Mineral sinks within ripening grape berries (*Vitis vinifera* L.)

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Summary

Trends in the accumulation of mineral elements into the grape berry components give information about vascular flow into the berry. Shiraz berries were dissected into receptacle, skin, pulp, brush and seeds and the accumulation of 10 mineral elements into these components was followed through development. The elements were separated into two categories according to their accumulation pattern into the berry. The first group of elements continued to accumulate throughout berry growth and ripening, and was comprised of phloem-mobile potassium, phosphorus, sulphur, magnesium, boron, iron and copper. The second group of elements accumulated mostly prior to veraison, and included the xylem-mobile minerals calcium, manganese and zinc. These results indicate that the xylem contribution to berry growth diminished after veraison. Berry fresh weight, dry weight, as well as berry sugar content, were all highly correlated with berry potassium content. While the pulp and skin were the strongest sinks for potassium and boron, seeds were the strongest sinks for calcium, phosphorus, sulphur, manganese and zinc. With the exception of calcium and manganese, seeds ceased to accumulate most elements during late ripening. The berry receptacle and brush did not accumulate any of the elements to levels above those of the other berry components at any stage of development. Therefore, they did not act as sinks for xylem- or phloem-mobile elements as vascular flow to the pulp and skin slowed.

K e y   w o r d s : grape berry, xylem, phloem, mineral elements, nutrients.

Introduction

The development of the grape berry is concomitant with an influx of water, carbon and a number of mineral elements. There are 17 elements which are considered to be essential for the growth and development of plants, with some having a role in the structure of compounds while others are involved in enzyme activation, or as a charge carrier and osmoregulator (Marschner 1995). At the vine level, high levels of calcium in the berry increases disease resistance (Chardonnet and Donèche 1995), while deficiencies in phosphorus, zinc, manganese and molybdenum result in a reduction of fruit set and a deficiency in potassium results in unevenly ripened berries (Mullins *et al*. 1996). The mineral nutrient status of the grape berry is of concern not only to the viticulturist, but also to the oenologist because there is a direct impact of berry nutrition on juice and must composition. For instance, potassium, the most predominant cation in grape juice, has an influence on the pH of the wine and tartrate stability (Mpelasoka *et al*. 2003), while copper can delay fermentation during wine making (Soumalainen and Oura 1971).

Elements can be classified as having high mobility in the phloem (magnesium, phosphorus, potassium and sulphur), low phloem mobility (calcium and manganese), and variable or conditional phloem mobility (boron, copper, iron and zinc) (Welch 1986). This conditional mobility is dependent on plant species, environmental influences, plant tissue and growth stage (Welch and Rengel 1999). Elements with low mobility in the phloem can be transported through the xylem. By monitoring the accumulation of phloem- and xylem-mobile elements into tissues, information can be derived on the contribution of the vascular streams to the development of a tissue. For instance, berry growth is dependent on both the xylem and phloem streams through the pedicel (Lang and Thorpe 1989, Greenspan *et al*. 1994, 1996), however, relative contributions through veraison and late ripening remain unclear (Rogiers *et al*. 2001). Potassium and calcium are commonly used to infer vascular flow to the berry, but further information can be obtained from assessing the accumulation patterns of other elements. Within the berry, xylem tracer studies have established that both the peripheral and the central vascular bundles to the skin and pulp and to the seed, respectively, remain functional up to veraison. With further berry development, the tracer studies suggest peripheral xylem bundles become non-functional while supply to at least the brush continued. How these changes in vascular function influence elemental accumulation within the different berry tissues remains unknown.

An understanding of the patterns of mineral element accumulation into the berry components would not only aid in vine nutrition, harvest and grape processing decisions but also provide further information on the roles of the vascular streams both, to and within the berry during development. Thus, the objective of the present study was to

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quantify the trends in accumulation of 10 mineral elements in 5 tissue components of Shiraz berries during growth and development. The aim was to assess the hypothesis that changes in berry vasculature function during development would be reflected in the accumulation patterns of the xylem- and phloem-mobile elements.

Material and Methods

B e r r y   s a m p l i n g   a n d   p a r t i t i o n i n g : This study was carried out on 11 year-old own-rooted, spur-pruned *Vitis vinifera* L. cv. Shiraz clone PT23/N/Griffith vines at the Charles Sturt University Vineyard, Wagga Wagga, NSW, Australia. Berries were sampled three times weekly from flowering to harvest in the 2000-2001 season. At each sampling date, 5 bunches were harvested at random. For the mineral element analysis, 50 berries were cut from each bunch, combined into a single sample, and frozen at -20 °C. The fresh weight of a second 50 berry sample from each bunch was determined. Subsequently the sample was juiced and total soluble solids (TSS) measured using a hand-held refractometer (ATAGO, Tokyo, Japan).

Berries were partitioned for mineral element analysis at 5 developmental stages. The dates were 24/12/2000 (pre-veraison, 23 d after flowering (DAF)), 6/01/2001 (pre-veraison, 48 DAF), 18/01/2001 (post-veraison, 60 DAF), 2/02/2001 (mid-ripening, 75 DAF), 16/02/2001 (late ripening, 89 DAF). The frozen 50 berry samples from each of the 5 bunches were separated into receptacle, skin, pulp, seeds and brush (vascular and associated parenchyma tissue in the central, proximal half of berry). The brush was punched out of the frozen berry using a capillary pipette, and subsequently the skin was peeled from the pulp using tweezers. The seeds were removed from the pulp and rinsed in de-ionized H_2O.

