

The Influence of the Leaf to Yield Ratio on Carbohydrate Reserves in Grapevine *Vitis vinifera* L. 'Qizil Uzom'

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ABSTRACT

Changes in reserves carbohydrates in relation to the leaf to fruit ratio were measured over three years at different grapevine phenological stages in one year old canes, trunks and roots of the cultivar 'Qizil Uzom' in Horticultural Research Station of Kahriz, Urmia, Iran. Soluble carbohydrates represented only a small part (<6.5 % of dry weight) of the total non-structural carbohydrates. In the roots and trunks, the starch content fluctuated during the growing season, reaching the lowest values between bud-break and flowering depending on the year, and the highest values between veraison and leaf fall. The vines maintained at 0.85 m canopy height had a leaf area of around 2.7 m².vine from the end of flowering until harvest, while the vines with a 1.35 m tall canopy had a leaf area of about 4.9 m².vine. The leaf/fruit ratio (source-sink), expressed by the "light-exposed leaf area.kg⁻¹ fruit", not only substantially influenced the soluble sugar content in berries but also the starch and total non-structural carbohydrates concentrations in the trunks and roots at pre-harvest time. Lowest leaf/fruit ratios resulted in decreased starch and total non-structural carbohydrates concentrations in the trunks and roots, which attained the maximum values when the leaf-fruit ratio neared 4.25 m² of light-exposed leaf area.kg⁻¹ fruit. The TSS content in the berries was reduced by 23 % when the leaf-fruit ratio was around 0.5m².kg⁻¹, in comparison to the maximum TSS values with a leaf-fruit ratio at 4.5m².kg⁻¹. A similar relation was observed between the leaf-fruit ratio and starch content in the trunks. Canopy height and leaf area had no predominant influence on the soluble sugars, starch contents, or total non-structural carbohydrates in the permanent vine parts.

Key words: non-structural carbohydrates, carbohydrate reserves, trunk, source-sink ratio.

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INTRODUCTION

The leaf area to yield ratio is one way to quantify vine balance, which will help us determine the management requirements for our vineyard. Much canopy management research suggests that between 2.4 and 5.8 square feet of leaf area per pound of fruit is required for optimum ripeness^{9,27}, though this range may need to be adjusted upward for cool climates like western Oregon and low-yielding cultivars like Pinot noir. The carbohydrates are the building blocks of organic compounds, store energy, and form support structures, such as cellulose, hemi-cellulose, and gluco-protein. It has been shown that carbohydrates mobilized from reserves in perennial parts are used for rapid growth of annual shoots in early spring^{32,38}, including stems, leaves and emerging inflorescences²⁴. At budburst, the winter bud leaves its dormant state and continues its development during spring². At that moment, preformed leaves and inflorescences grow rapidly and their needs in carbohydrates are considerable³². Carbohydrates required for annual development originate from both accumulated reserves in perennial woody organs the year before and photoassimilates synthesized in leaves^{23,24,26,40}. It has been shown that carbohydrates mobilized from reserves in perennial parts are used for rapid growth of annual shoots in early spring^{32,38}, including stems, leaves and emerging inflorescences⁴⁰. Interruption of reserve replenishment has direct impact on reproduction the following year, both the number of inflorescences per plant and the number of flowers per inflorescence being affected^{3,17}. Carbohydrates reserves took part not only in vegetative and reproductive growth and energy requirements (respiration) but also in grapevine defense against biotic¹⁸ and abiotic stress. In grapevine, young developing leaves can perform photosynthesis in early spring²⁵, but they cannot be considered as photoassimilate exporters until they reach 50% of their final size³⁰. This means that carbohydrates accumulated in perennial organs during the previous year support the early development of

annual organs. The opposite sink/source transition in leaves and a root occurs at the onset of flowering during female meiosis^{31,40}. At that time, the sugars required for the developing inflorescences originate from three different sources including reserves and photosynthesis in mature leaves and young green inflorescences^{16,29}. The supply of carbohydrates during key steps of flower formation such as female meiosis is decisive in the achievement of functional flowers and in fruit set^{4,6}. Under cool-climate conditions, it has been shown that a minimum leaf/fruit ratio approaching 10 to 20 cm² leaf-area/g fruit²² or 1.0 to 1.4 m² light-exposed leaf area·kg⁻¹ fruit²⁶ is required for obtaining adequate grape maturation.

