Carbohydrate distribution during berry ripening of potted grapevines: Impact of water availability and leaf-to-fruit ratio

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ABSTRACT

Insufficient leaf photoassimilation could allow mobilized carbohydrate reserves to contribute to berry sugar accumulation. However, the extent of this contribution during rapid and slow berry sugar accumulation is undefined. The potential effect of leaf-to-fruit ratio and water availability on carbohydrate reserve distribution in potted Tempranillo grapevines was examined during berry maturation. Within each leaf-to-fruit ratio treatment (full and 50% leaves), vines were grown under full or 50% reduced irrigation regimes. Dry biomass development, and the starch and soluble sugar concentrations were determined in the roots, trunks, stems and leaves. Berry sugar and anthocyanin accumulation were also assessed. Under full irrigation, no starch remobilization from roots was observed, regardless of the leaf-to-fruit ratio. Under reduced water supply, starch remobilization from roots was concurrent with rapid berry sugar accumulation, especially in grapevines with low leaf-to-fruit ratio. Soluble sugar accumulation coincided with starch depletion in the roots of grapevines under reduced water availability. When berry sugar accumulation slowed, an increase in carbohydrates was observed in the roots. Sustained water constraints during rapid berry sugar accumulation resulted in a forced reliance on stored carbohydrates to support berry sugar accumulation, but did not significantly alter the tempo of berry sugar and anthocyanin accumulation. A reduced leaf-to-fruit ratio intensified the reliance of fruit sugar accumulation on stored carbohydrates. Besides the importance of post-harvest carbohydrate reserve replenishment when root carbohydrate reserves are depleted during berry maturation, the reserves are also refilled during maturation when berry sugar accumulation slows. This study showed distinctly that root carbohydrate replenishment could already start a few weeks before harvest, and this replenishment could be important when the post-harvest carbon assimilation period is ineffective.

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1. Introduction

Leaf-to-fruit ratio and vine water status are parameters likely to influence vine carbon balance during berry maturation (i.e. the berry sugar accumulation phase). Abiotic factors such as temperature, light intensity, and water could limit vine carbon assimilation by restricting leaf photoassimilation (Escalona et al., 1999), while reduced leaf-to-fruit ratios, up to a point, could result in an increase of leaf photosynthetic activity (Candolfi-Vasconcelos and Koblet, 1991). However, the importance of the contribution of root carbohydrate reserves to support berry sugar accumulation under differing leaf-to-fruit ratios and grapevine water status is still a research question.

Carbohydrates are synthesized by plants through leaf photosynthesis and the effect of the abiotic factors in association with the vine internal competition for carbon, can affect the dynamics of non-structural carbohydrate reserve storage within the grapevine (Holzapfel and Smith 2012). These reserves are distributed to the different plant organs, and the concentration and partitioning within different organs vary throughout the growing season. The distribution of carbohydrates could be affected by soil water availability (soil depth, root implementation and functioning) and soil

temperature (Dayer et al., 2013; Field et al., 2009; Rogiers et al., 2011a).

Studies have shown that grapevines with higher crop load, and those subjected to elevated water constraint, exhibit reduced carbohydrate reserve concentrations at budburst the following season. Water constraints during the growing season (pre-dawn leaf water potential values below −0.6 MPa), and high crop loads (leaf-to-fruit ratios below 8 cm^2 leaf area per gram of fruit), have been reported to cause reduced starch concentrations in grapevine trunks during the dormancy period (Dayer et al., 2013). Furthermore, defruiting at the onset of fruit ripening increases total non-structural carbohydrate (TNC) concentration in the roots in subsequent seasons, while a complete defoliation at harvest reduces it (Smith and Holzapfel 2009). No previous studies, to the best of our knowledge, have however investigated the potential effect of the interaction between water availability and leaf-to-fruit ratio on the distribution of non-structural carbohydrate content between the different grapevine organs, during the berry sugar accumulation phase.

Non-structural carbohydrates provide energy and carbon for grapevine growth, and/or are stored as reserves in perennial plant organs. The stored carbohydrates are used for early season vegetative growth until leaf photosynthesis becomes the primary source of carbon, generally around flowering for the grapevine (Zapata et al., 2004). Carbohydrate reserves are also utilized towards the reproductive development, including supporting berry sugar accumulation, as confirmed by ^{14}C tracing studies (Candolfi-Vasconcelos et al., 1994). Woody tissues, especially perennial roots, also start to accumulate carbohydrates from anthesis, and the crop load influences the continuation of the perennial reserve accumulation during grape maturation (Holzapfel et al., 2010). Due to the involvement of carbohydrate reserves in these various functions, strong competition is expected to exist between the different sinks from véraison (berry softening) and the end of berry sugar accumulation (Davies and Robinson 1996; Wang et al., 2003a).

Carbohydrates are mainly stored as starch in grapevine roots, and this starch can be hydrolyzed to form soluble sugars. During dry conditions, the activity of starch-degrading enzymes, such as α-amylase, is often found to increase in plant tissues, resulting in starch breakdown, and an increase in soluble sugar concentrations (Jacobsen et al., 1986; Li and Li 2005). The ratio of starch to soluble sugars has been reported to decrease in grapevine perennial organs during water constraints (Rogiers et al., 2011b), as well as following early (Bennett et al., 2005) or late season (Smith and Holzapfel 2009) defoliation.

Similar to sugar accumulation, fruit anthocyanin accumulation also commences at véraison, and normally continues throughout berry maturation (Boss et al., 1996). The accumulation of anthocyanins in the berries is an important contributor to the quality of the fruit from a wine quality perspective. The grapevine water status is one of the major factors known to affect sugar (Wang et al., 2003b) and anthocyanin (Ojeda et al., 2002) accumulation in ripening berries.

The aim of this study was to investigate the interactive effects of leaf-to-fruit ratio and vine water status during the berry sugar accumulation stage on carbohydrate partitioning in perennial and annual grapevine organs. Although starch and/or soluble sugar concentrations at certain stages of the annual grapevine growth cycle (mainly at dormancy, budburst or harvest) have been predominantly reported for the roots and trunks, the kinetic of whole-vine TNC content distribution during the berry sugar accumulation phase is still a research question. The first goal was to determine the combined effect of water constraint and limited leaf-to-fruit ratio on the TNC allocation to perennial and vegetative organs during the berry sugar accumulation phase. The second goal was to quantify the contribution of remobilized starch reserves towards berry sugar content when whole vine leaf photoassimilation becomes

insufficient for sink demands during berry maturation. The last goal was to investigate how the accumulation of fruit sugar and anthocyanins responds when a greater reliance is placed on the starch reserves to support berry sugar accumulation. Experiments were conducted on grapevines grown in large pots, allowing the analysis of whole grapevines and individual organ biomass, including the whole root systems, where carbohydrate distribution was determined as affected by the different treatments (leaf-to-fruit ratio and water availability).

