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Effect of rootstock on nutrition, pollination and fertilisation in 'Shiraz' (*Vitis vinifera* L.)

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Summary

Rootstocks have previously been shown to alter reproductive performance in grapevines. The concentration of nutrients associated with pollination and fertilisation in grapevines such as boron, calcium, zinc and molybdenum were determined in petiole and pollen tissue from vines from a 'Shiraz' (*Vitis vinifera* L.) rootstock trial at flowering. 'Shiraz' on own roots had a higher calcium concentration in the petioles across the three seasons than the rootstock treatments. This coincided with higher seeded berry number, total number of berries per bunch and berry weight compared to rootstock treatments. '1103 Paulsen' had a significantly higher amount of boron and a lower number of seedless berries and a lower millerandage index (MI). Zinc deficiency was observed for '110 Richter' and '140 Ruggeri' across the three seasons and when zinc was found to be deficient, coulure index (CI) was increased. In the third and final year of the analysis pollen nutrition was incorporated into the analysis. Deficiency of molybdenum in both pollen and petiole analysis resulted in reduced berry weight due to stenospermocarpy or seed shrivel. Rootstocks with the highest number of pollen grains on the stigma also had the highest number of ovules fertilised. Calcium, zinc, boron and molybdenum are nutrients essential for pollination and fertilisation in grapevines and rootstocks were found to affect the sequestration of nutrients which affected reproductive performance.

K e y w o r d s : nutrition, pollination, fertilisation, rootstocks.

Introduction

The grapevine flowering process has been extensively reviewed (Pratt 1971, Srinivasan and Mullins 1981, Vasconcelos *et al.* 2009). Flowering is initiated by the dehiscence of the cap to expose the anthers that release pollen grains that land on the stigma of the pistil and swell to germinate. Germination results in the production of a pollen tube that grows down from the stigma through the style to the central part of the ovary and the ovule. Once a pollen tube reaches the ovule, sperm cells move down the pollen tube to fertilise the ovule (Vasconcelos *et al.* 2009). This process is known as fertilisation (May 2004) and under optimal conditions, it should take place 2-3 d after pollination (Pratt 1971). For the majority of cases, a successful fertilisation will result in the development of a seed; there may be up to 4 seeds developing within the ovules, two in each carpel (Vasconcelos *et al.* 2009). The exception to this occurs under stenospermocarpy, where pollination stimulates fruit development but ovules abort without producing mature seeds (Pratt 1971, Iland *et al.* 2011), this is the case for seedless grape varieties and seedless berries of seeded varieties (May 2004). The success of pollen germination and pollen tube growth is dependent on the presence of both macro and micro elements, in particular, nitrogen, calcium, boron, zinc and molybdenum (Brewbaker and Kwack 1963, Chen *et al.* 1998, Kaiser *et al.* 2005, Longbottom 2007, May 2004, Robinson 1994, Treeby 2001). The role of nutrition in flowering and fruitset of grapevines is relatively well known (May 2004). However, when a nutrient is deficient, for example, in the case of molybdenum deficient 'Merlot' (Williams *et al.* 2004, Longbottom 2007), limited attention has been paid to the effects of rootstocks on nutritional status and grapevine reproduction.

Millerandage and coulure are abnormal reproductive phenomena of fruitset that can have a negative impact on final yield (Dry *et al.* 2010). Millerandage occurs when a high proportion of flowers develop abnormally into either seedless berries or live green ovules (LGO) (May 2004, Collins and Dry 2009). Coulure results when a high proportion of flowers fail to develop into a berry or LGO, also defined as excessive shedding of ovaries or young berries (May 2004, Collins and Dry 2009). To measure the expression of coulure and millerandage, two indices have been developed: Millerandage Index (MI) and Coulure Index (CI) (Collins and Dry 2009). For these indices, the higher the numerical value, the greater the incidence of the condition. The use of rootstocks '140 Ruggeri' and 'Schwarzmann' on 'Merlot' have been shown to reduce the incidence of millerandage compared to own roots (Kaiser *et al.* 2005). Also, a decrease in millerandage and coulure for 'Merlot' vines grafted to rootstocks compared with own roots have been reported (Kidman *et al.* 2013). However, further work to quantify the nutritional status, fertilisation and fruitset for rootstocks is required.

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The objective of this study was to determine the influence of different rootstocks on pollination and germination and ovule fertility in the cultivar 'Shiraz' and compare this with own roots. In addition, calcium, boron, zinc and molybdenum were analysed to determine the influence of rootstocks on the sequestration of these nutrients. The cultivar 'Shiraz' was chosen for this study as the most planted cultivar in Australia representing 46 % of all vineyard area planted to red wine grapes (www.abs.gov.au/ausstats/abs@.nsf/DetailsPage/mf/1329.0.55.002).