Thus, there is a subtle equilibrium between organs accumulating or releasing sugars and those assimilating carbon through photosynthesis during the annual cycle. In grapevine, the influence of the source/sink balance has been investigated^{5,8,19,32,34}. Additionally, leaf area to fruit ratios required for adequate ripening under cool-climate conditions may be between 10 and 20 cm² leaf area/g of fruit²². Murisier²⁶ demonstrated a linear correlation between leaf area to fruit ratio and the formation of carbohydrate reserves in woody parts and roots. Moreover, few studies have focused on both source (defoliation) and sink (fruit removal) limitations on the same cultivar and on their impacts on reproductive parameters and physiological traits^{17,34,37,28}. The aim of this study was to investigate the influence of source/sink alteration at veraison, i.e. berry ripening, on reserve restoration in whole plant and, for the first time, its impact on flowering and fruit set during three successive growing seasons. In this view, the impact of total defoliation and fruit removal was estimated each year by plant weight and non-structural carbohydrate content in entire plant (roots, trunk and two years canes), and description of the reproductive development. The influence of the leaf/fruit ratio on the TNC content in the permanent parts of the grapevine at harvest was also investigated.

MATERIAL AND METHODS

The experiment was conducted between 2008 and 2010 on *Vitis vinifera* L. 'Qizil Uzom' (owen-rooted, planted in 1992) at the Horticultural Research Station Kahriz, Urmia, Iran (45°10'N, 37°35'E). Vines were pruned in a vertical shoot positioning system (cane pruning), including 10 shoots per vine. one planting densities, 1600 vines.ha⁻¹ (2 × 3 m) each comprising two heights of canopy (0.85

and 1.35 m) with trunk height (0.6 m), were compared. The soil of the vineyard in Kahriz is sand-loam, no deep and fertile, with a low water holding capacity estimated to be above 200 mm on 1.5 meters soil depth. Annual and monthly precipitations are reported in Tab. 1. The climatic data were collected from the weather station located in the very plot used for this experiment (www.irimo.ir).

Table 1: Monthly precipitation totals (mm) at the experimental site in Kahriz, Iran, during the three study years in comparison to long-term averages (1990-2010)

Months	Precipitation (mm)			
	2008	2009	2010	Ø 30 years
January	28.2	10.5	17.3	29.3
February	45.6	7.5	25.5	33.2
March	9.1	73.1	60.4	51.5
April	17.2	24.5	93.0	61.3
May	11.1	4.8	78.5	44.3
June	9.2	32.7	3.3	14.2
July	1.7	0.0	0.0	5.5
August	0.6	0.0	27.2	2.4
September	8.3	29.9	0.1	4.7
October	120.8	10.3	11.0	24.3
November	13.8	69.6	0.0	39.6
December	8.4	28.9	12.9	28.6
Annual Total	247	291.8	329.2	338.9

Leaf/fruit ratio was manipulated by canopy heights (0.85 and 1.35 m) and by varying the yield)) maintained throughout the season by successive toppings. The first topping was conducted at the end of flowering on 52-61days after bud burst; the shoots were re-topped every three weeks. Also, two levels of yield were compared by keeping 1 or 2 fruit clusters per shoot. Cluster dropping was

completed when the grapes were pealized on 69-78 days after bud burst), depending on the year. Total leaf area per vine was determined non-destructively several times during the growing season by multiplying leaf area per shoot and total number of shoots per vine. All primary and lateral leaves on two shoots per vine (10 shoots per treatment) were measured to estimate the average leaf area per shoot.

The leaf-fruit or source-sink ratio was estimated using the ratio of light exposed leaf area to crop load per vine (light-exposed leaf area·kg⁻¹ fruit).

Samples of one-year-old canes (fruit canes), trunks and roots were collected from each vine plant during the main development stages of the grapevine following Acimovic *et al.*¹ development scale (winter dormancy, budbreak, flowering, veraison, harvest and leaf fall). At each phenological stage, three vines per treatment (12 vines total) were mechanically excavated, extracting the maximum possible quantity of roots. Approximately 800g of roots of all lengths and diameters were collected, immediately washed and frozen with liquid nitrogen. The roots were then stored at -20 °C. Wood samples were also collected destructively with pruning shears. Approximately 500g of each of the two wood types (one-year-old canes and trunk) was collected from each vine, frozen with liquid nitrogen, and stored at -20 °C for analysis. All root and wood samples were weighed before freezing in the field to determine their fresh weight, and then before freeze-drying at Horticulture laboratory, Azad university of Mahabad (Iran). Soluble sugars (including glucose, fructose and sucrose) and starch were analyzed enzymatically and then measured spectrophotometrically at 340 nm as described by Gomez *et al.*¹⁵.

