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Charles Sturt University

The Limestone Coast Grape & Wine Council Inc.

How can we influence potassium (K) levels in the vineyard?



FINAL REPORT FOR INCUBATOR PROJECT

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Abstract

This project investigates the feasibility of using rootstocks to lower berry potassium concentrations ([K]) in Cabernet Sauvignon grapevines grown in the Coonawarra wine region. The overall aim is to achieve lower pH and higher titratable acidity in grape juice so as to maintain wine stability and bring down the cost of acid adjustment during winemaking. The objective is to provide new insights into the potential of particular rootstocks to modify K uptake by Cabernet Sauvignon grapevines grown in 'Terra Rossa' soil and their partitioning and accumulation into grape berries. To achieve this objective, the soils of a replicated rootstock trial located in the Limestone Coast of South Australia were characterised and plant tissue, grape and juice nutrient content were assessed at oenological maturity for 8 different rootstocks. Rootstock had a significant impact on cations of the vegetative tissue of Cabernet Sauvignon, with M5512 having the lowest petiole [K]. The concentrations of major cations in the berry were, however, not significantly altered by rootstock. While no particular rootstock stood out in its ability to limit Cabernet Sauvignon berry K accumulation, Börner berries 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 titratable acidity was higher for the rootstocks 140RU and 110R, and juice pH tended to be lower for the rootstocks 110R, 140RU, M5512 and M5489. There was no effect of rootstock on total soluble solids.

Executive summary

Excess [K] in grapes reduces the free tartaric acid concentration in the juice and can modify the pH of both the juice and wine. A common amelioration strategy is tartaric acid addition during winemaking, which can be costly. High [K] can lead to precipitation of natural, as well as tartaric acid added during winemaking, into potassium bitartrate which further increases cost. Industry observation, in the Limestone Coast, found that the [K] level in the juice varies amongst vintages; excess [K] in the juice appears to be exacerbated by stressful growing conditions such as heatwaves.

In this project, we characterised the concentrations of soil, petiole, rachis and grape K, calcium (Ca), and magnesium (Mg) cations of an established rootstock trial in the Coonawarra. The trial is composed of own rooted Cabernet Sauvignon and Cabernet Sauvignon on 7 rootstocks. This new database provides links between soil,

vine, berry and juice nutrient and composition at flowering and harvest. This project characterised the three major cations temporally and spatially in the soil-vine complex. It provided a better understanding of K uptake and partitioning in Cabernet Sauvignon grapevines as affected by rootstocks to allow vineyard managers to make informed decisions on rootstock selection for Cabernet Sauvignon wine production in this region.

The characterisation of the soil established that the cation profile and other soil compositional parameters were consistent, across the trial site at the sampled depths. Notably, rootstock was able to modify petiole [K] and [Mg] at flowering and [K] and [Ca] at harvest. Although concentrations of K, Ca and Mg in the whole berries were not affected by rootstocks, change in juice pH at harvest was evident. The two rootstocks released by CSIRO, M5512 and M5489, were successful at achieving both low [K] and low pH of the juice. Additionally, we observed that K, Ca and Mg accumulation into grape berries coincided with each other. A nutrient analysis of rachis affected by bunch stem necrosis is also presented.

Aims and background

K is the most abundant cation in the grape berry. It plays 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 (Coetzee, et al. 2017,Coetzee, et al. 2019,Rogiers, et al. 2006). From an applied perspective, [K] strongly influences juice pH and titratable acidity. High [K] in juice and wine reduces 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 also has limited solubility and this declines with increasing ethanol concentration. The K bitartrate precipitates can form on the cork or accumulate in the bottom of the bottle and decrease consumer acceptance of a wine.

Australian soils are naturally high in [K] and in some regions, excessive levels may result in problems at the winery as outlined above (Kodur 2011,Mpelasoka, et al. 2003). Several grapevine rootstocks have been shown to influence potassium uptake (Kodur, et al. 2011,Ruhl, et al. 1988). Rootstocks have been identified that can lower [K] in grape juice and wine in Shiraz and Chardonnay grown in other Australian viticulture regions (Walker and Blackmore 2012). Furthermore, rootstocks can affect K uptake in Shiraz grapevines (Kodur 2011). Both M5489 and M5512 are low to

medium vigour rootstocks that have previously been shown to lower K uptake and lower Shiraz juice and wine pH (Dry 2007). How the Cabernet Sauvignon scion interacts with rootstocks growing in the Coonawarra region remains unclear. K uptake by the plant can also be limited by the antagonistic interaction with Ca and Mg in the soil (Jakobsen 1993,Ohno and Grunes 1985). It is uncertain how K, Ca and Mg interact with each other at the root-soil interface, within the perennial storage components of the vine or within the must to ultimately affect the precipitation process. For these reasons we have opted to examine the interaction of these three cations from the field to the winery.

This project investigated the rootstock effect on K uptake and juice pH of Cabernet Sauvignon grapevines grown in the unique 'Terra Rossa' soil of the Limestone Coast region. It focused on an existing rootstock trial planted in 2009 and 2010 in Coonawarra in partnership with Vinehealth Australia, Treasury Wine Estates and Coonawarra Vignerons. Further, concentrations of other elements potentially antagonistic to K uptake in grapes, mainly calcium (Ca) and magnesium (Mg), were determined.

A more comprehensive understanding of the interaction between this regional soil and rootstocks and its effect on grape and wine potassium will facilitate the development of more sustainable wine production practises in the Limestone Coast region. Topics addressed in this report had been identified and developed in consultation with industry partners. The knowledge acquired here provides new leads into regional rootstock selection to optimize Cabernet Sauvignon production better suited to the Limestone Coast and potentially to other regions with excess potassium levels.

Project aims

	Output	Target Date	Activities
a	Finalised project plan	01/09/2018	Low K uptake rootstock (1) selection* and vine allocation (including own-rooted and selected rootstock) at Coonawarra vineyard. Finalise project design, experimental plan and timeframe for data collection.
b	Field trial implemented	01/10/2018	Temperature and humidity sensor installation.
c	Regional partner informed of progress	01/10/2018	Verbal report to the Limestone Coast Grape and Wine Council Technical Committee.
d	Regional partner informed of progress	31/01/2019	Verbal report to the Limestone Coast Grape and Wine Council Technical Committee.
e	Dataset linking soil, leaf and grape	01/04/2019	Canopy ambient temperature. Collect samples at key phenological points for K, Ca, Mg determination in soil, leaves and berries. Send samples to external lab for inorganic nutrient analysis.
f	Regional partner informed of progress	01/04/2019	Verbal report to the Limestone Coast Grape and Wine Council Technical Committee.
g	Data set linking rootstocks with wine composition	01/05/2019	Conduct small-scale winemaking^ with fruit from own-rooted vine and selected rootstock and assess links between soil, berry and wine chemical composition. Analysis of wine composition (pH, TA, K, Ca, Mg, anthocyanins). If differences in wine chemical composition are apparent, carry out wine tasting assessment.
h	Final Report	31/07/2019	Collate and analyse data. Submit Final Report to Wine Australia.
i	Regional partner informed of project outcomes	20/11/2019#	Present verbal report of project findings and implications to Regional Partner.

