Factors Influencing Diurnal and Seasonal Fine Root Growth in Grapevines

By

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Certificate of Authorship

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Editorial Note

The English style in this thesis is mainly Australian English. This thesis is based on a series of papers, but has been re-formatted in accordance with Charles Sturt University’s academic manual available at [http://www.csu.edu.au/acad_sec/academic-manual/hcontm.htm](http://www.csu.edu.au/acad_sec/academic-manual/hcontm.htm) (section 4: Regulations for presentations of print theses, other examinable print works and the written component of examinable multi-media work).

The thesis has been referenced in accordance to the American Psychological Association (APA 6th edition), in the line with Charles Sturt University’s referencing style. The APA 6th edition is available at:

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I would like to begin my acknowledgment in the name of Allah, the beneficent, and the merciful. I thank my God, my Lord Allah, for giving me an opportunity to do a PhD in Australia and make life easier for me and my family to get through all the difficulties.

I wrote my Master thesis in Chinese that is my second language. Now I am finalising my PhD thesis in a third language, in English. If you are told writing anything in a second or third language is easy, they are either too genius, or have not tried it themselves. For a person like me who has not had an English education background, writing is a big challenge to overcome. However, it seems I am getting there.

I would like to express my special gratefulness and thanks to my supervisors Dr. Bruno Holzapfel, Dr. Jason Smith, Dr. Suzy Rogiers, Dr. Yann Guisard, you all have been tremendous mentors for me. I thank Bruno for always supporting me on both research as well as on my personal life and future career, your advice always be priceless. I would like to thank Jason for your great support on every single part of my experimental design, for your guidance towards correct research approach and for also a lot of techniques you have taught for research. I would like to thank Suzy for your encouraging of my research and your generosity, your time and ideas. I greatly appreciated your sincere support during these years I have been at CSU. I would like to thank Yann your invaluable advice on my research, especially developing critical and logical thinking skills in research and writing. Overall, all of you are very supportive and great advisors.

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handling, complicated data analysis and learning new skills R software and Asreml in R, without your support I could not achieve my goals in data analysis. In addition, I have had great time in cRrow meetings where I have learnt a lot of data handling skills in R from you, David Luckett and Christopher Lisle. Many thanks again to all of you.

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Statement of Contribution to Publications

This thesis is a collection of papers resulting from the research undertaken by Mr. Kare Palwan Mahmud on Factors Influencing Diurnal and Seasonal Fine Root Growth in Grapevines. The candidate, Mr. Kare Palwan Mahmud contributed to these manuscripts as follows:

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Paper 1 provides information about diurnal root growth dynamics of different grapevine species in relation to soil temperature under natural conditions, suggesting that soil temperature contributes to diurnal fine root elongation dynamics in grapevine and indicating also that genotypic dynamics differ. Paper 2 provides information about how grapevine cultivars would behave at different times of a day if soil temperature keeps consistent or increases under different photoperiod conditions. This is supporting the hypothesis that diurnal root growth dynamics are driven by internal factors such as carbon supply or external factors such as soil temperature. Paper 3 provides information about whether the pronounced diurnal fine root growth dynamics found in pots also can be observed in bigger vines. Paper 4 provides information about seasonal root growth dynamics of grapevine rootstocks in relation to air temperature, soil temperature and soil moisture in a commercial warm climatic vineyard, indicating the link between short-term observations and long-term root growth observations. The paper 5 provides extra information about post-harvest root growth in relation to early irrigation and late irrigation approach in the period.

The preparation and development of papers were performed by the candidate under the guidance and supervision of all members of the supervisory team. Major contributions from other than the supervisory team were acknowledged by co-authorship.

I, as principal supervisor and co-author, confirm that the level of contribution by the candidate indicated above is accurate.
Statement of Contribution to Publication

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Abstract

Introduction: Grapes are one of the world’s most economically important fruit crops. Most of the grape cultivars are grafted onto rootstocks derived from one *Vitis* species or hybrids of two or three species. Commonly used rootstock varieties in vineyards do not only contribute to the management of aboveground parts such scion vigour, fruit composition and yield, but also allow for the genotype specific root system to adapt to a particular soil environment. The genotypic variation in root distribution provides an opportunity to select rootstocks based on suitability of root traits to certain environmental conditions. The aim of this study was to ascertain whether grapevine root systems have a clear diurnal and seasonal growth dynamics and whether it is varied across different rootstocks in response to soil temperature, air temperature or a circadian clock, or a combination of these factors.

Material and methods: Three separate pot experiments were carried out over a two year period and two field experiments were conducted over a seven year period. The pot experiments utilized one-year-old non-grafted vines of Shiraz (*Vitis vinifera*), and the rootstocks Ramsey (*V. champinii*), 140 Ruggeri (*V. berlandieri x V. rupestris*) and Schwarzmann (*V. rupestris x V. riparia*). These species were grown in 3.2 L tapered rectangular pots with two sides made of transparent acrylic. Each pot was in turn placed into a larger container filled with wet sand in order to exclude light from the transparent sides. Each pot could be temporarily removed so that root growth could be imaged using a flatbed scanner. Pot Experiment 1 was undertaken outside under natural conditions while Experiment 2 was conducted in controlled environment chambers under a constant temperature of 22 °C with progressively shifting and shortening day length. The vines were exposed to a typical ambient photoperiod and a consistent
temperature of 22 °C in the first two days and the last three days of the experiment. However, temperature was increased to 32 °C from day 3 to day 7 for monitoring root growth responses to soil temperature. Pot Experiment 3 utilized six-year-old Shiraz vines grown under field-like conditions in 780 L soil-filled plastic bins. Root growth was monitored using minirhizotron tubes installed in each bin. The experiments ran for 5 to 10 days, with root growth assessments carried out 3 to 5 times daily. In the field rootstock trial, root growth was monitored in the same four genotypes, grafted to Shiraz vines, and 169,600 images were collected by using a minirhizotron camera over the 2007/2008, 2008/2009, 2009/2010, 2012/2013 and 2013/2014 growing seasons. The trial was located at the National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, NSW Australia (35° 05'S 147° 35'E). Air temperature was recorded over the seven-year period while soil temperature and soil moisture sensors were monitored at the soil depths of 10, 30 and 60 cm over the 2012/2013 and 2013/2014 growing seasons. For the post-harvest study, root growth in response to three post-harvest irrigation strategies were compared using mature field-grown Shiraz. These included no post-harvest irrigation (NPHI), early post-harvest irrigation (EPHI) and late post-harvest irrigation (LPHI). The EPHI treatment maintained soil moisture in the readily available range for a period of 15 days after harvest. The LPHI treatment was applied at 30 days after harvest, and similarly maintained soil moisture for a period of 15 days. Minirhizotron tubes were used to monitor root growth across all three treatments. The data of the five experiments were then analysed using Asreml in R software (version 3.2).

Results: Under both pot and field-like conditions of these root growth studies, the elongation rate of actively growing roots was found to have a pronounced diurnal dynamics. Maximum growth rates, which ranged from 0.15 to 0.38 mm hour^{-1} across x
the three experiments, were highest in the afternoon and two hours after darkness, growth rates declined through the night and reached a minimum the next morning. This dynamics was observed across all genotypes with different levels of growth between them. In addition, these growth dynamics were evident in the small potted vines and the larger container grown Shiraz. Under the naturally fluctuating environmental conditions of Experiment 1, root growth was positively correlated with temperature. However, under the controlled environmental conditions of Experiment 2, a diurnal growth dynamics was observed during a typical photoperiod, regardless of temperature (constant or increased). Interestingly, the maximum root extension rate decreased when the soil temperature was increased to around 31 °C. However the rate of shoot growth rate increased in this higher temperature zone, suggesting that shoot dynamics are dominated by genotype, but root dynamics are dominated by temperature related variables, in the context of the typical photoperiod treatment. Under the conditions of progressively shortening days, continuous darkness was reached after 7 days. This caused a concurrent reduction in daily root growth rate, although root growth did not completely cease until early on day 10. During the last three days, a reduced diurnal fluctuation was still present despite continuous darkness. Under the progressively shortening photoperiod conditions, genotype played a dominant role only on average root length, while soil temperature was a main factor regulating average root length/growth rate. These findings suggest that the decline in root growth rates in response to a decreasing photoperiod may potentially be related to the dependence of fine root growth on carbohydrate supply from photosynthesis. The maintenance of a reduced diurnal growth dynamics into the period of continual darkness may also suggest a contribution from mechanisms that maintain physiological processes in alignment with the diel cycle.
Abstract

Under field conditions, vertical root distribution dynamics through the soil profile were genotypic specific. Roots of 140 Ruggeri were found in the top 10 cm to 60 cm while most of the roots of Ramsey were scattered between 20 cm to 40 cm. The root system of Schwarzmann was mainly distributed between 10 cm and 40 cm while own rooted Shiraz was dominantly found growing at depths between 20 cm to 60 cm. In terms of root population, 140 Ruggeri had the highest number of roots followed by Ramsey, Schwarzmann and own rooted Shiraz had the lowest number. The seasonal root growth dynamics varied between genotypes. While there was some seasonal variation in the periods and extent of root growth, some overall trends were apparent. Strong new root flushes occurred around bud break, and were most pronounced at flowering with the least growth occurring after harvest. Surprisingly, some root growth activity was still apparent in winter when the aboveground components of the vine were dormant. These seasonal dynamics were significantly related to air temperature, development stage and genotype.

**Conclusion:** In summary, fine root growth rates of grapevines were found to have a pronounced diurnal dynamics that was independent of genotype. Soil temperature was found to modify the amplitude of this dynamics, but it was hypothesised that carbon supply from photosynthesis and possibly the circadian clock may play more dominant roles in its regulation. The variation in seasonal root growth dynamics and distribution was dependent on genotype, air temperature, development stage and growing season. The mechanisms linking diurnal root growth dynamics at different phenological stages and its relationships with seasonal root growth dynamics and carbon supply needs further investigation. In addition, the elucidation of genotypic variation in root growth behaviour is also relevant to determining their suitability for particular environments.
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Chapter 1 General Introduction

Grapevines are one of the first domesticated fruit species among woody deciduous perennials and are the most economically important fruit crop in the world. Grapes can be eaten fresh as table grapes or they can be used for making wine, jam, juice, jelly, grape seed extract, raisins, vinegar, and grape seed oil. Grape cultivars are commonly grafted onto rootstocks, with 90% of *V. vinifera* scions grafted to less than 10 different rootstock varieties (Keller, 2010). Rootstocks are being used predominantly because of their resistance to phylloxera and nematodes, but also because they have tolerance to restrictive soil conditions, and influence vine vigour and grape maturity. Rootstocks allow the fusion of diverse genotypes into one functional system. Rootstocks are species derived from diverse climatic regions and belowground conditions and their root growth dynamics vary accordingly. (Smart et al., 2006). Like in other woody deciduous perennials, the roots of grapevines are a major storage organ for nutrients and carbohydrates that can be mobilized and transported to other organs of the vine when required. In addition, roots are growing in different environments and utilize a comparatively different pool of resources compared to the aboveground tissues. Therefore, to estimate timing of diurnal and seasonal vine root growth in relation to short- and long-term fluctuation of belowground environmental factors is important. Root growth dynamics of grapevines can be influenced by daily earth rotation as well as changes in soil moisture and temperature. These abiotic changes may result in timing of nutrient and water uptake and these root dynamics consequently impact on grapevine growth, productivity and fruit quality. Optimization of grapevine management under present and future climatic conditions demand an enhanced understanding of root growth behaviour of different rootstocks and their root growth dynamics under changing environmental conditions.
Past research associated with grapevine management and fruit quality has been predominantly undertaken on the aboveground parts, since studies on the physiology of roots are more complex. The standard book on root research by Böhm (1979) stated that the root research under natural field conditions remains in the early stage of science, especially timing of belowground processes and internal (e.g. growth regulators, photoassimilate supply, nutrient status) and external cues (e.g. soil temperature, soil moisture, day length) in relation to root growth are not well understood (Comas et al., 2005). Most of the known methods are time consuming and the accuracy of results is often limited. In recent years root researchers have developed new root observation methodologies, but some of them are either too expensive or only suitable for controlled environments and annual plants. The minirhizotron root observation laboratory is widely used for tree root growth observation (Lehnart et al., 2008; Linsenmeier et al., 2010; Wells, Glenn, & Eissenstat, 2002), which allows root detection without destruction of the root system. However, the diurnal and seasonal regulation of grapevine root growth influenced by the endogenous or exogenous rhythms requires still further clarification.

Recently, it has been suggested that endogenous rhythms permit the plants to estimate diurnal and seasonal environmental changes and synchronize their physiological process to optimise their growth (Dodd et al., 2005; Graf et al., 2010). Most diurnal growth rhythms have been investigated in vascular plants (Walter, Silk, & Schurr, 2009) and very few in woody plants (Head, 1965; Hilton & Khatamian, 1973). If the internal and external factors that influence the endogenous rhythm can be determined, the maximal growth rate could be predicted. Among the internal factors, carbohydrate availability is critical for root growth, while for the external factors, temperature is one of the
important determinants of root growth dynamics. For example, higher temperatures result in earlier bud break, shoot growth and seasonal canopy development, root growth is all stimulated (Callejas, Canales, & de Cortazar, 2009; Graham et al., 2002; Keller & Tarara, 2010; Rogiers et al., 2011; Skene & Kerridge, 1967; Zekelle & Kliewer, 1979). For the belowground parts, root growth dynamics can vary depending on climatic region and these differences are strongly associated with changes in temperature, soil type or rootstock genotype (McMichael & Burke, 1998, Psarras et al., 2000). Moreover, in some studies temperature appears to be the predominant factor manipulating observed fluctuation in plant root growth if all the other factors that influence root development are equal. (Matos et al., 2014).

Therefore, a better understanding of fine root growth behaviour of different grapevine genotypes will assist in rootstock selection based on their root traits to particular environments and would also be important for long-term sustainability of grape production.

1.1 Research aims and objectives

The aim of this study was to characterize the diurnal and seasonal fluctuations of fine root growth in grapevines and to determine if they are driven by genotype, soil temperature, photoperiod, circadian clock or a combination of these factors. The overall focus was to separate these factors and determine which of these were the main drivers of root growth under specific above and below-ground conditions. The specific objectives of this study were as follows:
1. To examine if grapevine rootstocks (genotypes) display clear diurnal dynamics of fine root growth under natural soil temperature fluctuations in potted conditions (Chapter 3).

2. To determine the impact of different light periods on diurnal fine root elongation dynamics of grapevine genotypes and to understand the relationship between soil temperature and root growth under controlled environments (Chapter 4).

3. To explore whether mature grapevines display the same diurnal dynamics of root elongation under field-like conditions (Chapter 5).

4. To characterise the long-term response of grapevines to air and soil temperature and the seasonal root growth dynamics of different rootstocks under field conditions (Chapter 6).

5. To test the effect of different irrigation treatments on post-harvest root growth dynamics under field conditions (Chapter 7).

1.2 References


Chapter 2 Literature Review

Synopsis

This chapter provides a review of past research on root growth in grapevines and other perennial and annual species. Several research gaps have been identified as the result of this review, and these have gone on to form the basis of the research that was undertaken in this thesis. The experimental work in this thesis focused on diurnal and seasonal root growth dynamics of different grapevine genotypes in relation to soil temperature.

Key contents

- Overview of grapevine root system
- Techniques for the study of root distribution and root growth
- Environmental influences on root growth
- Nutrient uptake
- Conclusions
2.1 Overview of grapevine root system

2.1.1 Root biomass and distribution

In a plant, the aboveground organs and belowground root system grow in contrasting environments, giving them the potential to utilize a comparatively different pool of resources available in these two environments (Ruts et al., 2012). This unique circumstance dictates that plants need to adjust and adapt to the changing environments on either side of the soil surface. The root system of the grapevine not only supports aboveground plant tissues physically but it also provides access to water and nutrients, and acts as a carbohydrate storage (source-sink relationship). Moreover, grapevine roots are a resource of plant hormones, which can alter the physiology, growth and development of the aboveground tissues (root-shoot interaction). The distribution of the roots within the soil is dependent on Vitis species, cultivars (Perry, Lyda, & Bowen, 1983b) as well as soil type and structure (Bauerlele et al., 2008; Comas et al., 2005). Vine root physiology and growth have lately received more research attention in various grape growing areas of the world, including Australia (Barnard, 1932), United States (Winkler, 1963), France (Seguin, 1972), Yugoslavia (Pantic, 1974), Israel (Safran, Bravado, & Berstein, 1975), and South Africa (Zyl & Huyssteen, 1980). Despite this recent interest, literature on grapevine root dynamics is still limited.

The root system of the grapevine penetrates into the soil, resulting in an extensive distribution of the available soil volume. It is highly exploratory and therefore, relative to other species, it has low density (Morano & Kliewer, 1994; Nagarajah, 1987). Exploratory roots generally grow faster and their growth rates may reach 1cm per day in late spring (Hilton & Khatamian, 1973). About 150 mm from the tip, these extension
roots may thicken while lateral roots elongate and branch to produce laterals of higher orders. High order roots in grapevine, as in apple (Head, 1973), grow slower than low order roots. Thus, proliferation of laterals results in short, fine roots, which utilize the water in the soil and stored nutrients in roots. Normally, the overall number of fine (1mm diameter) roots of a 15-year-old ‘Shiraz’ vine is about 10,000 (Randall & Coombe, 1978). However, mortality of fine roots is common in all woody perennial plants including the grapevine. Many fine roots die within weeks of their emergence but are continuously replaced by newly emerging laterals wherever soil conditions are favourable (Reynolds, 1975). These roots that survive eventually commence secondary thickening and contribute to the structural framework of root system over the growing season. Root diameters differ according to their age but are around 6-100 mm. The thickest main roots of *V. Vinifera* generally appear at a distance of 300-350 mm from the soil surface and their number does not increase after the third year from planting (Barnard, 1932).

The orientation and depth of the main roots can vary with different species of *Vitis*. Perold (1927) showed that the main roots of *V.rupestris* grows vertically with smaller angles and diffused more deeply, whereas roots of *V.riparia* commonly form larger angles and stays at a shallower position. These differences are further influenced in grafted plants by various stock/scion combinations (Richards, 1983). From the main framework, smaller (2-6 mm diameter) permanent roots arise and grow either horizontally or vertically. Roots of mature vines averaged 3 m horizontally (but occasionally reached 10 m) resulting in considerable overlapping with roots from neighbouring vines (Barnard, 1932; Smart et al., 2006). Other cultivars grown in a wide variety of soil types, had a lateral spread of roots ranging generally between 4 to 8 m (Richards, 1983), even reaching 30 m when the soil environment was favourable (Galet,
2000; Lehnart et al., 2008). However, grafted grapevines grown under different irrigation systems had root systems that extended only 1 m vertically and horizontally from the trunk in a coarse quality soil. It was noted that most of the roots were found at a depth of 0.4 m and 0.6 m horizontally from the trunk (Bassoi et al., 2003; Silva, Honorato, & Bonomelli, 1991) and these root growth dynamics varied if applying different irrigation system. For example, the roots are more spread with the micro sprinklers than with the drip irrigated vines. Furthermore, roots with diameters shorter than 2 mm occupied 80% of the total root length (Bassoi et al., 2003).

Root growth in grapevines is dependent on the period within the growing season. It initiates soon after bud-break and reaches quickly to an upper limit at anthesis, and then declines gradually through to berry maturity. However, during the postharvest period, new root production can initiate again (Van Zyl, 1988) depending on environmental conditions, before decreasing in winter as vines enter dormancy in cooler climates (Callejas, Canales, & de Cortazar, 2009). Certain roots can grow continuously throughout the dormancy (Comas et al., 2005).

2.1.2 Root anatomy

Grapevine roots grown from seeds initially consist of embryonic roots. These develop into a primary root system, from which secondary roots or lateral roots can extend (Osmont, Sibout, & Hardtke, 2007). Conversely, roots can arise from the cambium zone of woody canes if vegetative propagation is applied (Pratt, 1974). The young root system of the grapevine usually emerges as creamy-white with a root tip 2-4 mm long. Proximal to these root tips, root hairs occur in a zone that extends for 10-30 mm. Normally they are 200 µm long and 15 µm in diameter and they may reach a density of
300-400 hairs/mm² (Litvinov & Stapkin, 1965; Pratt, 1974), however their occurrence in the soil may be sporadic. Soil pH affects root hair production. There were 2.5 times more hairs on vines grown at pH 5.7 than at pH 7.5, but this had no effect on root growth or nutrient uptake (Winkler, 1963). Similar to many other crops, grapevines do not develop root hairs in solution culture (Litvinov & Stapkin, 1965). As roots of woody deciduous plants develop and age, they not only alter in their physical size and appearance but also undergo internal changes in the organization and differentiation of their tissues.

The anatomy of the grapevine root during primary development is similar to that of many other woody plants. The cortex consists of 8-10 layers of parenchyma cells rich in starch and with large intercellular spaces. Some cortical cells contain calcium oxalate crystals (raphide cells) and tannins. In young-white roots the endodermis suberises in a zone usually between one and several centimetres from the tip (Richards & Considine, 1981). Although additional development and thickening of the endodermis has been reported for apple roots (Mackenzie, 1979), this is not apparent in grapevine roots. Young roots of grapevines may be either white or light brown. The brown colour is probably due to oxidation of phenols released from vacuoles of dead or collapsed epidermal cells. When this occurs, the next layer of cells, the hypodermis, may develop suberin lamellae inside its cell walls (Richards & Considine, 1981). The onset of this “peripheral suberization” is not associated with collapse of the cortex; factors affecting “peripheral suberization” are not well documented. In grapevines the speed and level of suberization is most rapid in mid-summer if soil temperature is elevated and soil moisture is low (Freeman & Smart, 1976). Some roots suberize entirely to the tip when the soil becomes dry, but after long periods, can resume growth by regeneration from
their suberized tips or by emergence of new lateral primordia once soil conditions become favourable (Pratt, 1974).

Secondary thickening of roots begins with the development of a vascular cambium and a cork cambium (phellogen). When the vegetative season finishes, the damaged cortex cells and endodermis dry out and slough off. The following season, the vascular cambium resumes actively producing a ring of secondary wood internally and secondary phloem externally. Cambium derivatives extend exiting medullary rays and produce new rays, which are not in the same alignment. The cork cambium forms deeper in the non-functional secondary phloem (Galet, 1970). Reactivation of cambia in vines is often discontinuous and irregular along the length of the root. Thus a root that has undergone secondary development appears more uneven and thicker than a stem of similar age (Esau, 1948).

2.1.3 Fine root growth dynamics

Understanding factors regulating fine root (a diameter < 2 mm) growth dynamics and functioning can lead to more accurate and beneficial vineyard management, because fine roots, which can be replaced a number of times every year, stand for the most dynamic segment of the whole root system, playing an significant role in water and nutrient uptake (Basile et al., 2007; Comas, Eissenstat, & Lakso, 2000; Volder et al., 2004a). Therefore, to understand fine root dynamics for the optimization of soil and nutrient management is critical.

Considerable research is in progress to uncover the fundamental ‘rules’ of fine root growth (productivity, longevity and mortality). Previous studies on root systems were
based on the conception that most fine roots have similar root formation and physiological functioning as they belong to single class of roots, while other investigations have indicated that some physiological distinctions between fine roots may be related to variations in diameter (Pregitzer et al., 1998; Wells & Eissenstat, 2002). In terms of fine root productivity, Basile et al (2007) showed that fine root growth in peach rootstocks was lowest in winter and declined during the last stages of fruit growth. Fine roots formed during spring were longer than those formed later in the season. The seasonal fine root growing dynamics was similar in different rootstocks, but thickness varied depending on the rootstock. Some grapevines created most of the fine roots at the flowering stage with an additional smaller flush of growth during the postharvest period if growing conditions were favourable (Freeman & Smart, 1976). It is also obvious that growth of fine roots varies with different pruning systems, and this is especially relevant for cooler climates (Basile et al., 2007; Comas et al., 2005). Warming of soil will lead to earlier root production in the spring and is crucial for root growth (Clarke et al., 2015; Pregitzer et al., 2000) with greater root branching in warm soils. Respiration rates (Pregitzer et al., 1998), nitrogen concentrations (Pregitzer et al., 1997) and also root mortality of “thin” fine roots were higher (Wells, Glenn, & Eissenstat, 2002) than “thick” fine roots. In addition, fine roots of phylloxera resistant rootstocks have different root growth rates and lifespan (Bauerle et al., 2007). Some fine roots die quickly, within 3-4 weeks (Bauerle et al., 2008; Wells & Eissenstat, 2002; Wells et al., 2002; Withington et al., 2003). Therefore, these findings may make it possible to model root growth dynamics and function throughout the entire season in relation to soil temperature, moisture, soil nutrients and root respiration to better understand vine and environmental regulations over root growth dynamics (Comas et al., 2005; Comas et al., 2000; Pregitzer et al., 2000).
These studies on seasonal root growth dynamics of grapevine and other fruit crops have shown that the dynamics of root growth can be influenced by genotypic difference of rootstocks, developmental stages, cultural practices and soil environmental conditions (Comas, Bauerle, & Eissenstat, 2010). However, findings on seasonal dynamics of grapevines root growth vary between studies. For example, historic seasonal root growth dynamics showed that root growth is most pronounced between bloom and after harvest (Freeman & Smart, 1976). However, recent studies revealed that these dynamics are influenced by season and vineyard management (Eissenstat et al., 2006). Timing of root growth in response to internal or external factors still remains unclear (Comas et al., 2005). In a changing environment, diurnal rhythms of light and temperature are the most predictable factors, and plants may adjust their growth and physiology to anticipate their surrounding environmental changes using an endogenous circadian clock. For example, tap root length of Zea mays was decreased around 42 % when soil temperature was reduced 20 to 10 °C (Nagel et al., 2009).

There were few studies which attempted to monitor the diurnal dynamics of root growth. The first daily root growth study was undertaken on cherry trees (Prunus avium) using time-lapse movies at four- hour intervals over several days (Head, 1965). It showed that the maximum rates in root growth appeared between 4 pm and midnight and the lowest root growth rate occurred during the daytime between 8 am to 4 pm. Unfortunately the environmental variables such as day length, soil temperature or moisture were not determined. In a study the root elongation rates of five woody plants were measured, including the grapevine. It was detected that the root elongation rate of grapevines at dusk was greater compared to day growth (Hilton & Khatamian, 1973). Similarly, no statistical evidence was provided. In annual plants such as Arabidopsis, it was
suggested that the different genotypes of *Arabidopsis thaliana* had various root growth dynamics and their diurnal growth dynamics and growth rate were affected by photoperiod or by additional sugar supply under a consistent temperature (Yazdanbakhsh & Fisahn, 2011). Moreover, while the strong diurnal rhythms in the rates of root extension were regulated by carbon supply, it was also suggested that the circadian clock coordinates carbon allocation and root development (Yazdanbakhsh et al., 2011).

### 2.1.4 Rootstocks

Grapevine cultivars and rootstocks are derived from the *Vitis* genus, which belong to *Vitaceae* family (Galet, 1988; Mullins, Bouquet, & Williams, 1992). The first use of rootstocks were developed in response to the introduction of the aphid-like insect phylloxera (*Daktulosphaira vitifoliae*) to Europe in 1860’s. These rootstocks were either individual selections of *Vitis* species or hybrids of two or more species. There are 29 American *Vitis* species based on their anatomical and morphological characteristics, and three of these (*V. riparia*, *V. rupestris* and *V. berlandieri*) form the parentage of common of rootstock varieties in use at present (Galet, 1988). Due to their dioecious origins, these rootstocks can be male or female plants. The female rootstocks include *Kober 5BB*, *101-14 Millardet at de Grasset*, and *Fercal*, whereas male rootstocks include *Teleki 5C* and *SO4* and *Reparia gloire de Montpellier* (Meneghetti, Gardiman, & Calo, 2006). Rootstock species hybrids from *V. berlandieri* × *V. rupestris* and *V. berlandieri* × *V. riparia* were known to have higher resistance to phylloxera and were easy to propagate from cuttings and would graft well with the *V. vinifera* wine grape varieties. In terms of different climates, the former group has a higher vigour and is better adapted to warm and dry climates, and the latter less vigorous and are more suited to cooler and wet climates. However, none of these species have tolerance of higher soil
lime content. Moreover, the genetic resources of worldwide rootstocks are fairly narrow; 90% of all *V. vinifera* vines are grafted to no more than 10 different rootstocks. This could pose a risk from mutant strains of soil injurious insects such as phylloxera, with probably negative effects if the resistance of these rootstocks can be overcome (Keller, 2010a).

Nowadays, Grapevine rootstock varieties are also used for their resistance to or tolerance of constraining soil conditions such as saline, drought, water logging, lime and acidic soils (Keller, 2010a; May, 1994; Pongrancz, 1983). Management of vine growth can be influenced by rootstocks; the impact on scion vigour is a critical consideration in rootstock selection (Dry & Coombe, 2004; Galet & Smith, 1998). Variation of root growth dynamics is generally related to the grapevine cultivar or rootstock (Morano & Kliewer, 1994; Nagarajah, 1987; Perry, Lyda, & Bowen, 1983a). Some studies showed that the root distribution of the grapevine is mostly related to soil conditions, nutrient and water accessibility rather than an inherent characteristic of rootstock genotypes (Smart et al., 2006). However, Southey and Archer (1988) noticed that the soil environment determined the specific root growth arrangement within the soil profile, but root density, specifically, was determined by rootstocks of grapevine. Sultana grafted to Ramsey had a larger root system than ungrafted Sultana, with more fine roots, extensive root density and more total root length (Nagarajah, 1987).

2.2 Techniques for the study of root distribution and root growth

Unlike aboveground plant components, plant roots are ‘hidden’ under the ground and in order to observe them in their natural environment special procedures or equipment are used. Most root research is carried out under controlled conditions in laboratories.
However, studies on root systems under field conditions are still scarce because most of the methods used are extremely time-consuming, tedious, and destructive, particularly in large perennial plants including fruit trees and grapevines. Generally, there are four approaches for root growth studies, excavation of the root system, direct monitoring methods, labelling methods, and indirect methods (Black et al., 2010).

### 2.2.1 Rhizotrons and mini-rhizotrons

#### 2.2.1.1 Rhizotrons

The observation window technique used to study the roots through large reinforced glass or plastic windows installed in a trench was first used by Sachs (1873) and evolved into simple underground boxes known as rhizotrons (Böhm, 1979). Installations using transparent ‘walls’ to study roots in soil are termed rhizotrons. They are often large and can even be walk-in chambers. Minirhizotrons were developed subsequently using small transparent tubes inserted into the soil and were first employed by Waddington in 1971. Rhizotrons are among the common methods of observing roots (Futsaether & Oxaal, 2002), and most current knowledge of roots and the rhizosphere still come from experiments with plants growing in rhizotrons and modified rhizotrons (Neumann, George, & Plassard, 2009). The main advantage of this type of system is that it allows a continuous study of the roots of the plants during a complete life cycle. However, some disadvantages with the rhizotron type were also evident. It is costly, causing leakage, difficult to take samples during the experiment and it is not suitable for larger plants, especially for field experiments.

There are three different rhizotrons (underground glass chambers) that have been employed for root observation studies, such as the classical rhizotron, rhizolab and soil
biotron. The classical rhizotron is a tunnel installed with glass plates on the soil walls. The rhizolab is similar to a rhizotron except that the above and belowground environmental conditions are completely controlled. It is a root observation laboratory and basically used for the long-term measurement of root development with the same experimental treatment plant roots, combining controlled conditions with the advantages of field-oriented investigation (Box, 1996; Sackville et al., 1991; Upchurch & Taylor, 1990). The rhizolab is an automatic modified rhizotron, which can be used for building models of the most favourable crop conditions under completely controlled environmental situations. It allows the measurement of diverse parameters simultaneously in relation to root and shoot interactions with the soil and atmosphere. However, it is difficult to provide a realistic picture of field situations since the growing conditions are often artificial (Box, 1996; Smit, Groenwold, & Vos, 1994; Van de Geijn et al., 1994). The soil biotron is located within a natural ecosystem and it is a good alternative to field trials on root-soil interactions under artificially designed underground laboratory conditions. This method allows the measurement of roots adjacent to minirhizotrons and can be applied to soil fungi (Fogel & Lussenhop, 1991; Pregitzer, Hendrick, & Fogel, 1993).

