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Using rootstocks to lower berry potassium concentrations in 'Cabernet Sauvignon' grapevines

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Summary

Potassium is the most abundant cation in grape berries. It has important roles in grapevine physiology and winemaking. This study investigates the feasibility of using rootstocks to lower berry potassium concentrations ([K]) in 'Cabernet Sauvignon' grapevines. The ultimate target is to achieve lower pH and higher titratable acidity (TA) in grape juice so as to bring down the cost of acid adjustment during winemaking. The specific objective here is to provide new insights into the potential of particular rootstocks to modify K uptake by 'Cabernet Sauvignon' grapevines and their partitioning and accumulation into grape berries.

The vineyard soils of a replicated rootstock trial located in the Limestone Coast of South Australia were characterised. Petiole, berry and juice nutrient content were assessed at oenological maturity of 'Cabernet Sauvignon' grown on eight different rootstocks. Rootstock had an impact on cations of the vegetative tissue of 'Cabernet Sauvignon', with Merbein 5512 having the lowest petiole [K]. The concentrations of major cations in the berry were, however, not altered by rootstock. While no particular rootstock stood out in limiting 'Cabernet Sauvignon' berry K accumulation, berries grown on the 'Börner' rootstock tended to have slightly lower concentrations (< 10 %) relative to vines on their own roots.

Across the rootstocks, juice pH tended to increase with greater juice [K], while juice TA tended to decrease with greater juice [K]. It was found that juice TA was higher for the rootstocks 140 Ruggeri and 110 Richter, and juice pH tended to be lower for the rootstocks 110 Richter, 140 Ruggeri, Merbein 5512 and Merbein 5489. There was no effect of rootstock on total soluble solids.

K e y w o r d s : grape; 'Cabernet Sauvignon'; rootstock; potassium; *Vitis*.

Introduction

Potassium (K) has important physiological and biochemical roles in the grapevine (Rogiers *et al.* 2017). K is integral to phloem transport and thus sugar loading into grape berries (Rogiers *et al.* 2006b, Coetzee *et al.* 2017 and 2019). From an applied perspective, potassium concentration ([K]) strongly influences juice pH and titratable acidity (TA). High [K] in juice and wine can reduce the amount of free tartaric acid, resulting in potassium bitartrate formation and eventually affects the final pH of the grape juice, which is one of the most important factors that determine the quality of grape juice and wine (Boulton 1980, Kodur 2011). Potassium bitartrate has limited solubility and this declines with increasing ethanol concentration (Berg and Keefer 1958). The precipitates can form in the wine and accumulate on the cork or on the bottom of the bottle and decrease consumer acceptance of a wine.

Glasshouse studies have shown that genetic differences in rootstocks can affect K accumulation and transport in both own-rooted and grafted grapevines due to the differences in root development, vigour and dry matter partitioning of the plant (Kodur *et al.* 2010a and b). In a field study, K accumulation in the scions' vegetative parts of grafted grapevines, was shown to be affected by the genotype of the rootstock (Rühl 1991). It has been found that juice pH, associated with K accumulation in the grapes, can also be affected by the scion/rootstock combination as well as the regional location of the trial (Rühl *et al.* 1988). Low to medium vigour rootstocks, such as Merbein 5489 and Merbein 5512 (referred to as M5489 and M5512, respectively) have been shown to have lower K uptake and lower 'Shiraz' juice and wine pH in field trials conducted in the Sunraysia region of south-eastern Australia (Walker and Clingeleffer 2009). In a further trial in the Padthaway region of south-eastern South Australia, M5489 and M5512 resulted in reduced K but higher Ca accumulation in laminae relative to that in 140 Ruggeri, Paulsen 1103, 110 Richter, 101-14 and Ramsey but there were no differences among the rootstocks on K

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accumulation in grape juice (WALKER *et al.* 2019). How the 'Cabernet Sauvignon' scion interacts with rootstocks, including M5489 and M5512, growing in the calcareous soils of the Coonawarra region with regard to K accumulation in vegetative tissue, grape juice and wine remains unclear.

K uptake by the plant can sometimes be limited by the antagonistic interaction with Ca and Mg in the soil (OHNO and GRUNES 1985, JAKOBSEN 1993). For grapevines, alkaline soils (pH > 7.0) are associated with reduced K availability due to potentially increased calcium (Ca) and magnesium (Mg) uptake (HANNAN 2011). It is uncertain how K, Ca and Mg interact with each other at the grapevine root-soil interface, within the perennial storage components of the vine or within the wine must/juice to ultimately affect the precipitation process. Furthermore, 'Cabernet Sauvignon' is susceptible to bunch-stem necrosis (BSN) (KRASNOW *et al.* 2010). Nutritional factors, such as imbalance amongst K, Ca and Mg, might be associated with this physiological disorder (CHRISTENSEN and BOGGERO 1985, COCUCCI *et al.* 1988, CAPPS and WOLF 2000). For these reasons we have opted to examine the interaction of these three cations. This study investigated the rootstock effect on K uptake and juice pH of 'Cabernet Sauvignon' grapevines. Concentrations of other elements potentially interactive to K uptake in grapes, mainly Ca and Mg, were also determined.

Material and Methods

Plant material and experimental design: The site was located at the Alexander vineyard, Treasury Wine Estate, in the Coonawarra region in South Australia (37°16'51.6"S 140°49'50.7"E). The trial consisted of 'Cabernet Sauvignon' (clone CW44) vines grafted to 8 rootstocks including 110 Richter (110R) (*V. berlandieri* × *V. rupestris*), Ramsey (*V. champinii*.), 1103 Paulsen (1103P) (*V. berlandieri* × *V. rupestris*), Börner (*V. riparia* × *V. cinerea*), 140 Ruggeri (140RU) (*V. berlandieri* × *V. rupestris*), Merbein 5489 (M5489), Merbein 5512 (M5512) (both Merbein rootstocks are selections from a cross of *V. berlandieri* × *V. berlandieri*) and own roots (*V. vinifera*). Planted in 2009-2010, the vines were spaced 3.35 m apart and in rows that were 2 m apart, spur-pruned and trained along a single horizontal wire above a bilateral cordon. Rows were north-south oriented. The trial site consisted of 7 rows of the 8 rootstocks in a replicated and randomized vineyard design, with each row having 30 panels, and each panel consisting of 3 vines. Each rootstock was replicated 11 times within the trial site. Four replicates, each consisting of 6 vines for each rootstock, were used in this study.

