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Spatiotemporal changes in the accumulation of sugar and potassium within individual 'Sauvignon Blanc' (*Vitis vinifera* L.) berries

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Summary

It has been speculated that there may be a link between the transport of sugar and potassium into grape berries during ripening as they exhibit similar accumulation patterns. It is unclear if this proposed link is apparent in individual grape berries and in the grape berry compartments. Single grape berries were therefore analysed for sugar and potassium content and concentration within the skin, seeds and the pulp from pre-véraison until harvest. Sugar and potassium had similar accumulation patterns and positive relationships were confirmed between the sugar and potassium content within individual berries and compartments. The sugar content in the grape berry, however, increased 5-fold during ripening whereas the potassium content only doubled. Both sugar and potassium increased with berry size, suggesting a ternary relationship with berry water. The high variability in sugar and potassium contents between berries however affirms plasticity in their accumulation within individual berries.

K e y w o r d s : potassium; soluble sugars; 'Sauvignon Blanc'; grape berries; berry compartments.

Introduction

In the grapevine, sucrose is the main photoassimilate translocated by the phloem from the leaves to the sink organs, with assimilate partitioning prioritized towards the reproductive sinks, the grape berries (Keller, 2010). The allocation is, however, dependent on competing sink strengths and the stage of berry development (Conde *et al.* 2007, Keller 2010). At the phloem unloading point, the majority of the sucrose is hydrolysed by invertase into glucose and fructose. The hexose sugars accumulate mainly in the vacuoles of the pulp cells roughly in equal amounts (Conde *et al.* 2007). Several sucrose and hexose transporters have been identified that facilitate the unloading and translocation of these soluble sugars within the grape berry (Lecourieux *et al.* 2014).

Potassium (K^+) is the most abundant macronutrient in the grapevine and grape berries, and is essential for growth and development (Conde *et al.* 2007). Unlike sugar, K^+, which is xylem and phloem mobile, accumulates during early berry development with the import rate increasing after véraison (Rogiers *et al.* 2006a). The roles of K^+ within the grapevine and grape berry include: enzyme activation; contribution towards turgor control and cell expansion through osmotic potential regulation as the major ion; neutralization of soluble and insoluble anions in the cytosol to optimise the cytosolic pH for enzyme reactions; and regulating the rate of loading and long distance transport of assimilates through the phloem from the sink to the source organs (Marschner 1995, Mpelasoka *et al.* 2003).

It is speculated that there may be a functional link between the transport of sugar and K^+ into grape berries during ripening due to the positive relationship observed between berry K^+ and sugar (reviewed by Mpelasoka *et al.* 2003) and in view of their similar accumulation patterns (Rogiers *et al.* 2006b). The supposed link has thus far been attributed to the already established role of K^+ in the loading, long distance transport and unloading of sugar from the phloem in other plant systems (Holbrook and Zwieniecki 2005) and the co-regulation of sugar and K^+ transporters and channels (Ache *et al.* 2001, Deeken *et al.* 2002). The relationship between sugar and K^+ previously observed in grape berries was, however, determined on berries that were usually pooled according to common attributes such as the sampling date or the number of seeds (Bertoldi *et al.* 2011, Rogiers *et al.* 2006a, Walker *et al.* 2005). Given the heterogeneity of berries within a sampling population and their asynchronous and independent ripening (Coombe 1992, Ollat *et al.* 2002), assessing a pooled berry population may mask significant differences in the accumulation patterns

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between individual berries. The primary objective of this study was thus to investigate how the accumulation patterns of sugar and K^+ relate to each other within individual berries during ripening and the main compartments, specifically the skin (exocarp), pulp (mesocarp, endocarp) and seeds, which differ in morphology and function (Mpelasoka *et al.* 2003). The sugar and K^+ contents and concentrations were therefore determined on single 'Sauvignon Blanc' grape berries sampled regularly from prior to véraison until harvest from field-grown vines.

