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Vitis vinifera root and leaf metabolic composition during fruit maturation: implications of defoliation

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Grapevine (*Vitis vinifera*) roots and leaves represent major carbohydrate and nitrogen (N) sources, either as recent assimilates, or mobilized from labile or storage pools. This study examined the response of root and leaf primary metabolism following defoliation treatments applied to fruiting vines during ripening. The objective was to link alterations in root and leaf metabolism to carbohydrate and N source functioning under conditions of increased fruit sink demand. Potted grapevine leaf area was adjusted near the start of véraison to 25 primary leaves per vine compared to 100 leaves for the control. An additional group of vines were completely defoliated. Fruit sugar and N content development was assessed, and root and leaf starch and N concentrations determined. An untargeted GC/MS approach was undertaken to evaluate root and leaf primary metabolite concentrations. Partial and full defoliation increased root carbohydrate source contribution towards berry sugar accumulation, evident through starch remobilization. Furthermore, root *myo*-inositol metabolism played a distinct role during carbohydrate remobilization. Full defoliation induced shikimate pathway derived aromatic amino acid accumulation in roots, while arginine accumulated after full and partial defoliation. Likewise, various leaf amino acids accumulated after partial defoliation. These results suggest elevated root and leaf amino N source activity when leaf N availability is restricted during fruit ripening. Overall, this study provides novel information regarding the impact of leaf source restriction, on metabolic compositions of major carbohydrate and N sources during berry maturation. These results enhance the understanding of source organ carbon and N metabolism during fruit maturation.

Abbreviations – 25L, 25 leaves treatment; C, carbon; FL, full leaf area treatment; GABA, γ-amino-n-butyric acid; GC/MS, gas chromatography/mass spectrometry; LSD, least significant difference; N, nitrogen; NL, no leaf treatment; SSC, soluble solid content; TCA, tricarboxylic acid; TSS, total soluble solids; V, véraison; V + 9, 9 days after véraison; V + 18, 18 days after véraison; V + 27, 27 days after véraison; V + 37, 37 days after véraison;V + 46, 46 days after véraison.

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Introduction

Maturing post-véraison grapevine (*Vitis vinifera*) berries are sinks for non-structural carbohydrates and N (Roubelakis-Angelakis and Kliewer 1992, Davies and Robinson 1996). Carbohydrates are ultimately derived from leaf photoassimilation, but reserve carbohydrate remobilization from perennial tissues can provide an alternative carbon (C) source if photosynthetic supply is limited (Candolfi-Vasconcelos et al. 1994). Among the different grapevine organs, root starch is often the largest pool of storage carbohydrates, and this rapidly depletes if the period of high berry sugar demand after véraison coincides with restricted canopy photoassimilation (Rossouw et al. 2017a, 2017b). After the slowing of fruit sugar accumulation in the later ripening period, the roots instead become a C sink, and root starch storage initiates (Rossouw et al. 2017a). Soil N uptake is limited during the berry maturation period, and redistribution of N from roots, shoots, and leaves is thought to contribute to fruit N (Conradie 1991). Mature leaves and the roots are, additionally, the major sources of amino N in higher plants (Rentsch et al. 2007). Grapevine roots and leaves are, therefore, important sources of C and/or N during fruit sugar and N accumulation. The extent of the contribution of primary compound metabolism towards root and leaf C and N source activity requires further research.

While starch is often the predominant non-structural carbohydrate, sucrose, glucose, fructose, and less abundant (minor) sugars and sugar alcohols (e.g. raffinose and *myo*-inositol) also contribute to the carbohydrate pool of higher plants (Noiraud et al. 2001, Valluru and Van den Ende 2011). Conditions of high-carbohydrate demand, such as those created by reducing the leaf area of fruiting grapevines, induce enzymatic starch breakdown, and subsequent carbohydrate exportation from reserve storage (Eveland and Jackson 2012). Breakdown of starch reserves is therefore expected to alter the relative composition of root non-structural carbohydrates, such as the different sugars, and influence the metabolism of other C containing compounds (e.g. organic acids). Organic acids, such as malic and citric acid, are important intermediates during C metabolism (López-Bucio et al. 2000). The C skeletons of these tricarboxylic acid (TCA) cycle intermediates, are also utilized during N assimilation and amino acid biosynthesis (Popova and Pinheiro de Carvalho 1998). Restricting the leaf C source may cause altered root source activity by impacting on C flux through different pathways of primary root metabolism. The C flux through the shikimate pathway, which can represent up to 20% of the available C in plants (Haslam 1993), is for example, likely affected by limited C availability. As the origin of many amino acids and secondary metabolites (e.g. phenolic compounds), changes in C flux through the shikimate pathway could have crucial consequences on plant C and N source organ metabolic composition.

Grapevine N reserves are stored as proteins and amino acids (mainly arginine), with the largest proportion located in the root system and mature leaves (Roubelakis-Angelakis and Kliewer 1992). Amino acids provide a soluble source of organic N which can be transported between sources (leaves and roots) and sinks (the fruit) (Lam et al. 1996). As the fruit normally accumulates N during the post-véraison period (Roubelakis-Angelakis and Kliewer 1992), the metabolism of N containing compounds (e.g. amino acids) in N source organs are potentially altered by limited post-véraison N availability. By subsequently restricting the availability of organic N, defoliation may induce protein degradation in remaining N sources such as the roots (Volenec et al. 1996). Protein degradation in the roots will subsequently promote root amino N accumulation, which becomes available for further N translocation to sinks.

Monitoring changes in the abundance of primary metabolites in grapevines may provide an insight into metabolic pathway responses to abiotic conditions, and to canopy or crop load manipulations that are commonly used in commercial viticulture. Methods such as gas chromatography/mass spectrometry (GC/MS) allow the profiling of a wide range of plant metabolites, including soluble sugars, sugar alcohols, organic acids and amino acids from a single sample preparation (Lisec et al. 2006). In relation to C and N containing storage compounds and their associated metabolism, the responses of vegetative tissues is of particular interest. However, recent literature concerning grapevines has focused more on comparative studies between genotypes. Where the implications of abiotic conditions such as water or heat constraints have been examined, most of the related analyses were conducted in fruit samples (Hochberg et al. 2013, 2015a, 2015b, Cuadros-Inostroza et al. 2016). To the best of our knowledge, no previous studies have conducted a detailed profiling of grapevine root and leaf metabolite composition during the post-véraison period.

This study assessed the effects of post-véraison defoliation on the contents of non-structural carbohydrates and N in the fruit and in major C and N sources (roots and leaves), in conjunction with the source organ abundance of primary metabolites. By changing leaf C and N source availability, the main objective was to profile the primary metabolic composition (including soluble sugars, amino acids and organic acids) of remaining leaves and/or the

roots. A further objective was to link specific alterations in source organ primary metabolite concentrations to carbohydrate or N distribution between the source and sink organs, as influenced by leaf source availability.

Materials and methods

Experimental design and sample collection

Forty own-rooted *V. vinifera* cv. Shiraz (clone EVOVS12) grapevines, grown in 30 l pots containing commercial potting mix, were used for the study during the 2015/16 growing season. The grapevines were enclosed in a bird-proof cage in the hot climate Riverina grape growing region of New South Wales, Australia. The 3-year-old grapevines were spur-pruned to 5 two-bud spurs in the winter and distributed in four rows with 10 vines each. Shortly after budburst, the grapevines were fertilized every 3 weeks with 250 ml of 1:50 diluted complete liquid fertilizer (MEGAMIX Plus, Rutec, Tamworth, Australia). In total, approximately 3.2 g N was applied to each vine after budburst, and the fertilization events were ceased 1 month prior to the start of the experiment, aiming to avoid excessive soil N uptake during the experiment. After budburst the vines were trained where possible to 10 shoots, and the number of bunches per vine was counted at fruit set. All vines naturally contained between 13 and 19 bunches, and individual vines were subsequently classified as either containing low (13–15), medium (15–16) or high (16–19) number of bunches. This classification was later only used to minimize vine cropping variability among treatments and harvest dates. Nine days after the first sight of berry softening (i.e. véraison +9 days; V + 9), four vines, one out of each row, and from each of the bunch classes (two from the medium class) to ensure the collection of vines to be as unbiased as possible, were destructively harvested to represent the population of grapevines prior to the start of the experiment. The remaining nine vines per row were separated into three replicates, each representing a bunch number class, and randomly allocated a specific treatment and harvest date. This resulted in three replicates, spread over a four row, nine column randomized block design.

