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The Effect of Rootstock and Water Stress on the Reproductive Performance of Vitis vinifera L.

by
Catherine Mary Kidman

Thesis submitted to School of Agriculture, Food and Wine
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Doctorate of Philosophy

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Abstract

The reproductive process in grapevines could arguably be the most important, as its success determines the yield for the current season and sets the potential crop for the coming season. In regions where poor reproductive performance exists, for example, poor fruitset in cool climates or in environments where water restrictions are likely, reproductive performance of grapevines may potentially be managed through the use of American *Vitis* rootstocks. The aim of this research was to assess the effect of American *V.* rootstocks on the reproductive performance of *V. vinifera* scions.

Three scion cultivars commonly used in Australian viticulture, Cabernet Sauvignon, Shiraz and Merlot were investigated. The research identified that cultivars differ in their reproductive performance when grafted to the same rootstock. For Cabernet Sauvignon, rootstocks improved fruitfulness, for Merlot, rootstocks improved fruitset, while for Shiraz, the incidence of coulure— (an abnormal condition of fruitset), was more pronounced in rootstocks than for own-roots.

A detailed investigation of rootstocks which incorporated analysis of carbohydrates, pollination, fertilisation, fruitfulness and fruitset on the cultivar Shiraz— (the most commonly planted red cultivar in Australian viticulture) was performed. Results showed that the quantity of pollen grains present on the stigma was important for successful fertilisation. As such, rootstocks associated with higher quantities of pollen grains on the stigma had higher percentage fruitset and seeded berry number. In addition, the levels of carbohydrates in roots and trunk were greater in rootstocks associated with greater vegetative growth. This research highlighted the importance of balance between vegetative and reproductive growth, as an imbalance in favour of reproductive growth was at the expense of carbohydrate accumulation which affected fruitfulness in the following season.

The absence of irrigation affected reproductive development through yield losses which were attributed more to weight loss in bunches and berries through effects on berry size and dehydration,

rather than low fruitset. A detailed analysis of the three cultivars enabled classification of rootstocks and cultivars based on their reproductive performance which will help identify reproductive traits for rootstock and cultivar combinations.

In addition, there was an opportunity to assess the effect of rootstock and irrigation on grape and wine composition and wine sensory attributes of Shiraz. A novel sensory analysis technique enabled the discrimination of wine attributes between treatments and correlation with traditional wine quality assessments. For example, in one season, 1103 Paulsen with wine colour density, wine pH and the attributes “rich” and “black fruit”. 110 Richter with grape TA, wine phenolics and alcohol % and the attributes “astringent” and “black fruit” in the other season. In addition, low quality rootstocks Ramsey and 99 Richter were associated with ‘light’ and ‘simple’ attributes used by the expert winemaker panel. These findings have implications for rootstock selection management decisions and how we assess research wines for commercial application.

This study has led to a greater understanding of how rootstocks impact reproductive performance and will help the Australian and international wine industries make decisions on rootstock selection, especially for sites and cultivars where reproductive development could be limited.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any University or any other tertiary institution and, to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference has been made in the text.

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Journal of Papers Published as part of this Research:

Kidman, C.M., Dry, P.R., McCarthy, M.G. and Collins, C. (2013)

Reproductive performance of Cabernet Sauvignon and Merlot (*Vitis vinifera* L.) is affected when grafted to rootstocks.

Australian Journal of Grape and Wine Research 19 (3): 409:421

Presented in Chapter 3

Kidman, C.M., Olarte Mantilla, S.M., Dry, P.R., McCarthy, M.G. and Collins, C. (2014)

The effect of water stress on the reproductive performance of Shiraz (*Vitis vinifera* L.) grafted to rootstocks.

American Journal of Enology and Viticulture In publication Vol 65 (1) March 2014.

Presented in Chapter 4

Kidman, C.M., Dry, P.R., McCarthy, M.G. and Collins, C. (2014)

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

Vitis In publication Vol 53, July 2014

Presented in Chapter 5

Cox, C.M., Favero, A.C., Dry, P.R., McCarthy, M.G. and Collins, C. (2012)

Rootstock effects on bud fertility, primary bud necrosis and carbohydrate storage in Shiraz.

American Journal of Enology and Viticulture 63 (2): 277-283

Presented in Chapter 6

Kidman, C.M., Olarte Mantilla, S.M., Dry, P.R., McCarthy, M.G. and Collins, C.

Assessment of grape and wine chemical composition and sensory differences with rootstock an irrigation treatments in Shiraz (*Vitis vinifera L.*)

[prepared manuscript]

Presented in Chapter 7

Each of these manuscripts is displayed in the thesis in either published or submitted form according to the instructions to author of the specific journal

This Thesis has been prepared according to the University of Adelaide's specifications for "PhD by publications" format

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Conference Proceedings and Industry Publications

Cox, C.M, Dry, P.R., McCarthy, M.G. and Collins, C. (2008)
Maintenance of grafted vine health and productivity under forced zero irrigation.
In poster proceedings 8th International symposium on grapevine physiology and biotechnology, 23-28th November, Adelaide, Australia

Cox, C (2010)
The effect of water stress on the performance of grafted vines with an emphasis on wine quality.
Australian Viticulture 14 (1) 19-22.

Cox, C.M, Dry, P.R., McCarthy, M.G. and Collins, C. (2010)
Reproductive development changes when Merlot and Cabernet Sauvignon are grafted to rootstocks in a cool climate region In poster proceedings 14th Australian Wine Industry Technical Conference, 3-8th July, Adelaide, Australia

Kidman, C.M., Olarte Mantilla, S.M., Dry, P.R., McCarthy, M.G. and Collins (2013)
The effect of water stress on the reproductive performance of Shiraz (*Vitis vinifera* L.) grafted to American *Vitis* rootstocks. In poster proceedings 18th International symposium GiESCO, 7-11th July, Porto, Portugal.

Kidman, C.M., Olarte Mantilla, S.M., Dry, P.R., McCarthy, M.G. and Collins (2013)
The effect of water stress on the reproductive performance of Shiraz (*Vitis vinifera* L.) grafted to American *Vitis* rootstocks. In poster proceedings 15th Australian Wine Industry Technical Conference, 14-18th July, Sydney, Australia

Abbreviations

ABA	Abscisic acid
ANOVA	Analysis of Variance
BOM	Bureau of Meteorology
CI	Coulure Index
CO_2	Carbon dioxide
CSIRO	Commonwealth Scientific and Industrial Research Organisation
cv	Cultivar
cvs	Cultivars
GWRDC	Grape and Wine Research and Development Corporation
IP	Inflorescence primordia
LGO	live green ovary
MI	Millerandage Index
PBN	primary bud necrosis
PGIBSA	Phylloxera and Grape Industry Board of South Australia
Ψ_l	Midday leaf water potential
Ψ_{pd}	Pre-dawn leaf water potential
Ψ_s	Midday stem water potential

Chapter 1. Introduction

The process of reproductive development in grapevines can be divided into a number of sequential stages that occur over two successive growing seasons. As a consequence, the growth and success of the crop is dependent on bunch initiation in the previous season, inflorescence development, flowering and fruitset and the development of seeds and flesh within a grape berry in the current season (Pratt 1971, Srinivasan and Mullins 1981, May 2004, Vasconcelos, et al. 2009).

The effect of water deficit on reproductive development has been well documented (Matthews, et al. 1987, Matthews and Anderson 1989, Poni, et al. 1993). Early and late season water deficits have been shown to be detrimental to both the development of the current and following season's crop (Matthews and Anderson 1989) and the effects of rootstock on vine performance under deficit irrigation has previously been examined through yield response (McCarthy 1997, Stevens, et al. 2008, Keller, et al. 2012). Water use efficiency is a critical issue for expansion and sustainability in Australian viticulture, however the influence of rootstocks to mitigate the detrimental effects to reproductive performance require further investigation over a wider cultivar spectrum.

Reproductive development of grapevines may potentially be managed through the use of rootstocks (Candolfi-Vasconcelos and Castagnoli 1995, Cirami 1999, Whiting 2003, May 2004, Dry 2007). The importance of rootstocks in viticulture is well documented; particularly in relation to yield, fruit composition, pest resistance, salinity, nutrition and water relations (Cirami, et al. 1984, McCarthy and Cirami 1990, Ezzahouani and Williams 1995, Nicholas 1997, Keller, et al. 2001, Zhang, et al. 2002, Whiting 2003, Soar, et al. 2006, Soar and Loveys 2007, Pech, et al. 2008, Stevens, et al. 2008). The use of grapevine rootstocks (American *V.*) in phylloxera-free regions of South Australia has increased steadily over the past 30 years yet rootstock use still accounts for only 20% of total plantings (Dry 2007).

As water use efficiency is a critical issue for expansion and sustainability in Australian viticulture, there is growing interest in the use of rootstocks to minimise the impact of possible future water shortages on production; however, limited attention has been paid to quantifying grapevine reproduction when vines, either on their own roots or grafted onto a rootstock, are returned to dry land conditions as may occur under future climate change.

This project was initiated by The Phylloxera and Grape Industry Board of South Australia in response to industry concern about water deficits during the 2006 drought and was supported by the Grape and Wine Research Development Corporation (GWRDC) with the expectation that outcomes would contribute to the awareness of rootstocks while also providing new information that will assist in management decisions for wine grape growers.

1.1 Objectives of the Research

The research objectives of this thesis were to: i) investigate varietal differences of grafted vines on reproductive development; ii) improve knowledge of the effects of rootstocks on reproductive development to assist wine grape growers in choosing suitable rootstocks; iii) examine the effect of prolonged water deficit on grapevine reproduction; iv) ascertain whether rootstocks could mitigate the effects of water deficit and v) determine the effect of both water deficit applied to rootstocks on wine quality and the influence of rootstocks on wine quality.

1.2 Linking Statement

The research in this thesis is presented in chapters, including five research chapters, four of which have been published in peer reviewed journals and one prepared manuscript intended for publication. The manuscripts are presented in the thesis to reflect the timeline / scope of the project rather than the date of publication of published work.

- Chapter 1 comprises the introduction to the thesis
- Chapter 2 is a review of the literature pertaining to grapevine reproduction, the use of American *V.* rootstocks in viticulture, and the effects of water stress on grapevine reproduction and wine

quality. A summary of the literature and aims of the research are presented at the end of this chapter.

- In Chapter 3, two cultivars (Merlot and Cabernet Sauvignon) were examined for the effect of rootstocks on reproductive development. The methodology to examine reproductive development used in this chapter was also used in subsequent chapters. The findings of this chapter provide a greater understanding of reproductive development as affected by rootstock and cultivar providing rationale for positioning it as the first chapter.
- Chapter 4 examined the effects of rootstocks on reproduction in an additional cultivar (Shiraz) along with the reproductive response of rootstocks to the absence of irrigation for a period of three years. The findings identified in this chapter were related to the findings of Chapter 4 as well as having significant influence on results obtained in subsequent chapters, providing rationale for its positioning as the second research chapter.
- Chapter 5 presents research on the influence of grapevine rootstocks on pollen tube growth and germination for Shiraz. The data for this chapter were collected simultaneously with data used in Chapter 4. These findings provide evidence as to why reproductive development differs within a cultivar grown on different rootstocks while also detailing a novel method for analysis of pollen nutrition.
- In Chapter 6, comparisons were made between two sites for the effect of rootstocks on bud fertility, and in particular, the incidence of primary bud necrosis. Also the ability of rootstocks to store carbohydrates, important for bud fertility and growth. The findings of this paper serve to substantiate the findings outlined in the earlier chapters.
- Research on the effects of both zero irrigation and rootstock on wine quality is presented in Chapter 7. Data for this study, including small lot wines, were collected during the first two years of the experiment. Due to adverse seasonal conditions, no wine was made from the third year experiment. Nonetheless, analysis of grape and wine composition and wine sensory

attributes from the trial enhanced the results obtained in previous chapters, providing rationale for its positioning as the last chapter of the thesis.

A general discussion of the research in this thesis is presented in Chapter 8 and follows a similar pattern to the layout of the thesis by publication. The effect of rootstocks on reproductive development of three cultivars (Merlot, Cabernet Sauvignon and Shiraz), followed by the effect of water stress on reproductive development in Shiraz (Chapters 3 and 4) are presented first. The remaining part of the discussion is related to the effect of grapevine rootstocks on reproductive performance, more specifically, pollen tube growth and fertilisation and bud fertility in Shiraz (Chapters 5 and 6), followed by the effects of rootstocks and water stress on wine quality (Chapter 7).

Chapter 2. Literature Review

This literature review will focus on four main themes which emerged through the reviewed literature. These themes are: the importance of reproductive development for *V. vinifera* L.; the influence of water stress on reproductive performance; the use of grapevine rootstocks in Australian viticulture and their effect on reproductive performance, and the effect of water stress on wine quality. Although the literature represents these themes in a variety of contexts, where possible, this review will focus on the effect of either water stress or rootstocks on grapevine reproduction.

2.1 Grapevine reproduction

Bud development

The grapevine is a perennial crop that in southern hemisphere produces a crop once a sea son. Shoot development in the current season produces via the shoot apical meristem, leaf primordia and a meristematic structure known as an anlage (plural anlagen) which are uncommitted primordia (Srinivasan and Mullins 1981, May 2004, Vasconcelos, et al. 2009). Anlagen may become either inflorescence primordia (IP), shoot primordia (SP) or tendril primordia (TP) (Srinivasan and Mullins 1981). Anlagen are initiated in the latent bud located in the axil of every leaf node. The latent bud (also known as the compound latent bud as it does not normally burst until the following season) (Vasconcelos, et al. 2009, Iland, et al. 2011). Anlagen that develop into IP begin with the development of the bract, which is located at a node on a stem, known as a prophyll. Buds that develop in the axil of the prophyll go on to form the “primary” bud (N+2 bud), when “N” defines shoot, which is located centrally within the compound bud, while the $N+3_1$ and $N+3_2$ buds, known as “secondary” buds have limited development (Iland, et al. 2011). The secondary latent bud may form an inflorescence while the tertiary is typically vegetative (Srinivasan and Mullins 1981). The formation of the anlagen from the apex is the earliest indication of reproductive development and there may be up to three inflorescence primordia and between six and ten leaf primordia per N+2 depending on the cultivar (Srinivasan and

Mullins 1981). The IP is formed by extensive branching of the anlage (Pratt 1971, Srinivasan and Mullins 1981, Vasconcelos, et al. 2009). After the formation of the IP, the latent bud enters dormancy (Srinivasan and Mullins 1981, Vasconcelos et al. 2009). The initiation and development of anlagen occurs in the same season, approximately 15 months prior to the harvest of the developing IP, depending on the cultivar (Srinivasan and Mullins 1981, May 2004).

Bud fruitfulness

Bud fruitfulness is influenced by light, temperature, water stress, trellis, pruning and training system, and carbohydrate reserves (Buttrose 1969a, Buttrose 1969b, Buttrose 1974, Vasudevan, et al. 1998, Dry 2000, Bennett, et al. 2005, Sanchez and Dookoozlian 2005). Excessive shoot growth (Lavee, et al. 1981, Dry and Coombe 1994) and canopy shading (May 1965, Perez and Kliewer 1990, Wolf and Cook 1992) can be detrimental to bud fruitfulness; a reduction in yield per node, through either a reduction in bunches per shoot or a reduction in shoots has been shown to occur (Dry 2000). Conversely, increased fruitfulness (greater number and size of inflorescence primordia) can occur under high light intensity (Buttrose 1969a). It is well known that rootstocks affect both vegetative growth and yield of the scion variety (Keller, et al. 2001, Sommer, et al. 2001, Dry 2007, Keller, et al. 2012). Rootstocks with high vigour, such as Ramsey have been shown to be detrimental to fruitfulness when compared with own roots due to the rootstock's capacity to develop dense canopies and increase shading to the developing buds (Sommer, et al. 2001).

Literature on the effect of deficit irrigation on fruitfulness is varied. Bud fruitfulness has been shown to decline under deficit irrigation for varieties Cabernet Franc, Shiraz and in one season, Thompson Seedless, through a reduction in shoot number or a low shoot internode number (Matthews and Anderson 1989, Petrie, et al. 2004, Williams, et al. 2010); however, fruitfulness has also been shown to increase under deficit irrigation (Williams, et al. 2010) and under minimal and mechanically pruned situations, as fruitful buds burst in preference to less fruitful buds for Shiraz vines increasing the number of inflorescence per shoot (Petrie, et al. 2004). Furthermore, a mild water deficit may increase

fruitfulness through improved light interception to the developing buds through a decrease in foliage (Keller 2005, Williams, et al. 2010).

Primary bud necrosis

Bud development is subject to a detrimental condition known as primary bud necrosis (PBN), which is a physiological disorder that results in the death of the primary bud within the latent compound bud during bud initiation (Dry and Coombe 1994, Vasudevan, et al. 1998, Collins, et al. 2006, Collins and Rawnsley 2008). A decrease in bunches per node and a reduction in bunch weight has been shown to be a consequence of this disorder, as the secondary buds— which develop more than normal and compensate for the loss of the primary bud— are less fruitful (Dry and Coombe 1994, Dry 2000). Although the fundamental causes of PBN remain uncertain, many theories have been proposed to explain why PBN may occur. These include excess shoot vigour, shading, reduced carbohydrates and predisposition of cultivar (Dry and Coombe 1994, Vasudevan, et al. 1998, Collins and Rawnsley 2008).

Dry and Coombe (1994) examined fifteen cultivars of *V. vinifera* for the presence of PBN and found that PBN is a significant cause of poor fruitfulness in Australian vineyards. Levels of PBN greater than 20% in a vineyard are considered to have a significant impact on fruitfulness and therefore final yield (Pool 2000). The cultivar Shiraz is reported to have one of the highest incidences of PBN in Australian vineyards (Dry and Coombe 1994, Dry 2000, Dry, et al. 2003).

A survey of the incidence of PBN in Australian vineyards showed that viticultural practices of deficit irrigation may also influence the predisposition of buds to necrosis (Collins and Rawnsley 2004).

Although the direct effects of water stress on PBN remain uncertain, there are reports of both positive and negative effects on fruitfulness as a consequence of water stress. On the one hand, water deficits have been shown to reduce fruitfulness (Buttrose 1974, Matthews and Anderson 1989, Williams 2010). Water stress can limit the number of IP initiated along with the degree of branching of the IP. Initiation, differentiation and maintenance of IP are sensitive to water stress and under circumstances of severe water deficit, abortion of the developing young inflorescences may occur (Buttrose 1974,

Srinivasan and Mullins 1981, Matthews and Anderson 1989, Rogiers, et al. 2004). In contrast, a mild water deficit has been shown to promote fruitfulness through a reduction in shoot vigour and an improved canopy microclimate and improved light interception to the developing buds (Collins and Rawnsley 2004, Keller 2005, Keller 2010).

Factors found to influence fruitfulness also influence the incidence of PBN. Although the effect of rootstock type on fruitfulness and PBN is not well understood, rootstocks that affect shoot growth and shading may influence fruitfulness and PBN (Dry, et al. 2003, Collins and Rawnsley 2004). In the limited literature on this topic, responses have been contradictory; for example, Dry et al. (2003) found no influence of rootstock on the incidence of PBN while in contrast, Collins and Rawnsley (2004) observed significant differences in the incidence of PBN due to rootstock for the cultivars Shiraz and Cabernet Sauvignon and as such, it remains unclear whether rootstock has an effect on fruitfulness or the incidence of PBN.