M i n e r a l   e l e m e n t   a n a l y s i s : Whole and partitioned berries were ground in liquid N_2, refrozen, and sent to a commercial laboratory (Waite Analytical Services, University of Adelaide) for analysis of mineral elements. The samples were digested in nitric acid with a hydrochloric acid finish and then analysed by inductively coupled plasma optical emission spectrometry (ICPOES, ZARCINAS and CARTWRIGHT 1983).

D a t a   a n d   s t a t i s t i c a l   a n a l y s i s : Cubic smoothing splines fitted as linear mixed models using the techniques of VERBYLA *et al.* (1999), were used to model trends in fresh weight, TSS and mineral elements over DAF. Natural logarithmic transformations of the data were taken because of increasing variance over time. Fixed terms in the model for a variable included an overall mean and a linear trend across DAF. Random terms included the mean level curvilinear trend (cubic smoothing spline) as well as deviations from the curvilinear trend at particular DAF. The significance of all fixed terms was assessed (at 5 %) using Wald tests and the significance of random terms was assessed using Residual Maximum Likelihood Ratio tests (VERBYLA *et al.* 1999). All analyses were performed using ASREML, a FORTRAN package which uses the Average Information (AI) algorithm with sparse matrix meth-

ods to fit linear mixed models using Residual Maximum Likelihood (REML) (GILMOUR *et al.* 2002). Rates of element accumulation were calculated from linear regressions over a particular phase of development using SigmaPlot® (version 8.0, SPSS, Chicago, IL).

Results

B e r r y   g r o w t h : At 20 DAF, average berry fresh weight was 0.26 ±0.02 g (Fig. 1). Fresh weight increased to 0.60 ±0.05 g at about 30 DAF and then stabilized at this level over the next 20 d. Veraison, and resumption of berry growth, followed at 55 DAF. Fresh weight ceased increasing beyond 85 DAF and by 94 DAF the bunches were commercially harvested at an average fresh berry weight of 1.5 ±0.1 g, dry weight of 0.40 ±0.03 g and soluble solids of 24.1 ±0.3 °Brix. The trend in TSS was very similar to that of fresh weight accumulation, except near harvest when the rate of fresh weight accumulation was slower than the increase in TSS.
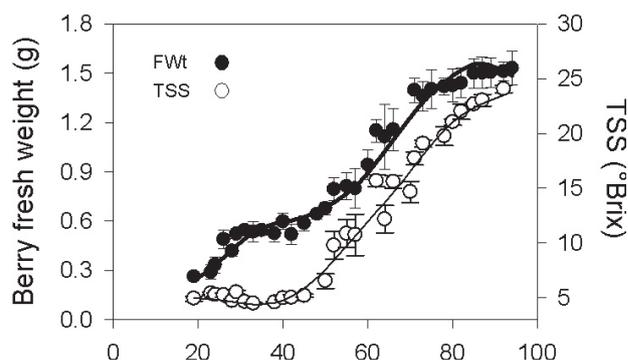

Fig. 1: Fresh weight (g) and TSS (°Brix) during development and ripening of Shiraz berries. Bars indicate ± standard error of the means.