Soil characterization and mineral analysis: The principal soil type was 'Terra Rossa', well-drained, a reddish clay to silty-clay. Soil samples were taken at flowering and harvest. At flowering, samples from all rootstock panels were taken at around 15 cm underneath the surface with a hand-held auger and stored at -20 °C until analysis at the end of the season. At harvest, samples were taken from own roots and Merbein 5512 panels at 10 cm underneath the surface. Soil samples were air dried at 60 °C for 5 d, ground and sieved (2 mm mesh size). A 2.5 g soil sample was used to analyse exchangeable cations (Al, Na, K, Ca and Mg) (spectroscopy method details below). Soil cation exchange capacity (CEC_{bases}) was calculated as the sum of the base cations. Exchangeable Sodium Percentage (ESP %) was calculated using equation (1). Exchangeable Potassium Percentage (EKP %) was calculated using the same equation but by swapping the numerator [Na] in equation (1) with [K].

$$\frac{[Na]}{[Na]+[Al]+[K]+[Ca]+[Mg]} \times 100\% \quad (1)$$

Soil electrical conductivity ($EC_{1:5}$) (SevenCompact Cond meter, Mettler Toledo, Port Melbourne, Australia) and pH $1:5_{(water)}$ (Hanna precision pH meter Model pH 211, Hanna instruments, Melbourne, Australia) were also determined.

Water input, fertilizer applications and composition of irrigation water: Rainfall (> 10 mm·d^{-1}) data were obtained from the Australian Bureau of Meteorology Coonawarra weather station (1.5 km from the trial site) (suppl. Fig. S1). The vines were drip irrigated; 63 mm of water was applied throughout the season (suppl. Fig. S1). Fertilizers were applied (suppl. Tab. S1). Concentrations of K, Ca and Mg of irrigation water were 7.83 mg·L^{-1}, 77.8 mg L^{-1} and 40.4 mg·L^{-1} respectively.

Petiole sampling: Petioles were sampled at flowering and harvest opposite the basal inflorescences and bunches, respectively. At flowering, 10 petioles per replicate were randomly sampled and stored at -20 °C before transport and processing for nutrient analysis at the end of the season. At harvest, 12 leaf petioles were collected from each replicate. The frozen 'flowering-time' samples and the fresh 'harvest' samples were packed in ice and transported back to the laboratory in Wagga Wagga, Charles Sturt University, within 24 h after harvest sampling and stored in a cold room (4 °C) for around 48 h before oven-drying and grinding the dried material for ion analysis.

Bunch sampling at harvest, winter pruning weight and trunk circumference measurement: Grapes were harvested at a targeted total soluble solids (TSS) of around 24.8 °Brix (13.8 °Baumé). Twelve healthy bunches, 2 from each of the 6 replicate vines of each rootstock were sampled randomly. Five bunches with whole-bunch BSN within each replicate were also sampled at the same time. Samples were packed in ice and transported back to the laboratory in Wagga Wagga, Charles Sturt University within 24 h, and stored in a cold room (4 °C). Individual bunch fresh weight was determined before sub-sampling in the laboratory. For each replicate, 30 berries from the 12 bunches were subsampled randomly. Berry fresh weight and dry weight were obtained. Another 120 berries were subsampled for nutrient analysis which were frozen until analysis. Thirty berries from BSN affected bunches were subsampled for BSN berry fresh and dry weight measurements. Total bunch number and number of bunches with BSN on each vine were recorded to determine yield and percentage of BSN bunches. Trunk circumference (cm) at irrigation line height, cordon length (m) and winter pruning wood weight of all sampled vines were determined.

Juice TSS, pH, TA, yeast assimilable nitrogen (YAN), L-malic acid and

L-tartaric acid: After subsampling the grapes, the rest of the grape samples were juiced. Fresh juice samples were analysed for TSS with a digital refractometer (Pocket PAL-1, Atago, Japan). Juice pH and TA were accessed using an autotitrator (Metrohm Fully Automated 59 place Titrando System, Switzerland). Frozen juice samples were used for YAN, L-malic and L-tartaric acid analyses. Using a Konelab 20XT Analyser (Thermo Fisher Scientific, Scoresby, Australia), the ammonia concentration was determined with an enzymatic test kit and α-amino acid concentration (NOPA) was determined with a colorimetric test kit. L-malic acid and L-tartaric acid were quantified using the Analyser with an enzymatic kit and a colorimetric test kit, respectively. All test kits were from Thermo Fisher Scientific.

K, Ca and Mg analysis: All K, Ca and Mg analyses were carried out using inductively coupled plasma atomic emission spectroscopy (ICP-AES) applying the standard method according to Rayment and Lyons (2011), at the Environmental and Analytical Laboratories at Charles Sturt University, Wagga Wagga. Intact berries (120 berry subsample) were defrosted and homogenized. A 500 mg (fresh weight) sample of the homogenate was used for K, Ca and Mg analysis. Petioles and rachises were dried and ground and 100 mg of ground tissue was analysed using ICP-AES, as were frozen juice samples and dried soil samples. The extracting solution used was 1 M ammonium chloride (pH 7.0).

Statistical methods: One-way ANOVA was applied to TSS, pH, TA, YAN, L-malic acid, L-tartaric acid, berry fresh weight, berry dry weight, yield, trunk circumference, pruning weight, soil exchangeable cations (Ca, Mg, Na and K), $CEC_{bases,}$ ESP, pH, $EC_{1:5}$, plant tissue and juice nutrients with rootstocks as the main source of variation. Tukey's multiple comparisons test was applied when significant rootstock effect was found. Correlation (Pearson's *r*) was calculated between rachis [K] and petiole [K] as well as between [K] and [Ca], between [K] and [Mg] and between [Ca] and [Mg] in both grape and juice. Two-way ANOVA, followed by Sidak's multiple comparison test was applied to nutrient analysis of necrotic and healthy rachises. All analyses were carried out using GraphPad Prism 8 (GraphPad Software, San Diego, California, USA). Principal Component Analysis (PCA) (mean centring standardized) was carried out using MATLAB R2018b (The MathWorks Inc. Natick, Massachusetts, USA), to obtain an overview of correlations amongst tissue [K], [Ca] and [Mg], juice TSS, TA, [K], [Ca], [Mg], malic and tartaric acid concentrations, YAN, bunch weight, yield and vigour of the grapevine (pruning weight and trunk circumference), and in relation to the rootstocks. The 3D scatter plots, for relationships between [K], [Mg] and [Ca] in grape and juice, were graphed using SigmaPlot 14 (Systat Software Inc., San Jose, California, USA)

Results

Soil exchangeable cations, CEC_{bases}, ESP, pH and $EC_{1:5}$: Soil exchangeable cations and soil cation exchange capacity (CEC_{bases}) are presented in Tab. 1. Exchangeable Sodium Percentage (ESP %), pH and electrical conductivity ($EC_{1:5}$) are presented in Tab. 2. Despite the size of the trial site and potentially varying depth of limestone layers, concentrations of the soil exchangeable cations were consistent across the sampled area. ESP, $EC_{1:5}$ and pH were not statistically different across all sampled locations at flowering. At harvest, all soil parameters were also the same for the two sampled rootstock locations (own roots and M5512) (Tabs 1 and 2). The soil was considered high in Ca and K, moderate in Mg and Na and high in soil cation exchange capacity according to Nicholas (2004).