2. Materials and methods

2.1. Experimental design and treatments

Forty own-rooted *Vitis vinifera* L. cv Tempranillo (clone D8V12) grapevines were used in the 2013/2014 growing season, planted in commercial potting mix soil in 50 L pots. The grapevines were grown in an outside bird proof cage in the warm to very warm climate of the Riverina region (Wagga Wagga, New South Wales, Australia). The three-year-old grapevines were spur pruned to four two-bud spurs in the winter, left with eight primary shoots each, and distributed in four rows of ten vines each, with a three-wire trellis system installed to support the vegetative growth. At fruit set, the total amount of bunches and berries per vine were counted, and vines were crop thinned just after fruit set so that all grapevines were left with six to seven bunches, totaling 400 berries per vine. Prior to the application of the treatments at the onset of véraison (very first sign of berry softening), four randomly selected vines, one per row, were destructively harvested in order to represent T$_0$ for the population of grapevines. After removal of the four initial vines through destructive harvesting, the nine remaining vines per row were evenly spaced out in the row, resulting in a four row by nine column array, containing three treatment replicates. Two irrigation treatments, two defoliation treatments, and three destructive harvest dates were randomized in the block design. Pressure compensated drip emitters (4 L/hr each) were used for irrigation during the experiment. Rainfall, atmospheric temperature, and relative humidity were recorded and collected from an on-site weather station and vapor pressure deficit (VPD) was calculated. Environmental conditions were summarized for three fortnightly intervals during the experiment, referred to as intervals 1, 2 and 3.

In order to study the interaction between either low or high leaf-to-fruit ratios, and either low or high water availability throughout the berry sugar accumulation phase, four distinct treatments were applied, i.e., low leaf-to-fruit ratio and low water availability (LowL/F:50%); low leaf-to-fruit ratio and high water availability (LowL/F:100%); high leaf-to-fruit ratio and low water availability (HighL/F:50%); high leaf-to-fruit ratio and high water availability (HighL/F:100%). Vines with a low leaf-to-fruit ratio were left with 50% less leaves than those with a high ratio (40 vs 80 leaves). Every second leaf from the base of each shoot was removed until each vine had the desirable amount of leaves. Vines were irrigated three times a day (0730, 1400 and 1800 h), with equal water volume applied each time of the day, ranging between 12 and 20 min of irrigation application time per irrigation event. The higher water availability treatment was conducted with the aim of watering pots each day just to the point of first visual free draining during the midday irrigation, via two irrigation emitters located to the left and right of a vine near the middle of each pot. In the lower water availability treatment, 50% of the water was delivered over the same period, through one irrigation emitter in the middle of the pot. Vines of both leaf-to-fruit ratio treatments received the same water volume within each irrigation treatment. Secondary shoots (laterals), and any newly formed leaves from primary shoots during the course of

the experiment, were removed daily as soon as the regrowth was observed.

At the fortnightly destructive harvest dates, i.e., véraison (27 Dec 2013, i.e., V), V + 14 (10 Jan 2014), V + 27 (23 Jan 2014) and V + 40 (5 Feb 2014), the pre-selected grapevines were dismantled. Whole root systems, trunks, spurs, stems, petioles, and leaves were separated, collected and washed with phosphate-free detergent and rinsed with deionized water. Leaves were collected in the morning between 0800 and 1000 h on each of the destructive harvest dates. The fresh weights of these organs were determined, and the samples were oven dried at 60 °C until a constant dry weight was reached.

2.2. Leaf-to-fruit ratio and berry composition

The total leaf area of all the leaves that were sampled from each individual vine at the respective destructive harvest dates was measured using a leaf area meter (LLI-3100C, LI-COR Biosciences Inc., Lincoln, Nebraska, USA). The total fruit weight of each grapevine was also recorded, and the leaf-to-fruit ratios determined. A 50 berry subsample per vine was oven dried at 60 °C until constant weight, and total vine fruit dry weight determined. The average soluble solid content per berry per vine was determined in a subsample of 50 representative berries, on the basis of berry fresh weight and juice soluble solid concentration (°Brix).

Berry anthocyanin concentration was analyzed from a 50 berry subsample per vine. The whole berries were homogenized (Ultra-Turrax T25, IKA, Selangor, Malaysia), and the phenolic compounds extracted from 1 g homogenate in 10 mL 50% ethanol (pH 2) for two hours. The samples were centrifuged at 3000 rpm for 10 min, and 1 mL supernatant was added to 9 mL HCl and left for 3 h at room temperature. The absorbance was measured at 520 nm (μQuant universal spectrophotometer MQX200, Bio-Tek, Winooski, VT, USA), to determine berry total anthocyanin concentrations (Iland et al., 2000).

2.3. Grapevine water status and leaf gas exchange

Weekly measurements of soil water content were made directly under an irrigation emitter in the midday period (between 1200 and 1400 h), using a time-domain reflectometry (TDR) probe (Trime®-FM3, Imko GmbH, Ettlingen, Germany). Measurements of stem water potential (SWP) were started one week after the treatments were initiated, and undertaken once a week, with a single leaf removed per vine per measurement to minimize the impact on vine leaf-to-fruit ratio. Following the method outlined by Choné et al. (2001), one leaf from each vine on a main shoot was enclosed in a zip-lock plastic bag covered with aluminum foil, and left covered for 30 min in the midday period (between 1200 and 1400 h) to allow stomatal closure. The leaves were then removed by a single cut of the petiole using a sharp blade, the bags removed, and the leaves were immediately placed in a pressure chamber for measurement (Model 1000, PMS instruments, Albany, Oregon, USA).

A portable photosynthesis system instrument (LCA-4, ADC Bio-scientific Ltd., Hoddesdon, Hertfordshire, UK) was used to measure comparison values of leaf temperature, stomatal conductance (g_s) and photosynthesis rates (A). Two fully intact leaves were chosen weekly on each vine between the 4th and 7th shoot node position from the base, to be used for measurements during midday periods (between 1200 and 1400 h) on clear, non-cloudy days.

2.4. Non-structural carbohydrates

Whole, dried plant material, collected during the destructive harvest dates (roots, trunks, spurs, stems, petioles and leaves), were ground through a heavy duty cutting mill (Retsch SM2000,

Hann, NRW, Germany) to 5 mm and a subsample was then ground through a 0.12 mm sieve using an ultracentrifugal mill (Retsch ZM200, Hann, NRW, Germany).

Following the method outlined in Smith and Holzapfel (2009), the starch concentration was determined in a 20 mg subsample by a commercial enzymatic assay (K-TSTA, Megazyme International, Bray, Ireland). In short, soluble solids were first extracted using three 1 mL portions of 80% (v/v) aqueous ethanol, two at 80 °C and one at room temperature for ten minutes. The extracts were centrifuged after each wash, the supernatants collected together, and then used for later soluble sugar analysis. The remaining dried plant material was suspended in 200 μL dimethyl sulfoxide and heated at 98 °C for 10 min. Starch was then hydrolyzed with α-amylase (30 units) and amyloglucosidase (33 units), and the starch content was calculated from the concentration of released glucose in the sample.

For the soluble sugar analyses, the combined extracts prepared during the starch analyses were diluted to 10 mL with deionized water, and used for determination of the concentrations of sucrose, D-glucose, D-fructose and total sugars, with a commercial enzymatic assay (K-SUFRG, Megazyme International, Bray, Ireland), as outlined in Smith and Holzapfel (2009). In this method, each sugar was converted into glucose-6-phosphate (G6P), and quantification of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) was performed, following oxidation in the presence of nicotinamide-adenine dinucleotide phosphate (NADP+) and G6P-dehydrogenase.

2.5. Statistical analysis

Data were analyzed using Statistica 12 (Statsoft Inc., Tulsa, OK, USA), with the analysis of variance (ANOVA) used to test the significance of each variable. Fisher's least significant difference (LSD) test was used to identify significant differences between means ($P < 0.05$). Significant differences in table columns and rows are indicated by upper case and lower case letters, respectively.