Material and Methods

Experimental site: In 2008, a three year experiment was established at Nuriootpa, South Australia, Australia (34°48'S, 139.01°'E). The vineyard was planted in 2001 at 1481 vines per hectare, vine spacing and row spacing 2.25 m x 3 m respectively and trained to a bilateral cordon. Row orientation was east/west. All grafted and ungrafted vines were sourced from a commercial nursery. Certified 'Shiraz' (clone BVRC30) and rootstocks were tested for virus. Prior to grafting, rootstocks were hot water treated for 30 min at 50 °C. Vines were spur pruned to approximately forty nodes per vine (approximately 19 nodes per meter) to match the commercial pruning level of the vineyard. Vines were drip irrigated, using either bore water or water from the Murray River via the Barossa Infrastructure Limited (BIL) scheme. Scheduling of irrigation was based on Gbug (gypsum block) sensor assessments and was approximately 10^6 $L \cdot ha^{-1}$ (100 mm) each season. Meteorological conditions were monitored using daily temperature and rainfall data derived from the Bureau of Meteorology weather station, located at the trial site. Long term average temperature and rainfall data were calculated from weather data archived on the Bureau of Meteorology website (http://www.bom.gov.au/climate/averages/tables/cw_023321.shtml). Annual rainfall for the region for the years 2008-2011 was 434 mm, 502 mm, 589 mm, and 640 mm, respectively. The long term average rainfall for the region is 500 mm (http://www.bom.gov.au/climate/averages/tables/cw_023321.shtml). The site is located within a phylloxera-free region that allows for the use of ungrafted *Vitis vinifera* vines. The soil is typically a Light Pass fine sandy loam A horizon overlying a red brown earth B horizon (NORTHCOTE 1954).

Experimental design: Measurements were taken over three consecutive seasons starting in the 2008/2009 growing season. 'Shiraz' was grafted to six rootstocks: 'Ramsey' (*Vitis champinii*), 'Schwarzmann' (*Vitis riparia* x *Vitis rupestris*), '1103 Paulsen' (*Vitis berlandieri* x *Vitis rupestris*), '140 Ruggeri' (*Vitis berlandieri* x *Vitis rupestris*), '99 Richter' (*Vitis berlandieri* x *Vitis rupestris*) and '110 Richter' (*Vitis berlandieri* x *Vitis rupestris*) and compared to own roots 'Shiraz' (*Vitis vinifera*). The experiment was performed across ten rows for each rootstock and own roots control. Within each plot, there were three replicate blocks of vines, consisting of seven treatment vines.

Pollen tube growth: At the commencement of flowering five flowers per inflorescence were collected from two randomly selected inflorescences per vine, per treatment replicate. Flowers were collected approximately three days after opening, and placed into labelled vials filled with Carnoys fluid (60 % ethanol (CH_3CH_2OH), 30 % chloroform ($CHCl_3$) and 10 % glacial acetic acid ($C_2H_4O_2$)). A total of two inflorescences per vine from two vines per treatment replicate were assessed for pollen tube growth. Sample processing was continued on a sub-sample of thirty flowers (five flowers from each of the two inflorescences from the three replicate blocks) selected from the initial sample. Pollen tube growth was analysed as described by EBADI (1996). Flowers were fixed for a minimum of 2 h in Carnoys fluid. Samples were then hydrated in 70 % ethanol for 30 min followed by 30 % ethanol for 30 min and then in water for a minimum of 1 h. Samples were softened for analysis using 0.8 M sodium hydroxide (NaOH) until the flowers changed from green to black (approximately 10 min). Flowers were then stained using 0.1 % water soluble aniline blue in 0.1 M potassium phosphate (K_3PO_4) for a minimum of 1 h before being placed onto a glass microscope slide with a cover slip and sectioned longitudinally down the centre to expose the stylar transmitting tissue and the ovules. Pollen and pollen tubes were prepared for microscopic observation with ultra-violet illumination using the Zeiss AX10 microscope (Oberkochen, Germany) at 10x 40 magnification.

The number of pollen grains on the stigma, the number of pollen tubes in the style and lower ovary and, the number of pollen tubes that had penetrated the ovule were scored for each flower.

Pollen viability: The procedure to evaluate pollen viability was as described by PETERSON and TABER (1987). Pollen was harvested from two basal inflorescences selected randomly from a vine; from two vines per treatment replicate, by gently tapping the inflorescence onto a small Petri dish to catch the pollen grains. A fine haired paint brush was then used to sweep the extracted pollen into labelled vials and then stored in a -20 °C freezer until analysis. Pollen viability was assessed using a fluorescein diacetate stain (2 $mg \cdot mL^{-1}$) in 100 ml acetone (C_3H_6O). One drop of fluorescein diacetate was added to a microscope slide and allowed to set for approximately 60 s or until solution went cloudy; to account for the acetone evaporation. During this period, approximately 10 drops of 10 % w/v sucrose ($C_{12}H_{22}O_{11}$) solution was added to the sampled pollen grains and thoroughly mixed. One drop of the sucrose pollen mixture was added to the fluorescein diacetate on the slide and a cover slip placed over the solution on the slide. Pollen grains were prepared for microscopic observation with ultra-violet illumination using the Zeiss AX10 microscope at 10 x 20 magnification. Pollen grains were scored on percent viability of fluorescing pollen.