Table 1

Soil exchangeable cations (Ca, Mg, Na, K) (cmol[+]·kg^{-1}) and soil cation exchange capacity (CEC_{bases}) (cmol[+]·kg^{-1}). Means are presented with standard error ($n = 4$). There was no significant effect of rootstock on these soil parameters at flowering or harvest

Rootstock	Ca Flowering	Ca Harvest	Mg Flowering	Mg Harvest	Na Flowering	Na Harvest	K Flowering	K Harvest	CEC_{bases} Flowering	CEC_{bases} Harvest
1103P	24.4 ± 0.7		4.5 ± 0.1		0.8 ± 0.06		1.4 ± 0.1		31.1 ± 0.6	
110R	22.4 ± 2.4		4.4 ± 0.2		0.7 ± 0.06		1.4 ± 0.1		28.9 ± 2.5	
140RU	26.0 ± 4.0		4.9 ± 0.5		0.8 ± 0.07		1.3 ± 0.1		33.0 ± 4.7	
Börner	23.7 ± 0.9		4.7 ± 0.2		0.7 ± 0.04		1.1 ± 0.1		30.1 ± 1.1	
M5512	23.4 ± 2.0	23.9 ± 2.1	4.5 ± 0.3	3.9 ± 0.2	0.9 ± 0.04	1.5 ± 0.1	1.3 ± 0.1	1.7 ± 0.1	30.1 ± 3.6	32.2 ± 1.8
M5489	22.5 ± 3.1		4.4 ± 0.4		0.7 ± 0.02		1.2 ± 0.1		28.7 ± 3.6	
Own	22.7 ± 2.1	25.1 ± 1.4	4.4 ± 0.2	4.0 ± 0.2	0.7 ± 0.05	1.2 ± 0.2	1.2 ± 0.1	1.6 ± 0.2	28.9 ± 2.3	30.8 ± 2.3
Ramsey	24.6 ± 1.4		4.7 ± 0.2		0.8 ± 0.08		1.2 ± 0.1		31.5 ± 1.6	

Table 2

Exchangeable Sodium Percentage (ESP), pH and $EC_{1:5}$ of each rootstock block at flowering and harvest. Means are presented with standard error ($n = 4$). There was no significant effect of rootstock on these soil parameters at flowering or harvest

Rootstock	ESP (%) Flowering	ESP (%) Harvest	Soil pH Flowering	Soil pH Harvest	$EC_{1:5}$ ($dS \cdot m^{-1}$) Flowering	$EC_{1:5}$ ($dS \cdot m^{-1}$) Harvest
1103P	2.51 ± 0.24		8.06 ± 0.04		0.20 ± 0.01	
110R	2.64 ± 0.82		8.15 ± 0.08		0.18 ± 0.02	
140RU	2.57 ± 0.44		8.18 ± 0.04		0.16 ± 0.02	
Börner	2.19 ± 0.32		8.06 ± 0.02		0.18 ± 0.01	
M5512	2.97 ± 0.24	4.56 ± 0.20	8.11 ± 0.03	7.60 ± 0.03	0.21 ± 0.02	0.51 ± 0.04
M5489	2.51 ± 0.32		8.08 ± 0.03		0.17 ± 0.04	
Own	2.45 ± 0.16	4.47 ± 0.86	8.01 ± 0.05	7.82 ± 0.10	0.20 ± 0.02	0.50 ± 0.14
Ramsey	2.62 ± 0.23		7.98 ± 0.05		0.21 ± 0.02	

Soil pH in this site ranged from 7.60 to 8.18 sampled at both times, therefore it is mildly alkaline. Soil $EC_{1:5}$ ranged from 0.16 to 0.21 $dS \cdot m^{-1}$ at flowering across the site and was averaged at 0.5 $dS \cdot m^{-1}$ at harvest. These values indicate that the soil was non-saline to slightly saline (CASS *et al.* 1996). Mean ESP (%) was 2.6 % and 4.5 % at flowering and harvest, respectively, across the sampled locations, indicating a generally stable soil structure and confirming the non-sodicity of the soil (NICHOLAS 2004).

Petiole [K], [Ca] and [Mg]: At flowering, Ramsey had the highest petiole [K] of 2.84 ± 0.28 $g \cdot 100\ g^{-1}$ dw, while M5512 was the lowest (1.25 ± 0.16 $g \cdot 100 g^{-1}$ dw) (Tab. 3). No statistical difference was found in petiole [Ca] amongst rootstocks. 1103P had the highest petiole [Mg] at 0.55 ± 0.13 $g \cdot 100\ g^{-1}$ dw, while Börner had the lowest (0.26 ± 0.01 $g \cdot 100\ g^{-1}$ dw). At harvest, M5512 had lower [K] (2.25 ± 0.22 $g \cdot 100\ g^{-1}$ dw) compared to that of own roots (3.98 ± 0.25 $g \cdot 100\ g^{-1}$ dw) and 1103P (4.05 ± 0.19 $g \cdot 100\ g^{-1}$ dw). M5489 had higher [Ca] (2.38 ± 0.09 $g \cdot 100\ g^{-1}$ dw) than own roots (1.54 ± 0.08 $g \cdot 100\ g^{-1}$ dw) and Ramsey (1.80 ± 0.09 $g \cdot 100\ g^{-1}$ dw) (Tab. 3). [Mg] was not statistically different amongst the rootstocks at harvest.

Rachis [K], [Ca] and [Mg]: Both the [K] and [Mg] of healthy rachises at harvest were consistent across all rootstocks (Fig. 1). Börner had the highest [Ca] (0.54 ± 0.02 $g \cdot 100\ g^{-1}$ dw) in healthy rachises amongst all tested rootstocks (Fig. 1b, suppl. Tab. S2). Rootstock had no effect on [K] or [Mg] concentrations of those rachises affected by BSN either. BSN rachises of own roots had lowest [Ca] (0.36 ± 0.03 $g \cdot 100\ g^{-1}$ dw) whereas M5512, M5489 and Ramsey all had higher [Ca] (Fig. 1b, suppl. Tab. S2). Comparing each element between healthy and BSN rachises, [K] in healthy rachises did not differ from [K] in rachises affect with BSN for each rootstock (Fig. 1a). Noticeably, 110R, M5512, M5489 and Ramsey all had higher [Ca] in their necrotic rachises when compared to each of their healthy rachises (Fig. 1b). In contrast, for Börner, higher [Ca] was found in healthy instead of BSN rachises (Fig. 1b). Only Ramsey had higher [Mg] in BSN rachises compared to healthy ones (Fig. 1c). M5489 had the highest percentage of BSN, while own roots had the lowest (Fig. 1d).