Material and Methods

Experimental vineyard and sampling: Twelve 'Sauvignon Blanc' (*Vitis vinifera* L. clone F4V6) own-rooted vines, located in the National Wine and Grape Industry Centre replicated variety vineyard, Wagga Wagga, Australia (35°3'38.57"S; 147°21'42.89"E; 121 m), were used in the study. The vineyard was planted in 2005 in a randomized block design of ten varieties in six rows. Each variety was repeated six times in a three vine panel with one panel of each variety per row. Row and vine spacing were 3.0 m by 1.5 m and a north-south row direction. Vines were trellised as a double cordon sprawling system on a single wire and hand pruned to two buds per spur in winter. Plants were of intermediate vigour and carried on average 14 spurs per vine, 28 buds per vine, 7 buds per metre of row. Main phenological stages (Dry and Coombe 2005) were visually determined daily and noted as the mean date for the 12 vines. Soil moisture was continuously monitored by a Yara ZIM technology soil moisture sensor (Yara Water Solution, Hennigsdorf, Germany) and the irrigation applied according to real-time data (Yara Water Solution software), maintaining the volumetric soil water content at ≈ 20 % during ripening. The mesoclimate (solar radiation, air temperature, relative humidity and rainfall) was monitored at 30 min intervals during the ripening period with a Campbell Scientific automated weather station situated adjacent to the vineyard.

Samples were collected weekly (S1 to S7) from 1 January 2014 until 12 February 2014 at 51, 57, 64, 71, 78, 85 and 93 days after flowering (DAF). Sixteen representative bunches (four bunches per replicate) were identified per sampling date through randomly generated duplex codes. The first code (1 to 6) identified the vine number and vine face (1 to 3 indicating the east face of vine 1 to vine 3, or 4 to 6 indicating the west face of vines 1 to 3) within a replicate block. The second code (0 to 100) indicated the bunch position on the vine, with 0 and 100 representing the far left and far right of the cordon arms respectively, and 50 the position directly above the trunk. This scale was partitioned into 10 segments. No distinction was made between distal and basal bunches. Three berries were sampled per identified bunch (n = 48 per sampling date), ensuring berries were selected randomly and were representative of the proximal, intermediate and apical as well as the internal/external positions on the bunch. Berries were transported to the neighbouring laboratory and stored at -20 °C until further analyses.

Berry classification and partitioning: Each individual berry (n = 336) was weighed (Sartorius TE214S, Sartorius AG, Goettingen, Germany) and the fresh mass (FM) recorded. Berries were then classified according to their diameter (Šuklje *et al.* 2012) which resulted in four categories (C1 to C4) with the number of berries per category generally following a normal distribution within sampling dates (supplementary Tab. 1). Each frozen berry was then carefully separated into the skin, pulp and seeds and placed in 2 mL Eppendorf tubes, ensuring tissues remained frozen, and the number of seeds and the mass per berry compartment (from here on referred to as the FM) recorded. The relative contributions of the pulp, skin and seeds to the total berry FM (supplementary Tab. 2) was in agreement with published literature (Bertoldi *et al.* 2011).

Sample preparation and analyses: Samples were homogenized with a Benchmark D1000 handheld homogenizer (Benchmark Scientific, Edison, USA) using a 5 mm generator head for the skin and pulp and a 7 mm generator head for the seeds. To facilitate homogenization, 0.5 mL deionized water was added to the skin and seed samples.

Total K^+ analyses: Homogenized samples (0.05 mL) were pipetted (MICROMAN positive displacement pipette, Gilson Inc., Middleton, USA) into 3 mL borosilicate glass culture tubes, 1 mL 70 % (w/w) nitric acid (Univar, USA) added, and digested in a fume hood in a water bath at 95 °C until orange fumes were no longer visible (≈ 24 h). The digested samples were made up to 10 mL with deionized water and 0.1 mL of 1 $mg \cdot mL^{-1}$ aqueous caesium chloride (BDH Analytical Chemicals, UK) added as an ionization suppressor. The total K^+ concentration was determined through flame atomic absorption spectrometry with a Varian SpectrAA 50B spectrometer (Agilent Technologies, Santa Clara, USA) equipped with a Na/K hollow cathode lamp (S. and J. Juniper and Co., Essex, England) and an air-acetylene burner. Instrument parameters were set as recommended by the manufacturer (Agilent Technologies, Santa Clara, USA) and the total K^+ concentration in the skin and pulp determined at 769.9 nm and in the seeds at 766.6 nm due to the differing K^+ concentrations within the compartments.