Flower development

The commencement of budburst in the following growing season coincides with the growth of flower initials on the IP (Iland, et al. 2011). IP branches divide many times to produce the flower initials (Srinivasan and Mullins 1981, May 2004). The inflorescence is a conical panicle structure that comprises grapevine flowers that house the sepals, petals, stamens and carpels (May 2004, Vasconcelos et al. 2009). The formation of floral organs and floral initials on the immature inflorescence initiates with sepal primordia and calyx development. Five petals connect with each other to make a corolla or cap (Boss, et al. 2003) and housed within the cap are the male androecium and female gynoecium. The androecium contains stamens, and each stamen has an anther with two pollen sacs (Vasconcelos, et al. 2009). The gynoecium contains the pistil which comprises the stigma, style and the ovary (Vasconcelos, et al. 2009). The ovary, located at the base of the style, consists of two fused carpels. Within the carpels are locules which contain the ovules. The two carpel walls meet at the centre of the ovary; the outer epidermal layers of the carpels form the central column of the ovary, the

septum or transmitting tissue. The septum is the central canal of the style that pollen tubes grow down and into (Vasconcelos, et al. 2009). The stigma and style are only required for the process of pollination whereas the ovary continues to develop into the flesh of the berry and the ovules develop into the seeds (Pratt 1971, May 2004).

For *V. vinifera,* flowering occurs when shoots on a vine have between 15 and 19 separated internodes, although 70% of flowering is said to occur between 15 and 17 internodes (Pratt and Coombe 1978, May 2004). The process of flowering is initiated by the dehiscence of the cap to expose the anthers that release the pollen grains which land on the stigma (Vasconcelos, et al. 2009). Temperature and relative humidity are paramount to this process as an increase in temperature and a drop in relative humidity are required for the release of the pollen grains from the anthers (Vasconcelos, et al. 2009). Flowering may take longer if weather is cool or wet (Vasconcelos, et al. 2009). Low temperatures of 14°C / 9°C or less have been shown to inhibit flowering while temperatures above 10°C are required for a period of at least 20 days in order for flowering to occur (Buttrose and Hale 1973). Conversely, exposure to high temperature for short periods does not hasten the flowering period. Rather, extreme day temperature of > 35°C and night temperature of 19°C to 35°C result in a decrease in percentage of flowers that are able to set berries (Buttrose and Hale 1973).

Pollination and fertilisation

Upon dehiscence of the flower cap, the pollen grains germinate, resulting in the production of a pollen tube (May 2004, Vasconcelos et al. 2009). The pollen tube contains sperm cell nuclei that grow down through the style from the stigma through the central part of the ovary towards the ovule. This process is known as fertilisation (May 2004) and under optimal conditions, fertilisation is 2-3 days after pollination (Pratt 1971). Pollen tubes must attain certain lengths (1.7-2.0 mm) to successfully span the distance between the stigma and micropyle (May 2004). Once the pollen tubes reach the ovules, sperm cells move down the pollen tube to fertilise the ovule (Vasconcelos, et al. 2009). Germination, pollination and growth of the pollen tube are sensitive to temperature. Low temperature (10°C to 13°C)

at anthesis can inhibit pollen tube growth and ovule fertilisation (Roubelakis and Kliewer 1976). However, more recently, the effect of low temperature during flowering has been shown to more severely affect ovule / seed development, and as a consequence, fruitset and berry growth, than pollen germination and pollen tube growth (Ebadi, et al. 1995).

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 with 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). Parthenocarpic ovules also result in seedless fruit when ovules fail to be fertilised (Iland, et al. 2011). Further to this, the cultivar can also influence the proportion of ovules that become seeds (May 2004); commonly two seeds are found in each locule rather than four seeds, as ovule growth in locules may inhibit the growth of the other ovule (May 2004).

Flowering and fruitset

The developing grape berry undergoes cell division, followed by cell enlargement. Most of the cell division occurs in the pericarp 5-10 days after flowering. Cell division occurs in the berry up until veraison where at this point, cell enlargement becomes responsible for the final berry size (Pratt 1971).

Flower number is an important determinant of yield as it sets the upper limit on the potential number of berries that can develop to maturity (Dunn and Martin 2000). The number of flowers per branch on an inflorescence decreases exponentially from the most proximal to distal position (May 2004). Environmental conditions at budburst have been shown to affect the degree of branching of the inflorescence and this can affect flower development (May 2004). As a result, the variation in flower number can be explained by the number of branches on the inflorescence (Dunn and Martin 2000). Evidence exists with other fruit species that percentage of flowers that are able to set declines with increasing flower number (Stover 2000). For navel orange, high flower numbers increase competition

for supply of nutrients and metabolites, which can result in abscission of the smaller, later developing fruitlets when demand exceeds supply. This in turn reduces the number, percentage set and growth rate of fruitlets (Guardiola, et al. 1984).

Optimal temperatures for flowering have been shown to be between 15°C and 23°C. This range ensures maximum flowering on or about the 5th and 6th day after the commencement of flowering (Staudt 1999). Lower temperatures will delay the beginning and prolong the process of flowering (Ebadi, et al. 1995a). Under optimal conditions, flowering of an inflorescence may take between 4 and 8 days (Vasconcelos, et al. 2009). Temperatures at flowering greater than 32°C have previously been shown to interrupt reproductive development of young floral buds in pea (*Pisium sativium*), followed by abscission several days after the cessation of the temperature stress (Guilioni, et al. 1997). For grapevines, an effect of high temperature (>32°C) on reproductive development has also been reported (Alexander 1965, Kliewer 1977, Greer and Weston 2010). Temperature regimes between 32°C to 40°C for twelve-hour days between flowering and fruitset markedly reduced fruitset by 42% at 40°C, and 23% at 35°C and increased the number of seedless berries within the cluster compared with treatments at 25°C in Pinot Noir. Similar reductions were observed for Carignane and Cabernet Sauvignon (Kliewer 1977).

Fruitset is defined as the conversion of an ovary into a berry (Ebadi 1996). Fruitset is a key determinant of yield as it is a measure of the proportion of flowers that are able to successfully develop into berries. Annual yield variation in grapevines can be as high as 30% between seasons (Boss, et al. 2003, Krstic, et al. 2005), and while much of this variation may be attributed to differences in climate and canopy management practices, the number of inflorescences per vine, flower number and fruitset are major contributing factors to this variation (Boss, et al. 2003). Flowers that undergo pollination but not fertilisation form live green ovaries (LGOs) (Friend, et al. 2003, Longbottom 2007). LGOs make up less than 1% of total bunch weight (Collins and Dry 2009). Typically, LGOs are seedless, remain small, green and hard (Collins and Dry 2009).

Millerandage and coulure are important, 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 LGOs (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). Coulure can result from deficiencies in soluble and insoluble sugar concentrations and may be caused by a disturbance in concentration of growth regulators (Lebon, et al. 2008 and authors therein). 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 both indices the higher the numerical value, the greater the incidence of the condition.

Recently, a review of flowering and fruitset indices was conducted by Collins and Dry (2009). As a result, the expression of coulure and millerandage were defined and separated (Collins and Dry 2009). Furthermore, this allowed for more accurate explanation of the poor fruitset of certain varieties. Prior to this analysis, commonly, fruitset was solely determined by the number of berries per bunch and this led to mis-classification of fruitset of many varieties (Coombe 1959). For accurate determination of fruitset, the accepted method now requires counts of flower numbers per inflorescence, followed by determination of berry class (seeded, seedless or LGOs) as a proportion of total berry number per inflorescence (Collins and Dry 2009).

The classification of wine grapes based on reproductive parameters revealed that certain varieties were more susceptible to poor fruitset than others; however, the cause of this poor fruitset, due to CI or MI or both, also differed by cultivar (Collins and Dry 2009, Dry et al. 2010). For example, Cabernet Sauvignon and Merlot are grouped together as they are both susceptible to poor fruitset due to a high incidence of both millerandage and coulure. In contrast, Chardonnay has been shown to have better fruitset than either Merlot or Cabernet Sauvignon (May 2004, Dry, et al. 2010). Chardonnay is regarded as having a low CI but a high MI (Collins and Dry 2009). Dry et al. (2010) were further able to

demonstrate that Shiraz is a cultivar that has more coulure than millerandage compared with other varieties such as Merlot where the converse is true. The ability to distinguish the expression of CI and MI as factors of poor fruitset demonstrate the highly complex role of fruitset in reproductive performance. Similarly, some varieties such as Chardonnay, Shiraz and Tempranillo are classified as having lower flower numbers than Cabernet Sauvignon and Merlot. These varieties typically have good fruitset, characterised by low millerandage yet despite this, also have low berry number similar to Cabernet Sauvignon and Merlot due to the low flower number, rather than poor fruitset and high flower number as is the case for both Cabernet Sauvignon and Merlot (Dry, et al. 2010).

2.2 Grapevine Rootstocks

Viticulture and winemaking in South Australia has been a long established industry with the arrival of vine cuttings from overseas during the 1830s (Bell 1988). In 1877 phylloxera (*Daktulosphaira vitifoliae)* was identified in Victoria, and shortly after in New South Wales (NSW) and Queensland (QLD) (May 2001). Phylloxera feeds on the roots of *V. vinifera* causing vine decline and ultimate death (Mullins, et al. 1992, Dry 2007). The initial spread of phylloxera throughout Europe and thereafter in Victoria, NSW and QLD was thought to be a consequence of the importation and use within Europe of the American *V.* vines and associated cultivars (May 2001). Phylloxera is a native pest of the American *V.* species, and as such, some species have developed resistance. Grafting a *V. vinifera* scion to an American *V* root system was the preferred method of control used in Europe and elsewhere, as grafting was found to be advantageous in not only providing resistance to the pest, but also ensuring the characteristics of the scion were maintained (Boehm 1996).

In addition to phylloxera tolerance, the importance of rootstocks in viticulture is well documented; particularly in relation to yield, fruit composition, pest resistance (e.g phylloxera and nematodes), salinity, nutrition and water relations (Cirami, et al. 1984, McCarthy and Cirami 1990, Ezzahouani and Williams 1995, Nicholas 1997, Keller, et al. 2001, Zhang, et al. 2002, Whiting 2003, Soar, et al. 2006, Soar and Loveys 2007, Pech, et al. 2008, Stevens, et al. 2008). Rootstocks used for

Australian viticulture are hybrids of the following American *V* species: *V. riparia, V. rupestris, V. berlandieri* and *V. champinii* (Dry 2007). There are approximately 20 “American” rootstocks used in Australian viticulture (Cowham 2003) and these hybrids retain certain parental characteristics (Dry 2007) that may be advantageous under particular environmental conditions; for example, lime tolerance with calcareous soil types (Whiting 2003).

Origins of the main rootstock hybrids used in Australia

The species used for American rootstocks originated from vastly different environments within America, and as such, have different growth characteristics that make them suitable or unsuitable under certain environmental situations (Whiting 2003).

Vitis riparia is commonly known as the riverbank grape (http://plants.usda.gov), and is native to a wide range of geographical locations which include Canada, Texas, Louisiana and the Rocky Mountains (Whiting 2003, Dry 2007). It is commonly found on or near riverbanks in moist deep and fertile soils (Whiting 2003) and as such, is less suited to drought environments. The species has a short vegetative cycle (Whiting 2003, Dry 2007).

Vitis rupestris is found from southwest Texas to Pennsylvania was found on gravelly banks of mountain streams (Whiting 2003, Dry 2007), although almost extinct, it is now only found in Southern Missouri. Known as the sand grape (http://plants.usda.gov), *V. rupestris* has a long vegetative cycle (Dry 2007), but is prone to poor performance under drought conditions on shallow soils (Whiting 2003).

Vitis berlandieri is native to southern North America in particular the limestone hills of south-central Texas (Whiting 2003, Dry 2007). Because of its geographic origin, *V. berlandieri* is lime tolerant, suitable for hot climates and has some drought tolerance and a long vegetative cycle (Whiting 2003).

Vitis champinii is a hybrid of *V. candicans (V. mustangensis)* × *V. rupestris,* and is native to Texas (http://plants.usda.gov). This hybrid species is vigorous and has been used both as a direct rootstock, for example, Dog Ridge and Ramsey, and also as a parent for Harmony and Freedom (http://iv.ucdavis.edu/Viticultural_Information).

Rootstocks and fruitset

An attempt to classify rootstocks according to their fruitset potential has been previously reported by several authors (Candolfi-Vasconcelos, et al. 1994, Cirami 1999, Whiting 2003, Dry 2007). Candolfi-Vasconcelos and Castagnoli (1995) reported that rootstocks significantly improved fruitset at two of five sites for Pinot Noir. However recent studies suggest that rootstock has no effect on fruitset, rather the complex interaction between the cultivar and rootstock has more effect on fruitset than just the rootstock alone (Tandonnet, et al. 2010, Keller, et al. 2012). A summary of previous literature by Dry (2007) details the influence of rootstocks on fruitset. Based on this summary, rootstocks 5C Teleki, Schwarzmann, 101-14 MGT, SO4, 3309C, 420A and Riparia Gloire are said to improve fruitset while 1103 Paulsen and 110 Richter were not recommended for poor set scion varieties. Further studies have shown the use of rootstocks 140 Ruggeri and Schwarzmann with the scion cultivar Merlot reduced the incidence of millerandage compared to own roots (Kaiser, et al. 2005).

Rootstocks and drought tolerance

One of the key priorities for rootstock choice in Australia is for drought tolerance or improved water use efficiency (Walker and Clingeleffer 2009). Of the top five most common rootstocks planted in Australia in the last decade, three are considered to be drought tolerant: 1103 Paulsen, Ramsey and 140 Ruggeri (http://www.phylloxera.com.au/resources/rootstocks).

Carbonneau (1985) defined drought tolerance of rootstocks by the ratio of stomatal conductance to total active leaf area of the plant (referred to as transpiration index). This classification is based on the water-extracting ability of the rootstock to maintain transpiration, a higher ratio indicated higher drought tolerance. Based on these calculations, Carbonneau described five classes of rootstocks; however, for the purpose of this review only rootstocks pertinent to Australian viticulture will be described. Class 1, highly drought resistant rootstocks: 110 Richter, 140 Ruggeri; Class 2, resistant rootstocks: 1103 Paulsen, 99 Richter and SO4; Class 3, less resistant rootstocks: 3309C, 420A, 5BB

Kober, 101-14 MGT. Class 4, susceptible: none relevant to Australia. Class 5, highly susceptible: none relevant to Australia.

Since Carbonneau (1985), there have been other studies to determine the drought tolerance of rootstocks. Ezzahouani and Williams (1995) examined the seasonal and diurnal patterns of leaf water potential to describe drought tolerance for rootstocks where the higher the water potential, the more drought tolerant the rootstock. Their results are in general agreement with those of earlier work by Carbonneau (1985), however, the later study ranks 1103 Paulsen as being as drought tolerant as 110 Richter and 140 Ruggeri. The inclusion of 1103 Paulsen as a highly drought tolerant species differs from Carbonneau (1985) as measures of tolerance were based on leaf water potential rather than stomatal conductance. Furthermore, Ezzahouani and Williams (1995) conceded that stomatal conductance better reflected soil water content than leaf water potential and this must be taken into account when assessing the relative ranking of rootstocks to soil water content and water stress.

A possible model to select for drought tolerance was proposed by Iacono and Peterlunger (2000) using ABA flux and net photosynthesis under water stressed conditions. Drought tolerance was defined as an ability to produce dry matter under water stress conditions. In this study, the varieties Muller Thurgau and Riesling had improved drought tolerance when grafted onto hybrid rootstocks.

Drought tolerance of rootstocks under Australian conditions has been defined as the ability of the rootstock to maintain yield and produce dry matter under water stressed environments (McCarthy, et al. 1997, Iacono and Peterlunger 2000). According to the literature, it is generally agreed that rootstocks Ramsey, 110 Richter, 140 Ruggeri, 1103 Paulsen, and 99 Richter are considered drought tolerant (Carbonneau 1985, Dry 2007, Walker and Clingeleffer 2009, Williams 2010). Rootstocks 5BB Kober, 5C Teleki and SO4 are considered moderately susceptible to drought and K51-40, 101-14MGT, Schwarzmann and 3309C are reportedly susceptible to drought (Nicholas 1997, Dry 2007). However, there are some exceptions to this, in particular when compared against an ungrafted control. Using yield as the main covariate, McCarthy, et al. (1997) found an approximate 50% reduction in yield in

unirrigated treatments of Shiraz grafted to a series of rootstocks. In that study, Shiraz vines grafted to Ramsey yielded more than other unirrigated rootstocks, but not significantly so when compared to own rooted vines, 99 Richter or 140 Ruggeri. Conversely, both 110 Richter and 1103 Paulsen performed poorly in the absence of irrigation (McCarthy, et al. 1997). In the absence of irrigation, Shiraz vines on own roots were as drought tolerant as rootstocks.

Keller, et al. (2012) attributed the geographic origin of *V. vinifera* as having an influence on drought tolerance, inferring that *V. vinifera* was likely to be more drought tolerant than some American *V.* species. Assessment of Shiraz grafted to various rootstocks, found scion genotypes rather than rootstock genotypes influenced the degree of drought tolerance in a grapevine (Virgona, et al. 2003). This theory is further supported by more recent studies (Keller, et al. 2012) over a wider range of grape varieties including Chardonnay, Merlot and Shiraz. In agreement with past observations, the rootstock effect on yield depended on the scion cultivar, but the authors did observe higher yields for rootstocks 3309C, 5C Teleki and own roots than 140 Ruggeri, 1103 Paulsen and an unnamed Cornell University rootstock 101-CU (Keller, et al. 2012).

A grafted vine's ability to cope with water stress may be attributed to the root system (Soar, et al. 2006). Soar, et al. (2006) demonstrated that rootstocks with a genetic ability to grow thicker root systems were better able, under dry conditions, to maintain yields and canopies compared with own roots than rootstocks with a genetic makeup that resulted in roots of finer structure (Williams and Smith 1991, Southey 1992). The ability to grow a thick, plunging root system would be an advantage in dry conditions to increase the potential to explore large volumes of soil for water (Soar, et al. 2006, Dry 2007).

2.3 Rootstocks and wine quality

Wine grape quality has long been evaluated by the composition of soluble solids, organic acids and pH in the berries and wine (Jackson and Lombard 1993). These important constituents are further influenced by maturity, quantity and distribution throughout the berry, all of which will influence the style

and quality of the wine (Olarte Mantilla, et al. 2012). To date, methods that are easy to assess, including soluble solids, organic acids and pH, along with assessment of colour, phenolics and tannins are the main quality parameters documented in the literature (Ough, et al. 1968, Downton 1977, Cirami, et al. 1984, Matthews, et al. 1990, Jackson and Lombard 1993, Cirami 1999, Gawel, et al. 2000, Petrie, et al. 2004, Roby, et al. 2004, Roby and Matthews 2004, van Leeuwin, et al. 2007, Bindon, et al. 2008, Bindon, et al. 2011, Walker and Blackmore 2012). According to a survey of South Australian grapegrowers an impediment to rootstock use has been associated with a negative stigma for wine quality (Hathaway 2001). Indeed, early research with rootstocks showed an increase in grape potassium and pH which was negatively correlated with wine quality (Hale and Brien 1978). As such, a negative stigma associated with rootstocks due to early wine quality work on rootstocks has continued (Hathaway 2001), this is despite limited differences in wine quality scores reported between rootstocks and own roots (Ewart, et al. 1993). Previous studies have highlighted the influence of rootstocks on wine potassium and pH levels (Walker, et al. 1998). pH is one of the more important quality parameters, as juice pH can affect fermentation rates (Ough, et al. 1968). Further to this, must with a higher pH have been shown to produce wines with low colour density, duller hue and lower ionized anthocyanins from grafted compared with ungrafted vines (Cirami, et al. 1984, Walker, et al. 1998, Gawel, et al. 2000). Increased additions of tartaric acid at the winery to adjust pH are estimated to cost the Australian wine industry millions of dollars per annum (Walker and Blackmore 2012). It is therefore not surprising, that the majority of previous work concerning rootstocks and wine quality has been in some way linked to the effects of increased potassium and wine pH; with very few using formalized sensory assessments. Of the initial studies to examine the effect of rootstock on wine quality, higher potassium content and higher pH have been reported as a result of using the following rootstocks: Ramsey, Harmony, Schwarzmann, 1613, Dog Ridge, Freedom, Rupestris du Lot (Ough, et al. 1968, Downton 1977, Cirami, et al. 1984, Ruhl, et al. 1988, Ewart, et al. 1993, Walker, et al. 1998). In more recent times, with the exception of Schwarzmann and Ramsey, rootstock use of these varieties has

diminished, for example, there have been no recorded new plantings of Freedom or Rupestris du Lot in the last decade and accordingly, only minimal plantings of Dog Ridge and Harmony remain in South Australia; 2.28 ha and 0.6 ha respectively (www.pgibsa.com.au). In contrast, some rootstocks have been reported to decrease pH of wine; these rootstocks include 140 Ruggeri, 1202C, 5A Teleki, SO4, 101-14 and 5C Teleki (Ruhl, et al. 1988, Ewart, et al. 1993). Typically, rootstocks that produce higher yields may also be considered higher in vegetative growth. For example, Walker, et al. (1998) attributed an increase in potassium for Ramsey due to variations in canopy density and shading— known to result in musts with higher potassium concentration— as a result of grafting to this vigorous rootstock cultivar (Walker, et al. 1998). In addition, organic acids such as malic and tartaric are affected by certain rootstocks— according to Ruhl, et al. (1988) a relationship exists between potassium and malate and pH. Rootstocks that cause a high malate have low tartrate concentrations and vice versa. However, the sum of individual concentrations of malate and tartrate are less influential on juice and wine pH than the ratio according to Ruhl, et al. (1988).