Table 3

Petiole [K], [Ca] and [Mg] at flowering and harvest of 'Cabernet Sauvignon' on eight different rootstocks. Means are presented with standard error ($n = 4$). Different lower case letters indicate statistical difference amongst rootstocks (One-way ANOVA, $P < 0.05$). There was no rootstock effect on petiole [Ca] at flowering or petiole [Mg] at harvest

Rootstock	Petiole [K] Flowering ($g \cdot 100 g^{-1}$ dw)	Petiole [Ca] Flowering ($g \cdot 100 g^{-1}$ dw)	Petiole [Mg] Flowering ($g \cdot 100 g^{-1}$ dw)	Petiole [K] Harvest ($g \cdot 100 g^{-1}$ dw)	Petiole [Ca] Harvest ($g \cdot 100 g^{-1}$ dw)	Petiole [Mg] Harvest ($g \cdot 100 g^{-1}$ dw)
1103P	2.64 ± 0.36 ab	2.25 ± 0.47	0.55 ± 0.13 a	4.05 ± 0.19 a	1.85 ± 0.04 bc	0.51 ± 0.01
110R	1.71 ± 0.09 bc	1.63 ± 0.04	0.30 ± 0.01 bc	2.89 ± 0.58 ab	1.91 ± 0.11 ac	0.57 ± 0.13
140RU	1.91 ± 0.16 ac	2.01 ± 0.08	0.51 ± 0.24 ab	3.10 ± 0.40 ab	1.88 ± 0.13 ac	0.64 ± 0.10
Börner	2.41 ± 0.18 ab	1.65 ± 0.05	0.26 ± 0.01 c	3.07 ± 0.20 ab	2.12 ± 0.14 ab	0.36 ± 0.03
M5512	1.25 ± 0.16 c	1.73 ± 0.16	0.45 ± 0.05 ac	2.25 ± 0.22 b	2.11 ± 0.14 ab	0.52 ± 0.02
M5489	2.01 ± 0.19 ac	2.22 ± 0.14	0.37 ± 0.03 ac	2.64 ± 0.23 ab	2.38 ± 0.09 a	0.47 ± 0.02
Own	2.53 ± 0.10 ab	1.60 ± 0.15	0.51 ± 0.02 ab	3.98 ± 0.25 a	1.54 ± 0.08 c	0.60 ± 0.06
Ramsey	2.84 ± 0.28 a	1.43 ± 0.05	0.36 ± 0.02 ac	3.50 ± 0.52 ab	1.80 ± 0.09 c	0.49 ± 0.03

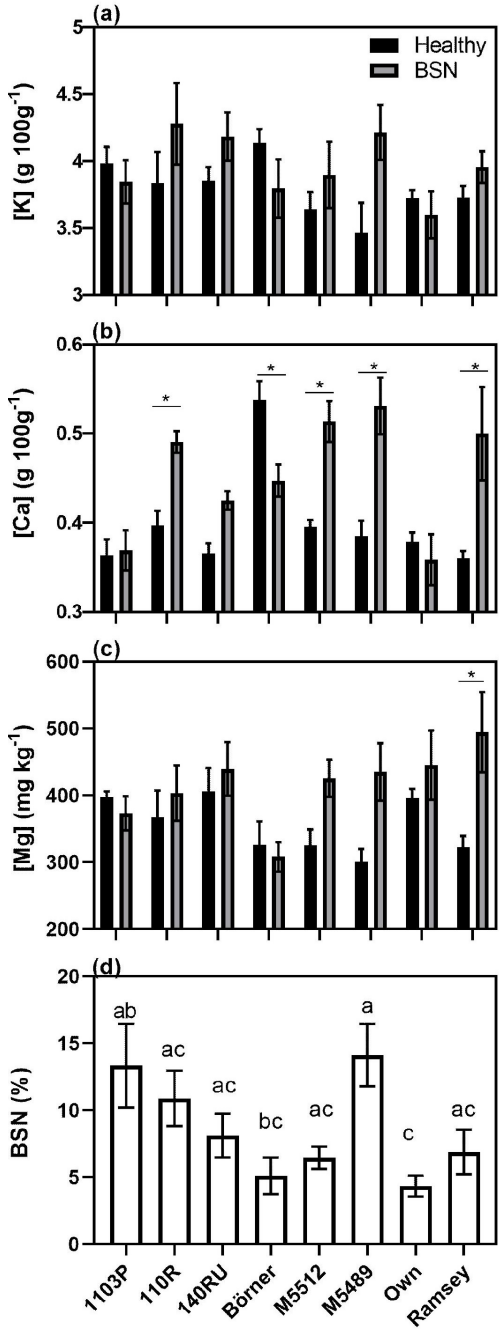

Fig. 1: Comparison of [K] (**a**), [Ca] (**b**) and [Mg] (**c**) between healthy and BSN rachises within each rootstock. Means are presented with standard error ($n = 4$). * indicates statistical difference. (Sidak's multiple comparisons test, $P < 0.05$). Comparison of percentage of BSN bunches per vine (**d**). Means are presented with standard error (n = 9). Different lower case letters indicate statistical difference amongst rootstocks (One-way ANOVA, $P < 0.05$).

Table 4

Effect of rootstock on 'Cabernet Sauvignon' juice TSS, pH, TA, YAN, L-malic acid and L-tartaric acid at harvest. Means are presented with standard error ($n = 4$). Different lower case letters indicate statistical difference amongst rootstocks. (One-way ANOVA, $P < 0.05$). There was no significant effect of rootstock on TSS

Rootstocks	TSS (°Brix)	pH	TA ($g \cdot L^{-1}$)	YAN ($mg \cdot L^{-1}$)	L-malic acid ($g \cdot L^{-1}$)	L-tartaric acid ($g \cdot L^{-1}$)
1103P	24.6 ± 0.2	3.86 ± 0.05 ab	3.84 ± 0.20 ab	182.0 ± 17.8 ac	1.65 ± 0.10 ab	4.58 ± 0.04 b
110R	24.2 ± 0.2	3.63 ± 0.05 c	4.48 ± 0.20 a	173.4 ± 21.2 ac	1.56 ± 0.05 abc	5.08 ± 0.12 a
140RU	23.8 ± 0.9	3.65 ± 0.07 bc	4.75 ± 0.42 a	221.3 ± 21.1 a	1.82 ± 0.20 a	4.92 ± 0.08 ab
Börner	23.5 ± 0.2	3.84 ± 0.06 ac	3.49 ± 0.08 b	153.1 ± 30.7 ac	1.14 ± 0.11 cd	4.66 ± 0.04 ab
M5512	24.4 ± 0.3	3.66 ± 0.04 bc	4.07 ± 0.09 ab	143.6 ± 12.9 bc	1.33 ± 0.03 bd	4.66 ± 0.17 ab
M5489	24.4 ± 0.2	3.64 ± 0.03 bc	4.15 ± 0.07 ab	124.5 ± 21.5 c	1.36 ± 0.04 bd	4.98 ± 0.09 ab
Own	23.4 ± 0.2	3.94 ± 0.04 a	3.29 ± 0.15 b	216.7 ± 12.0 ab	1.01 ± 0.06 d	4.69 ± 0.08 ab
Ramsey	23.9 ± 0.2	3.76 ± 0.04 ac	3.98 ± 0.16 ab	199.9 ± 9.6 ac	1.34 ± 0.07 abd	4.65 ± 0.10 ab