2.2.1.2 Minirhizotron (MR)

Minirhizotron inspection methods enable investigators to monitor and record roots directly and frequently in field conditions using a nondestructive process to give general information about the lifecycle of the root system, allocation, diameter and total root length using a minirhizotron camera system which can be used for non-destructive root observation (Bartz Technology Crop, Santa Barbara, CA, USA) (Hendrick & Pregitzer, 1992; Reid, Sorensen, & Petrie, 1993). Minirhizotron root studies have made available
practical data about the timing and amount of root turnover in perennial fruit plants (Eissenstat et al., 2000; Psarras et al., 2000; Wells et al., 2002), even if it has some limitations related to sampling and statistical variation between different treatments (Eissenstat & Yanai, 1997; Yao, Merwin, & Brown, 2006).

2.2.2 Whole plant / vine excavations

Excavation is one of the methods that can explore the biomass (Böhm, 1979), architecture and morphological characteristics of individual plants through measuring weights, length, diameters, volume and counting root tips. For perennial species, in general, excavation of the root system has been applied mainly for observing root biomass, root density and carbon distribution. Excavation depth is normally dependent on the capacity of the roots to break through the soil profile to a particular depth. Digging is stopped when no more roots can be found (Metcalfe et al., 2007). If the rootsystem is large, a trencher or a backhoe can be used for excavation, otherwise the excavation must be done using manual utensils such as hoes, shovels, and pressure water or air to retrieve the roots from the soil. This technique has largely been used for studying perennial plants (Bates, Dunst, & Joy, 2002; Hunter, 1998; Weinbaum et al., 2001). However, excavation of roots destructively does not provide useful information in relation to the lifecycle or turnover of the fine root system (Yao, Merwin, & Brown, 2009).

2.2.3 Soil core sampling approximation

Soil core sampling is mainly based upon the sampling of undisturbed soil for determining the distribution of roots and to calculate parameters such as biomass, root tip numbers and the volumetrical relationship between fine roots. This method is appropriate for soft soils with no stones and it is possible to make cores with a range of
Most samples can be taken by using manual or machine driven steel tubes into the root-zone, but it is necessary to keep the edge well sharpened. In stony or rocky soils, core samples need to be taken by a drilling appliance. Nowadays, grinding caps up to 100 mm in diameter can be found. The resulting data can be useful for many investigational aspects of root analysis, but sampling design, approach and equipment may vary when using soil core sample methods (Mackie-Dawson & Atkinson, 1991; Upchurch & Taylor, 1990; Vogt & Persson, 1991). In addition to this, another method, referred to as an in-growth core (mesh Bag), is also suitable for observing root biomass, chemical composition of roots. The mesh bags are incubated just after seed germination. This methodology is especially used for studying seasonal roots dynamics in the topsoil in annual and perennial plants (Steen & Atkinson, 1991; Vogt & Persson, 1991; Vogt, & Bloomfield, 1998).

2.2.4 Staining of the root profile

a. Quantifying root distribution and biomass

Non-destructive dye techniques have been used for quantifying root distribution and biomass. In this technique, dye must largely be absorbed by the root tissue, not by the soil. Rhodamine water tracing (RWT) is a relatively useful method for staining roots and it is combined with infrared photography. Generally, the root profile is covered with dye solution (1%) and the profiles are photographed with infrared colour film after the roots turn into a dark colour against a more lightly stained soil matrix. Then total areas of root cross section, length of roots, biomass and branching of roots can be measured by using computer software (Ruark & Bockheim, 1988).

b. Staining techniques for quantifying root growth rates
Various coloured dye solutions can be used for visualizing and measuring root growth rates. To achieve reasonable staining results, the dying material, buffering system, staining period, rooting media, and leachate type need to be considered carefully and not be toxic for plants. For estimating plant root growth rates, it has been suggested to use the red, navy and golden chlorotriazinyl (C.I.) dyes in a non-destructive staining method (Carman, 1982; Tennant, 1975).

### 2.3 Environmental influences on root growth

Regulation of plant functions such as nutrient uptake, carbohydrate reserve mobilisation or restoration, photosynthesis and carbon partitioning are related to the soil environment and soil types (Bowen, 1991). These different soil environment and soil types can result in either a lack of nutrient acquisition or on excess of nutrient uptake, leading to nutrient deficiencies or toxicity, respectively. Like other plants, in grapevines, climate change has a major effect on both vine growth and grape production. In hot climatic regions of Australia, extreme temperature is widely recognized to affect grapevine phenology, vegetative cycles and grape quality. Examples include advanced harvest times, dry and longer post-harvest period, increased grape sugar concentrations that lead to high wine alcohol levels, lower acidities and aroma compounds (Ramón, 2010). Root growth dynamics can be influenced by different soil conditions, cultural practices or rootstock genotypes and also climatic change-related effects (Psarras et al., 2000). These influences can be insightful and will be critical in understanding the root growth dynamics of grapevines.

#### 2.3.1 Soil temperature

Generally, soil temperature is lower than that of the air (McMichael & Burke, 1998), particularly in the deep soil layers. However, a change in both daily and seasonal
fluctuations of atmospheric and soil temperatures are likely to be a cause of global warming and the average yearly temperature on the Earth’s surface might raise if the regularity of hot days is expected to increase (Atkin, Edwards, & Loveys, 2000; Hulme, 1999; Long & Hutchin, 1991; Wigley, 1989). Therefore, it is necessary to characterize the root growth dynamics in relation to soil temperature. Soil temperature significantly affects the growth and development of root system (Abbasalani & Hay, 1983; Cooper, 1973; Kaspar & Bland, 1992) and root growth tends to increase to an optimum temperature (Cooper, 1973; Gomez et al., 1991; Taylor, Pearson, & Ratliff, 1970). In addition, morphological changes in roots, characterized by differences in root length and biomass, are associated with soil temperature (McMichael & Burke, 1998). Moreover, roots develop earlier at higher soil temperatures in both annual crops (Kaspar & Bland, 1992) and perennial plants (Bevington & Castle, 1985; Larson, 1970; Wilcox & Ganmore-Neumann, 1975). McMichael and Quisenberry (1993) found that the most favourable temperature for root development in cotton (Gossypium hirsutum L.) was between 28 to 35 °C, but 23 to 25 °C in sunflower (Helianthus annuus L.). McMichael and Burke (1998) also presented data from Lipiec (1990) about optimum temperature for root growth (elongation and dry mass) in different crop species. Root growth can be affected if the soil temperature deviates significantly from the optimum. For example, temperatures higher than the optimum may reduce elongation rates (Arndt, 1937) or induce more branches (Nielsen, 1974). Whereas, roots grown at low temperatures may reduce branching (Brouwer, 1964; Clarke et al., 2015) and cause the death of the root cortex (Christiansen, 1963). For, example, in a temperate climate, lower or extreme soil temperature often limits the root growth rate and rate of rooting-depth (Nielson, 1974). Root mortality rates also may increase with soil temperature (Forbes, Black, & Hooker, 1997; King, Pregitzer, & Zak, 1999).
Bonomelli and Bonilla (2012) showed that the first growth peak in roots of cherry trees occurred at 43 days after full bloom, with accumulated degree days (326 ADD) in soil at 20 cm depth. Plastic covering did not impact noticeably on the root growth dynamics of cherry trees as the temperature prevailing during the season was generally low at the soil surface. Conversely, it may have negative influences on root growth, since it can cause excessively high soil temperatures (Bonomelli, Bonilla, & and Nuñez, 2009). However, using plastic mulches in regions with low soil temperature has certain benefits associated with higher root zone temperature (Lamont 2005). For instance plastic mulch increased the dry matter of roots in broccoli plants (*Brassica oleracea* L.) (Díaz-Pérez, 2009) and increased the root growth in black currants (*Ribes nigrum* L.) (Larsson & Jensén, 1996).

Root growth of Sultana vines was observed for eight weeks from budburst. At 11 °C there was little growth, while at 20 °C roots stopped growing when vines flowered and roots at 30 °C had continues growth throughout the experiment, suggesting that the optimal soil temperature for grapevines is close to 30 °C (Woodham & Alexander, 1966) or somewhere between 25 to 30 °C (Kliwer, 1975). The base temperature for the root growth is around 13.3-16.9 °C (Clarke et al., 2015). It has been described that root growth rate of grafted peach trees was highest at a 20 °C root zone temperature (RZT) (Malcolm et al., 2006). Field, Smith et al (2009) observed that 6 % and 12 % of root biomass was lost at 13 and 23 °C respectively due to losses of carbohydrate. Researchers highlighted that grapevine roots were longer and thinner in diameter at 30 °C than those at 20 °C as well (Skene & G. Kerridge, 1967). Ibacache and Lobato (1995) estimated that root growth intensity of the grapevine cv. Gold had a significant linear relationship with soil temperature at a depth of 20 and 50 cm. While root growth
of ‘Thompson Seedless’ was consistent with an increase in soil temperature, the maximum intensity of annual root growth was not related to annual soil temperature but soil thermal diffusivity (Callejas et al., 2009).

It has been well known that soil temperature is one of the crucial factors which can regulate aboveground physiology. Evidence has been proposed that if all the factors that affect root development are equal, soil temperature would be the main modifier of root growth. However, there are still not enough studies available examining the effects of soil temperature on root development of deciduous, perennial fruit plants including grapevines. There are no known studies linking soil temperature with rootstock fine root growth, and scion growth. Hence, it is important to evaluate these observations under variable conditions, such as controlled and field conditions and different rootstocks.

2.3.2 Soil moisture (drought and water logging – irrigation practice)

Efficiency of water-use affects grapevine performance and lack of water will cause limitations to plant growth and yield (Kramer & Boyer, 1995). Water usage depends not only on precipitation rates but also rainfall frequency, VPD and plant transpiration, and how rapidly it evaporates under natural conditions. Moreover, soil water-holding capacity and root distribution associated with soil characteristics such as texture, structure, depth and organic matter also affect water availability. Presumably, variation in soil moisture related to water – holding capacity will affect the root zone significantly and impact vine root growth both between and within vineyards (Soar & Loveys, 2007). Irrigation methods in vineyard management have a significant effect on moisture diffusion within the soil profile, resulting in different root growth dynamics and water use efficiency of the grapevine (Araujo et al., 1995; Clothier & Green, 1997; Morano &
Kliewer, 1994; Van Zyl, 1988). For example, Soar and Loveys (2007) showed that the total root biomass at 20 – 25 cm below the surface and root diameter between 1 to 4 mm diameter had significantly increased in vines under sprinkler irrigation and then converted to drip irrigation, compared to vines maintained under sprinklers throughout. In addition, roots of grafted vines were mainly distributed under the vine row under drip irrigation, whereas root amounts were greater and spread over a larger planting area under microjet irrigation (Bassoi et al., 2003; Stevens & Douglas, 1994). Under the same irrigation system, the roots reached to a 1 m depth with most of the roots present to a 0.6 m depth, and a significant increase in the upper 0.4 m soil (Bassoi et al., 2002). Therefore the 0.4 m depth may be the effective rooting depth for soil moisture management.

Grapevines can also grow well in regions of little to no summer rainfall (Champagnol, 1984; Mullins et al., 1992), and also can stay alive in climate zones with limited water availability (Comas et al., 2010). Plant roots absorb this limited resource by altering root distribution (Stasovski & Peterson, 1991; Taleisnik et al., 1999) and physiology (Westgate & Boyer, 1985). In addition, plants (Bauerle et al., 2008; Smart et al., 2006) grown under field conditions, change their root growth dynamics according to the layers of available soil moisture (Brown et al., 1985), with increased new root production and longevity in source-limited environments (Bauerle et al., 2008; Pregitzer et al., 1993). Grapevine roots in deeper layers of soil have a longer lifespan than those roots that grow at the soil surface (Anderson et al., 2003), whereas there is no lifespan difference between irrigated and rainfall fed roots (Anderson et al., 2003; Bauerle et al., 2008).

Root growth rates of grapevine rootstocks may decline under dry soil without supplemental irrigation, whereas some rootstocks continuously create novel roots under
mild water stress (Comas et al., 2005) and may generate larger root populations (Bauerle et al., 2008; Ruiz et al., 2005). Bauerle and Smart et al. (2008) studied two contrasting rootstocks in Mediterranean climates and showed that the faster growing rootstock formed the bulk of roots throughout the summer and grew rapidly in irrigated soil zones, whereas slower growing rootstocks tended to produce the bulk of its roots in wet winter seasons and shifted the root growth to deeper soil profiles, where soil moisture was comparatively consistent. Hydraulic redistribution may alleviate water stress when soils tend to dry (Bauerle et al., 2008; Richards & Caldwell, 1987). The lifespan of grapevine roots under dry soil can be increased because of nocturnal hydraulic redistribution (Kuhns et al., 1985; Piatek & Allen, 1999). Grapevines also have reasonable larger xylem vessels than other plants, which allow low hydraulic resistance (Smart et al., 2004).

2.3.3 Root growth in response to carbon acquisition

For all plants, including grapevines, photosynthesis is essential for obtaining energy. During the photosynthesis processes organic compounds are produced by enzymatically incorporating CO₂ and H₂O. The carbon assimilated by the grapevine is ultimately partitioned to the reproductive structures to determine yield and grape composition (Alberto et al., 2010). However, just as important, the carbon that is derived from photosynthesis is used to drive the vegetative growth aboveground organs and also the below ground structures that anchors and sustains the rest of the plant (Jahnke et al., 2009).

2.3.4 Light absorption

The carbon balance of grapevines can be affected by management methods and climatic factors which impact on photosynthesis capacity during the growing season (Holzapfel 26
et al., 2010). Light intensities and air temperatures can impact on both leaf characteristics and the biological components of the photosynthetic machinery (Berry & Bjorkman, 1980). Under controlled environments, most of the V. vinifera cultivars perform well with high temperature and high light conditions, but a few (e.g. Riesling) perform better with low temperature and low light intensity (Butrosse, 1970; Sánchez & Dokoozlian, 2005). The decline in photosynthesis efficiency may be the result of so-called photo-inhibition, which limits CO$_2$ fixation (Takahashi & Murata, 2008). Other studies have demonstrated that environmental stresses such as temperature, drought, and salinity all impact on photosynthetic fixation of CO$_2$ and increase the speed of photo-inhibition (Demmig-Adams & Adams Iii, 1992; Murata et al., 2007; Powles, 1984).

Palliotti et al (2009) demonstrated that photosynthesis and carbon uptake may vary by position within the canopy. Shoots in the lower position close to the fruiting zone were influenced by photo-inhibition while the leaves at the upper position had higher photosynthetic activity and water use efficiency. The light-use efficiency of grapevine leaves is related to direct and diffuse light conditions within the canopy. In general, diffuse light penetrates further into the grape canopy than direct light. Therefore, diffuse light is distributed more extensively through the canopy, enabling a larger amount of the light to be used. Under diffuse conditions, the side of the canopy that would generally be shaded under clear skies received more light (Petrie et al., 2009). However, increasing shade noticeably decreased ripening, carbohydrate (sugar) and mineral nutrition (especially for N metabolism) in potted vines. Shade reduced photosynthesis, stomatal conductance, and leaf nitrate reductase activity (Smart, Smith, & Winchester, 1988). Thus, while the overall growth of roots depend on the carbon supply, the photoperiod is also involved in carbon allocation and growth.
2.3.5 Carbohydrate reserves

Grapevines mainly store carbohydrate reserves in the form of starch, which consists of amylose and amylopectin. There is also a smaller soluble sugar fraction, which consists mostly of glucose, fructose and sucrose (Sepúlveda & Kliewer, 1986). Grapevines roots, trunks, and canes are the major carbohydrate storage organs and contain the highest concentrations (Holzapfel et al., 2010). In roots, starch is stored in both phloem and xylem ray parenchyma cells during dormancy and are mostly depleted from phloem cells after bud-break (Smith & Holzapfel, 2005; Zapata, Deléens, Chaillou, & Magné, 2004).

Studies show that carbohydrate reserve concentrations in grapevine roots vary significantly during the season (Bates, Dunst, & Joy, 2002; Bennett et al., 2005; Cox et al., 2012; Holzapfel & Smith, 2012; Rogiers et al., 2011). There are several factors affecting carbohydrate reserves, including grapevine varieties and rootstocks, seasonal management, fruit load and environmental factors, (Holzapfel et al., 2010). In Merlot and Pinot Noir the mobilization of carbohydrates varied at different growth stages. Starch levels in the perennial tissues of Merlot were greater than in Pinot Noir at dormancy, and then declined rapidly in the roots of both cultivars until early flowering. At the bloom stage, starch began to accumulate in Pinot Noir but not in Merlot (Zapata et al., 2004). Roots were the main carbohydrate reserve organ and about 84 % of the starch was stored in roots during early spring in ‘Concord’ vines (Bates, Dunst, & Joy, 2002).

In cold climate conditions, pruning system can affect carbohydrate storage and mobilization in Riesling vines (Weyand & Schultz, 2006). Soil temperature during the
early spring period influences the rate of carbohydrate mobilization, shoot elongation, onset of flowering and veraison in Shiraz vines (Field et al., 2009; Clarke et al 2015). It was proposed that cool soils inhibited the enzymatic activity for starch hydrolysis and that C starvation resulted in slowed canopy and reproductive growth. Average total nonstructural carbohydrate (TNC) concentration can be altered with seasonal climatic factors and cultural practice such as fruit removal, canopy adjustment and deficit irrigation in Shiraz (Holzapfel & Smith, 2012; Rogiers et al., 2011). Carbohydrate reserves were also dissimilar in Shiraz vines grafted onto three different rootstock types under deficit irrigation conditions (Cox et al., 2012). However, the specific effects of different rootstock genotypes on carbohydrate storage and mobilization are not yet well understood and further investigations are warranted considering the important role that carbohydrate reserves have on overall growth and development of the vine and berries.

2.3.6 Respiration of fine and structural roots

Respiration is a critical metabolic process that converts carbon energy sources into readily useable energy rich molecules such as ATP and NADPH. More specifically, aerobic respiration is the chemical reaction used to release energy from glucose. Respiration provides the energy for the maintenance of cellular activity and fuels growth. Respiration is thus a normal consequence of root maintenance and growth and is highly dependent on temperature.

Between one– and two-thirds of carbohydrates stored in the roots is consumed via respiration (Lambers, Atkin, & Millenaar, 2002). It is well established that root respiration is an crucial fraction of soil CO$_2$ efflux (Moyano et al., 2009). Approximately 8-52 % of all carbohydrates formed each day by photosynthesis may be respired in the roots with greater values with increasing plant age and at a low nutrient
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supply (Boot, Schildwacht, & Lambers, 1992; Millar et al., 1998). Moreover, usage of these carbohydrates in root respiration is larger not only among nodulated roots but also symbiotic plants comparing with nonnodulated roots and nonsymbiotic plants (Lambers et al., 2002).

Respiration measurements have been carried out on fine and coarse roots and the soil of forest trees under short and long-term growth conditions (Burton & Pregitzer, 2003; Davidson, Belk, & Boone, 1998; Desrochers, Landhäusser, & Lieffers, 2002; Jia & Zhou, 2009; Kutsch et al., 2001; Lee et al., 2003) and different fruit trees such as apple (Buwalda & Lenz, 1992) and citrus (Bryla et al., 2001). In grapevines, some short-term root respiration rates have been estimated (Comas et al., 2000; Morinaga et al., 2003; Volder et al., 2005). Franck, Morales et al. (2011) investigated seasonal root respiration in *Vitis vinifera* (Chardonnay) and found that fine roots had higher respiration rates than coarse roots at 20 °C and root respiration rates decline noticeably with fine root age. This result is similar to other vine cultivars (Comas et al., 2000) and apples and oranges (Bouma et al., 2001). Variations in root respiration is generally due to differences of species, management (Morinaga et al., 2003), growth rate of the roots (Volder et al., 2004b) and environmental conditions (Amthor, 1984; Moyano et al., 2009). Still, there are limited data available on respiration models of lateral roots, especially amongst different rootstocks, which may have particular importance because of their restricted growth period. It is also pertinent to determine the percentage carbon lost through respiration by roots on a whole plant basis.

Root respiration and soil microorganisms are the main sources contributing to CO$_2$ efflux from soils. Root respiration is defined by Wiant (1967) as the respiration from
living root tissue. Other definitions that tackle CO$_2$ efflux from soils, including rhizomicrobial respiration, rhizosphere respiration, mycorrhizal respiration and mycorrhizosphere respiration are sometimes used differently by various researchers. For instance, Dilly & Bach et al. (2000) and Kutsch & Staack et al. (2001) defined rhizomicrobial respiration as respiration of both roots and associated micro-organisms, whereas Kuzyakov & Cheng (2001) and Nguyen (2003) defined it as only the latter. Subsequently, Moyano & Atkin et al. (2009) have summarized and defined these respirations according to the figure below (Fig 2-1).

**Fig 2-1** Diagram showing the sources of root derived carbon respiration - respiration from the living root tissue, respiration of rhizodeposits by micro-organisms in rhizosphere and respiration from mycorrhizal hyphae- and their grouping into rhizosphere and mycorrhizosphere respiration. Litter and soil organic matter (SOM) respiration are the carbon fluxes not deriving from live roots (Moyano et al., 2009).
2.3.6.1 Field root respiration methods

Excision methods, intact root chamber methods and large coarse root respiration methods are involved in quantifying root respiration. In these methods, infrared gas analysers (IRGAs) have been used to measure the increase in CO$_2$ concentration due to respiration.

In the excision method, roots have been freshly removed from the soil profile and placed in a cuvette for a quick determination of CO$_2$ efflux using IRGAs (Moyano et al., 2009). The cuvette is specifically designed for root respiration and its volume must be adequate for the desired size of the sample (Burton & Pregitzer, 2003). When roots are separated from the soil, it is however unavoidable to alter plant-microbe interactions; there is a loss of relatively active root hairs, which lessens the nutrient supply (Moyano et al., 2009). Moreover, root excision increases respiration rates due to a wounding response (Cabrera & Saltveit, 2003). To minimize the detrimental effects of excision, root respiration methodology is limited to four hours (Bloom & Caldwell, 1988; Lee et al., 2003; Lipp & Andersen, 2003; Marshall & Perry, 1987), single excisions are used or intact root mats containing many fine root segments are sampled (Burton & Pregitzer, 2003) and this was elaborated in the study of Moyano et al. (2009).

Large coarse root respiration can be measured under lab conditions, using cut root sections or under field conditions by using chambers installed over a root trial (Moyano et al., 2009). The first method may be suitable for measuring the respiration rate variation within and between the trees under controlled conditions (Kimura, Mototani, & Hogetsu, 1968), but it is impossible to obtain seasonal dynamics in respiration on the sample because of the destruction that it involves (Sprugel, Ryan, Brooks, Vogt, &
Martin, 1995). Therefore, it is difficult to distinguish maintenance respiration from growth respiration. In intact-root chamber methods, roots are placed into a sealed or open-top chamber within the soil profile without excision (Bryla et al., 2001; Cropper Jr & Gholz, 1991; Fahey & Yavitt, 2005) to reduce potential artefacts such as gas flow rates (Burton & Pregitzer, 2003), the soil medium (Cheng et al., 2005; Fahey & Yavitt, 2005; Kutsch et al., 2001), soil temperature and moisture (Moyano et al., 2009).

The latter approach is to fix chambers permanently onto intact large coarse roots and to monitor CO\textsubscript{2} efflux throughout prolonged periods of time (Benecke, 1985; Linder & Troeng, 1981; Wieser & Bahn, 2004). The disadvantage of this method is that it involves complex equipment to maintain air flow in the chamber, to monitor flow rates and changes in the CO\textsubscript{2} concentration. Due to these disadvantages, usually only a small sample size can be measured (Moyano et al., 2009). Thus, removable chambers which are clamped onto a root section can be a better choice to solve the problem of a small sample size (Ryan et al., 1996). Meanwhile it allows the measurement of root respiration within a single day at the same site over a growing season (Moyano et al., 2009).

### 2.3.6.2 Laboratory methods

In the laboratory, root respiration can be measured by using Clark –type oxygen electrodes or an infrared gas analyser. Using ‘Oxygen Cuvettes’ excised roots are bathed in buffered hydroponic medium (e.g. 10 mM HEPES AND 10 mM MES, pH 5.8) located in liquid phase chambers or in cleaned root fragments located in gas phase chambers. Temperature – controlled water baths are often utilized in both cases (Burton, et al., 1996). Estimation of root respiration is easy without any signal from microbial respiration with using these methodologies (Moyano et al., 2009).
2.3.7 Phylloxera

The life cycle of phylloxera involves an asexual component that can be maintained indefinitely and a sexual component that can arise when winged phylloxera emerge and lay male and female eggs. Asexual phylloxera feed on both roots and leaves of grapevines, with radicicole on roots and gallicole on leaves. Feeding on either tissue creates galls. Root galls have two types—nodosities (formed on new roots) and tuberosities (formed on mature roots). Leaf galls form on American *Vitis* species (depending on phylloxera strain), but generally not on the leaves of *V. vinifera*. However, the roots of *V. vinifera* are highly susceptible to gall formation and show extensive development of nodosities. This happens particularly in tuberosities when infected with phylloxera. These feeding sites disrupt root function and reduce the ability of the root system to provide nutrients and water to the aboveground parts. Damage caused by infections with secondary pathogens is also thought to contribute to the decline in vine health, and eventual death, after infection with phylloxera (Granett et al., 2001).

The American *Vitis* species are widely tolerant to phylloxera but show variations in susceptibility to root feeding (Grzegorczyk & Walker, 1998). *Vitis rotundifolia* and *V. cinerea* are regarded as immune to infection (Schmid, Manty, & Rühl, 2002), and in an in vitro assay, phylloxera made no attempt to feed on the roots of tissue cultured vines (Kellow et al., 2002). The root of *V. riparia* and *V. rupestris* are both resistant the phylloxera, but species such as *V. monticola* and *V. labrusca* have only moderate root galling (Galet & Smith, 1998). Amongst phylloxera populations, the predominance of asexual reproduction has led to distinct clones or biotypes (Corrie, Buchanan, &...
These biotypes show variations in their ability to infect grapevines, with Schwarzmann found to be completely immune to one biotype, but susceptible to another in tissue culture (Kellow et al., 2002). Corrie, van Heeswijck et al. (2002) found similar variations in rootstock resistance to phylloxera biotypes in field grown vines. Under confounding effects of environmental conditions on the viability of infected vines; it is difficult to assess the effect of phylloxera infection on the performance of grafted vines except under controlled conditions. However, in a pot study where the impact of phylloxera was quantified (Bates et al., 2001), it was observed that phylloxera reduced the growth of moderately resistant Concord by 21 %, and a further 34 % when an additional drought treatment was applied in pot experiments. Overall, vineyards are at risk from mutant stains of soil pests including phylloxera. That is why grapevine rootstocks were used for tolerance and resistance to root parasitise and other environmental stresses.

2.3.8 Soil physical and chemical properties

Soil physical and chemical properties can influence the growth and function of the grapevine root system, and both interact intimately. The type of soil affects root depth (Van Zyl, 1988). Increase in bulk density, poor water infiltration, and soil acidity decrease the number of roots (Conradie, 1988; Morlat & Jacquet, 1993; Van Zyl, 1988). Chemical, physical and biological properties of soil with which the roots interact will also affect the respiration rate as assessed by both the excision and in situ methods to measure root respiration (Moyano et al., 2009). Thus, the initial assessment of the vineyard soil prior to planting is important for grape growing because soil properties cannot be readily changed.
2.3.8.1 Physical properties of grape growing soils

Soil physical properties including soil colour, soil texture and water holding capacity affect root growth dynamics of grapevines (Myburgh, Cass, & Clingeleffer, 1998; Penkov, 1974). Soil surface colour has broad relationships with their organic matter content. Generally, the darker soil has higher organic matter content (Maschmedt, 2005). Soil texture is probably the most important of all soil properties as it impacts on rooting dynamics, water-holding capacities and nutrient retention (Brady & Weil; Maschmedt, 2005). For example, root systems grown in fine textured soils are smaller and shallower than in coarse -textured soils (Jackson, Sperry, & Dawson, 2000; Sperry et al., 2002). However, relationships between texture and horizontal or vertical distribution in natural ecosystems have not been found (Schenk & Jackson, 2002).

2.3.8.2 Chemical properties of grape growing soils

The chemical properties of soil naturally influence the nutrient uptake of plants. Grapevine can grow in variable soils so that it can survive and obtain adequate moisture and nutrition under a variety of chemical soil conditions such as salinity, soil acidity and limited nutrient levels.

2.3.8.2.1 Salinity

Salinity is one of the most critical aspects inhibiting growth and yield of plants. Salinity issues normally take place in regions where water loss from the soil goes beyond rainfall and salts dissolved in the soil solution are likely to accumulate at the surface of the soil. For irrigated vineyards, however, salinization has much more threat than unirrigated vineyards as irrigation water causes relatively higher concentration of salt on soil surface (Keller, 2010b). Salinity affects mostly grapevines grown in warm and dry climatic regions, such as Australia, Chile, California and south eastern Asia. It has an
adverse effect on the growth, anatomy, morphology and physiological characteristics of roots, even if the roots are less sensitive to salt stress than the shoots (Cheeseman, 1988; Munns & Tester, 2008; Shani et al., 1993).

Roots are the first vine component to experience the adverse effects of high salinity. This environmental stress reduces their ability to absorb water and nutrients. Studies have shown that grapevine root growth is less sensitive to salt stress than the growth of shoots (Hawker & Walker, 1978). It also impairs root respiration and water uptake, decreasing vine growth, yield and fruit quality (Shani & Ben-Gal, 2005; Shani et al., 1993). Among the dominant salt ions in the soil Na\(^+\) and Cl\(^-\) mostly affect the salinity damage in grapevines. Moreover, water uptake by roots can be reduced with the increasing of accumulation of these ions in the soil solution (Homaee, 1999; Shani et al., 1993). Stomatal conductance is also influenced by the deactivation of K\(^+\) uptake (Yamaguchi, 2008) and the increased production of ABA in roots under salinity, which in turn lessens photosynthesis and transpiration. The reduction in assimilates reduces the delivery of sugar to other plant tissues (Downton, Loveys, & Grant, 1990; Shani & Ben-Gal, 2005).

It is a challenge for grapevines growing under salt stress to uptake essential nutrient ions while excluding toxic ions such as Na\(^+\) and Cl\(^-\). Roots do have the ability, however, to pick the nutrient ions from the toxic soil solution and prevent over 95 % of Na\(^+\) and Cl\(^-\) from infiltrating the xylem (Munns & Tester, 2008). Some rootstocks derived from *V. riparia, V. berlandieri, V. candicans* and *V.champinii* (e.g., Ramsey, 140 Ruggeri, 1103 Paulsen, 110 Richter, and 101-14 Mgt ) can exclude a larger amount of the salt from root uptake and also restrict the root-to-shoot transport (Antcliff, Newman, & Barrett, 1983; Walker, Read, & Blackmore, 2000). Thus, highly concentrated salt in the
soil has a marginal effects on some of these rootstocks and scions grafted to these species (Downton, 1985; Stevens & Walker, 2002). Tregeagle and Tisdall et al. (2006) found that some of the rootstocks (Ramsey, 1103 Paulsen and 101-14 Mgt ) gradually lose their ability to reject salt and may become less salt tolerant under long-term exposure to the saline environment.