Soluble sugar analyses: The homogenized samples were centrifuged for 3 min at 16 000 x g (Eppendorf 5415C Microcentrifuge, Eppendorf AG, Hamburg, Germany) and 0.1 mL of the supernatant pipetted into a 1.5 mL Eppendorf tube. Samples were made up to 1.5 mL with deionized water and filtered through 0.22 µm Milex-GP sterile disposable syringe filters (Millipore, Bedford, MA, USA). Glucose and fructose were individually analysed with an automated spectrophotometer (Konelab Arena 20XT photometric analyser, Thermo Fisher Scientific Inc., Vantaa, Finland) with commercial D-glucose and D-fructose system reagents (Thermo Fisher Scientific Inc., Vantaa, Finland) designed and validated for this equipment. Glucose and fructose were generally present at ratios ≈ 1:1 (data not shown), so total sugars (the sum of glucose and fructose) were used for data interpretation.

The total soluble solids (TSS) of the pulp was determined from the supernatant with a digital handheld refrac-

tometer (ATAGO PAL-1, ATAGO Co. Ltd, Tokyo, Japan). The TSS, measured as °Brix, was used to calculate pulp density (ρ) where °Brix = (220 x (ρ – 1)) + 1.6 (Rotter 2016). The *ρ*-value in turn was used to determine the mass of the 0.1 mL pulp sample in order to calculate the content of the sugar per fresh mass of pulp.

Data and statistical analyses: The contents of complete berries were calculated as the sum of the separate compartments. Data were analysed with the use of statistical (STATISTICA® 12, StatSoft Inc., Dell Software, Round Rock, TX, USA) and graphing (SigmaPlot 11, Systat Software Inc., San Jose, CA, USA) softwares. Significant differences between sampling dates were determined by repeated measure analyses of variance (ANOVA) and bivariate relationships by Pearson's correlation coefficients. Means are presented ± the standard error (SE).

Results and Discussion

Climate and phenology: The mean day (6:00 am to 8:00 pm) and night (8:30 pm to 5:30 am) mesoclimatic temperatures for the period of ripening (37 d in total from véraison to harvest) were 29.6 and 25.6 °C, respectively, with one discernible (> 1.5 mm) rain event of 13.3 mm occurring 74 DAF (24 January 2014). The mean day temperature was above 30 °C for 51 % of the ripening period (supplementary Fig. 1).

The mean budburst date (E-L 4) was September 18, 2013 and flowering (E-L 23) occurred on November 11, 2013. The date of véraison (E-L 35) was estimated to be January 7, 2014 (57 DAF) which coincided with the second sampling date (S2). Berries were determined to be harvest ripe (E-L 38) on February 12, 2014 (93 DAF), the final date of sampling (S7).

Fresh mass, sugar and potassium accumulation patterns during the ripening period: Berry FM increased steadily during ripening (Fig. 1 (a: i)), suggesting that vines experienced minimal water constraint during the ripening period (Deloire *et al.* 2004). The slight decrease in the mean berry FM at S7 (≈ 4 %, significant at $p < 0.09$, Fig. 1 (a; i)) was likely due to reduced phloem unloading resulting in a reduction of the net hydraulic rate with a continued transpiration rate (Coombe and McCarthy 2000, Rogiers *et al.* 2004, Tilbrook and Tyerman 2009) and/or backflow through the xylem (Keller *et al.* 2006, Tilbrook and Tyerman 2009). The increase in the mean sugar concentration without an increase in the mean sugar content points towards a loss of water from the berry, resulting in the concentration of sugar. Further evidence for the presence of backflow was apparent in the K^+ content data, where this element decreased on the final sampling date.

Berry sugar and K^+ contents increased steadily from S1 to S6 (Fig. 1 (a: ii) and (a: iii)) as berry FM increased, consistent with at least an indirect relationship in their accumulation during ripening. Sugar accumulation per berry abated between S6 and S7 (Fig. 1 (a: ii)), corroborating the idea that phloem flow decreased towards the end of ripening as alluded to before. Sugar concentration continued to increase throughout ripening (Fig. 1 (a: ii)) but K^+ accumulation was temporarily interrupted between S4 and S5 (Fig. 1 (a: iii)).

