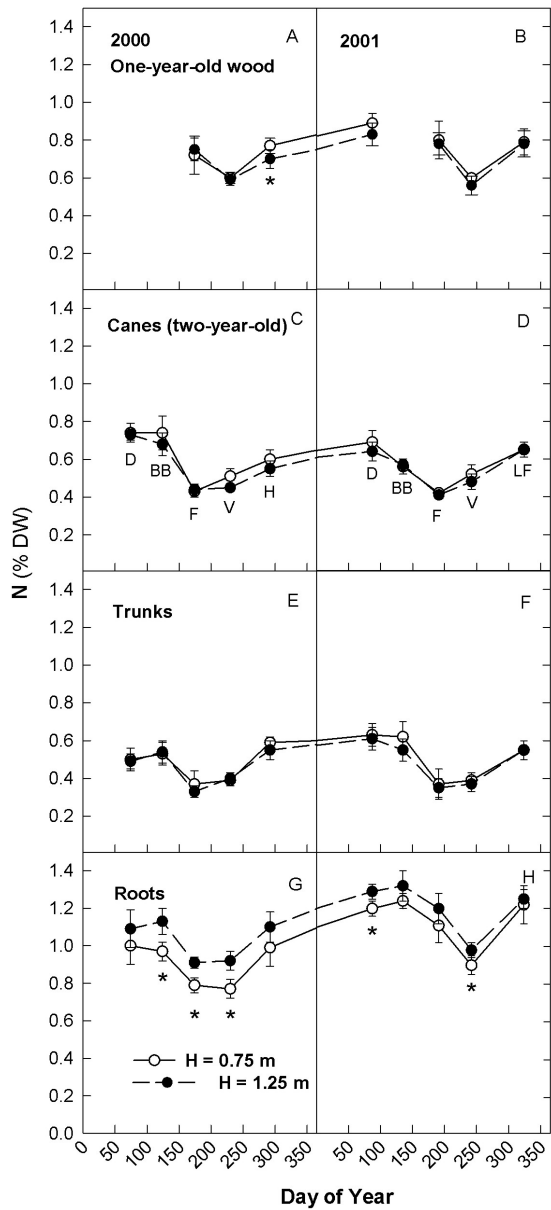


Fig. 2: Seasonal evolution of organic nitrogen concentration (N) in one-year-old wood, canes, trunks and roots for two foliage heights (H = 0.75 m and 1.25 m) of 'Chasselas' grapevines in Pully, Switzerland in 2000 and 2001. The means \pm standard deviation (n = 6) are presented. DW: dry weight. * denotes statistical significance at $p < 0.05$. D: dormancy, BB: budburst, F: flowering, V: veraison, H: harvest, LF: leaf fall.

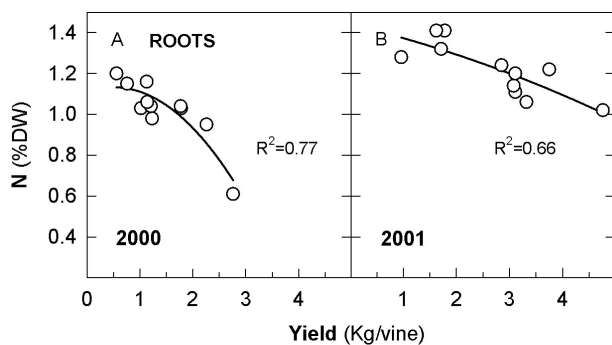


Fig. 3: Influence of crop load (yield per vine) on organic nitrogen concentration (N) measured in the roots at harvest (n = 12) in 'Chasselas' grapevines in Pully, Switzerland, 2000-2001.