The impact of salinity on grapevines can also be dependent on soil type, soil management and irrigation. Waterlogging, for instance, as a result of unnecessary irrigation, can increase the incidence of salinity and the vine may be unable to exclude Na\(^+\) and Cl\(^-\) (Stevens & Walker, 2002). Vine death may occur under exposure to long-term saline soil water (Shani & Ben-Gal, 2005). However, soil salts can be leached below the root-zone, and root functions and growth can be recovered quickly by using fresh irrigation water (Shani et al., 1993).

2.3.8.2.2 Soil pH

The potential unfavourable effects of acid soils on growth, productivity, and nutritional uptake of grapevines has been investigated in various geographical regions, such as Italy (Fregoni & Bavaresco, 1984), France (Delas & Juste, 1975), Germany (Booss, Kolesch, & Hoefner, 1982), South Africa (Conradie, 1983) and North America (Bates et al., 2002). Like other agricultural plants, grapevines prefer neutral pH levels. Generally, soil pH is controlled by the cation exchange complex associated with the clay minerals and the organic fraction of the soil (Maschmedt, 2005). One study indicated that *Vitis labruscana* cultivars in western New York were quite tolerant of the 4.5 pH or lower acidic vineyard soils as compared to *V. vinifera* cultivars in this area (Himelrick, 1991). Cummings and Lilly (1984) highlighted that the yield of muscadine grapes (*V.
rotundifolia) were reduced by lower soil pH (5.5), but higher pH levels did not appear to influence yield. Low soil pH reduced root dry mass and shoot dry mass of some rootstocks. This is because toxicity of Al and Mn concentration at soil pH 5.5 can affect root growth and development (Fey, Chancy, & White, 1978; Kochian, 1995). For example, Himelrick (1991) found that nine grape cultivars (Vitis vinifera L., V. labruscana Bailey and French-American hybrids) grown under a 4.8 pH soil condition, resulted in shoot weight reduction of around 27 %, a root weight decline by 13 %, and root volume decline by 21 % compared to vines grown at a 6.7 pH soil. Thus, soil pH plays important role in root growth, function and nutrient uptake.

2.3.9 Soil carbon dynamics
Carbon stored in soil stands for the largest terrestrial carbon pool in nearly all terrestrial biomes (Batjes, 1996; Bolin et al., 2001). Carbon in soil can improve the physical and chemical properties of the soil. For example, it may raise the cation exchange capacity (CEC) and water-holding ability of sandy soil. It adds to the structural strength of clay soils by assisting to combine particles into aggregates. Among organic matter in soil, carbon is a main component, holding a great amount of nutrients, cations and trace elements that are significantly important to crop growth. Carbon can also reduce leaching of nutrients and is essential to the organic acids that assist plants to absorb mineral elements from the soil. Soil pH can be maintained stable because of the carbon buffering role (Leeper & Uren, 1993). It is generally believed that the carbon in soil is a major factor in overall plant health (Leu, 2007).

2.4 Nutrient uptake
Aside from anchoring the plant in the soil, roots have the predominant role of supplying plants with inorganic nutrients. The storage capacity of the soil for nutrients and the
mobility of these nutrients depend on soil texture, organic matter content, soil temperature, soil moisture and pH. For instance, water availability is higher in loamy soils compared to sandy soils. Organic matter in both types of soil can increase the water and nutrient storage capacity as it has a favourable influence on soil structure. Nevertheless, nutrient availability is lower in the dry soil than in the wet soil even if its concentration is higher in dry soils (Marschner, 1995). Thus the movement of both macro- and micronutrients is dependent on soil moisture (Christensen, 1984; Mullins et al., 1992; Schreiner, Scagel, & Baham, 2006). In addition, the variety and rootstocks as well as root distribution and functioning are important for accessibility (Holzapfel & Treeby, 2007; Keller, 2010b; Pradubsuk & Davenport, 2011).

2.4.1 Macronutrient movement and uptake from soil

The tissue concentrations of the macronutrients, nitrogen (N), calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K) are the key indicators for the nutritional status of plants. Annual available soil nitrogen (N) fluctuates considerably during the growing season, ranging from 30 kg·ha\(^{-1}\) (Bates, Dunst, & Joy, 2002; Conradie, 1980, 1981) to around 80 kg·ha\(^{-1}\) (Hanson & Howell, 1995; Williams & Biscay, 1991). The availability of other macronutrients, such as relatively mobile calcium (Ca) and magnesium (Mg), and low mobile phosphorus (P) and potassium (K), are more stable (Nord & Lynch, 2009). Between 20 % and 40 % of the annual N requirement for the early canopy development can be derived from stored reserves of the trunk and roots (Bates, Dunst, & Joy, 2002; Conradie, 1980; Hanson & Howell, 1995). In pots, however, less than 10 % of annual needs for P, K, Ca or Mg can be supplied from stored reserves of the trunk and roots (Conradie, 1981).
Nutrient uptake increases just after bud break, but most uptake of nutrients from soil occurs between bloom and veraison in both concord grapevines (Vitis labrusca L.) and wine grapes (Vitis Vinifera L.) (Bates, Dunst, & Joy, 2002; Conradie, 1980, 1981; Hanson & Howell, 1995). Some studies demonstrated that an increase of N uptake after bloom is also associated with the new root production (Freeman & Smart, 1976; Mullins et al., 1992; Van Zyl, 1988). In addition, mature roots can uptake P and K, whereas Ca and Mg most likely can be taken by fine roots. Furthermore, while the fine roots are most effectively involved in nutrient uptake, the coarse structural roots of vines often create symbiotic units with mycorrhizal fungi, which can uptake water and nutrients at a significant distance from the roots (Bucher, 2006; Possingham & Obbink, 1971). These microorganisms are basically known for their ability to promote P and Zn uptake and transfer these nutrients to the roots (Marschner, 1995; Smith, Dickson, & Smith, 2001). Absorption of N, K, Ca, and Mg can also be assisted by these microorganisms under certain circumstances (Keller, 2010b).

Soil temperature is an important factor that impacts on nutrient uptake and the growth of the plant (Baghour et al., 2003). Higher soil temperatures increases the mineralization of N if the water potential of soil does not restrict the activity of microorganisms (Piatek & Allen, 1999; Zak et al., 1999). Warmer soil temperatures also lead to higher root respiration, root growth and nutrient uptake, provided the plant physiological activity is not limited by other factors, such as carbon acquisition and water supply (Bassirirad, 2000; Burton et al., 1998; Oertli, 1996).

2.4.2 Micronutrients and uptake from soil

The micronutrients, manganese (Mn), boron (B), copper (Cu), iron (Fe), molybdenum (Mo) and zinc (Zn) are also essential and necessary for plant growth, even though they
are found in only low concentration in plant tissues and in soil solution. Micronutrient deficiencies are common, especially deficiencies of B, Fe, Mn, and Zn are often observed in vineyards (Mullins et al., 1992) and subsequently result in chronic chlorosis, limited root growth or function and yield losses under certain conditions (Davenport & Stevens, 2006). Since Cu, Fe, Mn, and Zn exist in very low concentrations in the soil solution, it is a challenge to meet plant requirements (Cass, 2005).

Diffusion and mass flow account for the movement of nutrients in the soil solution (Brady & Weil, 1999). Mass flow normally meets the demand for B in plant growth (Tinker & Nye, 2000), whereas the movement of Zn in soil solution appears mainly to be by diffusion and occurs very close to root hairs (Melton, Mahtab, & Swoboda, 1973). Unlike other crops, grapevines seem to have low root densities (Schreiner & Linderman, 2005), but have a more lateral and vertical root distribution (Smart et al., 2006) and fine roots are important for water and nutrient uptake (Morlat & Jacquet, 1993).

Pradubsuk and Davenport (2011) studied the distribution of Concord vine micronutrients B, Fe, Mn, Cu, and Zn within the plant. They found that Fe, Cu, and Zn had highest concentrations in fine roots, but the actual values varied across seasons. The maximum concentration of B was present at bloom and Mn at harvest in leaf blades, shoot tips, as well as in petioles. The seasonal dynamics of Fe, Cu, and Zn were variable between two years, whereas seasonal dynamics of B and Mn had equivalent trends over both years. B uptake was most pronounced between bloom and veraison, whereas that of Mn occurred between bloom and harvest. The concentration of these micronutrients
in soil solution was associated with soil pH and soil organic matter content. When soil pH increased, micronutrient availability decreased with the exception of Mo (Mullins & Hansen, 2006).

2.4.3 Process of uptake and its relations to root age

Individual roots of different order and position in the soil profile have different ability for water and nutrient uptake (Volder et al., 2005), however root function is also dependent on root age. It has been determined that with increasing root age, respiration rate and P uptake in fine roots of fruit trees decreased significantly (Bouma et al., 2001). Similar results also have been reported in grapevines (Volder et al., 2004b). In some studies, a reduction of hydraulic conductivity or nutrient uptake with root age also have been observed (Kramer & Bullock, 1966; Nobel, Schulte, & North, 1990). Yet, these findings leave some uncertainty as the investigators ignored to compare between roots at the same order, only old and young roots were observed. In addition, there is still not enough information available about the seasonal changes in the physiological activity of lateral roots as they move toward senescence. Moreover, limited data exists on the capacity for nutrient uptake in the first couple of days of fine root life and also the carbon costs of fine root function in relation to nutrient uptake and growth.

2.4.4 Nutrient uptake and partitioning to perennial reserves

Most initial studies on quantifying seasonal uptake and partitioning of mineral nutrients mainly focused on aboveground organs only, with not taking the role of the perennial structure (trunk and root) into consideration (Alexander, 1958; Lafon et al., 1965; Marocke, Balthazard, & Correge, 1976). Since the late 1980s, some studies, basically aimed at N, have been carried out for entire field-grown vines (Bates, Dunst, & Joy, 2002; Conradie, 1980, 1981, 1986; Hanson & Howell, 1995; Schreiner et al., 2006;
Treeby & Wheatley, 2006; Williams, 1991). They found that the N partitioning in perennial parts differ from annual parts and these were evident at different developmental stages and cultivars. It might be possible to apply nutrients in the correct amounts, at the correct times when seasonal nutrient uptake dynamics have been quantified and nutrient availability can be better predicted. Thus, it is important to understand the nutrient partitioning between the annual and perennial tissues of the grapevine during the growing season with the purpose of predicting vine nutrient requirements more accurately.

The grapevine, in general, is more likely to respond to N than P and K fertilization (Williams, 1946). The phenological development of the grapevine is dependent on the partitioning of N between the annual aboveground tissues throughout the growing season (Conradie, 1990, 1991, 1992; Glad et al., 1994; Peacock, Christensen, & Broadbent, 1989; Wermelinger & Koblet, 1990), and leaves and shoots are the main sinks for N prior to anthesis, with this requirement being met by N reserves from the permanent structure (root, trunk and cordon) initially (Löhnertz, 1991). Between budburst and the end of bloom, between 20 % and 40 % of N is remobilized from the roots, cordons, and trunk to shoots, leaves, and clusters (Conradie, 1980; Williams, 1991). From bunch closure to veraison, the permanent structures (root and trunk) regain some of this lost N (Bates, Dunst, & Joy, 2002; Conradie, 1980; Hanson & Howell, 1995; Löhnertz, 1991), but mainly the N is allocated to the developing clusters, with approximately 12 % being deposited in the leaves (Conradie, 1991). Different dynamics of N uptake was observed between the veraison and harvest period in a trial conducted in South Africa (Conradie, 1980), suggesting that 34 % of N observed in post-harvest period and most of the N was stored in roots. However, the partitioning of N remained
fairly consistent both the annual and perennial organs at the time of harvest. In pot-grown Chenin blanc (Conradie, 1980), the fraction of N varied between the different organs with greatest amounts in the new shoots and leaves (40 %), followed by the clusters (37 %), trunk and cordons (6 %), and roots (17 %). Similar results were found with mature Concord vines, at 53 %, 28 %, 8 %, and 11 % respectively (Hanson & Howell, 1995).

In warmer areas, active shoot growth slows down around fruit-set, but roots can also grow vigorously after harvest. This results in active uptake of N, and the largest percentage (68 %) of the N absorbed in this stage is retained in roots, trunk, and cordons, with the rest being lost through leaf fall and pruning (Conradie, 1986). In cooler regions, leaf canopies stay active for a shorter period after harvest (Hanson & Howell, 1995) relative to the warmer regions as leaf senescence and N deficiency may occur due to lower temperatures (Keller & Koblet, 1994). Most N usually moves from the senescing leaves back to the perennial structure during this period (Bates, Dunst, & Joy, 2002), with some lost through leaf abscission (Hanson & Howell, 1995).

The seasonal uptake of P, K, Ca, and Mg has been less intensively studied relative to N. Conradie (1981) reported that less than 10 % of the annual vine requirement for P, K, Ca, and Mg remobilizes from stored reserves in the trunk and roots of pot-grown vines. However in mature field-grown, rain fed ‘Pinot Noir’ grapevines it has been reported that about 50 % of canopy demands for N and P migrated from reserves in the trunk and roots during the period of fruit maturity, but only 15 % of canopy K and < 5 % of canopy requirement of Ca and Mg were derived from stored reserves (Schreiner et al., 2006). It was also reported that the concentration of soil NO3-N reached a maximum value between 520 and 550 degree days, suggesting that N availability from organic
sources in vineyards can be predicted using a degree-day-type model (Davenport, Bair, & Stevens, 2012).

The impact of N and other mineral nutrients on vine growth and reproductive development is dependent on the initial soil N status (Spayd et al., 1994), cultivar/rootstock (Treeby, Holzapfel, & Walker, 1996), climate (Löhnertz et al., 2000) and cultivation practices (Conradie, 2001). Therefore, nutritional studies in different regions are essential to better comprehend the consequence of nutrient application on vine development under different environmental conditions (Christensen, 1969).

2.4.5 Methodology to study nutrient uptake and partitioning- stable isotopes

Stable isotope techniques are valuable as they are nonradioactive tracers and non-destructive integrators which allow to study the response of plant growth to their abiotic and biotic environmental conditions. Specifically, it is a useful method to understand plant–resource acquisition, plant interactions with important plant resources such as carbon, water and nitrogen.

Stable isotopes including oxygen (\(^{18}\)O), carbon (\(^{13}\)C), nitrogen (\(^{15}\)N), hydrogen (D or \(^{2}\)H) and sulfur (\(^{34}\)S) can be estimated in plant tissues (Janina & Nino, 2002). Advanced technologies in mass spectrometry (i.e. multiple-collector inductively coupled plasma mass spectrometry) make it simple to measure isotope ratios in heavier stable elements, including Fe, Cu, Zn, Mo and others (Janina & Nino, 2002).

2.4.6 Methodology to study nutrient movement- sap collection

Xylem sap can be collected from grapevines in spring from cut spurs exuding sap as the result of root pressure (Nikolaou, Koukourikou, & Karagiannidis, 2000; Skene &
Antcliff, 1972) or from topped plants (Skene & Kerridge, 1967). Sap can also be collected from grapevine shoots and trunks under vacuum conditions (Bollard, 1953; Keller et al., 1995; Keller, Kummer, & Vasconcelos, 2001a). Although the vacuum extraction and bleeding sap collection are still used for studying xylem composition in field grown grapevines, both have limitations. The collection of bleeding sap is limited to several weeks either side of bud-burst, after that collection is strongly effected by environmental conditions. The vacuum extraction from excised shoots, is destructive and the sap may be contaminated from cells destroyed at the cut surface.

Alternatively, root–pressure chambers have been used to collect xylem sap from potted vines by gradually increasing the pressure on the roots until sap flows from a cut surface of a shoot or petiole. By using this method, large and clean xylem sap samples can be collected. Root pressure chambers have been applied for annual plants such as wheat (Passioura, 1988), sunflower (Schurr, 1998), cotton (Yong et al., 2000) and perennial plants such as apples (Atkinson & Else, 2001) and grapevines (Keller, Smith, & Bondada, 2006; Soar et al., 2008; Soar et al., 2004). Sap collected by this method can then be used to quantify organic and inorganic components and to gain a better idea of the mobilisation of these substances in response to seasonal changes or environmental conditions (Müller et al., 2015).

2.5 Conclusions

In viticulture, the belowground environment and its management are important for growth, productivity and fruit quality. Soil temperature is a critical determinant of root growth dynamics, with higher temperatures resulting in earlier bud break, elevated root growth. Dynamics of root growth can vary depending on climatic region, soil type or rootstock genotype and these differences are associated with changes of soil...
temperature. Moreover, soil temperature is critical for root development and shorter term fluctuations or longer term changes under global warming may have a significant influence on the root environment.

Past research associated with grapevine management and fruit quality has been predominantly undertaken on the aboveground parts, since studies on the physiology of roots are more complex. In addition, observations of root growth dynamics in higher plants either through non-destructive uninterrupted monitoring or destructive observation are complex. However, recent advances in technology and improved techniques have made it easier to monitor higher plant root systems without disturbances to the soil profile and with greater control. Techniques that take advantage of digital images utilising root cameras or root scanners in minirhizotrons (root growth monitoring systems) are substantially better methods for measuring long-term root growth dynamics at different soil depths for fruit tree species in natural field conditions.

In the hot grape growing regions of Australia, short-term and long-term root growth responses to the soil environment are of practical interest where manageable factors such as root-zone temperature and moisture can fluctuate significantly over a period of hours and days. The possibility of genotypic variation is also relevant when the widespread use of grafted grapevines in viticulture provides an opportunity to further select rootstocks based on suitability of root traits to particular environments. From a mechanistic perspective, a more detailed examination of the diurnal root growth dynamic would also allow links with carbon supply and the day-night 24 h (diel) cycle to be investigated.
This information is crucial to understanding what aspects of rootstock behavior in diurnal and seasonal root growth can be controlled by grapevine management, and what aspects need to be assessed for selecting rootstock varieties for new plantings. Assessing the responses of rootstocks coming from a range of environments to an altered soil temperature, would indicate the capacity to adapt in a warmer climate and would also be of importance for long-term sustainability of grape production.

2.6 References


Chapter 2 Literature Review


Chapter 2 Literature Review


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Chapter 3 Circadian Dynamics of Fine Root Growth in Grapevines

Paper 1: Circadian Dynamics of Fine Root Growth in Grapevines. Kare Palwan Mahmud, Yann Guisard, Suzy Yvonne Rogiers, Sharon Nielsen, Jason Patrick Smith, Bruno Peter Holzapfel. *Plant and Soil* (submitted and under review)

Synopsis

Previous studies on grapevine root systems have focused on root distribution and seasonal root growth dynamics. However, no information on diurnal root growth dynamics has yet been reported. This manuscript provides information on diurnal dynamics in root growth of one year-old own-rooted Shiraz and three *Vitis* rootstocks under natural environmental conditions. These vines were grown in transparent acrylic pots so that changes in root length could be scanned non-destructively at several points during the day. Linear mixed models were used to analyse the effect of soil temperature, genotype and other related parameters on diel root extension dynamics.

Key contents

- Diel root elongation profiles
- Different light period
- Constant warm soil temperature
- Linear mixed model
3.1 Abstract

Aims Circadian rhythms have been well described in above-ground plant growth, but far less for below-ground processes. The aim of this study was to characterize the diurnal fine root growth dynamic of four grapevine genotypes and to assess the impact of soil temperature on these dynamics.

Methods One-year-old vines of Shiraz (Vitis vinifera), and the rootstocks Ramsey (V. champinii), 140 Ruggeri (V. berlandieri x V. rupestris) and Schwarzmann (V. rupestris x V. riparia) were grown in 3.2 L tapered rectangular pots with transparent acrylic windows to assess diurnal root growth dynamics.

Results Genotypes displayed pronounced diurnal root growth dynamics that positively followed daily soil temperature fluctuations. Maximum growth rates were highest in the afternoon, declined through the night and reached a minimum the next morning. While diurnal root growth rates were influenced by soil temperature, this was not the only impacting factor. There were differences between these genotypes in the amount of root growth and diameter.

Conclusions Fine root growth rates of grapevines were found to have a pronounced diurnal dynamic that was independent of genotype. Therefore, the greater growth rate of fine roots in the afternoon could be a consequence of the interaction between endogenous circadian rhythms in these genotypes and environmental conditions.

Key words: Rootstocks, fine root dynamics, genotype, diurnal dynamics, soil temperature
3.2 Introduction

The root system is the most important carbohydrate and nutrient storage organ of grapevines (Bates et al., 2002). It has various other important functions, including anchorage, water and nutrient uptake. There are many environmental factors affecting diurnal and seasonal vine root growth. The seasonal dynamics of root growth is important in vineyard management as it may have consequences for the most appropriate timing of fertilization and therefore optimum growth and reproductive development (Eissenstat et al., 2006a). However, our current knowledge of how environmental factors regulate the timing of root growth is limited, particularly that of grapevine fine roots.

Fine roots play a significant role in water and nutrient uptake as the ability of nutrient uptake declines with root age (Comas et al., 2000; Volder et al., 2004). They stand for the most dynamic segment of the whole root system which can be replaced a number of times every year. Studies on the timing of fine root growth are limited because most of the methods used are time consuming, tedious, and destructive, particularly in perennial plants such as fruit trees. The grapevine root system is extensive and most of the past observational studies have been based on root biomass, root distribution and root anatomy (Callejas-Rodriguez et al., 2012; Galet 2000; Lehnart et al., 2008; Perry et al., 1983; Randall and Coombe 1978). Recent root observation techniques including minirhizotron inspection methods enable investigators to monitor and record roots directly and frequently in field conditions with a non-destructive process and give general information about the lifecycle of the root system, the location and diameter of roots, and total root length (Hendrick and Pregitzer 1992; Wells et al., 2002).
Chapter 3 Circadian Dynamics of Fine Root Growth in Grapevines

The seasonal alterations of root growth in grape as typically described by Mullins et al., (1992) showed that root growth in the grapevine is most pronounced between flowering and veraison and after harvest. In order to monitor seasonal root growth dynamics, roots have been observed fortnightly or monthly (Comas et al., 2005; Eissenstat et al., 2006b). However, root growth is also highly responsive to temporal changes in temperature (Walter et al., 2002) and it is therefore very important to predict the time of root growth in relation to soil temperature at critical development stages. More recent studies indicate that root elongation dynamics are influenced by different climatic and soil conditions and cultural practices (Eissenstat et al., 2006b). In these environmental conditions, soil temperature and moisture were, however, the most critical factors (Callejas et al., 2009; Franck et al., 2011; Pregitzer et al., 1993; Pregitzer et al., 2000).

Soil temperature has been recognized as the main modifier of root growth if all other factors that impact root growth are favorable. The root elongation rates of soybean decreased around 10% when the soil temperature was reduced by 10 °C from 25 °C to 15 °C, suggesting that the maximum root growth rate may be related to the maximum temperature in the afternoon (Passioura 2006). When the root-zone temperature was reduced from 20 to 10 °C, the induction of lateral roots of Zea mays was inhibited with a 60% reduction in lateral root growth and tap root length also was also decreased by approximately 42% eight days after sowing (Nagel et al., 2009). At a low root temperature, root water uptake declines because of decreased transmembrane transport in soybean and broccoli (Markhart et al., 1979), and this has a direct effect on the creation of adventitious fine roots associated with increased carbon fixation rates (Jesko et al., 1971; Pradubsuk and Davenport 2011). Some plants have an ability to adjust their root growth to new temperature zones within several minutes (Walter et al., 2002). Woodham and Alexander (1966) found that the optimal soil temperature for grapes is
around 30 °C, which was supported afterwards by Kliewer (1975). A study revealed that the daytime maximum and the night-time minimum in soil temperature can be offset from the air temperature (Walter et al., 2009); in this instance the air temperature varied by 16 °C and reached a maximum around 2 pm, whereas soil temperature at 10 cm depth changed by only 3 °C and reached a maximum at 4 pm. Soil management practices such as mulching impact on soil temperature dynamics and likely also affect root growth (Fourie and Freitag 2010).

There are few studies which have attempted to monitor the diurnal dynamics of root growth. The first daily root growth study was undertaken on cherry trees (*Prunus avium*) using time-lapse movies at four-hour intervals over several days (Head 1965). It was observed that the maximum growth rate appeared between 4 pm and midnight and the lowest growth rate occurred during the daytime between 8 am to 4 pm. However, environmental variables such as day length, soil temperature or moisture were not determined. In a different experiment the root elongation rates of five woody plants were assessed, including the grapevine. It was observed that the root growth rate of grapevines in the dark period was higher than during the day (Hilton and Khatamian 1973), but no statistical evidence was provided. Additionally, it was suggested that genotypes of *Arabidopsis thaliana* had different root growth dynamics and their diurnal growth dynamics and growth rate were affected by photoperiod or by additional sugar supply (Yazdanbakhsh and Fisahn 2011). Moreover, while the strong diurnal rhythms in the rates of root extension were regulated by carbon supply, it was also suggested that the circadian clock coordinates carbon allocation and root development (Yazdanbakhsh et al., 2011).
In the warm grape growing regions of Australia, root-zone temperature and moisture can fluctuate significantly over a period of hours and days, particularly in the upper soil layers. These fluctuations can impact on short-term root developmental responses to the soil environment, impacting on root processes such as nutrient uptake. Genotypic responses are also relevant to study of fine roots since grafted grapevines provide an opportunity to select rootstocks based on suitability of root traits to particular environments. The aim of the study was to understand the diversity of diurnal fine root growth dynamics across several different genotypes and to assess the impact of soil temperature on fine root growth. To understand the influence of soil temperature and the day-night cycle, a pot experiment was designed using four grapevine genotypes to characterize their diurnal fine root extension dynamics over a five day period.

### 3.3 Materials and methods

#### 3.3.1 Plant materials and experimental setup

The experiment was undertaken in an outside bird-proof enclosure located at the National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, New South Wales (Australia). One year-old Shiraz (*V. vinifera*) and three ungrafted rootstocks were used for the study, namely 140 Ruggeri (*V. berlandieri* × *V. rupestris*), Ramsey (*V. champinii*) and Schwarzmann (*V. riparia* × *V. rupestris*).

In 2012, the virus tested cuttings (Shiraz and the three rootstocks) were sourced from the Murrumbidgee Irrigation Area Vine Improvement Association (Griffith, NSW). The vines were propagated in a 100 % perlite mixture at approximately 22-26 °C under glass house conditions. After 40 days, the plants had developed sufficient roots and were planted into 5 L pots using as commercial potting mix for further plant establishment.
The vines were watered daily to field capacity using an automatic irrigation system during the summer and fertilized with 50 ml of 10:1 diluted complete liquid fertilizer (MEGAMIX PLUS)® fortnightly per vine. The vines were sprayed with sulphur (5 g/L) every 14-28 days during the growing season to prevent powdery mildew and grapevine blister-mite infections.

Prior to bud-burst in the 2013/14 growing season, pots were specifically designed to allow root observations. These pots were 310 mm in height and the non-visible sides had a width of 100 mm while the width of the visible sides narrowed from 130 mm at the top to 100 mm at the bottom of the pot. Therefore the sides of the visible panes slope from a wider top to a narrower base, generating a surface for downward growing roots to hit. (Fig 3-1 a). For the visible sides a 2 mm thick clear acrylic plastic was used, while the other two sides and the base section was composed of 10 mm thick grey PVC. The clear acrylic plastic was screwed onto the grey PVC plastic, the pot number and rootstock name were written on the right corner of both visible sides by using water proof labels (Fig 3-1 b). All the vines were pruned to two buds during dormancy in August 2013-14, and well developed, evenly sized vines were selected from each genotype (48 in total). The vines were removed from their pots and the root system was carefully washed. Based on visual observations at this point in time, Schwarzmann and Shiraz had similar root architecture with horizontal branching and more lateral roots while 140 Ruggeri and Ramsey had fewer lateral roots (Fig 3-1 d). The roots were trimmed to the same length (about half of their original length) for stimulation of root growth. After the trimming, the plant fresh weight was determined. They were subsequently repotted into the designed pots with premium garden mix. It was noted (prior to the experiment) that there appeared to be differences between genotypes. To confirm this observation, an analysis of plant fresh weight was done using a one-way
ANOVA in R. Schwarzmann had a higher fresh weight than the other three genotypes ($p = .001$) (Fig 3-2). Four pots, each containing a different genotypes, were placed into a larger black container (50 L) with drainage holes. Four vines were allocated randomly to each large black container filled with a 5 cm layer of river gravel (10-13 mm) at the bottom to provide free drainage (Fig 3-1 c). The container was then filled with red brick sand in order to keep the smaller pots in position and protect the acrylic plastic faces and emerging roots from direct sunlight. In addition, the sand provided a thermal buffer against daily air temperature variations. The large containers were evenly spaced in a grid measuring 6 m × 6 m and randomly positioned. The vines were sprayed with copper and Sulphur sprays during the growing season to prevent mite and fungus infections according to standard practice for the establishment phase. Each vine was fertilized with 100 ml of 10:1 diluted complete liquid fertilizer (MEGAMIX PLUS)® every 15 days during the growing season and watered to field capacity manually twice a day. All selected vines pruned to one short trunk with two spurs. Then two shoots were vertically trained and laterals were removed.

3.3.2 Soil temperature

Soil temperature was monitored with thermocron sensors (iButton thermochrons, range -30 °C to 85 °C, resolution 0.5/0.0625 °C) (OnSolution Pty Ltd, Baulkham Hills, NSW Australia) during the experiments. Sensors were programmed to collect temperature hourly, then they were placed into plastic bags in order to protect them from oxidization and inserted into the small pots at the depth of 10 cm. Data was retrieved using a thermocron logger connected to the Etemperature software (OnSolution Pty Ltd, Baulkham Hills, NSW Australia) via USB after the completion of the experiment. For the analysis, each individual potted plant contained one sensor and soil temperature was averaged for that particular pot since the last acquired image.
3.3.3 Aboveground plant measurements

Bud break was recorded at the start of the season, and was defined as the date at which the first bud on each vine reached growth stage 4 (green tip visible) according to the E-L system modified by Coombe (1995). Vegetative growth measurements were conducted every three days on all vines during the experiment. Shoot growth rates were determined by recording the two shoots per vine from the base at the spur junction to shoot apex. Total leaf area per vine was determined non-destructively at the end of the
experiment by measuring the length of the mid primary veins of each leaf as predictive variable. The leaf area was calculated using the following allometric relationship; Shiraz \((y = 0.06 x + 1.56 x^2)\), 140 Ruggeri \((y = 1.99 x + 0.72 x^2)\), Schwarzmann \((y = 1.86 x + 0.67 x^2)\) and Ramsey \((y = - 0.14 x + 1.19 x^2)\) (Smith 2004). Post-hoc analysis was applied on the original data for comparing the total leaf area by using a one-way ANOVA in R.

![Graph showing fresh weights of dormant Shiraz (SHZ), 140 Ruggeri (RUG), Schwarzmann (SCH) and Ramsey (RAM) plants prior to replanting after root pruning.]

**Fig 3-2** Fresh weights of dormant Shiraz (SHZ), 140 Ruggeri (RUG), Schwarzmann (SCH) and Ramsey (RAM) plants prior to replanting after root pruning.
3.3.4 Root image collection

After re-potting, root appearances through the plexiglass windows were assessed every afternoon at approximately 6 pm. At each assessment, 12 pots were taken out randomly from the black containers and the visible two sides were cleaned with soft paper towel to allow for clear observations. Once the fine roots started appearing (October 13), images of 48 plants were recorded at three day intervals until the start of the experiment. These images were obtained by using a modified scanner (Epson perfection 4990 photo, Seiko Epson Corp, Japan), with a high resolution setting of 600 DPI. The scanner had the lid removed and a 5 mm PVC frame was placed on the surface of a scanner to keep the pots at a fixed position during scanning. A 30 cm ruler and a flat digital watch were taped onto the scanner surface as a back-up record of the length and date/time of each image.