* 8 rootstocks, including own roots, have all been tested in this study instead of own roots and 1 selected rootstock.

^ Small-scale winemaking was conducted by Limestone Coast Grape and Wine Council and AWRI to free up funding for additional rootstocks tested.

This is to coincide with a tasting of the experimental wines at the regional meeting hosted by the Limestone Coast Grape and Wine Council on 27th of Nov. 2019.

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), Ramsey, 1103 Paulsen (1103P), Börner, 140 Ruggeri (140RU), M5489, M5512 and own roots. Planted in 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 9 rootstocks (M6262 not used in this report due to low canopy vigour) in a replicated and randomized vineyard design, with each row having 15 panels, and each panel consisting of 6 vines. Each rootstock was replicated 11 times within the trial site. 4 replicates, each consisting of 6 vines for each rootstock, were used in this report, unless stated otherwise.

Soil

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 rootstocks were taken at around 15cm underneath the surface with a hand-held auger, samples were stored at -20 °C until analysis at the end of the season. At harvest, samples were taken from own roots and M5512 at 10cm underneath the surface. Soil samples were air dried at 60 °C for 5 days, ground and sieved (2mm 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 with swapping the numerator [Na] in equation (1) with [K].

$$\text{Exchangeable Sodium Percentage} = \frac{[\text{Na}]}{[\text{Al}]+[\text{Na}]+[\text{K}]+[\text{Ca}]+[\text{Mg}]} \times 100 \qquad \text{Equation (1)}$$

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

Water input, fertilizer applications and composition of irrigation water

Effective rainfall (defined as >10mm per day) was obtained from the Australian Bureau of Meteorology Coonawarra weather station (1.5 km from the trial site) (Supplementary Fig. 1). The vines were drip irrigated; 63 mm of water was applied throughout the season (Supplementary Fig. 1). Fertilizers were applied (Supplementary Table 1). 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. 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 opposite a bunch were collected from each replicate. The frozen flowering samples and the fresh harvest samples were packed in ice and transported back to the laboratory in Wagga Wagga, Charles Sturt University, within 24 hours after harvest sampling and stored in a cold room (4 °C) for around 48 hours before oven-drying and grinding the dried material for ion analysis.

Bunch sampling at harvest

Grapes were harvested at a targeted total soluble solids (TSS) of around 24.8 °Brix (13.8 °Baumé). 12 healthy bunches, 2 from each vine within the 6 vine replicate were sampled randomly. 5 necrotic bunches (BSN) within each replicate were also sampled at the same time. Samples were packed in ice and transported back to laboratory in Wagga Wagga, Charles Sturt University within 24 hours, and stored in cold room (4 °C). Individual bunch fresh weight was determined before subsampling 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 and frozen until analysis. 30 BSN berries from the necrotic bunches were subsampled for BSN berry fresh and dry weight measurements. Total bunch number per metre and BSN bunch number per metre were recorded to determine yield and percentage of BSN bunches.

Juice TSS, pH and TA

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).

K, Ca and Mg analysis

All K, Ca and Mg analyses were performed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) at the Environmental and Analytical Laboratories at Charles Sturt University, Wagga Wagga. Intact berries (120 berry subsample) were defrosted and homogenized. 500 mg 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.

Juice analysis prior to winemaking

Juice chloride concentrations ([Cl]) and berry colour ripeness, including free anthocyanins, total phenolic compounds and total tannins were determined at the winemaking facility at AWRI prior to acid adjustment. All tests were carried out on the one whole harvest sample and two technical replicates were applied to the tests. Berry colour analysis was performed by using a spectroscopic method developed from research of Somers and Evans (1974). [Cl] concentration in grapes was measured by silver ion titration with a chloridometer (Model 442–5150, Buchler Instruments, Lenexa, Kansas, USA).

Statistical methods.

One-way ANOVA was applied to total soluble solids, pH, TA, berry fresh weight, berry dry weight, yield, soil nutrient and composition, plant tissue and juice nutrients with rootstocks as the main source of variation. Tukey's multiple comparisons test was applied when significant rootstock effects were found. Linear regressions was applied rachis [K] and petiole [K], [K] and [Ca], [K] and [Mg] and [Ca] and [Mg] in 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 performed using GraphPad Prism 8 (GraphPad Software, San Diego, California, USA), except Principal Component Analysis (PCA) was performed using SigmaStat 4.0 (Systat Software Inc., San Jose, California, USA), to obtain an overview of correlations amongst tissue nutrient and juice composition, and their relationships 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 and Discussion

Soil exchangeable cations, CEC_{bases}, ESP, pH and $EC_{1:5}$

Soil exchangeable cations and soil cation exchange capacity (CEC_{bases}) are presented in Table 1. Exchangeable Sodium Percentage (ESP %), pH and electrical conductivity ($EC_{1:5}$) are presented in Table 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 the locations at flowering. Therefore any differences in plant tissue levels should reflect the ability of the rootstock and/or plant in affecting the ion uptake or partitioning. At harvest, all soil parameters were also the same for the two sampled rootstock locations (own roots and M5512) (Table 1 & 2). The soil was high in Ca and K, moderate in Mg and Na. It also had high soil cation exchange capacity (Nicolas, 2004). Soil pH in this site ranges from 7.6 to 8.18 sampled at both times, therefore it is moderately alkaline. However this elevated pH level has a minor effect on nutrient availability for grapevine growth (Edwards, 2018). A high [Ca] could, however, potentially affect and compete with K uptake by the plant (Jakobsen 1993,Overstreet, et al. 1952). Soil $EC_{1:5}$ ranged from 157.5 to 212 µS cm^{-1} at flowering across the site and was averaged at 504 µS cm^{-1} at harvest. These values indicate that the soil was non-saline to slightly saline, again with likely only minor repercussions on vine growth or root function (Nicolas, 2004). 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).