3. Results

The fortnightly periods between the four destructive harvest dates are referred to as intervals 1, 2 and 3. Intervals 1 and 2 represent the rapid berry sugar accumulation period, while interval 3 represents the slow berry sugar accumulation period.

3.1. Environmental conditions

The average daily temperature and vapor pressure deficit (VPD), and the total rainfall data collected during each interval of the experiment are shown in Table 1.

3.2. Overview of non-structural carbohydrate assimilation and allocation

For all treatments, the soluble sugar content (SSC) per berry initially accumulated significantly between V and V + 27 (rapid accumulation), and slowed down between V + 27 and V + 40, when there were no significant changes in berry SSC (slow accumulation) (Fig. 1). The tempo (mg/berry/day) of berry sugar accumulation did not differ significantly between the treatments during both, the rapid and slow berry sugar accumulation periods (Fig. 1).

The leaf stomatal conductance (g_s) of all treatments decreased significantly between intervals 1 (V to V + 14) and 2 (V + 14 to V + 27) for all treatments, while leaf photoassimilation rates (A) also significantly decreased during this stage, except for treatment LowL/F:100% (Fig. 1B). Further reduction in g_s took place during interval 3 (slow berry sugar accumulation) for grapevines under

Table 1
Periodic rainfall, daily mean, minimum and maximum atmospheric temperature and vapor pressure deficit (VPD) averages, and berry juice soluble solid concentration (°Brix) during the experimental period.

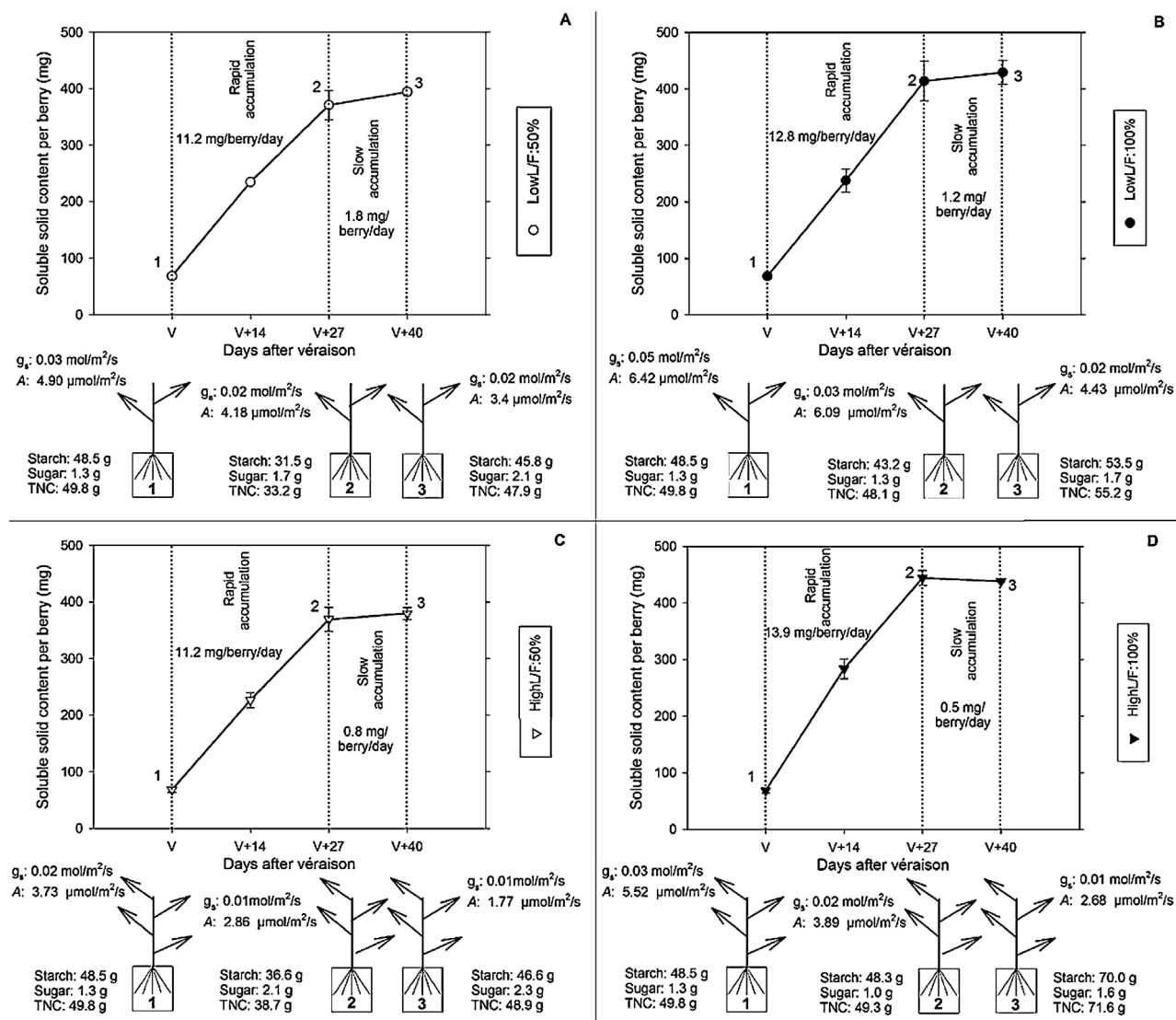|		V to V + 14 (Interval 1)	V + 14 to V + 27 (Interval 2)	V + 27 to V + 40 (Interval 3)
Temperature (°C)	Daily mean	22.9	29.6	28.0
	Mean min	15.6	21.8	20.1
	Mean max	29.3	36.7	35.0
VPD (kPa)	Daily mean	2.1	3.3	2.9
	Mean min	0.8	1.5	1.1
	Mean max	3.4	5.4	4.9
Rainfall (mm)	Total	1.9	1.2	14.8
Total soluble solids (°Brix)	Mean change	7.3–15.4	15.4–21.6	21.6–23.2

Fig. 1. Effects of the different irrigation and leaf-to-fruit ratio treatments (A: LowL/F:50%, B: LowL/F:100%, C: HighL/F:50% and D: HighL/F:100%) on the soluble solid content accumulation per berry, leaf stomatal conductance (g_s) and photosynthesis (*A*), and root starch, soluble sugar and total non-structural carbohydrate (TNC) content evolution per vine (n = 3).

higher water availability, while *A* also reduced significantly for all treatments except LowL/F:50% (Fig. 1A), during slow fruit sugar accumulation.

Under higher water supply, the root starch content per vine was not significantly affected during rapid berry sugar accumulation (Fig. 1B + D). However, under reduced irrigation, significant depletion in root starch content occurred during rapid berry sugar

accumulation (Fig. 1A + C). During slow berry sugar accumulation, the starch content in these roots increased significantly, and back to their initial levels at V. The root soluble sugar content of treatment HighL/F:50% increased significantly during rapid berry sugar accumulation, and was significantly higher than that of vines with high water availability at V + 27 (Fig. 1C). The root sugar content

of treatment LowL/F:50% showed a similar increasing trend during rapid berry sugar accumulation (Fig. 1A) (P = 0.07).

Reduced irrigation induced significant root TNC content depletion during rapid berry sugar accumulation (Fig. 1A + C). These TNC contents then increased significantly during slow berry sugar accumulation.

