Grapevine (*Vitis vinifera* L., cv. Pinotage) responses to water deficit modulated by rootstocks

by

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Declaration

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Summary

Water scarcity is a key limiting factor for viticulture in dry regions. Traditionally drought sensitive varieties have the potential to grow in dry areas, however in most situations, through the use of rootstocks. Drought-tolerant rootstocks are expected to improve grapevine response to water deficit by improving the water uptake and transport and by reducing the water loss in leaves by root-to-shoot signalling. The mechanisms of rootstocks' tolerance to drought are not yet fully understood. The main aim of this study was to improve the understanding of the rootstock/scion-cultivar interaction in the regulation of grapevine water use and leaf stomatal behaviour. Irrigated field vines without any water constraint were compared to rain-fed grapevines subjected to moderate water constraint. To better manage vine water status, reduce variability, and compare more rootstocks, greenhouse trials were also conducted where plants were well watered or subjected to severe water constraints. Pinotage grapevines (*Vitis vinifera* L.) grafted onto 110 Richter, 140 Ruggeri and 1103 Paulsen rootstocks were used for field experiments whereas Pinotage grapevines grafted onto 99 Richter, 110 Richter, 140 Ruggeri, 1103 Paulsen and Ramsey were used for greenhouse experiments. Our study suggested the influence of rootstocks on scion-cultivar water status and leaf stomatal size and density and gas exchange of the scion, implying an influence on water uptake and transport and a tight regulation of the stomatal conductance. Our data supported the hypothesis that the influence of rootstock in response to drought seemed to be higher under increasing water deficit up to a point where the plant water status is the main driver of the stomatal conductance and therefore photosynthesis regulation, considering the plant water status thresholds. In addition, the results suggested that stomatal development is affected by light, drought and possibly by rootstocks. Nevertheless, it is still not clear how the rootstock affects stomatal development and the link with scion-cultivar water use. It seems that the transpiration rate of leaves is more related to stomatal size than density. Thus one possible mechanism of Pinotage leaf adaptation to water constraints was structural during leaf growth, with a reduction in pore size to reduce plant water loss. The results showed that the rootstock is regulating the cultivar's stomatal size (anatomical changes during leaf growth) and functioning (stomatal regulation) through a complex signalling process. The effect of light on stomatal development is interesting in the context of canopy microclimate and canopy manipulation (choice of the vine architecture vs canopy size, in the context of climate change versus the possible increase in drought and water scarcity). The use of rootstocks is a long term investment which aims to provide resistance to soil pests and pathogens and to confer to the scion-cultivar drought and salt tolerance. The use of drought tolerant rootstocks is actually one of the most relevant practical solutions in dry terroir – units and in situations where water availability is limited. The understanding of the physiological and genetic mechanisms which govern scion-cultivar drought tolerance/behaviour induced by rootstocks is critical in terms of rootstocks choice in interaction with the scion-cultivar and is critical to assist breeding programs to create/select drought tolerant rootstocks.

This dissertation is dedicated to my wife Marcela and my children Iñaki, Catalina and Agustín

In Memory of my Father who died on 27 December 2010

Biographical sketch

Ignacio Serra was born in Santiago, Chile on 20 June 1973. Eighth months later the family moved to San Ramón, Costa Rica, where he later on began his schooling at the Escuela Laboratorio. Later they moved back to Chile where he continued his education at Colegio Preciosa Sangre in Pichilemu and Colegio Inglés George Chaytor in Temuco. He enrolled at Universidad de La Frontera, Temuco, Chile for an engineering degree in Agronomy and graduated in 2000. Ignacio trained at the Universidad Politécnica de Madrid, Spain in the course Master in Viticulture and Enology and graduated in 2002. He completed his MSc Agric (Viticulture) degree in 2010 on the topic “Influence of soil parameters and canopy structure on root growth and distribution” at the Stellenbosch University. He has been employed as a lecturer in viticulture and enology at the Department of Producción Vegetal, Universidad de Concepción, Chile, since 2003.