Measurement of reproductive performance: Reproductive performance was measured as described by COLLINS and DRY (2009). Briefly, three basal inflorescences per vine were selected for uniformity before flowering. Each sample inflorescence was enclosed in a fine mesh bag, secured with a plastic tie and labelled according to the method described by COLLINS and DRY (2009). After flowering was complete, the bags were re-

moved and collected flower caps counted as a representation of the number of flowers per inflorescence. At harvest a measurement of bunch number and bunch weight per meter of cordon was determined. In addition, the corresponding sample bunches that were chosen for flower cap assessment were collected at harvest, weighed and assessed for the number of seeded, seedless and LGOs within the bunch. Yield components and a measure of millerandage and coulure using the indices described by Collins and Dry (2009) were also calculated.

Nutrient analysis: Approximately 100 petioles from each replicate and treatment were collected at flowering for elemental analysis. Petioles used for the analysis were taken from the leaf opposite the basal bunch. Petioles were dried in brown paper bags in a fan-forced oven at 60 °C for a period of no more than one week. Dried samples were then ground into particles of < 1 mm in diameter. Elemental analysis on the petioles was performed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) for the following nutrients: boron, zinc, calcium, and in 2011, molybdenum (Waite Analytical Services Adelaide, Australia). For analysis of pollen, three basal inflorescences per vine from the eastern side of row on a north south oriented trellis system were collected at flowering from the replicates and treatments. Pollen was also harvested as described above and frozen at -20 °C for further analysis. 0.3 g of pollen was used for ICP-OES for the following nutrients: boron, zinc, calcium and molybdenum (Waite Analytical Services Adelaide, Australia).

Statistical analysis: A block design with seven vines per block replicated three times per treatments was used. The data analysis package Genstat (13th Edition 13.1.0.72 Lawes Agriculture Trust, United Kingdom 2007) was used to analyse the data using a one-way analysis of variance (ANOVA). The significance of the difference between treatment means was determined by using Fischer's least significant difference (LSD) test calculated at the 5 % level.

Results and Discussion

For successful reproductive development in many angiosperms, there is a requirement for adequate levels of both calcium and boron (Brewbaker and Kwack 1963). Calcium is a requirement for pollen tube growth and germination in a wide range of plant species as calcium aids in the rigidity and straightness of the pollen tube (Brewbaker and Kwack 1963). In each of the three seasons we did not see an effect on pollen tube growth or germination for any of the rootstock treatments. In grapevines, adequate calcium concentrations in the petiole range from 1.2-2.5 $mg \cdot kg^{-1}$ (Robinson 1994). Calcium concentrations in the petiole differed significantly between seasons and treatments but were all within the adequate range (Tab. 2). In 2009 and 2011, petiole calcium levels were significantly lower for both '1103 Paulsen' and '140 Ruggeri' rootstocks compared to own roots 'Shiraz'. In addition, '110 Richter' also had lower calcium concentrations in 2010 and 2011 when compared with own roots 'Shiraz'. In the 2009 season, when calcium was limited in '1103 Paulsen' and '110 Richter', these rootstocks had lower seeded berries, berry numbers and berry weights. In 2011, when calcium was limited in '110 Richter', berry weight was also reduced. Interestingly, own roots 'Shiraz' in all seasons had high calcium concentrations, which corresponded with higher berry number, total berry number per bunch and berry weight (Tab. 2). Boron aids in stigma receptivity, pollen germination and pollen tube growth as a result of sugar borate complexes that promote sugar absorption, sugar translocation and metabolism of sugars in the pollen (Chen *et al.* 1998, May 2004, Lee *et al.* 2009). Adequate concentrations of boron in the petioles of grapevines at flowering are between 30-100 $mg \cdot kg^{-1}$; however, toxicity in grapevines is reported at > 80 $mg \cdot kg^{-1}$ in leaf tissue (Robinson 1994, Yermiyahu *et al.* 2006). Boron deficiencies in grapevines can result in reduced set, with a high proportion of seedless berries (Robinson 1994, Creasy and Creasy 2009). No treatments were found to be either deficient or toxic for boron throughout the analysis; however, own roots 'Shiraz' had the lowest boron concentrations as a mean across the three seasons of the analysis compared to all other treatments. In contrast, '1103 Paulsen' and '110 Richter' had the highest concentrations as a mean of the three seasons of analysis. However, this had no effect on pollen viability, pollen tube growth or ovule fertilisation (Tab. 1), presumably because boron was not limiting and below the toxic threshold. Nonetheless, all rootstocks with higher boron levels had a lower number of seedless berries compared with own roots 'Shiraz', indicating a possible interaction between rootstock and boron for the reduction of seedless berries.