There are conflicting responses on the influence of rootstock on sugar accumulation. On the one hand, no significant differences in sugar accumulation were observed as a result of using rootstocks (McCarthy and Cirami 1990, Ewart, et al. 1993, Walker, et al. 1998, Gawel, et al. 2000, Keller, et al. 2012). While some authors report differences that are clearly cultivar and location specific (Ruhl, et al. 1988, Walker and Blackmore 2012), other studies confirm that rootstocks result in rapid sugar accumulation (Kaserer, et al. 1996). For Grüner Veltliner, the use of 140 Ruggeri rootstock resulted in a more rapid sugar accumulation than rootstocks such as 5C Teleki, 5BB Kober and SO4. Further to this, of the many studies to examine rootstocks and wine quality, few have formalized sensory assessments performed on the wines. Sensory assessments are vital to determine whether treatments impose a negative or positive impact on wine and whether this impact is due to appearance, aroma, flavour or a combination of all three (http://www.awri.com.au/researchanddevelopment/grapeandwinecomposition/sensory). Although more

recent studies now involve such assessments, much of the work on rootstocks is limited in regards to sensory analysis and description. Early sensory work by Gawel, et al. (2000) identified a difference in aroma between treatments, with 5C Teleki producing greater aroma intensity than ungrafted wines and wines made from Ramsey. Ramsey was considered to have the lowest flavour intensity of all the treatments. The authors also noted that differences between sensory treatments declined as the wine aged.

From most of the work performed on rootstocks for wine quality, it is clear that a combination of cultivar, location and soil type impact the resultant wine (Ruhl, et al. 1988, Ewart, et al. 1993, Walker and Blackmore 2012).

Rootstocks and wine sensory evaluations

To definitively define wine quality is complex and dependant on whether the definition is from a technical, productive or consumer viewpoint (Verdu Jover, et al. 2004). Technical methods often require sophisticated and expensive instruments for chemical analysis and few wineries have the access to or time to make such analyses (Olarte Mantilla, et al. 2012). More recent advancements in both berry sensory and wine sensory evaluations have been made possible through the use of various statistical models and evaluations (Verdu Jover, et al. 2004, Perrin, et al. 2005, Siegrist and Cousin 2009, Cadota, et al. 2010, King, et al. 2011, Olarte Mantilla, et al. 2012, Johnson, et al. 2013). One such technique is descriptive analysis (DA). DA quantifies sensory attributes to define various grape and wine characteristics; for example, berry, eucalyptus, fruit or black pepper (Heymann and Noble 1987, Olarte Mantilla, et al. 2012). This technique involves sourcing available and interested panellists of varying age and gender to participate. Typically, research projects requiring DA rely on volunteers at approximate costs of $40 per hour for facilitators and $25 per hour per panellist. This can equate to upwards of $20,000 for a three month project (Dr. Sue Bastian pers. comm). In addition, specialist computer software such as FIZZ biosystemes (France) is required to interpret the data. Training sessions are an essential requirement of the DA panel in order to generate appropriate descriptive

terms of the trial wines to be used in the study (Heymann and Noble 1987). A minimum of 6-8 training sessions for a period of two hours is preferred for a minimum of eight-twelve panelists (Lawless and Heymann 2010). Furthermore, a considerable volume of trial wine is needed as each trial wine is required to be assessed a minimum of two times during the formal training assessments for comparison with reference standards used to define the descriptive terms. As part of the training, the panel are trained to discuss the intensity of each descriptive term in each wine and after group consultation, assign an appropriate descriptive term to define differences between the wines selected (Heymann and Noble 1987). Although, the DA panel is a well reputed form of sensory assessment (Murray, et al. 2001, Lawless and Heymann 2010), the monetary and personnel costs associated with this method are likely to be prohibitive to commercial wineries that perhaps lack the time, skills and software to perform such rigorous tests.

Significant differences have previously been detected in consumer preference between rootstocks: 140 Ruggeri produced the most preferred wine and SO4 the least preferred during a four year assessment (Kaserer, et al. 1996). However, as this study lacked an ungrafted control it is unclear how the sensory preferences would change with such a comparison. In a more recent study on aroma and sensory attributes of ungrafted and grafted rootstocks for Pinot Noir and Chardonnay, wine quality was lower for rootstocks 140 Ruggeri than 110 Richter and SO4 (Wooldridge, et al. 2010). Indeed, 110 Richter was shown to have the highest quality of all treatments. On the other hand, no significant differences were detected between the treatments for aroma; however, scion cultivar was predictably, different; Pinot Noir having a more predominant aroma than Chardonnay.

There is growing interest in the use of rootstocks in Australian viticulture; however, limited attention has been paid to quantifying the effect to wine quality through formalized sensory assessments. To date, typically, little is known about the effects to wine sensory and wine chemical composition as a consequence of grafting to rootstocks. It is clear that further investigations into

sensory assessments of rootstocks are required to advance our understanding of the effect of rootstocks on wine quality.

2.4 The effect of water stress on grapevine productivity

Grapevine growth and yield are responsive to irrigation and rainfall events. A severe water stress may be detrimental to production and quality at various stages of grapevine growth (Hardie and Considine 1976). Although vine water status declines throughout any given season (Hardie and Considine 1976, Matthews, et al. 1987), and irrespective of irrigation amount or amount of readily available water in the soil, water deficit to cause a degree of water stress may be detrimental to yield.

Fruitset and water stress

Early work by Alexander (1965), showed that water stress may reduce fruitset if severe water stress occurs at flowering. However, Matthews and Anderson (1989) failed to observe an effect of early deficit irrigation on fruitset. These authors attributed this to an absence of water stress on the vines at this phenological stage, even though water application was between 56% and 69% less than continually irrigated vines across the three years. Alexander (1965) proposed a phase in early grapevine development that was highly susceptible to water stress. The phase occurs at flowering and lasts for four weeks. Water stress during this susceptible phase between flowering and fruitset can result in fruit abscission and fewer berries per bunch (Rogiers, et al. 2004), along with bunch shrivel and berry and branch abscission (Alexander 1965). Early season water stress generally coincides with the first cycle of berry development when cell division and cell expansion occur (Alexander 1965). Water stress experienced at cell division affects the potential for berry growth through a reduction in cell number per berry (Matthews, et al. 1987). The effect of early season water stress is often explained by the detrimental effect on berry development, weight and size (Matthews, et al. 1987, Matthews and Anderson 1989, Poni, et al. 1993, McCarthy 1997).

Although fruitset is acknowledged to be affected by water stress (Alexander 1965, Hardie and Considine 1976, Matthews and Anderson 1989, Rogiers, et al. 2004), it is unclear whether this is due to

millerandage or coulure or both. The ability to use the quantifiable indices that characterise fruitset—Millerandage Index (MI) and Coulure Index (CI)— to determine the effect of water stress would be an important step in understanding the effects of water stress on reproductive development.

Response of yield and berry development to water stress

Yield losses due to deficit irrigation can be due to a reduction in berry weight (McCarthy, et al. 1997), bunch number or berry number per bunch (Ezzahouani and Williams 1995). Furthermore, a reduction in shoot and leaf growth (Keller 2005), along with yield losses, are visible symptoms of severe water deficits. As water stress increases, visible symptoms include tendril abscission, cessation of shoot tips, and abscission of basal and middle leaves. According to Alexander (1965) the bunch will become affected when leaves begin to wilt.

A reduction in berry size is dependent on the intensity of the water stress particularly during the first growth phase (between flowering and veraison) when cell division of the pericarp occurs; a degree of water stress during this phase will impact on the rate of cell division and the enlargement of the pericarp cells (Ojeda, et al. 2001). Although water deficit can reduce pulp weight, and therefore berry weight, skin weight is only affected during the first growth phase (Ojeda, et al. 2002).

Late season water stress post veraison may also have the potential to negatively impact on yield (Petrie, et al. 2004), typically, through a reduction in pulp weight (Ojeda, et al. 2002). Visually, water stress between veraison and harvest will result in degrees of defoliation depending on the severity of stress (Poni, et al. 1993). However, it is generally concluded that berry growth is less sensitive to water stress post veraison than early season as it is too late to affect cell division or pericarp expansion (Ojeda, et al. 2001). Although water stress is commonly associated with decreases in yield and yield components, previous authors have also demonstrated changes in duration of ripening phases for the berry. Water stress reportedly does not affect the onset of veraison or the timing of ripening and harvest (Matthews and Anderson 1989, Jackson and Lombard 1993). However, water deficits have been shown to modify the duration of the key grapevine growth phases (Ojeda, et al.

2001). Under water deficit, phase I (cell division and pericarp expansion) concluded 3-4 days early while phase II, (the lag phase) was also shortened by water deficit, and depending on the severity, was reduced between 14 and 6 days. In this particular study, phase III (ripening) commenced when water was re-supplied to the vine. However the resumption of growth was significantly slower for vines subject to water stress (Ojeda, et al. 2001), while other studies have shown that a permanent water stress will delay ripening (Matthews and Anderson 1989, Jackson and Lombard 1993).

Water stress and carbohydrates

Water stress after harvest and before the vines go into dormancy can inhibit the storage of carbohydrates. A lack of actively photosynthesising leaves can limit the amount of carbohydrates for storage for the start of next seasons growth (Candolfi-Vasconcelos and Koblet 1990, Candolfi-Vasconcelos, et al. 1994, McCarthy 1997, McCarthy 1998, Flexas, et al. 2002). Previous authors have identified that water stress may have a negative carryover effect on productivity in the following season (Matthews and Anderson 1989, Petrie, et al. 2004). It is well known that improved assimilate supply to developing inflorescences improves fruitset (Coombe 1959, Candolfi-Vasconcelos and Koblet 1990, Candolfi-Vasconcelos and Castagnoli 1995, Caspari, et al. 1998, Collins and Dry 2009, Rogiers, et al. 2011). Loss of mature leaves before dormancy will reduce photosynthetic capacity, to the detriment of yield and bunch weight by reducing berry set (Coombe 1959). As such it is postulated that a negative carryover effect of severe water stress for the next season's crop may be observed through a reduction in bud fertility, fruitset, yield and berry weight due to a lack of assimilate supply to the developing inflorescences (Candolfi-Vasconcelos and Koblet 1990, Rogiers, et al. 2011). Indeed, bud fertility for next year's crop, flower and fruitlet abscission has been associated with deficient and poor remobilisation of assimilates (Candolfi-Vasconcelos and Koblet 1990, Zapata, et al. 2003, Rogiers, et al. 2011). In a study by Rogiers et al. (2011), depletion of carbon dioxide (CO_2) limited photoassimilate to the inflorescence due to the concurrent demands on photoassimilates by competing root, shoot and storage structures. Under the reduced CO_2 conditions, flower abscission and inflorescence necrosis

was exacerbated as assimilate supply was preferentially supplied to shoots rather than developing inflorescence. The authors were able to confirm that under non limiting CO_2 conditions, an increase in the storage of photoassimilates and therefore, photoassimilate supply to the grapevine increased fruitset. Furthermore, some varieties are reliant on remobilisation of accumulated assimilate supply from the previous season, up until E-L 31, while other varieties at this point rely on concurrent photoassimilate supply. A lack of assimilate supply to susceptible varieties was shown to increase the likelihood of coulure (Zapata, et al. 2003).

Interaction between cultivar and rootstock hybrids to water stress

In the past, grapevines were considered to be poorly adapted to stresses associated with a lack of available water (Schultz 2003). However, it is now more commonly acknowledged that grapevines can adapt to various environmental conditions and react according to an environmental stimulus; for example, water stress. Recently, differences in stress response between different *V. vinifera* varieties have proven to have modified stress mechanisms (Schultz 2003). These responses are driven primarily by the stomata and the terms isohydric and anisohydric responses are used to categorise the differences in stomatal behaviour and the ability of a cultivar to respond accordingly to water stress (Schultz 2003). Isohydric behaviour to water stress is through stomatal closure to maintain steady leaf water potential to conserve evaporative demand. On the other hand, anisohydric behaviour results in the loss of leaf water potential with increased evaporative demand (Schultz 2003).

The vegetative and yield responses of rootstocks to deficit irrigation were examined in inland, irrigated regions of southern Australia (Pech, et al. 2008, Stevens, et al. 2008, Stevens, et al. 2010). In these studies, irrigation volume was reduced by approximately 30% for the varieties Shiraz and Chardonnay resulting in a yield reduction of 31% and 9% respectively. Yield was reduced for Shiraz under deficit irrigation due to a reduction in bunch number per vine for 1103 Paulsen and 110 Richter, compared to other rootstocks in the study along with a reduction in berry number per bunch for 1103

Paulsen and Schwarzmann (Stevens, et al. 2010). On the other hand, yield was reduced for Chardonnay under deficit due to a reduction in berry weight (Stevens, et al. 2008).

For Chardonnay, neither irrigation nor rootstock genotype affected midday leaf water potential (Ψl) and deficit irrigation did not modify the performance of the rootstocks, as all yields were reduced for Chardonnay under deficit irrigation (Stevens, et al. 2008). In contrast, Shiraz under deficit irrigation exhibited lower Ψl and, in general, Ψl was not affected by rootstock genotype except on one occasion, when 1103 Paulsen recorded a significantly lower Ψl than 110 Richter and Ramsey (Stevens, et al. 2010). In that study, all deficit irrigated Shiraz treatments had lower berry number per bunch than irrigated treatments and irrigated treatments had greater berry weights than deficit irrigation. Rootstocks Schwarzmann and Ramsey, under deficit, had the highest berry weights of the deficit irrigation treatments. In a recent deficit irrigation study of Chardonnay, Merlot and Shiraz, rootstocks 3309C, 5C Teleki and own roots had higher yields than 140 Ruggeri, 1103 Paulsen and 101CU, attributed to more bunches and berries per bunch (Keller, et al. 2012). In that instance, scion cultivar had a greater effect on yield than rootstock type and similarly, the reported change in shoot vigour between the treatments was a consequence of deficit irrigation rather than rootstock type.

2.5 Water stress and Wine Chemical composition

The effect of water stress on fruit and potential wine quality is commonly examined through changes to TSS, pH, tartaric and malic acid ratios, anthocyanin and phenolic concentrations (Matthews, et al. 1990, Jackson and Lombard 1993, Petrie, et al. 2004, Roby, et al. 2004, van Leeuwin, et al. 2004). For red wine grapes, it is commonly noted that a mild water deficit is seen as beneficial to wine quality (Hepner, et al. 1985, Matthews, et al. 1990, Roby and Matthews 2004, van Leeuwin, et al. 2007), as resultant wines typically have higher concentrations of anthocyanin and phenolics (Matthews and Anderson 1988, Matthews, et al. 1990, Ojeda, et al. 2002, van Leeuwin, et al. 2007, Bindon, et al. 2008, Bindon, et al. 2011).

The commencement of berry softening and veraison signify the second cycle (phase III) of grape berry growth. The ripening grape berry comprises both non-solutes (water) and solutes (sugar), the accumulation of these solutes is due to the phloem sap into the berry (Coombe and McCarthy 2000). Previous authors have identified that water stress can affect berry composition which may affect wine colour and sensory attributes (Roby, et al. 2004, Bindon, et al. 2008, Bindon, et al. 2011) and this may be independent of changes in berry size that can also occur as a consequence of water stress. Furthermore, a loss of water in the berry from transpiration can result in a loss of non-solutes within the berry while solutes remain constant (Coombe and McCarthy 2000).

Studies that examined water deficits, applied at key phenological stages of pre-veraison and post-veraison, identified that pre-veraison water deficit decreases pericarp volume and modifies the structural properties of the cell components, which limits the enlargement of the pericarp and this affects berry size (Ojeda, et al. 2001, Ojeda, et al. 2002, Roby, et al. 2004, Bindon, et al. 2011). The response of non-solute accumulation to water deficit has been shown to produce differing results; on the one hand, smaller berries may have a higher concentration of sugar accumulated in the berry relative to its size, but only a small increase in sugar content. In studies by Petrie, et al. (2004), a 30% increase in sugar concentration was attributed to berry shrinkage as a result of water deficit. Ojeda et al. (2002) reported that sugar concentration is less influenced by water deficit than claimed by Petrie, et al. (2004). These differing responses to water deficit for sugar concentration highlight the importance of the timing (Matthews and Anderson 1988, Matthews, et al. 1990) and severity of water deficit as to whether sugar concentration is affected or not. Further to this, yield also plays a significant part in the response to water deficit. In studies by van Leeuwin, et al. (2007), low yields under moderate water deficit enhanced berry sugar content, anthocyanin concentration and grape phenolics. However, high yield and severe water deficit depressed sugar content, and resulted in early berry shrivel and incomplete ripening (van Leeuwin, et al. 2007). It is commonly acknowledged that when water stress

becomes severe, sugar accumulation can be depressed as a result of limited photosynthesis and carbon assimilation (Freeman, et al. 1980, van Leeuwin, et al. 2007).

Water deficits have been shown to not only increase intensity of colour, but also an increase in aromas of blackberry, strawberry, raspberry and blackcurrant have been reported (Myburgh 2011). Anthocyanins and their derivatives in winemaking contribute to wine colour and undergo a variety of complex interactions that can include short-term self association or co-pigmentation with phenolic compounds such as flavan-3-ols and proanthocyanins (Fei, et al. 2012). Co-pigmentation in particular, leads to an increase in wine stability (Chalmers, et al. 2010). However in a recent study examining the effects of sustained deficit irrigation on wine colour, polymeric pigments appeared to have the greater influence on wine colour than monomeric pigments or co-pigmentation (Chalmers, et al. 2010). Although the content of anthocyanins decreases throughout wine ageing (Somers 1971), polymeric pigments form from the reaction of monomeric anthocyanins with tannins and flavin-3-ols (Adams 2001). The increase in total red colour after six months for treatments with reduced irrigation was due to increased polymeric pigments rather than monomeric or co-pigment complexes (Chalmers, et al. 2010). For the two varieties in this study, a reduction in irrigation of 50% resulted in an increase in polymeric pigments by approximately 30% relative to the control. Although the authors attributed the increase in colour to changes in anthocyanin and phenolic biosynthesis in the berry and changes in phenolic chemistry throughout winemaking, the authors did not discount the concentration effect as a result of decreased berry size due to reduced irrigation. Previous authors (Roby, et al. 2004, Petrie, et al. 2004) have attributed an increase in anthocyanins in response to water deficit as a result of a decrease in berry size. Grape skin is made up of flavanols and anthocyanins (Ojeda, et al. 2002) and approximately 15-20% of berry fresh mass is due to skin tissue (Roby and Matthews 2004). A water deficit that reduces berry weight will increase skin to pulp ratio (Ojeda, et al. 2002) and skin to flesh weight ratio (Bindon, et al. 2011) and may increase anthocyanin and phenolic concentrations.