Berry juice TSS, pH, TA, yeast assimilable nitrogen (YAN), L-malic acid and L-tartaric acid: Rootstock had no significant effect on berry sugar ripeness with TSS ranging between 23.4 and 24.6 °Brix (Tab. 4). pH and TA were, however, affected by the rootstock genotypes. Own roots berry juice had the highest pH (3.94 ± 0.04) while 110R berry juice had the lowest (3.63 ± 0.05). Both M5512 and M5489 berry juice had lower pH (3.66 ± 0.04 and 3.64 ± 0.03 respectively) than own roots berry juice. Juice of 110R and 140RU berries had highest TA amongst all rootstocks (4.48 ± 0.20 and 4.75 ± 0.42 $g \cdot L^{-1}$) while Börner and own roots berries showed the lowest (3.49 ± 0.08 and 3.29 ± 0.15 $g \cdot L^{-1}$). 140RU berries had the highest YAN (221.3 ± 21.1 $mg \cdot L^{-1}$) while M5489 berries had the lowest (124.5 ± 21.5 $mg \cdot L^{-1}$). 140RU berry juice also had higher L-malic acid concentration (1.82 ± 0.20 $g \cdot L^{-1}$) than Börner, M5512, M5489 and own roots berry juice (1.14 ± 0.11, 1.33 ± 0.03, 1.36 ± 0.04 and 1.01 ± 0.06 $g \cdot L^{-1}$ respectively). Juice of 110R berries had higher L-tartaric acid concentration (5.08 ± 0.12 $g \cdot L^{-1}$) than that of 1103P (4.58 ± 0.04 $g \cdot L^{-1}$).

Berry fresh and dry weight, yield, trunk circumference and pruning weight: Berry size was impacted by rootstock. 110R showed the greatest berry fresh weight (1.05 ± 0.03 g) while own roots had the smallest berries of 0.80 ± 0.08 g. When dried, 110R

and 140RU had higher berry dry weight while Börner had the lowest. M5512 had the highest yield at 8.23 ± 1.02 kg·vine^{-1} compared to own roots (3.35 ± 0.39 kg·vine^{-1}). 110R had the biggest trunk circumference of 24.3 ± 0.9 cm while own roots had the smallest (14.0 ± 0.7 cm). Vines grown on 1103P had higher mean pruning weight (2.80 ± 0.53 kg·vine^{-1}) than Börner and own roots (0.91 ± 0.04 and 0.88 ± 0.18 kg·vine^{-1} respectively) (Tab. 5).

Grape berry and juice [K], [Ca] and [Mg]: In homogenized whole berry samples, none of [K], [Ca] or [Mg] showed any statistical differences amongst rootstocks. However, when taking berry fresh weight into account, and presenting the results on a per berry basis, mineral content did differ for some of the elements (Tab. 6). Ca content per berry remained consistent amongst rootstocks. 140RU berries had higher K of 2559 ± 126 µg berry^{-1}, while Börner berries had relatively lower K content (1751 ± 56 µg berry^{-1}) due to the lower berry weight (Tabs 5 and 6). Similarly, Börner berries showed lower Mg (80 ± 2.5 µg·berry^{-1}), while berries of 140RU and 110R both had higher Mg content. Börner had higher juice Ca concentration of 85 ± 4.8 mg·L^{-1}, while 110R and 140RU 140 had lower concentrations of 60 ± 2.6 mg·L^{-1} and 58 ± 3.5 mg·L^{-1} respectively. Own roots had slightly higher juice [Mg] of 81 ± 3.8 mg·L^{-1}, while M5512, Börner and 110R all had lower [Mg] (Tab. 6). There were positive correlations between whole berry [Mg] and [K] (Pearson's $r = 0.75$; $P < 0.0001$), [Mg] and [Ca] (Pearson's

Table 5

Effect of rootstock on 'Cabernet Sauvignon' berry fresh (fwt) and dry (dwt) weight, yield (kg·vine^{-1} and kg·m$_{(cordon)}^{-1}$), trunk circumference and pruning weight. Means are presented with standard error ($n = 4$). Different lower case letters indicate statistical difference amongst rootstocks. (One-way ANOVA, $P < 0.05$)

Rootstocks	Berry fwt (g)	Berry dwt (g)	Yield (kg·vine^{-1})	Yield (kg·m$_{(cordon)}^{-1}$)	Trunk circumference (cm)	Pruning weight (kg·vine^{-1})
1103P	0.94 ± 0.05 ac	0.271 ± 0.016 ac	4.93 ± 0.59 ab	2.38 ± 0.27 ab	21.1 ± 0.7 ab	2.80 ± 0.53 a
110R	1.05 ± 0.03 a	0.293 ± 0.007 a	6.24 ± 1.32 ab	3.11 ± 0.58 ab	24.3 ± 0.9 a	1.92 ± 0.24 ab
140RU	1.02 ± 0.02 ab	0.284 ± 0.011 a	5.62 ± 0.96 ab	2.86 ± 0.53 ab	20.3 ± 1.1 ab	2.15 ± 0.82 ab
Börner	0.83 ± 0.04 bc	0.230 ± 0.011 c	5.44 ± 0.41 ab	2.65 ± 0.17 ab	16.8 ± 0.8 b	0.91 ± 0.04 b
M5512	0.93 ± 0.02 ac	0.266 ± 0.008 ac	8.23 ± 1.02 a	3.92 ± 0.47 a	19.9 ± 0.9 ab	1.82 ± 0.29 ab
M5489	0.94 ± 0.03 ac	0.268 ± 0.018 ac	5.74 ± 1.08 ab	2.86 ± 0.49 ab	19.6 ± 1.1 b	1.71 ± 0.08 ab
Own	0.80 ± 0.08 c	0.234 ± 0.006 bc	3.35 ± 0.39 b	1.60 ± 0.17 b	14.0 ± 0.7 c	0.88 ± 0.18 b
Ramsey	1.00 ± 0.04 ac	0.279 ± 0.011 ab	5.27 ± 0.73 ab	2.56 ± 0.32 ab	19.6 ± 1.3 ab	2.05 ± 0.43 ab