All grapes from each vine were harvested separately. The effective yield (crop load per vine) and berry weight (on 50 berries per vine) were measured. The total soluble solids (TSS) content of the juice was determined with a digital refractometer (Atago PR-1, Tokyo, Japan) and expressed as percentage³⁶. Titratable Acidity (TA) was measured by titration with 0.1 N NaOH to pH

8.1. The results were expressed as g tartaric acid/100 ml fruit juice. The maturity index was calculated as the TSS/TA ratio¹³.

All computation and statistical analyses were done using IBM® SPSS® Statistics 19, a statistical software (IBM, NY, USA).

RESULTS AND DISCUSSION

Seasonal dynamics of the soluble (sucrose, glucose and fructose) and insoluble (starch) carbohydrates in the roots, trunks and canes revealed that most the storage was in the form of starch (Fig. 1). In the roots, the soluble sugars showed small variations and represented less than 3 % of dry weight (DW) during the season, while the starch content fluctuated between 12 % and 20 % of DW depending on the season. During each year, the root starch progressively increased just before flowering, increasing further between flowering and veraison. The accumulation of root starch continued through harvest until leaf fall, except in 2008. A decrease of starch in the roots was observed just before budbreak, sometimes lasting nearly until flowering. In the trunks and canes, starch was the dominant form of stored carbohydrates during the growing season, but in a lower concentration than in the roots. Conversion of starch into soluble carbohydrates was observed during the winter, coinciding with lower temperatures. The starch level was the lowest around flowering period in the two-year-old cane wood. Soluble carbohydrate concentrations increased from 2-3 % to 7-8 % DW between DOY300 (leaf fall) and DOY 60 (during the dormancy period). The maximum level of TNC reserves was observed at different stages of growth in all parts of the vine, and the minimum level of soluble sugar reserves was observed just before flowering.