Four replicates (one pot is one replicate) of each of the four genotypes (16 pots) were randomly selected from the original set of 48 plants for intensive diurnal observation of the fine root growth. These observations were conducted three times a day, at 6 am, 2 pm and 8 pm over a period of 5 days and 10 hours. The images were obtained from 8 pm on October 25 to 8 pm on October 30 (2013) on both visible sides of each pot. After the images were acquired, the exact observation time was collected from each image file by using the MS-DOS directory, and then all the file names were renamed to suit the minirhizotron analysis software (Rootfly, Version 2.02.0, Clemson University, South Carolina, USA) and saved at a 200 DPI resolution. Dimensions of the images were calibrated in the Rootfly software using the ruler captured with each image in order to obtain a pixel/mm multiplier. After calibration each individual root length and diameter were traced carefully 16 times over the 5 days (Fig 3-3). Root diameters were measured at the middle of the root, root were classified according to their colour (white, light
brown, brown and dark) and root state (live or dead) were also recorded at the time of tracing. It is worth mentioning that roots are tapered at the growing tip, but very uniform in their diameter after the tip.

Fig 3-3 Screen shoot of the Rootfly software used to trace fine root growth in Shiraz and three rootstocks. The “tube” on the top right corner indicated the pot replicate (a). The scanned root image is from one side of the pot in the centre for root tracing (b) and the window on the left indicated the side of pot which was monitored; window 1 was side A (c) and window 2 was side B of the pot (d). A library of historical record on this pot is available below the main images (e). The ruler on the left side of the image was used for the calibration of pixels (f). A flat digital watch taped onto the surface to record the measurement date and time (g).
3.3.5 Data analysis

The obtained images provided information on a number of root parameters (root length, growth rate and diameter) which were assessed for relationships with each other and other factors (genotypes, soil temperature and day-night (light-dark)). Hourly root growth rate refers to the increase in root length per unit tip per unit time. Firstly, it was investigated if there was a relationship between total leaf area and root growth. In addition, it was assessed if the different type of roots by growth speed (classified by growth rates: fast, slow, stopped and no growth) were driven by genotypes, soil temperature or time of the day, or if this growth behavior was related to root color or diameter. The statistical software R (version 3.2.0) was used for data processing and analysis.

The response measurements were continuous and the relationships between the predictor variables and the response were complex. A linear mixed model in ASReml-R (asreml-3.0) was used to analyse the data. The italicized terms listed in the models below were fitted as random terms and observation time was nested within day in the repeated measurements all other predictor variables were fitted as fixed terms in the model. A different model was required for each aim; these can be symbolically written as:

a. Whole plant fresh weight ~ 1 + genotypes

b. Leaf area ~ 1 + genotypes

c. Total root length ~ 1 + soil temperature + genotypes + total root diameter + day and night + replicate + pot + observation time + day (and all the interactions of these variables)
d. Total root diameter ~ 1 + soil temperature + genotypes + total root length + day and night + replicate + pot + observation time + day (and all the interactions of these variables)

e. Hourly root growth rate ~ 1 + soil temperature + genotypes + total root diameter + day and night + replicate + pot + observation time + day (and all the interactions of these variables)

f. Hourly growth rate of different root types by rate of root growth ~ 1 + soil temperature + genotypes + root type + total root diameter + day and night + replicate + pot + observation time + day (and all the interactions of these variables)

The purpose of model f is to determine the factors that drive root growth for each root classification. The advantage of using this method over multiple regressions is that it keeps in line with the other models already assessed in this experiment. Using multiple regressions would be a different statistical approach, adding unnecessary complexity to the data analysis.

A significance level of 0.1, 1 and 5% were considered statistically significant in this analysis. The model assumptions were that the residuals were normally distributed; they had a constant variance and were independent. Also, that the factor level variances were equal for the treatments, this was tested using the Brown-Forsythe Test. The Shapiro-Wilk test of normality was used to determine if the residuals were normally distributed. All the assumption were tested in the analysis and unless otherwise mentioned were found to be met.
3.4 Results

3.4.1 Fine root population

Over the 5-days this experiment was conducted, a total population of 818 white or light brown fine roots were observed (Table 3-1). 140 Ruggeri and Schwarzmann had higher root numbers than Ramsey and Shiraz. 46.3% of all roots grew during the observation period. Those genotypes having fewer roots had a greater proportion of roots that were actively growing. Broadly, over half of the roots observed in Shiraz and Ramsey grew during the observation period as opposed to less than half of the Schwarzmann and 140 Ruggeri roots. A Chi-squared test of independence indicated that the number of actively growing roots was depended on genotypes ($p < 0.001$).

Table 3-1 The proportion of actively growing roots observed in Shiraz and three rootstocks over 5 days in well-watered plants grown in containers with Perspex viewing windows ($n = 4$ plants).

<table>
<thead>
<tr>
<th>Rootstocks</th>
<th>Total root number</th>
<th>Number of actively growing roots</th>
<th>Proportion of growing roots (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shiraz</td>
<td>84</td>
<td>47</td>
<td>55.9</td>
</tr>
<tr>
<td>140 Ruggeri</td>
<td>291</td>
<td>114</td>
<td>39.2</td>
</tr>
<tr>
<td>Schwarzmann</td>
<td>263</td>
<td>113</td>
<td>42.9</td>
</tr>
<tr>
<td>Ramsey</td>
<td>180</td>
<td>105</td>
<td>59.3</td>
</tr>
<tr>
<td>Total</td>
<td>818</td>
<td>379</td>
<td>46.3</td>
</tr>
</tbody>
</table>

3.4.2 ASReml model outputs

The parameters computed for the ASReml described in the Methods section are shown in Table 3-2. The outputs of each model is described in detail in the subsequent sections.
3.4.3 Leaf area

Schwarzmann had a significantly higher leaf area compared to the other three genotypes (Table 3-2), averaging at 591 cm$^2$ per plant ($p = .017$). There were no leaf area differences between Shiraz, Ramsey and 140 Ruggeri and averaged at 439, 400, 429 cm$^2$ per plant respectively (Fig 3-4). Furthermore, Schwarzmann and Ramsey displayed a much smaller interquartile range of leaf area than Shiraz and 140 Ruggeri. The leaf area measurement here comprised mostly of primary leaves with few lateral leaves. All leave areas were summed.
Chapter 3 Circadian Dynamics of Fine Root Growth in Grapevines

Table 3-2 Outcome of the ASReml analysis for six models described in the text. “—” indicates that the predictor variable was not used in the model. “***”, ** and * indicate significance at the 0.1, 1 and 5 % respectively. “ns” indicates no significant interaction when a term was used. The results of the random terms are not shown in this table.

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>Fresh weight</th>
<th>Leaf area</th>
<th>Total root length</th>
<th>Total root diameter</th>
<th>Hourly root growth rate</th>
<th>Hourly root growth rate of root types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>***</td>
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<tr>
<td>Soil temperature</td>
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<tr>
<td>Observation time</td>
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<tr>
<td>Day and night</td>
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<td>ns</td>
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<tr>
<td>Total root length</td>
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<td>***</td>
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<tr>
<td>Total root diameter</td>
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<td>—</td>
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<tr>
<td>Root type</td>
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<td>Genotypes : Soil temperature</td>
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<td>Total root diameter : Day and night</td>
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<td>Genotypes : Soil temperature : Day and night</td>
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<td>Genotypes : Soil temperature : Day and night : Total root length</td>
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<td>Genotypes : Soil temperature : Day and night : Total root diameter</td>
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<td>Genotypes : Soil temperature : Day and night : Total root diameter : Root type</td>
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</table>
Fig 3-4 Total leaf area per plant measured at the end of the root observation period. Both primary and lateral leaves were included in the total. SHZ = Shiraz, RUG = 140 Ruggeri, SCH = Schwarzmann, RAM = Ramsey. The central box presents the interquartile range of the observations, the horizontal marks the median and the lines extend to the largest and smallest observations.

3.4.4 Total fine root length and diameter

Total fine root length was a function of the combination of diameter, genotypes, soil temperature and the day-night (light-dark) period ($p = .008$) (Table 3-2). At the onset of the experiment the total fine root length differed between the four genotypes; 140 Ruggeri had the greatest total root length at 1223 mm, followed by Schwarzmann at 870
mm and Ramsey at 501 mm, while Shiraz had the smallest total root length at 264 mm. These differences were apparent over the duration of the experiment (Fig 3-5).

The diurnal timing of root elongation was similar across the four genotypes over the five days, regardless of the initial root numbers. Maximum root elongation and new root production occurred in the afternoon between 2 and 8 pm every day. There was no root growth observed between 6 am to 2 pm during the first three days and on the last day. However, growth of some individual roots occurred on the fourth day during this dark period. Because these roots were so few in number this is difficult to resolve in Fig 3-5. However for these few roots that did grow during the night, average growth rate was 0.08 mm/h.
Fig 3-5 Total root length of Shiraz and three rootstocks over five days (SHZ = Shiraz, RUG = 140 Ruggeri, SCH = Schwarzmann, RAM = Ramsey,). Bars indicate standard errors of the means for each observation time (n = 4).

Total root diameter varied significantly between genotypes (Table 3-2). Compared to Shiraz, 140 Ruggeri and Schwarzmann had greater total root diameter. Ramsey and Shiraz did not vary in all observation days. At the onset of the observations, Shiraz had 27 mm, Ramsey had 55 mm, 140 Ruggeri had 88 mm, and Schwarzmann had 75 mm. These differences were maintained throughout the 5 days despite an average 10% increase in root diameter. In addition, increases of total root diameter of Schwarzmann and Shiraz occurred on different days during the experiment. Schwarzmann maintained the same root diameter from the first day until third day at 2 pm, and then increased over the next two days, while Shiraz root diameter expanded only over the first three
days (Fig 3-6). Total root diameter was also significantly affected by total root length, genotypes, soil temperature and the day-night period ($p = .018$) (Table 3-2). Overall, the maximum enlargement of root diameter occurred between 2 pm and 8 pm, at the same time of root elongation (Fig 3-5).

![Graph showing changes in total root diameter over 5 days in four varieties.]

Fig 3-6 Changes in total root diameter as observed over 5 days in four varieties. SHZ = Shiraz, RUG = 140 Ruggeri, SCH = Schwarzmann, RAM = Ramsey. Bars indicate standard errors of the mean for each observation time ($n = 4$).

Total root length per observation window was positively correlated to the total root diameter per window with each genotype having a different linear relationship between these two parameters (Fig 3-7). Shiraz had the lowest total root length as it had the smallest total root diameter. Unlike other genotypes, 140 Ruggeri displayed a large number of roots with large diameter. This strongly affected the nature of regression for
this rootstock in Fig 3-7. As a result, 140 Ruggeri display a relationship between root diameter and root length that is significantly different from the other genotypes.

Fig 3-7 The relationship between total root diameters on total root length for four genotypes. The data presented are from each individual observation (plant and time). The equations of these four genotypes: SHZ = Shiraz ($y = -43.6 + 13.3 \times$, $R^2 = 0.927$); RUG = 140 Ruggeri ($y = -490.1 + 20.5 \times$, $R^2 = 0.930$); SCH = Schwarzmann ($y = 89.4 + 11.0 \times$, $R^2 = 0.957$); RAM = Ramsey ($y = -84.6 + 12.2 \times$, $R^2 = 0.866$).
3.4.5 Total fine root growth rate

In all genotypes, root growth rate was greatest in the afternoon, over the sampling interval between 2 and 8 pm, with the amplitude of the maximum root growth rate varying for each individual genotypes (Fig 3-8). The maximum growth rate differed between genotypes, 140 Ruggeri was consistently higher in the first four days than other genotypes. Ramsey had a higher maximum rate in the first three days than Schwarzmann, which increased in the last two days. Shiraz had a lowest maximum root growth rate in first and last two days of the experiment.

The total root growth rate was significantly affected by soil temperature ($p = .009$) and observation time ($p = .007$), but not by genotype ($p = .114$) or total root diameter ($p = .218$) (Table 3-2). The analysis does indicate that there was no significant effect of genotype, and this is likely because growth rates are minimal or even completely arrested during large parts of the day. The maximum soil temperature occurred between 3 and 6 pm every day simultaneously with the observed root growth peak throughout the experimental period. The minimum temperature appeared between 5 and 7 am (Fig 3-8), with no root growth observed during this period.

3.4.6 Fine root types by growth speed

Through image processing, it was found that roots could be classified according to their speed of growth using slopes of individual root length plotted over 5 day period. (a) Slopes $\geq 0.1$ mm were classified as fast growing roots, (b) $0.05 \leq$ slopes $<0.1$ mm were classified as slow growing roots, (c) $0.05 <$ slopes $> 0$ mm were classified as stopped growing, while (d) slopes$= 0$ mm were classified as not growing (Fig 3-9 a). Data from roots that were not growing were not further included in the growth rate calculations of this paper.
The growth rates of these root types were related to type of root \((p = .000)\), observation time \((p = .009)\), the day-night (light-dark) period \((p = .001)\) and soil temperature \((p = .003)\). There were no relationships apparent between the growth rates of genotypes \((p = .860)\), root diameter \((p = .114)\) (Table 3-2). Those roots that were classified as not growing occurred around approximately 54% of total root numbers (see Table 3-1) and represented with small root populations (Shiraz and Ramsey) had fewer non-growing roots than genotypes with large initial root population (Ruggeri and Schwarzmann). Interestingly, fast, slow and stopped growing roots had similar diurnal root growth dynamics (Fig 3-9 b, c and d).
3.4.7 Growth dynamics and soil temperature

Growth rate dynamics were positively related to soil temperature for all genotypes (Fig 3-10 a), but as described in Table 3-2, soil temperature was not the only predictor for root growth. Soil temperature during the experimental period showed maximum values between 2 pm to 6 pm (Fig 3-10 b). Root growth rates tends to display a potential threshold at 20 °C with little growth occurring below that temperature and no growth was observed below 12 °C. However, there were some individual roots which did not
grow or elongated slowly while the soil temperature was above 20 °C. The data that are presented indicate that 20 °C is a potential threshold but further work is required to substantiate this.

3.5 Discussion

Root zone temperature varies across grape growing regions in response to their differing climatic conditions. In addition, there are seasonal and daily fluctuations in these temperatures, which are particularly pronounced in the upper soil layer, impacting on short-term root growth. The genotypic diversity in rootstocks potentially allows for selection based on suitability to soil temperature in terms of root growth, and thus water and nutrient uptake. Since the time-lapse cinematography study on diurnal fine root growth of cherries by Head (1965), this study is the first to describe the diurnal dynamics of fine root growth of fruit crops, in particular grapevines.
Fig 3-9 Root classification by root accumulative length of Shiraz and three rootstock (SHZ = Shiraz, RUG = 140 Ruggeri, SCH = Schwarzmann, RAM = Ramsey) over five days (a); root growth rates of three different root types as classified by considering the slopes of individual roots (see in the text), fast growing roots (b), slow growing roots (c) and roots that stopped growing (d). Error bars indicate standard errors of the means for each calculated growth rate.
Fig 3-10 Relationship between soil temperature and hourly root growth rate of Shiraz (SHZ), 140 Ruggeri (RUG) and Schwarzmann (SCH), Ramsey (RAM). The vertical line at 20 °C indicates a potential threshold of the soil temperature, where root growth activity is significantly higher above 20 °C than between 10 °C to 20 °C (a). Comparison of hourly root growth rate at different period of a day under different soil temperature regimes (b). Each symbol represents an individual root (n = 818) over a five day period.

3.5.1 Fine root population

Results of this experiment indicated that there were three broad categories in total root numbers of these four genotypes (large, medium and small root system) (Table 3-1).
140 Ruggeri and Schwarzmann had large fine root systems, Ramsey had medium size root population and Shiraz had a small root system. These differences were apparent despite root pruning to the same root length across the four genotypes only 6 weeks earlier. Several root researchers have reported that root populations vary between rootstocks. Southey and Archer (1988) concluded that 140 Ruggeri had the greatest root density and root diameter compared to other rootstock genotypes such as Ramsey, Teleki and 99 Richter; these findings were also consistent with our results. Nagarajah (1987) found that Thompson seedless vines grafted to Ramsey had a widespread root system with greater root density, more fine roots and higher total root length than non-grafted ones. Ramsey, in this study had a medium size root population, being considerably larger that of Shiraz. However, root population and distribution is not totally depend on the rootstocks, environmental and soil condition can override genetic differences.

There were only two broad categories of actively growing fine roots in this experiment. Ramsey, Schwarzmann and 140 Ruggeri had more actively growing roots than Shiraz. However, the proportion of growing roots out of the total number of roots were slightly greater than 50 % for Shiraz and Ramsey and only approximately 40 % for Schwarzmann and 140 Ruggeri. The proportion of non-growing fine roots was higher in those varieties which had larger root populations. It may be argued that roots counted as “non-growing” an artefact of our method, such that the root tip may be mistaken for as bends in roots. However, care was taken to ensure that the root tips of the non-growing roots were visible throughout of the experiment. These roots displayed growth at certain periods of time, and then ceased to grow. In addition, similar to findings on young pea plants (Gersani et al., 1998; Hodge 2004), most of the non-growing roots were observed in the middle of the pots where there were higher proportions of roots than on the side.
of the observation window. These results indicate that plants with a large root system had a small proportion of actively growing fine roots than their counterparts with a smaller fine root system, which could be due to carbohydrate supply limitations (Ericsson and Persson 1980; Loescher et al., 1990).

3.5.2 Leaf area

Schwarzmann had approximately 50% larger and far less variable leaf size than the other genotypes (Fig 3-4). Overall, Shiraz and Ramsey displayed a small and medium number of fine roots, respectively, with small total leaf areas; 140 Ruggeri had more fine roots with a small leaf area, while Schwarzmann was characterized with a large number of fine roots and a large leaf area. The allometric relationship between fine root population and leaf area is therefore inconsistent. Buttrose (1966) suggested that the root biomass of Gordo grapevines increased significantly with the increases of leaf area. In Vitis vinifera defoliation stimulated the number of fine and extension roots (Hunter et al., 1995). The findings in this experiment indicate that there is a genetic difference in the relationship between the fine root system and leaf area, which can be influenced by abiotic factors such as temperature in the root zone.

3.5.3 Total fine root length

Soil temperature, root diameter, rootstock and the day-night (light-dark) period were significantly related to total root length during this five day period. Root growth was most rapid between 2 pm and 8 pm every afternoon; the growth was more pronounced for 140 Ruggeri and Ramsey than for Schwarzmann and Shiraz. Some individual root growth was also observed at night time (8 pm to 6 am), while the slowest root growth occurred between 6 am to 2 pm. These findings indicate that there is a consistent root growth dynamics over the course of the day, which could be directly or indirectly
related to a circadian clock. Higher plants can predict the day–night cycle in their environment by way of a circadian clock (Harmer 2009). The growth of leaves (Nozue et al., 2007; Nozue and Maloof 2006) and roots (Yazdanbakhsh and Fisahn 2011; Yazdanbakhsh et al., 2011) of Arabidopsis thaliana have clear rhythmic dynamics with respect to the time of day. Similarly, cherry fine roots showed significant elongation rates at night in contrast to the day (Head 1965). Presence of diurnal rhythms in plants can regulate various biochemical and physiological factors. Although these 24 hour rhythms are endogenous, they are entrained to the surrounding environment by external cues which include light, temperature (Geiger and Servaites 1994). Therefore, circadian rhythms are not necessarily consistent across the season. A study presenting diurnal time-lapse root elongation on five perennials including grape, apple, poplar, quince and red pine in a field situation. Hilton and Khatamian (1973) showed that elongation rates during the day-night varied at different times of the season. In late-spring and midsummer, roots of five woody plants developed more in the night than during the day, but in late summer and autumn only quince roots grew faster in the night than during the day. These findings differed to the observation in this study which was carried out in spring; here the root elongation of grape genotypes was most rapid in the late afternoon.

The four grapevine genotypes of our study were planted on the same day, adjusted to the same root length and developed under the same conditions. However, at the start of the root observation (42 days after replanting), the initial total root length displayed a fourfold difference between the genotypes. These differences were not reflected in final total root length at the end of the experiment, 140 Ruggeri produced more roots and had a greater total root length, while Shiraz had the lowest, Ramsey and Schwarzmann were in between. The contribution to the total root length was also a function of root 104
numbers, but the two were not necessarily correlated. Similar findings were also found by Southey and Archer (1988), however, rooting dynamics likely do not only depend on rootstocks, but also the scion rootstock interaction with the root system (Daulta and Chauhan 1980).

### 3.5.4 Total root diameter

The findings confirmed that total root diameter per vine was related to total root length but also to genotypes, root temperature and the day-night period. As for the root length, the initial total root diameter and final root diameter displayed a fourfold difference between genotypes. The total root diameter was greater in 140 Ruggeri, Schwarzmann and Ramsey than in Shiraz. From these observations it was found that some individual roots of 140 Ruggeri had larger root diameter and longer root length range compared to other genotypes. In a separate study, the total root numbers of 140 Ruggeri in the 0.5-10 mm diameter zone doubled compared to other rootstocks such as Paulsen 1103 and others (Southey and Archer 1988). In addition, the root diameter increased at the same time as root elongation, but the fluctuation in root diameter in each genotype was different.

The four genotypes display genotypic differences in regard to the growth of their total root diameter. Under the conditions of this experiment, the increase in Shiraz total root diameter was not very significant. The relationship between total root diameter and total root length was more pronounced in 140 Ruggeri than in any of the other genotypes. Shiraz displayed the smallest root length and diameter; while the root diameter and length of Ramsey and Schwarzmann was larger, but less than that of 140 Ruggeri. Shiraz had a small fine root system (associated with a small leaf area); Ramsey had a fine root system of medium size (associated with a large leaf area); Schwarzmann’s fine
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root system was of medium size (associated with a small leaf area) while 140 Ruggeri had a large system of fine roots (associated with a small leaf area). Overall, the relationship between total root diameter and total root length (per pot) was inconsistent. This provides clear evidence of genetic differences in the relationship between total root length and diameter of the fine root system across the genotypes. These results indicate that when attempting to predict the physical characteristics of fine root systems, a genotype based approach is recommended.

3.5.5 Fine root growth rate and soil temperature

This diurnal fine growth study was conducted at the flowering stage of development, a stage where root growth in grapevines has been observed to be most pronounced (Eissenstat et al., 2006b; Freeman and Smart 1976). In general, the environmental conditions at this stage are conducive for plant growth. It is believed that soil temperature is a main impacting factor for root growth when other environmental conditions are favourable (Callejas et al., 2009). In this experiment, the rate of fine root growth increased with rising soil temperature. The maximum daily soil temperature corresponded with the maximum hourly root growth rate (length and diameter) in the afternoon between 2 pm to 8 pm. The minimum root growth rate also correlated with minimum soil temperature from 6 am to 2 pm. In this study, maximum root growth rate occurred between 20 to 25 °C and reached 2.3 mm per hour. This is comparable with annual crops, such as sorghum at approximately 4 mm and rice at 1.5 mm per hour (Iijima et al., 1998), but the growth rates of Arabidopsis thaliana was much less pronounced at 0.4 mm per hour (Yazdanbakhsh and Fisahn 2011). Growth rates greatly decreased to around 25 % between 10 and 20 °C and a minimum growth rate was reached when the temperature range was between 5 to 10 °C. This compares with a 10 % reduction in root growth rate of soybean (Passioura 2006). Lateral root induction of
Ricinus communis is strongly inhibited when root-zone temperature decreased from 20 to 10 °C (Poire et al., 2010) and, likewise, root branching in Shiraz grapevines increased with soil heat unit accumulation (Clarke et al., 2015). However, even under the higher temperature conditions that occurred in the mornings of the last 3 days of observation in our study, some individual roots in each genotype kept minimum or zero growth rates. Although the maximum daily growth rate consistently occurred between 2 pm and 8 pm, the absolute maximum root growth rate of these four genotypes varied over the experiment. The lowest rate was observed on the third day of the experiment and reached a maximum value on the fourth day. Interestingly, on this third day the minimum soil temperature was higher than the other days, but growth rate was lowest. Overall, it can be concluded that soil temperature is not the only factor that influences fine root growth in grapevines, but rather other endogenous and exogenous factors also likely play a role.

### 3.5.6 Classification of fine root types by rate of growth

Of all the root tips that were observed in these four genotypes more than 50 % did not grow over the 5 days. Over the same period of time, the growth rate of the actively growing roots was diverse; some roots were fast growing, some were slow while others were growing for a defined period and then stopped. Some roots only grew during the middle two or three days of the observation time. This characterisation of the roots by growth rate was also significantly influenced by the day-night cycle. It is likely that the divergent growth behaviour of these roots is a consequence of, at least partly, competition between individuals. Because of the uneven concentration and distribution of local nutrients in soil (Zhang et al., 1999), plant roots actively encounter and forage soil nutrient hot spots (Hodge 2004) and also avoid the areas with high root numbers (Gersani et al., 1998). These responses to nutrient availability may affect plant root
production and distribution. Fine root extension therefore tends to be faster than other types of roots when roots grow into a patch with nutrient higher and less root siblings and density. Furthermore, because of roots were hidden by the growing media, it was not clear where and when they are started to grow. The classification was made based on the visible roots once they can be seen on the monitoring window. It is difficult to identify where these visible roots originally came from, if they are main roots or lateral roots. However, this classification will suggest that subsequent studies should try to attempt to monitor the origin of the emergence of fine roots.

Combining fine root population, leaf area, fine root morphology (total length and diameter) and the classification data we can summarize the following. Firstly, Shiraz displayed a small root system (relative to the other rootstocks), composed of few roots and associated with a medium (even if variable) leaf area. Secondly, Schwarzmann and Ramsey had medium sized root systems associated with either a medium (Ramsey) or large (Schwarzmann) leaf area. There were interactions between total root length, total root diameter, genotype, soil temperature and day-night. However, the maximum or minimum growth rate of fine roots was only affected by soil temperature. No doubt, other internal and external factors also need to be taken into consideration in further diurnal root studies. Further research defining the integration and control of circadian rhythms, carbon supply from photosynthesis and/or reserve mobilization, nutrient uptake and root elongation is required.

3.6 Conclusions

In this diurnal fine root growth study of grapevine rootstocks, we observed that the fine root population differed from one genotype to another and the proportion of active growing roots were higher in genotype which had less roots. The total fine root length
was influenced by genotype, soil temperature, fine root diameter and day-night. However, diurnal fine root elongation rate correlated only with soil temperature and there were no significant differences between genotypes. The maximum rate of root growth occurred in the afternoon between 2 pm and 8 pm with the minimum between 6 am and 2 pm. Most interestingly, these growth dynamics were maintained throughout the observational period regardless of genotypes or speed of root growth. The daily fluctuations in soil temperature was not the only factor to impact fine root elongation as some individual roots underwent slow growth rates even under higher temperatures. Overall, during the 24 h day-night cycle, grapevine fine root growth was defined with clear extension dynamics regardless of genotypes. The influence of soil temperature on fine root elongation rates is likely to be the source of considerable variability in this dynamics. Therefore, characterizing root behaviour and diurnal root growth dynamics in grapevines will provide grape growers the practical means to select rootstocks and to understand the implications of short-term environmental effects on root growth dynamics.

3.7 Acknowledgments

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3.8 References


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Chapter 3 Circadian Dynamics of Fine Root Growth in Grapevines


Chapter 4 Diel Root Growth Cycles in Grapevines are Controlled by a Circadian Clock

**Paper 2:** Diel Root Growth Cycles in Grapevines are Controlled by a Circadian Clock.
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*Annals of Botany* (under submission)

**Synopsis**
In our previous findings, grapevines displayed a clear diel root growth dynamics under naturally fluctuating environmental conditions and root growth was positively correlated with soil temperature. This manuscript reports a novel study on diel root elongation dynamics under different photoperiod regimes where soil temperature was held constant or increased. Linear mixed models were used to analyse the effect of photoperiod, soil temperature, genotype and other related parameters on diel root extension dynamics.

**Key contents**
- Diel root elongation profiles
- Different photoperiod period
- Consistent and warm soil temperature
- Linear mixed model
4.1 Abstract

While extensive research has focused on grapevine root growth at weekly to seasonal time scales, relatively little attention has been given to diel dynamics in root growth. Grapevines have a pronounced diel rhythm in fine root growth but the internal and exogenous factors that regulate this daily cycle in fine root growth have not yet been characterized.

In this particular study, the fine root system of grapevines was monitored over a period of ten days with a high resolution scanner, under constant soil moisture and three different photoperiod regimes: (1) typical fixed photoperiod (14 h light/10 h dark) with oscillating soil temperature (22 °C → 30 °C → 22 °C), (2) delayed photoperiod (shifted later by a 4 h interval every second day) under constant soil temperature (22 °C) and (3) shortening day length (shortened by a 4 h interval every second day) under constant soil temperature (22 °C). Digital root images and shoot length measurements were collected and diel root and shoot elongation dynamics were characterised.

Shiraz and Ramsey exhibited pronounced diel rhythms in shoot and root growth rates under a typical, fixed photoperiod. Maximal root growth rate occurred between 6-10 pm, equivalent to 1-2 hours prior to and 2 hours after the onset of darkness. Subsequently, during the latter part of the dark period, root growth rate decreased and reached minimal values 6-10 am in the early morning, at the onset of the light period. In the typical photoperiod, the 30 °C soil temperature negatively affected root growth by about 50 % while it positively influenced shoot growth. Notably, the shoot extension rate peaks shifted from late afternoon to midnight at this higher temperature zone.
In the delayed photoperiod and progressively shortening photoperiod studies, the diel changes in root growth rate followed the same dynamics as in the fixed photoperiod study with the maximum occurring at 6-10 pm and the minimum occurring at 6-10 am, regardless of whether the plant was in light or dark. This suggests that light was not the direct impacting factor for stimulating root elongation. Maximum shoot growth rates were not fixed to a clock, however, and shifted into the dark period, while minimum rates always occurred at the end of the dark period regardless of the time of day.

Overall, fine root growth rate of grapevines were found to have a pronounced diel dynamics that was independent of genotype. An internal circadian clock, independent of light/dark cues, appears to be a major determinant of this diel rhythm. Soil temperature was found to modify the amplitude of this dynamics, but we argue here that carbon supply from photosynthesis is also required to maintain maximum root growth.

**Key words:** Grapevine, diel cycle, fine root growth, diurnal rhythms, image analysis, shoot growth

### 4.2 Introduction

Due to the rotation of the Earth, diel (24 h) cycles have evolved within living organisms (Buijs & Escobar, 2007). Plant growth is greatly rhythmic with respect to the time of day and accordingly many physiological and biochemical parameters are modulated in concert with the diel cycle (Geiger & Servaites, 1994; Head, 1965; Achim Walter, Silk, & Schurr, 2009; Yazdanbakhsh, Sulpice, Graf, Stitt, & Fisahn, 2011). The circadian clock, synchronized with daily environmental changes, allows plants to adjust and adapt to a changing environment on either side of the soil surface.
Chapter 4 Diel Root Growth Cycles in Grapevines are Controlled by a Circadian Clock

The first study on diel dynamics of fine root growth in plants dates back to 1965, focusing on diurnal root extension rates of cherry (Head, 1965). Using time-lapse movies, with 4 hour intervals, it was found that the maximum root extension rate appeared between late afternoon (4 – 8 pm) and at night (8 pm -12 am) with minimum root growth occurring between morning (8 am – 12 pm) and noon (12 pm- 2 pm). Unfortunately, in this early study of cherry roots, there were no exogenous data offered to interpret the observed dynamics. A subsequent study presenting day-night root elongation data on five perennials including grape, apple, poplar, quince and red pine in a field situation (Hilton & Khatamian, 1973) revealed that elongation rates during the day-night varied at different times over the growing season. In late spring and midsummer, roots of all five woody plants developed more during the night than during the day, but in late summer and autumn only quince roots grew faster during the night than during the day. More recent studies on the diel oscillations in root tip growth in Arabidopsis thaliana indicated highest growth rates one hour before dawn and a minimum at dusk (Yazdanbacksshi and Fisahn 2010). These dynamics continued under constant conditions in this species as well as in a number of annual plants such as Oryza sativa (Iijima, Oribe, Horibe, & Kono, 1998), Sorghum bicolor (Iijima et al., 1998), Zea mays (Walter et al., 2002), and Nicotina tabacum (Achim Walter & Schurr, 2005).