Concentration of organic acids contributes to the acid taste and influences colour and microbial stability (Esteban, et al. 1999). The main organic acids of wine are tartaric and malic acids which accumulate in both the skin and flesh of the berry and are synthesized during berry formation. After veraison, malate content declines through respiration, while tartaric acid remains constant (Esteban, et al. 1999, Iland, et al. 2011). Titratable acidity is closely related to the malic acid concentration more so than the tartaric acid concentration (van Leeuwin, et al. 2004). Throughout ripening, the acid concentration decreases from veraison through to harvest, but this decrease is always greater for malic than tartaric acid (Esteban, et al. 1999). Tartaric acid is more stable than malic acid and this may be due to the chemical characteristics of tartaric acid that enables the acid to remain constant throughout ripening (Esteban, et al. 1999). Water deficit reduces titratable acidity through a reduction in malic acid. Degradation of malic acid was higher in the absence of irrigation, with reduction between 42% and 71% compared with 34%-62% for the irrigated control. On the other hand, tartaric acid decreased by between 27% and 45% for unirrigated treatments and between 31% and 36% for irrigated treatments (Esteban, et al. 1999). The increased malic acid degradation in the absence of irrigation is likely due to an increase in bunch exposure to solar radiation and increased berry temperature that occurred as a consequence of smaller, more open canopies resulting from water stress, compared with more shaded canopies of the irrigated controls (Esteban, et al. 1999, Bindon, et al. 2008). This in turn, results in an increase in tartaric to malic acid ratio (Matthews and Anderson 1988).

2.6 Summary

The reproductive process in grapevines could arguably be the most important, as its success determines the yield for the current season and sets the potential crop for the coming season. The process of reproductive development in grapevines can be divided into a number of sequential stages that occur over two successive growing seasons. As a consequence, the growth and yield of the crop is dependent on bunch initiation in the previous season, inflorescence development, flowering and fruitset

and the development of seeds and flesh within a grape berry in the current season (Pratt 1971, Srinivasan and Mullins 1981, May 2004, Vasconcelos, et al. 2009). Factors affecting these processes may include the cultivar (Longbottom 2007, Dry, et al. 2010), climatic conditions (Buttrose 1969a, Buttrose 1969b, Dunn and Martin 2000, Sommer, et al. 2000, Sommer, et al. 2001), shoot growth (Lavee, et al. 1981, Dry and Coombe 1994), canopy shading (May 1965, Perez and Kliewer 1990, Wolf and Cook 1992) and choice of rootstock through an effect on scion vigor (Keller, et al. 2001, Sommer, et al. 2001, Dry 2007). Further to this, in regions where poor fruitset is of concern, reproductive development of grapevines may potentially be managed through the use of rootstocks (Candolfi-Vasconcelos and Castagnoli 1995, Cirami 1999, May 2004, Whiting 2004, Dry 2007). The use of grapevine rootstocks (American *V.*) in phylloxera-free regions of South Australia has increased steadily over the past 30 years yet rootstock use still accounts for approximately 20% of total plantings (Dry 2007). The importance of rootstocks in viticulture is well documented; particularly in relation to yield, fruit composition, comparative pest resistance, salinity, nutrition and water relations (Whiting 1988, McCarthy and Cirami 1990, Cirami, et al. 1994, Ezzahouani and Williams 1995, Nicholas 1997, Walker, et al. 2002, Zhang, et al. 2002, Keller, et al. 2001, Whiting 2003, Soar, et al. 2006, Dry 2007, Soar and Loveys 2007, Pech, et al. 2008, Stevens, et al. 2008). However, at present, comparisons between rootstocks and non-grafted *V. vinifera* scions for their effects on grapevine reproduction are limited or often lack a comparison with a non-grafted control. Further to this, in regions where poor fruitset is of concern, reproductive development of grapevines may potentially be managed through the use of rootstocks (Candolfi-Vasconcelos and Castagnoli 1995, Cirami 1999, May 2004, Whiting 2004, Dry 2007).

Water use efficiency is a critical issue for expansion and sustainability in Australian viticulture. The likelihood of future drought and consequent water restrictions throughout many grape growing regions of the world highlight the requirement to determine if grafting to a drought tolerant rootstock provides an advantage in a water stressed environments. As such, Australian viticulturists need to

prepare to sustainably operate with reduced water resources. The uncertainty of water availability has therefore led to increased interest in rootstocks, particularly those with reported drought tolerance.

In general, a mild water deficit is seen as beneficial to wine quality through an increase in the concentration of anthocyanin and phenolics. When water stress becomes severe, sugar accumulation can be depressed; while a reduction in titratable acidity is also often reported. This in turn may have serious ramifications for both harvest time and final wine quality. Typically, little is known about the effects of prolonged water stress on wine chemical composition and quality. Moreover, of the many studies to examine rootstocks and wine quality, few have had formalized sensory assessments performed on the wines, and although more recent studies now involve such assessments, much of the work on rootstocks is limited in regards to sensory analysis and description or lack an ungrafted control for comparison.

Chapter 3. Published Article: Reproductive performance of Cabernet Sauvignon and Merlot (*Vitis vinifera* L.) is affected when grafted to Rootstocks

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Contribution to the Paper	Kidman designed and conducted the research experiments, analysed the data and drafted and constructed the manuscript.
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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 2.25m x 3m 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 1 $mL \cdot ha^{-1}$ (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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Assessment of rootstock and irrigation effects on Shiraz (*Vitis vinifera* L.) using grape and wine chemical compositional measures and a novel sensory analysis technique

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Abstract

Rootstock use for water management in viticulture is a key practice used internationally. However, little is known about the effect on grape and wine composition and sensory attributes of wine. An experimental trial comparing 6 rootstocks with an own rooted control; with and without irrigation was established in the Barossa Valley, Australia in 2008. Grape and wine composition measures were assessed and a novel sensory analysis technique using sensory attribute word frequency data was used to compare treatments. Significant differences in grape TSS, pH, Titratable acidity (TA), colour and phenolic levels were found between rootstocks. Generating sensory attribute word frequency data from expert wine judges allowed discrimination between treatment wines. Principal component analysis of sensory attributes revealed consistent findings between treatments for both vintages. Rootstock had a greater effect than irrigation on both composition and sensory attributes of wines. These findings have implications for rootstock selection management decisions and how we assess research wines for commercial application.

Key words: rootstocks, water stress, wine quality, wine sensory assessment, word frequency data

1. Introduction

The importance of rootstocks in viticulture is well documented; particularly in relation to yield, fruit composition, comparative pest resistance, salinity, nutrition and water relations (Cirami, et al. 1984, McCarthy and Cirami 1990, Ezzahouani and Williams 1995, Nicholas 1997, Walker, et al. 1998, Keller, et al. 2001, Zhang, et al. 2002, Whiting 2003, Dry 2007, Stevens, et al. 2008, Kodur, et al. 2010, Stevens, et al. 2010, Keller, et al. 2012, Walker and Blackmore 2012, Whiting 2012). In limited studies in this field, rootstock type has affected consumer preference. 140 Ruggeri produced the most preferred wine and SO4 the least preferred during a four year assessment (Kaserer, et al. 1996). However, as this study lacked an ungrafted control it is unclear how the sensory preferences would change with such a comparison. In a more recent study on aroma and sensory attributes of ungrafted

and grafted rootstocks with Pinot Noir and Chardonnay, wine quality was lower for 140 Ruggeri than 110 Richter or SO4 (Wooldridge, et al. 2010). Indeed, 110 Richter was shown to have the highest quality of all treatments. On the other hand, no significant differences were detected between the treatments for aroma; however, scion cultivar was predictably, different; Pinot Noir having a more predominant aroma than Chardonnay. It is clear further work in this field is required as the current literature reporting the effect of rootstocks on grape and wine chemical composition and sensory attributes are limited or often lack comparisons with own rooted controls.

In Australia, rootstock use is limited, compared with Europe and elsewhere, where phylloxera necessitates the use of rootstocks. For example, in South Australia, approximately 80% of vineyards are planted to own roots. According to a survey of South Australian grapegrowers an impediment to rootstock use has been associated with a negative stigma for wine quality (Hathaway 2001). Indeed, early research with rootstocks showed an increase in potassium and pH which was negatively associated with wine quality (Hale and Brien 1978). Despite minimal differences in wine quality scores between rootstocks and own roots (Ewart, et al. 1993).

Previous studies have highlighted the influence of rootstocks on wine potassium and pH levels (Walker, et al. 1998). pH is one of the more important quality parameters, as juice pH can affect fermentation rates (Ough, et al. 1968). Further to this, rootstocks with a higher pH have been shown to produce wines with lower colour density, duller hue and lower ionized anthocyanins than ungrafted wines (Cirami, et al. 1984, Walker, et al. 1998, Gawel, et al. 2000). Increased additions of tartaric acid at the winery to adjust pH are estimated to cost the Australian wine industry millions of dollars per annum (Walker and Blackmore 2012). It is therefore not surprising, that the majority of previous work concerning rootstocks and wine quality has been in some way linked to the effects of increased potassium and wine pH; with very few using formalized sensory assessments.

It is commonly acknowledged that for red wine grapes, a mild water deficit is seen as beneficial to wine quality (Hepner, et al. 1985, Matthews, et al. 1990, Roby and Matthews 2004, van Leeuwin, et al.

2007). Resultant wines typically have higher concentrations of anthocyanin and phenolics (Matthews and Anderson 1988, Matthews, et al. 1990, Ojeda, et al. 2002, van Leeuwin, et al. 2007, Bindon, et al. 2008, Bindon, et al. 2011) and an increase in desirable aromas such as blackberry, strawberry, raspberry and blackcurrant (Myburgh 2011). As water use efficiency is a critical issue for expansion and sustainability in Australian viticulture, there is growing interest in the use of rootstocks to minimise the impact of possible future water shortages on production; however, limited attention has been paid to quantifying the effect on wine quality when vines, either on their own roots or grafted onto a rootstock, are returned to dry land conditions as may occur under future climate change. To date, little is known about the effects of prolonged water stress on wine chemical composition and wine quality from a sensory perspective.

Wine grape quality has long been evaluated by the composition of soluble solids, organic acids and pH in the berries and wine (Jackson and Lombard 1993). These important constituents are further influenced by maturity, quantity and distribution throughout the berry, all of which will influence the style and quality of the wine (Olarte Mantilla, et al. 2012).

To definitively define wine quality is complex and dependant on whether the definition is from a technical, productive or consumer viewpoint (Verdu Jover, et al. 2004). Technical methods often require sophisticated and expensive instruments for chemical analysis and few wineries have the access to or time to make such analyses (Olarte Mantilla, et al. 2012). To date, methods that are easy to assess, including soluble solids, organic acids and pH, along with assessment of colour, phenolics and tannins are the main quality parameters documented in the literature (Ough, et al. 1968, Downton 1977, Cirami, et al. 1984, Matthews, et al. 1990, Jackson and Lombard 1993, Gawel, et al. 2000, Petrie, et al. 2004, Roby, et al. 2004, van Leeuwin, et al. 2004, Bindon, et al. 2008, Bindon, et al. 2011, Walker and Blackmore 2012). More recent advancements in both berry sensory and wine sensory evaluations have been made possible through the use of various statistical models and evaluations (Verdu Jover, et al. 2004, Perrin, et al. 2005, Siegrist and Cousin 2009, Cadota, et al. 2010, King, et al. 2011, Olarte

Mantilla, et al. 2012, Johnson, et al. 2013). One such technique is descriptive analysis (DA). DA quantifies sensory attributes to define various grape and wine attributes; for example, berry, eucalyptus, fruit or black pepper (Heymann and Noble 1987, Olarte Mantilla, et al. 2012). This technique involves sourcing available and interested panellists of varying age and gender to participate. Typically, research projects requiring DA rely on volunteers at approximate costs of $40 per hour for facilitators and $25 per hour per panellist. This can equate to upwards of $20,000 for a three month project (Dr. Susan Bastian pers. comm). In addition, specialist computer software such as FIZZ biosystemes® (France) is required to interpret the data. Training sessions are an essential requirement of a DA panel in order to generate appropriate descriptive terms of the trial wines to be used in the study (Heymann and Noble 1987). A minimum of 6-8 training sessions for a period of two hours is preferred for a minimum of eight-twelve panellists (Lawless and Heymann 2010). Furthermore, each trial wine is required to be assessed a minimum of two times during the formal training assessments for comparison with reference standards used to define the descriptive terms. As part of the training, the panel are trained to discuss the intensity of each descriptive term in each wine and after group consultation, assign an appropriate descriptive term to define differences between the wines selected (Heymann and Noble 1987). Although the DA panel is a well reputed form of sensory assessment (Murray, et al. 2001, Lawless and Heymann 2010), the monetary and personnel costs associated with this method are likely to be prohibitive to commercial wineries that perhaps lack the time, skills and software to perform such rigorous tests.

The aims of this research were twofold; firstly to assess the effect of rootstock type on wine quality using grape and wine chemical composition and sensory attributes of wines made from Shiraz grafted to American *Vitis* rootstocks; and secondly, to determine whether rootstock use could mitigate the effects of severe water stress with limited effect on wine quality and sensory attributes. To assess differences in wine sensory attributes, a novel sensory analysis technique has been developed using word frequency data generated from expert winemaker panels.

2. Materials and Methods

2.1. Experimental site

In 2008, a three year experiment was established at Nuriootpa, South Australia, Australia (34.80˚S, 139.01°E). The vineyard was planted in 2001 at 1481 vines per hectare, vine spacing and row spacing was 2.25 m by 3 m respectively and trained to a bilateral cordon. The site is located within a phylloxera-free region that allows for the use of own rooted *V. vinifera* vines. The vineyard was planted on a uniform site with little soil variation. The soil is a Light Pass fine sandy loam A horizon overlying a red brown mottled clay B horizon (Northcote 1954). The fine sandy loam A horizon (A1+A2) was 30 cm deep averaged across a 40 m2 sampling grid pattern with structural roots to one metre (Rudd 1975). All plant material (Shiraz clone BVRC30 and rootstocks) were certified by South Australian Vine Improvement Incorporated (SAVII®) nurseries as being free from known virus and diseases. Prior to grafting, rootstocks were hot-water-treated for 30 minutes at 50 °C, and all vines were planted as potted one-year-old plants. Vines were spur-pruned to approximately forty nodes per vine to match the commercial pruning level of the vineyard. Vines were drip-irrigated, using irrigation sourced from either bore water (water sourced from below ground aquifer) or water from the Murray River via the Barossa Infrastructure Limited (BIL) scheme. The values used to schedule an irrigation event were based on soil water content measured with capacitance probes that recorded soil water hourly. Based on the capacitance sensors the theoretical average total soil water content to 1.1 m depth for September 2008, September 2009 and September 2010 was 236 mm, 270 mm and 265 mm respectively.

When the soil water content to 1.1 m depth declined to a predetermined value in each season, irrigation commenced. Irrigation of control plants was intended to mimic the strategy for high quality wine production in the Barossa and therefore control vines were subject to a degree of water stress throughout the season. Further to this, due to irrigation supply flow constraints, all rootstock

combinations received the same volume of irrigation on a weekly basis as a single irrigation. Applied water was 1.1 ML/ha (110 mm) in 2008/2009 and 1.3 ML/ha (128 mm) in 2009/2010. All unirrigated treatments had no irrigation applied for the duration of the experiment and only received water through precipitation. A Bureau of Meteorology weather station, located approximately 500 m to the west of the trial site, was used for climate data. Total rainfall for the site for the years 2008-2010 was 434 mm, 502 mm and 589 mm respectively. Growing season rainfall (September to April) was 227 mm in 2008/2009 and 267 mm in 2009/2010. Long term annual rainfall for the site is 500 mm (http://www.bom.gov.au/climate/averages/tables/cw_023373.shtml).

2.2. Experimental design

V. vinifera L. Shiraz (clone BVRC30) was grafted to six American *V.* rootstocks: Ramsey (*V. champinii*), Schwarzmann (*V. riparia X V. rupestris*), 1103 Paulsen (*V. berlandieri X V. rupestris*), 140 Ruggeri (*V. berlandieri X V. rupestris*), 99 Richter (*V. berlandieri X V. rupestris*) and 110 Richter (*V. berlandieri X V. rupestris*) and compared to ungrafted, own roots Shiraz (*V. vinifera*). The experiment was performed across 10 rows for each rootstock (whole plot consisting of 42 vines per rootstock). Within each plot, there were three replicate blocks of irrigated and three randomised, replicate blocks of unirrigated vines (subplots), consisting of 7 treatment vines (split plot design). The unirrigated treatment was established in August 2008 prior to the start of the 2008-2009 growing season. Treatments undergoing zero irrigation, and adjacent buffer rows had their drip irrigation lines bypassed.

2.3. Berry compositional measurements

Maturity assessments were undertaken from veraison to harvest. One hundred berries per plot were sampled each week for analysis of total soluble solids (TSS, °Brix), pH and titratable acidity (TA, g

tartaric acid equivalents/L). Berries were collected from the top, middle and bottom of random bunches and placed in sealed polyethylene bags and stored on ice before analysis in the laboratory. Berry weights were recorded and berries were crushed using a manual press and all free run juice was collected in 10 mL polyethylene centrifuge tubes. The samples were centrifuged at 3000 rpm for 5 minutes. Clarified juice samples were used to determine TSS using a digital refractometer (BRX-242 ERMA, Japan). Juice pH and TA were measured by titration (Crison Compact Titrator 08328 Alella, Crison, Spain) as described in (Iland, et al. 2004). In addition, a further 50 berries were collected at harvest for mean berry weight and frozen for analysis of total anthocyanins and total phenolics (Iland et al. 2004)

2.4. Winemaking

Shiraz grapes were harvested by hand between 22° and 24° Brix and each treatment pooled into three 30 kg replicates for winemaking. Each winemaking replicate was comprised of randomly selected bunches of each treatment. A crusher/destemmer (Enoitalia, ENO-15, Italy) was used to process each replicate and juice/must pumped directly into 30 L food grade plastic open fermenters with screw top lids (Winequip products, Magill, South Australia). During crushing 50 mg/L of sulphur dioxide (SO_2) was added as a 20 % solution of potassium metabisulphite (PMS) to all the sampling units. Tartaric acid additions were made to the ferments prior to yeast inoculation to adjust the pH to 3.5. Diammonium phosphate (0.5 g/L) was added as well at the time of yeast inoculation when the ferments were between 18-20 ^{o}C. Each ferment was then co-inoculated with 25 g/hl reconstituted dried yeast (Maurivin® AWRI 796, Mauri Yeast Australia, Sydney, Australia) and 1 g/hL *Oenococcus oeni* VP41 LAB (Lallemand, Underdale, Australia) following the manufacturer's instructions. All fermentations were maintained at 15 °C ± 2 °C and the cap manually plunged every 12 hours for a period of 6 days or until fermentations had reached 2˚ Baume (plunged approximately 18 times). Once below 1° Baumé (Bé),

the wines were pressed using a bladder press (Diemme 130 L Laboratory Press, JB Macmahon Pty Ltd, Forestville, Australia) operated using the following protocol; 0.2, 0.4, 0.6, 0.8 and 1 bar each held for five minutes with two crumples between presses. The wine was transferred to 10L glass demijohns (Winequip products, Magill, South Australia) and stored at 20°C. When primary fermentation was completed, the ferments were racked off gross lees into 5L glass vessels to avoid ullage with the addition of glass marbles. SO_2 (to reach 80ppm Total SO_2), copper sulphate and tartaric acid (1 g/L if pH was higher than 3.55) additions were made to ferments that had completed malo-lactic fermentation (<0.05 g/L malic acid by enzymatic test kit, [Roche, Castle Hill, Australia]), and kept at 0^{o}C for 3 weeks for cold stabilization. After cold stabilization the finished wines were filtered using a pad filter (Colombo-Rover pump & 6 pad filter, Italy) provided with 0.8 µm Z6 cellulose filters pads (Ekwip, NSW, Australia) and bottled into 375 ml bottles with screw cap closures. The wines were then stored at a constant temperature of 15 ^{o}C for future wine sensory and chemical evaluations.