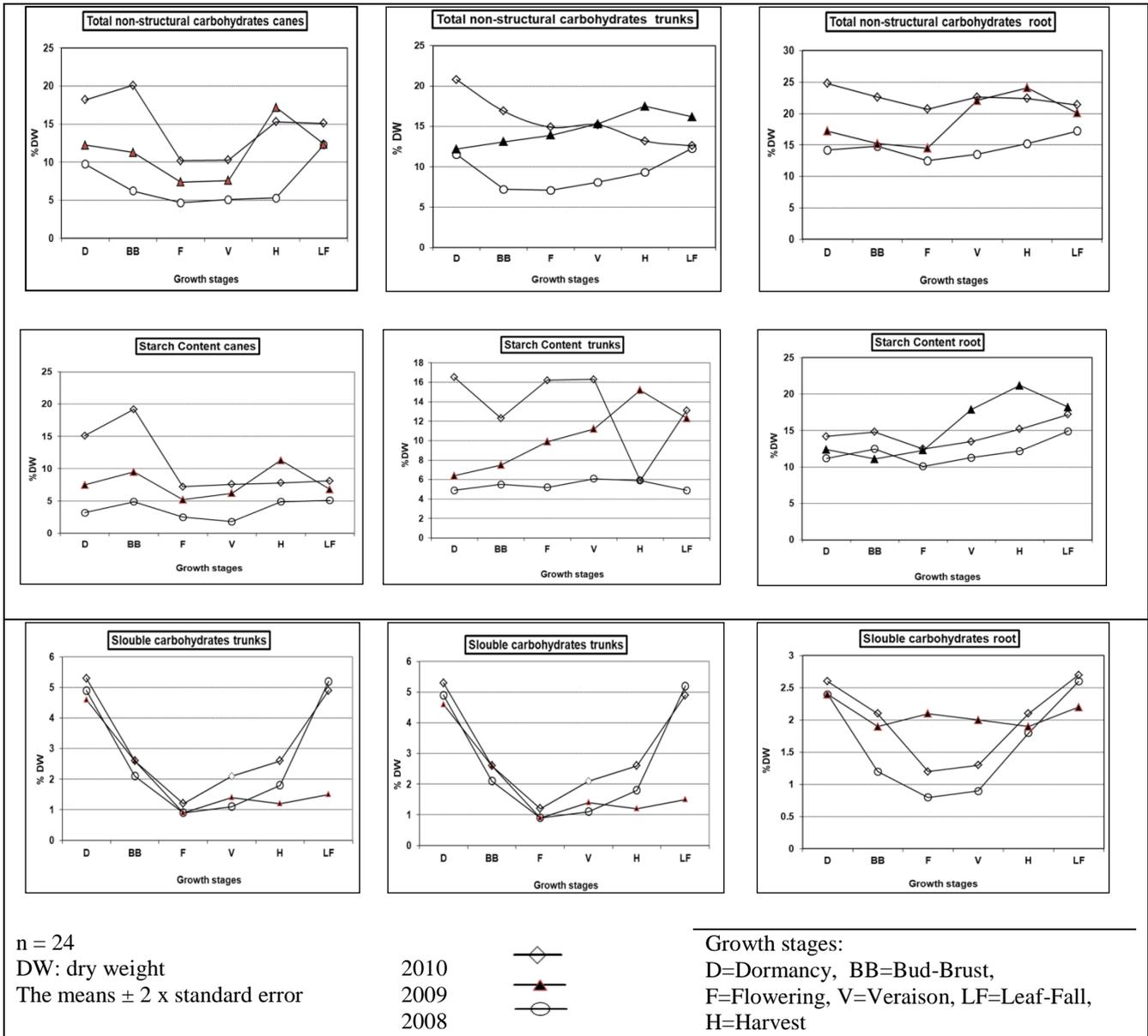


Fig. 1: Growth stages evolution of soluble carbohydrates (sucrose, glucose, and fructose), starch and total non structural carbohydrate (TNC) contents in the canes, trunks and roots of 'Qizil Uzom' grapevines in Urmia, Iran from 2008-2010

Leaf–fruit ratio effect: Fig. 2 represents the seasonal evolution of leaf area per vine in 2010. The vines maintained at 0.85 m canopy height (HC1) had a leaf area of around 2.7

m².vine from the end of flowering until harvest, while the vines with a 1.35 m tall canopy (HC2) had a leaf area of about 4.9 m².vine.

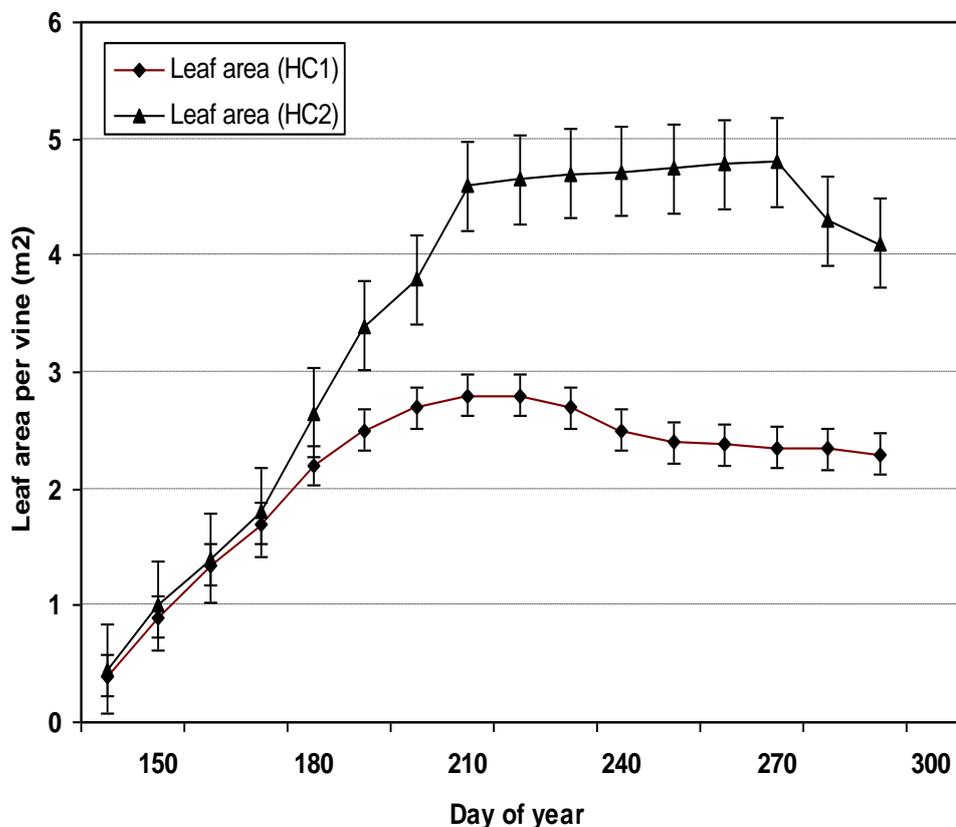


Fig. 2: Seasonal evolution of estimated leaf area per vine during the 2010 growing season for two different foliage heights (H = 0.85 m and 1.35 m) of 'Qizil Uzum' grapevines in Kahriz, Iran in 2010. The means ± 2 x standard error, n = 6.