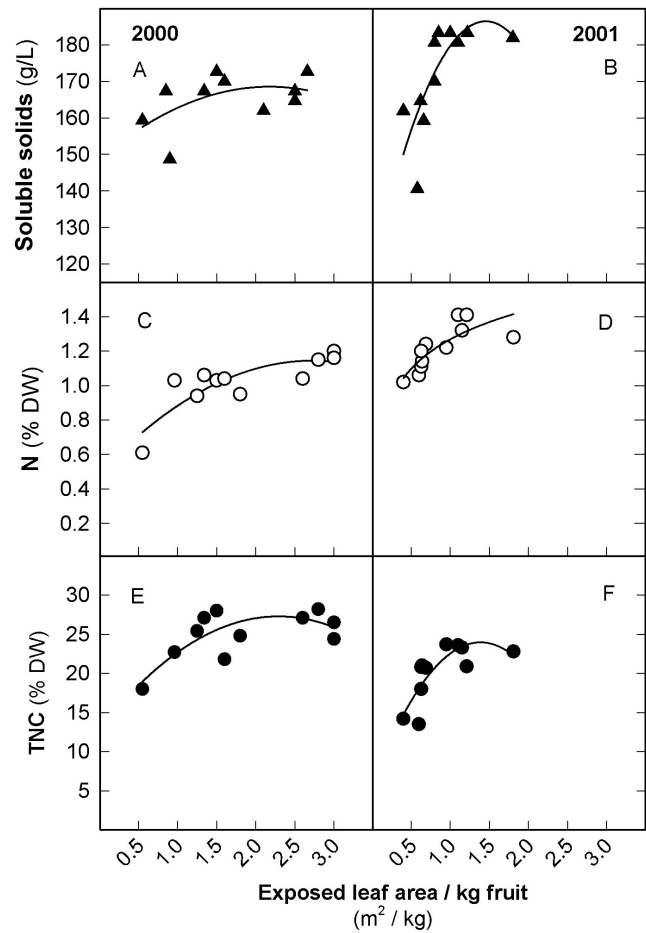


Fig. 4: Influence of the leaf to fruit ratio (light-exposed leaf area per kg fruit) on soluble solids accumulation in the berries, organic nitrogen (N) and total non-structural carbohydrate (TNC) concentration measured in the roots of 'Chasselas' grapevines in Pully, Switzerland, at harvest in 2000-2001.

Discussion

Seasonal N and TNC variations: Seasonal N and TNC dynamics showed that grapevine ('Chasselas') stored N and TNC preferentially in roots but also in all wood fractions. In our study, the highest N concentrations were measured at budbreak in the woody parts, and later in the roots. Reserves stored in perennial plant parts are available as a buffer during periods of low supply and/or high N and TNC demand, especially during spring growth (KELLER 2010).

Indeed, N and TNC reserves are widely used to support leaf area development and root growth at the beginning of the season (KELLER and KOBLET 1995, CHENG *et al.* 2004, HOLZAPFEL *et al.* 2010, PRADUBSUK and DAVENPORT 2010, HOLZAPFEL and SMITH 2012). The N reserve pool used shortly after budbreak usually reaches a minimum level at flowering (LÖHNERTZ *et al.* 1989, SCHALLER *et al.* 1989, ZAPATA *et al.* 2004), which sometimes persists until veraison, confirming the results presented by BATES *et al.*

(2002). Due to strong shoot and root growth during spring, nutrient demand is more important between budbreak and flowering, although soil N uptake occurs mainly after flowering (CONRADIE 1986, LÖHNERTZ 1988, 1989, PEACOCK *et al.* 1989). A limited availability of N and TNC reserves due to incomplete recovery during the previous year may strongly affect not only vegetative growth but also bloom initiation/development and berry set (KELLER and KOBLET 1994, BENETT *et al.* 2005, CELETTE *et al.* 2009, DAYER *et al.* 2013, VAILLANT-GAVEAU *et al.* 2014).