Table 6

Grape and juice potassium (K), calcium (Ca) and magnesium (Mg) concentrations and content per berry at harvest of 'Cabernet Sauvignon' on various rootstocks. Means are presented with standard error ($n = 4$). Different lower case letters indicate statistical difference amongst rootstocks (One-way ANOVA, $P < 0.05$)

Rootstock	Grape [K] (mg·L^{-1})	Grape [Ca] (mg·L^{-1})	Grape [Mg] (mg·L^{-1})	Grape K (µg·berry^{-1})	Grape Ca (µg·berry^{-1})	Grape Mg (µg·berry^{-1})	Juice [K] (mg·L^{-1})	Juice [Ca] (mg·L^{-1})	Juice [Mg] (mg·L^{-1})
1103P	2361 ± 199	238 ± 15	105 ± 12	2247 ± 266 ab	226 ± 23	99 ± 14 ab	2280 ± 128	64 ± 4.9 bc	67 ± 4.6 ab
110R	2372 ± 146	248 ± 12	115 ± 6.7	2474 ± 108 ab	259 ± 7.2	120 ± 4.2 a	2039 ± 82	60 ± 2.6 c	64 ± 1.6 b
140RU	2518 ± 102	229 ± 14	117 ± 4.6	2559 ± 126 a	233 ± 18	119 ± 6.6 a	2037 ± 44	58 ± 3.5 c	65 ± 5.5 ab
Börner	2135 ± 135	267 ± 29	97 ± 7.5	1751 ± 56 b	217 ± 13	80 ± 2.5 b	2124 ± 122	85 ± 4.8 a	61 ± 2.6 b
M5512	2252 ± 37	268 ± 6.0	115 ± 2.1	2103 ± 18 ab	250 ± 5.3	107 ± 1.4 ab	2115 ± 19	68 ± 1.5 bc	61 ± 1.7 b
M5489	2527 ± 208	288 ± 15	120 ± 7.8	2397 ± 254 ab	273 ± 20	114 ± 10 ab	2107 ± 108	75 ± 4.8 ac	64 ± 3.7 ab
Own	2323 ± 154	245 ± 15	129 ± 9.0	1841 ± 168 ab	196 ± 23	103 ± 13 ab	2253 ± 129	79 ± 3.0 ab	81 ± 3.8 a
Ramsey	2391 ± 42	246 ± 15	116 ± 3.8	2407 ± 124 ab	247 ± 14	116 ± 5.2 ab	2288 ± 49	73 ± 3.6 ac	67 ± 2.6 ab

$r = 0.57$; $P = 0.0007$) and [K] and [Ca] (Pearson's $r = 0.41$; $P = 0.019$) across the entire population, regardless of rootstock type ($n = 32$) (Fig. 2a). Positive correlations were also found between juice [Mg] and [K] (Pearson's $r = 0.51$; $P = 0.003$), [Mg] and [Ca] (Pearson's $r = 0.49$; $P = 0.005$) and [K] and [Ca] (Pearson's $r = 0.40$; $P = 0.024$) (Fig. 2b).

Relationships between soil and various tissue [K]: No relationship was apparent between

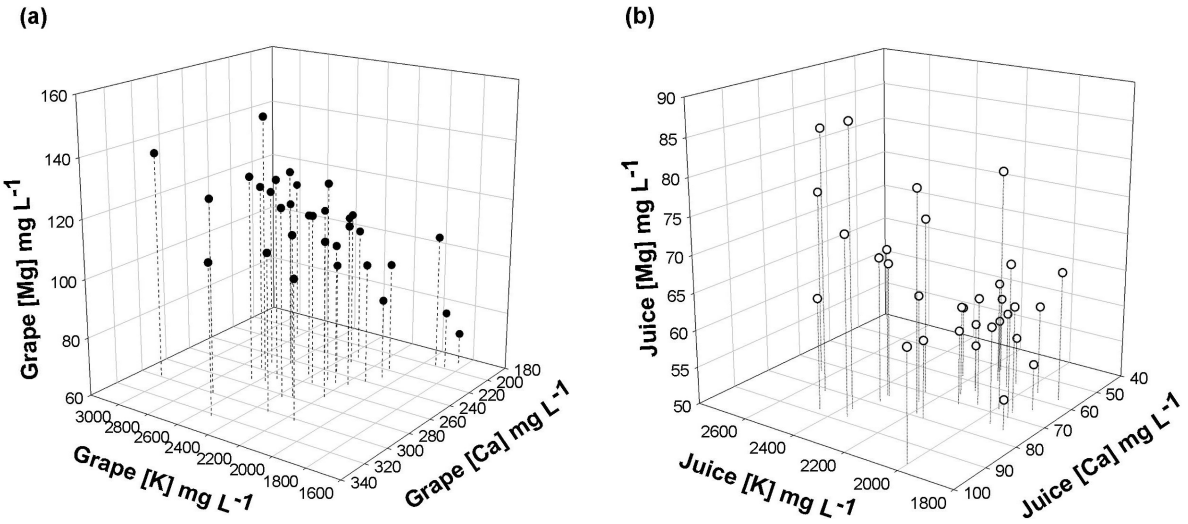

Fig. 2: 3D scatter plot of the relationship between grape [K], [Ca] and [Mg] at harvest ($n = 32$) (**a**). 3D scatter plot of the relationship between juice [K], [Ca] and [Mg] (**b**).