Table 1. Soil exchangeable cations (Ca, Mg, Na, K) (cmol[+]/kg) and soil cation exchange capacity (CEC_{bases}) (cmol[+]/kg). 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}	CEC_{bases}
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	$EC_{1:5}$ (µS cm^{-1}) Flowering	$EC_{1:5}$ (µS cm^{-1}) Harvest
1103P	2.51 ± 0.24		8.06 ± 0.04		201.6 ± 8.8	
110R	2.64 ± 0.82		8.15 ± 0.08		175.3 ± 18.8	
140RU	2.57 ± 0.44		8.18 ± 0.04		157.5 ± 23.8	
Börner	2.19 ± 0.32		8.06 ± 0.02		176.3 ± 9.3	
M5512	2.97 ± 0.24	4.56 ± 0.20	8.11 ± 0.03	7.60 ± 0.03	212 ± 18.0	506.8 ± 44.1
M5489	2.51 ± 0.32		8.08 ± 0.03		174.3 ± 17.6	
Own	2.45 ± 0.16	4.47 ± 0.86	8.01 ± 0.05	7.82 ± 0.10	202.5 ± 15.5	501.0 ± 143.0
Ramsey	2.62 ± 0.23		7.98 ± 0.05		210.5 2± 3.2	

Petiole [K], [Ca] and [Mg]

At flowering, Ramsey had the highest petiole [K] of 28383 ± 2835 mg L^{-1}, while M5512 was the lowest (12457 ± 1606 mg L^{-1}) (Table 3). No statistical difference was found in petiole [Ca] amongst rootstocks. 1103P had the highest petiole [Mg] at 5506 ± 1276 mg L^{-1}, while Börner had the lowest (2608 ± 86 mg L^{-1}). At harvest, M5512 had lower [K] (22512 ± 2201 mg L^{-1}) compared to that of own roots (39787 ± 2473 mg L^{-1}) and 1103P (40484 ± 1869 mg L^{-1}). M5489 had higher [Ca] (23802 ± 910 mg L^{-1}) than own roots (15389 ± 833 mg L^{-1}) and Ramsey (18018 ± 898 mg L^{-1}) (Table 3). [Mg] was not statistically different amongst the rootstocks at harvest. Whether petiole [K] at flowering can be used as an indicator for [K] in grape juice and wine is debatable (Ruhl 1993, Walker and Blackmore 2012). Nevertheless, the consistently lower petiole [K] in M5512 indicates its effectiveness at lowering K uptake and/or partitioning to the vegetative tissue on the trial site.

Table 3. Petiole [K], [Ca] and [Mg] at flowering and harvest. Means are presented with standard error (n = 4). Different lower case letters indicate statistical difference amongst rootstocks (One-way ANOVA, $p < 0.05$).

Rootstock	Petiole [K] Flowering (mg L^{-1})	Petiole [Ca] Flowering (mg L^{-1})	Petiole [Mg] Flowering (mg L^{-1})	Petiole [K] Harvest (mg L^{-1})	Petiole [Ca] Harvest (mg L^{-1})	Petiole [Mg] Harvest (mg L^{-1})
1103P	26413 ± 3589 ab	22528 ± 4658	5506 ± 1276 a	40484 ± 1869 a	18503 ± 418 bc	5077 ± 99
110R	17073 ± 937 bc	16315 ± 382	3006 ± 96 bc	28937 ± 5793 ab	19055 ± 1125 abc	5713 ± 1324
140RU	19094 ± 1613 abc	20104 ± 802	5050 ± 235 ab	31031 ± 3979 ab	18764 ± 1312 abc	6428 ± 1033
Börner	24070 ± 1753 ab	16491 ± 455	2608 ± 86 c	30683 ± 1996 ab	21169 ± 1394 ab	3631 ± 267
M5512	12457 ± 1606 c	17256 ± 1598	4464 ± 467 abc	22512 ± 2201 b	21146 ± 1394 ab	5221 ± 231
M5489	20144 ± 1889 abc	22199 ± 1367	3682 ± 330 abc	26367 ± 2271 ab	23802 ± 910 a	4687 ± 186
Own	25330 ± 978 ab	15964 ± 1529	5128 ± 230 ab	39787 ± 2473 a	15389 ± 833 c	6001 ± 596
Ramsey	28383 ± 2835 a	14343 ± 534	3568 ± 181 abc	35004 ± 5230 ab	18018 ± 898 c	4890 ± 304

Rachis [K], [Ca] and [Mg]

Both the [K] and [Mg] of healthy rachises were consistent across all rootstocks. Börner had the highest [Ca] (5382 ± 208 mg L^{-1}) in healthy rachises amongst all tested rootstocks (Table 4). Rootstock had no effect on the [K] or [Mg] concentrations of those rachises affected by BSN either. BSN rachises of own roots had lowest [Ca] (3588 ± 284 mg L^{-1}) whereas M5512, M5489 and Ramsey all had higher [Ca] (Table 4).

Comparing each element between healthy and BSN rachises, noticeably, 110R, M5512, M5489 and Ramsey all had higher [Ca] in their necrotic rachises when compared to each of their healthy rachises (Figure 1b). In contrast, for Börner, higher [Ca] was found in healthy instead of BSN rachises (Figure 1b). Only Ramsey had higher [Mg] in BSN rachises compared to healthy ones (Figure 1c). M5489 had the highest percentage of BSN, while own roots had the lowest (Figure 1d).

Table 4. [K], [Ca] and [Mg] at harvest in healthy rachises and those affected by BSN. Means are presented with standard error (n = 4). Different lower case letters indicate statistical difference amongst rootstocks (One-way ANOVA, $p < 0.05$).