2.5. Wine compositional measures

Standard chemical measurements (SO_2 (ppm), pH, TA (g/L), volatile acidity (g/L), alcohol (%) and residual sugar (g/L)) were performed on the wines at the time of sensory evaluation, following the methodologies described in Iland et al. (2004). Wine samples were analysed for density (au), hue, total anthocyanins (mg/L), and total phenolics (au) as described by Iland et al. (2004) and modified for use with 96-well ultraviolet transparent microtiter plates (Greiner, Sigma-Aldrich, Sydney Australia). Wine samples (50 µL) for total anthocyanin and total phenolic determinations were added to 1 M HCl (5 mL) and incubated for a minimum of three hours at room temperature before aliquots (300 µL) were transferred to 96-well microtiter plates and read at 520 nm (total anthocyanins) and 280 nm (total phenolics) using a Quant Microplate spectrophotometer (Thermo Scientific Multiskan Spectrum, USA).

Density and hue were calculated from absorbance values of neat wine (150 µL aliquots in 96-well microtiter plates) read at 420 nm and 520 nm.

2.6. Sensory evaluation of wines

Wines from all treatments and replicates (42 wines total) were assessed by expert winemakers in 2009 and 2010. Eight winemakers (6 male, 2 female) participated in the panel in 2009 and 16 winemakers (11 male, 5 female) in 2010. The approximate age group ranged from 25 to 60 years and most (>95%) had tertiary training and/or greater than ten years winemaking experience.

Thirty ml of each wine was served in coded, INAO (ISO standard) 215 ml tasting glasses (Arcoroc Viticole, Cardinal International, France). A random three-digit code (generated using Design Express®, Version 1.6, Qi Statistics, United Kingdom) was given to each replicate x treatment and presentation randomised to prevent first order carry-over effects (Macfie, et al. 1989). To avoid palate fatigue and cleanse their palate, the assessors were provided with filtered water and plain water crackers (Arnotts®, Australia) to have between wine samples. Every participant was then asked to individually assess each of the 42 randomly assigned wines. Each wine was firstly assessed using the Australian wine show standards twenty point score system (Ewart, et al. 1993, Dunphy and Lockshin 1998). Briefly, three points were awarded for colour, seven points for aroma and ten points for palate. Judges were then asked to provide a written description of attributes that best describe the wine. All attributes and final wine quality scores used by each judge for every wine were then entered into Excel (Microsoft Excel (Version 2011), Redmond, Washington, USA). Where similar terms for certain attributes were used these were grouped together and shown in Table 1. The final list of attributes from all judges for every wine were then imported from Excel into Nvivo 10 (Version 10, QSR International, Victoria, Australia) to calculate the frequency a certain attribute was used by the panel of judges for each individual wine. The full list of attributes used to describe the wines for each vintage is presented in Table 2.

2.7. Statistical analysis

Statistical analyses of berry and wine compositional measures and wine quality scores were performed using a split plot design (rootstock x irrigation) analysis of variance (ANOVA), using Genstat (13th Edition 13.1. Lawes Agriculture Trust 2010, United Kingdom). ANOVA was also performed on all sensory attribute word frequency data generated from wine evaluations using XLSTAT Version 2012 1.01 (Addinsoft SARL, France). Attributes that were significantly different between treatments were then subjected to principal component analysis (PCA) using XLSTAT Version 2012 1.01 (Addinsoft SARL, France) and presented as biplots. Details of individual analyses are provided in the text or captions.

3. Results and Discussion

3.1. Maturity assessments

Significant differences in final berry weight were observed between all treatments in both seasons and have been reported in (Kidman, et al. 2014). As shown in Figs. 1 and 2, these differences were consistent from veraison, through the ripening period. Berry weight increases between the first and second sampling period (7 days) for irrigated treatments was on average 7 mg berry $^{-1}$ day $^{-1}$ and 0 mg berry $^{-1}$ day $^{-1}$ for unirrigated treatments in the 2009 season and 5.4 mg berry $^{-1}$ day $^{-1}$ and 2.7 mg berry $^{-1}$ day $^{-1}$ for the irrigated and unirrigated treatments respectively in 2010 (11 days).These results are significantly smaller than previously reported. According to Esteban, et al. (1999), the greatest increase in berry weight occurs at the onset of veraison and shortly afterwards. These authors reported an average increase of 34 mg berry $^{-1}$ day $^{-1}$ and 26 mg berry $^{-1}$ day $^{-1}$ for irrigated and non irrigated Tempranillo vines immediately following veraison. Esteban et al. (1999) attribute the differences in berry weight as the main factor influencing TSS and reported an increase of 6.8 °Brix and 7.1 °Brix between

the veraison and post veraison time points reported— which is far greater than the 4 °Brix and 3.7 °Brix recorded for the 7 days after veraison for irrigated and non irrigated treatments respectively in 2009, and 3.8 °Brix and 4.5 °Brix for 11 days after veraison for irrigated and non irrigated treatments, respectively, in 2010. The two studies differ in both variety and climatic growing conditions. Both factors can affect the rate of TSS accumulation; GDD (budburst to harvest) was lower in 2009 than for Esteban et al. (1999), while in 2010, the converse was true. Tempranillo is also an early ripening variety (Kerridge and Antcliff 1999) which may have also contributed to these differences in accumulation of TSS during the ripening period. Further to this, the irrigated vines were subject to a mild degree of water deficit to reflect the strategy for high quality wine production in the Barossa (Kidman et al., 2014) and this would have influenced differences in berry weight and sugar accumulation.

TSS values followed a similar pattern between the treatments for the 2009 season, with Shiraz on own roots with and without irrigation having consistently lower TSS throughout the ripening period. Indeed, both the own roots Shiraz treatments were picked at a sub optimal TSS value in 2009, nonetheless, the decision to harvest was made as TSS values began to plateau due to the heavy crop load in these treatments for that year (Kidman, et al. 2014). In 2010, there were small differences between treatments for TSS accumulation; most treatments, with the exception of irrigated Schwarzmann, increased TSS at similar rates. Irrigated 1103 Paulsen and 140 Ruggeri were harvested approximately one week earlier than all other treatments, as these treatments reached the desired TSS level (24-25 °Brix). Furthermore, irrigated 140 Ruggeri and 1103 Paulsen had higher TSS values throughout the ripening period than all other treatments. Ramsey without irrigation had the lowest TSS for the 2010 season (Figure 2) but was still harvested at a higher TSS value than both the irrigated and non irrigated own-rooted Shiraz treatments from the 2009 season (Figures 1 and 2). Ramsey has previously been shown to delay maturity in two out of three seasons in an experiment conducted on Cabernet Sauvignon (Gawel, et al. 2000). The authors attributed the effect of high crop load and vigour on the inability of Ramsey to ripen when compared with Cabernet Sauvignon on own roots. In the

present study, yield of Ramsey was similar to own- rooted Shiraz treatments; however, lower vegetative growth coupled with lower midday leaf water potential than own rooted Shiraz treatments (Kidman, et al. 2014) are likely to have limited photosynthesis and carbon assimilation (Freeman, et al. 1980, van Leeuwin, et al. 2007) and hence the ability of Ramsey to ripen a large crop.

pH values increased rapidly for all treatments in 2009 between week one and three of ripening (Figure 1). Thereafter, pH increase but at a reduced rate. However, at week four of ripening (22nd February) pH for all treatments decreased. It is likely the measures of pH on this date were erroneous, potentially due to equipment problems, as all treatments suffered this decline, while berry weight, TSS values and TA values did not mirror this trend. At harvest, most treatments were picked with a pH between 3.5 and 3.9 (grape pH) (Table 2). In 2010, the increase in pH was more gradual than in 2009, and values for pH for all treatments were initially higher than 2009. Changes in pH were correlated with seasonal climate variations (Kidman, et al. 2014). For example, the climate of 2009 was, on average, cooler than 2010; however, the season was characterised by extreme weather events, in particular, higher temperature following veraison and just prior to harvest. This corresponded with spikes in TSS, pH and TA at these time points (Figure 1). In contrast, 2010 was characterised by a more constant increase in TSS, pH and TA and a more constant temperature accumulation for that season, particularly from veraison through to harvest (Kidman, et al. 2014).

3.2. Grape and wine compositional measures and wine quality scores

Rootstock and irrigation treatments had a significant effect on some grape and wine composition parameters. An interaction between rootstock and irrigation was observed for alcohol, wine pH, wine colour density wine colour hue and wine phenolics in 2009 (Table 3). There was no significant interaction between rootstock and irrigation treatments for grape juice pH, TA, TSS, grape colour and phenolics (Table 3). In 2010 a significant interaction between rootstock and irrigation was observed for

alcohol % and wine phenolics and in addition, grape juice TA. No significant interaction was observed for other grape and wine compositional measures.

Shiraz on own roots had significantly lower harvest TSS and pH compared to the rootstock treatments and higher grape TA compared to all other rootstocks apart from 110 Richter in 2009 (Table 3). Wine quality observations for each rootstock were similar in 2009. These findings support Ewart et al. (1993) wherein few differences in wine quality score, based on the Australian wine show 20 point system, were observed between rootstock treatments when compared with Chardonnay on own roots. In 2010, the trend was not the same. 99 Richter had lower harvest grape TA than all other treatments apart from irrigated Ramsey and unirrigated 140 Ruggeri. 99 Richter and Ramsey had lower TSS than all other treatments and Shiraz on own roots had lower TSS than 110 Richter, 1103 Paulsen and 140 Ruggeri. In 2010, Ramsey and 99 Richter had significantly lower wine quality scores compared with other rootstocks and Shiraz on own roots. No significant difference in grape pH was observed.

No consistent difference in grape colour and grape phenolic levels were observed between vintages. However, wine colour density and wine phenolic levels tended to be lower for Shiraz on own roots and Ramsey in both vintages compared to other rootstock treatments (Tables 3 and 4). Must and wine composition of Cabernet Sauvignon grafted to a series of rootstocks has previously been reported (Gawel, et al. 2000). Comparison of the same rootstocks by Gawel et al. (2000) with those found in the present study identified lower wine colour density in 110 Richter and Ramsey when compared with Schwarzmann or Cabernet Sauvignon on own roots. Those authors attributed the low wine colour and wine phenolic levels to lower grape TSS. In the present study, grape TSS was significantly lower with lower wine colour and wine phenolics. Further to this, Walker et al. (1998) demonstrated that lower wine colour from vines grafted to Ramsey were due to higher potassium concentration, which was shown to be positively correlated with wine pH and negatively correlated with wine TA. Although we did not measure potassium concentration in the berries, we did not observe a low juice pH nor high wine pH which would indicate the effects of high potassium for Ramsey rootstocks which has previously been

reported (Ewart, et al. 1993). However, we believe moderate to high yields and vigour associated with both Ramsey and own roots Shiraz over the two years and the associated low wine TA may infer an increase in potassium values and resultant low wine colour and quality. It is clear a greater understanding of the relationship between potassium and wine colour is required.

Very few compositional differences were observed between irrigation treatments, this is likely due to the fact that irrigation of control plants was intended to mimic high quality wine production in the Barossa and therefore subject to a degree of mild water deficit (Kidman et al., 2014). Higher wine alcohol concentration in irrigated treatments was found in both years of analysis while grape TSS was only significantly higher in irrigated treatments in 2009, not 2010 (Table 3 and Table 4). Previous studies have reported a 30% increase in TSS as a consequence of water deficit (Petrie, et al. 2004) whilst other studies report the opposite (Ojeda, et al. 2002). These conflicting responses to water deficit for TSS highlight the importance of the timing (Matthews and Anderson 1988, Matthews, et al. 1990) and severity of water deficit as to whether TSS is affected or not. It is curious as to why differences were only observed in 2009 as in both seasons unirrigated treatments suffered yield decline of 22% and 23% respectively (Kidman, et al. 2014). In studies by Van Leeuwin et al. (2007), low yields under moderate water deficit enhanced berry TSS and anthocyanin and phenolic concentration. However, high yield and severe water deficit depressed TSS, and resulted in early berry shrivel and incomplete ripening as a result of limited photosynthesis and carbon assimilation (Freeman, et al. 1980, van Leeuwin, et al. 2007). In 2009, water stress of both irrigated and non irrigated treatments was more severe than in 2010 (Kidman, et al. 2014) and this may have contributed to the differences in accumulation of sugar. Further to this, vegetative growth as measured by pruning weight showed a reduction of 18% for unirrigated treatments in 2009 and 3% in 2010 (Kidman, et al. 2014), which may have contributed to the different response between years in the ability of the unirrigated vines to ripen the crop.

Wine phenolics tended to be higher for non irrigated treatments in both seasons. Wine colour density was observed to be higher in non irrigated treatments than irrigated treatments in the 2010 season. In contrast, wine colour hue was lower in non irrigated than irrigated treatments for the 2010 season (Table 4). In some instances, a water deficit that reduces berry weight will increase skin to pulp ratio (Ojeda, et al. 2002) and skin to flesh weight ratio (Bindon, et al. 2011), which may increase wine anthocyanin and wine phenolic concentrations.

No effect of irrigation on wine quality score was observed for either the 2009 or 2010 vintage. Notwithstanding, differences in wine sensory attributes of Cabernet Franc subject to changes in vine water status have previously been reported (Matthews, et al. 1990), with visual differences able to be more readily detected than flavour through the response of anthocyanin synthesis to water deficit. Furthermore, (Matthews, et al. 1990) found that changes to aroma were more readily detected than taste, as volatile constituents were observed to change under water deficit more readily than soluble constituents. The opposite was found in this study as more attributes describing the taste of the wines than aroma were used by winemakers to describe each of the experimental wines as seen in Table 2.

3.3. Sensory attribute assessments and principal component analysis

Ten of 27 attributes used by winemakers to describe wines tended to be affected by rootstock and/or irrigation treatments in 2009 and 10 of 25 attributes in 2010. The proportion of judges that identified the same attribute in a given wine from each treatment is shown in Tables 5 and 6 for 2009 and 2010, respectively.

Principal component analysis was used to analyse those sensory attributes that tended to differ between treatments. These findings have been presented as a biplot to visually represent findings. The first and second principal component (PC) accounted for 49.43% and 21.06% of the variance in the PCA of the data for the 14 treatments, respectively in 2009 (Fig. 3). As illustrated in Fig. 3, PC 1

separated wines based on the attributes; rich, elegant, bitter, vibrant, simple, red fruit and light bodied. Own rooted Shiraz with and without irrigation, 99 Richter with and without irrigation and 140 Ruggeri with irrigation were described more often by winemakers as simple, light bodied and bitter. Irrigated 99 Richter was found to have greater red fruit characters by a greater proportion of winemakers. Wines made from Shiraz grafted to 1103 Paulsen with and without irrigation were described as being richer and more elegant. 110 Richter with and without irrigation and irrigated Schwarzmann were found to be more vibrant than other treatments. The second PC separated irrigated Schwarzmann, 140 Ruggeri and 1103 Paulsen wines from other treatments as a greater proportion of winemakers described these wines as having black fruit, crimson and good length attributes.

The first and second principal component (PC) accounted for 52.70% and 21.71% of the variance in the PCA of the data for the 14 treatments, respectively in 2010 (Fig. 4). As seen in Fig. 4, PC 1 separated wines based on red fruit, confectionary, and elegant attributes. Wines made from Shiraz with and without irrigation were described more often as elegant, confectionary and red fruit. The opposite was found for Schwarzmann and 110 Richter with and without irrigation which were more often described as astringent with greater black fruit characters. 1103 Paulsen with and without irrigation was described as being rich with good length and high bitterness by more winemakers. The second principal component separated wines based on the attributes simple and light bodied compared to rich and astringent. Ramsey and 99 Richter with and without irrigation were again described as being simpler and lighter bodied than other treatments and these attributes were associated with lower wine quality in 2010.

In both seasons, wines described as being rich and having black fruit had higher levels of wine phenolics and alcohol. In 2009, wine colour density and pH correlated with black fruit and rich attributes. Wine colour hue correlated with red fruit.

Water deficits have been shown to not only increase intensity of wine colour, but also increased aromas of blackberry, strawberry, raspberry and blackcurrant have been reported (Myburgh 2011). A

greater proportion of winemakers described wines made from unirrigated 140 Ruggeri, 99 Richter and Ramsey as having black fruit characters.

4. Conclusions

The effect on grape and wine composition and sensory attributes of wine made from 2008/2009 and 2009/2010 seasons identified some effects of irrigation and rootstock on grape and wine composition and sensory assessment in both years of analysis, namely differences in TSS, pH, TA, colour and phenolic levels. However, on the whole, rootstock type more than irrigation affected wine composition and sensory analysis. It is likely this result is due to the irrigated vines also being under a degree of mild water deficit in both seasons. A novel sensory analysis technique, using sensory attribute word frequency data, enabled discrimination of attributes between the treatments across the two years of the analysis. This technique identified a number of word attributes associated with either rootstock or irrigation treatment or both. In 2010 wine quality differed significantly between the treatments. Word frequency data associated with the lower quality wines were associated with the attributes of “light” and “simple” further to this these wines were significantly lower in TSS than other treatments and this may have contributed to the lower wine quality score. These findings have implications for rootstock selection management decisions and how we assess research wines for commercial application.

There is potential to use expert winemaker panels to generate preliminary data on research trials using word frequency data before running a descriptive analysis panel. This has potential to save time and money.

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Table 1
List of sensory attributes and synonyms used by winemakers to describe wines made from a rootstock x irrigation trial in the Barossa Valley, Australia.

Sensory attribute	Synonyms
acidic	high acidity, high acid, sour, sharp, crisp, aggressive acid
alcoholic	alcohol warm, high alcohol, hot,
aromatic	fragrant, scented, pungent
astringent	high astringency, dry, drying
balanced	good weight, even
bitter	highly bitter, high bitterness, bitter characters
black fruit	dark fruit, blackberry, black cherry, black currant, blueberry
chalky	powdery, chalky tannins, dusty tannins
complex	complexity
confectionary	lollies, candied, confected, boiled lollies
cooked fruit	jammy, over ripe, cooked, stewed cherry,
crimson	deep pink
earthy	dirty, dusty, forest floor
elegant	delicate, attractive
floral	perfumed, violet, rose
full bodied	full,
good length	long finish, lingering
green	grassy, unripe, herbal, herbacious, vegetal, mint, menthol, eucalyptus, green edge
light bodied	light, lean, thin, light red, light depth, lighter colour depth
medium bodied	medium weight, medium red, medium body, medium deep colour
pepper	peppery, white pepper, black pepper
red fruit	raspberry, red plum, plum, red cherry, red berries, red currant, red fruit aromas, red berry fruit
rich	concentrated, deep, intense
ripe	ripe fruit, riper
savoury	salty, savoury flavours,
simple	plain, austere, lacking, dull
soft	soft tannins,
spicy	intense spice, spice, cloves, christmas spice, cinnamon, chocolate, coffee, anise
sweet	sweet fruit, fruit sweetness,
unbalanced	poor structure, flabby
varietal	
vibrant	bright, brilliant, exciting, fresh, lively, lifted, lifted fruit

Table 2
List of sensory attributes generated by expert winemakers to describe wines from rootstock and irrigation trial, Barossa Valley, Australia in 2009 and 2010.