As expected, sugar and K^+ accumulation patterns differed between the berry compartments. The skin and pulp had relatively similar sugar concentrations at S7, however, sugar contents were five times higher in the pulp than in the skin (Fig. 1 (c: ii) and (d: ii)). Potassium was present in the berry compartments prior to the onset of véraison (Fig. 1 (b: iii), (c: iii) and (d: iii)) as K^+ accumulates in the berry throughout development (Davies *et al.* 2006, Rogiers *et al.* 2006a). During the pre-véraison period, K^+ fulfils a number of roles, for example, in cell expansion following cell division (Davies *et al.* 2006). Potassium concentration was highest in the skin (Fig. 1 (c; iii), followed by the pulp (Fig. 1 (d; iii) and seeds (Fig. 1 (b; iii), with the largest K^+ content located in the pulp, followed by the skin and minor quantities in the seeds (Fig. 1 (b; iii) to (d; iii)). This is in accordance with previous observations (Bertoldi *et al.* 2011, Mpelasoka *et al.* 2003, Rogiers *et al.* 2006a, Walker *et al.* 2005).

Seeds have distinct cell structures and morphology (Gerós *et al.* 2012) and hence the mechanisms for the import and storage of sugars and K^+ are expected to differ from the remainder of the grape berry tissues. The seeds reached a maximum mean FM (Fig. 1 (b: i)) at S2 (véraison) signifying the end of seed maturation and the start of seed desiccation (Fait *et al.* 2006, Gouthu and Deluc 2015, Gutierrez *et al.* 2007). The increase in sugar content within the seeds (Fig. 1 (b: ii)) was interrupted by a lag phase from S2 to S6, but resumed during the late ripening period (S7). This lag phase may be associated with carbohydrate diversion from accumulation in seeds to reserve restoration within the woody tissues as noted by Rogiers *et al.* (2006a). Potassium accumulation generally closely followed the accumulation pattern of sugar, in content and concentration. The K^+ concentration however remained stable from S4 to S7 (Fig. 1 (c: iii)), indicating that K^+ was accumulated at a similar rate as the increase in seed FM (Fig. 1 (c: i)).

Sugar content steadily increased in the skin as ripening progressed, in accordance with previous studies (Possner and Kliewer 1985). A rapid increase in K^+ concentration also occurred within the skin from S1 to S2 (Fig. 1 (c: iii)), corresponding to véraison. Potassium may play a role at the initiation of véraison in the loosening of the cell walls of the berry skin to permit rapid berry enlargement (Taiz 1984). The decrease in K^+ content in the skin from S2 to S3 following véraison (Fig. 1 (c: iii)), also observed at this developmental period by Iland and Coombe (1988), could potentially be due to the redistribution of K^+ towards the pulp to aid in the initial rapid cell expansion and sugar influx into the grape berry following véraison (Fig. 1 (d: i) and (d: ii)).

The accumulation patterns of sugar and K^+ in the pulp, as well as the change in FM, closely follow those of the complete berry (Fig. 1 (d: i) to (d: iii)) which is expected considering this compartment is the largest contributor to the berry mass (supplementary Tab. 2).

Rates of sugar and potassium accumulation during the ripening period: Although the patterns of sugar and K^+ accumulation in the grape berry