The three experimental treatments: full leaf (i.e. control, 100 primary shoot leaves per vine and all laterals, FL), 25% leaf (25 primary shoot leaves, with no lateral leaves, 25L), and no leaves (NL), were established at a stage (V + 9) when berry sugar accumulation was expected to occur rapidly. All the leaves were removed on NL vines, while the 25L l vines were left with 25 primary leaves each, adjacent to a bunch, and additionally on one node above or below a bunch when required. When more than 100 primary shoot leaves were present, the leaves on FL vines were reduced to 100 per vine. The leaf-to-fruit ratio of FL vines was adjusted to approximately 8 cm^2 leaf area per g fresh fruit weight, while that of 25L vines was adjusted to 2 $cm^2\ g^{-1}$. The adjusted FL ratio is on the lower end of a range (8–12 $cm^2\ g^{-1}$) suggested to, in a given climatic region, contribute towards maximum grapevine fruit sugar accumulation capacity (Kliewer and Dokoozlian 2005). For NL and 25L vines, any new vegetative growth was removed as soon as the regrowth of leaves and lateral shoots was observed.

A pressure compensated drip emitter (4 l h^{-1}) was installed in the middle of each pot, close to the vine trunk, and irrigation events were scheduled four times daily (08:00, 11:30, 14:30 and 18:00 h). All vines received the same amount of water during each irrigation event. The irrigation event duration was the same at each application per day, and ranged between 13 and 20 min per event throughout the experiment (depending on daily atmospheric conditions), aiming to always allow visual free water drainage from all pots during each irrigation event. Three vines from each treatment were destructively harvested every 9–10 days after the start of the experiment. At the destructive harvest dates, i.e. December 28, 2015 (V + 9), January 6, 2016 (V + 18), January 15, 2016 (V + 27), January 25, 2016 (V + 37), and February 3, 2016 (V + 46), the pre-selected grapevines were dismantled. Whole root systems, leaf blades and all fruits were collected from each vine, and washed with phosphate-free detergent and rinsed with deionized water. The fresh weights of these organs were determined, and the root and leaf samples were oven-dried at 60 °C until a constant dry weight was reached. During the destructive harvests, subsamples of the roots, leaf blades and berries were collected. The root subsamples consisted of full-length root parts taken from within 10 cm from the basal part of the trunk, always between 2 and 6 mm in diameter, with at least 50 g in total tissue fresh weight. Soil particles were shaken off and the roots rapidly rinsed with deionized water, prior to freezing in liquid N. Leaf subsamples consisted of 20 leaves, taken adjacent to a bunch, or from the shoot node directly above or below a bunch when required, and frozen in liquid N. Berry subsamples consisted of 100 berries per vine, immediately frozen in liquid N after their removal from the vine. The snap-frozen subsamples were stored at −80°C until further processing. The periods between the different destructive harvest dates are referred to as Intervals 1, 2, 3 and 4, respectively.

Vegetative and reproductive development

The total area of the leaves collected from each individual vine at the respective destructive harvest dates

was measured using a leaf area meter (LI-3100C, LI-COR Biosciences Inc., Lincoln, NE). The leaf subsample areas were measured immediately after removal of the leaves, prior to the snap-freezing of these leaves. Total fruit weight of each grapevine was recorded, and subsamples of 50 berries per vine were used to determine the fresh weight per berry and juice total soluble solid (TSS) concentration. Berry soluble solid content (SSC) was calculated on the basis of berry fresh weight and TSS. The total fruit sugar content per vine basis was subsequently calculated and is henceforth referred to as the fruit sugar content.

Total tissue dry weight of whole root systems and leaves were calculated for each vine by combining the weights measured from the dried main samples with estimated dry weights of the sub-samples. A subsample of 50 frozen berries per vine was ground to a fine powder under liquid N using an analytical mill (A11 basic analytical mill, IKA, Selangor, Malaysia), and freeze-dried (Gamma 1–16 LSC, Christ, Osterade am Harz, Germany) until a constant weight was reached. Total fruit weight per vine was estimated from the weight loss during drying. Root and leaf structural biomass per vine were estimated by subtracting the non-structural carbohydrate content (total starch and soluble sugar content) of these tissues from their total dry weight.

Non-structural carbohydrate determination

The root and leaf subsamples for each vine were taken from −80°C storage and ground to a fine powder under liquid N, using an analytical mill (A11 basic analytical mill, IKA). Frozen ground tissues of each sample were then freeze-dried (Gamma 1–16 LSC, Christ) until a constant weight was reached. Starch and total soluble sugar concentrations in a 20-mg freeze-dried sample of ground tissue were determined following the methods outlined in Smith and Holzapfel (2009).

Total nitrogen (N) determination

Nitrogen concentrations were determined in finely ground, freeze-dried samples of roots, leaves and fruit. N concentration in 200 mg of a representative sample was determined by the LECO method (Standard methods of Rayment and Lyons, Soil chemical methods, Australasia, Dumas Combustion Method 6B2b), using a LECO CNS TruMAC analyzer (LECO Corporation, St. Joseph, MI).

Metabolite extraction and analysis

Extraction and derivatization of untargeted metabolites in freeze-dried root and leaf subsamples were performed using the method outlined in Lisec et al. (2006) with some modifications. Firstly, 100 mg ground tissue were homogenized with 1.4 ml 100% (v/v) methanol and 30 µl internal standard solution (1 g l^{-1} of each, adonitol, L-hydroxyproline and adipic acid, dissolved in 50% v/v methanol). The homogenate was shaken at 70°C for 10 min (Thermomixer 5436, Eppendorf, North Ryde, Australia), before being centrifuged at 11 000 *g* for 10 min. The supernatant was transferred to a glass vial, and mixed with 0.75 ml chloroform and 1.4 ml ultrapure water. The mixture was centrifuged at 2200 *g* for 15 min, and 150 µl of supernatant (polar phase) collected and dried under a constant stream of pure N_2 gas.

Derivatization of the extracted metabolites was initiated by adding 40 µl of 20 mg ml^{-1} methoxyamin hydrochloride in pure pyridine, to the dried extracts. Samples were then shaken at 37°C for 2 h, before being centrifuged at 5000 *g* for 2 min. *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA, 70 µl) was added, and samples were shaken again for 30 min at 37°C, before being centrifuged at 5000 *g* for 2 min. Solutions of analytical grade standards (10 µg l^{-1} in 50% v/v methanol), obtained from Sigma-Aldrich (Sigma, St. Louis, MO), consisted of soluble sugars: Sucrose, D(+)-glucose, D(−)-fructose, D(+)-mannose, L-rhamnose, D(−)-mannitol, galactinol dehydrate, D(+)-raffinose, melibiose, D(+)-turanose, D(+)-melezitose, D(+)-cellobiose, D(−)-ribose, D(−)-arabinose, D(+)-trehalose, maltose monohydrate, D(+)-galactose, D(+)-xylose, dulcitol (galactitol), L-fucose, and *myo*-inositol; amino acids: L-glutamic acid, L-arginine, L-proline, L-glutamine, γ-amino-n-butyric acid (GABA), L-threonine, L-methionine, β-alanine, L-lysine, L-asparagine, L-aspartic acid, L-leucine, L-isoleucine, L-valine, L-alanine, L-serine, glycine, L-tyrosine, L-phenylalanine, L-tryptophan, L-histidine, L-cysteine and L-cysteine), and miscellaneous compounds (L-ascorbic acid and protocathechuic acid), and were prepared in order to assist in the retention index and spectra identification of these compounds.

Extraction and analysis of samples were randomized, and after every tenth sample, a quality control root sample was injected. GC/MS analyses were conducted by injecting 1 µl into the GC column (30 m × 0.25 mm, 0.25 µm HP-5MS, Agilent, Santa Clara, CA), in both split-less and split mode (300:1, to allow the measurement of more abundant metabolites). The GC/MS system consisted of a 7683B series autosampler, 7890A gas chromatograph and 5975C mass spectrometer with an electron impact ionization source and a quadruple analyzer (all from Agilent). The injection port was set at 250°C, the transfer line at 280°C, the ionization source at

Table 1. Effect of defoliation treatments (full leaf – FL, 25% leaves – 25l and no leaf – NL) on grapevine leaf-to-fresh fruit weight ratio, root and leaf structural biomass per vine, and total fruit dry weight per vine at the destructive harvests (V + 9, V + 18, V + 27, V + 37 and V + 46; mean ± SE, n = 3). Means are separated within rows and columns using Fisher's LSD test, significant differences are indicated at $P < 0.05$. Where different lower case letter appears in a row, values differ significantly between dates. Where different upper case letter appears in a column, values differ significantly between treatments.