E l e m e n t s   w i t h   a c c u m u l a t i o n   t h r o u g h o u t   b e r r y   d e v e l o p m e n t : Potassium, phosphorus, sulphur, magnesium, boron, iron, and copper accumulated in the berry during all stages of development, however rates of accumulation were highest after veraison (Fig. 2). In spite of orders of magnitude differences in amounts of each element, the accumulation patterns of potassium, phosphorus, boron and copper were almost identical. These elements accumulated steadily prior to veraison (32 µg potassium, 4.2 µg phosphorus, 0.08 µg boron, and 0.019 µg copper berry^{-1} d^{-1}). However, accumulation rates per berry were highest between veraison and the fresh weight plateau (110 µg potassium, 8.1 µg phosphorus, 0.14 µg boron and 0.030 µg copper berry^{-1} d^{-1}). This equates to between 1.6 and 3.4 fold increases in the rates of accumulation, respectively, after veraison. Beyond 82 DAF, accumulation rates of these 4 elements slowed. Magnesium accumulation increased steadily until 70 DAF at 2.6 µg berry^{-1} d^{-1} and then slowed to 1.0 µg berry^{-1} d^{-1}, while iron and sulphur accumulation rates were steady at 0.054 and 1.80 µg berry^{-1} d^{-1} through until harvest. Over the entire sampling period (75 d), the total amount of each element per berry increased by 6 to 16 fold.
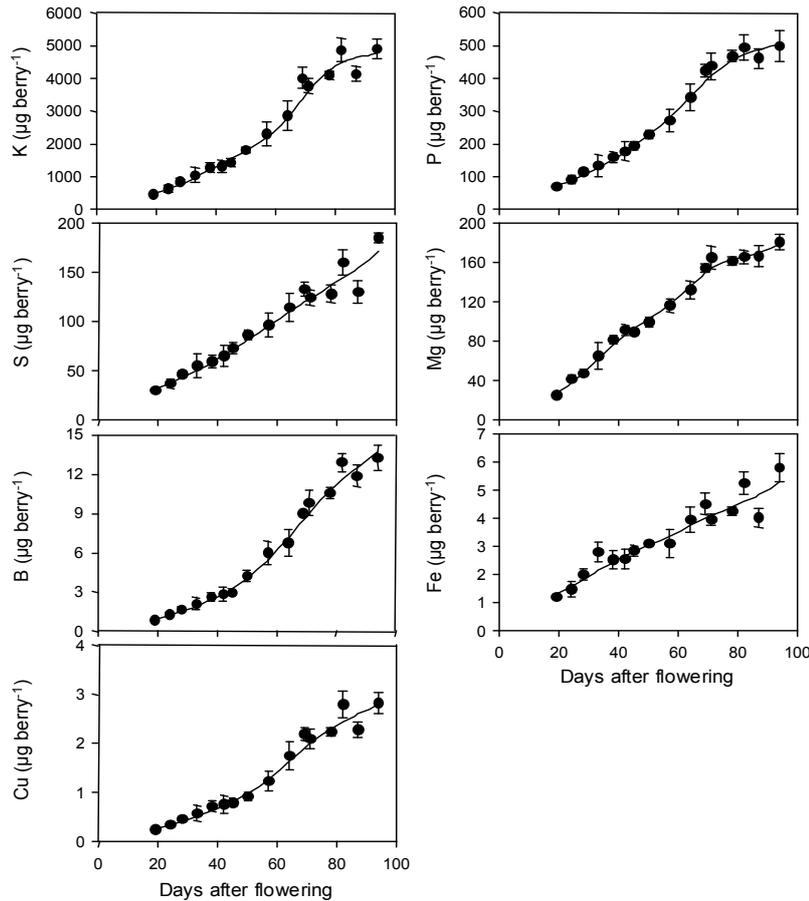
Fig. 2: Accumulation of potential phloem-mobile elements K, P, S, Mg, B, Fe, and Cu (µg berry^{-1}) during development and ripening of Shiraz berries. For details: Fig. 1.

E l e m e n t s   a c c u m u l a t e d   o n l y   p r i o r   t o v e r a i s o n : Calcium, manganese and zinc accumulated strongly (8.45, 0.028, and 0.026 µg berry^{-1} d^{-1}) until 50, 70 or 45 DAF, respectively, but accumulation stopped as berries continued to develop further (Fig. 3). Between 20 and 95 DAF, berry calcium in total increased 3.7 fold, while manganese increased 2.3 fold and zinc 2.4 fold.

Sodium did not accumulate over time and amounts were quite variable (up to 30 µg berry^{-1}) (data not shown).

B e r r y   m i n e r a l   c o m p o s i t i o n   a t h a r v e s t : Within the berry (Figs 2 and 3), potassium was the most abundant mineral element (4.91 ±0.32 mg), equivalent to 0.3 % of the berry's fresh weight at harvest. Phosphorus and calcium were the next two most abundant elements at harvest (499 ±46 and 473 ±15 µg berry^{-1} respectively), approximately 10 % of the amount of potassium. Sulphur and magnesium levels were similar, at 185 ±5 and 181 ±8 µg berry^{-1}, respectively. This was followed with boron (13 ±1), sodium (12.4 ±2.7), iron (5.8 ±0.5), copper (2.8 ±0.2), manganese (2.3 ±0.2) and zinc (1.6 ±0.2 µg berry^{-1}). Five other elements were detected in minute amounts in the Shiraz berries. They include aluminium (<1 µg g^{-1}), nickel (<0.3 µg g^{-1}), molybdenum (<0.2 µg g^{-1}), cobalt (<0.2 µg g^{-1}) and cadmium (<0.09 µg g^{-1}).

C o r r e l a t i o n s   o f   b e r r y   w e i g h t   a n d s u g a r   w i t h   b e r r y   p o t a s s i u m   a m o u n t s : Changes in potassium content and berry fresh weight over the growing season were highly correlated in a linear pat-
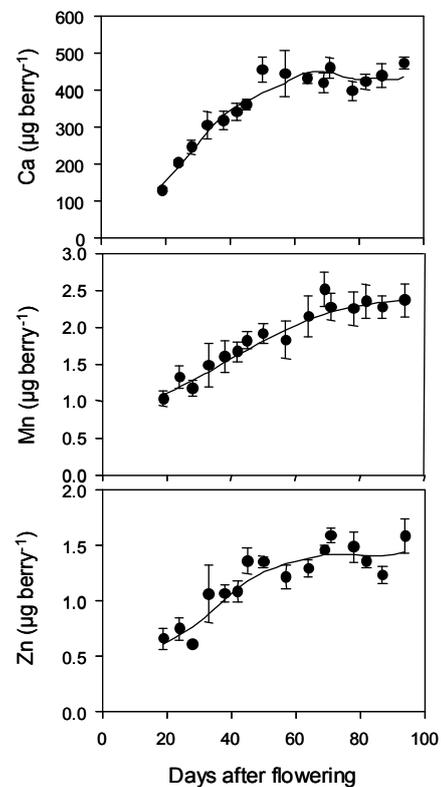

Fig. 3: Accumulation of potential xylem-mobile elements Ca, Mn and Zn (µg berry^{-1}) during berry development. For details: Fig. 1.

tern ($r^2 = 0.99$, P<0.01), while berry dry weight and sugar content were also correlated with potassium, although in a more sigmoid pattern ($r^2 = 0.99$, P<0.01 for both regressions) (Fig. 4). However, it was notable that at increases in potassium content above about 2 mg berry^{-1} both dry weight and sugar content increased linearly. This suggests accumulation of potassium and the growth processes of the berry may be mechanistically linked.