3.3. Soil water content and vine water status

Reduced water supply induced significantly lower soil water content (Table 2). However, the leaf-to-fruit ratio treatments also affected the soil water content of vines that received more irrigation, where the high leaf-to-fruit ratio significantly caused 30% lower average soil water content compared to the treatment with lower leaf-to-fruit ratio, despite receiving the same water volume. Under reduced irrigation, the leaf-to-fruit ratio did not significantly impact the soil water content.

The stem water potential (SWP) was significantly affected by the irrigation regime and the defoliation treatments (Table 2). Higher water supply resulted in less negative SWP values, and corresponded to a moderate to weak overall grapevine water constraint according to published classification thresholds (Van Leeuwen et al., 2009); however, higher leaf-to-fruit ratio treatments showed more negative SWP values. During interval 2 (V + 14 to V + 27), SWP values became significantly more negative for treatment lowL/F:100%. Reduced water supply resulted in a moderate to severe grapevine water constraint, according to previously suggested thresholds (Van Leeuwen et al., 2009).

3.4. Leaf and fruit structural development

Vines with a high leaf-to-fruit ratio lost 19 and 24% of their leaf area between V and V + 40 under higher and lower irrigation supply, respectively (Fig. 2A). No significant differences in leaf area among the different destructive harvests were observed for grapevines with low leaf-to-fruit ratio, irrespective of the irrigation regime.

Total fresh fruit weight per vine increased significantly from V to a maximum at V + 27 for all treatments, except HighL/F:100%, where the maximum fresh weight was observed at the end of interval 1 (V + 14) (Fig. 2B). After the initial increase in fresh weight, total fresh fruit weight decreased for all treatments to varying degrees (significantly for treatments with high leaf-to-fruit ratio), with grapevines from treatment HighL/F:50% showing the largest total fresh fruit weight loss towards the end of interval 3 (25% of overall decrease). There was no significant difference in leaf-to-fruit ratios at the final destructive harvest date (V + 40) (Fig. 2C).

3.5. Leaf gas exchange

Leaf stomatal conductance (g_s) and photosynthesis rates (A) were significantly affected by both water availability, and grapevine leaf-to-fruit ratio (Table 2). Grapevines generally exposed to the highest seasonal water constraints, according to the SWP values (treatment HighL/F:50%), showed the lowest g_s and A. Under both irrigation regimes, the low leaf-to-fruit ratio treatments showed significantly higher g_s and A values than the corresponding high leaf-to-fruit ratio treatments. Treatment LowL/F:100% resulted, on average, in 34% higher A values than treatment HighL/F:100%, while treatment LowL/F:50% resulted in 43% higher A values than treatment HighL/F:50%. Vines from treatment LowL/F:100% showed the highest A, and treatment HighL/F:50% the lowest A (P < 0.05).

The average mid-day leaf temperature per interval was constantly measured above 35 °C during the experiment (Table 2).
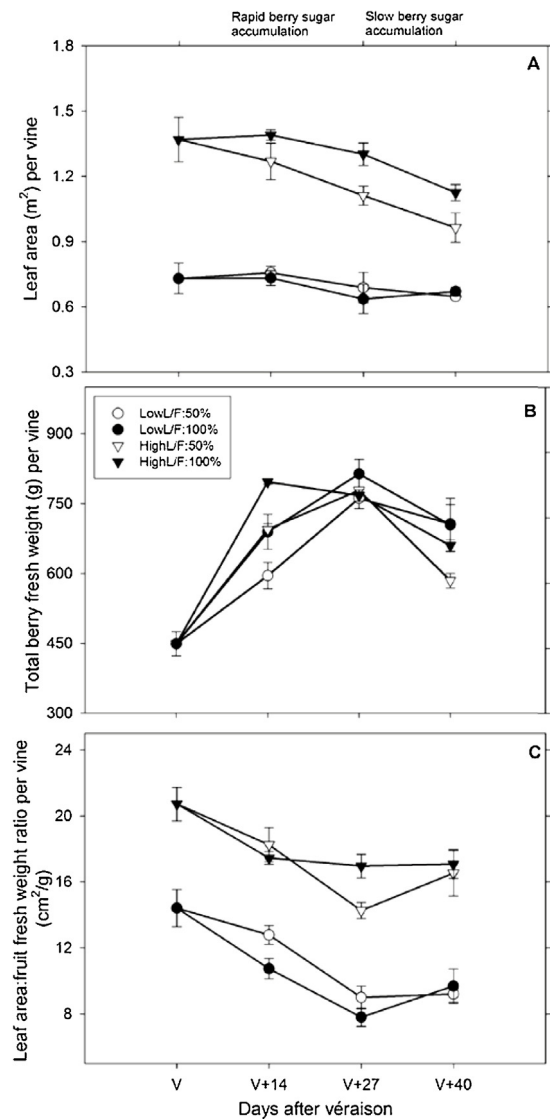

Fig. 2. Effects of the different irrigation and leaf-to-fruit ratio treatments on total grapevine leaf area (A), total berry fresh weight per vine (B), and leaf-to-fresh fruit weight ratio per vine (C) (mean ± SE; n = 3).

3.6. Berry composition

Treatment HighL/F:50% induced significantly lower berry soluble solid content (SSC) at V + 40 than the grapevines under high water availability, furthermore, the berries of treatment LowL/F:50% had significantly lower SSC than treatment HighL/F:100% at V + 40 (Fig. 3A). The berry juice soluble solid concentrations at V + 40 ranged from 22.3°Brix for treatment LowL/F:100% to 24.6°Brix for treatment HighL/F:100%, but did not significantly differ between any of the treatments at this stage (data not shown).

Seasonal berry dry weight evolution followed a similar pattern than that observed with berry sugar accumulation (Fig. 3A). Rapid berry dry weight increase was observed during rapid berry sugar accumulation (V to V + 27), with a slower berry dry weight increase during slow fruit sugar accumulation (V + 27 to V + 40). The average fresh weight per berry at V + 40 ranged from 1.62 g for treatment HighL/F:50% to 1.93 g for treatment HighL/F:100%, and the differences were significant between these treatments. There were no other significant differences in fresh weights per berry between any treatments (data not shown).

Table 2
Average leaf temperatures during the different intervals of the experiment, and the influence of different irrigation and leaf-to-fruit ratio treatments on soil water content, stem water potential, and leaf stomatal conductance and photosynthesis rates.