Neither canopy height nor leaf area had any impact on the TNC content in the canes (Fig. 3 A). However, in the trunks of the 0.85 m canopy vines, the TNC content measured in 2010 was lower in the winter (before budburst)

and at budburst compared to the 1.35 m canopy vines (Fig. 3 B), and it was higher at veraison stage. Also, this result can see in roots (Fig. 3 C).

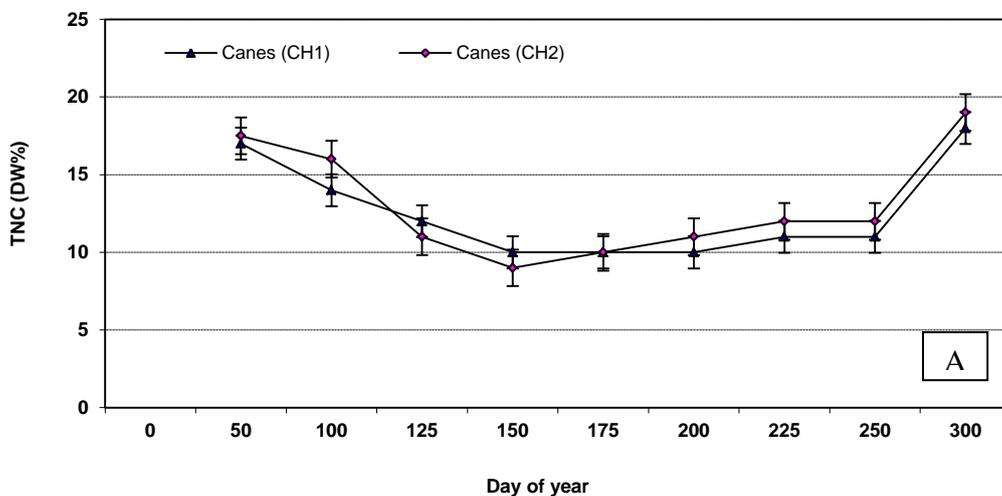
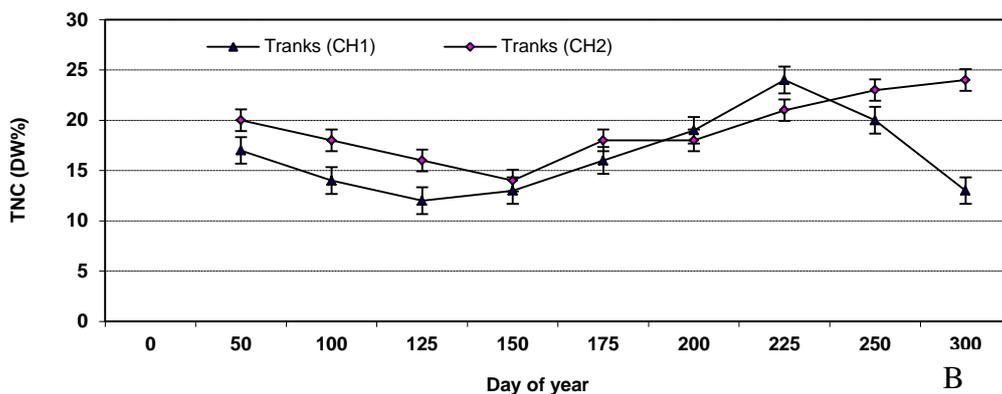
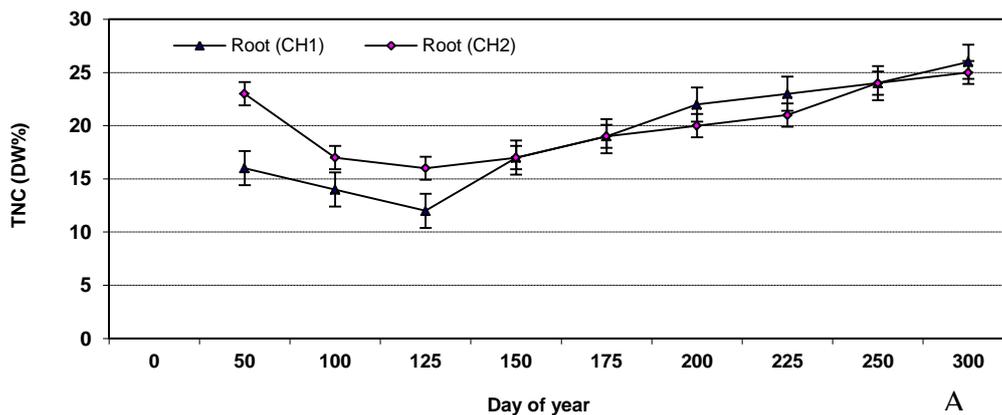


Fig. 3: Seasonal evolution of total non structural carbohydrates (TNC) in the canes, trunks and roots for two foliage heights (CH1=0.85 m and CH2=1.35 m) of 'Qizil Uzom' grapevines in Kahriz, Iran in 2010. The means \pm 2 x standard error, n = 6. DW: dry weight. *denotes statistical significance at $p < 0.05$.