Rootstock	Rachis [K] Healthy (mg L^{-1})	Rachis [Ca] Healthy (mg L^{-1})	Rachis [Mg] Healthy (mg L^{-1})	Rachis [K] BSN (mg L^{-1})	Rachis [Ca] BSN (mg L^{-1})	Rachis [Mg] BSN (mg L^{-1})
1103P	39851 ± 1246	3637 ± 180 b	397 ± 8	38467 ± 1610	3692 ± 226 bc	373 ± 25
110R	38386 ± 2305	3970 ± 166 b	367 ± 40	42803 ± 3049	4908 ± 122 ab	404 ± 41
140RU	38535 ± 1024	3656 ± 115 b	406 ± 35	41843 ± 1805	4253 ± 103 abc	440 ± 40
Börner	41386 ± 1018	5382 ± 208 a	326 ± 35	37968 ± 2197	4475 ± 179 abc	308 ± 22
M5512	36387 ± 1326	3959 ± 76 b	325 ± 24	38980 ± 2485	5137 ± 229 a	426 ± 28
M5489	34660 ± 2253	3852 ± 174 b	301 ± 19	42163 ± 2049	5313 ± 319 a	435 ± 43
Own	37265 ± 583	3788 ± 102 b	396 ± 14	36000 ± 1762	3588 ± 284 c	445 ± 52
Ramsey	37271 ± 887	3606 ± 77 b	323 ± 17	39567 ± 1176	5001 ± 523 a	495 ± 60

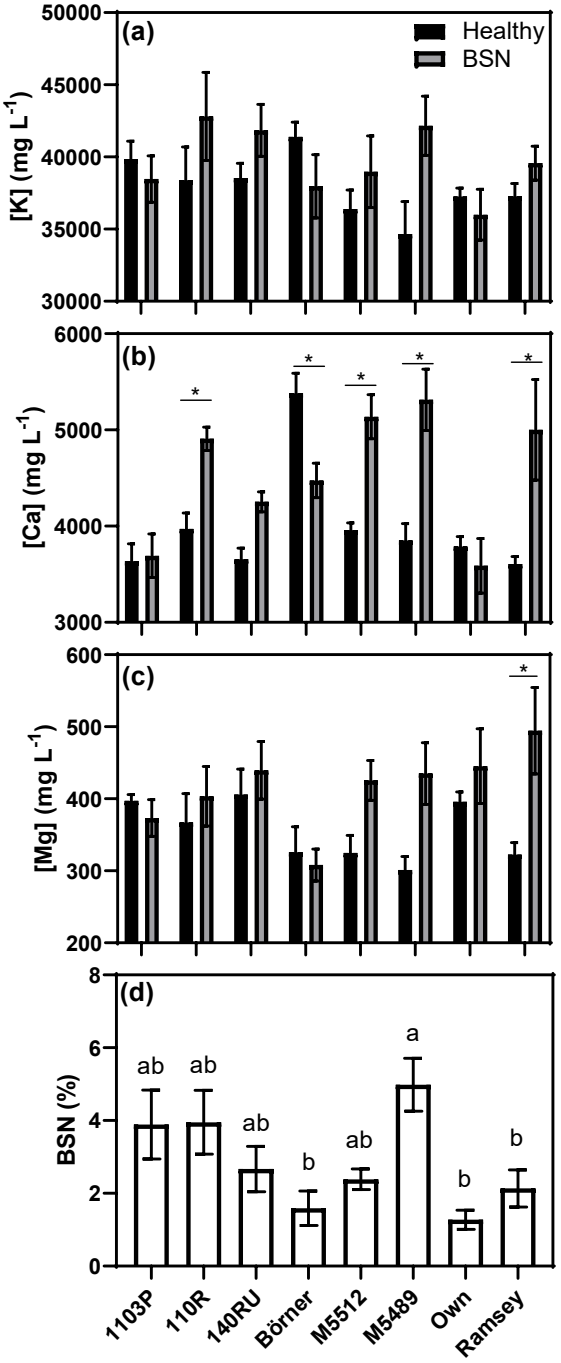


Figure 1. Comparison of [K] (a), [Ca] (b) and [Mg] (c) between healthy and BSN rachises within each rootstock. Means are presented with standard error (n = 4). * indicates statistical difference. (Sidak's multiple comparisons test, $p < 0.05$). Comparison of percentage of BSN bunches per vine (d). Means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

Grape juice composition, berry fresh and dry weight and yield at harvest

Rootstock had no significant effect on berry sugar ripeness with TSS ranging between 23.4 and 24.6 °Brix (Table 5). pH and TA were however altered by the rootstocks. Own roots had the highest pH (3.94 ± 0.04) while 110R had the lowest (3.63 ± 0.05). Both M5512 and M5489 (3.66 ± 0.04 and 3.64 ± 0.03 respectively) had lower pH than own roots. 110R and 140R had highest TA amongst all rootstocks (4.48 ± 0.20 and 4.75 ± 0.42 g L^{-1}) while Börner and own roots showed the lowest (3.49 ± 0.08 and 3.29 ± 0.15 g L^{-1}). Berry size was also impacted by rootstock. 110R had the greatest berry fresh weight (1.05 ± 0.03 g) while own roots had the smallest of 0.80 ± 0.08 g. When dried, 110R and 140RU had highest berry dry weight while Börner had the lowest. M5512 had the highest yield at 3.92 ± 0.47 kg m^{-1} compared to own roots (1.6 ± 0.17 kg m^{-1}). Juice [Cl], free anthocyanins, total tannins and total phenolic compounds of juice collected immediately after crushing, are presented in Table 6. Colour ripeness appeared to be consistent across all rootstocks. These data indicated the capability of all rootstocks in achieving desirable and similar colour ripeness.

Table 5. Effect of rootstock on berry TSS, pH, TA, berry fresh (fwt) and dry (dwt) weight and yield 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 L^{-1})	Berry fwt (g)	Berry dwt (g)	Yield (kg m^{-1})
1103P	24.6 ± 0.2	3.86 ± 0.05 ab	3.84 ± 0.20 ab	0.94 ± 0.05 ab	0.27 ± 0.02 ac	2.38 ± 0.27 ab
110R	24.2 ± 0.2	3.63 ± 0.05 c	4.48 ± 0.20 a	1.05 ± 0.03 a	0.29 ± 0.01 a	3.11 ± 0.58 ab
140RU	23.8 ± 0.9	3.65 ± 0.07 bc	4.75 ± 0.42 a	1.02 ± 0.02 ac	0.28 ± 0.01 a	2.86 ± 0.53 ab
Börner	23.5 ± 0.2	3.84 ± 0.06 abc	3.49 ± 0.08 bc	0.83 ± 0.04 bc	0.23 ± 0.01 c	2.65 ± 0.17 ab
M5512	24.4 ± 0.3	3.66 ± 0.04 bc	4.07 ± 0.09 ab	0.93 ± 0.02 ab	0.27 ± 0.01 ac	3.92 ± 0.47 a
M5489	24.4 ± 0.2	3.64 ± 0.03 bc	4.15 ± 0.07 ab	0.94 ± 0.03 ab	0.27 ± 0.01 ac	2.86 ± 0.49 ab
Own	23.4 ± 0.2	3.94 ± 0.04 a	3.29 ± 0.15 bc	0.80 ± 0.08 b	0.23 ± 0.01 bc	1.60 ± 0.17 bc
Ramsey	23.9 ± 0.2	3.76 ± 0.04 ac	3.98 ± 0.16 ab	1.00 ± 0.04 ab	0.28 ± 0.01 ab	2.56 ± 0.32 ab

Table 6. Effect of rootstocks on juice [Cl], free anthocyanins, total tannins and total phenolic compounds.