	Treatment	V to V+14	V+14 to V+27	V+27 to V+40	Treatment main effect
		Rapid berry sugar accumulation		Slow berry sugar accumulation	
Leaf temperature (°C)	Average	35.1	36.0	35.8	–
Soil water content (%)	LowL/F:50%	C [a] 9.0 a [b]	B 9.2 a	B 9.0 a	C 9.08
	LowL/F:100%	A 14.7 a	A 16.6 a	A 14.6 a	A 15.32
	HighL/F:50%	C 7.9 a	B 8.9 a	B 8.8 a	C 8.40
	HighL/F:100%	B 10.8 a	B 10.7 a	B 10.3 a	B 10.68
Stem water potential (-MPa)	LowL/F:50%	C 1.21 a	BC 1.34 a	B 1.24 a	C 1.26
	LowL/F:100%	A 0.66 a	A 0.97 b	A 0.87 a	A 0.80
	HighL/F:50%	D 1.38 ab	C 1.57 b	B 1.32 a	D 1.43
	HighL/F:100%	B 1.00 a	AB 1.15 a	A 0.93 a	B 1.04
Stomatal conductance (mol/m²/s)	LowL/F:50%	B 0.03 a	B 0.02 b	B 0.02 b	B 0.02
	LowL/F:100%	A 0.05 a	A 0.03 b	A 0.02 c	A 0.04
	HighL/F:50%	C 0.02 a	C 0.01 b	C 0.01 b	C 0.01
	HighL/F:100%	B 0.03 a	B 0.02 b	BC 0.01 c	B 0.02
Photosynthesis rate (μmol/m²/s)	LowL/F:50%	B 4.90 a	B 4.18 b	B 3.40 b	B 4.39
	LowL/F:100%	A 6.42 a	A 6.09 a	A 4.43 b	A 5.94
	HighL/F:50%	C 3.73 a	C 2.86 b	C 1.77 c	C 3.06
	HighL/F:100%	B 5.52 a	B 3.89 b	B 2.68 c	B 4.44

[a] Means separated within columns using Fisher's LSD test, significant differences are indicated at P < 0.05. Where the same capital letter appears in a column, values do not differ significantly.

[b] Means separated within rows using Fisher's LSD test, significant differences are indicated at P < 0.05. Where the same lower case letter appears in a row, values do not differ significantly.
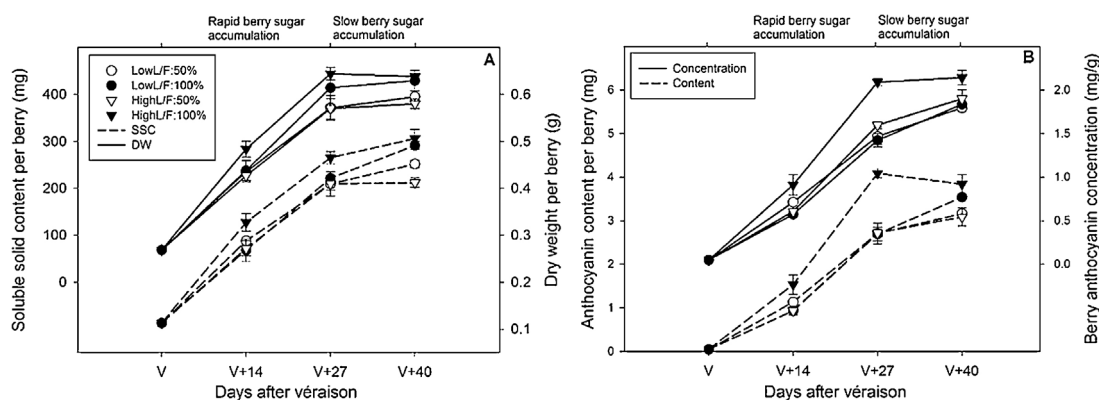

Fig. 3. Impact of irrigation and leaf-to-fruit ratio on A: soluble solid content (SSC, left axis) and dry weight (DW, right axis) per berry, and B: anthocyanin content per berry (left axis) and berry anthocyanin concentration (right axis) during the experimental period (mean ± SE; n = 3).

The berry anthocyanin concentration increased significantly for all treatments during intervals 1 and 2, when rapid berry sugar accumulation also took place (Fig. 3B), and continued to increase significantly during interval 3 for all treatments, except HighL/F:100%. At V+27, the fruit of treatment HighL/F:100% had significantly higher anthocyanin concentration than that of all other treatments. The total anthocyanin content per berry increased significantly during rapid berry sugar accumulation, and especially accumulated rapidly for treatment HighL/F:100% during interval 2 (Fig. 3B). The anthocyanin content per berry continued to increase significantly during interval 3 for treatment LowL/F:100%. At V+40, the anthocyanin content per berry did not differ significantly between the treatments, although the berries of treatment HighL/F:100% tended to have higher anthocyanin content than that of treatments HighL/F:50% (P = 0.06) and LowL/F:50% (P = 0.08).

3.7. Non-structural carbohydrates

Whole vine (excluding the fruit) and perennial tissue TNC content evolution is represented in Fig. 4. In Table 3, organ-specific starch and soluble sugar concentrations are reported, while organ-specific total dry biomass and TNC content development is reported in Table 4.

3.7.1. Whole-vine and perennial TNC content

The TNC content for the combined perennial and vegetative seasonal organs (total vine TNC) (Fig. 4A) was reflective of the overall perennial tissue TNC content (Fig. 4B). Under higher water availability, the total vine and perennial tissue TNC content did not significantly change during rapid berry sugar accumulation (V to V+27), but increased significantly during slow berry sugar accumulation (V+27 to V+40) when vines also had a high leaf-to-fruit ratio. Under lower water availability, total vine and perennial TNC contents reduced significantly during rapid berry sugar accumulation (Fig. 4). This was especially obvious in vines with low leaf-to-fruit ratio, where both TNC contents decreased by 30%. The decrease was most pronounced during interval 2 (V+14 to V+27), and especially for vines with low leaf-to-fruit ratio. During slow berry sugar accumulation, significant TNC replenishment took place, regaining the initial TNC contents observed at V.

Table 3
Influence of different irrigation and leaf-to-fruit ratio treatments on the starch and sugar concentrations in the roots, trunks, stems and leaves (% DW) at the various destructive harvest dates (n = 3).

			Rapid berry sugar accumulation		Slow berry sugar accumulation			Rapid berry sugar accumulation		Slow berry sugar accumulation	
		Treatment	V	V + 14	V + 27	V + 40		V	V + 14	V + 27	V + 40
Starch%	Roots	LowL/F:50%	25.3 a	B[a] 20.7 b[b]	B 18.4 b	B 25.1 a	Trunk	7.1 ab	A 9.3 a	B 6.2 b	A 8.2 ab
		LowL/F:100%	25.3 a	A 26.8 a	A 25.3 a	AB 29.3 a		7.1 a	A 8.6 a	A 9.3 a	A 9.5 a
		HighL/F:50%	25.3 a	AB 22.7 ab	B 18.9 b	B 24.7 a		7.1 a	A 8.0 a	B 6.7 a	A 8.1 a
		HighL/F:100%	25.3 b	AB 25.5 b	AB 20.3 b	A 34.0 a		7.1 b	A 7.4 b	A 10.2 ab	A 11.9 a
Sugar%		LowL/F:50%	2.5 b	B 2.4 b	AB 2.9 ab	A 3.8 a		1.5 a	BC 1.3 a	A 1.6 a	A 2.3 a
		LowL/F:100%	2.5 b	B 1.7 c	B 2.3 bc	A 3.1 a		1.5 a	C 0.8 c	B 0.9 bc	A 1.4 ab
		HighL/F:50%	2.5 b	A 3.1 ab	A 4.0 a	A 4.0 a		1.5 b	A 2.1 a	A 2.0 ab	A 1.7 ab
		HighL/F:100%	2.5 b	B 2.4 b	B 2.4 b	A 3.4 a		1.5 a	AB 1.6 a	AB 1.4 a	A 1.4 a
Starch%	Stems	LowL/F:50%	3.4 b	A 4.8 a	B 3.8 ab	B 4.7 a	Leaves	0.14 a	C 0.06 b	B 0.06 b	B 0.15 a
		LowL/F:100%	3.4 b	A 4.7 a	B 4.7 a	B 4.8 a		0.14 a	AB 0.10 a	B 0.14 a	B 0.22 a
		HighL/F:50%	3.4 b	A 4.6 ab	B 3.5 b	B 5.4 a		0.14 a	BC 0.05 b	B 0.06 b	B 0.14 a
		HighL/F:100%	3.4 c	A 5.5 b	A 6.7 ab	A 8.0 a		0.14 b	A 0.12 b	A 1.32 a	A 0.55 ab
Sugar%		LowL/F:50%	2.1 a	A 1.9 a	A 2.2 a	B 1.9 a		4.9 a	A 2.6 b	B 2.4 b	A 3.6 ab
		LowL/F:100%	2.1 ab	B 1.2 b	B 1.2 b	AB 2.8 a		4.9 a	A 2.6 b	AB 2.9 b	A 3.3 b
		HighL/F:50%	2.1 b	A 2.5 a	A 2.5 a	AB 2.4 ab		4.9 a	A 2.7 b	AB 3.0 b	A 3.2 b
		HighL/F:100%	2.1 b	A 2.0 b	A 2.2 b	A 3.1 a		4.9 a	A 3.4 b	A 4.4 ab	A 3.6 b