In the grapevine industry, understanding the timing of root growth in the short and long-term is an important aspect of sustainable vineyard management as it has consequences for nutrient and water uptake by the vine. In the hot grape growing regions of Australia, short-term root growth responses to the soil environment are of practical interest where manageable factors such as root-zone temperature and moisture can fluctuate significantly over a period of hours and days. Genotypic variation is also relevant because the widespread use of grafted grapevines in viticulture provides an opportunity.
to select rootstocks based on suitability of root traits to specific environments. Unfortunately, fundamental information on root physiology and viticultural recommendations on best practises for optimal root function is inadequate (Eissenstat et al., 2006; Mullins, Bouquet, & Williams, 1992). This is in part because, unlike aboveground plant tissues, the observation of root growth dynamics in higher plants either through non-destructive uninterrupted monitoring or destructive observation is difficult and therefore more complex. Recent studies demonstrated that advanced technologies and techniques have made it easier to monitor higher plant root systems without disturbances to the soil. Techniques such as minirhizotron root camera (Bartz Technology Corp, CA, USA) or CI-600 root growth monitoring system (CID, Inc. Camas, WA, USA) are found to be appropriate modern methods for measuring root biomass and long-term root growth dynamics in field conditions (Anderson et al., 2003; Jose et al., 2001; Muñoz-Romero, López-Bellido, & López-Bellido, 2011; Wells, Glenn, & Eissenstat, 2002).

Our previous study on young potted plants in outdoor conditions demonstrated that the grapevine root system has a pronounced diel root growth dynamic and that it was positively correlated to soil temperature with a maximum root growth rate between 20 – 27 °C. However, we found that some individual roots had lower growth rates compared to others, even when exposed to optimum soil temperature regimes. From a mechanistic perspective, a more detailed examination of the diel root growth dynamic may reveal other factors driving root growth. This paper summarizes the results of three controlled environment studies on two grapevine species. These three studies were designed to better understand the driving factors for the daily cycles in fine root growth. The first study addresses the impact of temperature on root growth rates by exposing vines to a fixed 14 h photoperiod and an increase in 10 °C soil temperature over 5 days. The
second study investigates the role of the day/night cue by delaying the light period by 4 h every second day but maintaining a 14 h light period and constant soil temperature. This was achieved by increasing the night period by 4 h every second day. The third study examines the role of day length by shortening the light period by 4 hours every second day until vines were grown in constant darkness. These vines were also maintained at a constant soil temperature. All vines were watered precisely to field capacity four times a day to remove any confounding effects of soil moisture.

4.3 Materials and methods

4.3.1 Culture of plants

In this experiment, we used own rooted Shiraz (V. vinifera) and ungrafted rootstock Ramsey (V. champinii). In 2012, the virus tested cuttings were sourced from the Murrumbidgee Irrigation Area Vine Improvement Association (http://www.avia.org.au/). The vines were propagated in a 100 % perlite mixture at approximately 22-26 °C under glass house conditions. After 40 days, the plants had developed sufficient roots and were planted into 5 L pots using a commercial potting mix for further plant establishment. Vine fertilizer application, irrigation regime and disease management are as described in the Materials and Methods of Chapter 3.

In August 2013-14, all the vines were pruned to two buds during dormancy. Well developed and evenly grown vines were selected from each variety (18 plants in total). Before re-potting the vines with premium garden mix, into their specific pots, they were washed carefully and the roots were trimmed to half of the original length. Subsequently, the fresh mass of the whole bare rooted plants was assessed. Total plant fresh mass of Shiraz was highly significantly different from Ramsey vines (p = .0043).
Ramsey had less fresh weight than Shiraz. Once re-potted, the vines were sprayed with preventive copper and sulphur sprays to prevent mite and fungus infections and were fertilized using 100 ml of 10:1 diluted complete liquid fertilizer (MEGAMIX PLUS) every 15 days.

4.3.2 Transparent pot assembly

Prior to bud-burst in the 2013/14 growing season, purposely-built pots were designed to allow for the visual observation and measurement of growing roots. The pot design is as described in Chapter 3 Materials and Methods. The pot number and cultivar names were written on the right corner of both visible sides by using water proof labels (Fig 3-1 B). After plants were potted in these transparent pots, they were placed into larger black light-proof containers (50 L) in sets of four (Fig 3-1 C). The vines were allocated randomly in a position in the large black containers on a 5 cm layer of river gravel (10-13 mm) to provide free drainage. The remaining space in the black containers between the four inserted pots was carefully filled with red brick sand in order to keep the smaller pots in position and to protect the acrylic plastic and emerging roots from direct sunlight.

4.3.3 Controlled environment conditions

This experiment was conducted in three large walk-in growth chambers (TH 6000, Thermoline Scientific, Smithfield, NSW, Australia) at the National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, New South Wales (Australia). Light intensity was measured with light sensors (Apogee, Logan UT, USA) at mid canopy height. The photon flux density (PFD) was 350 µmol m⁻² s⁻¹ and provided by a lighting system comprising of four 1 kW Metal Halide lamps (HQ I-T 1000D, Osram Powerstar, Germany). The relative humidity in the three chambers was kept between
approximately 40 and 45 % day and night. Carbon dioxide concentrations were not controlled in the growth cabinets.

The soil temperature was monitored with thermocron sensors (iButton thermochron, range -30 °C to 85 °C, resolution 0.5/0.0625°) (OnSolution Pty Ltd, Baulkham Hills, NSW, Australia) during the experiments. The sensors were programmed to collect soil temperature hourly, then were placed into plastic bags in order to protect them from oxidization and inserted into the transparent pots at a depth of 10 cm. Data were retrieved from these temperature sensors using a thermocron logger connected to the Etemperature software (OnSolution Pty Ltd, Baulkham Hills, NSW Australia) via USB after the completion of the experiment (Fig 4-1). For the analysis, one sensor was placed in each individual potted plant and soil temperature was averaged for that particular pot since the last acquired image.

Each individual pot was watered precisely to field-capacity four times a day during the experiment. Prior to the onset of observations, the pot was over-watered until excess water drained from the bottom of the pot. The pot was then allowed to drain until the point where no more water exited and its mass was recorded. With each root scan, the pot was weighed and the amount of water lost over the last 4 hours was replaced with an equivalent amount plus an additional 10 % of the original weight of the pot to account for increases in biomass.

Plants were exposed to the same typical photoperiod (14 h light/10 h dark) four days prior to the experiment in each chamber. The experiment was started from the fifth day under the same conditions when roots in all three chambers were monitored for two days. Chamber A (typical, fixed photoperiod) was used as an environment in which the
day/night period was typical of the ambient environment at this time of the year (14 h light/10 h dark) (Fig 4-1 A). The air and soil temperature of this chamber was kept constant for the first two days and last three days of the experiment at 22 °C. From day 3 to day 7 air and soil temperature was elevated to around 30 °C in order to investigate the response of higher temperature on fine root growth (Fig 4-1 A).
**Fig 4-1** Soil temperature fluctuations in each chamber. The soil temperature was constant at around 22 °C with fluctuations between 20 to 23 °C in the first two days and last three days in chamber A, and was elevated to around 30 °C from day 3 to day 7 with a fluctuation of 29 °C to 32 °C (figure A). The soil temperature fluctuations in chamber B and C were maintained at around 22 °C with fluctuations between 20 to 23 °C (figure B and C). Each data point is an average of hourly soil temperature recorded from the pots. Typical = Typical photoperiod, Shifting = Delayed photoperiod, Shortening = Progressively shortening photoperiod. Dark grey shadings indicate the dark period.

Plants in Chamber B and C were also exposed to a 14 h light /10 h dark photoperiod for the first two days of measurement. The photoperiod was delayed in Chamber B by increasing the dark period by 4 h every second day. The light period was maintained at
14 h. Over the ten days the plants experienced the following conditions: 14/10 (L / D), 14/10, 14/14, 14/10, 14/14, 14/10, 14/14, 14/10, 14/10, 14/10. The photoperiod was shortened by 4 h intervals every two days in Chamber C until continual darkness: 14/10, 14/10, 10/14, 10/14, 6/18, 6/18, 2/22, 2/96. Over the 10 day period soil temperature was held constant at 22 °C in Chambers B and C (Fig 4-1 B and C).

4.3.4 Digital root image collection

A modified photo scanner (Epson perfection 4990 photo, Seiko Epson Corp, JAPAN) was used to capture the root images from the potted vines. The lid of the scanner was removed and a 5 mm PVC frame was placed on the surface of a scanner to keep the pots at a fixed position during the scan. To obtain high resolution images, a 600 DPI setting was used for scanning. A 30 cm ruler and a flat digital watch was taped onto the surface to record the length and exact measurement date and time. Digital root image collections of Shiraz and Ramsey vines (n = 9 in each specie) with three replicates were started two days prior to the photoperiod treatment. Images were taken simultaneously at four-hour intervals from 0600 h to 2200 h every day over a period of 10 days. Following image capture, the exact observation time was collected from each image file by using the MS-DOS directory, and then all the file names were renamed to accommodate for the Rootfly (Version 2.02.0) image processing software (Rootfly, Clemson University, South Carolina, USA) which was released under the GNU General Public License (GPL) (Clemson University, South Carolins, USA). This software is typically used for minirhizotron image analysis. The original high resolution images (600 DPI) were then converted to 200 DPI for root tracing in the Rootfly software. Before starting root tracing, the measurement units in the software were calibrated (pixels per mm) using the ruler image in each scan. Ten active roots (5 roots per window) were randomly chosen and increases in root length were monitored four times.
daily so that results are in mm/root tip/unit time. Each individual root length and
diameter were traced manually 50 times over the 10 days as shown on Fig 3-3. Root ID,
root colour (white, light brown, brown and dark) and root state (live or dead) were also
recorded at the time of tracing. This image information was exported into Microsoft
Excel for further analysis.

4.3.5 Aboveground plant measurements
 Shoot length was recorded simultaneously with the root scan. For the shoot growth
measurements, bamboo sticks were tightly fixed in the pots and used as references for
the shoot length measurements. Shoots were tied to these sticks in a vertically straight
fashion and on the first time of the measurement, shoot tips were carefully laid on the
sticks and a mark was made to indicate the initial growing point. Every 4 hours
thereafter the shoot length was measured from this initial point with a ruler and recorded.

4.3.6 Data analysis
 The following concept was tested:
 Diel fine root growth dynamics (as described by average root length, average shoot
length, hourly root and shoot growth rate) is driven by photoperiod, genotype, root
diameter, soil temperature, and time of day or combination of these factors.

The response measurements in this experiment were found to be continuous and the
relationships between the predictor variables and the response were complex. A linear
mixed model in ASReml-R (cite R and ASReml R) was used to analyse the data.
The models were organised in three blocks of repeating models. The first block (models a to d) excluded the period of 30 °C in chamber A (typical photoperiod) and aimed at comparing periods of similar soil temperature regimes. The second block (models e to h) included the period of 30 °C in the chamber A and therefore examined the entire experimental period over the three chambers. This period of varying temperature was treated as two distinct variables. Soil temperature (ST) represented the period aligned with the other chambers and CTEMP represented the period of increased temperature in chamber A. The last block of models (i to l) included the period of 30 °C in the chamber A, but excluded the treatments as a factor. This was done to better examine the dynamics of the response variable within each treatment (Table 4-1).

For example, the full syntax for model a is as follows (note that underlined random terms are common to all models and observation time was nested within day in the repeated measurements; all other predictor variables are fitted as fixed terms in the model):

a. Average root length ~ 1 + treatment (typical photoperiod, shifting and shortening) + soil temperature (not including 30 °C ) + genotypes + average shoot length + average root diameter + replicate + pot + observation time + day (and all the interactions of these variables)

For the model assumptions, a significance level of 5 % has been used in this paper. The model assumptions are that the residuals are normally distributed; they have a constant variance and are independent. Finally, the factor level variances are equal for the treatments, as tested using the Brown-Forsythe Test. The Shapiro-Wilk test of normality
is used to determine if the residuals are normally distributed. All the assumptions were
tested in the analysis and unless otherwise mentioned were found to be met.

Table 4-1 Different models used for different aims. All the random terms in the
model not shown in this table. RS = Genotypes; ST = Soil temperature; LGZ =
Root or shoot (length or growth rate) (it depends on response); DIA = Root
diameter.

<table>
<thead>
<tr>
<th>Model</th>
<th>Response Variable</th>
<th>Treatment (TRT)</th>
<th>Soil Temperature (ST)</th>
<th>Genotype (RS)</th>
<th>Average shoot length</th>
<th>Average root diameter (DIA)</th>
<th>Average root length</th>
<th>Hourly shoot growth rate</th>
<th>Hourly root growth rate</th>
</tr>
</thead>
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<td>a</td>
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<td>✔️ (LGZ)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>b</td>
<td>Average shoot length</td>
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<td>✔️ 2</td>
<td>✔️</td>
<td>✔️ (LGZ)</td>
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<tr>
<td>c</td>
<td>Hourly root growth rate</td>
<td>✔️ 1</td>
<td>✔️ 2</td>
<td>✔️</td>
<td>✔️</td>
<td></td>
<td></td>
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<td>(LGZ)</td>
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<td>d</td>
<td>Hourly shoot growth rate</td>
<td>✔️ 1</td>
<td>✔️ 2</td>
<td>✔️</td>
<td>✔️</td>
<td></td>
<td></td>
<td></td>
<td>(LGZ)</td>
</tr>
<tr>
<td>e</td>
<td>Average root length</td>
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<td>✔️ 3</td>
<td>✔️</td>
<td>✔️ (LGZ)</td>
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<td></td>
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<tr>
<td>f</td>
<td>Average shoot length</td>
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<td>✔️ 3</td>
<td>✔️</td>
<td>✔️</td>
<td></td>
<td></td>
<td></td>
<td>(LGZ)</td>
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<td>✔️ 3</td>
<td>✔️</td>
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<td></td>
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<td>✔️ 3</td>
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<td>j</td>
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<td>k</td>
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<td>l</td>
<td>Hourly shoot growth rate</td>
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</table>

1typical photoperiod or shifting or shortening (as factor levels); 2not including 30°C; 3including 30 °C; 4typical photoperiod or shifting or shortening
4.4 Results

4.4.1 ASReml model outputs

The parameters computed for the ASReml Models described in the Methods section (Table 4-1) are shown in Tables 4-2, 4-3 and 4-4. The outputs of each model are described in detail in the subsequent sections.

The ASReml models a-d for root and shoot growth under different photoperiod treatments (not including the hot temperature period in typical photoperiod) (Table 4-2) showed that the treatment and genotype or their combinations with other factors significantly affected root length (model a). Root growth rate (model b) was the function of soil temperature and root diameter and displayed small interactions of root diameter with soil temperature or shoot growth rate. The average shoot length however (model c), was affected by root diameter or influenced by combination of all the predictors with relatively strong interactions. The shoot growth rate was not impacted by any predictors except the combination of treatment and soil temperature. Hence, we suggest that different illumination treatments had only significant effects on root length and other responses through interactions. For example, root length was a function of treatment (TRT) and genotype (RS) and root growth rate was influenced by interaction of soil temperature (ST) and root diameter (DIA).
Table 4-2: Outcome of the ASReml analysis from the model of a, b, c and d described in the table 1. This result is including three treatments with the exception of the data during the hot temperature zone. “***; ** and *” indicate significance at the 0.1, 1 and 5 % respectively. “Blank” indicates no significant interaction when a term was used. The results of the random terms are not shown in this table. TRT = different photoperiod treatment; RS = genotype; ST = soil temperature of 22 °C; LGZ = Root or shoot (length or growth rate) (it depends on response); DIA = Root diameter.

<table>
<thead>
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<th>Predictor variables</th>
<th>Root length a</th>
<th>Root growth rate c</th>
<th>Shoot length b</th>
<th>Shoot growth rate d</th>
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<tr>
<td>DIA</td>
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<td>LGZ : DIA</td>
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</tbody>
</table>
Chapter 4 Diel Root Growth Cycles in Grapevines are Controlled by a Circadian Clock

When the hot soil temperature zone (CTEMP) was included in the typical photoperiod treatment (Models from e-h; Table 4-3), root length, root growth rate and shoot length were significantly affected by CTEMP or its interactions with other factors, but not treatment. For root growth models (e-h), the models are similar to (a-b), with the addition of root or shoot length (LGZ) and hot temperature zone in typical photoperiod condition (CTEMP) for the e model and CTEMP for the f model. For root models (g-h) the inclusion of CTEMP as a predictor change the nature of the model c, d. TRT became a significant predictor of shoot growth rate (h) when compared to (e), even though CTEMP is a not significant predictor. Overall, the Models from a to d and the Models from e to h suggest that in order to better understand the nature of the relationship between predictor and response variables, the data could be broken down by treatment (TRT) (Table 4-4).

The linear mixed models (ASReml models i-l for the root growth parameters in typical photoperiod; Table 4-4) indicate that the growth rate of shoots (model l) was only influenced by the genotypic factor, with a small interaction with soil temperature. The average shoot length however (model k), was influence by all predictors, with some interactions, all including the genotype predictor. We therefore suggest that genotype is a strong predictor factor for average shoot growth and growth rate in this treatment (typical photoperiod) where temperature varied over the experiment. The soil temperature was a significant factor in all treatments for root length and root extension rate. Shoot length or root length (LGZ) was also significant in all treatments for root growth or shoot growth. Moreover, root growth rate strongly correlated with soil temperature even it fluctuated in a small range and was also influenced by a combination of genotype and shoot growth rate under the conditions of the shortening photoperiod.
Table 4-3 Outcome of the ASReml analysis from the model of e, f, g and h described in the table 1. This result includes the data from the elevated temperature zone in the typical photoperiod treatment. “***”, ** and * indicate significance at the 0.1, 1 and 5 % respectively. “Blank” indicates no significant interaction when a term was used. The results of the random terms are not shown in this table. TRT = different photoperiod treatment; RS = genotypes; ST = soil temperature of 22 °C; LGZ = root or shoot (length or growth rate) (it depends on response); DIA = root diameter. CTEMP = soil temperature higher zone in typical photoperiod treatment; “—” indicates the variable or interaction should not be considered for analysis as it was not included in the model.

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<th>Predictor variables</th>
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<th>Shoot length c</th>
<th>Shoot growth rate d</th>
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</table>
Table 4-4 Outcome of the ASReml analysis from the models of i, j, k and l as described in Table 1. This result shows the interactions in a treatment individually. “—”indicates that the predictor variable was not used in the model. “***; ** and *” indicate significance at the 0.1, 1 and 5 % respectively. “Blank” indicates no significant interaction when a term was used. The results of the random terms are not shown in this table. TRT = different photoperiod treatment; RS = genotypes; ST = soil temperature of 22 °C; LGZ = root or shoot (length or growth rate) (it depends on response); DIA = root diameter; DN = day and night; TOD = Observation time of day. CTEMP = soil temperature during the higher zone in the typical photoperiod treatment.

<table>
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<td>ST:LGZ:DIA</td>
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<td>RS:ST:CTEMP</td>
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<td>RS:ST:TOD</td>
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<td>LGZ:DIA:CTEMP</td>
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4.4.2 Diel root and shoot elongation dynamics of grapevines under typical photoperiod conditions

Under a typical photoperiod, both Shiraz and Ramsey species clearly displayed the presence of highly pronounced diel rhythms in root growth under both consistent (22 °C) and higher temperature (30 °C) regimes (Fig 4-2). The maximum growth rate occurred between late afternoon and two hours after dusk; then root extension during darkness declined and the minimum rates in root elongation appeared shortly after re-illumination. The maximum root elongation rates decreased by around 50% from day 3 to day 7 where root-zone temperature increased from approximately 22 °C to 30 °C. However, after the soil temperature returned to 22 °C, the root growth increased again and maximum growth rate normalized. Shiraz vines displayed greater root development rates compared to Ramsey upon returning to 22 °C (Fig 4-2). Overall, as a factor however, these difference were not significant over the duration of the experiment.

Diel rhythms of shoot growth in these two species differed slightly from those of the root growth. In general, shoots of Shiraz exhibited maximal growth activity late in the light period and early in the dark period in the first five days and last three days (somewhat similarly to root behaviour). However, maximum shoot elongation shifted into the absolute darkness period on day 6 and 7 in Shiraz and on day 4 and 8 in Ramsey. The growth dynamics of shoots of both genotypes displayed a positive relationship with CTEMP temperature. Ramsey consistently displayed a greater shoot growth rate than Shiraz (Fig 4-2).

Average root length (model i) and its associated root growth rate (model j) were significantly influenced by temperature of the soil (ST and CTEMP) as well as the length/rate of shoot growth (LGZ) (Table 4-4). Genotype was however involved in a
range of interactions, suggesting that it is an important variable in these models, but not as a specific factor and could therefore have a moderation effect. Moreover, these data indicate that shoot dynamics are dominated by genotype, but root dynamics are dominated by temperature related variables, in the context of the typical photoperiod treatment. They also demonstrate that the significance of the root length: shoot length relationships was highly significant in all cases (Table 4-4).

**Fig 4-2** Average root and shoot extension rate under a typical photoperiod which was similar to the natural photoperiod outside (14/10 h L/D). Average root growth rate was calculated using the active growing roots \((n = 10)\); two sides of each pot were observed and on each side \((n = 5)\) roots were selected randomly. Average shoot growth rate was calculated on two shoots per plant \((n = 6)\); \(T = 22 \, ^\circ\text{C} \) (first two days and last three days); \(T = 30 \, ^\circ\text{C} \) (day 3 to day 7). RS = genotype; RAM = Ramsey; SHZ = Shiraz. Error bars indicate average standard errors (SE) for each observation time for root growth across all the days.
4.4.3 Diel root and shoot elongation dynamics of grapevines under a delayed photoperiod

Plants grown under a delayed photoperiod showed no genotypic (RS) differences in both root and shoot length/growth rate (model I, j, k, l) (Table 4-4). Prior to the experiment, all vines were growing in similar day-night conditions (14/10 h L/D) for two days. The delayed light period was commenced on the evening of the second day. In order to shift the day period by 4 hour intervals and to keep the day length the same at 14 hours, the night period was increased to 14 hours every second day. Shiraz and Ramsey exhibited clear diel root growth dynamics in the first two days, showing maximum root extension rates at the onset of the dark period, at 8 pm while minimal root growth peaks occurred at the end of the dark period and early morning at 6-10 am (Fig 4-3). This dynamics in root growth became less defined once the first shift had taken place, however the general trends remained the same. Over the next 6 days, maximum root growth rates in both genotypes tended to occur consistently at around 6-10 pm, and minimums at 6-10 am, but less so in Shiraz over the last two days. Importantly, because of the delay in the onset of the light period root growth was no longer co-ordinated with photoperiod. For example, on day 5, 6 and 7, the maximum root growth occurred in the middle of the light period and was lowest near the beginning of the dark period. By day 9 and 10 the maximum root growth occurred in the middle of the dark period, in complete contrast to the first days prior to the first shift.

Diel shoot elongation dynamics mimicked that of the root elongation dynamics over the first two days of the study with a maximum just at the onset of the darkness, at around 6-10 pm, and a minimum at the termination of darkness at 6-10 am (Fig 4-3). Similar to the roots, the diel cycles in shoot growth were also less defined once the first shift had taken place. However, in contrast to root growth rates, shoot growth rates tended to shift
along with the photoperiod so that maximum growth rates consistently occurred at some point during the dark period and minimums occurred during the early part of the light period. This resulted in periods where maximum root growth rates coincided with minimum shoot growth rates.

Fig 4-3 Average root and shoot extension rate in response to a delayed photoperiod; the photoperiod was kept similar to natural outdoor conditions for the first two days (14/10 h L/D) and from this point shifted forward by 4 h every second day (14/14 h L/D). Average root growth rate was calculated by using only the actively growing roots (n = 10); two sides of the pots were observed and on each side five roots were selected randomly. Average shoot growth rate was calculated on two shoots per plant (n = 6); T = 22 °C. RS = genotype; RAM = Ramsey; SHZ = Shiraz. Error bars indicate average standard errors (SE) for each observation time for root growth across all the days.
4.4.4 Diel root and shoot elongation rates of grapevines under progressively shortening light periods

All the factors and some of their combinations significantly affected average root length (model i). Shoot length, however, was influenced by average root length and its interaction with genotype and diameter under this condition (model k). Finally, soil temperature was a main factor regulating root growth rate (model j) (Table 4-4). Overall, soil temperature was significant to root growth, but not shoot growth; genotype was significant to root length. Only root growth rate and shoot growth rate (LGZ) was consistently significant to root length and shoot length.

In this treatment, progressively shortening the photoperiod while maintaining a constant temperature gradually shifted the maximum root elongation rate peaks towards the central part of the dark period (Fig 4-4). As in the other two studies, maximum growth rate tended to occur at around 6-10 pm, and minimums at 6-10 am. These dynamics were consistent regardless of shortening day light. Slowly and consistently, the magnitude of maximum rate tended to decline with the increasing length of the dark period. Notably, a diel root growth dynamic was still observed in progressively shortening day lengths beyond continual darkness at day 7 and root growth did not completely cease until early on day 10. During the intervening three days, a reduced diel fluctuation was still evident. Overall, Ramsey had higher root growth rates relative to Shiraz roots.

Conversely, Shiraz and Ramsey exhibited identical shoot growth dynamics (Fig 4-4). The maximum and minimum shoot elongation rates occurred at about the same time as root growth throughout the entire observation period. Unlike the decline in root growth rate from Day 5, shoot growth rate only began to reduce gradually once absolute
darkness was initiated. On the last day, it was also observed that the apical meristem of the shoots started wilting despite ample soil moisture. This suggests a preferential allocation of growth resources toward the shoot rather than root. Even in total darkness, the shoots continued to grow for a longer period than the roots.

![Graph](image)

**Fig 4-4** Average root and shoot extension rate in a progressively shortening photoperiod; the dark period was increased by 4 h every second day from Day 3. The chambers were kept under a constant temperature of 22 °C. The average root elongation rate was calculated from the actively growing roots only \((n = 10)\); two sides of the pots were observed and on each side five roots were selected randomly. The average shoot growth rate was calculated on two shoots per plant \((n = 6)\); \(T = 22 \, ^\circ\text{C}\). RS = genotype; RAM = Ramsey; SHZ = Shiraz. Error bars indicate average standard errors (SE) for each observation time for root growth across all the days.

Overall significant differences were found in root and shoot growth across the treatments. Regardless of the exclusion or inclusion of the warm temperature zone (CTEMP) in the typical photoperiod treatment, the root length was affected by
Chapter 4 Diel Root Growth Cycles in Grapevines are Controlled by a Circadian Clock

treatment (TRT) and genotype (RS) (Table 4-2 and Table 4-3). CTEMP was a significant factor affecting shoot length (Table 4-3). Genotype did not display any difference in root and shoot growth rate in those two different analysis (CTEMP included and excluded). However, root growth rate had a significant difference when the hot temperature was included or excluded (Table 4-2 and Table 4-3). Most of the other interactions contributed mainly to root and shoot length and very few combinations affected root and shoot growth rate. Data in Table 4-4 clearly demonstrate that the root length and shoot length relationship was significant in all treatments, while root diameter and its interaction with other factors contributed to root length and shoot length in this progressively shortening photoperiod condition.

4.5 Discussion

In our preliminary study on diel root elongation dynamics of young potted grapevines under outdoor conditions (as outlined in Chapter 3), it was found that different genotypes exhibited similar diel root extension dynamics despite dissimilar root populations. It was evident that these diel root elongation dynamics were associated with changes in temperature, but it was surmised that this may not be the only factor regulating diel root growth dynamics. For elucidation on the potential role of light in the diel rhythmicity of grapevine roots, plants were grown under three different photoperiod regimes. The role of root zone temperature on these dynamics was also clarified. The interaction between clock time, light and soil temperature was investigated here in two grapevine genotypes under constant ample soil moisture. The three sets of models used to analyse root growth rate allowed the assessment of the various factors across the three studies and within each study. These studies were only 10 days in duration and therefore few, if any, ontogenic changes were likely, thus assuring that the outcomes of the statistical analyses were robust.
Under a photoperiod typical to what a field grapevine may experience, the elongation rate of actively growing roots was found to have a pronounced diel dynamics. The maximum growth rates, which ranged from 0.10 to 0.38 mm hour$^{-1}$ across the three treatments, were highest in the late afternoon and early darkness (6 - 10 pm), then declined through the night and reached a minimum in the next morning (6 - 10 am). This dynamics was observed across both genotypes and resembles those described in cherry by Head (1965). The maximum rate of root growth also occurred late in the afternoon and after dusk in this perennial species. The reduction in root growth rates at the end of the night period and the first part of the re-illumination period may be explained by reduced carbon availability (Gibon et al., 2004; Thimm et al., 2004).

4.5.1 **Grapevines displayed a diel root growth dynamics under a typical photoperiod irrespective of soil temperature increases.**

Maximum root growth rates of Ramsey and Shiraz vines declined by 50 % when exposed to 30 °C relative to 22 °C temperatures. Despite this drastic decline in growth rates the diel fluctuations were still evident with a minimum occurring at 6 - 10 am. In our previous experiment, we found that the grapevine root system had little growth when soil temperature was at 11 °C and it reached maximum growth at 25 °C (Fig 3-8 and 3-10). There is no conflict between the results of Chapter 3 and this chapter. In chapter 3, we found that the maximum root growth occurred between 20 °C to 25 °C (Fig 3-10) and 30 °C was not examined. In chapter 4, root growth was greater at 22 than at 30 °C and therefore when the data of the two studies are considered together, a decline in root growth appears to occur at some point between 25 and 30 °C. Others have shown that the optimal soil temperature for grapevines is close to 30 °C (Woodham & Alexander, 1966) or between 25 to 30 °C (Kliwer, 1975). However,
Skene and Kerridge (1967) highlighted that roots were longer and thinner in diameter at 30 °C than those at 20 °C. There were no such relationship between root length and diameter found in this study (Table 4-3).

Notably, the diel fluctuations in shoot growth matched with that of the root growth with the maxima and minima occurring at the same time. Unlike the decline in root growth rates during the 30 °C temperature period, however, shoot growth rates were elevated in both Shiraz and Ramsey. Both air and soil temperature were increased within the chambers and therefore alterations in shoot growth may be the consequence of air or soil temperature, or both. Bevington and Castle (1985) demonstrated that in citrus trees the number of growing roots and rate of root growth decreased during the period of shoot elongation. This result supports our findings in this treatment, suggesting that shoot growth could be overriding root growth at these higher temperatures, possibly because shoots are a preferential sink to roots.

4.5.2 Grapevine root growth continued to follow a 24 h ‘time’ clock despite a delayed photoperiod

Roots continued to grow at maximum rates at 6-10 pm and minimum rates at 6-10 am regardless of the light cycle. As a result of the shift in the photoperiod, maximum root growth occurred in the middle of the light period as well as the dark period. This indicates that the day/night cue was not regulating the daily cycling in root growth in grapevines. Soil moisture was also not driving this rhythm because this variable was carefully controlled at four points during the day, so that it remained constant throughout the 24 h cycle. Similarly, soil temperature was maintained within a tight window. Moreover, as a result of the shift in photoperiod, maximum root growth rates coincided with minimum shoot growth rates, thus indicating that the two growth
dynamics were not linked. As such, it appears that newly fixed photoassimilates were not acting as a signal to drive increased root growth. Having removed the effect of light under constant soil moisture and temperature we can suggest that root growth follows a ‘time’ clock rather than a ‘light’ clock.