Rootstock	Juice [Cl] (mg L^{-1})	Free anthocyanins (mg g^{-1})	Total tannins (mg g^{-1})	Total phenolic compounds (a.u. g^{-1})
1103P	30.0	1.81	7.60	179.0
110R	29.3	1.82	6.68	165.7
140RU	28.8	1.91	7.10	175.3
Börner	33.5	2.47	8.76	218.9
M5512	27.2	2.06	7.24	181.1
M5489	29.2	2.13	7.70	189.9
Own	45.2	2.17	7.90	196.6
Ramsey	32.9	2.01	7.56	185.2

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 (Table 7). 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 (Table 5 & 7). Similarly, Börner berries showed lower Mg (80 ± 2.5 µg $berry^{-1}$), while berries of 140RU and 110R both had higher Mg content.

In contrast, juice [K] did not differ amongst the rootstocks. Because K accumulation in grapes is mainly through the phloem and coincides with the rapid sugar accumulation during ripening (Rogiers, et al. 2017) and grape juice [K] is also positively correlated with juice TSS (Ramos and Romero 2017,Walker, et al. 2000), the similar juice [K] measured in this trial also reflected the consistent TSS across all rootstocks (Table 5). For Ca in the juice, Börner had higher concentration of 85 ± 4.8 mg L^{-1}, while 110R and 140RU 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] (Table 7). There were positive correlations between whole berry [Mg] and [K] (r^2 = 0.56; $p < 0.0001$), [Mg] and [Ca] (r^2 = 0.32; $p < 0.0001$) and [K] and [Ca] (r^2 = 0.17; $p < 0.0001$) across the entire population, regardless of rootstock type (n = 32) (Figure 2a). Positive correlations were also found between juice [Mg] and [K] (r^2 = 0.26; p = 0.003), [Mg] and [Ca] (r^2 = 0.24; p = 0.005) and [K] and [Ca] (r^2 = 0.16; p = 0.024) (Figure 2b). It had been shown that a given concentration of calcium may increase or decrease the absorption of potassium depending on the concentration of potassium in the external media (Overstreet, et al. 1952). It is uncertain if the three tested cations might interact with each other at the soil-plant interface and have antagonistic effects on their accumulation in the plant tissue (Jakobsen 1993). This interaction does not seem to apply in this case either since all three cations exhibited positive correlations in accumulating in the grapes, at least in the current concentration ranges.

Table 7. Grape and juice potassium (K), calcium (Ca) and magnesium (Mg) concentrations and content per berry at harvest of various rootstocks. Means are presented with standard error. 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 bc
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 c
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 bc
Börner	2135 ± 135	267 ± 29	97 ± 7.5	1751 ± 56 bc	217 ± 13	80 ± 2.5 bc	2124 ± 122	85 ± 4.8 a	61 ± 2.6 c
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 c
M5489	2527 ± 208	288 ± 15	120 ± 7.8	2397 ± 254 ab	273 ± 20	114 ± 10 ab	2107 ± 108	75 ± 4.8 abc	64 ± 3.7 bc
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 ab
Ramsey	2391 ± 42	246 ± 15	116 ± 3.8	2407 ± 124 ab	247 ± 14	116 ± 5.2 ab	2288 ± 49	73 ± 3.6 abc	67 ± 2.6 bc