[a] Means separated within columns using Fisher's LSD test, significant differences are indicated at P < 0.05. Where the same capital letter appears in a column, values do not differ significantly.

[b] Means separated within rows using Fisher's LSD test, significant differences are indicated at P < 0.05. Where the same lower case letter appears in a row, values do not differ significantly.

Table 4
Influence of different irrigation and leaf-to-fruit ratio treatments on the dry biomass of total fruit per vine, and the dry biomass and total non-structural carbohydrate (TNC) content per organ.

			Rapid berry sugar accumulation		Slow berry sugar accumulation	
		Treatment	V	V + 14	V + 27	V + 40
Biomass (g)	Fruit	LowL/F:50%	55.0 c	B[a] 106.2 b[b]	B 170.4 a	AB 179.7 a
		LowL/F:100%	55.0 c	B 118.4 b	AB 185.6 a	AB 180.7 a
		HighL/F:50%	55.0 d	B 118.9 c	AB 182.5 a	B 161.0 b
		HighL/F:100%	55.0 c	A 157.0 b	A 196.3 a	A 194.0 a
Biomass (g)	Roots	LowL/F:50%	191.5 a	A 197.6 a	B 171.0 b	A 181.8 ab
		LowL/F:100%	191.5 a	A 168.7 a	B 182.0 a	A 182.6 a
		HighL/F:50%	191.5 a	A 190.5 a	AB 192.7 a	A 183.2 a
		HighL/F:100%	191.5 b	A 163.3 b	A 236.1 a	A 207.2 ab
TNC content (g)		LowL/F:50%	49.8 a	A 42.2 a	A 33.2 b	B 47.9 a
		LowL/F:100%	49.8 a	A 44.2 a	A 48.1 a	B 55.2 a
		HighL/F:50%	49.8 a	A 45.1 a	A 38.7 b	B 48.9 a
		HighL/F:100%	49.8 b	A 42.9 b	A 49.3 b	A 71.6 a
Biomass (g)	Stems	LowL/F:50%	54.7 a	AB 69.2 a	A 58.2 a	A 63.6 a
		LowL/F:100%	54.7 a	B 55.8 a	A 70.8 a	A 67.9 a
		HighL/F:50%	54.7 a	AB 61.1 a	A 55.2 a	A 57.4 a
		HighL/F:100%	54.7 b	A 77.9 a	A 74.8 ab	A 77.4 a
TNC content (g)		LowL/F:50%	3.3 a	AB 4.6 a	B 3.5 a	B 4.2 a
		LowL/F:100%	3.3 a	B 3.3 a	B 4.2 a	B 5.0 a
		HighL/F:50%	3.3 b	B 4.2 ab	B 3.3 b	B 4.5 a
		HighL/F:100%	3.3 c	A 5.9 b	A 6.6 ab	A 8.6 a
Biomass (g)	Trunk	LowL/F:50%	84.7 a	A 106.7 a	A 81.8 a	A 93.3 a
		LowL/F:100%	84.7 a	AB 94.5 a	A 87.8 a	A 99.6 a
		HighL/F:50%	84.7 a	B 78.7 a	A 86.3 a	A 85.4 a
		HighL/F:100%	84.7 a	AB 97.9 a	A 94.5 a	A 95.9 a
TNC content (g)		LowL/F:50%	7.3 b	BC 11.4 a	A 6.4 b	A 9.8 ab
		LowL/F:100%	7.3 b	C 9.0 ab	B 8.8 ab	A 10.8 a
		HighL/F:50%	7.3 a	A 7.9 a	A 7.5 a	A 8.4 a
		HighL/F:100%	7.3 c	AB 8.8 bc	AB 11.0 ab	A 12.6 a
Biomass (g)	Leaves	LowL/F:50%	0.7 b[c]	AB 0.9 a	A 1.0 a	A 1.0 a
		LowL/F:100%	0.7 b	AB 0.9 a	A 0.9 a	A 1.0 a
		HighL/F:50%	0.7 b	B 0.8 a	A 0.8 a	B 0.8 a
		HighL/F:100%	0.7 b	A 1.0 a	A 0.9 a	AB 0.9 a
TNC content (g)		LowL/F:50%	0.03 a[c]	A 0.02 a	B 0.02 a	A 0.04 a
		LowL/F:100%	0.03 a	A 0.02 a	AB 0.03 a	A 0.04 a
		HighL/F:50%	0.03 a	A 0.02 a	AB 0.02 a	A 0.03 a
		HighL/F:100%	0.03 b	A 0.04 ab	A 0.05 a	A 0.04 ab

[a] Means separated within columns using Fisher's LSD test, significant differences are indicated at P < 0.05. Where the same capital letter appears in a column, values do not differ significantly.

[b] Means separated within rows using Fisher's LSD test, significant differences are indicated at P < 0.05. Where the same lower case letter appears in a row, values do not differ significantly.

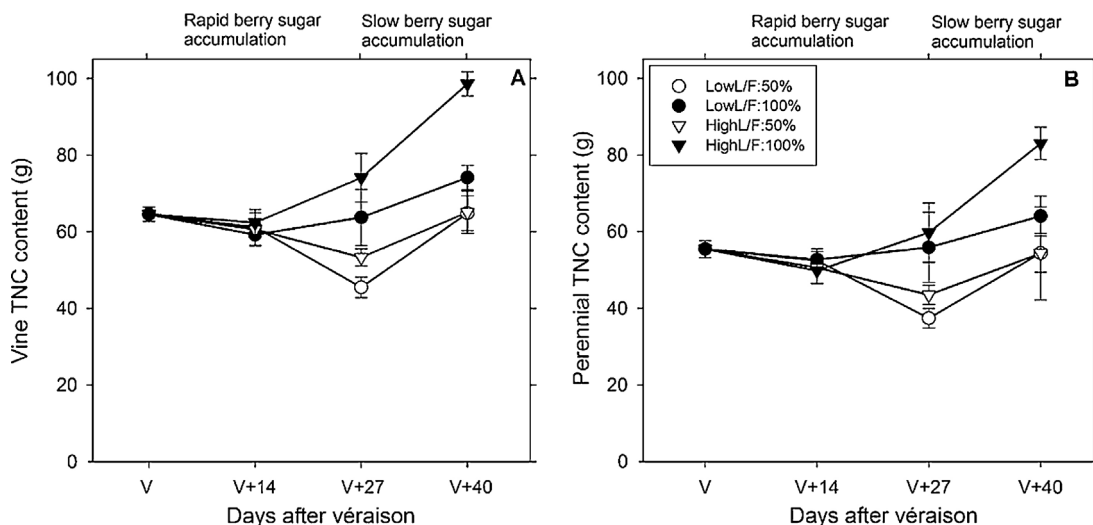[c] Leaf biomass and TNC content indicated as per leaf per vine.