4.5.3 **Grapevines presented a 24 h diel root growth cycle under gradually shortening day periods and this cycle was present in absolute darkness.**

The circadian clock of plants is known to persist in continuous light (Yazdanbakhsh and Fisahn 2009) and dark (Dunlap, Loros, & DeCoursey, 2005). As such, the presence of the diel dynamics in absolute darkness indicates that grapevine root growth follows a 24 h endogenous circadian clock. That said, this growth rhythm became weaker and then absent on the second and third day of absolute darkness, suggesting that illumination is necessary for further rhythmic growth beyond this point.

Shortening the day length to 6 h from the fifth day in this study caused a concurrent reduction in daily root and shoot growth rate, although root growth did not completely cease until early on day 10. The decline in root growth rates in response to a shorter photoperiod suggests that root growth was resourced by a carbohydrate supply from photosynthesis. The starch metabolism mutants and wild-type plants of *A. thaliana* grown under three different photoperiods exhibited dissimilar growth dynamics in their diel kinetics that were also influenced by photoperiod. Unlike grapevines, *A. thaliana* root growth is highest at night and this appears to be fuelled by the degradation of starch (Yazdanbakhsh & Fisahn, 2011). It has been shown that starch degradation occurs steadily through the night to support root growth (Graf et al., 2010, Yazdanbakhsh et al., 2011). However, when plants of *A. thaliana* were placed in an equal day/night cycle, starch depletion occurred around 4 h before the next dawn (Graf et al., 2010). The
differences in root elongation rates during the night period between small annual plants and perennial fruit crops, could possibly be related to the greater demand for starch and its rapid subsequent degradation after the onset of dark in the larger plants.

4.6 Conclusion

Grapevine root growth exhibits a reproducible endogenous circadian rhythm. The onset and length of each individual cycle was not entrained by an altered photoperiod over several days. The amplitude of maximum root growth was, however, strongly affected by photoperiod and soil temperature and it is suggested that carbon supply may be an underlying factor.

4.7 Acknowledgment

This work was supported by the National Wine and Grape Industry Centre, Charles Sturt University and New South Wales Department of Primary Industry. We thank Rob Lamont and Helen Pan for their contributions.

4.8 References


Chapter 4 Diel Root Growth Cycles in Grapevines are Controlled by a Circadian Clock


Chapter 4 Diel Root Growth Cycles in Grapevines are Controlled by a Circadian Clock


Chapter 5 Diurnal Root Growth Dynamics in Mature Grapevines


Synopsis

In our previous two experiments characterizing root growth behaviour in young potted vines, we observed pronounced diurnal growth dynamics across all genotypes and this was apparent under naturally fluctuating environmental conditions and also in controlled environments. The emphasis of this work was to determine if this diurnal dynamics was also evident in large container-grown mature Shiraz vines.

Key contents

- Six year-old Shiraz vines
- Field-like conditions
- Diurnal root elongation profiles
- Air, canopy and soil temperature
5.1 Abstract

Six-year-old Shiraz vines were grown under field-like conditions in 780 L soil filled plastic bins. One year after transplanting, root growth was monitored non-destructively using minirhizotron tubes. Images of the roots were collected four times a day at sunrise, midday, sunset and midnight over six consecutive days during flowering. It was found that the vines displayed a diurnal dynamic in root growth with a maximum in the afternoon and evening, and a minimum in the morning. While it was not possible to separate the effects of day-night, light and temperature a positive relationship between soil temperature and root elongation was observed. The diurnal dynamics of root growth was consistent with earlier observations in young potted vines, and is the first indication that mature vines also exhibit a pronounced diurnal dynamics of fine root growth. The possible influence of carbon supply and the role of circadian clock in these diurnal growth dynamics are discussed.

Key words: Diel root profile, root extension rate, Shiraz, field-like conditions, temperature

5.2 Introduction

Root observation studies in grapevines have clearly indicated that root density (Daulta and Chauhan 1980; Southey and Archer 1988), root distribution (Bassoi et al., 2002; Morano and Kliewer 1994; Pradubsuk and Davenport 2011) and seasonal root growth dynamics (Bonomelli et al., 2012; Eisenstat et al., 2006; Mullins et al., 1992) are influenced by the soil environment (Callejas et al., 2009; Comas et al., 2010; Stevens and Douglas 1994), genotype (Swanepoel and Southey 1989) and aboveground phenology (Bonomelli et al., 2012). A significant amount of information is available in
the grapevine literature on root growth dynamics at weekly to seasonal time scales. However, understanding factors that influence root growth dynamics over the season requires a better understanding of factors determining short-term root growth and initiation.

A study on day vs. night root growth dynamics of five woody species showed that the root growth rate of all five plants was higher at night than during the day (Hilton and Khatamian 1973). In addition, time-lapse cinematography was used to study diurnal behaviour in cherry roots. It was found that maximum root elongation rates appeared between 1600 and 2400 h and reached minimum growth rates between 0800 and 1600 h (Head 1965). Environmental factors were not, however, analyzed in these two studies. Complementing these root elongation reports, diurnal variation in root diameter was examined in relation to net radiation in cotton lateral roots. It was found that root diameter increased late at night when water appeared, then shrinkage occurred when roots lost water faster than they could absorb it, especially on sunny days (Huck et al., 1970). For grapevines, daily changes in root growth were studied at different times in the growing season (Hilton and Khatamian 1973), describing daily mean root elongation and soil temperature over a nine day period. There has been no information on diurnal root growth dynamics in relation to diurnal fluctuation of soil temperature reported.

Very few studies have attempted to characterize short-term root growth dynamics under natural conditions in any species. In our previous work on young potted grapevines we found pronounced diurnal dynamics and we believe this is the first report on this phenomenon in *Vitis vinifera* (Chapter 3 and 4). This diurnal dynamics was characterized with a maximum growth rate in the afternoon and a minimum growth rate
in the morning. Moreover, it was determined that those dynamics were closely related to the soil temperature. In order to characterize the root growth dynamics of more mature grapevines with an expanding root system, diurnal root growth was monitored using minirhizotron techniques in six-year-old Shiraz vines transplanted from large pots one year previously into large bins.

5.3 Materials and methods

5.3.1 Plant material and minirhizotron tube installation

This study was conducted in a bird proof enclosure at the national Wine and Grape Industry Centre located at Charles Sturt University, Wagga Wagga, New South Wales, Australia. In two concrete trenches 12.2 m long × 1.2 m wide × 1m deep six 780 L plastic bins were installed in each trench, spaced 60 cm apart. Similar sized four-year-old own rooted Shiraz vines, previously grown outdoors in large pots, were selected and one was planted in each of the 12 bins in August 2013 (Fig 5-1). Two polycarbonate tubes (100 cm length × 5.5 cm in diameter) were installed in a north-south direction at a 30° angle in each bin to a depth of 60 cm. This allowed for miniature camera system access (Bartz Technology Crop, Santa Barbara, CA, USA) and thus frequent root observation non-destructively (Fig 5-1). The length of the tube below ground level was 80 cm and the distance from the center line of the tube to the inside edge of the bin was 10 – 12 cm. After installation of the plants, tubes and soil temperature sensors, the bins were filled with a garden soil mix and watered. The vines were six years old and had been in the bins for one season prior to onset of root-imaging from 4th to 9th November 2014.
5.3.2 Radiation and soil temperature sensor installation and data collection

A photosynthetically active radiation (PAR) sensor was installed above canopy height prior to the experiment, logging at 15 minute intervals. The PAR readings were used to divide up the 24 h measurement period into meaningful intervals across the day/night cycle (Fig 5-2 b). T-type thermocouples (Jonley, Pty Ltd, NSW, Australia) were used to
measure air, canopy and soil temperature in one of the 12 bins. Air and canopy temperature was monitored with the thermocouples placed 50 cm above the canopy and within the middle of the canopy midway along one cordon. The other thermocouples were distributed at 22 cm, 44 cm, 80 cm and 99 cm horizontally and at 15 cm, 30 cm, 45 cm and 60 cm vertically. In this experiment, 9 thermocouples in close proximity to the tubes were used for monitoring soil temperature at four depths (Fig 5-2 a). All thermocouples were connected to a 12 Channel Temperature Recorder (Lutron BTM-4208SD, InstrumentCatalog™, SA, Australia) in a water-proof container, the temperature was recorded on an hourly basis.

Vines were irrigated by hand on a daily basis with a 2-gallon watering can. In order to maintain soil moisture at field capacity, TDR probes were installed in each bin at the depth of 8 cm and 48 cm in soil profile. Then soil moisture was monitored at each observation time by a computer via a RS-232 port connected to a TDR MiniTRASE Kit (ICT, international, Australia). Fertilizer application was applied manually by using liquid fertilizer (MEGAMIX PLUS)® every 15 days during the growing season.
Fig 5-2 Diurnal cycles in air, canopy and soil temperature at four soil depths (a); Diurnal cycle of photosynthetically active radiation (PAR) (b).

5.3.3 Root observation

Five days prior to the onset of the experiment, 12 tubes were selected from 12 vines and root growth was monitored once a day during the flowering period. Based on these five days of observation, root distribution dynamics were assessed with depth, with an even number of windows per depth across the vines, and actively growing roots were identified ($n = 68$). Diurnal root elongation was monitored by capturing images (Fig 5-2 b) at midnight (0030 to 0130 h), sunrise (0530 to 0630 h), midday (1230 to 1330 h) and sunset (1930 to 2030 h) over a period of six days. The first observation occurred at noon
on the 4th of November and the last one at noon on the 9th of November 2014. Average root length and growth rate was calculated per window.

A minirhizotron camera (Bartz Technology Crop, Santa Barbara, CA, USA) was used for image collection. Before capturing the root images, the focus of the camera was optimized and the same pixel resolution was used over the entire experimental period. After digital image collection, root images were processed by Rootfly software (Version 2.02.0) (Rootfly, Clemson University, South Carolina, USA). The image dimensions were calibrated by using a reference image inside the case of the minirhizotron camera before tracing the root growth dynamics. After calibration, all the growing roots were traced and assessed manually in the images using the software above. Root diameters were measured at the middle along the vertical length of the roots. All growing roots were classified as either (1) continuously growing roots, (2) roots that had been growing and then stopped growing or (3) new growing roots for subsequent data analysis. The continuously growing roots were those that were present at the start and kept growing through the entire period. New growing roots were those that appeared in the imaging windows during the experiment. Data from all 50 windows, including images captured at the greatest depth, have been included in the analyses. All the results were exported to Excel to further analysis with Asreml (asreml-3.0) in R (version 3.2.0).

5.3.4 Statistical analysis

In this experiment, the collected images provided information on fine root length and root growth rate, both which were continuous responses. The relationships between the root growth profiles and other factors such as soil temperature, soil depth and day-night were assessed.
Prior to statistical analysis of diurnal root growth in relation to soil temperature, the soil temperature data (recorded at hourly intervals) between each observation time was averaged to match the observation time. Then a linear mixed model in ASReml-R (cite R and Asreml R) was used for further analysis. The italicized terms listed in the models below are fitted as random terms; all other predictor variables are fitted as fixed terms in the model. These can be symbolically written as:

**Average root length** ~ depth + root type (continuously growing roots, newly merged roots) + day and night + soil temperature + \( observation \ time \) + day (and all the interactions of these variables)

**Average root growth rate** ~ depth + root type (continuously growing roots, newly merged roots) + day and night + soil temperature + \( observation \ time \) + day (and all the interactions of these variables)

For the model assumptions, significance levels of 0.1, 1 and 5 % were used. The model assumptions are that the residuals are normally distributed; they have a constant variance and are independent. Also, that the factor level variances are equal for the treatments, this is tested using the Brown-Forsythe Test. The Shapiro-Wilk test of normality is used to determine if the residuals are normally distributed. All the assumption were tested in the analysis and unless otherwise mentioned were found to be met.

### 5.4 Results and discussion

In this study, total root numbers, average root length and root growth rates were studied in mature ungrafted Shiraz vines across a 14 h photoperiod over six days. Clear diurnal
elongation dynamics in fine root growth were evident, with maximum root growth rates in the afternoon and minimum extension rates in the morning.

5.4.1 Root distribution with soil depth

Roots were vertically distributed along the depth of the bin down to 52 cm. Root presence differed through the soil profile, with the greatest number of roots as well as total root length at the 48-52 cm soil depth, followed by 16-20 cm and 36-40 cm (Fig 5-3). The root distributions that were observed in these large vines grown in spacious bins under a natural outdoor temperature regime are similar to those profiles that were observed in mature vines grown in field conditions (Fig 6-4). Furthermore, root size, color and branching order were very similar to the field observations. These vines were growing in a reasonable soil volume and this particular non-destructive monitoring facility allowed the adoption of the same camera system as in the field. The vines had only been transplanted one year previously, which would have initiated many new growing roots, this made them easy to find using the minirhizotrons. The garden mix was uniform, dark and also provided good contrast against the white roots relative to the red field soil. That said, these data may indicate that the roots had reached the bottom of the bin and then grew horizontally and this may suggest a depth restriction of the bin system relative to field conditions. However, under this condition, the soil is much easier to penetrate, that might have been another reason that they got down relatively quickly.
Fig 5-3 Root distribution dynamics with depth. Roots counts and total length per window in minirhizotron viewing areas by depth. Data present the average of 12 minirhizotron tubes where 178 roots in total across the 12 tubes were monitored.

5.4.2 Classification of root types according to growth behaviour

In the preliminary set of observations, a total of 178 white fine roots were visible along the surface of the 12 minirhizotron tubes. No growth measurements were possible on those root tips that were partially veiled by soil particles or turned away from the tube surface. Only 68 of these fine roots were identified as potentially growing and were continuously photographed over the six-day period (Fig 5-3). During the observation
period, at both soil depth zones, a total of 17 fine roots were defined as not growing, 34 fine roots were continuously growing, five roots were growing at some point during the six days and then stopped, and finally 12 new roots appeared (Table 5-1). Most (about double) of the roots that were growing were located in the 0-30 cm relative to the 31-60 cm depth. Likewise about 5 fold more new roots appeared in the top layer. These findings indicate that, at the flowering stage under these conditions, Shiraz had more root growth activity close to the soil surface than deeper in the soil. It was noted that the number of new roots produced during the observation period did not agree with the existing vertical root distribution dynamic prior to the monitoring. This may be explained by seasonal differences in soil temperature at the different depths or, alternatively, soil moisture due to differences in rainfall dynamics.

Table 5-1 Total fine root numbers at two soil depth zones. Roots were classified based on their growth status over six days of observation. See table 5-2 for outcomes of the analysis.

<table>
<thead>
<tr>
<th>Root type</th>
<th>Not growing roots</th>
<th>continuously growing roots</th>
<th>newly appeared roots</th>
<th>stopped growing roots</th>
<th>Total root number</th>
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<tr>
<td>Soil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-30 cm</td>
<td>14</td>
<td>23</td>
<td>10</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>30-60 cm</td>
<td>3</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>34</td>
<td>12</td>
<td>5</td>
<td>68</td>
</tr>
</tbody>
</table>
Fig 5-4 Example of root growth behaviour. One root was followed over 6 days and images were taken approximately every 6 hours. The images show the growth of a continuously growing root and also the appearance of 4 new roots as they develop from the main axis (the images are ordered from left to right).

An example of root elongation dynamics that were classified as continuously growing, stopped growing or newly appearing is provided in Fig 5-4. At both depths, all three categories of roots were present. Average root length over the six day period were greater in the top layer of soil than the bottom layer, and this was evident for both continuously growing roots and newly appearing roots, but not those roots that had stopped growing (depth : root type: \( p < 0.001 \), see Table 5-2 for outcomes of
significance testing). Average root growth rate was also greater in the top soil zone ($p < 0.01$) but these rates were not affected by root type.

While assessing total root growth is very informative, tracing of each individual root in a fully mature grapevine root system would have been an enormously time consuming and tedious task. The Rootfly software used in our root research is not an automated program and diurnal root growth dynamics of every individual root was traced manually. The root growth data collected in Chapter 3 was from a much smaller root system in a potted situation using a scanner. In this chapter, we used a different approach to deal with such a large root system- the minirhizotron tubes. Aside from the impractical nature of monitoring each individual root, the visible surface of the minirhizotron tubes are narrow or were covered by soil particles. Therefore, it is difficult to trace the history of every individual root from the images accurately.
5.4.3 Diurnal root growth dynamics

Root growth rates were dependent on the day/night cycle ($p < 0.001$, Table 5-2) and soil temperature ($p < 0.01$). Low growth rates occurred from midnight (00 00 h) to sunrise (around 0600 h) and sunrise to noon (1300 h), while the greatest elongation rates
Chapter 5 Diurnal Root Growth Dynamics in Mature Grapevines

occurred in the afternoon from 1300 h to 2000 h and after sunset (2000 h) to midnight (00 00 h) in both the 0-30 cm and 30 -60 cm soil depth zones (Fig 5-6).

These pronounced differences in growth rates across the day followed the diurnal changes in PAR \((p < 0.001)\) and soil temperature \((p < 0.01)\) (Table 5-2). In a 24 h cycle, soil temperature had sinusoidal oscillations with PAR, air and canopy temperature (Fig 5-2). However soil temperature was delayed and had a smaller range between the maximum and minimum relative to the air and canopy temperatures. Soil temperature ranged between 16 and 24 °C in the upper soil zone and 18 to 24 °C in the lower soil zone (Fig 5-7). The soil temperatures were greater when the fine root elongation rates reached a maximum. It is clearly apparent that root growth rate is significantly higher during the late day period and lowest during the late night and early morning (Fig 5-6). This was supported by statistical analysis (Table 5-2, day- night ***). The lowest growth rates corresponded to the lowest soil temperatures which also occurred during the late night and early morning (Fig 5-7). For these reasons it appears that temperature has an influence on root growth (Fig 5-8). Root growth was greater in the 21 to 23 °C range relative to the 18 to 21 °C range or the 15 to 18 °C range (Fig 5-8). Maximal root growth rates did not exceed 0.04 mm/h in the lowest temperature range, but nearly doubled to 0.08 mm/h in the highest temperature range.

Matos et al. (2014) surmised that soil temperature is the main driver for the oscillations in root growth rate in *Brachypodium*. They observed a considerably more rapid growth rate at 28 °C relative to 12 °C, irrespective of the presence or absence of light. Similarly, diurnal root growth mechanisms were investigated under constant day-night temperature conditions in *Arabidopsis thaliana* (Yazdanbakhsh and Fisahn 2011) and no diel distinction of root elongation was detected (Head 1965; Iijima et al., 1998;
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Walter and Schurr 2005). In the results of the Shiraz vines presented here, it is difficult to ascertain whether the day/night cycle or the soil temperature is the predominant driver. However, it is interesting to note that on Day 5 soil temperatures were at their highest within the six day period, yet this did not translate into greater maximum root growth rates at the lower soil depth or for the continuously growing roots in the upper fraction of the soil. This would indicate that soil temperature is certainly not the only driver in determining root elongation rates.

The day-night (diel) rotation of the earth and diurnal rhythms in plants have been found to have consequences on physiological and biochemical parameters in aboveground tissues and roots (Acevedo et al., 1979; Walter et al., 2009; Zeeman et al., 2007). Diurnal growth rhythms originate from both internal and external factors (Farré 2012; Harmer 2009; Walter et al., 2009). In this study the role of temperature and light was assessed. However, internal factors such as nutrient and carbon availability should also be considered. Starch accumulation as a result of photosynthesis during the day and its degradation in darkness is a critical switch in the energy supply for plants during the daily light-dark periods (Geiger and Servaites 1994; Gibon et al., 2004). Such an energy switch may be controlling the day/night cycle in root growth that was observed in these large Shiraz vines in bins. We may hypothesise that as the energy from starch reserves becomes depleted during the latter part of the night and early morning, root growth slows and then regains again after photoassimilates are exported from the leaves later in the day. Further work examining the role of carbohydrates, both photoassimilates and carbon reserves, on root growth is warranted.
Fig 5-6 Diurnal growth dynamics of continuously growing existing roots and new roots over six days at soil depths of 0-30 cm and 30-60 cm. The bars indicate standard errors of the mean between each observation time. The dark gray boxes refer to the dark period.
Fig 5-7 Diurnal fluctuations in average soil temperature at 0-30 cm and 30-60 cm. The dark grey boxes indicate the night period.
Fig 5-8 Relationship between soil temperature and hourly root growth rate of Shiraz. Each symbol represents an individual roots ($n = 11$) over the six days.
### Table 5-2 Outcome of the ASReml analysis for a model. “***”, ** and *” indicate significance at the 0.1, 1 and 5 % respectively. “ns” indicates no significant interaction when a term was used. The results of the random terms are not shown in this table.

<table>
<thead>
<tr>
<th>Predictor variables</th>
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<th>Average root growth rate</th>
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<tr>
<td>Depth</td>
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</table>

### 5.5 Conclusion

Both time of day and soil temperature regulated root growth in this non-destructive observational study and this was also found in the controlled environment experiment outlined in Chapter 4. In addition, it is evident that mature vines exhibited similar pronounced diurnal root growth dynamics as the young potted vines of the study described in Chapter 3. These similarities across vine age and size provide further confidence in the assertion that grapevine root growth is cyclic and that abiotic factors have a role in the extent of root growth that occurs during the day and night.
5.6 Acknowledgments

The authors would like to thank National Wine and Grape Industry Centre, Charles Sturt University and New South Wales Department of Primary Industry for their support. We also thank Rob Lamont and Helen Pan for their technical support during the study.

5.7 References


Chapter 5 Diurnal Root Growth Dynamics in Mature Grapevines


Chapter 6 Seasonal Rooting Dynamics of Grapevine Rootstocks Over Seven Years under Field Conditions

Chapter 6 Seasonal Rooting Dynamics of Grapevine Rootstocks Over Seven Years under Field Conditions


Synopsis

Over a shorter time course, diurnal root growth dynamics of potted and mature grapevines have been studied. It was found that grapevines roots have diurnal rhythmicity and this was related to a circadian clock and soil temperature fluctuations. Those findings on the diel time scales may also hold true on weekly to seasonal time scales. This manuscript entails a comprehensive study on seasonal root growth profiles of grafted Shiraz on own rooted Shiraz and other three common rootstocks in relation to natural air and soil temperature fluctuations at different soil depths over a period of seven years. Overall, the periodicity of root growth in short-term and long-term scales is critical to determining the timing of irrigation and fertilization for sustainable vineyard management.

Key contents

- Root distribution dynamics in different depths and years
- Root elongation dynamics of genotypes
- Soil moisture, air and soil temperature
Abstract

Minirhizotron tubes were installed to monitor root growth dynamics of mature Shiraz grapevines that were grafted on Shiraz, Ramsey, 140 Ruggeri and Schwarzmann in a rootstock trial established in the hot climate Riverina region of New South Wales, Australia. The vertical root distribution and seasonal root growth dynamics of those genotypes were studied in five seasons across a seven year period. New root production were significantly influenced by genotype, soil depth, season, phenological stage and year over the seven years. Soil moisture and soil temperature were monitored at 10, 30 and 60 cm in the last two seasons and it was found that soil moisture at 30 cm as well as soil temperature at all three depths were significant predictors of root growth. New root numbers were significantly higher in 140 Ruggeri than the other rootstocks, and the roots was evenly distributed from the top soil down to the depth of 52 cm in the soil profile, whereas the majority of roots of Schwarzmann, Shiraz and Ramsey were located in the soil at 10-40 cm, 20-40 cm and 20-52 cm respectively. Across the genotypes the peak of root growth tended to occur at flowering. Root growth also occurred during the other phenological stages, however this was dependent on genotype. In some years, root growth was observed in early and late winter at rates exceeding that of autumn, and this was associated with warmer temperatures during this period. Overall, seasonal rooting dynamics were responsive to abiotic factors but dominated by genotype.

Key words: Genotype, seasonal root growth, vertical root distribution, soil depth, Soil temperature, soil moisture
Chapter 6 Seasonal Rooting Dynamics of Grapevine Rootstocks Over Seven Years under Field Conditions

6.1 Introduction

Optimization and predictions of fruit tree management under present and future changing environments necessitates enhanced understanding of belowground processes. Aboveground growth and development of woody perennials can be impacted on by timing of root growth, root distribution and root interactions with their surrounding environment (Contador et al., 2015; Eissenstat et al., 2006; Mar Alsina et al., 2011; Norby & Jackson, 2000). For example, shallow rooting has negative consequences on fruit tree performance in dry years but a positive influence in a wet spring (Contador et al., 2015). In addition, the manipulation of root growth through rootstocks (Cox, Favero, Dry, McCarthy, & Collins, 2012) can reduce excessive shoot growth (Atkinson & Else, 2001), carbohydrate metabolism and whole plant growth (Robbins & Pharr, 1988). Tree performance and productivity may be related to constraints in fine root growth and root functioning. In addition, perennial fruit tree root growth may be genetically controlled by rootstocks or the interactions between rootstocks and scions (Basile et al., 2007; Di Filippo & Vila, 2011a; Keller, 2010). For example, commonly used rootstocks in grapevines are derived from diverse climatic backgrounds and have genotypic differences (Comas et al., 2005; Keller, 2010). These variations may generally impact both root and shoot growth, and eventually the sustainable productivity of fruit trees (Keller, Mills, & Harbertson, 2012). Because of their genetic diversity, rootstocks can effect scion vigor (Tandonnet et al., 2010), flower induction (Di Filippo & Vila, 2011b), may even alter mineral and water uptake (Bassoi et al., 2002) due to their different root anatomy. Therefore, better understanding of root dynamics within fruit crops, such as root growth dynamics and root lifespan, implications for fertilizer and irrigation management.
Understanding how this might vary in relation to common rootstocks could open possibilities towards building a broad range of advancements to fruit tree management as well as provide solutions for current problems in sustainable agriculture.

Unlike the aboveground tissues, root growth can continue in mature trees all through the year (Bhar, Mason, & Hilton, 1970; Callejas, Canales, & de Cortazar, 2009; Lyr & Hoffmann, 1967; Teskey & Hinckley, 1981). There is very little information about root growth dynamics in woody plants, including grapevines. Dynamics of grapevine root growth generally found in text books are based on inadequate evidence (Eissenstat et al., 2006; Mullins, Bouquet, & Williams, 1992). Root growth studies are scarce as methods are complex, time consuming and tedious either through non-destructive direct monitoring or by destructively observation. Long-term studies on larger woody plants are particularly uncommon. However, it has been observed that root growth dynamics of grapevines vary between cultivars, vine age, management practises and environmental stress. Freeman and Smart (1976) found that Shiraz vines had rapid root growth 10 weeks after bud break with maximum root development when shoot growth had ceased and also after harvest. In a study with five woody cultivars, grape root activity occurred in early spring prior to bud break and during bud break, and in mid to late summer when shoot growth had ceased (Hilton & Khatamian, 1973). More recently, Callejas et al. (2009) showed that grapevine roots displayed several growth peaks; they were most pronounced at flowering, veraison and harvest and these dynamics were different at three soil depths.
The varying root growth dynamics at different soil depths results in different root distribution dynamics. Bassoi et al. (2002) found that grapevine rootstocks had a similar rooting dynamics under micro-sprinkler irrigation conditions. Roots were present to a 100 cm depth and approximately 90 % of the roots were distributed down to a 60 cm depth, with a larger root occurrence in the first 40 cm. Some studies have shown that the distribution of roots in the soil profile can be related to genetic factors which also regulate the density of roots (Southey & Archer, 1988; Williams & Smith, 1991). In addition, the distinct distribution of grapevine roots is a result of different soil environments (Morlat & Jacquet, 1993; Nagarajah, 1987), particularly soil temperature and soil moisture (Atkinson, 2011; Callejas et al., 2009; Lyr & Hoffmann, 1967; McMichael & Burke, 1998). When soil temperature is not a limiting factor, soil water content may regulate root development (Bauerle et al., 2008; Monselise, 1947). However, when all the factors that impact on root growth are optimal, soil temperature is the main impacting factor of root development. It was reported that the ideal range of soil temperature for maximum root growth of walnut is between 21 and 24 °C (Kuhns et al., 1985). In citrus, maximum root growth was observed around 29 °C and declined below 22 °C (Bevington & Castle, 1985). Woodham and Alexander (1966) and Kliwer (1975) indicated that the optimal soil temperature for grapevine root growth is close to 30 °C. The warm soils can stimulate root growth and nutrient uptake of grapevines (Clarke et al., 2015; Rogiers et al., 2011).

In Australia, South Burnett, Riverina and Hunter Valley are the warmest grape growing regions with median growing season temperatures of 22.9, 21.5 and
Chapter 6 Seasonal Rooting Dynamics of Grapevine Rootstocks Over Seven Years under Field Conditions

20.7 °C, respectively (Hall & Jones, 2010). Wagga Wagga, Riverina, NSW is the second warmest grape growing region in Australia. However, this study would be the longest hot climate study that has been attempted since studies on root lifespan in Merlot vineyard at Oakville, California, USA (Bauerle et al., 2008). Therefore, in hot climates, it would be the interest in seasonal root growth and distribution in hot soils (Huang, Lakso, & Eissenstat, 2005). In addition, In terms of vineyard management, potential genotypic differences between rootstocks in seasonal fine root growth dynamics and root distribution also another important aspect. Therefore, two of the most widely planted rootstocks (Ramsey and Schwarzmann) and another important grapevine rootstock (140 Ruggeri) was selected as they are believed to be the most favourable rootstocks for Shiraz vineyards in warm and hot climates (Dry, 2007) and relevant to the Australian industry. Finally, a two-stage seasonal root growth study was conducted in a four years comparison of rootstocks, and then temperature and soil moisture was monitored in the last three years.

6.2 Materials and methods

6.2.1 Location and vines

The field experiment was carried out in a Shiraz rootstock field trial at a commercial vineyard located at the National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, NSW Australia (35°05'S 147° 35'E) over the seven year period from 2007, 2008, 2009, 2010, 2012, 2013 and 2014. The Shiraz vines and other rootstocks were planted in 1998 and the following year the Shiraz scion was chip grafted on own rooted Shiraz and other different rootstocks. Vines were trained into a bilateral cordon and vines were pre-pruned
mechanically, but final pruning was by hand. For this study, four-vine replicates
of own rooted Shiraz (V. vinifera), 140 Ruggeri (V. berlandieri × V. rupestris),
Ramsey (V. champinii), Schwarzmann (V. riparia × V. rupestris) were used (total
16 vines). The four genotypes were distributed randomly in ten rows in a
Randomized Complete Block Design (RCBD). The vines were planted in 1998 at
a density of 1666 vines ha⁻¹ (2 × 3 m vine and row spacing respectively). Vines
were watered with a drip irrigation system. During the root observation period the
areas around the vines were kept weed free through mechanical means. Nutrition,
pest management, and other vineyard operations were consistent with common
commercial vineyard practices. The major phenological stages were recorded

6.2.2 Installation of minirhizotron tubes

Two clear polycarbonate (minirhizotron) tubes of 100 cm length with an external
diameter of 55 mm were installed on two sides of each vine at an angle of 30° in
autumn 2007, three months prior to the start of the experiment. This resulted in 4
vines per genotype or 8 observations tubes per genotype. There were two tubes
per vine replicate in each genotype. The tubes were positioned within the vine
rows and 50 cm away from the centre of the trunk in order to avoid damage from
machine harvesting or other mechanical activities in the vineyard. The bottom of
the tubes were sealed with a 2.5 cm thick clear acrylic plastic plug. The top of the
tubes that extended above the soil surface were painted with black colour and
enclosed with caps, made from white UPVC (23 cm long × diameter of 66 mm)
in order to reflect sunlight and prevent water and insects entering the tubes. These
caps were held in place with black sticky rubber seals (24 mm wide × 3 mm thick).

### 6.2.3 Installation of soil moisture sensors

Soil moisture was recorded during the 2012/2013 and 2013/2014 seasons with 16 MEA Gbugs (Measurement Engineering Australia Pty. Ltd, SA), installed every second replicate of each rootstock. They were fixed to the post for protection from machine damage during pruning and harvest. The data loggers recorded soil moisture in the root zone at a 10, 30 and 60 cm depth every 2 hours. Gypsum blocks (MEA’s GBHeavy, MEA2176) were also installed at 10 cm, 30 cm and 60 cm, as these are best suited to a tension range of 50 to 500 kPa. These sensors were equally spaced on the circumference of a 10 to 15 cm radius centred directly under the dripper.