Element concentration during berry development: Because the rate of gain in berry weight varied throughout development, trends in element concentrations (mg of element per kg of fresh berry tissue) were often very different from the trends in element accumulation per berry. The concentrations of potassium, boron and copper continued to increase throughout development (1.8, 2.5 and 1.9 fold, respectively) (Fig. 5). By contrast, the concentrations of the other phloem-mobile elements either remained steady (phosphorus) or declined markedly (magnesium, sulphur and iron) (7-40 %) to concentrations below that occurring prior to veraison. This decline occurred despite post-veraison accumulation rates exceeding pre-veraison rates. In all likelihood, the decrease in concentration of these elements could be attributed directly to the gains in berry fresh weight (Fig. 1). A similar effect occurred with the xylem-mobile elements (Fig. 6), that is, concentrations declined throughout berry development (manganese) or after veraison had occurred (calcium and zinc).
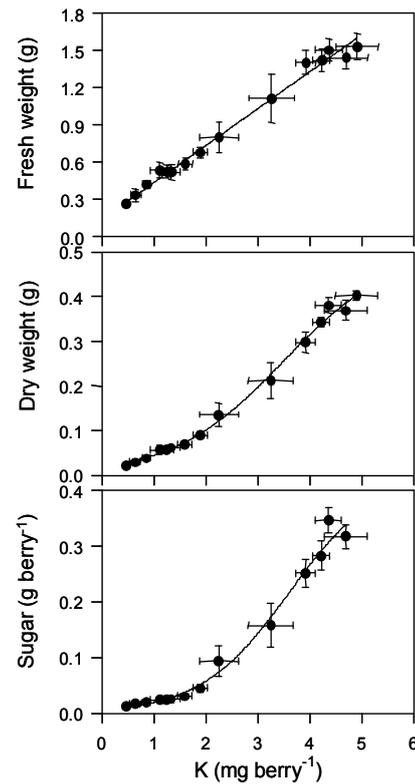

Fig. 4: Berry fresh weight (g), dry weight (g) and sugar (g berry^{-1}) correlated with potassium (mg berry^{-1}) content of berries. For details: Fig. 1.
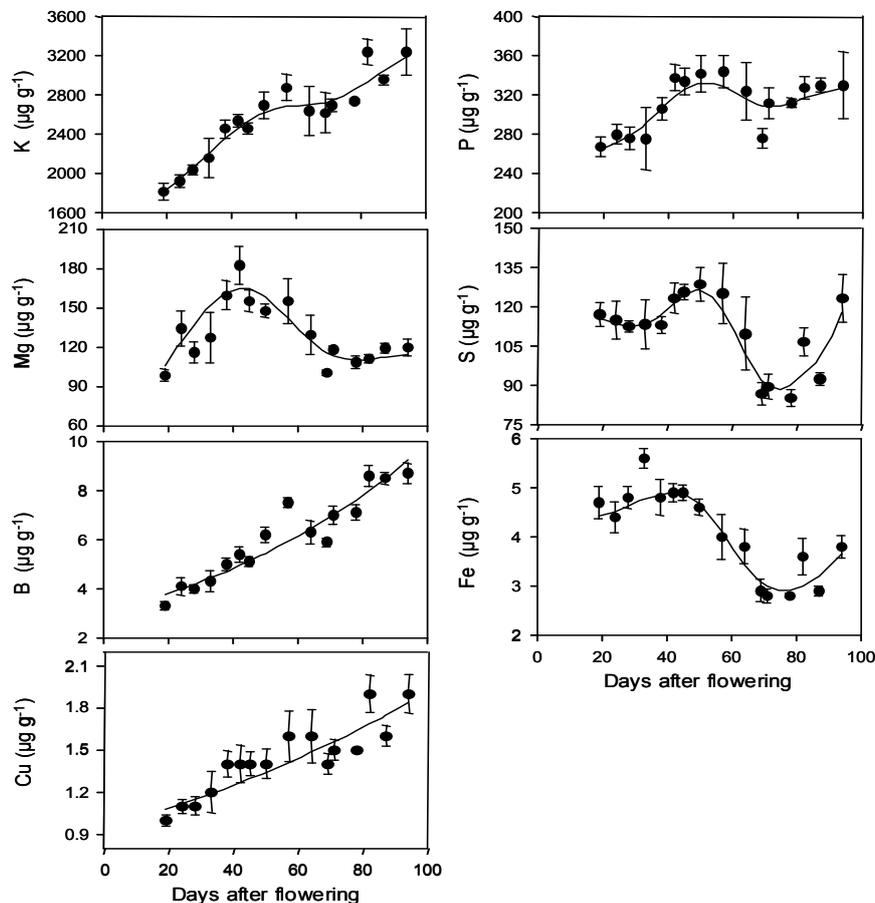

Fig. 5: Changes in concentration of potential phloem-mobile elements K, P, Mg, S, B, Fe and Cu (μg g^{-1}) during berry development. For details: Fig. 1.