			Interval 1			Interval 2			Interval 3			Interval 4		
	Treatment	V + 9			V + 18			V + 27			V + 37			V + 46	
Leaf-to-fresh fruit weight ratio ($cm^2\ g^{-1}$)	FL	11 ± 2	a	A	8 ± 1	ab	A	6.9 ± 0.6	b	A	7.0 ± 0.8	b	A	7.5 ± 0.4	b
	25L	11 ± 2	a	B	1.9 ± 0.2	b	B	1.9 ± 0.2	b	B	1.6 ± 0.1	b	B	1.5 ± 0.1	b
	NL	11 ± 2			–			–			–			–	
Root structural biomass ($g\ vine^{-1}$)	FL	172 ± 6	b	A	226 ± 19	a	A	226 ± 19	a	A	224 ± 19	a	A	201 ± 5	a
	25L	172 ± 6	a	A	182 ± 17	a	A	182 ± 17	a	A	168 ± 16	a	B	147 ± 13	a
	NL	172 ± 6	a	A	197 ± 19	a	A	197 ± 19	a	A	199 ± 24	a	B	155 ± 13	a
Leaf structural biomass ($g\ vine^{-1}$)	FL	76 ± 3	b	A	76 ± 2	b	A	82 ± 3	ab	A	82 ± 4	ab	A	91 ± 3	a
	25L	76 ± 3	a	B	19 ± 1	b	B	19 ± 3	b	B	22.8 ± 0.4	b	B	22 ± 2	b
	NL	76 ± 3			–			–			–			–	
Total fruit dry weight ($g\ vine^{-1}$)	FL	203 ± 20	c	A	338 ± 53	b	A	457 ± 25	ab	A	544 ± 46	a	A	575 ± 51	a
	25L	203 ± 20	c	A	292 ± 19	b	B	356 ± 11	ab	B	365 ± 7	ab	B	408 ± 49	a
	NL	203 ± 20	b	A	272 ± 37	ab	B	301 ± 24	a	B	316 ± 17	a	B	322 ± 8	a

230°C and the quadrupole at 150°C. The helium carrier gas was set at a constant flow rate of 1.3 ml min^{-1}. The column temperature program was set at 65°C for 2 min, followed by a 6°C min^{-1} ramp to 300°C, where it was held for 25 min. The ionization energy was set at 70 eV. Mass spectra were recorded in full mode at 2.66 scans s^{-1} with a mass-to-charge ratio of 50 to 600 amu. Spectral deconvolution (signal-to-noise ratio threshold = 10; mass absolute height ≥ 2000; compound absolute area ≥ 10 000) allowed the identification of co-eluting chromatographic peaks, and was conducted through the MassHunter Workstation software (Qualitative Analysis, version B.07.00, Agilent). Acquired MS spectra were searched for, and identified by using the National Institute of Standards and Technology algorithm (NIST, Gaithersburg, MD). The retention index for each compound in the analyzed samples was calculated by using the retention times of a series of alkanes (C8–C28) in an injected retention index solution (Fluka, Buchs, Switzerland).

Statistical analysis

Datasets regarding grapevine vegetative and reproductive development (Table 1), non-structural carbohydrate (Fig. 1) and total N distribution (Fig. 2) and primary metabolite concentration (Figs 3 and 4, with Supporting Information in Tables S1 and S2) were analyzed using Statistica 13 (Dell Inc., Tulsa, OK). For each variable, both treatment differences at a single time and how a treatment changed over time were of interest. It was also recognized that residual variance at each time may differ and that the interventionist nature of the treatments may also lead to reduced residual variance for some treatments. To facilitate these comparisons, univariate ANOVA at each date (all treatments) and for each treatment (all dates) were conducted. An average Fisher's least significant difference (LSD) test was used to identify significant differences between means ($P < 0.05$). Significant differences in table and heat map columns and rows are indicated by upper case letters (between treatments) and lower case letters (between dates), respectively.

For each of the grapevine organs (roots and leaf blades), at each harvest date after the initial harvest (V + 18, V + 27, V + 37 and V + 46), a linear mixed model was fitted for every unequivocally identified primary metabolite (78 for roots and 75 for leaves) using ASReml-R (Butler et al. 2007). Each model included Treatment as a fixed effect and Replicate as a random effect. For the roots, Treatment included FL, 25L and NL, while for the leaves Treatments included FL and 25L. The significance of treatment effects was assessed using approximate *F*-tests using the techniques of Kenward and Roger (1997). For each vine organ, primary metabolites which had significant treatment effects ($P < 0.05$) for any of V + 18, V + 27, V + 37 and V + 46 were retained.

To test the relationship between the concentration of root starch and *myo*-inositol, a cubic smoothing spline was fitted using the linear mixed model methods of Verbyla et al. (1999). The fixed effects included intercept effects for the overall mean and treatments, and effects for linear trend including overall linear trend and trend due to treatment. The random effects included overall spline curvature and curvature due to treatment as well as effects due to replicates at each

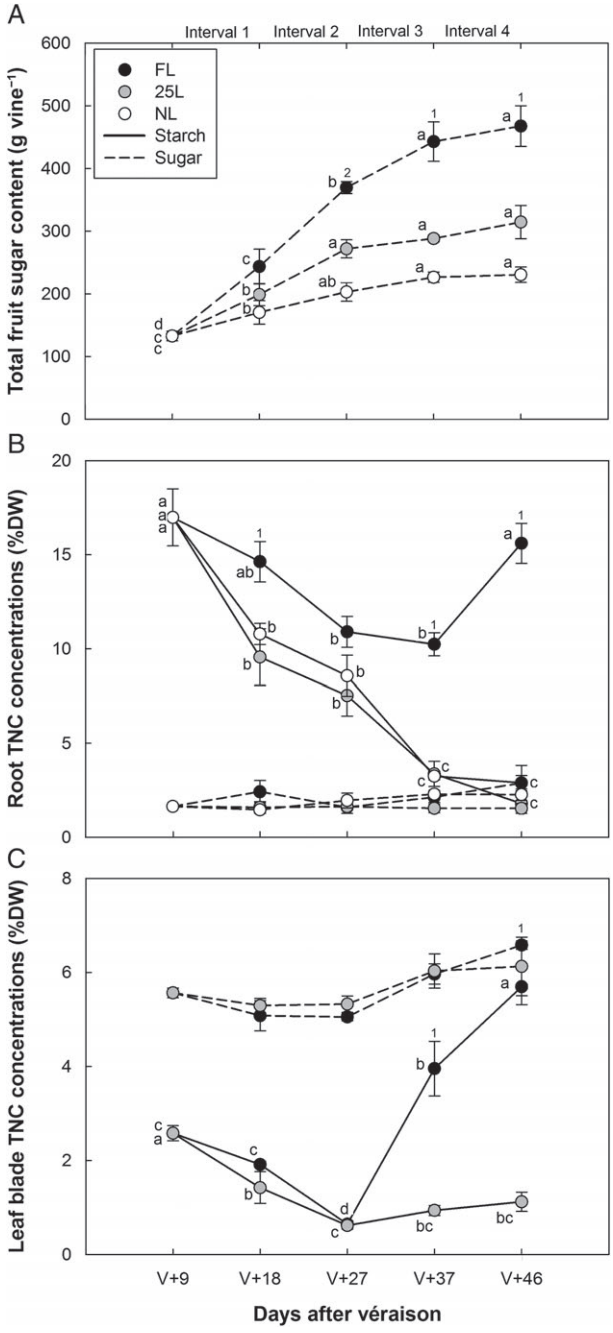

Fig. 1. Impact of defoliation (full leaf – FL, 25% leaves – 25 l and no leaf – NL) on total fruit sugar content per vine (A), root starch and total sugar (total non-structural carbohydrates, TNC) concentrations (B), and leaf starch and total sugar (TNC) concentrations (C) during the experimental period (mean ± SE; n = 3). Significant differences ($P < 0.05$) between harvest dates for each treatment are indicated by different lower-case letters. Significant differences ($P < 0.05$) between treatments at each harvest date are indicated by different numerals [1: FL > (25 l and NL) and 2: FL > 25 l > NL]. To allow clarity of the most important results, these significant differences are indicated for fruit sugar content (A), and root (B) and leaf (C) starch concentrations only.