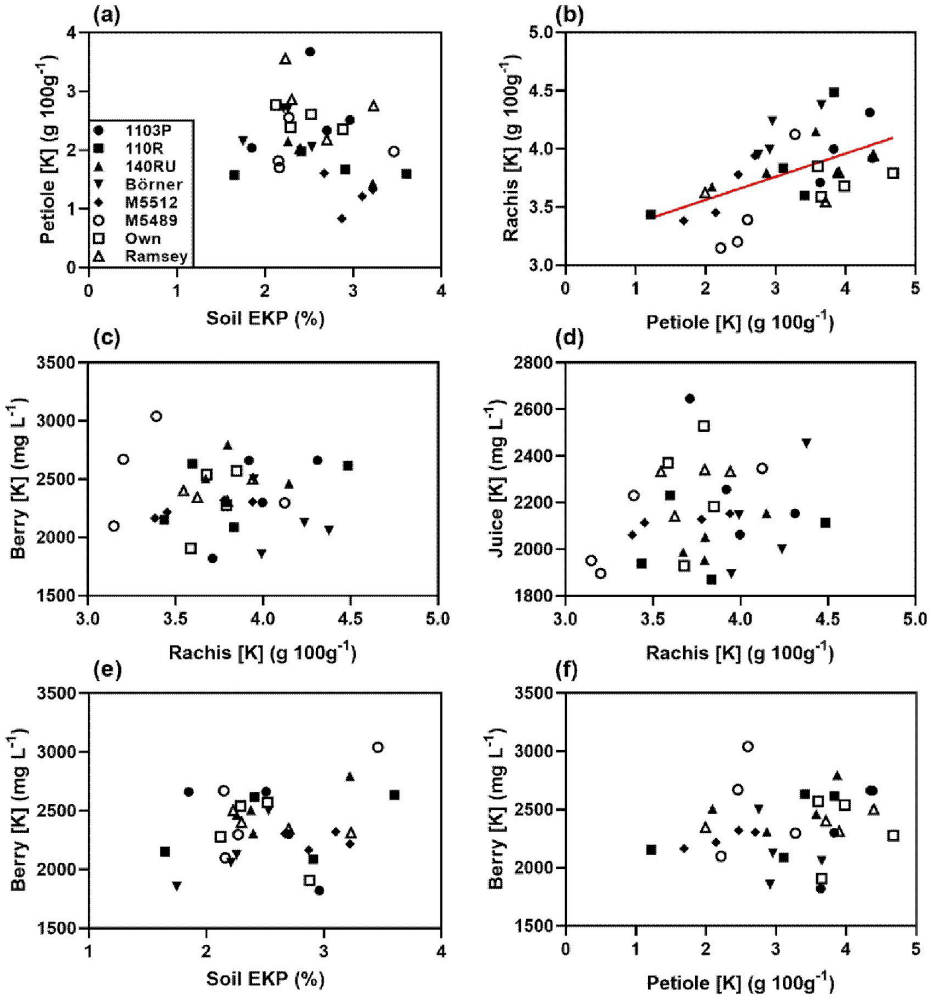

Fig. 3: Relationships between petiole [K] and soil EKP of individual replicate at flowering (**a**), between rachis [K] and petiole [K] (**b**), berry [K] (**c**) and juice [K] (**d**), berry [K] and soil EKP (**e**) and berry [K] and petiole [K] (**f**) at harvest. Points are individual replicates. A positive linear correlation (red line, Pearson's $r = 0.53$, $P = 0.002$) was found between rachis [K] and petiole [K] (**b**).

petiole [K] and soil exchangeable K percentage (EKP) at flowering (Fig. 3a). A positive linear correlation between rachis [K] and petiole [K] with Pearson's $r = 0.53$ ($P = 0.002$) was found (Fig. 3b). No linear relationship existed between berry [K] and rachis [K] (Fig. 3c), juice [K] and rachis [K] (Fig. 3d), berry [K] and soil EKP (Fig. 3e) or berry [K] and petiole [K] (Fig. 3f).

Principal Component Analysis (PCA): PCA applied to all sample replicates and variables was undertaken to more clearly determine the relationship between rootstocks and measured attributes. Six PCs each with eigenvalue greater than 1 were retained (Fig. 4a). Clear clusters of rootstocks were evident in the biplot (Fig. 4b) with the first two principle components accounting for around 49 % of data variance. The rootstocks M5512 and M5489 were tightly grouped and located at the right bottom of the plot and were separated from own roots and Börner on the PC1 axis. 140RU and 110R were mostly positioned in the top right quadrant and were diagonally opposed to Börner. Through inspection of the loadings (Fig. 4b), it can be inferred that PC1 was largely influenced by TA, concentrations of L-malic acid and L-tartaric acid, fresh and dry berry weight, trunk circumference, bunch weight and yield (positive) as well as pH, juice [Ca], juice [K], juice [Mg] and petiole [K] (negative). PC2 was heavily influenced by petiole [Ca], grape [Ca] (negative) and petiole [K], juice [K] and YAN (positive). From inspection of the loadings and scores, it can be inferred that Börner might be associated with relatively higher juice [Ca] compared to all other rootstocks. Own rooted vines were associated with high levels of petiole [K], juice [K] and [Mg] and pH. It was also indicated that the grouping of M5512 and M5489 replicates and their separation from the others, in particular own roots and Börner in PC1 might be contributed by their relatively lower juice [K] and [Mg] and pH. Higher order PCs' grouping did not reveal any sample grouping consistent with the experimental design (data not shown).

Discussion

This study investigated the effectiveness of using rootstocks in the Limestone Coast growing region to limit K uptake by 'Cabernet Sauvignon' grapevine, in order to manage acidity of berry juice, and ultimately of the must and wine. Furthermore, this study also tested the interactive link between tissue [K], [Ca] and [Mg], the three most predominant cations in the grapevine

Soil composition and nutrient availability was consistent across the block and thus the site was appropriate for studying the effect of rootstock on nutrient uptake by the plant. Despite the soil being moderately alkaline and slightly saline, the elevated pH level as well as the EC are both known to have minor effects on nutrient availability for grapevine growth (NICHOLAS 2004, EDWARDS 2018).

It is debatable whether petiole [K] at flowering can be used as an indicator for [K] in grape juice and wine (WALKER and BLACKMORE 2012). Nevertheless, the consistently lower petiole [K] in M5512 indicates its effectiveness at lowering K uptake and partitioning to the vegetative tissue on this trial site, consistent with results reported by WALKER *et al.* (2019). There were no strong overall relationships between soil K, petiole [K] and berry [K], thus indicating the complexity in regulating K uptake by the roots, and to partition K between the vegetative and reproductive tissues. A more careful examination of the vertical soil mineral gradient,