Zinc deficiency in grapevines has been associated with coulure and millerandage (Robinson 1994, Creasy and Creasy 2009) as zinc is required for both pollen and fruit development (Marschner 1986). An adequate level of zinc in grapevine petioles is > 26 $mg \cdot kg^{-1}$ (May 2004, Robinson 1994). Zinc deficiency was observed for '110 Richter' and '140 Ruggeri' across the three seasons and for '1103 Paulsen' in two out of the three seasons and '99 Richter' and 'Schwarzmann' in one season (Tab. 2). When zinc was found to be deficient in these rootstocks, CI was higher rather than MI. As previously mentioned, 'Shiraz' is a cultivar that has more coulure than millerandage (Dry *et al.* 2010). It is therefore not surprising, that low millerandage was expressed in relation to low zinc concentrations, yet notable differences for coulure when zinc was deficient. These results support the essential role of zinc in fruit development to prevent excessive development of coulure and millerandage.

In the third and final year of the analysis, in addition to petiole nutrition, pollen nutrition was incorporated into the analysis. Also, molybdenum was assessed along with boron, zinc and calcium. Although molybdenum has previously been measured in the inflorescence at flowering (Longbottom 2007), to the best of our knowledge, this appears to be the first time pollen nutrition has been analysed in grapevines. For each of the elements, the concentration was always higher in the pollen than in the petiole, with the exception of calcium (Tab. 2). A comparison between petiole and pollen nutrition was performed for the 2011 data

Table 1

Reproductive performance of 'Shiraz' on different rootstocks at Nuriootpa, South Australia (2009-2011)