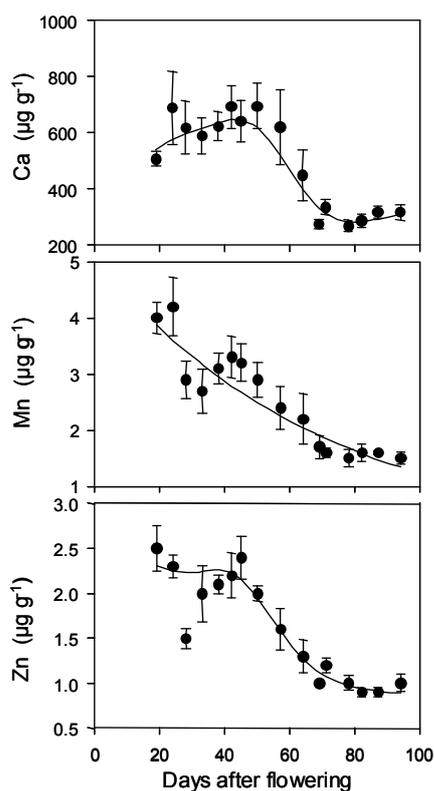Fig. 6: Changes in concentration of potential xylem-mobile elements Ca, Mn and Zn (µg g⁻¹) during berry development. For details: Fig. 1.

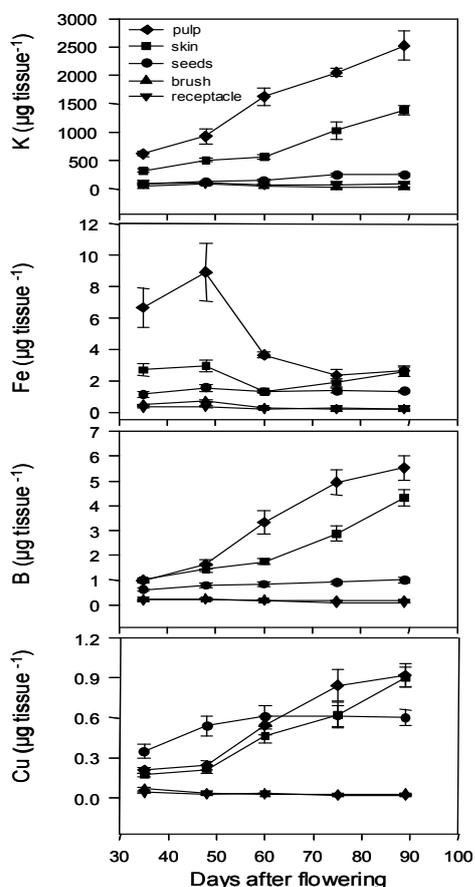Fig. 7: Accumulation of K, Fe, B and Cu (µg tissue⁻¹) in predominantly the pulp, but also the receptacle, brush, seeds and skin during berry development. For details: Fig. 1.

Partitioning into tissues

Content: Potassium, boron, iron and copper were predominantly deposited in the pulp and skin of the berry (Fig. 7). Potassium accumulation into the pulp and skin increased up to 90 DAF (the last sampling date for the partitioning analysis). At this time, the pulp contained 59 % of the total berry amounts of potassium, while the skin contained 32 %, and the seeds contained 6 % of the total potassium in the berry. Similar ratios were apparent for the element boron. By contrast, at 90 DAF, iron and copper amounts were equal in the pulp and skin (at 37 % for iron and copper), and seeds contained 19 % (iron) and 24 % (copper) of the total berry amounts.

On the other hand, calcium, phosphorus, magnesium, sulphur, manganese and zinc were primarily accumulated in the seeds (Fig. 8). By 90 DAF, the seeds contained 61, 41, 35, 34, 55 and 42 % of the berry's calcium, phosphorus, magnesium, sulphur, manganese and zinc, respectively. The content of the elements remained more or less constant following veraison, however the accumulation of calcium, manganese and zinc appeared to increase from about 75 DAF into the seeds, skin, pulp and brush. The seeds ceased to accumulate sulphur, phosphorus, potassium, and magnesium between 50 and 60 DAF.

Relative to other berry components, the brush and receptacle did not accumulate any of the elements examined in this study to any great extent (Figs 7 and 8), and amounts were generally less than 5 % (potassium, phosphorus, sulphur, boron and copper) or 15 % (magnesium) of the total berry amounts at 90 DAF. Percentage of calcium in the brush remained at 1 %, and in the receptacle at 10 %, of the total calcium within the berry. Potassium and magnesium amounts remained constant in the receptacle throughout berry development, while phosphorus, boron, sulphur and copper amounts declined. In the brush, amounts either increased (magnesium), decreased (phosphorus, boron, copper), or did not alter (sulphur, potassium).

Concentration: Throughout berry development, the seeds contained markedly higher concentrations of calcium, phosphorus, magnesium, sulphur, manganese and copper compared with the other berry components (Fig. 9). The concentrations of phosphorus, magnesium, sulphur and copper increased up to 75 DAF, however, the concentrations of calcium and manganese increased 1.2-2.6 fold after being relatively steady between 40 and 75 DAF. The receptacle also contained high concentrations of calcium, magnesium and manganese. Potassium and zinc were most concentrated in the receptacle, while iron and boron were most concentrated in the brush of the berry (Fig. 10).