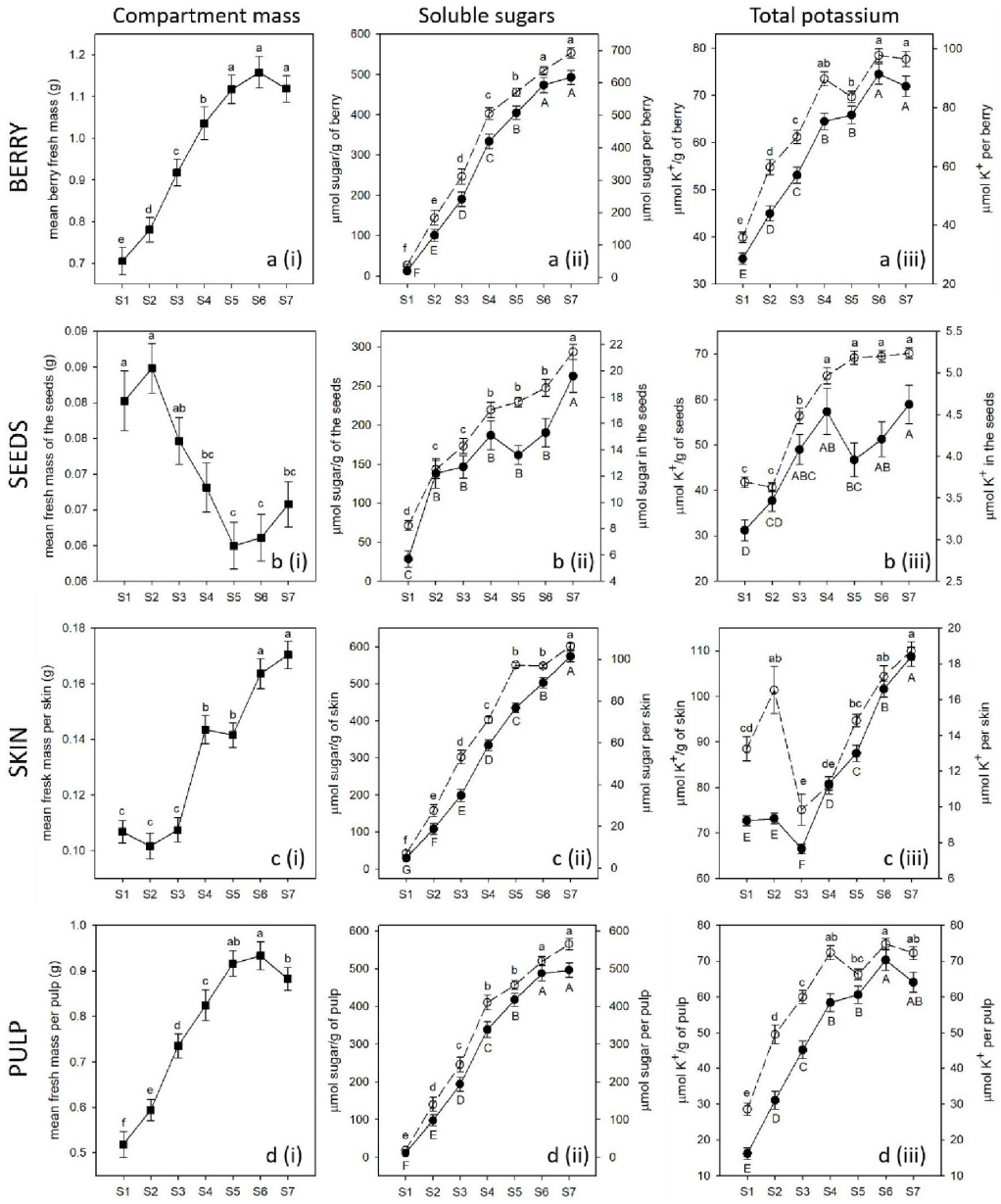

Fig. 1: Fresh mass (FM) per berry or berry compartment (**i**) and accumulation patterns of soluble sugars (**ii**: glucose and fructose) and total potassium (**iii**: K^+) across the sampling period for the complete berry (**a**: n = 226), seeds (**b**: n = 287), skin (**c**: n = 285) and pulp (**d**: n = 286). Values are means ± SE for each sampling date (S1 to S7). Concentration (µmol·g^{-1} of fresh tissue) is indicated with a hollow symbol and broken line, while the content (µmol per tissue) is indicated with a solid line and filled symbol. Mean values assigned with different letters per sampling date indicate significant differences ($p < 0.05$) between sampling dates, with lower case letters assigned to the concentration and capital letters for the content.

were generally similar during ripening (Fig. 1), it was evident that there were differences in the accumulation rates of sugar and K^+ between sampling dates for the total berry population and within the berry size categories (Tab. 1).

Total berry population per sampling date: The daily sugar accumulation rate for the total population increased from véraison onwards, attaining a maximum rate of 52.20 µmol sugar per berry per day between S3 and S4 (Tab. 1). The daily rate thereafter decelerated, slowing down to only 10.85 µmol sugar per berry per day towards the end of ripening (S6 to S7) (Tab. 1) when a disruption in the phloem flow is said to occur (COOMBE and MCCARTHY 2000, ROGIERS *et al.* 2004). As some sugar is still accumulated in the berry at this stage, it indicates that phloem flow could not have been completely disrupted towards the end of ripening in this study (Tab. 1).