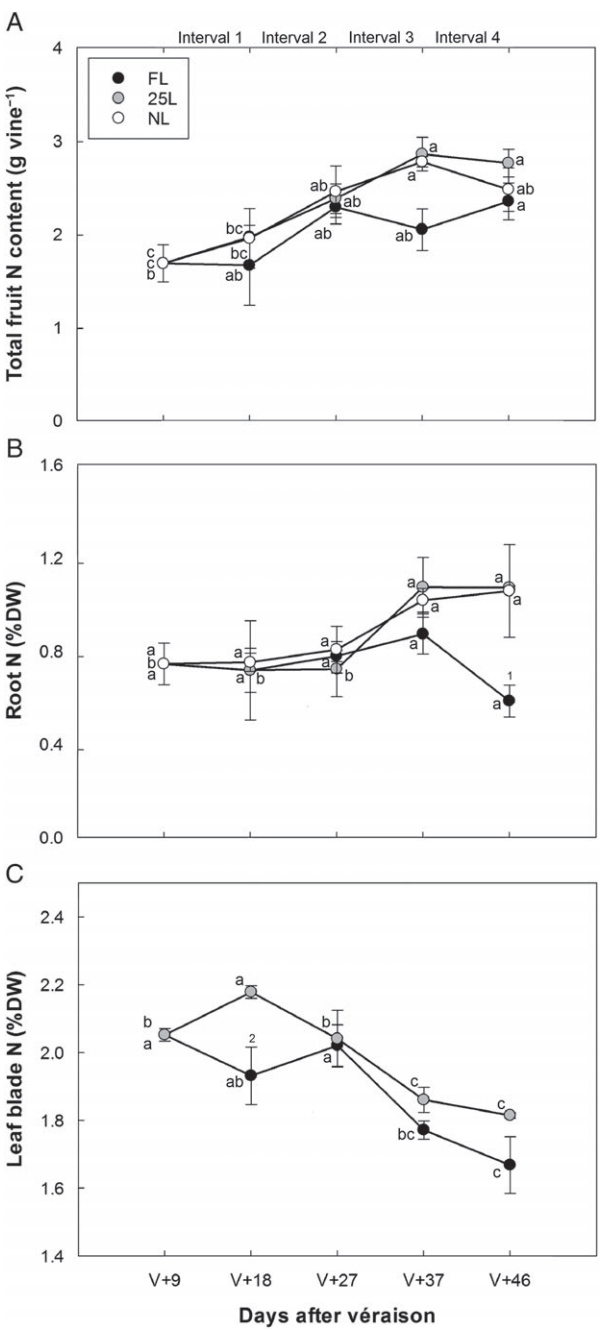

Fig. 2. Impact of defoliation (full leaf – FL, 25% leaves – 25 l and no leaf – NL) on total fruit nitrogen (N) content per vine (A), root N concentration (B), leaf N concentration (C) during the experimental period (mean ± SE; n = 3). Significant differences ($P < 0.05$) between harvest dates for each treatment are indicated by different lower-case letters. Significant differences ($P < 0.05$) between treatments at each harvest date are indicated by different numerals [1: FL < (25 l and NL) and 2: FL < 25 l].

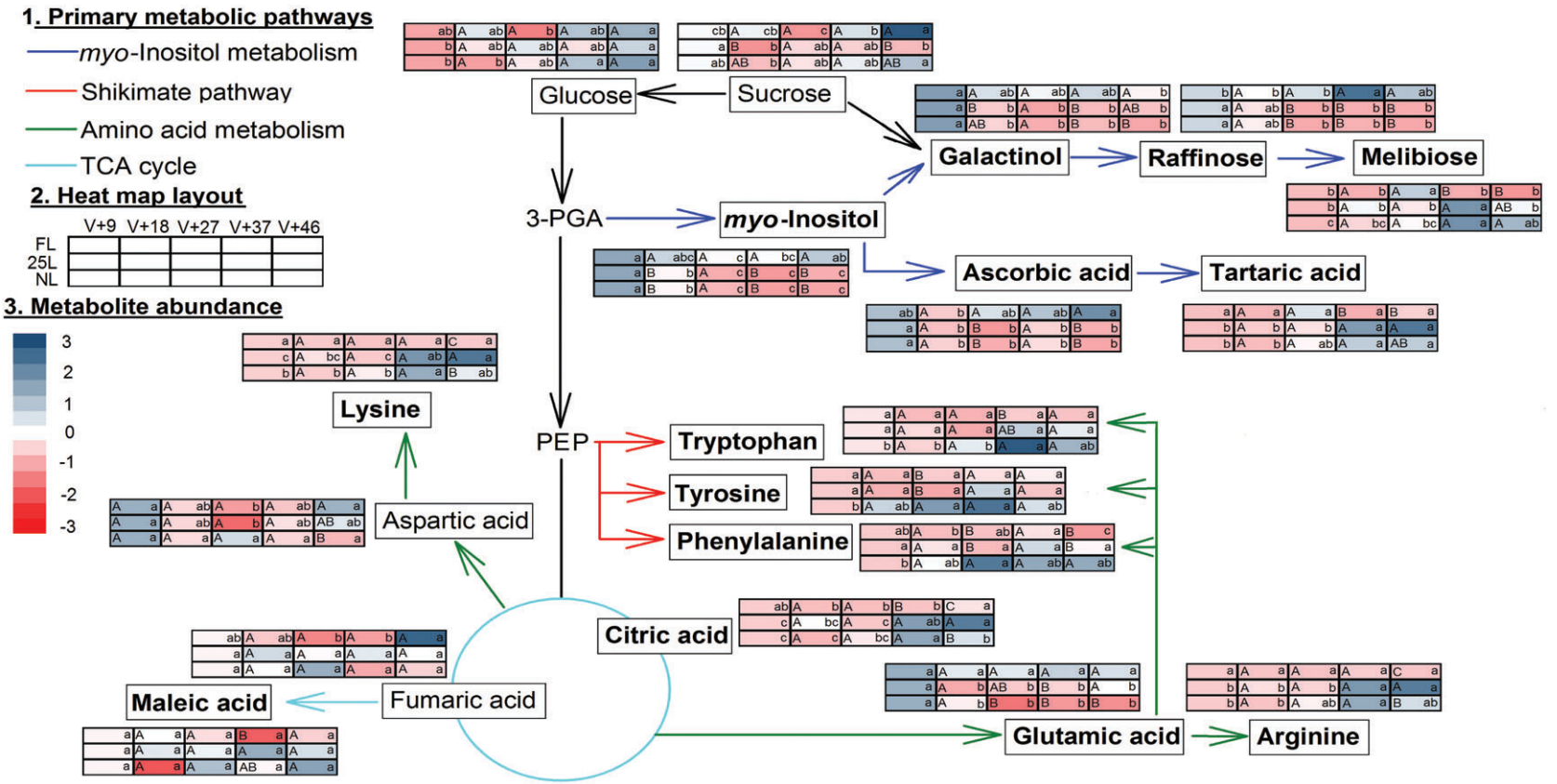

Fig. 3. Simplified pathway response of the root primary metabolites significantly affected by the treatments (full leaf: FL; 25% leaves: 25l; no leaf: NL) and other metabolites directly involved in the pathways (1). Significant differences are indicated at $P < 0.05$, heatmap columns indicate the three treatments, while heatmap rows indicate the harvest dates (V + 9, V + 18, V + 27, V + 37 and V + 46). Where different upper-case letters appear in heatmap columns (2), values differ significantly between treatments. Where different lower-case letters appear in a row, values differ significantly between harvest dates. Average metabolite abundance is color coded according to the scale on the left (3). 3-PGA: 3-phosphoglyceric acid; PEP: phosphoenolpyruvic acid.