The leaf-fruit ratio (light-exposed leaf area/kg fruit) had a great impact on berry sugar content at harvest during the four years of this study

(Fig. 4). The highest berry sugar contents were observed when the leaf-fruit ratio was above 1.5 m².kg.

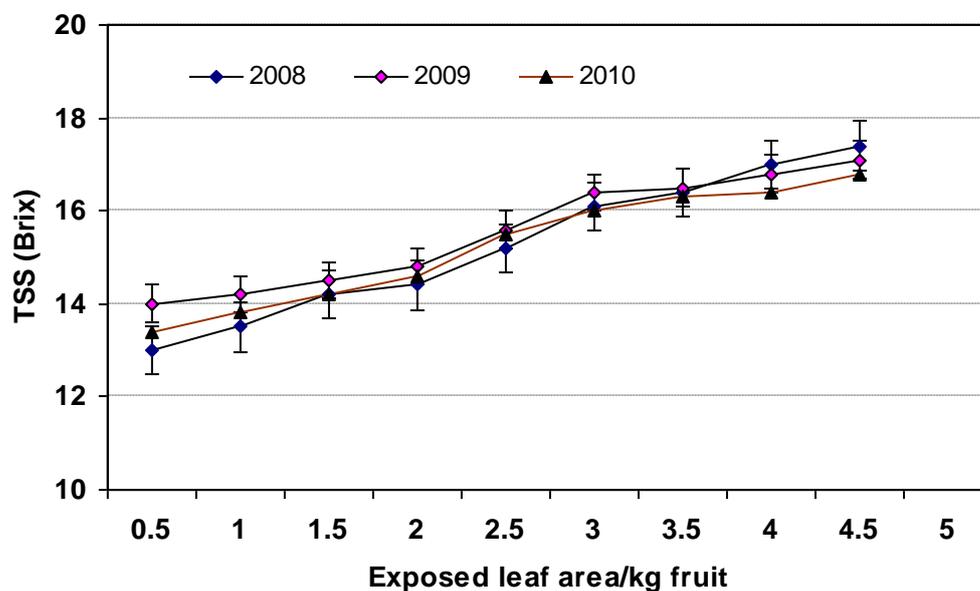


Fig. 4: Influence of the leaf to fruit ratio (light-exposed leaf area/kg fruit (m² . Kg⁻¹) on sugar accumulation in the berries in 'Qizil Uzom' grapevines in Kahriz, Iran from 2008-2010.
TSS: Total solid soluble

The TSS content in the berries was reduced by 23 % when the leaf-fruit ratio was around 0.5 m².kg⁻¹, in comparison to the maximum TSS values with a leaf-fruit ratio at 4.5 m².kg⁻¹

(Fig. 4). A similar relation was observed between the leaf-fruit ratio and starch content in the trunks (Fig. 5).