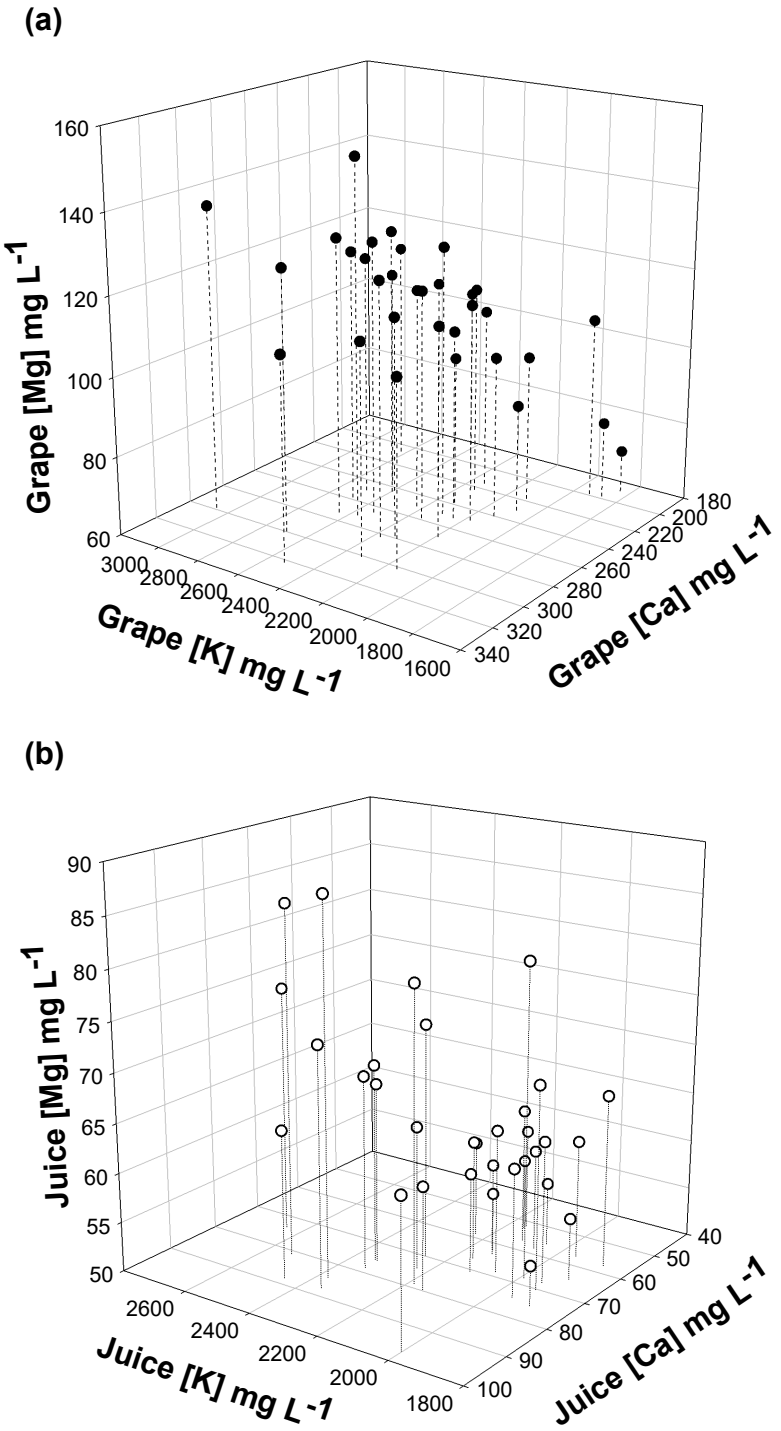


Figure 2. The relationship between grape [K], [Ca] and [Mg] at harvest (n = 32) (a). Slopes of linear regressions (not shown) are all positive, with $r^2 = 0.56$ between [Mg] and [K], $r^2 = 0.32$ between [Mg] and [Ca] and $r^2 = 0.17$ between [K] and [Ca] ($p < 0.0001$). The relationship between juice [K], [Ca] and [Mg] (b). Slopes of linear regressions (not shown) are all positive, with $r^2 = 0.26$ between [Mg] and [K], $r^2 = 0.24$ between [Mg] and [Ca] and $r^2 = 0.16$ between [K] and [Ca] ($p < 0.05$).

Relationships between soil and various tissue [K]

No relationship was apparent between petiole [K] and soil exchangeable K percentage (EKP) at flowering (Figure 3a). A positive relationship between rachis [K] and petiole [K] with $r^2 = 0.28$ ($p = 0.002$) was found (Figure 3b). No linear relationship existed between berry [K] and rachis [K] (Figure 3c), juice [K] and rachis [K] (Figure 3d), berry [K] and soil EKP (Figure 3e) or berry [K] and petiole [K] (Figure 3f).

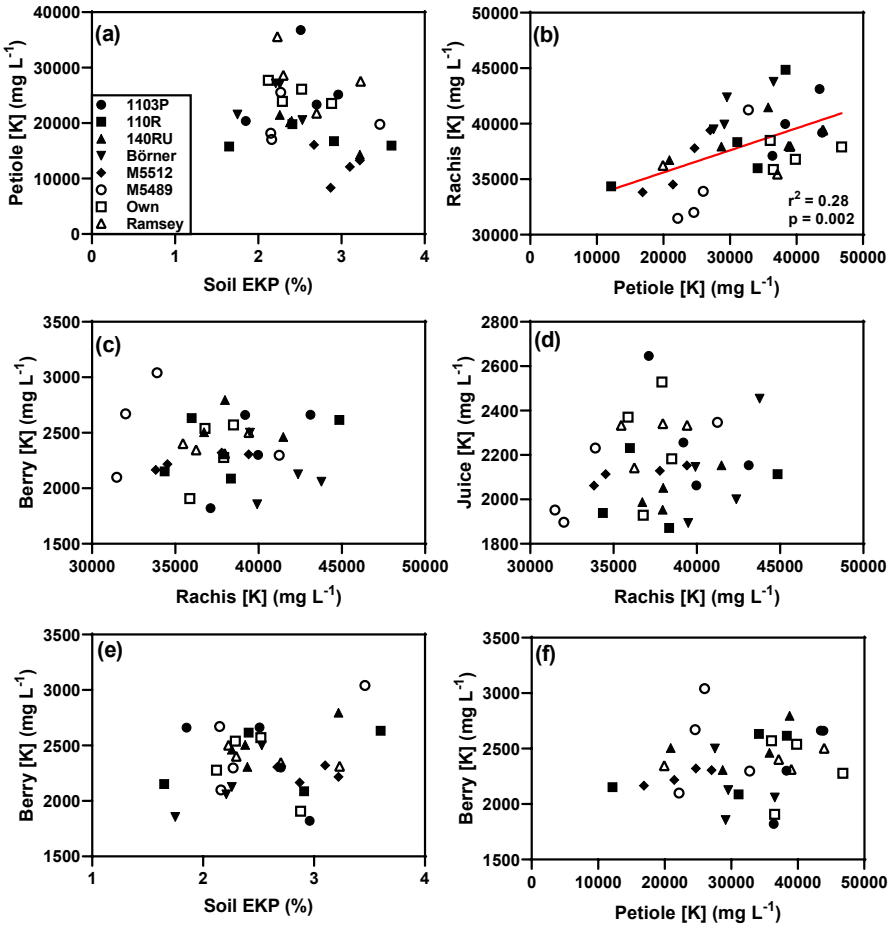


Figure 3. Relationships between petiole [K] and soil EKP of individual replicate at flowering (a), between rachis [K] and petiole [K] (b), berry [K] (c) and juice [K] (d), berry [K] and soil EKP (e) and berry [K] and petiole [K] (f) at harvest. Points are individual replicates. Linear regression (non-zero slope, $p < 0.05$) is fitted in (b).

Principal Component Analysis

PCA applied to all sample replicates and variables was undertaken to more clearly determine the relationship between rootstocks and measured attributes. Clear clusters of rootstocks was evident in the scores plot (Figure 4) with the first two principle components accounting for around 54% of data variance. Rootstocks M5512 and M5489 were tightly grouped and located at the left bottom of the plot and were

separated from own roots and Börner on the PC1 axis. 140RU and 110R were positioned in the top left quadrant and were diagonally opposed to Börner.

Through inspection of the loadings (Figure 5), it can be inferred that PC1 was largely influenced by TA, fresh and dry berry weights (negative) and juice [Cl], pH, juice [Ca], total phenolic compounds, total tannins and free anthocyanins (positive). PC2 was heavily influenced by petiole [Ca], grape [Ca] (negative) and petiole [K], juice [Mg] and juice [K] (positive). From inspection of the loadings and scores, it can be inferred that Börner had relatively higher levels of anthocyanins, phenolic compounds and juice [Ca] compared to all other rootstocks. Own rooted vines had high levels of petiole [K], juice [K], [Cl] and [Mg] and pH. M5512 and M5489 had lower juice [K], [Mg] and [Cl] and pH. These 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 2) K may had been precipitated out during juicing. The grouping of 140RU, 110R, M5512 and M5489 in the PCA scores plot, indicated all had relatively low juice pH and [K] and higher TA. 140RU is known to have low translocation efficiency of K from roots to shoot (Kodur, et al. 2010) due to retention of K in root vacuoles (Ruhl 1993) and potentially high re-translocation of K from shoot to roots (Kodur, et al. 2010).