### 6.2.4 Installation of soil temperature sensors and data logger

Soil temperature was recorded over the 2012/2013 and 2013/2014 seasons with T-type thermocouples (Jonley, Pty Ltd, NSW, Australia). Marks were made at 10, 30 and 60 cm on the T-type thermocouples before the sensors was inserted under the drip line and also half way between the drip lines in their inter-row area. All thermocouples were connected to a 12 Channel Temperature Recorder (Lutron BTM-4208SD, InstrumentCatalog™, SA, Australia), which was installed in a water proof container and connected to a rechargeable battery that was charged with a BP-solar panel-1205 (Energy Matters-A SunEdition Company, Victoria, Australia). Soil temperature data was recorded on an hourly basis and the data was retrieved every month.
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6.2.5 Air temperature data collection

The air temperature data from 2007 to 2014 were collected at a weather station located at the New South Wales Department of Primary Industries in Wagga Wagga. This weather station was located approximately 1.5 km from the experiment. Maximum, average and minimum air temperature were recorded on a daily basis.

6.2.6 Root observation and digital image collection

A minirhizotron camera system were used for non-destructive root observation (Bartz Technology Crop, Santa Barbara, CA, USA) of the four genotypes, from the soil surface to a depth of 60 cm. Digital images were captured fortnightly from 50 consecutive 1.2 cm fixed windows in each tube over the period of August 2007 to October 2010 and June 2012 to June 2014. Throughout these five seasons, 169600 digital images were collected. To confirm accuracy of root history, the image location (window), the tube number and image history (session) were checked using Rootfly software (Rootfly, Version 2.02.0, Clemson University, South Carolina, USA).

For root image analysis the roots were counted rather than traced. The reason for this were two fold. Root images from these seven years were numerous and root tracing of every individual root would have been too time consuming. It not only requires reasonable time but it is difficult to trace the history of individual roots from the images accurately, because due to progressive fine layer of clay covering some windows, it was not possible to see all the roots properly. This could be related to the soil type in this vineyard; and that possibly wasn’t
encountered in other studies. The tubes were in the ground seven years by the end of the study, and perhaps this is just an un-avoidable aspect of minirhizotron studies. Furthermore, these polycarbonate tubes, with a slightly less smooth surface than acrylic also added to the problem of tracing roots accurately.

Secondly, it was a very long-term study with multiple rootstocks, and it was decided that characterising the new root production periods was of greatest interest. For the root counting Rootfly software was used. The root images with no new root growth were treated as zero growth. The results were exported to Excel (Version 2007) for further analysis.

6.2.7 Statistical analysis

6.2.7.1 Analysis across all observation dates

Based on the objectives of this research, it was decided to classify a root as growing or not growing to enable the investigation of growth dynamics across the soil profile and seasons. Roots that were ‘not growing’ were those that were not visibly growing as assessed by the minirhizotron images. To analyse this binomial data a generalised linear mixed model was used, in the form of a binomial logistic regression. This model was fitted in the statistical software R (version 3.2.0) using the ASReml-R (asreml-3.0) package. The model used for this analysis (Model 1) can be symbolically written as:

\[
\text{Binomial growth} \sim \text{average air temperature} + \text{genotype} + \text{season} + \text{phenological stages} + \text{year} + \text{observation date} + \text{replicate} + \text{tube} + \text{soil depth} \quad \text{(and all the interactions of these variables)}
\]
Chapter 6 Seasonal Rooting Dynamics of Grapevine Rootstocks Over Seven Years under Field Conditions

To match the root observation data to the daily air temperature data, averages for the period until the observation date were calculated.

An alternative assessment of the data was achieved by concentrating on only the growing roots and newly appearing roots. The number of new roots at each observation date was recorded and used as the response variable in a linear mixed model fitted in the statistical software R (version 3.2.0) using the ASReml-R (asreml-3.0) package. It was necessary to log transform this data, so the model assumption of homogeneity was met. The model used for this analysis (Model 2) can be symbolically written as:

\[
\ln (\text{Number of new roots}) \sim \text{average air temperature} + \text{genotype} + \text{season} + \text{phenological stages} + \text{year} + \text{observation date} + \text{replicate} + \text{tube} + \text{soil depth} \quad \text{(and all the interactions of these variables)}
\]

A log-likelihood ratio test was used to determine whether the random terms were significant in both Models 1 and 2. Year refers to the calendar year while season refers to spring, summer, winter or autumn. Phenological stage refers to the developmental stage of the vine and observation date refers to the day the image was taken. Soil temperature was recorded at the depth of 10, 30 and 60 cm. It was categorical. However, root images are collected from the top soil to 60 cm and it was a continuous variable. Soil depth is included as a random factor in our Model because the root image locations\(^\wedge\) were a continuous variable in this experiment.
6.2.7.2 Analysis across last three years of observation dates

Soil temperature and moisture in the last three years of data collection were registered at hourly intervals and air temperature was recorded on a daily basis, to match the root observation data to the environmental factors, averages for the period until the observation date were calculated. Before deciding which environmental factors to use in the analysis, correlation between the factors were calculated as one of the assumptions of the analysis methods used is that the independent variables need to be uncorrelated with each other.
Fig 6-1 Correlation map of air temperature, soil temperature and moisture at different depths. “av10 cm” = average soil temperature at 10 cm; “av30 cm” = average soil temperature at 30 cm; “av60 cm” = average soil temperature at 60 cm; “avt” = average air temperature; “SM10CM” = average soil moisture at 10 cm; “SM30CM” = average soil moisture at 30 cm; “SM60CM” = average soil moisture at 60 cm.

Soil moisture at 30 and 60 cm were correlated with each other (Fig 6-1), and therefore we decided to use 10 and 30 cm in our model. Air temperature and soil temperature were strongly correlated, therefore, only one of these measurements
could be used in the model. To keep this consistent with the analysis across all observation dates, air temperature was retained for this analysis. The model used for this analysis (Model 3) can be symbolically written as:

\[
\ln (\text{Number of new roots}) \sim \text{average air temperature} + \text{genotype} + \text{season} + \text{phenological stages} + \text{year} + \text{observation date} + \text{soil moisture at depth 10 cm} + \text{soil moisture at depth 30 cm} + \text{replicate} + \text{tube} + \text{depth} \quad \text{(and all the interactions of these variables)}
\]

A significance level of 5% was considered statistically significant in this analysis. The model assumptions were that the residuals were normally distributed; they had a constant variance and were independent.

6.3 Results

6.3.1 The model outcomes

The root parameters related to genotypes and other abiotic factors computed for the ASReml described in the Methods section are shown in Table 6-1 and 6-2. The outcomes of each model is explained in detail in the subsequent sections.
Table 6-1 Outcome from the analysis based on Model 1 and Model 2 described in the methodology. “***; ** and *” indicate significance at the 0.1, 1 and 5 % respectively. The non-significant interaction terms are not included in the table. In Model 1, significance is shown for the factors which resulted in no root growth. In Model 2, significance is shown for the factors that affected the number of new roots produced.

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<td>Genotype : Observation date</td>
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</table>
Table 6-2 Outcome from the analysis based on Model 3 described in the methodology. “***”, ** and *” indicate significance at the 0.1, 1 and 5 % respectively. “ns” indicates no significant interaction when a term was used. The non-significant interaction terms are not included in the table.

<table>
<thead>
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<tr>
<td>Genotype : Soil moisture at 30 cm</td>
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</table>

6.3.2 Soil moisture, soil and air temperature, phenological stages

Soil water content was higher at the depth of 10 cm compared to the other two depths, 30 cm and 60 cm. The soil became very dry once summer started with the driest period occurring during summer and autumn with some fluctuations at the depths of 30 cm and 60 cm (Fig 6-2 b). Notably, the soil water content at 30 cm was highly correlated with soil moisture at 60 cm (Fig 6-1). Soil water at 10 cm was higher due to its proximity to the point of irrigation.

Maximum summer soil temperatures in the 2013/2014 season were between 35-37 °C and greater by approximately 5 °C relative to the 2012/2013 season at the
10 cm and 30 cm depths. Soil temperatures in winter, spring and autumn were similar, in the range of 5-15 °C, 10-30 °C and 30-10 °C respectively (Fig 6-2 a). In addition the soil temperature at the depth of 30 cm was highly correlated to the temperature at the 60 cm depth (Fig 6-1). The maximum air temperature in 2009 reached 44 °C compared to other six years (Fig 6-3). The maximum air temperature was lower than 40 °C in year one and year five. However, no year to year difference was found in average air temperatures which was calculated based on the values between each two observation dates (data not shown in the table as those not significant terms were removed).

The timing of the major phenological stages varied between years, fluctuating between 4 to 10 days. However, the harvest date of 2009 was around 20 days earlier than the other years (Table 6-3) in this study and this could be related to the higher air temperature in that season and/or management factors.

Table 6-3 Date of onset of the major phenological stages in grapevine development over five seasons. These dates are based upon the Eichhorn and Lorenz system modified by Coombe (1995).

<table>
<thead>
<tr>
<th>Phenological stages</th>
<th>Season 1</th>
<th>Season 2</th>
<th>Season 3</th>
<th>Season 4</th>
<th>Season 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering</td>
<td>08/11/2007</td>
<td>29/10/2008</td>
<td>02/11/2009</td>
<td>30/10/2012</td>
<td>02/11/2013</td>
</tr>
<tr>
<td>Veraison</td>
<td>02/01/2008</td>
<td>07/01/2009</td>
<td>30/12/2009</td>
<td>08/01/2013</td>
<td>30/12/2013</td>
</tr>
<tr>
<td>Harvest</td>
<td>29/02/2008</td>
<td>10/02/2009</td>
<td>23/02/2010</td>
<td>06/03/2013</td>
<td>09/03/2014</td>
</tr>
</tbody>
</table>
Fig 6-2 Seasonal dynamics of soil temperature (a) and soil moisture (b) at 10 cm, 30 cm and 60 cm depths adjacent to minirhizotron tubes over two seasons (2012/2013 and 2013/2014). Values are means of four locations for each of the four genotypes. The colour of the symbol refers to winter, spring, summer and autumn.
Fig 6-3 Seasonal dynamics of maximum, mean and minimum air temperature over seven years. On the figure the “avt” is average air temperature; “maxt” is maximum air temperature and “mint” is minimum temperature. Values are daily air temperature from June 2007 to June 2014. The colour of the symbol refers to winter, spring, summer and autumn.
6.3.3 Total new root production in different seasons and phenological stages

Total new root numbers varied between genotypes. 140 Ruggeri had the greatest total root numbers compared to Ramsey, followed by Schwarzmann and Shiraz. The maximum new root production was observed in spring at bloom in each species while it reached a minimum in autumn at harvest. Interestingly, Ramsey had more new root production in winter and at bud-break compared to the other genotypes (Table 6-4). Notably, the new root population in winter was higher than autumn growth.
Chapter 6 Seasonal Rooting Dynamics of Grapevine Rootstocks Over Seven Years under Field Conditions

Table 6-4 A genotype comparison of the total number of new roots produced over a seven year period in each of the four seasons and around the major phenological stages.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Genotypes</th>
<th>Shiraz</th>
<th>140 Ruggeri</th>
<th>Schwarzmann</th>
<th>Ramsey</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seasons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td></td>
<td>1157</td>
<td>2621</td>
<td>1440</td>
<td>1603</td>
</tr>
<tr>
<td>Summer</td>
<td></td>
<td>254</td>
<td>470</td>
<td>414</td>
<td>333</td>
</tr>
<tr>
<td>Autumn</td>
<td></td>
<td>50</td>
<td>156</td>
<td>156</td>
<td>109</td>
</tr>
<tr>
<td>Winter</td>
<td></td>
<td>245</td>
<td>331</td>
<td>205</td>
<td>514</td>
</tr>
<tr>
<td>Bud-break</td>
<td></td>
<td>93</td>
<td>136</td>
<td>41</td>
<td>191</td>
</tr>
<tr>
<td><strong>Phenological stages</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flowering</td>
<td></td>
<td>594</td>
<td>1563</td>
<td>1075</td>
<td>972</td>
</tr>
<tr>
<td>Veraison</td>
<td></td>
<td>132</td>
<td>136</td>
<td>164</td>
<td>124</td>
</tr>
<tr>
<td>Harvest</td>
<td></td>
<td>99</td>
<td>203</td>
<td>81</td>
<td>69</td>
</tr>
</tbody>
</table>

6.3.4 New root production, vertical root distribution dynamics and seasonal root growth dynamics of grapevines

Substantial new root production was observed non-destructively through the visible sides of the minirhizotrons. Year to year variability was found in new root growth among the genotypes and new root populations also varied through the soil profile (Fig 6-4). New root numbers were significantly different between rootstocks ($p < 0.001$) (Table 6-2). In this vineyard, vertical root distribution was unique for each genotype over the period of seven years. Soil depth was one of the influencing factors in this new production ($p < 0.05$) (Table 6-1). Roots of 140 Ruggeri were distributed mostly between the top soil to a 52 cm depth while most of Shiraz roots were found below the 20 cm to 40 cm range of the soil profile. Higher root numbers were present in Schwarzmann between the 10 cm and 40 cm soil layers. However, there was a tendency for greater root numbers in...
Ramsey rootstocks below the 20 cm soil depth with only a few new roots in the upper part of the soil (Fig 6-5).

Distinctive seasonal root growth activity occurred over the seven years. Root growth was most pronounced around flowering, followed by veraison, bud-burst and harvest (Fig 6-4). New roots formed in spring were greater than those produced later in the season. The seasonal dynamics in root growth were similar in the different rootstocks, but the amount of roots varied between the rootstocks. Overall, when considering the significant interactions between new root production with direct or indirect related factors it appears that new root growth was significantly affected by genotype \((p < 0.01)\), air temperature \((p < 0.001)\), season \((p < 0.001)\), development stage \((p < 0.001)\), year \((p < 0.001)\) and observation date \((p < 0.001)\) (Table 6-2). In the last three years, it was found that new root production was also significantly related to soil temperature \((p < 0.01)\) and soil moisture at the depths of 30 cm or 60 cm \((p < 0.01)\). The combination of genotype with soil temperature \((p < 0.001)\) or with soil moisture at the depth of 10 cm \((p < 0.05)\) also influenced new root growth (Table 6-1).
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[Graph showing seasonal rooting dynamics of different grapevine rootstocks over seven years.]
Fig 6-4 Number of new roots and their seasonal distribution dynamics by soil depth as monitored in minirhizotron window positions over 2007/2008, 2008/2009, 2009/2010, 2012/2013 and 2013/2014. Values are means of new roots counted at each image location (depth), by observation date. SHZ = Shiraz, RUG = 140 Ruggeri, SCH = Schwarzmann, RAM = Ramsey. The italicized symbols on the left corner of the figure indicates phenological stages of grapevines and winter period (dormancy); B = Bud break, F = Flowering, V = Veraison, H = Harvest, W = Winter. No growth (zeros) not shown in this figure. Average new root numbers at each particular depth is shown on the figure legend as 1, 2 and 3 with the size of the symbol proportional to the number of new roots.
Chapter 6 Seasonal Rooting Dynamics of Grapevine Rootstocks Over Seven Years under Field Conditions

6.4 Discussion

6.4.1 Seasonal root growth dynamics of grapevines

New root production in grapevines was a function of biotic and abiotic factors such as genotype, phenological stage, and air or soil temperature, moisture, season and year. The seasonal dynamics in root production were consistent over the seven years of this study. New root production began in late winter and peaked at flowering in spring when soil temperature reached between 15 and 30 °C throughout the soil profile. The active root population declined in mid or late summer and after harvest. These periodic changes in new root growth over the seven years suggests that the initiation of new root production in grapevines could be partially influenced by soil temperature and/or plant demand for nutrient/water resources. Year to year difference were found in timing of root growth (Table 6-1) over the seven years. However, the separate analysis from the last three years which was including soil temperature and moisture in the model showed that new root production had no significant difference in the last three years and observation date (Table 6-1).

Unimodal dynamics of root production, with one clear flush of new roots over the year, have been found in several fruit crops including grapevines. This unimodal curve may relate to a temperate environment where dormancy starts soon after crop harvest (Atkinson, 2011; Comas et al., 2005; Contador et al., 2015; Head, 1967). In contrast, in our current study, the root measurement system was used quite different from other researchers as mentioned in the methodology. In this system, a multimodal distribution in new root production was evident with a
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major flush occurring at flowering and minor modes at other phenological stages. The amplitude between the major and minor modes varied between rootstocks. Unlike in other cooler grape growing regions, where dormancy begins shortly after harvest, the vine leaves in our warm climate vineyard remained functional for approximately two to three months prior to leaf senescence. This may provide vines with enough carbohydrates that go beyond root reserve replenishment requirements and allow more root flushes during the post-harvest period and even after leaf fall.

According to the previous study, maximum root growth in grapevines occurs before flowering and after harvest (Freeman & Smart, 1976). Van Zyl (1988) found that root production of grapevines began after bud-break and maximum growth rates occurred at the bloom stage, after which the growth rate decreases. However, in that study, a new root flush period started after harvest. In a different study, the main root flushes of Merlot and Concord were observed in summer between bloom and veraison and little root production occurred before harvest and during dormancy when conditions were favourable (Eissenstat et al., 2006).

In our present study, it was found that the main period of new root production occurred in spring, followed by summer, dormancy and autumn. This variability in when root production occurs may be related to varying soil temperatures and root carbon availability during the major phenological stages. The presence of new root production during dormancy can be explained by Bhar (1970). He showed that some grapevine roots can grow continuously during dormancy where the ambient soil temperature was 3 °C or higher. Furthermore, in a more recent study (Eissenstat et al., 2006), year to year differences were evident in the
specific timing of root production and dynamics appear to be influenced by cultural practices such as irrigation (Bassoi et al., 2003) and pruning (Comas, Eissenstat, & Lakso, 2000; Ferree, Scurlock, & Schmid, 1999).

### 6.4.2 Influence of soil temperature on grapevine root growth

When other abiotic factors are not limiting, soil temperature initiates root growth and distribution (Atkinson, 2011; Bonomelli, Bonilla, & Núñez, 2009; Callejas et al., 2009; Rogiers et al., 2011). We found that the soil temperature at 10, 30 and 60 cm was highly correlated to the air temperature (Fig 6-1). As stated in the Methods, because soil temperature and air temperature were highly correlated only one of these could be used in the model. In our study, soil temperature data was recorded in the last three years. Air temperature was thus used as this data was collected over the full 7 years. The very strong correlation between air and soil temperature at these two depths (Fig 6-1) indicate that there was likely no significant lag period between the two. Generally, the soil temperature is lower than the air temperature and the seasonal fluctuation occurred with depth depending on the changes of soil moisture and aboveground plant growth (McMichael & Burke, 1998). In grapevines, it was observed that the optimal soil temperature for root growth is close to 30 °C (Kliewer, 1975; Woodham & Alexander, 1966). The average annual root growth of table grapes (*Vitis Vinifera* L.) cv. Thompson Seedless was not correlated with the annual soil temperature and higher thermal diffusivity in soil profile favoured root production (Callejas et al., 2009). Based on our last three years of results, the maximum root growth appeared in spring at the flowering stage when the soil temperature was in the range of 15 to 30 °C and then new root production decreased over summer until
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autumn. However, the roots started to grow during early dormancy while the soil temperature was 10 to 15 °C. Interestingly, in autumn, there was little new root growth after harvest when soil temperature was around 10-30 °C, a similar temperature range during spring when root production was prolific. This could possibly be related to the root carbon availability during the different times of the year.

6.4.3 Influence of soil moisture on grapevine root growth

Efficiency of water-use affects grapevine performance and lack of water will cause limitations to plant growth and yield (Kramer & Boyer, 1995). There was no apparent influence of soil moisture at 10 cm on the production of new roots in our study, however there was an effect at 30 cm. Irrigation was successful at consistently wetting only the top 10 cm while most of the roots were further down the profile where the presence of irrigation water was more sporadic. The soils at 30 cm were probably sufficiently dry to limit root production at this depth. Irrigation methods in vineyard management have a significant effect on moisture diffusion within the soil profile, resulting in different root growth dynamics and water use efficiency of the grapevine (Araujo et al., 1995; Morano & Kliewer, 1994; Van Zyl, 1988). The data of the more recent years indicate that there are fewer new roots forming relative to the previous years and this might be the consequence of the irrigations not reaching the 30 cm depth due to less applied water. Unfortunately no irrigation records are available to verify this. Further studies wetting the entire soil profile would give a better indication of new root production in response to soil moisture, and this is a potential area for further study. Due to a very dry season, soil water content was very low (about 50 % of
the plant available water), therefore the soil moisture sensors were operating at their limit (-500 kPa). In future work, different soil moisture sensors with a greater range are recommended.

6.4.4 Influence of genotype on grapevine root distribution

The four different rootstocks of this study had different root production behaviours. While maximum root production peaks appeared at flowering in each rootstock. The rootstocks behaved differently at the other stages. For example, Schwarzmann and Shiraz had greater root populations at veraison than harvest and this was followed by bud-break, while 140 Ruggeri had a contrary result. Ramsey had greater new root production at bud-break, followed by veraison and harvest. Other fruit crops such as peach grafted on five different rootstocks had similar seasonal dynamics of new root production. Fine root populations reached a minimum in winter and declined during the final stages of fruit development (Basile et al., 2007). In the same study it was found that in spring some rootstocks produced greater root length compared to those produced later in the season.

The genotypes in this study had different amounts of new roots through the soil profile (Fig 6-5) and total new root production was significantly different at the different soil depths (Table 6-1). Information on these differing root growth behaviours can potentially be used to plan time of fertiliser application and irrigation amounts. A peach rootstock with a different genetic background (K119-50) formed a large amount of new roots below 69 cm unlike four other rootstocks (Basile et al., 2007). Similar to our study, different grapevine species had different root numbers. For example, the greatest root numbers were observed in
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Dogridge followed by Barbera and Concord (intermediate), then Noble with the smallest amount of roots (Perry, Lyda, & Bowen, 1983). In addition, the root distribution dynamics varied between cultivars. Noble had shallow roots, with approximately 35% of the total roots in the 0-15 cm depth whereas most of the roots of Dogridge occurred in the 90-105 cm soil profile. In the present study, new root production by 140 Ruggeri occurred in the 0-52 cm layer with the greatest root numbers between 10-30 cm. Shiraz had a maximum number of new roots in the 20-40 cm depth, whereas Schwarzmann had the greatest root populations between 10-40 cm. Ramsey had a very unique root distribution dynamic and most of the new roots occurred in the soil zone at 20–52 cm (Fig 6-5). The installation angle and the observation direction did not influence root counts (Linsenmeier et al., 2010). The wide range in root distribution and seasonal dynamics in root growth of grapevine species and rootstocks, as apparent in this study, suggests that they have adapted to the soil environmental conditions from which they originate.

6.5 Conclusion

We conclude from this study that grapevine rootstocks displayed genetic variability in root development in response to changes in soil depth, developmental stage, season, year, and temperature and soil moisture. These particular root distribution characteristics in different soil depths and seasonal root growth dynamics of grapevine rootstocks suggest that the genetic diversity of rootstocks could be drawn upon as a management tool for specific soil environments. In addition, climatic background of these rootstocks and their ability to adapt to different environmental conditions was one of the important
aspects contributing to these variations between rootstocks. These rootstocks with differing root growth dynamics may influence whole vine performance, carbohydrate reserves and nutrient and water uptake dynamics through the season. Most importantly, these findings can be added to models that estimate whole grapevine performance and yield in changing soil environments in those grape growing regions which are the hottest.

6.6 Acknowledgments

The authors would like to thank National Wine and Grape Industry Centre and Charles Sturt University and New South Wales Department of Primary Industry for their support. We also thank Rob Lamont and Helen Pan for their technical support during the study.

6.7 References


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Chapter 7 Root Growth during Post-Harvest Irrigation of Warm-Climate Grapevines


Synopsis

In hot grape growing regions of Australia, grapevine canopies can be functional for three to four months after harvest. Nevertheless, a deficient water supply during this period may inhibit new root production, nutrient uptake and root carbohydrate reserve replenishment, with consequences on whole vine performance in the following year. As outlined in Chapter 6, minimum root activity was apparent over the first four years of the study (2007, 2008, 2009 and 2010), especially in own-rooted Shiraz. In this trial, we examined the impact of three post-harvest irrigation regimes on Shiraz fine root growth in the 2012/2013 season. The irrigation strategies included: no post-harvest irrigation (NPHI), early post-harvest irrigation (EPHI) and late post-harvest irrigation (LPHI).

Key contents

- Post-harvest irrigation strategies
- Post-harvest root growth dynamics
- Canopy functioning
Abstract

In warm climate regions of Australia grapevines may retain leaves for up to four months after harvest. However, a dry post-harvest period may reduce root growth, nutrient uptake and the replenishment of carbohydrate reserves. This can impact on canopy function in the current season as well as canopy development and berry ripening in the following season. This research examined the role of the root environment, particularly soil temperature and moisture, on fine root growth after harvest in 2013. Three post-harvest irrigation strategies were compared using mature field-grown Shiraz. These included no post-harvest irrigation (NPHI), early post-harvest irrigation (EPHI) and late post-harvest irrigation (LPHI). The EPHI treatment maintained soil moisture in the readily available range for a period of 15 days after harvest. The LPHI treatment was applied at 30 days after harvest, and similarly maintained soil moisture for a period of 15 days. Minirhizotron tubes were used to monitor root growth across all three treatments. Fine root growth in EPHI increased nearly sixteen-fold 20 days after irrigation. Maximum root growth rates were observed at seven days after harvest in the EPHI treatment and these growth rates were maintained for seven days prior to declining. The root growth response to the LPHI treatment was much less pronounced. Soil temperature during EPHI was warmer by 6 °C when compared with LPHI. These results indicate that moisture but not necessarily soil temperature (in the conditions of this experiment) are critical factors influencing fine root growth in the post-harvest period.

Key words: Shiraz, post-harvest irrigation, root growth
7.1 Introduction

Grapevines grown in the warmer viticultural regions of Australia can maintain functioning canopies for one to four months after harvest. Significant new root growth can occur after harvest in these regions, which is important for further nutrient uptake and refilling the perennial reserves with nitrogen (N) and other nutrients. These accumulated nutrients are utilised in the following spring for vine development and growth, with up to 60 % of N requirements for these processes taken up in the post-harvest period (Bates, Dunst and Joy, 2002; Conradie, 1992; Van Zyl, 1984). A sufficient accumulation of nutrient and carbohydrate reserves in perennial organs after harvest is important for optimising vegetative and reproductive growth in the following season (Hunter, Skrivan and Ruffner, 1994; Tromp, 1983; Zapata, Deléens, Chaillou and Magné, 2004).

Annual root growth in perennial crops is closely related to environmental factors, particularly soil temperature and soil moisture (Cooper, 1973; Kaspar and Bland, 1992; Soar and Loveys, 2007). When other conditions are optimal, soil temperature is the main trigger for root growth, but in drying soil, uptake of water and nutrients becomes increasingly more difficult, even if the soil temperature is warm enough (Keller, 2005). This indicates that beyond a minimum temperature threshold (a “biological threshold”), water is the primary limiting factor. Therefore, it can be inferred that water stress after harvest may reduce root growth temporarily, hence affecting nutrient reserves in roots and associated grapevine performance in the following season.
Chapter 7 Root Growth during Post-Harvest Irrigation of Warm-Climate Grapevines

7.2 Materials and methods

7.2.1 Plant material and growing condition

The experiment was conducted in an own-rooted Shiraz (*V. vinifera*) vineyard located at the National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, NSW Australia (35º05’S 147º 35’E). The vines were planted in 1998 at a density of 1666 vines ha\(^{-1}\) (2 × 3 m vine and row spacing respectively), trained in a bilateral cordon, and were machine pruned in accordance with local commercial practice. For this study, nine panels of three vines were randomly selected across a section of the vineyard 15 rows wide by 30 vines long. After the grapes were machine harvested on March 5 2013, three irrigation treatments were implemented as described below. The irrigation regime in the vineyard prior to the applications of treatments was approximately 10 L per vine applied every second day before harvest. During the experimental period, the average daily air temperature varied from 27 °C to 10.9 °C, respectively. Average rainfall was relatively low with only 1.13 mm registered over the two month period after harvest.

7.2.2 Irrigation treatments

The three irrigation treatments implemented in the study included early post-harvest irrigation (E PHI), late post-harvest irrigation (L PHI) and no irrigation (N PHI). The water was applied to vines of both the E PHI and N PHI treatment using 60L plastic bins placed on either side of the vine with small holes in the base to deliver water at approximately the same rate and location as the drip irrigation system installed in the vineyard. The E PHI treatment began at harvest on March 5 and was repeated every three days for 15 days with the application of
200 L of water over 18 m² of soil surface area. This provided approximately 330 L of water per vine over the period of the irrigation treatment. The LPHI treatment started on April 5 and ran for 15 days with application of the same volume of water. The NPHI treatment received no irrigation over the whole post-harvest period (Fig 7-1). To assess canopy condition during the irrigation treatments, digital photographs were taken every week at the same time as the root image collection and visually assessed for percentage of green leaves.

7.2.3 Soil moisture and temperature

Nine soil moisture loggers (Measurement Engineering Australia Pty. Ltd, SA, Australia) were installed (one per replicate). The loggers captured data every 2 hours for 20 days. They were connected to gypsum block soil moisture sensors, functioning at a tension range of 50 to 500 kPa (suitable for this experimental site). The sensors were installed at 30 cm and 60 cm depths and were located in two positions 10 to 15 cm from the dripper towards the vine trunk. Soil temperature was monitored with Thermochron™ sensors (OnSolution, Baulkham Hills, NSW, Australia) during the experiment. Sensors were programmed to collect temperature data at 60 minutes intervals, placed into small plastic containers, and buried at depths of 10 cm, 30 cm and 60 cm (one per plot). Data was retrieved at the completion of the experiment.

7.2.4 Root observation and data collection

For miniature camera system access (Bartz Technology Crop, Santa Barbara, CA, USA) to the root zone, three polycarbonate tubes (1 m length) had been installed in the vine row at a 30° angle in each plot six years earlier. This allowed frequent
non-destructive root observation during the growing season of the same fixed root-zone to a depth of 60 cm. Root growth observations began on February 12, prior to the irrigation treatments. After harvest, root images were collected at 6-12 day intervals over the following two months until the end of April. Further intensive daily root growth observations were conducted over a ten day period on newly emerging roots in the EPHI treatment, starting one week after harvest. The collected data were processed using the Rootfly software (Version 2.02.0, Clemson University) as shown in Fig 7-2 and results were exported to Microsoft Excel (Version 2007) for further analysis.

7.2.5 Statistical analysis

Data analysis was performed with R software (Version 3.1.1). A repeated measurement ANOVA was applied on the data to determine the influence of soil moisture, soil temperature, and irrigation type on growth of roots. Roots in the NPHI group are used for comparison to roots in the EPHI and LPHI group, respectively. For the daily root growth analysis of EPHI (soil temperature and moisture on daily growth rate), a general linear model was applied by using soil temperature and moisture and level of irrigation treatment as predictor variables.

7.3 Results and discussion

The average soil moisture in the NPHI treatment was significantly lower throughout the post-harvest period when compared to other treatments. The soil moisture at 30 cm in both EPHI and LPHI increased very quickly after irrigation, demonstrating the effectiveness of the watering process and associated instrumentation. However, soil moisture at 60 cm in both treatments took approximately two to three days to reach the same level as at 30 cm depth (Fig 7-
3). There was no significant difference in soil moisture between the two depths following this equalisation process, over the duration of the experiment.