For direct comparison, the final concentrations of all elements in the three major sinks (seeds, pulp and skin) are shown in the Table.

Discussion

Patterns of nutrient accumulation into berries: The elements monitored in this study can be divided into two broad categories based on their accumulation patterns; (1) those elements that continued to accumulate throughout berry growth, and (2) those ele-
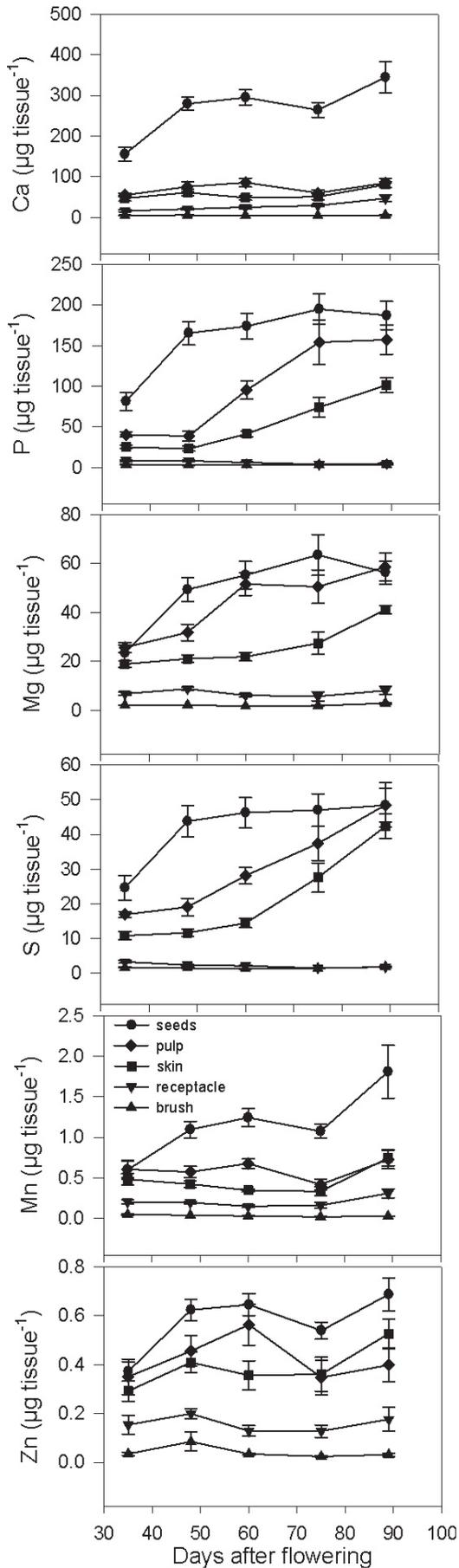
Fig. 8: Accumulation of Ca, P, Mg, S, Mn and Zn (μg tissue^{-1}) in predominantly the seeds, but also the receptacle, brush, pulp and skin during berry development. For details: Fig. 1.
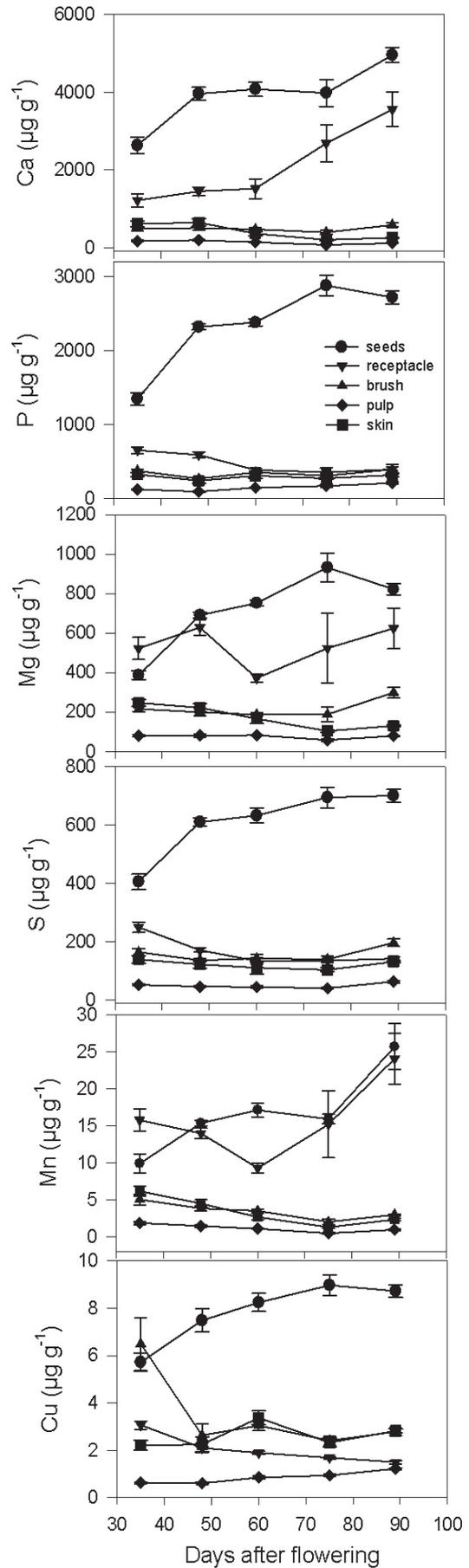
Fig. 9: Changes in concentration of Ca, P, Mg, S, Mn and Cu (μg g^{-1}) in berry seeds, and also the berry receptacle, brush, pulp and skin during berry development. For details: Fig. 1.
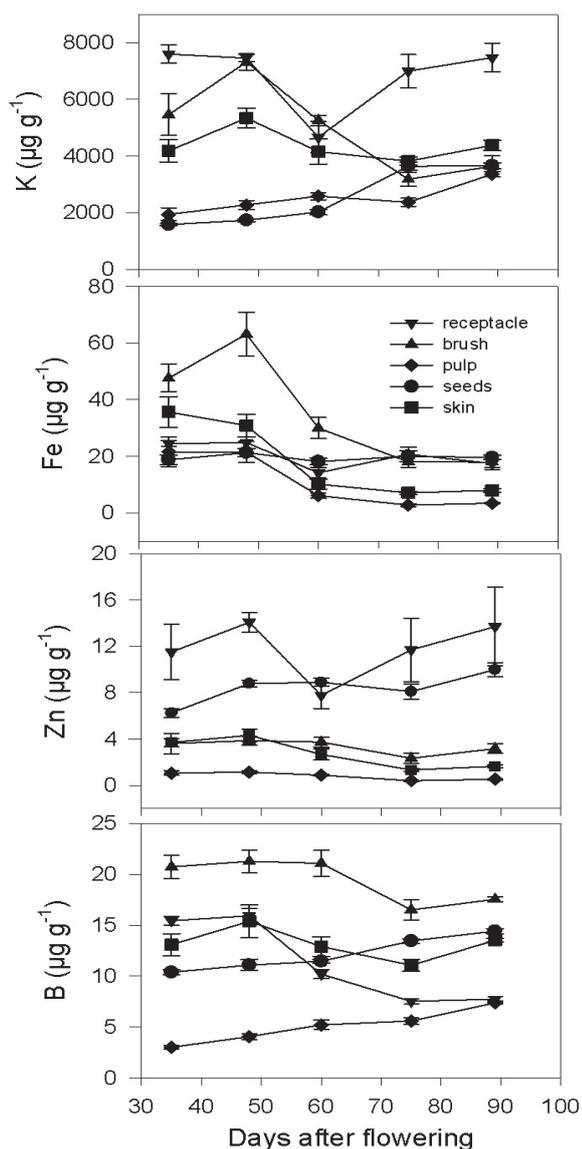
Fig. 10: Changes in concentration of K, Fe, Zn and B (µg g⁻¹) in receptacle, brush, pulp, seeds and skin during berry development. For details: Fig. 1.