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Preface

This dissertation is presented as a compilation of five chapters. Each chapter is introduced separately and is written according to the style of the journal Australian Journal of Grape and Wine Research to which Chapter 2 was submitted and accepted for publication.

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Chapter 1

General introduction and project aims

Chapter 1: General introduction

1.1 Introduction

Most of the grapevines cultivated around the world have to deal with drought, if irrigation is not applied, experiencing different levels of water constraint, from moderate to severe water deficits. Furthermore, drought tolerance became relevant under the prospects of water deficit due to higher temperatures linked to a possible evolution of the climate. Recently it has been shown that seasonal climatic conditions affect more the grapevine carbohydrate mobilization and storage than cultural practices (Holzapfel and Smith 2012). Grapevines are considered relatively tolerant to water deficit, while differences among cultivars (Schultz 2003) and rootstocks (Keller 2010) exist. The study of grapevine grafted onto rootstock adds more complexity to the system due to the resulting interactions. Rootstocks can affect scion vigour and resulting canopy size modification therefore affecting grapevine water use. Changes in root growth and functioning that affect water uptake and water transport, but also at the canopy level through long-distance signalling that affect water loss are also part of the rootstock effect on scion water uptake and demand. The scion-rootstock interaction depends on the scion characteristics (leaf anatomical characteristics, canopy size related to vigour, capacity to modulate root-to-shoot signalling at the shoot level, etc.), rootstock properties (root anatomy, morphology and growth, root functioning in terms of adsorption of water and minerals, root-to-shoot signalling related to hormone biosynthesis) and the quality of the graft union in terms of the connection of the vascular system and the cambium functioning and ability to differentiate proper xylem and phloem tissues.

Several studies showed that rootstocks can improve water uptake and transport through changes in root growth, hydraulic conductance and xylem embolism repair (Baiges et al. 2001, Galmés et al. 2007, Gambetta et al. 2012, Marguerit et al. 2012); in addition to a reduction of water loss through stomatal regulation by complex long distance signalling processes (Lovisolo et al. 2002, Schultz 2003, Soar et al. 2006, Rodrigues et al. 2008, Marguerit et al. 2012, Romero et al. 2012). The use of rootstocks to enhance drought tolerance in grapevine might be a feasible solution to face drought. Nevertheless, the ways the rootstock induce drought tolerance to the scion is still not fully understood, with some debate regarding the control of stomatal regulation (Chaves et al. 2010) and lack of information on the root system functioning.

Considering that grapevine water use is a key factor for the sustainability of the wine industry, the present study focuses on the grapevine drought tolerance modulated by the use of rootstocks.

Pinotage is a South African cultivar, used as scion in this study, which is known to be drought tolerant an is often cultivated in dry land, grafted on drought tolerant rootstocks and trained as Goblet (bush vine). To increase the productivity of Pinotage without compromising the fruit and wine quality, this cultivar is cultivated under irrigation and trained as Vertical Shoot Positioning. Very little physiological studies have been done on Pinotage water use efficiency, leaf functioning and anatomy versus stomatal size and density in interaction with rootstocks.

The goals of this study is to help the South African Wine Industry and nurseries to make the appropriate choice in terms of Pinotage/rootstock combination in relation with the wine industry gaols (i.e. yield per vine, fruit composition and wine quality), we have been funded by WINETECH to study the possible effect of some rootstocks on Pinotage water use efficiency, leaf functioning and anatomy in order to possibly give some recommendations in terms of rootstocks choices. This study was therefore conducted to improve the understanding of the possible effects of some rootstocks on Pinotage (*Vitis vinifera* L.) leaf functioning and stomatal size and density. The rootstocks have been chosen among the most used by the nurseries in South Africa to graft most of the scion cultivars, including Pinotage. Two classes of rootstocks have been considered: a) drought tolerant as 110 Richter, 99 Richter, 140 Ruggeri, and 1103 Paulsen, these rootstocks having different levels of drought tolerance, and b) drought sensitive as Ramsey which was chosen as a reference been drought sensitive and conferring vigour to the scion in watered situations.