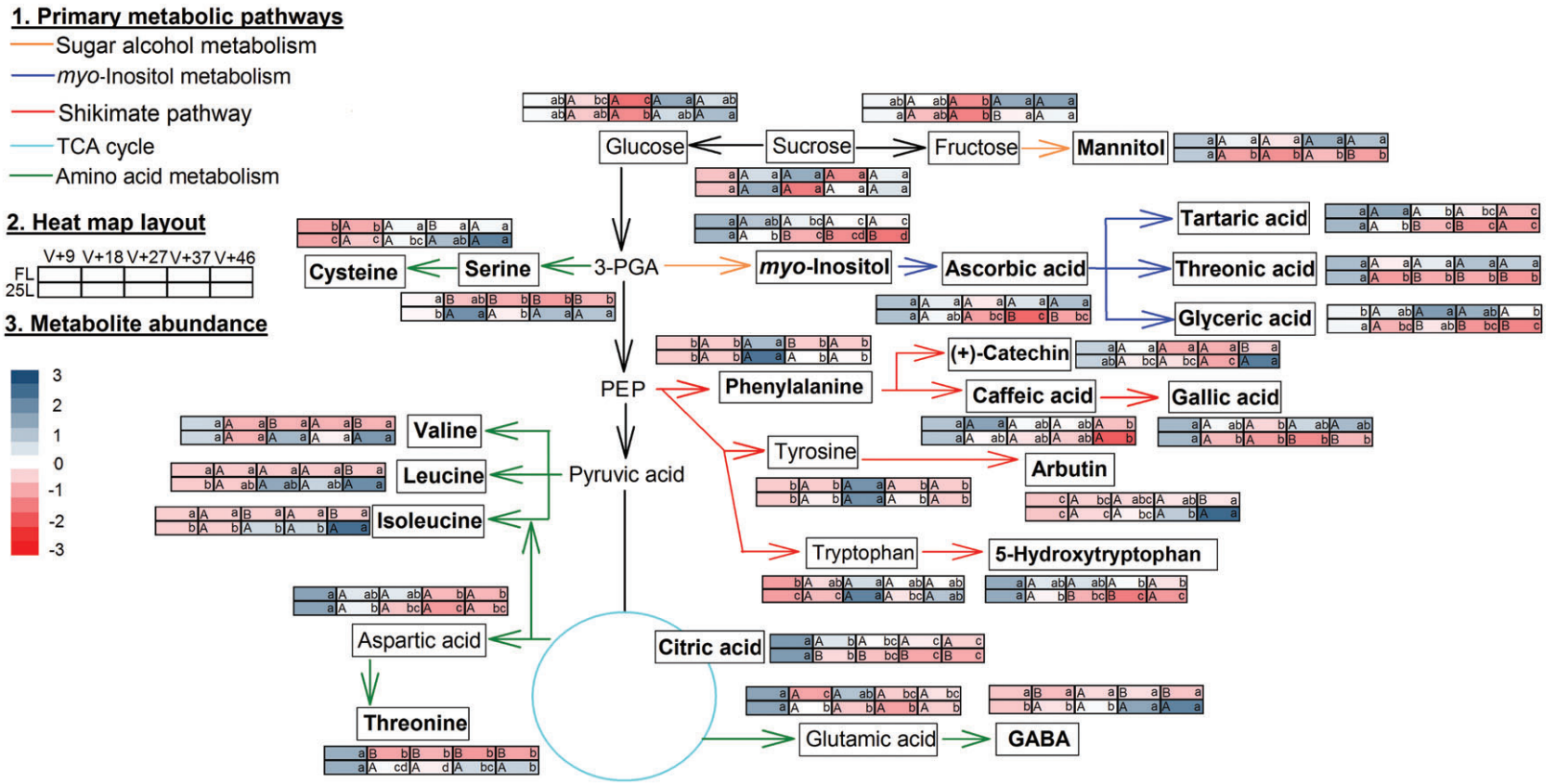

Fig. 4. Simplified pathway response of the leaf primary metabolites significantly affected by the treatments (full leaf: FL; 25% leaves: 25 l) and other metabolites directly involved in the pathways (1). Significant differences are indicated at $P < 0.05$, heatmap columns indicate the three treatments, while heatmap rows indicate the harvest dates (V + 9, V + 18, V + 27, V + 37 and V + 46). Where different upper-case letters appear in heatmap columns (2), values differ significantly between treatments. Where different lower-case letters appear in a row, values differ significantly between harvest dates. Average metabolite abundance is color coded according to the scale on the left (3). 3-PGA: 3-phosphoglyceric acid; PEP: phosphoenolpyruvic acid.

time of measurement. The significance of fixed treatment effects was assessed by the approximate *F*-tests using the techniques of Kenward and Roger (1997) and the significance of spline curvature was assessed by examining $0.5(1 - \Pr(\chi^2 \leq d))$ where d refers to models which differ in a single spline curvature term. This linear model was fitted using ASReml 3.0 (Gilmour et al. 2009).

Results

Vegetative and reproductive development

The total leaf area (data not shown) and corresponding leaf-to-fruit ratio per control (FL) vine were initially (at V + 9) adjusted to 1.4 m^2 and 8 cm^2 leaf area g^{-1} fresh fruit (Table 1), respectively, and these values did not change significantly during the experiment. The leaf area and leaf-to-fruit ratio of the 25-leaf treatment (25L) were adjusted to 0.6 m^2 and 1.9 cm^2 g^{-1}, respectively, with the latter being significantly lower than those of FL from V + 18.

The root structural biomass per FL vine increased significantly during Interval 1 (Table 1), while that of 25L and no leaf (NL) did not alter significantly. FL had significantly larger root structural biomass at V + 46 than 25L or NL. The FL leaf structural biomass per vine increased significantly between V + 18 and V + 46 (Table 1), while that of 25L decreased during Interval 1 due to the defoliation. After treatment implementations, FL leaf structural biomass was significantly larger than that of 25L at all harvest dates.

The FL and 25L total fruit dry weight per vine increased significantly during Interval 1 (Table 1). The FL total fruit dry weight continued to increase between V + 18 and V + 37, while that of 25L increased significantly between V + 18 and V + 46. The NL total fruit dry weight per vine increased significantly between V + 9 and V + 27. FL significantly induced the largest fruit dry weight per vine from V + 27.

Carbohydrate distribution

Fruit sugar accumulation: FL total fruit sugar content per vine increased rapidly during Intervals 1 (12 g day^{-1}) and 2 (14 g day^{-1}) (Fig. 1A). It continued to increase significantly during Interval 3 although at a reduced rate (7 g day^{-1}), and did not change significantly during Interval 4 (3 g day^{-1}). Although at rates lower than that of the control, 25L fruit sugar content per vine increased significantly during Intervals 1 (7 g day^{-1}) and 2 (8 g day^{-1}). The NL total fruit sugar content per vine increased at a slow but significant rate during Interval 1 (4 g day^{-1}) and between V + 18 and V + 37 (3 g day^{-1}). Among treatments, FL fruit had significantly higher sugar content from V + 27, and 25L fruit contained significantly more sugar per vine than those of NL at V + 27.

Root carbohydrate abundance: Starch concentration in FL roots decreased significantly between V + 9 and V + 27, and then increased back to its original concentration during Interval 4 (Fig. 1B). The 25L and NL root starch concentrations reduced significantly during Intervals 1 and 3. Among the treatments, FL root starch concentration was highest at V + 18, V + 37 and V + 46. The FL root total sugar concentration was significantly higher at V + 46 than at V + 9 and V + 27, and was significantly higher than that of 25L at V + 46 (Fig. 1B). The NL root total sugar concentration was significantly higher from V + 37 than at V + 18.

Leaf carbohydrate abundance: FL leaf starch concentration decreased significantly during Interval 2, and increased significantly during Intervals 3 and 4 (Fig. 1C). The 25L leaf starch concentration reduced significantly during Intervals 1 and 2. Among treatments, FL significantly induced the highest leaf starch concentration from V + 37. The only significant leaf sugar concentration changes occurred where the FL concentration increased during Intervals 3 and 4 (Fig. 1C). No leaf sugar concentration treatment differences occurred.

Nitrogen (N) distribution

Fruit N accumulation: FL total fruit N content per vine increased significantly between V + 9 and V + 46 (0.02 g day^{-1}) (Fig. 2A). The 25L and NL total fruit N contents per vine increased significantly between V + 9 and V + 27 (0.04 g day^{-1}). No significant treatment differences occurred in total fruit N content.

Root N abundance: The only significant root N concentration change occurred where 25L N increased during Interval 3 (Fig. 2B). Among treatments, root N concentrations of 25L and NL were significantly higher than that of FL at V + 46.

Leaf N abundance: FL leaf N concentration reduced significantly during Interval 3, and was significantly lower at V + 46 than before V + 37 (Fig. 2C). The 25L leaf N concentration increased significantly during Interval 1, and reduced significantly during Intervals 2 and 3. Among the treatments, 25L leaf N concentration was the highest at V + 18.

Metabolic adjustments

Primary metabolites from the roots and leaves were categorized as sugars, sugar alcohols, amino acids, miscellaneous acids, or others (including flavonoids and stilbenoids). Further information regarding the

Table 2. Proposed metabolic pathways related to the biosynthesis of significantly treatment affected root metabolites. All metabolites significantly differing among the defoliation treatments (full leaf, 25% leaves and no leaf) for any of the destructive harvest dates after treatment implementation (V + 18, V + 27, V + 37 and V + 46) are listed. The metabolites are categorized based on their chemical or structural properties (sugars, sugar alcohols, amino acids or miscellaneous acids).

Classification	Metabolite	Proposed primary pathway
Sugars	Sucrose	Primary carbohydrate metabolism
	Raffinose	*Myo*-inositol metabolism
	Melibiose	*Myo*-inositol metabolism
	Arabinose	Glucose-6-phosphate
Sugar alcohols	*Myo*-inositol	Glucose-6-phosphate
	Galactinol	*Myo*-inositol metabolism
	Mannitol	Fructose metabolism
	Arabitol	Glucose-6-phosphate
	Glycerol	Glycerate
Amino acids	Glutamic acid	α-Ketoglutarate
	Arginine	α-Ketoglutarate
	Glutamine	α-Ketoglutarate
	Tryptophan	Shikimate
	Phenylalanine	Shikimate
	Tyrosine	Shikimate
	Glycine	3-Phosphoglycerate
	Lysine	Oxaloacetate
	Threonine	Oxaloacetate
	Valine	Pyruvate
Miscellaneous acids	Ascorbic acid	*Myo*-inositol metabolism
	Tartaric acid	*Myo*-inositol metabolism
	Citric acid	Tricarboxylic acid cycle
	Maleic acid	Tricarboxylic acid cycle
	3-Hydroxyanthranilic acid	Shikimate
	Protocatechuic acid	Shikimate
	2-Keto-gluconic acid	Gluconate

Table 3. Proposed metabolic pathways related to the biosynthesis of significantly treatment affected leaf metabolites. All metabolites significantly differing among the defoliation treatments (full leaf and 25%) for any of the destructive harvest dates after treatment implementation (V + 18, V + 27, V + 37 and V + 46) are listed. The metabolites are categorized based on their chemical or structural properties (sugars, sugar alcohols, amino acids, miscellaneous acids and others).