Average soil temperature displayed similar fluctuations across all treatments. Soil temperature increased gradually at each depth in all treatments in the early stage of the experiment. Maximum temperature occurred 10 days after harvest at 30 cm (26.6°C) and 12 days after harvest at 60 cm (25.1°C). The decrease in soil temperature of the EPHI and NPHI treatments during the late stage of the experiment was more apparent at 10 cm relative to the 30 and 60 cm depths. However, the maximum soil temperature in the LPHI reached approximately 22.5 °C at both the 10 cm and 60 cm depths 10 days after LPHI and this peak appeared two days earlier than the soil temperature 20.5 °C at 30 cm (Fig 7-4). The maximum soil temperature during the EPHI treatment period was 6 °C warmer than during the later implemented LPHI treatment. Importantly, the soil temperature at all depths mirrored air temperature over the duration of the experiment and was not significantly affected by the application of irrigation water due to the various treatments. The maximum air temperature was 27 and this occurred 6 days after harvest and the minimum temperature was 10.9 °C, 50 days after harvest.

Canopy development observations for each treatment indicated that the majority of leaves in the canopies were green until one month after harvest (irrespective of irrigation treatments) and following month more than 50 % of leaf started
yellowing. It is also worth noting that approximately 90% of leaves had fallen off the vines by the end of the experiment in all treatments (April 30).

No root growth occurred in the weeks prior to the implementation of the irrigation treatments (Fig 7-5 b). Post-harvest root production in the EPHI treatment was significantly higher \( (p = .008) \) than in the LPHI or the NPHI treatments (Fig 7-5 a). A general observation across treatments was that the new growing roots were mainly distributed within 20-50 cm depth, essentially the zone where the soil temperature data was captured (data not shown). Root production after LPHI was not significantly different from the roots that received NPHI (Fig 7-5 a). In the EPHI treatment, a new flush of root growth occurred seven days after harvest and overall, root growth increased nearly sixteen-fold 20 days after irrigation before gradually decreasing (Fig 7-5 b).

Root length increased briefly immediately after the LPHI was applied. Maximum root growth rate was achieved seven days after harvest in the EPHI treatment and this growth rate was maintained for seven days, before declining (Fig 7-5 c). Vines that had received NPHI displayed a small flush of root growth seven days after harvest, synchronously with vines of the EPHI, but at a reduced scale. The soil tension of the NPHI treatment was very low at that time, around 300 kPa at 30 cm and 410 kPa at 60 cm, and this therefore likely contributed to the limited root growth.

To obtain more detailed information about the growth rates of individual roots, additional daily images were collected from three observation windows in the
EPHI treatment. These were windows with roots that appeared during the 10 day detailed observation period, and remained in frame for three or more days (Fig 7-5 d). The growth rates of the four roots observed in this period ranged from a maximum of 7.1 mm day\(^{-1}\) to a minimum of 0.2 mm day\(^{-1}\). The average rate of elongation for actively growing roots during this period was 1.8 mm day\(^{-1}\).

Various factors can have an influence on root growth, including pruning, irrigation and seasonal conditions (Comas, Anderson, Dunst, Lakso and Eissenstat, 2005; Eissenstat, Bauerle, Comas, Lakso, Neilsen, Neilsen and Smart., 2006; Mullins, Bouquet and Williams, 1992). It is interesting that in this experiment, vine responses to irrigation early or late after harvest both resulted in a flush of roots approximately 5 to 7 days after irrigation. However, the timing of irrigation in relation to harvest strongly influenced the total length of roots produced. This suggests root growth responses to environmental conditions may also vary in response to developmental stage.

7.4 Conclusion

This small experiment contributed to our understanding of root behaviour after harvest. Following harvest, vineyard managers should switch their focus from fruit quality and quantity to vine maintenance in order to optimise reserve accumulation prior to dormancy. This will help to optimise vegetative growth early in the following season in this perennial species.
This experiment has demonstrated that postharvest root growth in warm grape growing regions is primarily a function of water availability and timing of irrigation. Early irrigation promotes the production of more roots than late irrigation or no irrigation after harvest. Root growth lagged 5 to 7 days following the application of water and canopies reached a visually assessed arbitrary threshold of 50% of yellow leaves four weeks after harvest, regardless of the irrigation treatments.

Although this experiment is preliminary, replication of the conditions studied here could contribute to simple grower guidelines to optimise post-harvest root growth and management. A simple model including additional stresses such as increased soil temperature (usually as a result of increased air temperature but also influenced by soil physical characteristics), heavy crop loads and sub optimal nutrition would help refine post-harvest vine management.

### 7.5 Acknowledgments

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### 7.6 References

Chapter 7 Root Growth during Post-Harvest Irrigation of Warm-Climate Grapevines


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Figures

Fig 7-1 Schematic representation of the post-harvest irrigation treatments. Standard irrigation stopped 4\textsuperscript{th} of March 2013 just one day before harvest. The early post-harvest irrigation started in the evening just after harvest. The late post-harvest irrigation started one month after harvest.

Fig 7-2 Screen shoot from Rootfly software that was used for following fine root growth. The tube on the top right corner indicates the vine replicate (a). The observed root image is derived from a fixed position within the soil profile for root tracing (b) and the window on the left indicates the depth
which was monitored. The bottom images were taken at the same camera positions processed at other observation times (d).

![Soil moisture graph]

Fig 7-3 Average soil moisture over days after harvest within the three post-harvest irrigation treatments (LPHI, NPHI and EPHI) at two different soil depths (30 and 60 cm). Soil moisture data was collected from 1st of March till 30th of April 2013. Arrows indicate the onset of irrigation events (EPHI started 5th of March, LPHI started 5th of April).
Fig 7-4 Average daily soil temperature at three soil depths (10, 30 and 60 cm) from the three post-harvest irrigation treatments (LPHI, NPHI and EPHI) and air temperature throughout the experimental period. Arrows indicate the onset of irrigation events (EPHI started 5th of March, LPHI started 5th of April).
Fig 7-5 Total root length of three post-harvest irrigation treatments (LPHI, NPHI and EPHI) before receiving treatment (a). Accumulated new root length during the experiment (b), and corresponding mean daily increase in root length per tube (c). Daily observations of four actively growing roots (d), showing root length while visible in observation window, and corresponding growth rate (mm day\(^{-1}\)). Error bars ±SE Mean.
Chapter 8 General Discussion and Conclusions

Key contents

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8.1 General discussion

A successful and sustainable grapevine industry is reliant on fundamental knowledge and understanding of both above- and belowground processes within the grapevine. Roots anchor the plant within the ground, they act as a carbon and nutrient reserve and they synthesise a number of components critical to the functioning of the plant. Moreover, fine roots play a particularly significant role in the productivity of the grapevine as they are responsible for water and nutrient uptake (Comas et al., 2000; Volder et al., 2004).

Investigations of processes within plant components located below the soil surface are relatively scarce compared to studies of aboveground processes (Contador et al., 2015). This is because most of the methods are extremely time-consuming, tedious, and destructive, particularly when the subject of interest is a large perennial plant such as fruit tree or grapevine (Böhm, 1979; Hendrick & Pregitzer, 1992; Mackie-Dawson & Atkinson, 1991; Metcalfe et al., 2007). The sequence of studies presented in this thesis is based on non-destructive methods for root monitoring of plants grown in pots, large containers and in the field. This has resulted in new information on short and long term root growth dynamics in grapevines in relation to their soil environment.

The overall objective of this research was to examine the diurnal and seasonal timing of fine root growth of four grapevine genotypes in response to environmental conditions. This research was conducted in the Riverina region of NSW, a very warm grape growing region in Australia. We expect that the
information provided in this work will be extendable to other warm viticulture regions across the globe.

8.1.1 Diel and seasonal growth dynamics of grapevine roots

The diel rooting dynamics of fine roots in fruit crops was originally reported by Head (1965) in cherries but no studies are reported in grapevines. Our research was the first descriptive study on the diel dynamics of fine root growth of grapevines using a combination of purposefully designed small pots, large field-like containers and field conditions.

8.1.1.1 Fine grapevine roots display a strong diel dynamics in small and large pots

We found that the actively growing fine roots of grapevines displayed a clear and pronounced diel dynamics (Fig 3-8, Fig 4-2, Fig 4-3 and Fig 4-4 and Fig 5-6). However, the amplitude of this diel dynamic of root growth was affected by prolonged changes in photoperiod (Fig 4-3). This diel dynamic was evident in the small, young potted vines (Chapter 3 and 4) as well as the large containers with matured Shiraz vines (Chapter 5). Diel root growth dynamics were also observed in annual plants (Yazdanbakhsh & Fisahn, 2011; Yazdanbakhsh et al., 2011) and other perennial plants (Head, 1965; Hilton & Khatamian, 1973). However, the maximal and minimal diel rate of root growth varied between plants.

8.1.1.2 The maximum growth rate of fine root growth occurs late in the day

Irrespective of whether vines are grown in natural or controlled environments, root extension was greatest and slowest at approximately the same time of day.
The maximum root elongation rates observed in the outdoor young vine study occurred between early afternoon and sunset (Chapter 3). In small pots under a controlled typical photoperiod (Chapter 4), root growth was most rapid between late afternoon and two hours past sunset, whereas in large field-like containers (Chapter 5) it occurred from afternoon until midnight. The slight differences across the experiments may be related to the specific sampling time. In all the three experiments, root growth rate declined throughout the dark period and reached a minimum the next morning, before cycling again (Chapter 4). The timing of maximum and minimum root elongation rate observed in cherry trees (Head, 1965) generally aligns with our studies. Head (1965) showed that maximum root elongation rate of cherry occurred between 4 pm and 12 am and reached minimum rates between 8 am to 4 pm daily. In other root studies, Hilton and Khatamian (1973) reported that the night root growth rate was greater than the day rate in five woody plants and that these varied with seasons. Our study involved the continuous observation of root elongation and therefore is more detailed than the Hilton and Khatamian (1973) study which was limited to only one day and night observation rather than at several periods throughout the course of the day. Consequently, they were not able to compare the diurnal dynamics of root growth observed in our study except using a broad day/night classification.

8.1.1.3 The changes in hourly root growth rate was varied

In our study, the hourly root elongation rates ranged from 0.08 to 0.38 mm hour\(^{-1}\) across the pot experiments including field-like condition experiment, equating to a greater than four-fold daily change in the rate of growth. In cherry (Head, 1965), however, the hourly growth rate between 0.25 and 0.56 mm hour\(^{-1}\), showing a two-fold daily root growth rate. The hourly root growth rate of
germinating seeds of *Arabidopsis thaliana* also varied two-fold between 0.057 to 0.145 mm hour$^{-1}$ (Yazdanbakhsh et al., 2011). It seems that in grapevines there is greater fluctuation in growth throughout the course of the day and this could be related to a number of exogenous and internal factors which are detailed further below.

### 8.1.1.4 The diel cycle continues in altered photoperiods

Light is an environmental factor that is involved in the programming of rhythmic processes in animals and plants. In plants, many metabolic activities such as stomatal opening and nyctinastic movements of leaves are regulated by the quantity and quality of light. In the delayed photoperiod and progressively shortening photoperiod studies (Chapter 4), the diel changes in root growth rate followed the same dynamics as in the fixed photoperiod study with the maximum occurring at 6-10 pm and the minimum occurring at 6-10 am, regardless of whether the plant was in light or dark. When exposed to prolonged period of darkness (three days), a reduced diurnal fluctuation was still observed and root extension rate was reduced but did not completely cease until the third day (Fig 4-4). Dunlap et al. (2005) demonstrated that root growth in continual darkness maintained a 24 h cycle. Our study confirms this behaviour of grapevines in the dark (Table 4-4). That said, this dynamics became more erratic, over time and disappeared after several days in complete darkness, suggesting that light was perhaps required to resynchronize the daily rhythms.

### 8.1.1.5 The diel cycle of fine root growth may be fuelled by carbon

The weakening root elongation rates in response to decreasing photoperiod (Chapter 4) may be the result of shortage of carbohydrate supply (Yazdanbakhsh
et al., 2011). Shortening the day length caused a reduction in daily maximum root growth rate, suggesting that root growth was resourced by a carbohydrate supply. In the model plant Arabidopsis thaliana, the circadian clock orchestrates diurnal carbon allocation and growth (Graf et al., 2010; Yazdanbakhsh et al., 2011) and greatest root growth occurs during the dark through the process of starch degradation. Our results using grapevines suggests that starch degradation in fine roots may occur faster in woody crops than in annual plants as they demand more energy supply for growth due to a larger root system. The inhibition of root growth from midnight to next dawn also could be due to the change in energy metabolism or root signalling pathway during the night period (Smith & Stitt, 2007).

8.1.1.6 Grapevine roots grow mostly at flowering but pre- and post-harvest flushes can occur

In general, root growth in grapevines has been reported to be most pronounced before or during bloom (1988). Eissenstat et al. (2006) also reported that the main root flushes of Merlot and Concord occurred in summer between bloom and veraison and little root production occurred before harvest and during dormancy. In our 7 year study of field grapevines, we found that grapevine genotypes had consistent seasonal rooting dynamics despite different vertical root distribution characteristics (Chapter 6). We observed, that the new root growth activities started in late winter and peaked at bloom when the soil temperature reached between 15 and 30 °C at a soil depth of 10 cm (Fig 6-2 a). New smaller flushes of new root production were, however, apparent in mid or late summer and after harvest. Many seasonal root growth studies in fruit crops including grapevines showed that fruit crops had clear a unimodal dynamics of new root production
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Comas et al., 2005; Contador et al., 2015). These unimodal dynamics may be as a result of a temperate environment where dormancy starts soon after crop harvest (Atkinson, 2011; Comas et al., 2005; Contador et al., 2015; Head, 1967). Photoperiodism is a major means by which various developmental phases are regulated by the environment, especially in regions with distinct seasonal climates. However, in our study a multimodal rooting dynamics was evident with a major flush occurring at flowering and minor modes at other phenological stages. The vine leaves in our warm climate vineyard can remain functional for up to three months compared to cooler grape growing regions where dormancy begins shortly after harvest. The results in this study suggest that timing of new root production in grapevines could be partially influenced by soil temperature and/or plant nutrient and water resource availability (Bevington & Castle, 1985; Bonomelli et al., 2012; Callejas et al., 2009a). Our post-harvest irrigation study (Chapter 7) confirms that vines experiencing sufficient watering in the early stage of post-harvest can develop further root flushes during the post-harvest period. These results are in accordance with the findings of previous work on Shiraz conducted in the Riverina (Smith et al., 2009). It is uncertain if the root growth in response to post-harvest irrigation are in response to a flush of soil moisture (which would potentially bring nutrients with it), or simply the soil water availability. Unfortunately, the data presented here, even when considered as a whole, are not sufficient to assess this.

Our study indicated that the major new root flushes occurred around bloom in spring with some root growth at bud break, followed by veraison in summer, and dormancy at harvest in autumn. To some extent, the present study supports the
previous studies (Freeman & Smart, 1976; Van Zyl, 1988). However, Eisenstat et al. (2006) found that root growth is most pronounced between bloom and veraison and after harvest. The inconsistency in new root production could be due to varying soil temperatures and soil moisture during the major phenological stages. The presence of new root production during dormancy can be explained by Bhar (1970) who found that certain roots would produce continuously fine roots during dormancy where the ambient soil temperature was 3 °C or greater.

### 8.1.2 Influence of genotype on grapevine root growth

The characterization of genotypic diversity in rooting behaviour to specific soil environments is critical to the development of a sustainable viticulture industry that is responsive to climate change (Bassoi et al., 2002; Bates et al., 2001; Di Filippo & Vila, 2011; Harbertson & Keller, 2012). Worldwide, 90% of commercial vines are grafted to a rootstock adapted to specific soil or environmental conditions such as drought salinity, phylloxera and nematodes. Rootstocks also have the ability to curtail excess vegetative growth (Keller, 2010) and have an impact on yield and fruit composition. The successful choice of a particular rootstock is dependent on understanding its behaviour under particular environmental conditions, and this includes a consideration of its rooting dynamics and distribution (Smart et al., 2006).

#### 8.1.2.1 New roots production is influenced by a range of factors but is mostly genotypic

The results of our long-term field study (Chapter 6) demonstrated that the production of new roots was a function of air temperature, genotype, season,
phenological stage, year and observation time. The genotype factor however played a dominant role among these factors, particularly when two-way interactions were considered (Table 6-1). In this study, 140 Ruggeri produced the maximum number of new roots, followed by Ramsey, Schwarzmann and Shiraz. In our young vine pot study (Chapter 3), the four rootstocks produced different root numbers (Table 3-1) although they had similar diurnal root growth dynamics (Fig 3-8). Schwarzmann and 140 Ruggeri had larger fine root systems, followed by Ramsey and Shiraz (Table 3-1). These root population differences may be due to the age of plant in the pots compared to that of those in the field or to differences in soil texture and soil environmental conditions. Nevertheless, Shiraz and 140 Ruggeri seem to consistently be the most and least productive rootstocks, respectively, with Schwarzmann and Ramsey being moderate producers of roots.

In early studies it was reported that root populations vary between genotypes. For example, Southey and Archer (1988) reported that 140 Ruggeri had larger root density and root diameter compared to other genotypes such as Ramsey, Teleki and 99 Richter. Similarly, Perry et al. (1983) found that the greatest numbers of grapevine roots was in Dogridge, followed by Barbera and Concord, and finally Noble. Our results in pots and field experiments support these earlier observations that from an absolute and relative perspective, root systems are very different across genotypes. The performance of grafted vines is however affected by the behaviour of the scion. Nagarajah (1987) for example, reported that Thompson seedless vines grafted on Ramsey had a widespread root system with greater root density, more fine roots and higher total root length than ungrafted ones.
Therefore, different root characteristics of rootstocks influence scion growth, yield level and whole plant performance (Atkinson & Else, 2001).

8.1.2.2 Rootstocks differ in their vertical root distribution

In our pot experiment it was not possible to observe root distribution dynamics as the pots were too small. However, in large bins (Chapter 5) we observed that the maximum number of roots in Shiraz were located towards the bottom of the bin (52 cm), a characteristic not apparent in the field experiment. This may have been the result of the dimensions of the bin, soil depth or the location of the end of the minirhizotron tube. Nevertheless, root size and changes in root colour within the bins were similar to the root behaviour in the field rootstock trial. The genotypes in our field study exhibited diversity in vertical root distribution dynamics through the soil profile (Fig 6-4, Fig 6-5). The total new root production in each genotype was significantly different at different soil depths (Fig 6-5). New root production by 140 Ruggeri occurred between 0 and 52 cm with the greatest root numbers between 10 - 30 cm, whereas Schwarzmann had the greatest root populations between 10 and 40 cm. Shiraz had a maximum number of new roots in the 20-40 cm soil depth. Ramsey had a very unique root distribution dynamics and most of the new roots occurred between 20 and 52 cm. Ramsey is usually considered to have a long ranging root system, perhaps suggesting that a proportion of the Ramsey roots were below what could be measured. It would be worthwhile for future studies to extend the measurements beyond 50 cm as this was the limitation of our minirhizotron tubes. Perry et al. (1983) also indicated that the root distribution dynamics varied between Vitis cultivars. For example, in this study Noble had shallow roots, with approximately 35% of the total roots in the 0-15 cm soil depth whereas most of the roots of Dogridge occurred between
90-105 cm soil profile. In other fruit crops such as in peach, rootstock of K119-50 produced larger amount of new roots below 69 cm soil depth compared to other rootstocks (Basile et al., 2007). These differences illustrate the genotypic response of root distribution in similar soil conditions. Based on this knowledge, growers can therefore select specific rootstocks to match their soil characteristics and apply irrigation according to where most of the roots occur.

8.1.2.3 Root diameter differs between genotypes and is related to root length

Under the conditions of our pot experiment, the four genotypes exhibited differences in their total root diameter. The total root diameter per vine was correlated with total root length (Fig 3-7) and this was more obvious in 140 Ruggeri than in any of the other genotypes. Shiraz showed the smallest root length and root diameter; while the root diameter and length of Ramsey and Schwarzmann was larger, but less than that of Ruggeri 140 (Fig 3-7). In a previous study, (Southey & Archer, 1988) root diameter increased concurrently with root elongation in 140 Ruggeri and 1103 Paulsen, with some differences in root diameter between the genotypes. These results confirm that when attempting to predict the physical characteristics of fine root systems for the purpose of modelling a genotype based approach is recommended (Atkinson & Else, 2001).

8.1.2.4 Rootstocks differ in their seasonal rootgrowth dynamics

In our field study (Chapter 6), the four genotypes had different new root numbers at different phenological stages. All the genotypes produced the maximum number of new root numbers at flowering (Table 6-4), however root growth was also observed in all cultivars at other stages, but always in very modest numbers, when compared to flowering. For example, 140 Ruggeri displayed a larger
amount of roots at harvest, and (in similar numbers), at bud-break and veraison. Schwarzmann and Shiraz had root flushes at veraison in greater numbers than at harvest followed by bud-break. Finally, Ramsey produced strong root flushes at bud-break, followed by veraison and harvest. These differences may be the result of the origin of these vines in terms of the length of their growing seasons, the soil type, precipitation dynamics and temperature cycles throughout the season.

8.1.2.5 Genotypic differences in root system size were not related to vine leaf area

A range of studies suggest a stable allometric relationship between above- and belowground biomass. In our potted study, the allometric relationship between fine root population and leaf area was inconsistent and the aboveground leaf area did not display the expected relationship with the genotype’s root population. For example, 140 Ruggeri had the largest fine root population but a small leaf area. By contrast, Schwarzmann had a large number of fine roots associated with a leaf area significantly larger than any other genotype. Likewise, Ramsey and Shiraz exhibited a small and medium number of fine roots, respectively, with small total leaf areas. Our results in this experiment indicate that there is a genotypic difference in the biomass allocation between the fine root system and leaf area. Predictive allometric relationships should therefore be calibrated accordingly.

8.1.3 Influence of environmental factors on grapevine root growth

8.1.3.1 Soil temperature has an influence on root growth

In our pot studies both under naturally fluctuating environmental conditions as well as temperature and light controlled environments, the root populations and
circadian root growth dynamics were related to soil temperature. It has been well established that when other abiotic factors are non-limiting, soil temperature is the main driver to initiate root growth and distribution (Atkinson, 2011; Bonomelli et al., 2009; Callejas et al., 2009b; Rogiers et al., 2011). We observed that maximal new root production at the flowering stage (spring) in the field experiment when the soil temperature was between 15 to 30 °C (Fig 6-2). Our results suggest that the optimal soil temperature for root growth was between 20 and 28 °C (Fig 3-10). At soil temperatures greater than 28 °C, root growth decreased by 50 % (Fig 4-2). This is in accordance with Kliwer (1975) who showed that the optimum soil temperature occurred between 25 and 30 °C. At higher temperatures, our study observed increased shoot growth rate in combination with a decrease in root elongation rate (Fig 4-2). This indicates that shoot growth and root growth occur independently and have different temperature optimums. Some roots in each genotype did not show significant root growth rate under optimum temperature in our pot experiment. We therefore conclude that soil temperature is not the only factor influencing fine root growth in grapevines, but that other factors also play an important role.

The diel and seasonal fluctuations in soil temperature is particularly pronounced in the upper soil layer (Bonomelli et al., 2012) and this is likely to have a strong impact on the short term and long term root growth of irrigated vines where most of the fine roots are located near the soil surface. Even though vine roots grow and function in diverse environments, minor fluctuations in soil temperature can affect new root production and root growth rate, depending on the phenological stage (Bhar et al., 1970). In our five year seasonal root growth field study, soil
temperature and moisture data were recorded during the last two seasons (2012-2014). In these two years, the soil temperature at 10, 30 and 60 cm soil depth was highly correlated to air temperature (Fig 6-1). Therefore, air temperature was used as one of the factors in the root growth analysis, showing a significant influence in seasonal root growth dynamics (Table 6-1). New root production was greatest during spring and decreased during the summer period and this may be related to the high soil temperatures during this period which were recorded to average at 30 °C. However, we also observed that autumn growth was lower than the root growth during the dormancy period even though the soil temperature was about 10-30 °C at harvest. This again indicates that aside from temperature other factors are important and it has been even suggested that carbohydrate availability during the different times of the year can influence root growth (Bennett et al., 2005).

8.1.3.2 Soil moisture has an influence on root growth

Under natural conditions, water is provided by rainfall and is temporarily stored in soil profile for extraction by plant roots. In our study, it was found that soil water content at 30 cm soil depth had a significant impact on new root production (Table 6-2). There was no apparent effect of soil moisture at soil depth of 10 cm (Chapter 6 and 7) and 60 cm (Chapter 6) on the production of new roots. However, under our field conditions irrigation was only successful in wetting the top 10 cm. By contrast, most of the roots were further down the profile where the presence of water was more sporadic. The soil at 30 cm was sufficiently dry to limit root production at this depth. In our post-harvest field study, we found that vines were significantly responsive to irrigation applied after harvest (Fig 7-5). A flush of roots was observed approximately 5 to 7 days after irrigation. We
observed that the timing of irrigation can be a factor influencing total root production. For example, early irrigation had a significant influence in root growth compared to irrigation after harvest (Fig 7-5). This suggests that root growth responses to environmental conditions may also vary in response to developmental stage (Eissenstat et al., 2006). In addition, in warm grape growing regions of Australia, irrigation after harvest is critical for the initiation of fine root growth, because unlike in some regions, a functional canopy can be maintained for up to two to three months. In our field study on grapevines, soil moisture was recorded in the last two years of the study to evaluate the relationship between root growth and soil environment. No differences in the number of new roots between these two years were observed (Table 6-2). However, genotype and soil moisture were found to be critical factors in seasonal new root production. Furthermore, year to year differences were evident in new root production and seasonal dynamics. Those variations in root growth is influenced by cultural practices such as irrigation (Chapter 7) (Bassoi et al., 2003) and pruning (Comas et al., 2000; Ferree et al., 1999).

8.1.3.3 The dark component of the diel cycle of fine grapevines root growth aligns with previous studies

The maintenance of a reduced diurnal growth dynamics in a period of continual darkness was suggested as a mechanism to maintain physiological processes in alignment with the diel cycle (Ruts et al., 2012) in Arabidopsis. When exposed to prolonged period of darkness (three days), a reduced diurnal fluctuation was still observed and root extension rate was reduced but did not completely cease until day 10 (Fig 4-4). The growth rhythms were reduced but disappeared only in the 10th day of darkness, suggesting that re-illumination may regulate rhythmic
growth through photosynthetic carbon assimilation (Smith & Stitt, 2007). In addition, the slow growth during the night could be due to a lack of available carbohydrates (Gibon et al., 2004; Ruts et al., 2012; Wiese et al., 2007; Yazdanbakhsh et al., 2011). Dunlap et al. (2005) reported that the circadian clock is known to function in continuous darkness. They demonstrated that root growth in darkness maintained a 24 h cycle and that this growth was correlated with soil temperature. Our study confirmed this behaviour during the dark period for grapevines (Table 4-4).

8.1.4 Implications for vineyard management

Timing of root production may have significant implications on the future management of fruit crops (Eissenstat et al., 2006). In viticulture, grape growers undertake various management practices that coincide with critical phenological stages including, bud-burst, flowering, veraison and harvest (Eissenstat et al., 2006). Understanding periods of root growth in relation to these stages under changing environmental conditions is critical in scheduling the various vineyard management activities (Eissenstat et al., 2006). Similar, the planting and vineyard establishment phase will be impacted by root growth periods. Once the vineyard is established, timing of nutrient and water application, and other management issues such as cultivation and timing of organic matter, mulch and lime application may be critical to root growth. Timing of leaf trimming during canopy development may also have consequences on root growth through the competition of carbohydrates between root and shoot growth. The choice of cover crop and inter-row sward management may be important to grapevine root growth as these species compete for nutrients and water. Conditions such as
temperature, water availability and nutrient availability are important factors that can impact root growth (Kaspar & Bland, 1992; Keller, 2005; Yao et al., 2009).

In addition, the genetic diversity of grapevine rootstocks is also becoming increasingly significant in the context of favourable root growth under broad range of soil environments (Bonomelli et al., 2009; Keller, 2010). Quantifying the root growth dynamics of different vine genotypes in varying soil and climatic conditions can provide direction towards reforming vine management practices and improving overall grapevine performance and productivity.

8.2 Conclusions

- Under the conditions of the present study, the elongation rates of actively growing roots were found to have a pronounced diurnal dynamics. Maximum growth rates, which ranged from 0.15 to 0.38 mm hour\(^{-1}\) across the three pot experiments, were highest in the afternoon, declined through the night and reached a minimum the next morning. These results are early suggestions that the diel cycle is a regulated process for grapevines.

- The diurnal dynamics of root growth was seen across all genotypes and was evident in both the small potted vines and the larger container grown Shiraz. The significance of this finding is that it suggests the control of root elongation is conserved across genotypes and linked to fundamental physiological process that coordinate plant growth and metabolism with the diel cycle.
Under naturally fluctuating environmental conditions and controlled environment conditions, diurnal root growth dynamics were observed. Progressively shortening the day length until continual darkness caused a concurrent reduction in daily root growth rate. However, root growth did not completely cease until several days of complete darkness, and a reduced diurnal dynamics was still present. These results suggest that neither light nor temperature are the primary regulators of the diel cycle, although it does not exclude them from influencing physiological processes associated with it.

We argue that the decline in root growth rates in response to decreasing photoperiod suggests a dependence of fine root growth on carbohydrate supply from photosynthesis. The maintenance of a reduced diurnal growth dynamics into the period of continual darkness may also suggest a contribution from mechanisms that maintain physiological processes in alignment with the diel cycle.

Our study confirms the seasonal root growth dynamics of grapevines, and observed a maximum new root production at flowering relative to other phenological stages, however dormancy root flushes can also occur.

Root growth of grapevines in both our diurnal and seasonal studies was influenced by a range of internal and external factors, however the most significant statistical factor was “genotype”.

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- Under the warm climate conditions of this study, in all our experiments, the genotypes displayed different characteristics in their root population, seasonal root growth dynamics and vertical root distribution. These characteristics need appropriate calibration in modelling or predictive physiological studies.

- Under the conditions of our diurnal root growth study, root population was not related to vine leaf area. However, root length closely related to shoot length. Allometric relationships between above and below ground biomass therefore need to be cautiously verified.

- Soil temperature was found to modify root growth dynamics in the diel and seasonal cycles. The optimum soil temperature for root growth is between 20 and 28 °C. These results confirm the maximum fine root production temperature range.

- Soil moisture was discovered to be one of the critical factors in seasonal root development, particularly after harvest in hotter climatic regions. Soil water content at 30 cm soil depth has a significant effect on root growth. These results offer a vineyard management strategy for warm climate grape producers wishing to optimise post-harvest fine root growth for enhancing nutrient uptake.

8.3 Recommendations for Future Work
Understanding root growth and function is an important research topic and there are still essential questions to be explored. The findings of this study demonstrated that the diurnal and seasonal root growth dynamics differ from hours to years and diversity of rooting dynamics across genotypes involved in complex physiological and environmental processes. There have been numerous studies on the above ground parts of annual and perennial plants, but only a few have explored detailed root growth in woody plants, especially grapevines. The research recommendations are suggested as follows:

- Observation of detailed diurnal root activity to determine the exact starting and stopping point of root growth in time of day.

- Examination of comprehensive diurnal root development to clarify the lifespan of fine roots, in particular the conditions required for initiation, survival and death triggers.

- Evaluation of the role of carbohydrate in the circadian clock. In particular, overall starch turnover in fine roots during development.

- Assessment of carbohydrate availability and root respiration effects on root elongation dynamics between genotypes and under a broad range of soil temperature environment.
• Examination of root survival in relation to temperature and drought. Determine the thresholds of soil temperature which restrict root growth for different genotypes.

• Investigations of cultural practices on changes of fine root growth production and nutrient uptake, and ultimately vine productivity.

• Valuation of the importance of diurnal and seasonal root growth dynamics on nutrient uptake in fertiliser application.

8.4 References