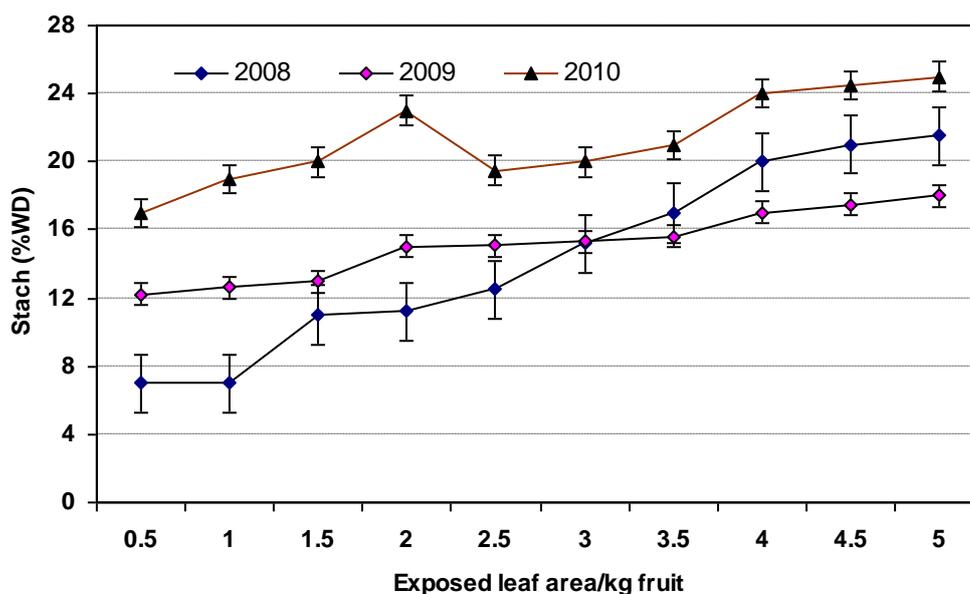


Fig. 5: Influence of the leaf to fruit ratio (light-exposed leaf area/kg fruit (m².Kg⁻¹) on starch accumulation in the trunks in 'Qizil Uzom' grapevines in Kahriz, Iran from 2008-2010. n=6
DW: Dry weight

The seasonal pattern of supply is relatively simple, peaking between bloom and veraison and varying primarily with total light interception. Since light interception depends on leaf area, higher shoot numbers will lead to a more rapid canopy development and an earlier peak in canopy photosynthesis⁴⁰. Seasonal TNC dynamics show that 'Qizil Uzom' stored carbohydrates as starch preferentially in roots but also in all wood fractions such as trunks and canes. Reserves stored in perennial plant parts are available as a buffer during periods of low supply and/or high TNC demand, especially during spring growth^{7,19}. Indeed, TNC reserves are widely used to support leaf area development and root growth at the beginning of the season^{8,17,20,28}. Soluble carbohydrates represented less than 7 % DW of TNC reserves in all grapevine parts, and even less in the roots (< 2 %). These results confirm earlier observations of different grapevine cultivars^{2,11,14,32,34,38,39,40} and on other woody species^{18,24}. In cool climate conditions, maximum TNC values were observed at around 13-16 % DW in dormant grapevines^{2,10,38}. In a hot climate, higher TNC values of above 30 % DW could be observed^{32,34,38}. In this study, intermediate TNC values (10-25 % DW) were observed in the trunks and roots. With 'Qizil Uzom', root starch concentration reached maximum values during dormancy and then started decreasing just before budbreak and continued to drop until flowering. The starch decrease during this period could be partially explained by the root necrosis process^{7,40} and by the loss caused by sap bleeding after pruning^{11,14}. Besides root necrosis and sap bleeding, decline in starch reserves indicate that carbohydrate reserves in roots play a key role in leaf and root growth during the period from budbreak to flowering^{1,26}. The rapid growth of shoots and leaves during this part of the season is mainly due to mobilization of TNC reserves and particularly starch^{2,40}, while photosynthesis covers only a part of growth and energy requirements during this period^{15,19,32}.

May *et al.*²⁵ demonstrated a direct effect of shading on crop development. Treatments with reduced light intensity had smaller bunches and smaller berries, although sugar accumulation was unaffected. Kliewer and Dookozlian²² showed that defoliation treatments reduced berry weight and soluble solids but increased acidity, particularly in the early stages of ripening and that removal of apical leaves had a greater impact than removal of basal leaves.

In trunks and canes, starch was converted into soluble sugars during the winter, improving the wood frost resistance^{16,27}. Because starch is osmotically inactive, it does not improve frost resistance by itself. Thus, grapevine acclimatization requires a conversion of starch stored in phloem and xylem parenchyma cells into soluble sugars (mainly sucrose), which allow the reduction of osmotic potential in the tissues and play the role of cell protectors^{12,19}. This conversion seems to be initiated by autumn temperatures below 5 °C²⁹ and by shorter days, and continues until the middle of winter. The phenomena is still present in this study, but less pronounced as per Keller and Loescher's²⁴ results on sweet berry. The starch content in wood fractions tended to increase between dormancy and budbreak (particularly in 2000) on 'Qizil Uzom', as observed by Weyand and Schultz³⁸ on 'Riesling'. These authors suggested that the starch increase was probably related to assimilation from sugars associated with an increase in ambient temperature before budbreak, which replenished starch depleted for maintenance of respiration in winter³⁹. However, starch content in the one- and two-year-old wood decreased after budburst and reached its minimum value at cluster closure as per Weyand and Schultz³⁸ results and Bates *et al.*². In trunks, however, starch was accumulated from budburst until harvest. Also, In this study, the source-sink relation expressed by the ratio of light exposed leaf area·kg⁻¹ fruit clearly influenced the starch and TNC concentrations in the permanent parts of the grapevines (trunks and roots) at harvest.