1.2 Project aims

To achieve these goals field and greenhouse experimentations have been conducted.

1) Field experimentations were done on the following Pinotage/rootstock combinations: 110 Richter, 140 Ruggeri and 1103 Paulsen. By doing field experimentations, not only the possible effects of rootstocks on Pinotage leaf functioning and vine water status were considered, but we took advantage of working on productive vines from a vineyard to study the possible interaction between drought and bunch microclimate on fruit growth and basic composition. This part of the PhD is actually a sub topic and not the core research of it, and will be described as such. The field experimentations were conducted only over two seasons because it appeared that the Stellenbosch University vineyard which was recommended to us as a dry land, in fact was not, thus we were not able to get the desired water constraint and stress.

2) Greenhouse experimentations were done to study the responses of Pinotage leaf functioning and vine water status using climatic controlled conditions and vines in pots

which allowed controlling the soil moisture and the vine water status in order to be able to get a progressive water constraint and stress. Five different rootstocks were chosen, the three used for the field experimentations and we added another drought tolerant rootstock 99 Richter and a water sensitive one Ramsey. This choice was made to get a range of drought tolerant rootstocks and compare them with Ramsey which is known to confer vigour to the scion.

The greenhouse experimentations allowed as well conducting some research on the possible responses of Pinotage leaf stomatal size and density combining the complex interaction between two abiotic factors (light and water) and the selected rootstocks. A discussion on the interaction rootstock/cultivar in terms of adaptation to sites (soil x climate) where drought and water scarcity are a concern is presented in the Chapter General Discussion and Conclusions.

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Chapter 2

Literature review

Review: the interaction between rootstocks and cultivars (*Vitis vinifera* L.) to enhance drought tolerance in grapevine

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Review: the interaction between rootstocks and cultivars (*Vitis vinifera* L.) to enhance drought tolerance in grapevine

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Rootstocks to enhance drought tolerance in grapevine

Abstract

Water scarcity is a key limiting factor in agriculture. Grapevines react at the physiological, biochemical and genetic levels to tolerate water constraints. Even though grapevines are considered relatively tolerant to water deficits, grapevine growth and yield can be seriously reduced under water deficit. Drought tolerant rootstocks are expected to enable the scion to grow and yield when water supply is limited. Genetic machinery allows rootstocks to control water extraction capacity and scion transpiration. Numerous works have demonstrated the positive role of drought-tolerant rootstocks on the control of cultivars' leaf stomatal conductance and therefore on canopy transpiration. The mechanisms, in terms of signalling and gene functioning, need further study. Furthermore, there is no standardised methodology to rank rootstocks in terms of their tolerance to drought. A potential effect of rootstocks on stomatal development is also discussed. This review will critically discuss the current knowledge of the mechanisms of drought tolerance afforded by rootstocks, taking into account the scion/rootstock interaction, and will present some of the challenges for future investigations.

Keywords: *climate change, drought tolerance, rootstocks, water deficit, water use*

2.1 Introduction

The use of rootstocks is common in most viticultural areas, and most rootstocks currently used around the world were developed before 1930 from American *Vitis* species in an effort to avoid the damage caused by phylloxera, which devastated the European vineyards in the last half of the 19th century (Granett et al. 2001). Currently, scion cultivars are grafted onto rootstocks that are either North American species or inter-specific hybrids (Mullins et al. 1992) (Figure 2.1) that have a limited genetic background due to the fact that 90% of all rootstocks used around the world originated from less than ten different rootstock cultivars (Keller 2010). Rootstocks are selected for their resistance to phylloxera, however, several other characteristics are also required, such as suitability for grafting, rooting and propagation; resistance to nematodes and Pierce's disease, tolerance to lime, drought, salinity and vigour conferred are also considered (Granett et al. 2001). Possible water scarcity in the near future (Intergovernmental Panel for Climate Change 2008) increases the interest in drought tolerance afforded by rootstocks.