Fig. 4. Total non-structural carbohydrate (TNC) content per vine in combined grapevine roots, trunks, spurs, shoots, leaves and petioles (A) and in total perennial tissues (B), as affected by the irrigation and leaf-to-fruit ratio treatments (mean ± SE; n = 3).

3.7.2. Starch and soluble sugar concentration distribution

Under higher water supply, the total root starch concentration was not significantly affected during rapid berry sugar accumulation (V to V + 27) (Table 3). However, the root starch concentration did significantly increase by V + 40 for the high leaf-to-fruit ratio treatment. Under reduced irrigation, the root starch concentrations of treatments with low and high leaf-to-fruit ratio reduced significantly by 27 and 25% respectively, during rapid berry sugar accumulation. During slow berry sugar accumulation (V + 27 to V + 40), the starch concentrations in these roots increased significantly. The root soluble sugar concentration of treatment HighL/F:50% increased significantly during rapid berry sugar accumulation, and were significantly higher than that of vines with high water availability at V + 27 (Table 3).

The trunk starch concentration of treatment HighL/F:100% was significantly higher at V + 40 than at V (Table 3). The trunk soluble sugar concentrations reduced significantly during rapid berry sugar accumulation for treatment LowL/F:100%, and was significantly lower than that of the reduced irrigated grapevines at V + 27 (Table 3).

In the stems, vines with a higher water availability exhibited a significant increase in starch concentration during rapid berry sugar accumulation, and this resulted in significantly higher stem starch concentration for treatment HighL/F:100%, compared to the other treatments at V + 27 (Table 3). All treatments showed significantly higher stem starch concentrations at V + 40 than at V. Stem soluble sugar concentration increased significantly for treatment HighL/F:50% during rapid berry sugar accumulation (Table 3).

The leaf starch concentrations for treatment HighL/F:100% increased significantly during rapid berry sugar accumulation (Table 3), and this treatment also induced significantly higher leaf starch concentrations at V + 40 than any of the other treatments. The leaf starch concentration of vines receiving reduced irrigation depleted significantly during rapid berry sugar accumulation. The leaf soluble sugar concentration also reduced significantly for all treatments, except treatment HighL/F:100% during rapid berry sugar accumulation (Table 3).

3.7.3. Organ dry biomass and TNC content

The total fruit dry weight per vine increased significantly for all treatments during rapid berry sugar accumulation (V to V + 27) (Table 4). The total fruit dry weight per vine remained constant during slow berry sugar accumulation for all treatments, apart from

treatment HighL/F:50% where it significantly reduced. At V + 40, treatment HighL/F:100% had significantly higher total fruit dry weight than treatment HighL/F50%.

The total root dry weight decreased significantly during rapid berry sugar accumulation for vines of treatment LowL/F:50%, while treatment HighL/F:100% induced significantly larger root dry weights at V + 27 than both of the low leaf-to-fruit ratio treatments (Table 4). Reduced irrigation induced significant root TNC content depletion during rapid berry sugar accumulation (Table 4). At V + 40, treatment HighL/F:100% exhibited significantly higher root TNC content than any other treatments.

The trunk total dry weights did not significantly change during the experiment for any of the treatments (Table 4). The trunk TNC content of treatment HighL/F:100% increased significantly during rapid berry sugar accumulation (Table 4).

The total stem dry weight of treatment HighL/F:100% was significantly higher at V + 40 than at V, but stem dry weights did not change significantly during the experiment for any of the other treatments (Table 4). Stem TNC contents increased significantly for treatment HighL/F:100% during rapid berry sugar accumulation (Table 4).

The dry weight per leaf increased significantly between V and V + 14 for all treatments, and remained constant thereafter (Table 4). Both low leaf-to-fruit ratio treatments had significantly higher dry weight per leaf than treatment HighL/F:50% at V + 40. The TNC content per leaf of treatment HighL/F:100% increased significantly during rapid berry sugar accumulation.

4. Discussion

To study the contribution of non-structural carbohydrate (TNC) reserves towards berry dry matter accumulation, two distinct treatments of leaf-to-fruit ratio in combination with two vine water supply regimes were implemented at véraison (onset of berry softening). The reduced water supply and/or leaf area treatments were aimed to reduce canopy photoassimilation enough so as for berry sugar accumulation to rely on remobilized stored carbohydrates from the perennial structure.

The relationship between the tempo of berry sugar accumulation and root TNC content, in potted grapevines subjected to moderate to severe water constraints, has been illustrated in the present study. Although it has previously been confirmed by ^{14}C tracing studies that root carbohydrates can be relocated towards

the berries during berry sugar accumulation (Candolfi-Vasconcelos et al., 1994), this is to the best of our knowledge, the first study to indicate an inverse relationship between the contents of root TNC and berry sugar, when leaf photoassimilation is limited during rapid fruit sugar accumulation. The clear replenishment of root TNC as the berry sugar accumulation tempo slowed down, is an additional original result. The roots, therefore, became a complementary source of TNC to supply towards the sink TNC demand of maturing fruit.

When comparing the TNC contents and concentrations between the roots, trunks, stems and leaves: the roots had the highest values, and the root TNC content represented, on average, 73% of the total TNC content in these organs. The net loss of combined root, trunk, stem and leaf TNC content (Fig. 4A) during rapid berry sugar accumulation, in grapevines under reduced water supply, can be attributed to root starch depletion. In fact, root starch remobilization accounted for 89% of the whole-vine (excluding the fruit) TNC loss during rapid berry sugar accumulation in treatment LowL/F:50%. An apparent hydrolysis of root starch took place during rapid fruit sugar accumulation in the reduced irrigated grapevines, corresponding to the soluble sugar accumulation in these roots. The starch hydrolysis was induced by a sugar deficit, prompted by the fruit (temporary TNC sink) sugar demand outweighing the leaf (TNC source) photoassimilate supply (Eveland and Jackson, 2012). Soluble sugars are transported from the roots in the phloem, and TNC can thereby be mobilized from the roots to the berries, contributing to the sink TNC demand. Further investigation is, however, needed to quantify the absolute amount of sugars relocated from the roots toward the fruit during rapid berry sugar accumulation.