Variable		Control (SHI)	110 Richter	1103 Paulsen	140 Ruggeri	99 Richter	Ramsey	Schwarz-mann	Season mean	*P* value	LSD (5 %)
Pollen grains on stigma	2009	47.0^{cd}	36.9^{abc}	58.4^{efg}	30.2^{a}	33.4^{a}	60.7^{fgh}	75.0^{i}	**48.8**	< .001$^{(R)}$	5.91
	2010	35.9^{ab}	47.6^{cd}	67.2^{ghi}	71.2^{hi}	60.7^{fgh}	45.0^{bc}	57.8^{defg}	**55.1**	< .001$^{(S)}$	4.30
	2011	104.0^{j}	103.2^{j}	52.6^{def}	61.3^{fgh}	138.4^{k}	74.0^{i}	102.0^{j}	**90.8**	< .001$^{(R*S)}$	11.01
	mean	**62.3**	**62.6**	**59.4**	**54.2**	**77.5**	**59.9**	**78.3**			
Viable pollen	2009	22.3^{cdef}	21.4^{cdef}	11.3^{a}	20.7^{cdef}	18.7^{bcdef}	25.3^{def}	12.8^{ab}	**18.9**	< .001$^{(R)}$	1.80
	2010	23.9^{ef}	24.5^{f}	15.7^{abc}	12.1^{ab}	21.1^{cdef}	34.1^{gh}	16.9^{abcd}	**21.2**	< .001$^{(S)}$	2.58
	2011	22.8^{def}	21.7^{cdef}	23.5^{def}	17.4^{abcde}	34.9^{h}	31.4^{gh}	48.1^{i}	**28.5**	< .001$^{(R*S)}$	6.61
	mean	**23.0**	**22.5**	**16.8**	**16.7**	**24.9**	**30.2**	**25.9**			
Pollen tubes in style	2009	10.1	9.8	10.8	9.0	10.3	9.4	9.7	**9.9**	0.337$^{(R)}$	n.s.
	2010	7.63	11.3	10.7	7.8	10.0	10.7	8.1	**9.5**	0.036$^{(S)}$	2.40
	2011	7.57	10.5	14.7	14.7	10.9	16.8	14.6	**12.4**	0.742$^{(R*S)}$	n.s.
	mean	**9.4**	**12.6**	**13.0**	**10.0**	**15.1**	**8.9**	**9.9**			
Ovule fertilisation	2009	0.26^{abcde}	0.20^{abcd}	0.36^{abcde}	0.33^{abcde}	0.40^{cde}	0.16^{ab}	0.13^{a}	**0.26**	< .001$^{(R)}$	0.128
	2010	0.42^{de}	0.45^{e}	0.29^{abcde}	0.31^{abcde}	0.47^{e}	0.40^{cde}	0.20^{abcd}	**0.36**	0.073$^{(S)}$	n.s.
	2011	0.33^{abcde}	0.40^{cde}	0.28^{abcde}	0.17^{abc}	0.73^{f}	0.14^{ab}	0.37^{bcde}	**0.34**	0.042$^{(R*S)}$	0.233
	mean	**0.34**	**0.35**	**0.31**	**0.27**	**0.53**	**0.23**	**0.23**			
Flower number	2009	168^{efg}	206^{h}	173^{fg}	156^{cde}	182^{g}	162^{cdef}	135^{a}	**180**	< .001$^{(R)}$	9.08
	2010	181^{g}	166^{def}	148^{abc}	137^{ab}	202^{h}	216^{h}	138^{ab}	**166**	< .001$^{(S)}$	10.47
	2011	154^{cde}	167^{efg}	152^{bcd}	155^{cde}	171^{fg}	138^{ab}	150^{bc}	**151**	< .001$^{(R*S)}$	14.53
	mean	**168**	**179**	**158**	**149**	**160**	**185**	**159**			
Seeded berries	2009	81.2^{cdef}	87.1^{efgh}	66.7^{bc}	68.0^{cd}	79.7^{cde}	47.9^{a}	52.0^{ab}	**68.9**	0.008$^{(R)}$	10.86
	2010	97.1^{fghi}	97.3^{fghi}	87.0^{efgh}	68.0^{cd}	99.1^{ghi}	104.8^{i}	101.7^{hi}	**93.6**	< .001$^{(S)}$	5.37
	2011	85.5^{efg}	83.5^{defg}	90.6^{efghi}	90.8^{efghi}	86.1^{efgh}	68.5^{cd}	98.5^{ghi}	**86.2**	< .001$^{(R*S)}$	15.97
	mean	**87.9**	**89.3**	**81.4**	**75.6**	**88.3**	**73.7**	**84.0**			
Seedless berries	2009	31.3^{ef}	11.8^{d}	38.6^{g}	35.4^{fg}	27.1^{e}	39.6^{g}	28.0^{e}	**30.2**	< .001$^{(R)}$	2.92