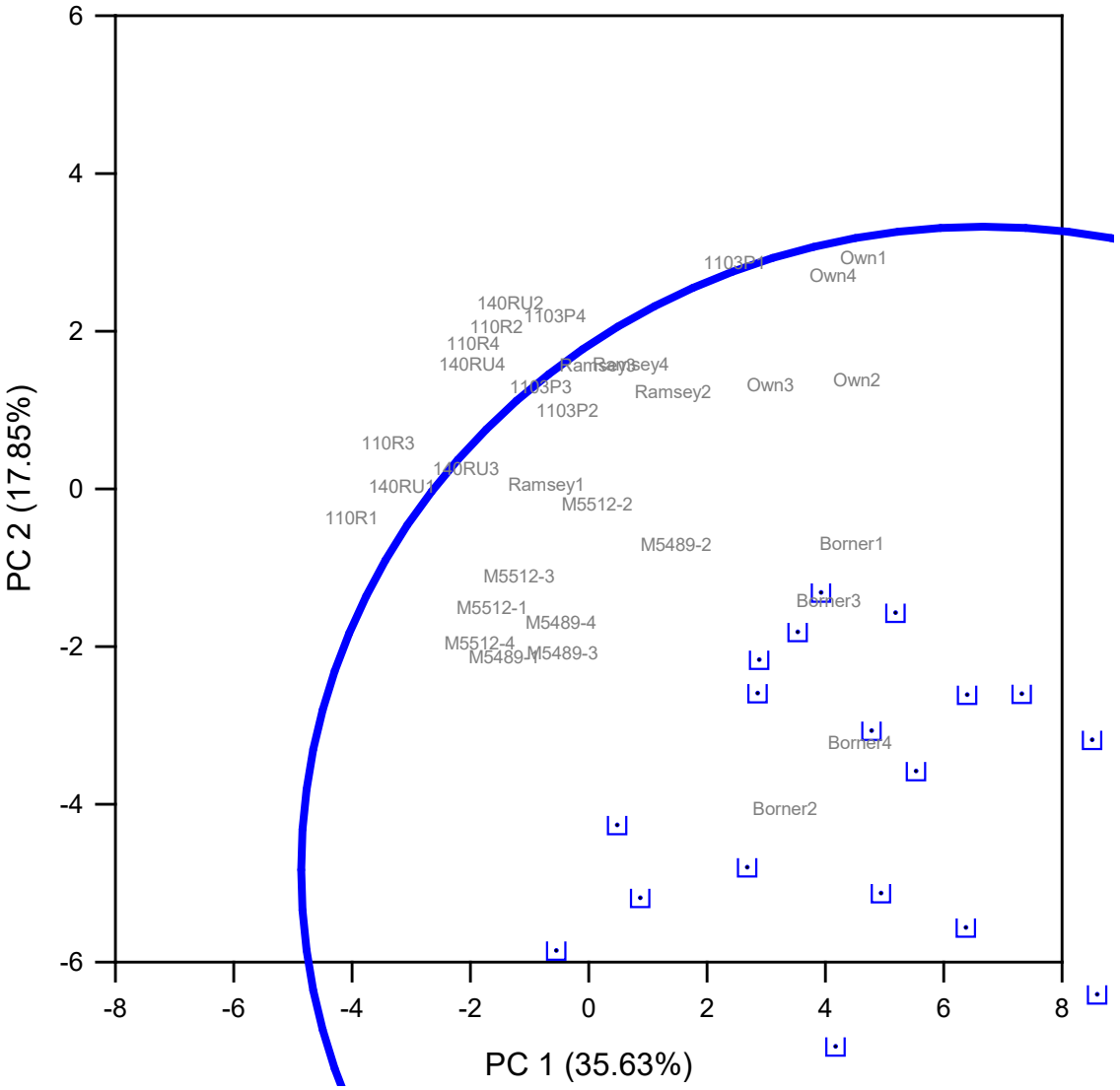


Figure 4. Extracted principal components as a function of 19 variables for 8 rootstocks each consisting of 4 replicates.