Table

Mineral element concentrations (µg g⁻¹) in the seeds, pulp and skin of Shiraz berries at 90 d after flowering. At this stage, average berry fresh weight was 1.5 g and TSS was 24.1 °Brix

	Seeds		Pulp		Skin	
Ca	4960	± 191	112	± 9	252	± 11
K	3660	± 98	3360	± 93	4380	± 185
P	2720	± 86	211	± 16	320	± 23
Mg	822	± 29	78	± 3	130	± 5
S	700	± 22	64	± 4	132	± 4
Mn	26	± 3	0.96	± 0.08	2.3	± 0.2
Fe	19.6	± 0.8	3.5	± 0.2	8.0	± 0.6
B	14.4	± 0.2	7.4	± 0.1	13.5	± 0.2
Cu	8.7	± 0.3	1.23	± 0.04	2.83	± 0.08
Zn	10.0	± 0.6	0.52	± 0.04	1.6	± 0.1

ments that accumulated mostly prior to veraison. Among the first group of elements were the macronutrients potassium, phosphorus, sulphur and magnesium, all of which are generally considered to be phloem-mobile elements (WELCH 1986). The high rates of accumulation of these elements after veraison is thus consistent with flow through the phloem into grape berries after veraison (LANG and THORPE 1989, GREENSPAN et al. 1994).

Potassium was the most abundant element with higher rates of accumulation after veraison compared to the other elements. Similar trends in potassium accumulation have been previously reported for various V. vinifera cultivars (SCHALLER et al. 1992, CREASY et al. 1993, OLLAT and GAUDILLÈRE 1996). Potassium uptake into the expanding cells may be required to maintain electrical charge balance inside the cells and to keep proton pumps active (STILES and VAN VOLKENBURGH 2004). A key role for potassium in cell expansion is supported by our results which indicate that potassium accumulated predominantly in the pulp and skin tissues, and furthermore, there was a strong relationship between potassium accumulation and berry fresh weight.

Potassium, being the main osmotically active cation in both the phloem sap and grape berries, would contribute to phloem sap flow (sugar import) by helping to establish an osmotic potential gradient between the leaves (source) and the berries (sink) (VÉRY and SENTENAC 2003). Again, the relationship between potassium and both berry sugar and dry weight accumulation support this assertion. It may be, therefore, that accumulation of potassium assists in the drive for the accumulation of sugars into the berry, but also contributes significantly to uptake of water, especially after veraison. After about 80 DAF, the rate of potassium accumulation into the berry declined in accordance with the rates of fresh weight and sugar accumulation also declining after this time. These changes in accumulation patterns conform with the hypothesized decrease in phloem flow into grape berries during late ripening (MCCARTHY and COOMBE 1999) and provide further support for the linkage between potassium and sugar accumulation.