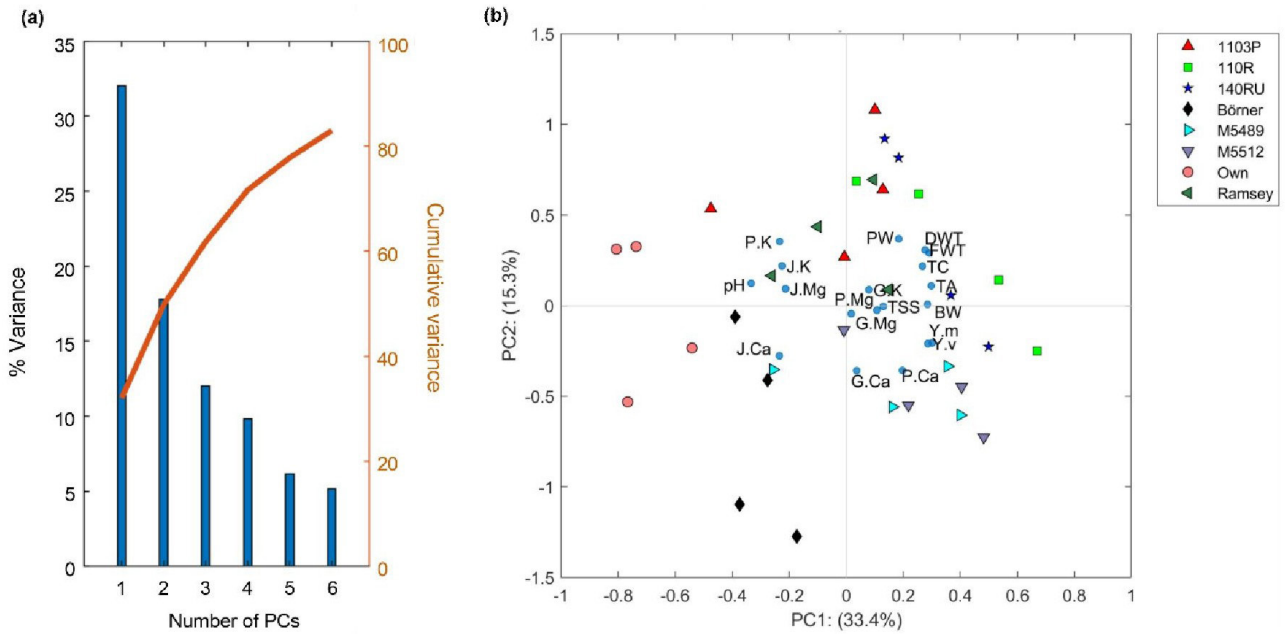

Fig. 4: Plot of % variance (left y-axis, blue column) and cumulative variance (right y-axis, orange line) of six principal components used in the PCA (**a**). Biplot of extracted principal components as a function of 22 variables for 8 rootstocks each consisting of 4 replicates and relations between the 22 variables (loadings) (**b**). (Variables: TSS: total soluble solids; TA: titratable acidity; DWT: berry dry weight; FWT: berry fresh weight; P.K: petiole [K]; P.Ca: petiole [Ca]; P.Mg: petiole [Mg]; G.K: grape [K]; G.Ca: grape [Ca]; G.Mg: grape [Mg]; J.K: juice [K]; J.Ca: juice [Ca]; J.Mg: juice [Mg]; BW: bunch weight; Y.v: yield kg·vine^{-1}; Y.m: yield kg·m^{-1}; TC: trunk circumference; PW: pruning weight; YAN: yeast assimilable nitrogen; LMA: L-malic acid; LTA: L-tartaric acid).

presence of soil mineral elements in a form unavailable for root uptake and root system characteristics will be helpful for assessing the overall performance of the rootstocks. The small differences in berry [K] between the rootstocks indicate that the reproductive tissues are a dominant sink for this nutrient, and that the grapevine is a responsive system that strives to maintain an equilibrium across the genus.

Because K accumulation in grapes is mainly through the phloem and coincides with the rapid sugar accumulation during ripening (Rogiers *et al.* 2017) and since grape juice [K] is also positively correlated with juice TSS (Walker *et al.* 2000, Ramos and Romero 2017), the similar juice [K] measured in this trial also reflects the consistent TSS across all rootstocks (Tab. 4). A positive correlation between juice pH and juice [K] was apparent across the tested rootstocks as shown for earlier studies (Walker *et al.* 1998, Walker and Blackmore 2012). It is noteworthy that own roots produced berries with higher pH relative to that for M5512, M5489, 110R and 140RU. Own roots also resulted in lower berry TA relative to that for 110R and 140RU, M5512 and M5489 have both shown some potential in lowering [K] in the petiole and juice as well as lowering juice pH, when compared to own roots (Walker and Clingeleffer 2009). Furthermore, at a different site, the effect was evident for laminae but not for grape juice (Walker *et al.* 2019). The lowest pH and highest TA were however found in berries from 140RU and 110R, both of which did not have particularly low berry [K]. These results indicate, as expected, that juice pH and TA are driven by not only the presence of K but other factors such as tartaric, malic and other acids and ions in the solution.

Furthermore, when [K] was high in the grape so were the concentrations of the other two elements at harvest, at least under the growing conditions of this study. It had been shown that a given concentration of Ca may alter the absorption of K by barley plants, depending on the concentration of K in the external media (Overstreet *et al.* 1952). The interactions of the three elements at the soil-plant interface in relation to their antagonistic effects on their accumulation in the plant tissue can be difficult to define (Jakobsen 1993). It is also possible that the level of antagonistic effect of Ca and K differed between the parts of the grapevine. As demonstrated in this study (Fig. 4), the petiole Ca and K concentrations are situated in opposite quadrants of the PCA biplot. Additional correlation analysis found that petiole [Ca] and [K] of individual replicates are indeed negatively correlated across all rootstock genotypes ($P < 0.005$). A negative correlation between petiole [Mg] and [K] was also found ($P < 0.05$). However, the variables loadings shown in Fig. 4 for juice minerals or grape berry minerals were closely aggregated. The accumulation of magnesium, a phloem-mobile mineral element also coincides with the accumulation of sugars in red grape berries (Rogiers *et al.* 2006a, Walker *et al.* 2019). All three cations exhibited positive correlations in grape and juice (Fig. 2) suggesting that any antagonistic accumulative interactions amongst cations does not apply to the berries, at least in the current concentration ranges, but perhaps can still exist in vegetative tissue such as the petiole. Future studies attempting to limit grapevine K uptake could focus on testing the antagonism between K, Mg and Ca with varying concentrations of these elements supplemented to the soil. While there were no obvious overall differences in rachis nutrient concentrations as a result of BSN, more Ca was harboured in the necrotic rachises of a few rootstock types. It must be said, however, that it is uncertain if this was contributing to- or the result of BSN (Christensen and Boggero 1985, Keller and Koblet 1995). Nonetheless, this may provide some further direction for investigations on the cause of BSN.

In summary, this one year trial of 'Cabernet Sauvignon' has generated some promising results in identifying suitable rootstocks for optimising pH and TA in grape juice. PCA results confirmed the potential of M5512 and M5489 in lowering juice [K] and pH and achieving higher TA. Interestingly, grape [K] and juice [K] did not show a good correlation, suggesting 1) K accumulation in grape seeds and skin might differ amongst rootstocks and/or 2) K may have precipitated out during juicing. 140RU is known to have low translocation efficiency of K from roots to shoot (Kodur *et al.* 2010b) due to retention of K in root vacuoles (Rühl 1993) and potentially high re-translocation of K from shoot to roots (Kodur *et al.* 2010b). The grouping of 140RU, 110R, M5512 and M5489 in the PCA scores plot indicate that these particular rootstocks had relatively low juice pH and [K] and higher TA.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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