It is difficult to determine the relative N sources during the pre-bloom period (BATES *et al.* 2002); indeed, thin and fine roots that have accumulated N during the early-season root flush become an N storage source as well as an N uptake source during this period. Moreover, the contribution of the N reserves from wood and roots during the period from canopy development until bunch closure may vary greatly in relation to climatic conditions (cool-hot climate), stress conditions (water scarcity) and training systems (pruning level), according to WEYAND and SCHULTZ (2006). Our study highlighted a correlation between changes in leaf area per vine and N concentration in the different organs and tissues (wood and roots) during the season. From budbreak to bunch closure, leaf area development was largely correlated with N reserve mobilization, confirming the observations of WEYAND and SCHULTZ (2006). N reserve accumulation essentially occurred when the plant requirements, such as growth and bunch development, were satisfied, *i.e.* when resource supply exceeded the demand. In fact, N accumulation in the shoots, canes and trunk occurred only after bunch closure (mid-July) and even later in the roots from veraison until leaf fall. Between veraison and leaf fall, nutrient (N, TNC) requirements for vegetative growth tended to decrease, except for the second peak of root N absorption at post-veraison in fruits and wood reserves (LÖHNERTZ 1988, PRADUBSUK and DAVENPORT 2010), which allows the vine to restore N and C reserves in the perennial parts (trunks, roots) (PELLEGRINO *et al.* 2014). In hot climates, soil N taken up from pre-harvest until a few weeks later is directly incorporated in the N reserve pool (CONRADIE 1986). In cool climates, the weather conditions (temperature, precipitation) at the end of the season largely determine the leaf photosynthetic capacity, leaf senescence process and soil N uptake, thus influencing the potential of nutrient accumulation (N, TNC) in wood tissues (BATES *et al.* 2002). In our study, the climatic conditions, especially during fall 2000 (low rainfall and high solar radiation in September-October), and to a lesser extent in 2001, allowed the vine to maintain a high photosynthetic capacity and the possibility to accumulate TNC in roots (ZUFFEREY *et al.* 2012) as well as N in the reserve tissues (canes, trunk, roots). Late in the season, during leaf senescence, N compounds are remobilized from proteins and nucleic acids and are translocated from leaves to perennial organs for storage in vines (CONRADIE 1986, KELLER 2010) or other plant parts (YANG *et al.* 2002). Climatic conditions and plant factors largely determine the N amount (in the form of amino acids or small peptides) transferred from leaves to the trunk and roots (WILLIAMS 1987, LÖHNERTZ *et al.* 1989, SCHALLER *et al.* 1989, CONRADIE 1990).

Leaf-fruit ratio: Our study showed that the leaf area per vine and yield per vine influenced N and TNC reserve recovery in perennial organs (ZUFFEREY *et al.* 2012). Vines with large leaf areas exhibited higher N concentrations in roots throughout the season compared with vines with smaller leaf areas. Nevertheless, no significant difference was observed in the N concentration of the trunks and canes with respect to canopy height. WEYAND and SCHULTZ (2006) also reported that the training system can influence the wood N content, particularly through the leaf area developed by the pruning system. For example, minimal pruned vines whose leaf area is clearly larger than that of conventionally pruned vines accumulate more N in the wood tissues. A higher transpiration rate of vines with a greater leaf area would increase N uptake in the whole plant, provided soil moisture conditions are adequate (no water restriction).

The yield per vine strongly affected the N reserve concentration at harvest. An increase in the crop load primarily decreased the root N content, and to a lesser extent, the trunk N content. The source-sink relationship, expressed as the light-exposed leaf area per kg fruit, clearly influenced the N concentration in the roots at harvest. Root N concentration increased with an increase in the leaf-fruit ratio. Similar observations were noted for root TNC concentration in relation to the source-sink ratio in previous studies (HOLZAPFEL *et al.* 2006, ZUFFEREY *et al.* 2012). Under cool climate conditions, it has been shown that a minimum leaf-fruit ratio, *i.e.* approximately 10-20 cm² of leaf area per g fruit (KLIEWER and DOKOOZLIAN 2005), or 1.0 to 1.4 m² of light-exposed leaf area per kg fruit (MURISIER and ZUFFEREY 1997), was required for adequate grape maturation. It appears that the leaf-fruit ratio also determines the nutrient demand and the distribution of nutrients to reserve organs.

Conclusion

The vines accumulated N and TNC reserves in the perennial organs, mainly in the roots. Important TNC mobilization occurs from budbreak to flowering in the canes, trunk and roots. N mobilization occurs at the same time as that of TNC in the canes and trunk, but occurs later in roots, *i.e.* at bunch closure or even at veraison in some cases. N accumulated in all plant wood fractions from flowering (and from veraison in the roots) until leaf fall. The greatest N reserves were observed in winter during dormancy until pre-budbreak. Vine leaf area as well as yield per vine played a major role in the root N concentration during the season and at harvest. The highest N concentrations were measured in vines with the largest leaf area ($H = 1.25$ m corresponding to 4.5 m² LA per m² of soil) and the lowest yield (approximately 1.0 kg per vine). The leaf-fruit ratio, expressed as the light-exposed leaf area per kg fruit, largely determined the N and TNC concentrations measured in the roots at harvest. The N and TNC concentrations increased with an increase in the source-sink ratio and reached maximum values at 2.0 m² of light-exposed leaf area per kg fruit. The highest berry sugar contents were observed when the leaf-fruit ratio was above 1.5 m² per kg fruit.

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