Classification	Metabolite	Proposed principle pathway
Sugars	Glucose	Primary carbohydrate metabolism
	Raffinose	*Myo*-inositol metabolism
	Melibiose	*Myo*-inositol metabolism
	Rhamnose	Fructose metabolism
	Melezitose	Sucrose metabolism
	Ribose	Glucose-6-phosphate
Sugar alcohols	Mannitol	Fructose metabolism
	Myo-inositol	Glucose-6-phosphate
Amino acids	Arginine	α-Ketoglutarate
	GABA	α-Ketoglutarate
	Serine	3-Phosphoglycerate
	Cysteine	3-Phosphoglycerate
	Valine	Pyruvate
	Leucine	Pyruvate
	Isoleucine	Pyruvate
	Phenylalanine	Shikimate
	Tryptophan	Shikimate
	5-Hydroxytryptophan	Shikimate
	Threonine	Oxaloacetate
Miscellaneous acids	Ascorbic acid	*Myo*-inositol metabolism
	Tartaric acid	*Myo*-inositol metabolism
	Threonic acid	*Myo*-inositol metabolism
	Glyceric acid	*Myo*-inositol metabolism
	Caffeic acid	Shikimate
	Gallic acid	Shikimate
	Lactic acid	Pyruvate
	Citric acid	Tricarboxylic acid cycle
	Fumaric acid	Tricarboxylic acid cycle
	2-Keto-glutaric acid	Tricarboxylic acid cycle
	Phosphoric acid	Tricarboxylic acid cycle
	Gluconic acid	Glucose metabolism
	Ribonic acid	Glucose-6-phosphate
	Nonanoic acid	Glycerol metabolism
	Palmitic acid	Glycerol metabolism
Other compounds	Arbutin	Shikimate
	Catechin	Shikimate
	Glycerol monostearate	Glycerol metabolism

metabolite abundance and MS spectra are indicated in Tables S1 and S2. Simplified listings of all metabolites which had significant treatment effects for any of the destructive harvest dates, and the associated biosynthetic pathway of each metabolite, are indicated for roots (Table 2) and leaves (Table 3).

Figs 3 and 4 illustrate the effects of the defoliation treatments on root and leaf metabolite concentrations, respectively. The concentrations of metabolites that exhibited significant treatment differences, and are involved in major C and N metabolic pathways, are indicated in the figures. For ease of interpretation, treatment effects and notable metabolite responses are described in further detail below. This description is structured in accordance to the simplified metabolic pathways for roots (*myo*-inositol metabolism, amino acid metabolism including shikimate pathway derived amino acids, and the TCA cycle) and leaves (sugar alcohol and further *myo*-inositol metabolism, the shikimate pathway including aromatic amino acids, the TCA cycle, and amino acid metabolism other than those related to the shikimate pathway).

Root metabolism

Myo-inositol metabolism: The FL root *myo*-inositol concentration decreased between V + 9 and V + 27, and then increased between V + 27 and V + 46. For the 25L and NL treatments root *myo*-inositol concentrations

decreased during both the first two intervals. FL roots subsequently contained more *myo*-inositol than those of 25L and NL at V + 18, and again from V + 37 (Fig. 3). While FL root galactinol decreased between V + 9 and V + 46, that in 25L and NL roots reduced during Interval 1. Among treatments, FL roots contained the most galactinol at V + 37, and additionally more galactinol than those of 25L and NL at V + 18 and V + 46, respectively. FL root raffinose increased during Interval 3, while that of 25L and NL reduced between V + 9 and V + 27. Among treatments, FL roots exhibited the highest raffinose concentration from V + 27. FL root melibiose increased during Interval 2 before decreasing during Interval 3, while that in 25L and NL roots increased between V + 9 and V + 37. Melibiose was subsequently more abundant in 25L and NL roots than those of FL at V + 37, while NL roots additionally contained more melibiose than those of FL at V + 46.

FL root ascorbic acid increased between V + 18 and V + 46, while that in 25L and NL roots reduced during Interval 1. Among treatments, FL roots contained the most ascorbic acid at V + 27 and V + 46. While FL root tartaric acid did not change significantly, that in 25L and NL roots increased between V + 9 and V + 37. The 25L and NL roots subsequently contained more tartaric acid than FL roots from V + 37 and at V + 46, respectively.

Amino acid metabolism: The FL root glutamic acid concentration never changed significantly, however, that in 25L and NL roots decreased during Interval 1. FL roots subsequently contained more glutamic acid than those of NL and 25L from V + 27 and at V + 37, respectively. Furthermore, 25L roots contained more glutamic acid than those of NL at V + 46. The FL root arginine concentration also did not change during the experiment, however, that in 25L and NL roots accumulated during Interval 3 and between V + 18 and V + 37, respectively. Among treatments, 25L contained most arginine at V + 46, when NL roots contained more arginine than FL roots.

For shikimate pathway derived amino acids, FL and 25L root tryptophan concentrations did not change during the experiment, however, that in NL roots accumulated during Interval 3. NL roots subsequently exhibited more tryptophan than those of FL at V + 37. Like with tryptophan, FL and 25L root tyrosine concentrations did not change significantly, however, NL roots accumulated tyrosine between V + 9 and V + 27. Among treatments, NL roots contained most tyrosine at V + 27. FL root phenylalanine increased between V + 18 and V + 37, before decreasing during Interval 4. While 25L root phenylalanine did not change significantly, that in NL roots increased between V + 9 and V + 27. NL roots subsequently contained more phenylalanine than those of FL and 25L at V + 27.

Aspartic acid reduced in FL roots between V + 9 and V + 27, before increasing towards V + 46. 25L aspartic acid reduced between V + 9 and V + 27, while NL root aspartic acid concentration did not alter significantly. FL roots contained more aspartic acid than those of NL at V + 46. FL root lysine did not change significantly, while that of 25L and NL increased during Interval 3. At V + 46, 25L roots contained the most lysine, while NL roots exhibited higher lysine concentration than FL roots.

TCA cycle intermediate metabolism: FL root citric acid increased during Interval 4, while that in 25L and NL roots accumulated during Interval 3, the NL citric acid then decreased during Interval 4. Among treatments, 25L and NL roots contained more citric acid than those of FL from V + 37, while 25L roots also contained more citric acid than NL roots at V + 46. No significant maleic acid concentration changes occurred, however, 25L roots contained more maleic acid than those of FL at V + 37.

Leaf metabolism

Sugar alcohol and further *myo*-inositol metabolism: While the FL leaf mannitol concentration did not change significantly, that of 25L leaves decreased during Interval 1. As a result, FL leaves contained more mannitol than those of 25L at V + 46 (Fig. 4).

FL *myo*-inositol decreased between V + 9 and V + 27, while that in 25L leaves gradually decreased from V + 9 to V + 46. Among treatments, *myo*-inositol concentration was lower in 25L than FL leaves, from V + 27. FL leaf ascorbic acid concentration did not change significantly, while that in 25L leaves decreased between V + 9 and V + 27. The 25L leaf ascorbic acid concentration was subsequently higher than that of FL leaves from V + 37. Tartaric acid decreased in FL leaves during Interval 2 and between V + 27 and V + 46. The 25L leaf tartaric acid decreased during Intervals 1 and 2, and among treatments, FL leaves contained more tartaric acid at V + 27 and V + 37. While the FL leaf threonic acid concentration did not change significantly, that in 25L leaves decreased during Interval 1. The 25L leaves subsequently exhibited more threonic acid from V + 27. FL leaf glyceric acid increased between V + 9 and V + 27, before decreasing towards V + 46. The 25L leaf glyceric acid decreased during Interval 1, and among treatments, FL leaves contained more glyceric acid from V + 27.

Shikimate pathway derivatives: For amino acids, leaf phenylalanine accumulated during Interval 2 regardless of the treatments, before decreasing during Interval 3. However, 25L leaves contained more phenylalanine than those of FL at V + 37. Leaf tyrosine also accumulated during Interval 2, before depleting with no

significant concentration differences among treatments. Likewise, tryptophan accumulated during Interval 2 before depletion in 25L leaves at V + 37, while increasing between V + 9 and V + 27 in FL leaves. In FL leaves, 5-hydroxytryptophan decreased between V + 9 and V + 37, while reducing during Interval 1 and between V + 18 and V + 37 in 25L leaves. Among treatments, FL leaves contained more 5-hydroxytryptophan at V + 27 and V + 37.

Caffeic acid depleted in the leaves of both treatments between V + 9 and V + 46, without significant treatment differences. On the other hand, gallic acid was significantly less abundant at V + 27 than at V + 9 in FL leaves, while 25L leaf gallic acid reduced during Interval 1. FL leaf gallic acid was more abundant than that of 25L leaves from V + 37. Arbutin accumulated in leaves of both treatments between V + 9 and V + 37, and additionally during Interval 4 in those of 25L. The 25L leaves subsequently contained most arbutin at V + 46. While FL (+)-Catechin did not change significantly, it decreased between V + 9 and V + 37 in 25L leaves, before increasing during Interval 4. The 25L leaves contained more (+)-catechin than those of FL at V + 46.