The TNC content in the roots strongly diminished when the leaf-fruit ratio was less than $1.5 \text{ m}^2 \cdot \text{kg}^{-1}$. The present results corroborate other studies^{12,17,34} that showed the leaf-fruit ratio as an important factor of the process of carbohydrate refilling in roots before and after harvest. Moreover, these studies reported that higher yielding grapevines were much more dependent on climatic conditions during post-harvest period in terms of TNC reserve replenishment compared to lower yielding grapevines^{11,14}. In cool-climate conditions without water restriction, the observed late-season vegetative growth probably represented a powerful sink on TNC dynamics between veraison and harvest. Indeed, grapevines with greater leaf area ($H = 1.35 \text{ m}$; $\text{LA} \cdot \text{vine}^{-1} = 4.9 \text{ m}^2$) accumulated less starch and less TNC in the trunks and roots around veraison in 2008 and 2010 compared to grapevines with smaller leaf area ($H=0.85 \text{ m}$; $\text{LA} \cdot \text{vine}^{-1} = 2.7 \text{ m}^2$). In this case, C-assimilation in grapevines with greater leaf area ($\text{LA} \cdot \text{vine}^{-1} = 4.5 \text{ m}^2$) was mainly allocated to vegetative growth of secondary shoots, which were more numerous than in the grapevines with smaller leaf area (unpublished data). Nevertheless, TNC reserves at harvest were similar between the two canopy heights ($H=0.85 \text{ m}$ and $H=1.35 \text{ m}$), showing an equilibrium between assimilation, translocation and C-storage in the permanent parts of grapevine. Different canopy manipulation experiments (tipping, leaf removal) performed by Vasconcelos and Castagnoli³⁵ have also illustrated that the leaf-fruit ratio could influence starch and TNC mobilization in the trunks of 'Pinot Noir' during dormancy. After two consecutive years of intensive defoliation (removal of all leaves of primary and secondary shoots), starch content in wood decreased markedly in contrast to the non-defoliated treatment in an experiment performed by Candolfi-vasconcelos and Koblet⁵. Moreover, a positive correlation was reported by these authors between the soluble sugar content in the berries and the starch reserves in the wood, showing that the fruits and wood were both

important sink organs for TNC storage during maturation, depending on the leaf-area per vine³⁰. The present study also showed a strong positive correlation between the starch reserve in the roots and the soluble sugar content in the berries at harvest ($r = 0.85$; $p < 0.01$) and they were dependent on the leaf-fruit ratio. Likewise, Murisier²⁶ also mentioned a positive correlation between the leaf-fruit ratio and TNC content (mainly starch) in the permanent parts of grapevine. In the present study, the low starch and TNC content in roots, measured at the end of the seasons 2009 and especially 2010, could mainly be due to a lower leaf-fruit ratio, in comparison to 2008, which reduced C- assimilation and storage. Another explanation for the lower starch content in roots could be the translocation of sugars from starch mobilization in roots and woody fractions to the berries during the ripeness when the weather conditions are unfavourable (high precipitations, low solar radiation)²⁸. The present results were obtained in well-watered grapevines, favorable to vegetative growth and even excess vigor in some years. The impact of water restriction represents a major factor not only in C assimilation, but also in TNC storage and allocation in the different sink organs of grapevines^{4,13,17}. Future research would be required to study the effects of water stress in relation to the source-sink ratio on TNC reserves by grapevine.

CONCLUSION

The vines accumulated TNC (mostly as starch) reserves in the perennial organs, mainly in the roots. Important TNC mobilization occurs from budbreak to flowering in the canes, trunk and roots. An important mobilization of starch from roots and trunks was observed during the period from budburst to flowering and was related to the decrease of TNC reserves in the same organs. The lowest level of TNC was measured in roots and trunks at flowering time. From flowering until leaf fall, starch accumulated in the roots, trunks and canes. The highest level of TNC was measured at harvest and sometimes even later at leaf fall. The TNC and starch contents in roots were

higher at the end of the season 2010 in comparison to the other years, probably due to the prevailing climatic conditions which were favorable to high canopy photosynthesis. The leaf-fruit ratio expressed by the ratio of light-exposed leaf area per kg fruit strongly determined the starch and TNC contents in the trunks and roots at harvest. The starch and TNC concentrations increased along with the source-sink ratio up to a maximum value of about 2.5 m² of light-exposed leaf area per kg fruit. The root starch content doubled from 12 to 25 % D.W., when the leaf-fruit ratio increased from 0.5 to 2.0 m² of light-exposed leaf area per kg fruit. On the other hand, the relationship between soluble carbohydrates and leaf-fruit ratio was less obvious.

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