	2010	34.9^{fg}	6.7^{bcd}	5.6^{abc}	6.5^{abcd}	4.6^{abc}	6.6^{abcd}	7.8^{cd}	**10.4**	< .001$^{(S)}$	2.21
	2011	1.6^{ab}	6.6^{abcd}	0.9^{a}	3.8^{abc}	3.5^{abc}	3.5^{abc}	3.1^{abc}	**3.3**	< .001$^{(R*S)}$	5.73
	mean	**22.6**	**8.4**	**15.0**	**15.2**	**11.7**	**16.6**	**13.0**			
LGOs	2009	4.1^{defg}	6.9^{hij}	9.7^{jk}	3.9^{cdefgh}	3.4^{abcdef}	4.3^{defgh}	2.1^{abcdef}	**4.91**	< .001$^{(R)}$	1.93
	2010	1.4^{abcd}	3.5^{abcdef}	2.1^{abcdef}	1.8^{abcde}	0.7^{abc}	0.2^{a}	0.5^{abc}	**1.39**	< .001$^{(S)}$	1.71
	2011	11.0^{k}	8.9^{ijk}	5.1^{efgh}	12.7^{k}	12.1^{k}	6.1^{ghi}	5.1^{efgh}	**8.71**	< .001$^{(R*S)}$	3.27
	mean	**5.51**	**6.44**	**5.61**	**6.13**	**5.40**	**3.39**	**2.55**			
% Fruitset	2009	62.5^{cdef}	60.1^{bcdef}	63.8^{def}	54.8^{abcd}	60.6^{cdef}	64.5^{defg}	65.7^{fg}	**57.1**	< .001$^{(R)}$	6.48
	2010	74.2^{gh}	76.0^{h}	64.7^{efg}	55.1^{abcdef}	47.5^{a}	58.3^{bcdef}	53.4^{abc}	**67.0**	< .001$^{(S)}$	3.38
	2011	59.0^{bcdef}	58.2^{bcdef}	61.8^{cdef}	61.6^{cdef}	50.7^{ab}	76.2^{h}	63.8^{def}	**60.5**	< .001$^{(R*S)}$	9.82
	mean	**65.2**	**64.7**	**63.4**	**57.2**	**63.6**	**53.1**	**63.5**			
Total berry number	2009	112^{i}	99^{defghi}	105^{efghi}	107^{ghi}	106^{fghi}	87^{abcd}	79^{abc}	**99.8**	0.017$^{(R)}$	11.23
	2010	132^{j}	104^{efghi}	92^{cdefg}	74^{ab}	103^{defghi}	111^{i}	109^{hi}	**104**	< .001$^{(S)}$	5.72
	2011	87^{abcd}	90^{bcdef}	91^{cdefg}	94^{cdefg}	89^{bcde}	72^{a}	101^{defghi}	**89.5**	< .001$^{(R*S)}$	16.71
	mean	**110**	**97**	**96**	**92**	**100**	**90**	**97**			
CI	2009	3.5^{defg}	3.6^{efg}	3.0^{abcde}	3.4^{cdefg}	3.8^{efghi}	3.5^{defg}	2.5^{abcd}	**3.96**	0.002$^{(R)}$	0.675
	2010	2.5^{abcd}	2.2^{a}	3.5^{defg}	4.3^{ghi}	4.7^{hi}	4.2^{fghi}	4.3^{ghi}	**3.20**	< .001$^{(S)}$	0.352
	2011	3.4^{cdefg}	3.7^{efgh}	4.3^{ghi}	3.0^{abcde}	4.8^{i}	2.3^{ab}	3.3^{bcdef}	**3.37**	< .001$^{(R*S)}$	1.024
	mean	**3.12**	**3.15**	**3.30**	**3.87**	**3.26**	**4.37**	**3.47**			
MI	2009	2.9^{gh}	1.9^{f}	4.3^{i}	3.3^{gh}	3.0^{gh}	4.3^{i}	0.7^{ab}	**3.40**	< .001$^{(R)}$	0.338
	2010	2.7^{g}	1.0^{abc}	1.3^{cde}	1.3^{cde}	0.6^{a}	1.1^{bcd}	4.1^{i}	**1.20**	< .001$^{(S)}$	0.205
	2011	1.3^{cde}	1.7^{ef}	0.7^{ab}	1.6^{def}	1.7^{ef}	0.8^{abc}	0.7^{ab}	**1.25**	< .001$^{(R*S)}$	0.563
	mean	**2.30**	**1.51**	**2.09**	**2.05**	**1.75**	**2.05**	**1.89**			
Berry weight (g)	2009	1.3^{gh}	0.8^{de}	0.5^{bc}	0.6^{cd}	0.3^{ab}	0.1^{a}	0.7^{cd}	**0.62**	< .001$^{(R)}$	0.166
	2010	0.8^{de}	0.9^{def}	0.8^{de}	0.9^{def}	0.8^{de}	0.2^{a}	0.7^{cd}	**0.73**	< .001$^{(S)}$	0.09
	2011	1.3^{gh}	1.0^{ef}	1.1^{fg}	1.3^{gh}	1.4^{gh}	1.5^{h}	1.0^{ef}	**1.23**	< .001$^{(R*S)}$	0.263
	mean	**1.11**	**0.89**	**0.80**	**0.97**	**0.82**	**0.62**	**0.80**			