Vintage	
2009	2010
acidic (T)	acidic (T)
alcoholic (T)	astringent (T)
aromatic (A)	balanced (T)
astringent (T)	bitter (T)
bitter (T)	black fruit (T)
black fruit (T)	complex (T)
chalky (T)	confectionary (A)
confectionary (A)	earthy (A)
cooked fruit (T)	elegant (T)
crimson (V)	floral (A)
elegant (T)	full bodied (T)
floral (A)	good length (T)
full bodied (T)	green (T)
good length (T)	light bodied (T)
light bodied (T)	medium bodied (T)
medium bodied (T)	pepper (A)
pepper (A)	red fruit (T)
red fruit (T)	rich (T)
rich (T)	ripe (T)
ripe (T)	savoury (T)
savoury (T)	simple (T)
simple (T)	soft (T)
spicy (A)	spicy (A)
sweet (T)	sweet (T)
unbalanced (T)	vibrant (T)
varietal (T)	
vibrant (T)	

Sensory attributes used by expert winemakers have been labelled as either being visual (V), aroma (A) or taste (T).

Table 3

Grape and wine composition measures and wine quality scores for Shiraz rootstock and irrigation treatments, Barossa Valley, Australia, 2009 vintage.

Variable 2009	Treatment	Treatment: Own roots Shiraz	110 Richter	1103 Paulsen	140 Ruggeri	99 Richter	Ramsey	Schwarz-mann	Irrigation mean	P value	LSD (5%)
Grape pH	Irrigated	3.49	3.68	3.88	3.79	3.85	3.8	3.78	3.75	<.001(R)	0.091
	Non irrigated	3.50	3.66	3.79	3.84	3.89	3.73	3.69	3.73	0.247(I)	
	Rootstock	3.49a	3.67b	3.84cd	3.81cd	3.87d	3.77c	3.74bc		0.433(R*I)	
Grape TA (g/L)	Irrigated	5.23	4.72	4.32	4.21	4.39	4.45	3.85	4.45	0.014(R)	0.617
	Non irrigated	5.27	4.85	4.35	4.22	4.13	4.72	4.18	4.53	0.529(I)	
	Rootstock	5.25c	4.78bc	4.33ab	4.21ab	4.26ab	4.59ab	4.02a		0.885(R*I)	
TSS (°Brix)	Irrigated	20.8	23.6	24.7	23.2	23.1	23.5	23.7	23.2a	<.001(R)	1.44
	Non irrigated	17.7	22.5	22.2	22.7	22.8	23.1	22.9	22.0b	0.004(I)	0.75
	Rootstock	19.2a	23.1b	23.4b	22.9b	23.0b	23.3b	23.4b		0.251(R*I)	
Grape colour mg/g berry	Irrigated	0.20	0.17	0.20	0.18	0.20	0.19	0.22	0.19	0.048(R)	0.038
	Non irrigated	0.21	0.16	0.20	0.23	0.19	0.19	0.25	0.20	0.415(I)	
	Rootstock	0.21bc	0.16a	0.20b	0.20b	0.19ab	0.19ab	0.24c		0.673(R*I)	
Grape phenolics (au/g)	Irrigated	0.61	0.59	0.68	0.66	0.72	0.63	0.72	0.66	0.013(R)	0.12
	Non irrigated	0.69	0.56	0.76	0.94	0.77	0.67	0.86	0.75	0.004(I)	0.057
	Rootstock	0.65ab	0.57a	0.72bc	0.80c	0.75bc	0.65ab	0.79c		0.146(R*I)	
Alcohol %	Irrigated	12.8cdef	14.4h	13.7g	12.8cdef	12.9def	12.2abc	13.2efg	13.2	0.003(R)	0.630
	Non irrigated	11.9ab	12.6cde	13def	12.7cdef	11.7a	12.4bcd	13.3fg	12.5	<.001 (I)	0.175
	Rootstock	12.3	13.5	13.3	12.8	12.3	12.3	13.2		<.001 (R*I)	0.686
Wine pH	Irrigated	3.38a	3.56e	3.66f	3.52cde	3.55de	3.44abc	3.44abc	3.51	<.001(R)	0.091
	Non irrigated	3.41ab	3.42ab	3.51cde	3.47bcd	3.57e	3.52cde	3.42ab	3.47	ns(I)	
	Rootstock	3.40	3.44	3.58	3.59	3.56	3.48	3.43		0.030(R*I)	0.088
Wine TA (g/L)	Irrigated	7.19	7.12	6.84	6.85	7.13	6.76	6.67	6.94	0.010 (R)	0.225
	Non irrigated	6.58	7.26	6.92	7.24	6.92	6.64	7.00	6.94	0.988(I)	
	Rootstock	6.88ab	7.19c	6.88ab	7.05bc	7.02bc	6.70 a	6.84 ab		0.148(R*I)	
Wine colour density mg/g berry	Irrigated	7.3a	13. 3d	10.9c	8.7ab	8.8ab	8.9ab	9.0abc	9.6	0.002(R)	1.38
	Non irrigated	7.3a	9.0abc	10bc	9.7bc	10.3bc	10.2bc	10.0bc	9.5	0.817(I)	
	Rootstock	7.3	11.2	10.5	9.2	9.6	9.6	9.5		0.019(R*I)	1.98
Wine colour hue (au/g)	Irrigated	0.87de	0.78abc	0.77ab	0.89de	0.90e	0.85cde	0.82abcd	0.84	0.011(R)	0.049
	Non irrigated	0.85cde	0.86de	0.88de	0.91e	0.84bcde	0.75a	0.87de	0.85	0.536(I)	
	Rootstock	0.86	0.82	0.82	0.90	0.87	0.80	0.85		0.035(R*I)	0.074
Wine Phenolics (au/g)	Irrigated	27.8abc	44.8h	33.4ef	32cdef	32.5def	29.9abcde	27.2ab	32.5	<.001R)	4.21
	Non irrigated	25.5a	30.4bcde	39.5g	38.4g	36.3fg	28.2abcd	38.4g	33.8	0.049(I)	1.28
	Rootstock	26.7	37.6	36.5	35.2	34.4	29.0	32.8		<.001(R*I)	4.65
Wine quality score	Irrigated	13.1	13.4	13.3	14.0	13.7	13.0	13.4	13.4	0.834(R)	
	Non irrigated	13.4	13.4	13.2	12.8	13.4	13.2	12.7	13.2	0.176(I)	
	Rootstock	13.3	13.4	13.2	13.4	13.5	13.1	13.0		0.139(R*I)	

The P value and least significant difference (LSD, P=.005) for rootstock (R), irrigation (I) and rootstock x irrigation interaction (RxI) comparisons are shown for the 2009 season.

Numbers within rows followed by the same letter are not significantly different from each other. For all rootstock and irrigation treatment each value represents the mean of twenty-one replicate samples for each rootstock and irrigation combination.

Table 4

Grape and wine composition measures and wine quality scores for Shiraz rootstock and irrigation treatments, Barossa Valley, Australia, 2010 vintage.

Variable 2010	Treatment	Treatment: Own roots Shiraz	110 Richter	1103 Paulsen	140 Ruggeri	99 Richter	Ramsey	Schwarz-mann	Irrigation mean	P value	LSD (5%)
Grape pH	Irrigated	3.93	3.90	3.98	3.87	3.97	3.93	3.93	3.93	0.125(R)	
	Non irrigated	3.82	3.94	3.90	4.03	3.92	3.79	3.75	3.88	0.097(I)	
	Rootstock	3.87	3.92	3.93	3.95	3.94	3.86	3.84		0.059(R*I)	
Grape TA (g/L)	Irrigated	4.53^{def}	4.32^{cde}	4.32^{cde}	4.98^{f}	3.73^{ab}	3.90^{abc}	5.01^{f}	4.40	0.003(R)	0.432
	Non irrigated	4.22^{bcde}	4.69^{ef}	4.04^{abcd}	3.93^{abc}	3.58^{a}	4.54^{def}	4.68^{ef}	4.24	0.125(I)	
	Rootstock	4.37	4.5	4.18	4.46	3.65	4.22	4.85		0.010(R*I)	0.550
TSS (°Brix)	Irrigated	24.6	25.9	25.5	25.2	23.3	22.9	25.2	24.7	<.001(R)	0.875
	Non irrigated	24.7	25.2	25.8	26.1	23.4	22.8	25.4	24.8	0.747(I)	
	Rootstock	24.6^{b}	25.6^{c}	25.7^{c}	25.7^{c}	23.4^{a}	22.8^{a}	25.3^{bc}		0.757(R*I)	
Grape colour mg/g berry	Irrigated	0.43	0.42	0.51	0.42	0.34	0.35	0.40	0.41	0.328(R)	
	Non irrigated	0.43	0.46	0.40	0.48	0.42	0.35	0.43	0.43	0.440(I)	
	Rootstock	0.43	0.43	0.44	0.46	0.45	0.38	0.35		0.177(R*I)	
Grape phenolics (au/g)	Irrigated	0.70	0.71	0.85	0.64	0.59	0.78	0.76	0.72	0.636(R)	
	Non irrigated	0.76	0.81	0.77	0.82	0.73	0.78	0.88	0.79	0.073(I)	
	Rootstock	0.73	0.76	0.81	0.73	0.66	0.78	0.82		0.603(R*I)	
Alcohol %	Irrigated	14^{ef}	14.5^{h}	13.5^{c}	14.1^{fg}	13.3^{c}	12.2^{a}	14.3^{gh}	13.7	<.001(R)	0.207
	Non irrigated	14.1^{fg}	14.3^{gh}	13.6^{cd}	14.2^{fg}	12.7^{b}	12.1^{a}	13.8^{de}	13.5	<.001 (I)	0.076
	Rootstock	14.1	14.4	13.5	14.2	13	12.1	14		0.002(R*I)	0.240
Wine pH	Irrigated	3.55	3.54	3.65	3.59	3.59	3.50	3.58	3.57	0.011(R)	0.074
	Non irrigated	3.43	3.59	3.65	3.60	3.57	3.47	3.61	3.58	0.780(I)	
	Rootstock	3.54^{ab}	3.57^{b}	3.65^{c}	3.60^{bc}	3.58^{bc}	3.48^{a}	3.59^{bc}		0.310(R*I)	
Wine TA (g/L)	Irrigated	6.43	6.81	6.33	6.98	6.46	6.17	6.99	6.60	0.014 (R)	0.406
	Non irrigated	6.74	6.99	6.22	6.84	6.46	6.41	6.65	6.62	0.779(I)	
	Rootstock	6.59^{abc}	6.90^{c}	6.28^{a}	6.91^{c}	6.46^{ab}	6.29^{a}	6.82^{bc}		0.205(R*I)	
Wine colour hue (au/g)	Irrigated	0.90	0.91	0.93	0.91	0.89	0.84	0.89	0.90^{a}	0.006(R)	0.048
	Non irrigated	0.90	0.89	0.86	0.81	0.92	0.77	0.90	0.87^{b}	0.037(I)	0.027
	Rootstock	0.90^{b}	0.90^{b}	0.90^{b}	0.86^{b}	0.91^{b}	0.81^{a}	0.90^{b}		0.131(R*I)	
Wine colour density (au/g)	Irrigated	9.06	12.5	10.8	11.1	9.7	8.0	11.4	10.4	<.001(R)	0.769
	Non irrigated	9.70	12.8	12.7	13.7	11.1	8.5	12.6	11.6	<.001(I)	0.467
	Rootstock	9.40^{b}	12.7^{e}	12.7^{e}	12.4^{de}	10.4^{c}	8.2^{a}	11.9^{d}		0.145(R*I)	
Wine Phenolics (au/g)	Irrigated	34.3^{a}	44.6^{de}	39.3^{bc}	42.3^{cd}	38.1^{b}	33.6^{a}	44.6^{de}	39.5	<.001(R(	2.39
	Non irrigated	39.4^{bc}	46.3^{ef}	49.4^{f}	47.1^{ef}	44.7^{de}	36.3^{ab}	45.1^{d}	44.1	<.001(I)	1.27
	Rootstock	36.8	45.5	44.4	44.7	41.4	35.0	44.8		0.019(R*I)	3.16
Wine quality score	Irrigated	15.0	15.4	15.7	15.0	14.6	14.6	15.2	15.1	0.007	0.56
	Non irrigated	15.3	15.4	15.2	15.7	14.6	14.2	15.5	15.1	0.658(I)	
	Rootstock	15.4^{b}	15.5^{b}	15.4^{b}	15.4^{b}	14.4^{a}	14.4^{a}	15.2^{b}		0.194(R*I)	

The P value and least significant difference (LSD, P=.005) for rootstock (R), irrigation (I) and rootstock x irrigation interaction (RxI) comparisons are shown for the 2010 season.

Numbers within rows followed by the same letter are not significantly different from each other. For all rootstock and irrigation treatment each value represents the mean of twenty-one replicate samples for each rootstock and irrigation combination.

Table 5
Word frequency scores for significantly different wine attributes from rootstock and irrigation trial, Barossa Valley, Australia, 2009 vintage.

Sensory attribute 2009	Treatment	Own roots Shiraz	1103 Paulsen	110 Richter	140 Ruggeri	99 Richter	Ramsey	Schwarz mann	Irrigation Mean	P value	LSD
	Irrigated	0.02#	0.60	0.73	0.02	0.08	0.35	0.13	0.32	0.002 (R)	0.18
astringent	Non irrigated	0.10	0.58	0.77	0.08	0.10	0.08	0.19	0.27	ns (I)	
	Rootstock	0.10 a	0.59 b	0.75 b	0.05 a	0.09 a	0.22 a	0.16 a		ns (RxI)	
	Irrigated	0.02	0.48	0.06	0.02	0.02	0.04	0.06	0.10	<0.0001	0.05
bitter	Non irrigated	0.02	0.56	0.06	0.06	0.02	0.00	0.08	0.11	ns (I)	
	Rootstock	0.02 a	0.52 b	0.06 a	0.04 a	0.02 a	0.02 a	0.07 a		ns (RxI)	
	Irrigated	0.23	0.48	0.98	0.48	0.29	0.44	0.73	0.52	0.002 (R)	0.22
black fruit	Non irrigated	0.21	0.65	0.90	0.85	0.27	0.38	0.69	0.56	ns (I)	
	Rootstock	0.22 a	0.57 b	0.94 c	0.67	0.28	0.41	0.71		ns (RxI)	
	Irrigated	0.08	0.13	0.13	0.13	0.04	0.00	0.04	0.08 a	0.044 (R)	0.12
confectionary	Non irrigated	0.98	0.06	0.00	0.06	0.35	0.02	0.00	0.21 b	0.046 (I)	0.06
	Rootstock	0.53 b	0.10 a	0.07 a	0.10 a	0.20 a	0.01 a	0.02 a		ns (RxI)	
	Irrigated	0.08	0.52	0.06	0.06	0.08	0.71	0.10	0.23	0.039 (R)	0.23
elegant	Non irrigated	0.06	0.54	0.00	0.00	0.06	0.79	0.08	0.22	ns (I)	
	Rootstock	0.07 a	0.53 b	0.03 a	0.03 a	0.07 a	0.75 b	0.09 a		ns (RxI)	
	Irrigated	0.67	0.79	0.79	0.19	0.06	0.06	0.15	0.39	<0.0001 (R)	0.17
good length	Non irrigated	0.63	0.83	0.13	0.69	0.19	0.13	0.15	0.39	ns (I)	
	Rootstock	0.65 c	0.81 c	0.46 b	0.44 b	0.13 a	0.10 a	0.15 a		ns (RxI)	
	Irrigated	0.25	0.10	0.10	0.19	0.83	0.75	0.15	0.34 a	0.002 (R)	0.18
light bodied	Non irrigated	0.13	0.15	0.04	0.02	0.71	0.50	0.08	0.23 b	0.048 (I)	0.08
	Rootstock	0.19 a	0.13 a	0.07 a	0.11 a	0.77 b	0.63 b	0.12 a		ns (RxI)	
	Irrigated	0.69	0.63	0.25	0.65	0.48	0.52	0.17	0.48	0.002 (R)	0.22
red fruit	Non irrigated	0.63	0.52	0.15	0.52	0.40	0.54	0.25	0.43	ns (I)	
	Rootstock	0.66 b	0.58 b	0.20 a	0.59 b	0.44 b	0.53 b	0.21 a		ns (RxI)	
	Irrigated	0.15 a	0.77 b	0.85 b	0.15 b	0.00 a	0.04 a	0.19	0.31 a	<0.0001 (R)	0.15
rich	Non irrigated	0.67 b	0.90 b	0.67 b	0.92 b	0.00 a	0.04 a	0.17	0.48 b	0.043 (I)	0.13
	Rootstock	0.41	0.84	0.76	0.54	0.00	0.04	0.18		0.046 (RxI)	0.25
	Irrigated	0.19	0.13	0.04	0.17	0.54	0.77	0.15	0.28	0.021 (R)	0.12
simple	Non irrigated	0.17	0.04	0.10	0.06	0.79	0.77	0.15	0.30	ns (I)	
	Rootstock	0.18 a	0.09 a	0.07 a	0.12 a	0.67 b	0.77 b	0.15 a		ns (RxI)	

#Word frequency score expressed as a proportion of judges (mean of 3 wine replicates) who perceived this attribute in the final wine (n=8 judges x 3 wine replicates x 7 treatments).

Table 6
Word frequency scores for significantly different wine attributes from rootstock and irrigation trial, Barossa Valley, Australia, 2010 vintage.