TCA cycle intermediates: Citric acid reduced in leaves of both treatments during Interval 1 and between V + 18 and V + 37. However, among treatments, FL leaves contained more citric acid from V + 18.

Amino acid metabolism: FL and 25L leaf glutamic acid decreased during Interval 1, before increasing in FL leaves during Interval 2. However, no leaf glutamic acid treatment differences were observed. While FL leaf GABA concentration did not change significantly, that in 25L leaves accumulated during Interval 3. The 25L leaves subsequently exhibited more GABA at V + 18, and from V + 37.

FL leaf aspartic acid decreased between V + 9 and V + 37, while that in 25L leaves decreased both, during Interval 1, and between V + 18 and V + 37. However, no leaf aspartic acid treatment differences were observed. Threonine decreased in leaves of both treatments during Interval 1, before accumulating in those of 25L during Interval 3. Among treatments, 25L leaves contained most threonine from V + 18. While FL leaf isoleucine concentration did not change significantly, that of 25L leaves accumulated during Interval 4. The 25L leaves subsequently contained most isoleucine at V + 27 and V + 46. FL leaf leucine concentration did not alter significantly, however, 25L leaf leucine accumulated between V + 9 and V + 46. Among treatments, 25L leaves contained most leucine at V + 46. Although no significant valine concentration changes occurred for both treatments, 25L leaves contained more valine at V + 27 and V + 46.

Serine decreased in FL leaves between V + 9 and V + 27, while increasing in 25L leaves during Intervals 1 and 3. The 25L leaves contained most serine among treatments from V + 18. Cysteine accumulated in FL leaves during Interval 2, while it accumulated in 25L leaves between V + 18 and V + 37. The 25L leaves contained more cysteine than those of FL at V + 37.

Relationship between root starch and *myo*-inositol

Changes in root starch and *myo*-inositol concentrations were similar over time (Fig. 5). In FL roots, both starch and *myo*-inositol significantly declined from V + 9 to V + 37 but recovered to the abundance at V + 9, by V + 46. For 25L and NL, root starch and *myo*-inositol declined significantly between V + 9 and V + 37, and plateaued during Interval 4. The relationship between starch and *myo*-inositol is additionally illustrated in Fig. S1.

Discussion

The current study evaluated implications of reduced carbohydrate and N source-sink biomass ratios during berry maturation for metabolite concentrations of the major source organs. Specific defoliation induced changes in root and leaf carbohydrate and N utilization, and linkages to primary metabolism, are explained below. Leading into the discussion, it is particularly noteworthy that decreasing source-sink ratios during berry ripening distinctly increased root carbohydrate source activity. Conversely, removing the leaf N source only had minor effects on total N re-distribution from leaves and roots. Defoliation did, however, alter N composition of roots and leaves, suggesting increased amino N source activity when the total vine N source-sink biomass ratio was reduced.

Root carbohydrate reserve remobilization

Following the removal of all leaves in the full defoliation treatment, root starch concentrations rapidly declined (Fig. 1B). However, the continued accumulation of berry sugar in the corresponding period, albeit at a lower rate than control vines, indicates a clear contribution of reserve carbohydrates to berry ripening (Fig. 1A). Such contributions from stored carbohydrates have previously been demonstrated by ^{14}C labeling, when carbohydrates from perennial tissues were translocated to fruit after defoliation during fruit sugar accumulation (Candolfi-Vasconcelos et al. 1994). The retention of some leaves on partially defoliated vines did not alter the rate of root carbohydrate mobilization relative to

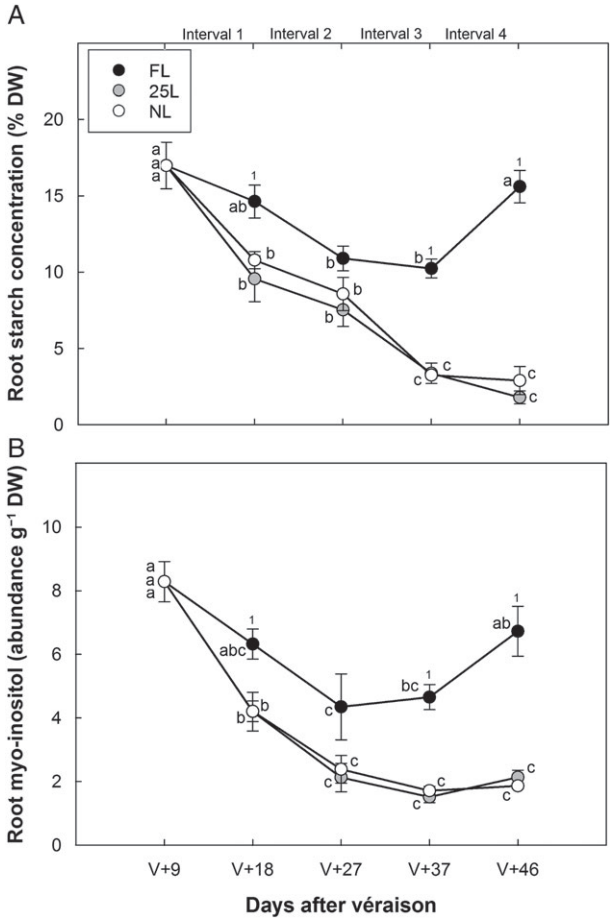

Fig. 5. Impact of defoliation (full leaf – FL, 25% leaves – 25 L and no leaf – NL) on root starch (A) and myo-inositol (B) concentration during the experimental period (mean ± SE; n = 3). Significant differences ($P < 0.05$) between harvest dates for each treatment are indicated by different lower-case letters. Significant differences ($P < 0.05$) between treatments at each harvest date are indicated by a numeral [1: FL > (25 L and NL)].

the fully defoliated vines, but the availability of extra carbohydrates from concurrent photosynthesis did allow an increased rate of berry sugar accumulation. Under full leaf area, root starch concentration reduced only during the phase of rapid fruit sugar accumulation, and then increased when fruit sugar accumulation slowed. This starch reduction may imply reserve remobilization towards the sugar-accumulating berries as these vines carried a substantial crop load. However, root reserves could also be utilized for respiration and structural development (Holzapfel et al. 2010), of which latter was observed during Interval 1 under full leaf area.

Total N distribution was unaffected by defoliation

Under full leaf area, fruit sugar content per vine increased by 252% in the 37-day period following the start of the experiment (Fig. 1A). During the same period fruit N content only increased by 39% (Fig. 2A), implying a proportionally greater importance of the véraison to harvest period for berry carbohydrate than N accumulation. The leaf N concentrations of 2.1% (Fig. 2C) were adequate according to published levels (Holzapfel and Treeby 2007), and suggest that the lower N accumulation reflected lower fruit sink demand rather than reduced availability of N from the vegetative parts of the vine.

Although fruit N accumulation rates varied somewhat across the five sampling dates, the fruit did not exhibit any significant N content treatment differences by the final harvest. Therefore, despite the reduction or complete removal of leaves as an N source (Rossouw et al. 2017b), fruit N accumulation was maintained. The lack of change in root N concentration after partial or full defoliation (Fig. 2B), suggests the roots did not become a significant net source during fruit N accumulation (Conradie 1991). Although N fertilization was ceased a month prior the experiment, it is likely that soil N uptake contributed to maintaining fruit N accumulation. Soil N uptake is not unusual shortly after véraison (Löhnertz 1991), and the limitation or absence of the leaf N source did not interfere with N allocation towards the fruit by the final harvest.

A central role for *myo*-inositol during root carbohydrate remobilization

The parallel changes of root starch and *myo*-inositol concentrations with treatment and time (Fig. 5), suggests a role for *myo*-inositol during root carbohydrate remobilization. The roles of *myo*-inositol in plants include signaling, involvement in the synthesis of cell wall polysaccharides, and precursor for other metabolites including galactinol, raffinose and ascorbic acid (Valluru and Van den Ende 2011). However, while *myo*-inositol has been found in the phloem sap of various plants, suggesting a potential long-distance transport role (Noiraud et al. 2001), *myo*-inositol is not generally considered a major C transport compound. The close similarities between starch and *myo*-inositol concentration profiles in the grapevine roots of the present study is an original result, and further investigation is needed to determine the underlying connection. The impact of defoliation on the decreased root concentrations of *myo*-inositol and its derivatives, i.e. galactinol and raffinose (Loewus and Murthy 2000) (Fig. 3), suggests that these metabolites play a role during carbohydrate reserve remobilization.