The daily berry K^+ accumulation rate was high during the period preceding véraison (S1 to S2, Tab. 1), most likely because K^+ is the main ion acting as an osmoticum during this period (DAVIES *et al.* 2006).

Simultaneous to the decrease in the sugar accumulation rate between S4 and S5, the K^+ accumulation rate plummeted by 86 % to only 0.31 µmol per berry per day (Tab. 1), supporting the hypothesis that sugar and K^+ accumulation in the grape berry may be linked. The sudden increase in the daily K^+ accumulation rate between S5 and S6 (Tab. 1), also observed in both skin and pulp (Fig. 1 (c: iii) and (d: iii)), may be explained by the rain event prior

Table 1

The daily rate of change of the fresh mass (FM) and sugar and potassium (K^+) accumulation (µmol per day) between sampling dates. The ratio between the rate of sugar and K^+ accumulation per sampling date is also indicated. C1 to C4 represent the berry size categories from the smallest to the largest berries

Berry population	Sampling date	Rate of change per berry per day			
		mg FM per day†	µmol sugar per day†	µmol K^+ per day†	Daily sugar to K^+ ratio
All	S1 to S2	10.69	28.59	2.41	11.84
	S2 to S3	19.59	33.53	1.85	18.16
	S3 to S4	16.86	52.20	2.26	23.11
	S4 to S5	11.72	24.26	0.31	77.92
	S5 to S6	5.73	22.49	2.36	9.53
	S6 to S7	-5.60	10.85	-1.60	-6.78
C1	S1 to S2	22.41	7.29	1.70	4.29
	S2 to S3	3.54	15.13	0.64	23.55
	S3 to S4	19.46	50.03	3.32	15.09
	S4 to S5	9.91	37.82	0.37	102.77
	S5 to S6	-3.49	5.95	1.14	5.23
	S6 to S7	14.55	34.93	-0.42	-82.59
C2	S1 to S2	39.68	23.04	3.41	6.76
	S2 to S3	1.82	19.85	0.61	32.38
	S3 to S4	38.50	79.51	3.89	20.44
	S4 to S5	-1.79	3.51	-0.72	-4.83
	S5 to S6	8.03	28.46	2.50	11.40
	S6 to S7	3.42	15.01	0.77	19.43
C3	S1 to S2	27.02	40.89	3.43	11.93
	S2 to S3	9.76	31.54	1.15	27.51
	S3 to S4	34.80	56.94	4.22	13.48
	S4 to S5	-4.61	13.39	-1.28	-10.46
	S5 to S6	17.31	24.38	2.58	9.45
	S6 to S7	-3.29	9.64	-1.71	-5.62
C4	S1 to S2	34.43	78.32	3.90	20.06
	S2 to S3	-0.90	4.69	1.19	3.94
	S3 to S4	59.20	81.11	3.89	20.85
	S4 to S5	-12.89	18.07	0.15	122.76
	S5 to S6	5.10	43.35	2.30	18.81
	S6 to S7	1.60	29.33	3.64	8.05

† Rate per day was calculated as the difference in the content between two consecutive sampling dates, divided between the number of days between the sampling dates (sampling dates were weekly from January 1st, 2014 to February 12, 2014).

to S5 (supplementary Fig. 1) considering that the presence of water, especially precipitation, favours the transport of mineral elements throughout the grapevine (Esteban *et al.*, 1999). The loss of 1.60 µmol K^+ per berry per day between S6 and S7 (Tab. 1) corroborates the potential efflux of this metabolite via the xylem from the grape berry as mentioned in the previous section.

Even though K^+ and sugar were present in the grape berry at a ratio of 0.64 at S1 (data not shown), sugar accumulated at an accelerated rate compared to K^+ from véraison (S2) onwards. Between S3 and S4, sugar accumulated ≈ 23 times faster than K^+ per berry per day (Tab. 1), and up to 77 times faster even after sugar and K^+ accumulation slowed down (S4 to S5), clearly indicating that the magnitude of increase in sugar content is not closely followed by that of K^+ in the berry. Freeman and Kliewer (1983) have stated that K^+ does not compete with sugars to reach berries, which is supported by observations in this study, suggesting that if there is a link, it is heavily skewed in favour of sugar accumulation over K^+ accumulation.