Sensory attribute 2010	Treatment	Own roots Shiraz	1103 Paulsen	110 Richter	140 Ruggeri	99 Richter	Ramsey	Schwarzmann	Irrigation Mean	P value	LSD
bitter	Irrigated	0.38	0.08	0.50	0.33	0.17	0.38	0.67	0.36	0.002 (R)	0.21
	Non irrigated	0.63	0.04	0.54	0.04	0.17	0.13	0.08	0.23	ns (I)	
	Rootstock	0.50 c	0.06 a	0.52 c	0.19 ab	0.17 ab	0.25 ab	0.37 bc		ns (RxI)	
black fruit	Irrigated	0.29	0.58	0.67	0.17	0.38	0.25	0.71	0.43	<0.0001 (R)	0.15
	Non irrigated	0.25	0.63	0.58	0.50	0.50	0.38	0.79	0.52	ns (I)	
	Rootstock	0.27 a	0.61 b	0.62 b	0.33 a	0.44 a	0.32 a	0.75 b		ns (RxI)	
crimson	Irrigated	0.17 a	0.71 bc	0.96 c	0.17 a	0.96 c	0.25 a	0.75 bc	0.57	0.002 (R)	0.27
	Non irrigated	0.21 a	0.75 bc	1.00 c	0.83 bc	0.29 a	0.38 ab	0.67 b	0.59	ns (I)	
	Rootstock	0.19	0.73	0.98	0.50	0.62	0.32	0.71		0.035 (RxI)	0.22
elegant	Irrigated	0.04	0.63	0.17	0.21	0.13	0.79	0.13	0.30	0.044 (R)	0.23
	Non irrigated	0.17	0.54	0.13	0.25	0.21	0.96	0.13	0.34	ns (I)	
	Rootstock	0.11 a	0.58 b	0.15 a	0.23 a	0.17 a	0.88 c	0.13 a		ns (RxI)	
good length	Irrigated	0.75	0.96	0.67	0.50	0.38	0.17	0.75	0.60	0.039 (R)	0.27
	Non irrigated	0.96	1.00	0.58	1.00	0.29	0.17	1.00	0.71	ns (I)	
	Rootstock	0.86 c	0.98 c	0.62 b	0.75	0.33 a	0.17 a	0.88 c		ns (RxI)	
light bodied	Irrigated	0.46	0.17	0.13	0.17	0.79	0.75	0.29	0.39	<0.0001 (R)	0.15
	Non irrigated	0.88	0.17	0.13	0.17	0.71	0.63	0.08	0.40	ns (I)	
	Rootstock	0.67 b	0.17 a	0.13 a	0.17 a	0.75 b	0.69 b	0.19 a		ns (RxI)	
red fruit	Irrigated	0.58	0.42	0.75	0.88	0.96	0.46	0.75	0.68	0.042 (R)	0.21
	Non irrigated	0.79	0.54	0.67	0.67	0.50	0.42	0.92	0.64	ns (I)	
	Rootstock	0.69 b	0.48 a	0.71 b	0.77 b	0.73 b	0.44 a	0.84 b		ns (RxI)	
rich	Irrigated	0.25	0.92	0.13	0.63	0.13	0.25	0.25	0.36	0.002	0.23
	Non irrigated	0.75	0.63	0.42	0.50	0.08	0.17	0.29	0.41	ns (I)	
	Rootstock	0.50 b	0.77 c	0.27 a	0.56 bc	0.10 a	0.21 a	0.27 ab		ns (RxI)	
simple	Irrigated	0.67	0.13	0.46	0.63	0.58	0.63	0.25	0.48	<0.0001 (R)	0.18
	Non irrigated	0.79	0.33	0.42	0.25	0.67	0.79	0.25	0.50	ns (I)	
	Rootstock	0.73 c	0.23 a	0.44 b	0.44 b	0.63 c	0.71 c	0.25 a		ns (RxI)	
vibrant	Irrigated	0.42	0.13	0.54	0.54	0.67	0.17	0.29	0.39	0.021	0.13
	Non irrigated	0.33	0.75	0.42	0.79	0.29	0.08	0.54	0.46	ns (I)	
	Rootstock	0.37 b	0.44 b	0.48 b	0.67 b	0.48 b	0.12 a	0.42 b		ns (RxI)	

#Word frequency score expressed as a proportion of judges (mean of 3 wine replicates) who perceived this attribute in the final wine (n=16 judges x 3 wine replicates x 7 treatments).

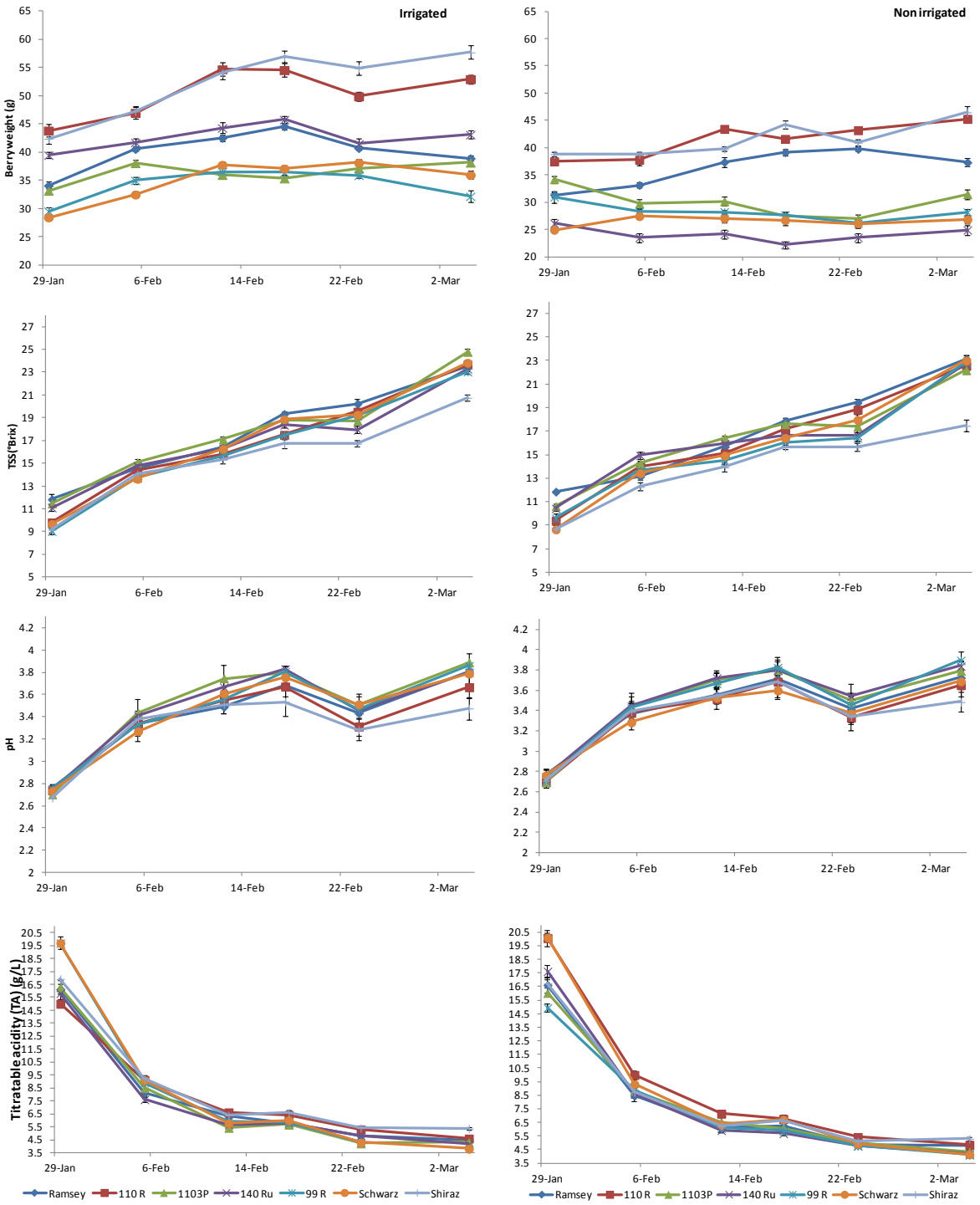

Fig. 1. Effect of rootstock and irrigation on berry weight, TSS, pH and Titratable acidity (TA) in developing Shiraz fruit from the Barossa Valley, Australia, 2009 vintage. Error bars represent the least significant difference (LSD, P=.005).

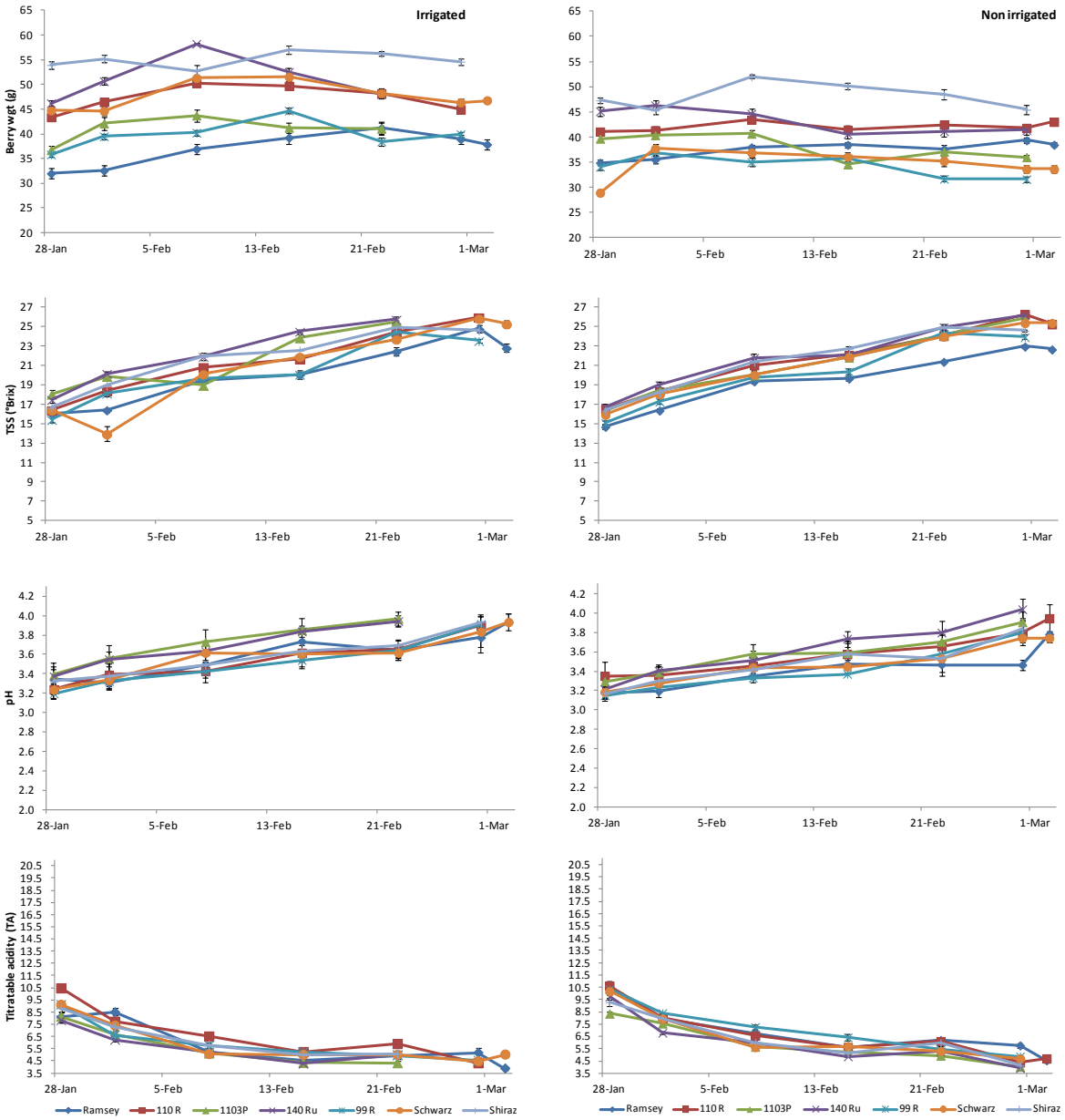

Fig. 2. Effect of rootstock and irrigation on berry weight, TSS, pH and Titratable acidity (TA) in developing Shiraz fruit from the Barossa Valley, Australia, 2010 vintage. Error bars represent the least significant difference (LSD, P=.005).

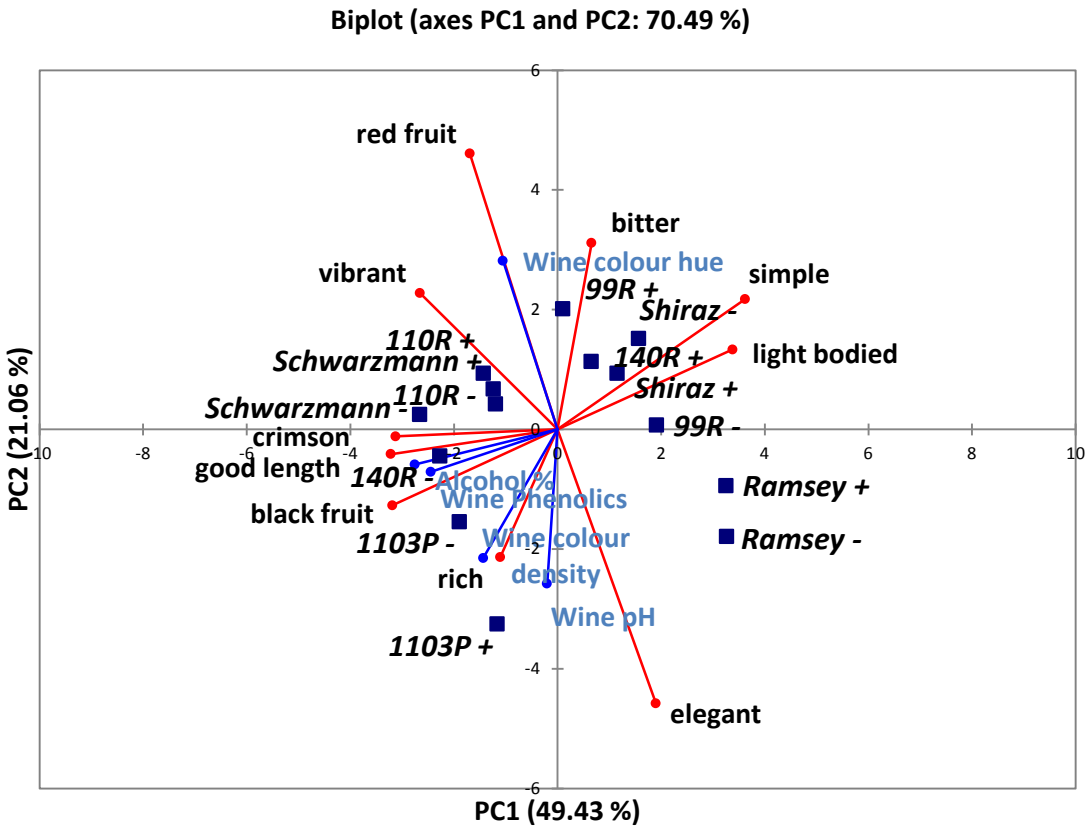

Fig. 3. Principal component analysis of the mean proportions of significantly different sensory attributes for Shiraz wines made from different rootstocks and irrigation treatments grown in the Barossa Valley, Australia in 2009.

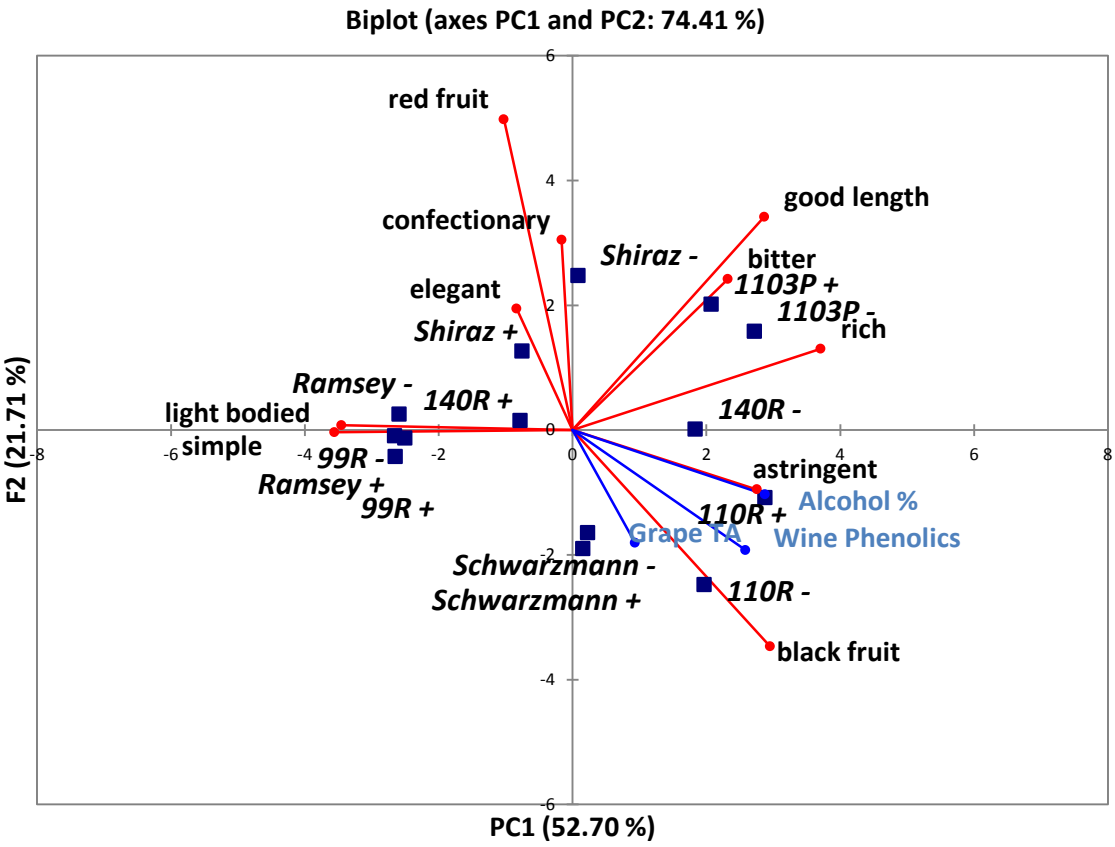

Fig. 4. Principal component analysis of the mean proportions of significantly different sensory attributes for Shiraz wines made from different rootstocks and irrigation treatments grown in the Barossa Valley, Australia in 2010.

Chapter 8. General Discussion

This study has provided new knowledge on the effect of American *Vitis* rootstocks on the reproductive performance of *V. vinifera* scion cultivars; Cabernet Sauvignon, Merlot and Shiraz. Cabernet Sauvignon and Merlot are two cultivars commonly regarded as being susceptible to poor fruitset due to a high incidence of both millerandage and coulure (May 2004, Dry et al. 2010). In contrast, Shiraz is regarded as having good fruitset due to moderate incidence of coulure and low incidence of millerandage (Dry et al. 2010). Nonetheless, the importance of Shiraz as the most planted cultivar in Australia, representing 46% of all vineyard area planted to red wine grapes warrants the investigation of the effects of rootstocks on this cultivar (www.abs.gov.au/ausstats/abs@.nsf/DetailsPage/mf/1329.0.55.002/).

Investigation of the reproductive performance of Cabernet Sauvignon, Merlot and Shiraz grafted to American *V.* rootstocks provides more detailed knowledge as to why fruit yields differ within a cultivar grown on different rootstocks and how this compares to the same cultivar grown on its own roots. A greater understanding of how rootstocks impact reproductive performance will help the Australian and international wine industries make decisions on rootstock selection, especially for sites and cultivars where fruit set could be limited.

Reproductive performance is most commonly quantified by fruitset (%), calculated as total berry number per bunch from the number of flowers on an inflorescence. More recently, it has been further quantified by determination of the incidence of coulure and millerandage (Collins and Dry 2009). An increase in the incidence of coulure results in a high proportion of flowers on an inflorescence that fail to develop into a berry, often through excessive shedding of ovaries or young berries. On the other hand, millerandage occurs when a high proportion of flowers develop abnormally into either seedless berries or live green ovaries (LGOs) (May 2004, Collins and Dry 2009). For this study, because of the perennial nature of the grapevine, reproductive performance encompassed bunch initiation in the previous season, inflorescence development, flowering and fruitset and the development of seeds and

flesh within a grape berry in the current season (Pratt 1971, Srinivasan and Mullins 1981, May 2004, Vasconcelos, et al. 2009).