Statistical significance of the effects of rootstocks on 'Shiraz' are given by $P < 0.05$, $P < 0.01$, $P < .001$ and not significant (n.s.) at a 0.05 level. For all treatments and seasons, each value represents the mean of between five and twenty-one replicate samples for each group. The 5 % LSD values listed are for comparison treatments (R) and for comparison seasons (S). Where there were no significant (R x S) interactions, the treatment means were compared using the (R) 5 % LSD and the season means were compared using the (S) 5 % LSD. Letters account for significant differences among treatments.

CI: Coulure Index, LGO: live green ovary, LSD: least significant difference, MI: Millerandage Index.

Table 2

Petiole nutrient status of 'Shiraz' on different rootstocks at Nuriootpa, South Australia (2009-2011) and pollen nutrient status of 'Shiraz' on different rootstocks (2011)

Variable		Control (SHI)	110 Richter	1103 Paulsen	140 Ruggeri	99 Richter	Ramsey	Schwarz-mann	Season mean	*P* value	LSD (5 %)
Petiole Calcium (mg·kg^{-1})	2009	1.9^{d}	1.4^{abc}	1.2^{s}	1.2^{s}	1.4^{sbc}	1.5^{bc}	1.4^{abc}	**1.4**	0.049$^{(R)}$	0.307
	2010	1.5^{bc}	1.2^{a}	1.4^{abc}	1.4^{abc}	1.5^{bc}	1.5^{bc}	1.7^{cd}	**1.4**	0.263 $^{(S)}$	n.s.
	2011	1.7^{cd}	1.2^{a}	1.3^{ab}	1.3^{ab}	1.4^{abc}	1.6^{bcd}	1.3^{ab}	**1.4**	< .001$^{(R*S)}$	0.326
	mean	**1.7**	**1.3**	**1.3**	**1.2**	**1.5**	**1.5**	**1.4**			
Petiole Boron (mg·kg^{-1})	2009	38.0^{bcde}	58.3^{j}	64.3^{k}	53.8^{ij}	54.2^{ij}	50.3^{hi}	45.4^{fgh}	**52.0**	< .001$^{(R)}$	4.46
	2010	33.6^{ab}	40.0^{cdef}	40.3^{cdef}	36.6^{abcde}	41.4^{efg}	31.3^{a}	34.6^{abc}	**36.8**	< .001$^{(S)}$	1.89
	2011	35.3^{abcd}	53.6^{ij}	56.3^{j}	45.0^{fgh}	47.0^{gh}	44.3^{fg}	41.1^{def}	**46.1**	0.007$^{(R*S)}$	5.74
	mean	**35.6**	**50.6**	**53.6**	**45.1**	**47.5**	**42.0**	**40.4**			
Petiole Zinc (mg·kg^{-1})	2009	87.7^{i}	24.8^{abcdef}	34.4^{defg}	26.6^{abcdef}	52.6^{h}	57.1^{h}	45.3^{gh}	**46.9**	< .001$^{(R)}$	10.93
	2010	31.7^{bcdef}	14.4^{a}	23.7^{abcd}	20.9^{abc}	26.0^{abcdef}	30.5^{bcdef}	26.0^{abcdef}	**24.7**	< .001$^{(S)}$	3.86
	2011	35.1^{defg}	20.7^{ab}	24.1^{abcde}	22.9^{abcd}	37.8^{fg}	34.2^{cdefg}	37.2^{efg}	**30.3**	< .001$^{(R*S)}$	13.43
	mean	**51.5**	**20.0**	**27.4**	**23.4**	**38.8**	**40.6**	**36.2**			
Petiole Molybdenum	2011	0.09^{d}	0.05^{ab}	0.06^{abc}	0.08^{cd}	0.05^{ab}	0.07^{bcd}	0.04^{a}		0.013	0.027
Pollen Calcium (mg·kg^{-1})	2011	1.11	1.00	1.11	1.05	1.11	0.91	1.03		0.087$^{(R)}$	n.s.
Pollen Boron (mg·kg^{-1})	2011	52.9^{a}	85.6^{c}	85.7^{c}	83.4^{c}	67.6^{b}	52.6^{a}	55.3^{a}		< .001$^{(R)}$	9.11
Pollen Zinc (mg·kg^{-1})	2011	52.4	47.7	57.1	55.8	52.7	48.9	57.5		0.836	n.s.
Pollen Molybdenum	2011	0.55^{d}	0.26^{b}	0.27^{bc}	0.38^{c}	0.24^{b}	0.33^{bc}	0.12^{a}		0.012$^{(R)}$	0.119

Statistical significance of the effects of rootstocks on Shiraz are given by P < 0.05, P < 0.01, P < .001 and not significant (n.s.) at a 0.05 level. For all treatments and seasons, each value represents the mean of three replicate samples for each group. The 5 % LSD values listed are for comparison treatments (R) and for comparison seasons (S). Where there were no significant (R x S) interactions, the treatment means were compared using the (R) 5 % LSD and the season means were compared using the (S) 5 % LSD. Letters account for significant differences among treatments.

and correlation co-efficients were assessed between petiole and pollen concentrations. In studies by Longbottom (2007), a strong correlation was observed between petiole nutrition and inflorescence nutrition for molybdenum (r^2 = 0.77). In the present study, a relationship between pollen and petiole concentration was observed for both boron and molybdenum, (r^2 = 0.73) and (r^2 = 0.67) respectively, while in contrast, there were no correlations for calcium or zinc between pollen and petiole concentrations.

The role of molybdenum in fruitset is well known, particularly with reference to the cultivar 'Merlot', where low levels of molybdenum have been shown to contribute to millerandage (Williams *et al.* 2004, Kaiser *et al.* 2005, Longbottom 2007). Severe expression of either millerandage or coulure contributes to reduced bunch weight and subsequent lower yield. Abnormal pistil formation, poor pollen tube growth due to abnormalities within the stylar transmitting tissue and abnormal ovule development are also associated with molybdenum deficiency, which are likely to contribute to the disorder of millerandage (Longbottom 2007). Elsewhere in other crops such as maize, molybdenum deficiency has resulted in abnormal flower formation, low pollen viability, and low pollen grain numbers (Agarwala *et al.* 1979). For grapevine, deficiency of molybdenum occurs when petiole concentrations are lower than 0.05 mg·kg^{-1} while adequate concentrations are between 0.05-0.09 mg·kg^{-1} in petioles analysed at flowering (Williams *et al.* 2004). The only treatment shown to have low petiole molybdenum was 'Schwarzmann' (0.04 mg·kg^{-1}) (Tab. 2). Although this was not significantly lower than '110 Richter', '1103 Paulsen' or '99 Richter', this value would be regarded as deficient according to petiole standards proposed by Williams *et al.* (2004). Similarly, pollen concentration was also lowest for Schwarzmann than the other rootstock treatments. In contrast, own roots 'Shiraz' had the highest concentration of both petiole and pollen molybdenum concentration compared with the other rootstock treatments (Tab. 2). Interestingly, 'Schwarzmann' did not have higher values for either CI or MI, and pollen number, pollen viability and ovule fertilisation were not significantly lower than the other rootstock treatments as a consequence of lower molybdenum concentration.