Within berry size categories: The daily rate of sugar, K^+ and FM accumulation during ripening differed between berry diameter categories (Tab. 1). Similar accumulation trends were apparent in C2 and C3 (Tab. 1), the categories generally contributing the largest number of berries to the total berry population (supplementary Tab. 1). Between S4 and S5, C2 and C3 exhibited a sudden interruption in the daily sugar accumulation rate to 3.51 and 13.39 µmol sugar per berry per day, respectively, which was not apparent in the total berry population (Tab. 1). The slight loss in berry mass and K^+ in C2 and C3 accompanying the decrease in the daily sugar accumulation rate (S4 to S5, Tab. 1) may be attributed to the equilibration of the homeostasis within the berry due to the sudden change in the osmotic potential after the sharp decline in sugar accumulation. This theory is strengthened by the negative sugar to K^+ accumulation rate ratio in C2 and C3 (-4.83 and -10.46 respectively) at this time (Tab. 1).

In general, trends in the daily accumulation rate of berry FM, sugar and K^+ for the largest berries (C4) were similar to those for C2 and C3, except that K^+ continued to accumulate between S6 and S7 in C4 (Tab. 1). An increase in the daily K^+ accumulation rate between S5 and S6 occurred in all berry diameters and coincided with an increase in berry FM in all categories except C1 (Tab. 1). This corroborates the possibility that K^+ accumulation responded to the rain event that occurred before S5 (supplementary Fig. 1).

The smallest berries (C1) reached the peak daily sugar accumulation rate between S3 and S4, similar in timing to berries from C2 and C3 (Tab. 1). Between S3 and S4, the C1 berries increased the least in FM per day (19.46 mg FM per berry per day) while also accumulating moderate amounts of sugar (50.03 µmol of sugar per berry per day). These berries may be smaller because fewer cells formed during cell division, resulting in a smaller total vacuolar volume per berry for solute accumulation (Fontes *et al.* 2011) which potentially explains the lower content of sugar and K^+ in these berries in relation to the other diameter categories (Tab. 1). The smallest berries (C1) may also have been phenologically delayed at fruit set and/or véraison in comparison to C2 and C3. The berries of C1 contained the least seeds per berry (1.21 ± 0.06, $p < 0.05$, data not shown) in comparison with the remainder of the berry diameter categories. It has been shown that the number of seeds affect the berry size (Walker *et al.* 2005), but contrasting results were found for the effect of the number and mass of the seeds on the timing of the onset of ripening between varieties (Gouthu and Deluc 2015).

Berry enlargement is a result of water influx due to the strong increase in the osmotic potential gradient as sugar accumulates in the vacuoles of mainly the pulp cells (Keller *et al.* 2015). Strong positive relationships were observed for the sugar and K^+ content per berry as the berry fresh mass increased (r = 0.82 and 0.89 respectively, supplementary Fig. 2A and 2B) but the tighter fit between berry FM and K^+ (supplementary Fig. 2B) suggests that changes in K^+ content