Boron is considered to have conditional or low mobility in the phloem (SHELP et al. 1995). This element is primarily transported in the xylem but is commonly translocated in phloem of sink organs that do not readily transpire (SHELP et al. 1995). Grape berries, including cv. Shiraz, transpire at much lower rates than are typical of leaves (ROGIERS et al. 2004) and the pattern of accumulation of boron in this study was consistent with mobility of this element in the phloem. Copper and iron have also been considered to have variable or conditional phloem mobility (WELCH 1986), but since their accumulation continued throughout berry development, the majority of their accumulation into the berry would apparently have been through the phloem.

Based on the patterns of accumulation being restricted prior to veraison, a second group of elements comprising calcium, manganese and zinc, could be discerned. Calcium and manganese are considered to have low phloem mobility (WELCH 1986) and, therefore, most likely have entered the berry through the xylem. Zinc, a variable phloem-mobile element (WELCH 1986), has also apparently entered the berry through the xylem. These three elements were

characterised by a marked decline in rates of accumulation from about 50 d after flowering. However, there is no *a priori* reason why the xylem flow rates across the whole vine in general should decline at this stage. It would appear most likely that the diminished xylem flow to the berry alone after veraison would account for the decline in accumulation of calcium, manganese and zinc. This conclusion conforms with other studies showing a decline in xylem flow after veraison (Düring *et al.* 1987, Findlay *et al.* 1987, Lang and Düring 1991). However, it has been recently hypothesised that a loss of a hydrostatic pressure gradient in the berry apoplast, rather than a loss of xylem function, leads to reduced xylem flow after veraison (Bondada *et al.* 2005). The results here conform with both possibilities.

Other studies have shown that calcium accumulation either ceased at veraison (Hrazdina *et al.* 1984) or continued until full ripeness (Ollat and Gaudillère 1996, Rogiers *et al.* 2000, Cabanne and Donèche 2003). It appears that in grapes, as in apple fruit (Saure 2005), calcium accumulation patterns are variable. This may have been caused by seasonal differences in vapour pressure gradients and thus transpiration (Marschner 1995). Since calcium is carried along the transpirational stream, any environmental factor which influences the flow rate of this stream would also influence the deposition of calcium into the transpiring organ. The soil characteristics, including moisture levels and mineral content, may also play a large part in determining nutrient translocation to the berries.

B e r r y   t i s s u e   s i n k s : The strongest berry tissue sinks for the phloem-mobile elements varied for each element. For example, on a tissue basis, potassium and boron accumulation in the pulp and skin was much higher than in the seeds, while most of the phosphorus accumulated in the seeds. High potassium concentrations in the skin conform with data reviewed by Mpelasoka *et al.* (2003). X-ray microanalysis has shown that potassium tends to be located in the hypodermal cells of the skin (Storey 1987).

With the exception of potassium, seeds had essentially stopped accumulating the phloem-mobile elements at about 50 DAF. This suggests that, in Shiraz berries, the seeds became isolated in some way after veraison from the phloem-mobile elements. This isolation, however, appeared to be limited to the seeds since the pulp and skin continued to accumulate most of the elements after this time. An apoplasmic mode of phloem unloading has been suggested into the grape berry pulp (Lang and Düring 1991) however unloading into the seed may follow a symplasmic pathway, as is the case for walnut fruit (Wu *et al.* 2004). It is notable that grape seeds acquire the ability to germinate by veraison, although storage reserves such as starch and lipids continue to accumulate in the endosperm after veraison (Currle *et al.* 1983). This contrast between sugar and nutrient accumulation suggested perhaps that unloading may be preferential, rather than a complete discontinuity in phloem flow. That none of these elements accumulated in either the brush or receptacle indicated that these two tissues were not a major depositing site for these elements.

Within the Shiraz berry, the seeds were the largest sink for the xylem-mobile elements. Most of the calcium, manganese and zinc were deposited into the seeds of the berry prior to veraison, however there was another increase late in ripening. Calcium content of seeds also increased during ripening of several other grape varieties (Cabanne and Donèche 2003). Assuming that this element is moved through the xylem, this suggested that xylem was continuously connected to the seeds. In legumes and grains, most of the nutrients are delivered to the seeds by the phloem because a discontinuity occurs between xylem vessels in the maternal tissue and the seeds (Welch 1986). In pre-veraison grape berries, however, xylem tracer studies indicated that xylem flow occurred along not only the bundles supplying the skin and pulp of the berry, but also the central bundles leading to the seeds (Rogiers *et al.* 2001), again suggesting continuous xylem flow to the seeds. After veraison, however, the xylem tracers were limited to the brush region of the central bundles (Düring *et al.* 1987, Findlay *et al.* 1987, Creasy *et al.* 1993, Rogiers *et al.* 2001). Further work is required to ascertain if after veraison, the dye, and for that matter xylem flow, is continuous along the vasculature leading directly to the seed.

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