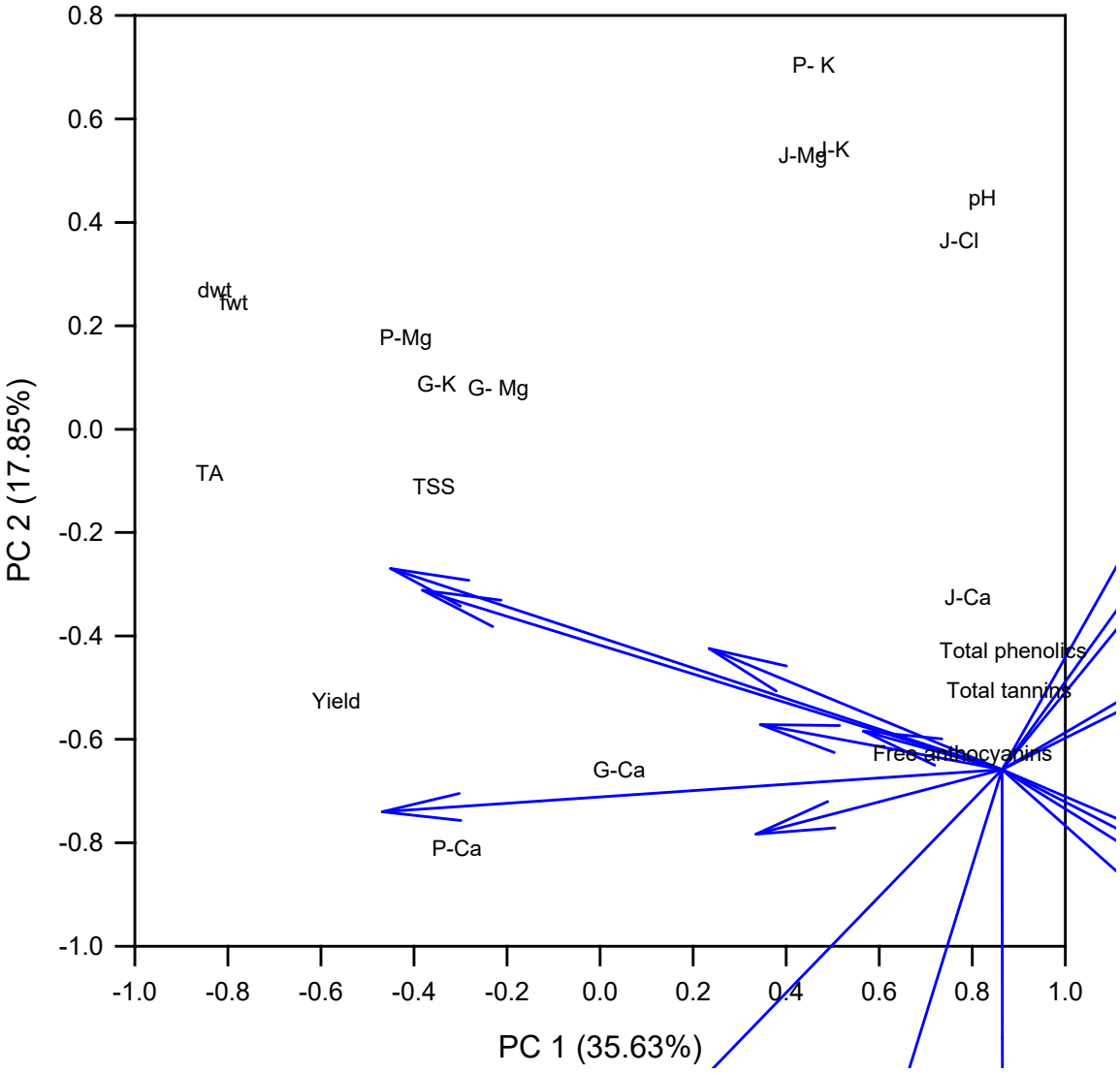
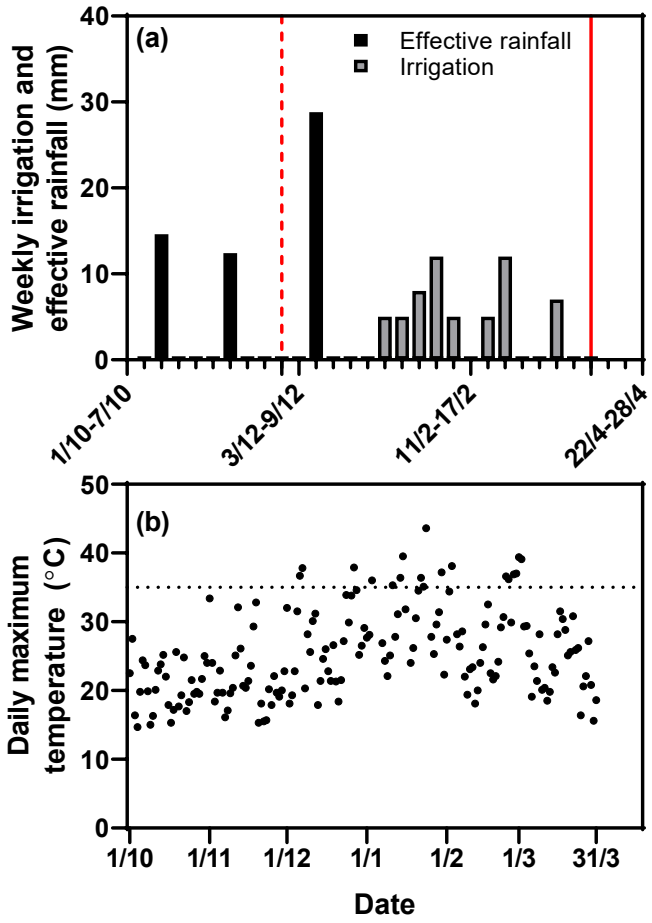


Figure 5. Relation between the 19 variables (loadings). (TA: titratable acidity; dwt: berry dry weight; fwt berry fresh weight; P-K: petiole [K]; P-Ca: petiole [Ca]; P-Mg: petiole [Mg]; G-K: grape [K]; G-Ca: grape [Ca]; G-Mg: grape [Mg]; J-K: juice [K]; J-Ca: juice [Ca]; J-Mg: juice [Mg], J-Cl: juice [Cl]; TSS: total soluble solids.)



Supplementary Figure 1. Weekly water input, effective rainfall (>10mm daily) and irrigation, from 1st of October to harvest (2nd of April, indicated by solid red line) (a). Flowering week was indicated by dashed red line, harvest by solid red line. Daily maximum temperature (b). Dashed line indicates 35 °C.

Supplementary Table 1. Fertilizer application during growing season.

Date	Product name	Nutrient	Application rate (L hectare^{-1})
18/10/2018	MaxiPhos Injecta 23	16% N, 23% P	46.28
13/11/2018	Bluestripe Magnesium Sulfate	6% Mg	2.75
5/12/2018	ZnMnMATE	5% Zn, 10% Mn	2.74

Conclusion and recommendations

This study investigated the effectiveness of using rootstocks in the Limestone Coast growing region to limit K uptake of Cabernet Sauvignon grapes, 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. This one year trial of Cabernet Sauvignon on the 'Terra Rosa' soil has generated some promising results in identifying the suitable rootstocks for optimising pH and TA in grape juice.

Soil composition and nutrient availability was consistent across the trial site as indicated by the soil composition analyses, and thus the site was appropriate for studying the effect of rootstock on nutrient uptake by the plant. There were no strong overall relationships between soil K, petiole [K] and berry [K], indicating the complex ability of the plant to regulate K uptake by the roots, and to partition K between the vegetative and reproductive tissues. The small differences in berry [K] between the rootstocks indicate that the reproductive tissues are a dominant sink for this nutrient and the grapevine is a responsive system that strives to maintain an equilibrium.

A positive correlation between juice pH and juice [K] was apparent across the tested rootstocks, while a negative correlation was observed between TA and juice [K]. It is noteworthy own roots has a higher pH to M5512, M5489, 110R and 140RU. Own roots also has a lower TA to 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, without affecting sugar and colour ripeness, when compared to own roots. M5512 also had higher yield than own roots. 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, Ca, Mg and K all accumulated in the grape at similar rates. That is, when [K] was high in the grape so were the concentrations of the other two elements, at least under the growing conditions of this study. Therefore future studies attempting to limit grapevine K uptake could focus on testing the antagonism between potassium, magnesium and calcium 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 calcium 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. Nonetheless, this may provide some further direction for investigations on the cause of bunch stem necrosis.

Extension

The outcomes of the project will be communicated through a seminar within the Limestone Coast region, an industry and scientific article. This project will be followed up by a longer Wine Australia funded study commenced in the Riverina and Orange regions in 2019, at the National Wine and Grape Industry Centre, Charles Sturt University and NSW Department of Primary Industries, focussed on the management of wine pH in a warming climate and different soil types (Project title: Managing wine pH in a changing climate). The outcomes of this Incubator Initiative project will be used to refine the objectives of the larger project.

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