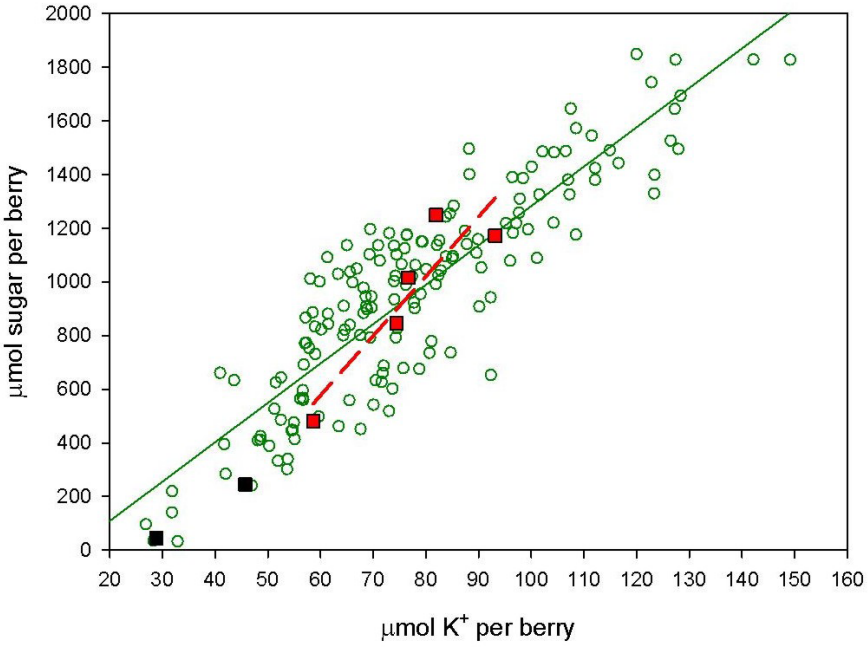

Fig. 2: Relationship between sugar and potassium (K^+) contents (µmol per berry) in individual berries (sum of the individual berry compartments) sampled from the third (S3) to the final (S7) sampling date (post-véraison period), with the fit shown for the complete population in green (O: —; n = 165; r = 0.87). Also indicated is the pooled values per sampling date in red (■: ---; n = 5; r = 0.95). Pre-véraison sampling dates (S1 and S2) are indicated (■: n = 2) but not included in the fit. Correlation coefficients were calculated through Pearson bivariate correlation analyses.

may be more closely related to changes in berry FM than to changes in berry sugar content. This observation, together with a concomitant increase in the content of sugar and K^+ per berry as the berry size increased (Tabs 1 and 2), point towards a possible ternary relationship between sugar, K^+ and water accumulation in grape berries during ripening.

Relationship between sugar and potassium within the berry compartments and individual berries: A strong positive relationship between the sugar and K^+ content was evident within each sampling date in the complete berry and the separate berry compartments from S2 onwards, except in the skin where a significant ($p < 0.001$) relationship only became apparent at S4 (Tab. 2). The negative correlation in the skin at S2 again confirms the possible redistribution of K^+ towards the pulp.

Significantly ($p < 0.001$) positive correlations were also apparent between sugar and K^+ contents in the berry compartments for the complete sampling period (Tab. 2), but displaying a reasonable spread of the data points from the fitted linear curve (data not shown). It is therefore evident that the accumulation of sugar and K^+ within the individual berry compartments is not tightly coupled.

Around one-third (35 %) of the berry K^+ content at S7 was however present at the onset of véraison (S2) while sugar was primarily accumulated from véraison (S2) onwards (Tab. 1). The presence of K^+ within the grape berry and berry compartments prior to the onset of active sugar accumulation and berry enlargement from véraison onwards is apparent from the weaker relationships seen in Tab. 2 at S1.

In individual berries (Fig. 2), the content of both metabolites increased during the ripening period (S3 to S7) and a significant positive (r = 0.87), although not linear (quadratic), relationship was apparent between the accumulated sugar and K^+. The discernible spread of the data points (Fig. 2) demonstrates the autonomous nature of the increase of these metabolites in individual grape berries between S3 and S7 (Coombe 1992, Ollat *et al.* 2002).

The strong relationship (r = 0.98) between the aggregated sugar and K^+ data per sampling date (Fig. 2), implies that the pooling of berry samples potentially masks biochemical and phenological differences between individual grape berries.

Conclusions

This investigation on the temporal and spatial accumulation patterns of sugar and K^+ within individual grape berries revealed similarities in the accumulation of these two components within the berry and its compartments during ripening. However, the high variability in sugar and K^+ content between single berries highlights plasticity in the accumulation of these metabolites. Although sugar and K^+ had comparable accumulation patterns, sugar accumulated at a continuously accelerated rate compared to K^+ for the first four weeks of ripening. From véraison to harvest, sugar content in the grape berry increased five-fold during ripening (from 246 µmol at véraison to 1240 µmol at harvest) whereas the potassium content only doubled (from 45 µmol at véraison to 82 µmol at harvest). This clearly indicates that sugar accumulation in the grape berry cannot solely be associated with K^+ accumulation. The increase in sugar

and K^+ contents as the berry size increased may suggest a ternary relationship of sugar and K^+ with the water content of the berry during ripening.

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