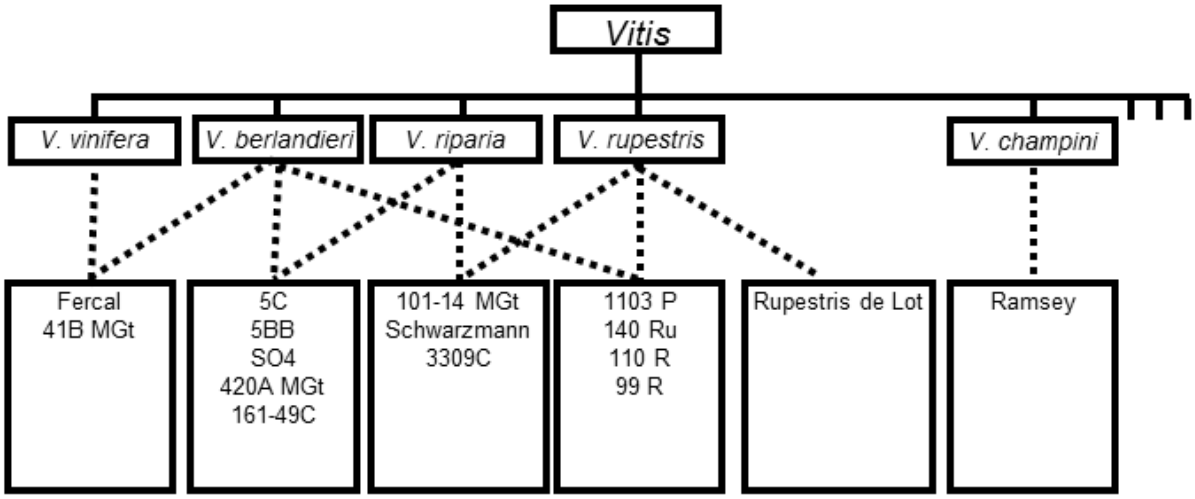

Figure 2.1 Genetic origin of some rootstocks used worldwide [adapted from Dry (2007)].

Drought induces senescence of older leaves (Jackson 1997), a decrease in growth, a decrease in plant water potential, stomatal closure, lower transpiration and photosynthetic rates (Yordanov et al. 2000). The drought responses of a plant involve a series of physiological and biochemical changes. Stomata are pores that control the gas exchange between leaves and the atmosphere (Hetherington and Woodward 2003), which is necessary for photosynthesis. In C_3 plants, during a mild water constraint, a reduction in photosynthesis is mainly due to stomatal closure, with a transition phase with stomatal and non-stomatal limitations, while during severe water deficit the non-stomatal limitation to photosynthesis is dominant (Lovisolo et al. 2010). This may include a decline in Rubisco activity (Dias and Brüggemann, 2010). Many studies have