Berry weight was shown to be lower for 'Schwarzmann' than the other treatments, but not significantly lower than '110 Richter' or '1103 Paulsen'. The lower molybdenum concentration for 'Schwarzmann', '110 Richter' and '1103 Paulsen' compared with the other rootstock treatments suggests a possible role of molybdenum on berry weight. Williams *et al.* (2004) described similar findings in studies on own rooted grapevines. It is well known that seed number is a key factor in berry size and weight of berries (Friend *et al.* 2009, Kliewer 1977). Although berry weight was lower for 'Schwarzmann' than the other treatments (with the exception of '110 Richter') 'Schwarzmann' had a higher number of seeded and total berries per bunch than 'Ramsey' and own roots, which had higher berry weights than

'Schwarzmann' in 2011. Previously, molybdenum deficiency has been shown to result in poorly developed seeds and shrivelled seeds for oat and wheat crops (Anderson 1956, Chatterjee and Nautiyal 2001). Further to this, studies by Kaiser *et al.* (2005) showed that berry weights at harvest were lower for 'Merlot' on own roots and 'Schwarzmann' than for '140 Ruggeri'. In that study, foliar applications of molybdenum increased berry weight in 'Schwarzmann' to equal that of '140 Ruggeri', and while own roots berry weight also increased, this was not to the extent of the weight increase observed for 'Schwarzmann'. Although it is unclear from this work whether molybdenum was deficient in 'Schwarzmann' and own roots, the low berry weight for 'Schwarzmann' in the absence of adequate molybdenum is similar to the findings of 'Kaiser' *et al.* (2005). As a consequence, we suggest that 'Schwarzmann' may have undergone late stenospermocarpy due to low molybdenum concentrations, so that ovules of 'Schwarzmann' were still able to be fertilised and begin to develop, and hence not impact on millerandage. The abortion of the embryo (Pratt 1971, Liu *et al*. 2007, May 2004), or potential shrivel of the seed could have resulted in smaller berries and lower berry weights due to the absence of large seeds in response to molybdenum deficiency.

The number of pollen grains on the stigma differed significantly between rootstocks and between seasons (Tab. 1). Previously, the average number of pollen grains on the stigma two days after flowering for 'Shiraz' was reported at 64.6 (Ebadi *et al.* 1995). In the present study, ungrafted 'Shiraz' had an average of 62.3 pollen grains on the stigma (three year mean) which is in accordance with previous literature, while for the rootstock treatments, average pollen grain number varied between 54 and 78 (Tab. 1). Rootstocks with the highest mean number of pollen grains on the stigma across the three seasons of the experiment were '99 Richter' and 'Schwarzmann' and the lowest of all the treatments across the three seasons of analysis was '140 Ruggeri' (Tab. 1). On average, both 'Schwarzmann' and '99 Richter' were found to have higher numbers of pollen grains, seeded berries and fruitset than '140 Ruggeri' or 'Ramsey' (Tab. 1). Consistently, across the three seasons '99 Richter' had the highest number of ovules fertilised than the other treatments. For a successful pollination, high quantities of pollen must be transferred to a receptive stigma (Sharafi and Bahmani 2011). Treatment means across the three seasons of the experiment resulted in high pollen grain number on the stigma and a higher proportion of ovules that were able to be fertilised for '99 Richter' compared with '140 Ruggeri'. This resulted in a higher number of seeded berries, higher fruitset and a lower number of seedless berries, and lower CI and MI for '99 Richter' when compared with '140 Ruggeri'.

Conclusion

The development and growth of berries is a function of ovule fertilisation that results in the production of a seed. Calcium, zinc, boron and molybdenum are nutrients essential for successful pollination and fertilisation and as demonstrated, deficiencies of these nutrients can result in inferior growth of the berry leading to uneven berry development. Zinc deficiency was observed for '110 Richter' and '140 Ruggeri' across the three seasons and when zinc was found to be deficient in these rootstocks, coulure was increased in 'Shiraz'. The role of molybdenum and berry weight was explored for 'Schwarzmann' as deficiency of molybdenum in both petiole and pollen analysis resulted in reduced berry weight due to stenospermocarpy or seed shrivel. Own roots 'Shiraz' had an inherently higher calcium concentration in the petioles across the three seasons and this may have resulted in higher seeded berry number, total number of berries per bunch and berry weight. On the other hand, '1103 Paulsen' had a significantly higher amount of Boron and this may have contributed to the lower number of seedless berries and a lower MI.

The data suggests that the number of pollen grains and pollen viability is indicative of the potential success of fertilisation, as evidenced by rootstock '99 Richter' with high pollen grain number, high pollen viability and high ovule fertilisation.

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