Similar to *myo*-inositol, root ascorbic acid depleted shortly after partial or full defoliation (Fig. 3). It has been established that *myo*-inositol metabolism provides

an alternative pathway for ascorbic acid biosynthesis (Lorence et al. 2004). The similarities in root *myo*-inositol and ascorbic acid concentrations over time, in terms of both, for example, exhibiting higher concentration in FL roots by V + 46, is therefore potentially related to *myo*-inositol providing the initial substrate towards an ascorbic acid biosynthetic route (Lorence et al. 2004, Valpuesta and Botella 2004). Furthermore, ascorbic acid is a precursor for tartaric acid, and defoliation led to a depletion of root ascorbic acid, while tartaric acid accumulated (Fig. 3). Therefore, root ascorbic acid catabolism potentially resulted in tartaric acid accumulation (DeBolt et al. 2006). Additionally, root citric acid also accumulated under reduced leaf area (Fig. 3). Intermediates of the TCA cycle, such as citric acid, play an essential role during C metabolism, supplying C skeletons for the biosynthesis of various metabolites, such as phenolic compounds and amino acids (Popova and Pinheiro de Carvalho 1998).

Defoliation induced root amino acid accumulation

Glutamic acid was the only root amino acid that depleted under partial and full defoliation (Fig. 3). The biosynthesis of amino acids in higher plants mainly occurs in roots and mature leaves, from where it is transportable to sinks, thereby facilitating the distribution of organic N between plant organs (Rentsch et al. 2007). Although defoliation did not affect the root total N concentration, it did impact on root amino N composition. As an essential amino-group donor during the synthesis of many other amino acids, glutamic acid plays a crucial role during N partitioning (Forde and Lea 2007). The depletion of root glutamic acid after partial and full defoliation may indicate its involvement in amino N repartitioning within these roots.

Various other amino acids accumulated in the roots of partially or fully defoliated vines, including arginine, lysine, phenylalanine, tryptophan, and tyrosine (Fig. 3). It can thus be proposed that glutamic acid metabolism was involved in the accumulation of these amino acids in the roots, either directly by providing a C skeleton for arginine synthesis (Berg et al. 2002), or as an amino donor towards the synthesis of the others. The accumulation of arginine in roots, only after partial or full defoliation may relate to a N transport role. In fact, arginine is characterized by a high N:C ratio, and is known to be transported in the xylem and phloem between source and sink organs, facilitating organic N distribution (Lea et al. 2007). When the leaves as a N source was restricted or limited, root amino acid accumulation presumably contributed to the root amino N source activity.

The aromatic amino acids (i.e. phenylalanine, tryptophan and tyrosine) only accumulated in the roots after full defoliation (Fig. 3). These amino acids originate from the shikimate pathway, and are precursors for many secondary metabolites, including phenolic compounds (Maeda and Dudareva 2012). Various secondary metabolites derived from the aromatic amino acids, including anthocyanins (Boss et al. 1996), accumulate in post-véraison grapevine berries. The removal of leaves as an amino acid source seemingly induced the biosynthesis of root amino acids through the shikimate pathway. Genes related to the aromatic amino acids are expressed in post-véraison grapevine berries (Berdeja et al. 2015). However, many amino acids, including phenylalanine, are also present in the vascular tissues of higher plants (as for example, indicated in *Trifolium repens* and *Lupinus albus*), where they accrete after defoliation (Hartwig and Trommler 2001). The possibility is, therefore, raised that grapevine leaves are important aromatic amino acid sources, from where they may be phloem translocate to the fruit to contribute to secondary metabolism. The roots may become an alternative aromatic amino acid source after exclusion of the leaf source.

Leaf sugar alcohols and organic acids depleted rapidly after partial defoliation

Myo-inositol and mannitol, some of the most prevalent sugar alcohols in higher plants (Noiraud et al. 2001), depleted rapidly in remaining leaves after partial defoliation (Fig. 4). Apart from sucrose as the principal plant transported sugar, some sugar alcohols and raffinose family oligosaccharides can also be important C transporters (Noiraud et al. 2001). Mannitol, unlike *myo*-inositol, is a known primary photosynthetic product in mature leaves, and a recognized transport compound (Noiraud et al. 2001). During the present study, mannitol was, therefore, presumably synthesized in leaves during photosynthesis, and its rapid depletion in leaves after partial defoliation suggests an important C transport role during limited canopy photoassimilation. Although there has been ambiguity around the transport role of *myo*-inositol (Noiraud et al. 2001), the current study suggests that root *myo*-inositol plays a central role during carbohydrate remobilization, and perhaps similarly in leaves.

In addition to the sugar alcohols mentioned above, various leaf organic acids rapidly depleted after partial defoliation (Fig. 4). Citric acid was among the organic acids depleted under reduced leaf area, and like other TCA intermediates, it is a vital metabolic branch point as its conversion provides C skeletons for N assimilation, in addition to playing an important role in plant energy and C metabolism (Popova and Pinheiro de Carvalho 1998). Leaf ascorbic acid concentration, like that of *myo*-inositol, was negatively impacted by

partial defoliation (Fig. 4). As a potential precursor, leaf *myo*-inositol metabolism may have affected the ascorbic acid concentration. Likewise, leaf tartaric acid, threonic acid and glyceric acid also depleted after partial defoliation (Fig. 4). These organic acids are derived from ascorbic acid (Loewus 1999), further indicating a change in ascorbic acid metabolism in remaining source leaves. The phenolic acids, caffeic acid and gallic acid, depleted towards the end of berry ripening in remaining leaves after partial defoliation, while arbutin and (+)-catechin increased (Fig. 4). These compounds are products of the shikimate pathway (Siegler 1998, Balasundram et al. 2006) and, therefore, like in roots, leaf metabolites yielded from the shikimate pathway were affected by post-véraison leaf source limitation.

Partial defoliation induced leaf amino acid accumulation

Various amino acids accumulated in remaining leaves after partial defoliation (Fig. 4). Amino acid synthesis in plants, as mentioned above, mainly occurs in roots and mature leaves, where they are utilized or stored, or exported to sinks to contribute to growth and secondary metabolism (Rentsch et al. 2007). Leaf proteins (e.g. Rubisco and chloroplast proteins) are extensively degraded during leaf aging, thereby producing free amino acids, outsourceable to sinks (Masclaux-Daubresse et al. 2010). In the present study, GABA, leucine, isoleucine, as well as cysteine and serine were among the amino acids that accumulated in the remaining leaves after partial defoliation (Fig. 4). After defoliation in the present study, the ratio of the leaf N source to the fruit N sink was drastically reduced. It could, therefore, be argued that the increased source requirement placed upon the remaining leaves of treatment 25L advanced its aging process, prompting amino acid accumulation and its subsequent exportation.

Conclusion

A study was conducted to determine the implications of reduced leaf carbohydrate and N source availability, during a period of considerable fruit C and N sink demand, on remaining leaf or root source activity. A focus was placed upon primary metabolite abundance responses in the source organs, ultimately with the goal of identifying metabolites that contribute to carbohydrate and N source functioning in roots and leaves. In terms of carbohydrate distribution, post-véraison leaf source absence slowed, but did not completely stop fruit sugar accumulation. In the absence of leaf C assimilation, root starch provided an alternative C source for berry ripening. Root *myo*-inositol concentrations were directly related to starch concentration, suggesting an important, if yet to be elucidated role, in starch metabolism. Furthermore, the depletion of *myo*-inositol metabolism derived metabolites (galactinol, raffinose and ascorbic acid) after defoliation, illustrates the contribution of this proposed pathway towards root carbohydrate source functioning. Compared to carbohydrates, defoliation did not have a considerable effect on fruit N content. In fact, vegetative N pools did not contribute to post-véraison fruit N requirements, which instead appear to have been met by root uptake from the soil and/or regulation of amino N composition in the leaves and/or roots. However, arginine and shikimate pathway derived root aromatic amino acids did accumulate after full defoliation, indicating there is a least a response in this pool in roots during leaf N absence. The remaining leaves also accumulated various amino acids (including GABA, leucine, isoleucine and serine) after partial defoliation, suggesting protein degradation could make a small N contribution to the fruit. Overall, this study has shown that *myo*-inositol metabolism and the flux through the shikimate pathway play central roles in grapevine carbohydrate and N source organs during fruit ripening. The findings of this study contribute to understanding leaf and root C and N metabolism and utilization during fruit maturation.

Authors' contributions

G.C.R. conducted the experiment, wrote the body of the paper, and carried out sample preparations, and laboratory and data analyses. K.Š. contributed to sample preparation and led the GC/MS analysis. B.A.O. contributed to the experimental layout and conducted statistical analyses. B.P.H. coordinated the project and supported the experimental planning. A.D. contributed to treatment planning and experimental design. J.P.S. and C.B. reviewed the methods and results. All authors reviewed, edited and approved the final version of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Root metabolite concentration and GC/MS information.

Table S2. Leaf metabolite concentration and GC/MS information.

Fig. S1. Linear and curvilinear trends of root starch concentration and *myo*-inositol concentration.

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