shown that grapevine response to water deficit involves a reduction in stomatal conductance and photosynthesis (Iacono et al. 1998, Koundouras et al. 2008, de Souza et al. 2003); a decrease in leaf expansion and internode elongation (Schultz and Mathews 1988, Cramer et al. 2007, Lovisolo et al. 2010); and a reduction in yield (dos Santos et al. 2003, Chaves and Oliveira 2004). Drought can cause cellular water loss, which induces osmotic stress that affects cell division and elongation and which, in turn, affects the growth of different organs (Bartels and Sunkar 2005). The degree of growth limitation can vary depending on the nature of the tissue, e.g. shoots, leaves or roots (Wu and Cosgrove 2000). The rate at which water constraints develop, i.e. gradually or abruptly, could also determine the extent of growth limitation (Christmann et al. 2007). Furthermore, the cell will have to deal with the production of reactive oxygen species that negatively affect the cell metabolism and cell wall structure (Bartels and Sunkar 2005). Therefore, the sensitivity of growth to drought will depend on regulation at the physiological, biochemical and genetic level that can control changes in the cell wall (tightening and loosening) (Moore et al. 2008). Turner (1986) suggested three mechanisms of plant adaptation to water deficit, namely drought escape, drought tolerance with low plant water potential and drought tolerance with high plant water potential. In terms of drought tolerance, rootstocks are expected to enable the scion to grow and function normally when water supply is limited. The mechanisms of tolerance to drought by rootstocks are not yet fully understood. In tomato, a higher scion fruit yield under salinity was related to a greater capacity of the rootstock to improve water flow to the scion, probably due to an enhancing vascular cylinder area and xylem cell lignification in comparison with a non-grafted variety (Asins et al. 2010). In apple, peach and cherry the effect of rootstock genotype on scion vigour has been related to the influence on the hydraulic conductance capacity (Atkinson et al. 2003, Tombesi et al. 2010, Zorić et al. 2012). Furthermore, in kiwi it was found that differences in phenology between scion and rootstock combinations appear to be responsible for the rootstock influence on shoot growth (Clearwater et al. 2007). In grapevine, high vigour rootstocks have higher fine-root hydraulic conductivity due in part to higher aquaporin expression and activity (Gambetta et al. 2012). Furthermore, rootstocks with higher inherent vigour perform better than low vigour rootstocks under water deficit conditions (Williams 2010). Nevertheless, the effect of vigour on the plant's drought tolerance is still not clear (Jones 2012). It has been postulated that using drought-tolerant rootstocks in grapevine can help to minimise the effect of water constraints via improved water uptake and transport (Carbonneau 1985, Soar et al. 2006) and by controlling the plant's transpiration through chemical signalling (Loveys and Kriedemann 1974, Stoll et al. 2000, Soar et al. 2006) and hydraulic signalling (Vandeleur et al. 2009). The aim of this review is to identify

and discuss the main advances in the understanding of the rootstock/scion interaction in the regulation of grapevine water use.

2.2 Root anatomy

In general, grapevines are considered relatively tolerant to water deficits, due in part to their relatively large xylem vessels in comparison with those of other plants (Comas et al. 2010), allowing a quick recovery from water constraints (Lovisolo et al. 2008a). Furthermore, grapevine roots have larger xylem vessels (Figure 2.2) in comparison with their stems, causing them to be more prone to xylem cavitation (Lovisolo et al. 2008a). Cavitation and embolism can affect whole-plant hydraulic conductance at different levels: leaves, stem and roots. It has been suggested that the sensitivity to cavitation and embolism might be related to plant mechanisms to adapt to water deficit conditions involving stomatal conductance regulation (Domec and Johnson 2012). In peach and cherry it was found that rootstocks that induce more vigour have larger xylem vessels and lower vessel density in comparison with the ones considered dwarfing rootstocks, resulting in different hydraulic conductance capacities (Tombesi et al. 2010, Zorić et al. 2012). In the same way, citrus rootstocks that have higher hydraulic conductance appear to have larger xylem vessels (Rodríguez-Gamir et al. 2010). In grafted grapevines, the anatomical characteristics of the xylem of the rootstocks might influence the water uptake and transport/conductance capacity. Besides differences in hydraulic architecture due to genetic origin, soil type can affect plant adaptation to drought in terms of changes in whole-plant hydraulic conductance by affecting xylem tissue development (Tramontini *et al.* 2012).