Rootstock influence on reproductive performance differed between the three scion cultivars. Merlot grafted to rootstocks had significantly higher fruitset and bunch parameters than own roots. Fruitset was 41% to 75% higher for Merlot grafted to rootstocks compared to own roots and this correlated with significantly higher seeded berry number per bunch. No significant change to fruitset for rootstocks was observed for Cabernet Sauvignon. The influence of rootstock on reproductive performance and yield in Cabernet Sauvignon was mainly due to an effect on fruitfulness and bunch number (see chapter 3). For Shiraz, rootstocks caused a higher incidence of coulure and a lower fruitset than own roots. Further to this, a regression analysis identified an inverse relationship between fruitset and coulure for Shiraz as one of the main influences of rootstocks on reproductive performance in Shiraz (R^2 0.936, chapter 4). Indeed, some varieties, for example Shiraz, are more susceptible to coulure than millerandage (Dry et al. 2010). The incidence of coulure is influenced by climate but can also result from deficiencies in the concentrations of carbohydrates (Lebon, et al. 2008). When coulure was high in Shiraz, pruning weights were low. The exception to this occurred for the wet 2010/2011 season when the incidence of coulure was high irrespective of vegetative growth, and this was particularly evident for rootstocks 140 Ruggeri and Ramsey. Conversely, when coulure was low, for example in own roots, pruning weights were higher than Ramsey, which may infer that for own roots, vegetative growth was not limiting for the production of carbohydrates for reproduction, while the converse was true for Ramsey. This is further substantiated by higher root and trunk carbohydrate concentrations for own roots than Ramsey (Chapter 6). In an attempt to identify the effect of carbohydrate concentration on reproduction, an analysis of the role of carbohydrates was performed for Shiraz. As previously mentioned, a reduction in carbohydrate has been shown to lead to problems with reproduction (Lebon, et al. 2008). Analysis of inflorescence development, as measured through

fruitfulness and abnormal conditions affecting fruitfulness such as primary bud necrosis (PBN), was assessed with respect to carbohydrate concentration (see chapter 6).

No relationship between the incidence of PBN and pruning weight was observed even though there were significant differences among treatments in other parameters measured. As such, the fundamental causes of PBN are still largely unknown, and require further investigation. Although it is well known that an increase in vigour associated with high shoot growth and shoot weight can influence PBN (Lavee, et al. 1981, Dry and Coombe 1994), vines in this study were not considered overly vigorous. In addition, a relationship between carbohydrate concentration and incidence of PBN was inconclusive, although carbohydrate supply differed between the treatments. Therefore, to confirm a relationship between PBN and bud fertility, further studies are required. Nonetheless, as a consequence of these findings, it is important for Shiraz wine grape growers using rootstocks, to maintain ample vegetative growth for the production of storage carbohydrates, but not to the extent that vegetative growth causes excessive shading of the renewal zone. Investigations into the appropriate management of vigor are needed to ensure this would be beneficial in Shiraz. In addition, these findings suggest potential fruitfulness measured at dormancy, is a good indicator of potential inflorescence number in the following spring, while actual fruitfulness can be indicative of yield.

The number of pollen grains present on the stigma was shown to be important for successful fertilisation. Shiraz grafted to rootstocks 99 Richter and Schwarzmann were found to have more pollen grains on the stigma than the other rootstocks which indicates potential genetic differences between rootstock hybrids (see chapter 5). Higher pollen grains on the stigma were associated with higher percentage fruitset and seeded berry number in these rootstocks and lower coulure and millerandage. In contrast, 140 Ruggeri had the lowest pollen grain number and a lower fruitset and seeded berry number. The data suggest that the number of pollen grains and pollen viability is indicative of the potential success of fertilisation. This is likely due to the fact that an increase in the number of pollen grains on the stigma increased the likelihood of germination through an increase in the percentage of

viable pollen for the receptive stigma, increasing the chances of germination. Further to this, research into the requirement of nutrients calcium, boron, zinc and molybdenum for fertilisation was assessed. The study correlated low seeded berry numbers and berry weights with low petiole calcium concentrations for the rootstocks 1103 Paulsen and 140 Ruggeri. Own roots Shiraz had an inherently higher calcium concentration in the petioles across the three seasons and was correlated with higher seeded berry number, total number of berries per bunch and berry weight. Low molybdenum concentration correlated with low seed weight for Schwarzmann. This investigation suggests that Schwarzmann may have undergone late stenospermocarpy, a condition where pollination stimulates berry development but ovules abort without producing mature seeds (Pratt 1971, Iland et al. 2011). This finding is supported by the fact that Schwarzmann had low molybdenum concentrations, yet Schwarzmann ovules were still able to fertilise and begin development and therefore not affect the incidence of millerandage. The assumed abortion of the embryo, or potential shrivel of the seed (Pratt 1971, May 2004, Liu, et al. 2007), resulted in smaller berries and lower berry weights due to the absence of large seeds in response to the low molybdenum concentration. However, an ability to assess the developing ovules through ovule sectioning would be beneficial to ascertain whether stenospermocarpy did in fact occur. It is clear further investigation of this nature is required in order to fully characterise this disorder in Schwarzmann along with other rootstocks.

Zinc is essential for both pollen and fruit development, as such, an increase in the expression of coulure was observed when zinc was found to be deficient. Previously, zinc deficiency in grapevines has been associated with coulure and millerandage (Robinson 1994, Creasy and Creasy 2009) as zinc is required for pollen and fruit development (Marschner 1986). These results support the essential role of zinc in fruit development to prevent excessive development of coulure.

In the third and final year of the study, in addition to petiole nutrition, pollen nutrition was examined. This novel analysis was performed to determine whether nutrient concentration differed between the petiole— the common mode of nutritional analysis— and pollen. 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 determined in grapevines. For each of the elements, the concentration was always higher in the pollen than in the petiole, with the exception of calcium. A comparison between petiole and pollen nutrition was performed for the 2011 data 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.

The comparison between rootstocks and cultivar, coupled with the indices for fruitset, enabled a thorough examination of rootstock effects on various components of reproduction. This thesis highlights the cultivar-specific interactions that occur for individual rootstocks and as a result, identified that cultivars can differ in their reproductive performance when grafted to the same rootstock. As such, the data were normalised and agglomerative hierarchical clustering (AHC) using Euclidean distance and Ward's method (Ward 1963) was performed on the rootstocks to determine reproductive classes for each cultivar rootstock combination studied.

A three-class solution was achieved and class centroids are displayed in Table 1. Rootstocks grouped together by AHC are circled in Figure 1 and notated by Classes I to III.

Class I can be characterised as having high flower number, CI, MI and seedless berries; and low fruitset and total berry number. The cultivars Cabernet Sauvignon (for all rootstocks and own roots) and Merlot own roots were both assigned to this class. It is interesting to note that both Cabernet Sauvignon and Merlot were classified similarly according to Dry et al. (2010). In this instance, for Cabernet Sauvignon, the cultivar had more of an effect on reproductive performance than did rootstock, yet for Merlot, only the own roots is classified as having poor fruitset due to high flower number, CI, MI and

seedless berries and as such, assigned to class I while grafting Merlot to one of the four rootstock combinations shifted Merlot to Class II.

Class II can be characterised as having high seeded and total berry number along with high LGOs. This class only had the cultivar Merlot grafted to rootstocks 5C Teleki, 1103 Paulsen, Ramsey and Schwarzmann. These rootstocks markedly improved total berry number and the number of seeded berries per bunch and lessened the incidence of CI and MI when compared with the Merlot own roots control. 5C Teleki has previously been associated with high berry number per bunch (Keller, et al. 2012). This classification further serves to strengthen the response of own roots Merlot to grafting to improve fruitset and also helps to substantiate the positive response of own roots Merlot to applications of molybdenum to improve fruitset (Longbottom 2007, Longbottom, et al. 2010).

Class III can be classified as having high bunch number per vine and high fruitset, lower flower numbers and lower incidence of CI and MI. All Shiraz rootstock combinations are represented in this class. Previously, the reproductive performance of the cultivar Shiraz was classified as having both low flower number and low MI (Dry et al. 2010) and it would appear this classification holds true for Shiraz irrespective of rootstock. The use of a hierarchical clustering enables wine grape growers to assess the various features of reproductive performance that classify a cultivar / rootstock group. To our knowledge, this is the first time that this type of analysis has been attempted for rootstock hybrids. This will benefit knowledge of reproductive traits of rootstocks and will assist in the appropriate cultivar / rootstock choice based on reproductive performance. The fact that the AHC grouped on cultivar more so than rootstock further serves to highlight the cultivar specific response, in particular for Merlot, over rootstock for measures of reproductive performance. Merlot was the only cultivar to be positively influenced by rootstock and as such, further work over a wider cultivar spectrum would be required to identify if any other cultivars are as responsive to rootstocks as Merlot.

The second component of the thesis was to evaluate the effect of water stress on reproductive performance of grafted vines. Early and late season water deficits have been shown to be detrimental

to the development of both the current and the following season's crop (Matthews and Anderson 1989, Petrie, et al. 2004). Early season water deficits can interfere with pollination and fertilisation and may cause poor fruitset and/or abscission of inflorescences (Alexander 1965, Keller 2005) which may result in fewer berries per bunch (Rogiers, et al. 2004). Although fruitset is acknowledged to be affected by water deficits (Alexander 1965, Hardie and Considine 1976, Matthews and Anderson 1989, Rogiers et al. 2004), the fundamental causes of poor fruitset remain uncertain. The aim of this chapter (see chapter 4) was to ascertain whether rootstocks could mitigate the detrimental effects of water deficit on yield through improved reproductive performance in the cultivar Shiraz.

For Shiraz, the absence of irrigation resulted in a reduction in yield due to lower bunch number, bunch weight and berry weight rather than lower fruitset or berry number. In addition, the absence of irrigation did not negatively affect the ability of any treatment to store carbohydrates in the perennial structures (see chapter 6). As such, the response to zero irrigation through yield reductions was in general not attributed to the reproductive parameters associated with fruitset; coulure and millerandage. It is apparent that in this study, water stress occurred post flowering and fruitset as yield losses were due to weight loss in bunches and berries through effects on berry size and cumulatively, in the following seasons though a reduction in fruitfulness. Matthews and Anderson (1989) also failed to show an effect of water stress on fruitset. These authors attributed this to an absence of water deficit at this phenological time point and our results support a similar finding. Own roots, unirrigated Shiraz in particular suffered a cumulative yield decline due to a reduction in bunch number and bunch weight. In the absence of irrigation, Ramsey was the best performing rootstock and maintained yield similar to irrigated treatments, with the exception of season one. McCarthy et al. (1997) also found that Shiraz vines grafted to Ramsey yielded more than other unirrigated rootstocks, but not significantly so when compared to 110 Richter or 140 Ruggeri. Overall, 1103 Paulsen, unirrigated in every season was associated with the lowest yields. 1103 Paulsen has previously been shown to perform poorly in the absence of irrigation (McCarthy et al. 1997). Despite this, it continues to be ranked as drought tolerant

for Australian conditions (Nicholas 1997, Dry 2007). With the exception of Ramsey, yield reduction of between 22% and 25% was experienced irrespective of rootstock in the absence of irrigation. Therefore, based on the results of this thesis, the classification of drought tolerant rootstocks, as defined by the ability to maintain yield (McCarthy et al. 1997), would include Ramsey and 110 Richter. In contrast, rootstocks susceptible to drought (i.e suffered significant cumulative yield decline in the three year absence of irrigation) include 1103 Paulsen, own-roots Shiraz,140 Ruggeri, 99 Richter and Schwarzmann. These findings have significant consequences for rootstock choice in grape growing regions of similar soil type faced with future drought and water allocation issues. It is likely the Light Pass fine sandy loam A horizon overlying a red brown mottled clay B horizon (Northcote 1954) influenced these drought tolerance rankings, in particular, the ability of 1103 Paulsen and 140 Ruggeri which elsewhere on soil types such as coarse sand (Carbonneau 1985), and presumably with less root restrictions, were classified as drought tolerant.

Table 2 describes the differences in income ($/ha) under different irrigation regimes and from the different rootstock hybrids. This table was generated from (a) the yield results extrapolated out to (t/ha) of the three year field study on Shiraz at Nuriootpa and (b) calculated average purchase value / tonne ($1,719 per tonne) for Shiraz grown in the Barossa region at 2013 prices http://www.phylloxera.com.au/media/WGCS-SA-Final-Report-2013.pdf.

The table identifies clear differences in the ability of the different rootstocks to maintain yield under zero irrigation and the effect of cumulative irrigation on income. Incorporation of wine quality information from the 2009 and 2010 vintages (Chapter 7) enables a thorough examination into the potential rootstocks that could be beneficial for use on Shiraz in the Barossa under conditions of ongoing drought. Maintance of yield and profitability under zero irrigation would support the use of rootstocks Ramsey and 110 Richter (Table 2). However, in light of quality preferences of Ramsey rootstock from this trial, 110 Richter would be most preferred for maintenance of yield and profitability under conditions of severe drought or water restriction. This rootstock was able to maintain yield even

after cumulative seasons of zero irrigation and was associated with desirable quality characteristics of black fruit and high wine phenolics (Chapter 7). Although income was highest for Shiraz as averaged across the three years of data, the high yields associated with own roots Shiraz impacted on the ability of the treatments to ripen to the desirable TSS value, particularly in 2009 and 2010. In addition, Ramsey was also unable to ripen to the desired TSS due to the lower vegetative capacity of Ramsey (chapter 4) and this had a negative impact on wine quality, particularly in 2010. Furthermore, both Shiraz and Ramsey showed poor wine colour density and phenolic levels in vintages where wine was made (chapter 7).

Analysis of grape and wine composition and wine sensory attributes revealed that both rootstock and irrigation treatments had a significant effect on some grape and wine composition parameters measured, however only rootstock significantly affect wine quality, and only in one year of the analysis. Very few compositional differences were observed between irrigation treatments and no effect of irrigation on wine quality score was observed in either the 2009 or 2010 vintages. It is likely this result is due to the control vines also being under a degree of mild water deficit in both seasons.

A novel sensory analysis technique enabled discrimination of wine attributes between treatments and correlation with traditional wine quality assessments, such as pH, alcohol, wine colour hue and phenolics. For example, wine phenolics and grape TA was associated with “black fruit”, wine colour hue was associated with “red fruit” and alcohol was associated with “good length” in one season and alcohol associated with “astringent” in the other season.

Based on these findings there is now a potential to use expert winemaker panels to generate preliminary data on research trials using word frequency data before running a descriptive analysis panel. These findings have implications for rootstock selection management decisions and how we assess research wines for commercial application

Table 1. Classification of reproductive classes for rootstock and cultivar combinations

Variables	Classes			*P-value*
	I	II	III	
	CAS 1103P CAS 5CT CAS Ramsey CAS Schwarz CAS Own MER Own	MER 1103P MER 5CT MER Schwarz MER Ramsey	SHZ Own SHZ 110R SHZ 1103P SHZ 140 Ru SHZ 99 R SHZ Ramsey SHZ Schwarz	
Bunch no	26a	29a	40b	<0.001
Flower no	362c	255b	166a	<0.001
Seeded	53a	93c	82b	<0.001
Seedless	24c	19ab	15a	0.021
LGOs	5b	7c	4a	<0.001
Fruit set (%)	23a	47b	61c	<0.001
Total berry no	77a	111b	97b	<0.001
CI	7.5c	4.9b	3.6a	<0.001
MI	3.4b	2.2a	1.9a	<0.001
Bunch wgt	69.3a	149.8b	69.0a	<0.001
Berry wgt	0.9a	1.3b	0.8a	0.002
Yield	1.9a	4.2c	2.7b	<0.001

Statistical significance of the effects of rootstocks and cultivar for components of reproduction according to ANOVA test are given by P<0.05, P<0.01, P<0.001 and not significant (ns). Data represents the mean value for each variable for the rootstock and scion combinations in each class derived from multiyear and site experiments. Letters account for significant differences among treatments using LSD at 5%.

Table 2. Recommended rootstock combinations for Shiraz based on predicted income ($/ha) under conditions of drought or water restrictions

Rootstock		1 year zero irrigation	2 year zero irrigation	3 year zero irrigation	Average
110 Richter	% change in yield	-39%	-17%	-2%	-19%
	Income ($/ha)	-$ 6,227.61	-$ 2,854.98	-$ 321.26	-$ 3,134.61
Ramsey	% change in yield	-9%	5%	-17%	-7%
	Income ($/ha)	-$ 813.46	$ 937.11	-$ 3,697.53	-$ 1,191.29
Shiraz own roots	% change in yield	-13%	-17%	-18%	-16%
	Income ($/ha)	-$ 3,198.66	-$ 3,562.66	-$ 3,618.92	-$ 3,415.98
1103 Paulsen	% change in yield	-29%	-22%	-23%	-24%
	Income ($/ha)	-$ 2,937.41	-$ 2,595.54	-$ 4,018.79	-$ 3,183.92
Schwarzmann	% change in yield	-12%	-30%	-36%	-29%
	Income ($/ha)	-$ 1,075.31	-$ 5,650.55	-$ 6,188.81	-$ 4,304.89
99 Richter	% change in yield	-39%	-32%	-20%	-29%
	Income ($/ha)	-$ 3,736.32	-$ 5,796.03	-$ 3,867.25	-$ 4,466.53
140 Ruggeri	% change in yield	-39%	-37%	-36%	-37%
	Income ($/ha)	-$ 5,688.13	-$ 5,323.23	-$ 9,637.82	-$ 6,883.06

Figure 1

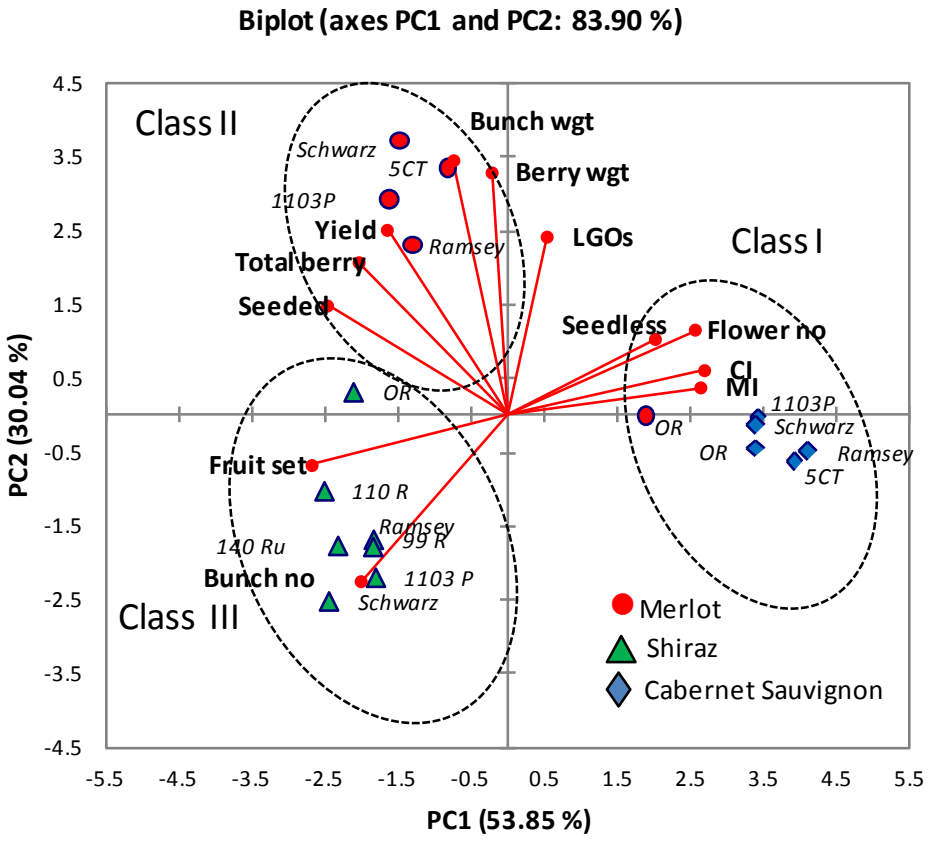

Figure 1. Principal component analysis of reproductive performance variables for three different winegrape cultivars and seven rootstock hybrids. Cultivar, rootstock and variable annotation are shown in Table 1. The cultivars and rootstocks grouped together by agglomerative hierarchical classification are circled and annotated by Class I, II, III.

Chapter 9. Literature Cited (Literature Review and General Discussion)

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