The net loss of TNC in the roots, trunks, stems and leaves during rapid berry sugar accumulation, and in grapevines under moderate to severe water constraints, potentially contributed up to 18 and 10% to total fruit dry biomass accumulation per vine for treatments LowL/F:50% and HighL/F:50%, respectively. As there were no significant biomass increases for any other organs in grapevines from these treatments during this period, it implies that significant amounts of stored TNC were unlikely used towards the structural development of other organs. While not quantified in the present study, it must however also be noted that some of the TNC could have been lost through respiration, although the whole-vine respiration rate is likely reduced under limited water availability (Escalona et al., 2012). In addition, the amount of carbon lost through respiration in relation to the total pool of carbohydrates is also thought to be very limited, as previously illustrated in grapevine berries (Romieu et al., 1992). It, therefore, seems likely that root reserve TNC made a significant contribution to the berry sugar content for the grapevines that received reduced irrigation, to compensate the limited leaf assimilation (Candolfi-Vasconcelos et al., 1994). To further clarify the relative contribution of root respiration to the change in TNC content during berry ripening, future studies could include the determination of root respiration rates.

When berry sugar accumulation slowed down, starch accumulated in the roots as the berry carbohydrate sink strength was reduced. The content in TNC and especially starch, at the end of berry maturation is an indicator of the starch reserve availability at budburst for the following season (Smith and Holzapfel 2009). The reserve TNC at budburst is utilized for early season vegetative and reproductive growth and development. Previous studies suggest that low carbohydrate reserve content at budburst is detrimental towards vegetative growth (Loescher et al., 1990), inflorescence and flower initiation and development, fruit set, and overall fruit yield (Bennett et al., 2005; Smith and Holzapfel 2009). It is, therefore, probable that the vegetative and reproductive development of grapevines from treatments with low water availability could be affected in the following season, especially if further reserve

accumulation is impaired due to a short post-harvest period. More work is however needed to quantify the post-harvest recovery of root TNC content following the depletion thereof during berry sugar accumulation.

The leaf-to-fruit ratio at the final destructive harvest date (V + 40) of grapevines with low leaf-to-fruit ratios, was found to be within a range (8–12 cm^2 leaf area per gram of fruit) estimated to, in a comparison of grapevines with a wide range of leaf-to-fruit ratios in a given climatic region, allow the maximum accumulation of berry soluble solids, as well as maximum development of berry fresh weight and skin anthocyanins, on a single canopy trellis-system (Kliewer and Dokoozlian, 2005). However, when using potted vines, the present study indicates that the water status of grapevines with the same leaf-to-fruit ratios can significantly impact on the berry soluble solid content (SSC) of mature fruit, as grapevines under reduced water supply and a high leaf-to-fruit ratio had inferior berry SSC than those under higher water supply, and the same leaf-to-fruit ratio. Nevertheless, the consistent pattern and tempo of berry sugar content accumulation between the treatments, and the lack of significant treatment differences in berry anthocyanin content and soluble solid concentration (°Brix) at V + 40, suggests that no treatment caused an inhibition of berry sugar import or skin anthocyanin biosynthesis. Water stress can cause alterations in the expression of genes involved in regulating plant sugar transportation between source and sink organs (Williams et al., 2000), as well as those involved in berry skin anthocyanin biosynthesis (Castellarin et al., 2007). A sustained water stress, for example, causes an up- or down-regulation of the genes encoding hexose transporters in grapevines (Medici et al., 2014), and may thereby affect the tempo of berry sugar accumulation. Likewise, water stress can inhibit or promote anthocyanin biosynthesis in grapevine berries (Ojeda et al., 2002). Because there were no significant alterations in the tempo of berry sugar accumulation and no significant differences in the berry anthocyanin content of mature berries in the present study, it can be assumed that the reduced water supply treatments (treatments lowL/F:50% and HighL/F:50%) induced a sustained water constraint rather than a stress. The water constraints, therefore, affected leaf photosynthesis (A), although not altering berry ripening in terms of sugar and anthocyanin accumulation.

Increased water availability and decreased leaf-to-fruit ratio improved mid-day leaf gas exchange rates during the present study. Although leaf stomatal conductance (g$_s$) and A declined as the experiment progressed, the gas exchange rates in the present study were determined by the treatments, rather than the variation in atmospheric temperatures and vapor pressure deficits (VPD) during the different intervals of the experiment. The gas exchange rates recorded in the present study, especially for grapevines under moderate to weak water constraints, were however, relatively low in comparison to values reported for field-grown Tempranillo grapevines (Medrano et al., 2003). Constantly high atmospheric VPD, and air and leaf temperatures during the midday periods when these measurements were conducted could attribute to these low values. Another contributor to the low gas exchange values is the fact that the scheduling of the irrigation events caused the soil moisture content in the pots to presumably reach its lowest volumes at the time of the day when these measurements were conducted, and thereby promoted stomatal closure. Furthermore, the measurements were conducted late in the growing season, and on older leaves towards the basal parts of the shoot, when leaf aging likely already impaired maximum leaf gas exchange rates (Poni et al., 1994).

Based on the observations from the present study, it is, however, important to note that limitations in whole-vine photoassimilation during berry ripening is thought to be overcome through TNC remobilization from storage tissues (Candolfi-Vasconcelos et al., 1994).

Under moderate to weak water constraints, no net whole-vine TNC remobilization was observed throughout the experiment (Fig. 4A), indicating sufficient canopy photoassimilation. However, under moderate to severe water constraints, reserve TNC remobilization took place during rapid berry sugar accumulation, suggesting that the net photoassimilation of these grapevines was insufficient to meet sink demands during berry maturation. The reduced leaf area of the low leaf-to-fruit ratio grapevines likely suppressed canopy transpiration, causing lower water constraints compared to that of the grapevines with full leaf area. However, the combination of reduced leaf area and low water supply induced the greatest suppression of canopy photoassimilation, resulting in the highest relative remobilization of TNC from the roots of treatment LowL/F:50%. This indicates that under limited water supply during rapid berry sugar accumulation, a higher leaf-to-fruit ratio could be beneficial towards maintaining root carbohydrate reserves prior to harvest. This would be important when a post-harvest reserve accumulation period is absent or insufficient, as often observed, for example, in cooler climate areas.

5. Conclusion

The effects of water availability and grapevine sink-source relations on carbohydrate reserve storage by dormancy, expressed on a concentration basis, have been studied previously. However, a novel approach was undertaken in the present study to investigate carbohydrate content distribution, specifically during the active berry sugar accumulation phase, and to quantify the contribution of carbohydrate reserves towards berry sugar accumulation. Moderate to severe water constraints resulted in less carbohydrate allocation to the perennial grapevine organs, although not altering the evolution of berry sugar and anthocyanin accumulation. Carbohydrate reserves were remobilized in reduced-irrigated grapevines to contribute to the berry sugar content. When berry carbohydrate sink demand decreased, carbohydrates were redirected towards the roots, and root starch accumulated. The largest relative contribution (up to 18%) of total perennial and vegetative seasonal organ carbohydrate mobilization towards berry dry matter accumulation, occurred for vines with low leaf-to-fruit ratios and under reduced irrigation. In these grapevines, root starch mobilization accounted for up to 89% of the loss of total perennial and vegetative seasonal organ carbohydrate content during rapid berry sugar accumulation. Moderate to severe water constraints can cause a greater reliance on TNC reserves to support berry dry matter accumulation, although seemingly not impacting on the effectiveness of berry sugar import or anthocyanin biosynthesis. Although reserve carbohydrate replenishment starts as soon as the berry sugar accumulation tempo slows (possibly even a few weeks prior to harvest), restricted water availability during berry maturation can cause lower carbohydrate reserve content in the roots by fruit maturity. In vineyards where no, or an ineffective post-harvest period occurs, the impact of this prevention of root carbohydrate reserve accumulation by fruit maturity is more severe.

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