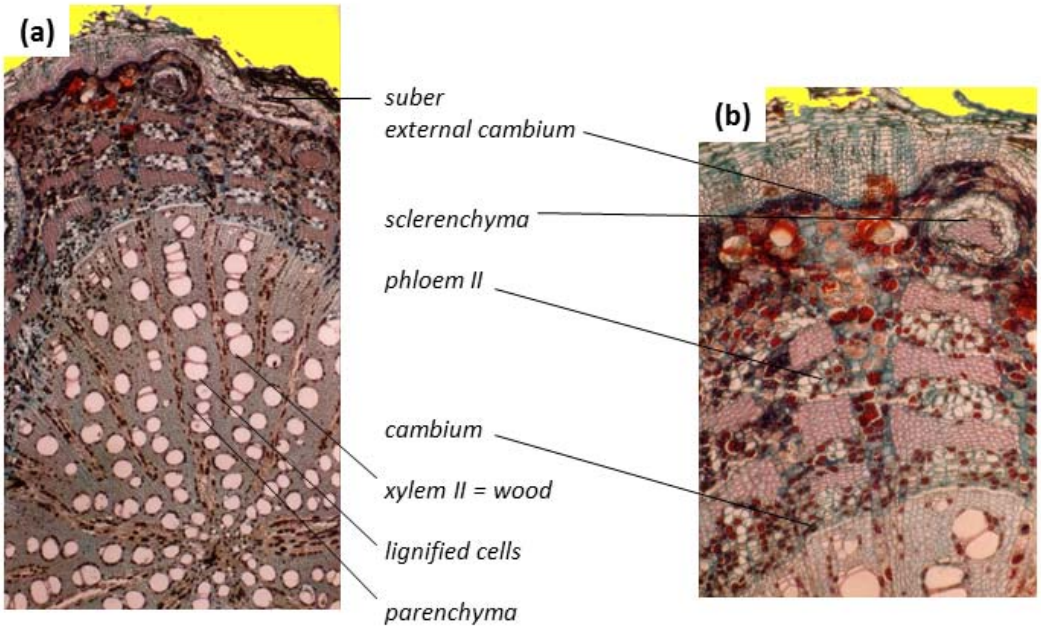

Figure 2.2 Cross section of *Vitis* sp. root (adapted from Bernard, Montpellier SupAgro, France, personal communication). (a) Cross section of a stained root; (b) higher magnification of the upper section shown in (a).

2.3 Root growth and development

Having a well-developed root system may improve water uptake by exploiting more efficiently the resources available in the soil. Most of the roots are found in the top 1 m of soil, although they can be found at a depth of up to 6 m (Seguin 1972) or more. The root system consists of the main framework roots (6 to 100 mm in diameter) and smaller, permanent roots (2 to 6 mm in diameter) (Mullins et al. 1992). Root density can be affected by soil water availability and type of irrigation (Soar and Loveys 2007), canopy manipulation (McLean et al. 1992, Hunter and Le Roux 1992, Serra-Stepke 2010), trellis system and vine spacing (Archer et al. 1988) and rootstock genotype (Southey and Archer 1988, Morano and Kliewer 1994). The pattern of new lateral root growth will depend on the climatic conditions where the vineyard is located. Grapevines in temperate and Mediterranean climates show root growth activity mainly between flowering and veraison, followed by some root growth during summer if the soil water content is favourable (Van Zyl 1984). In addition, a smaller postharvest growth of roots can occur in temperate climates. In subtropical climates, root growth occurs primarily postharvest, with no spring flush (Comas et al. 2010). Escalona et al. (2012) found that under irrigation, the estimated carbon losses due to respiration amounted to 47 to 65 g per plant. This equated to 30 to 50% of the total estimated gains due to photosynthesis. Furthermore, respiration by the root system represented 70 to 80% of the total carbon losses, illustrating the large requirements of this organ. The remaining proportion consisted of both leaf and stem respiration.

Early studies proposed that a genetic variability exists regarding rooting depth (Pongrácz 1983, Pouget 1987), e.g. *V. riparia* is described with a root system that is well branched and shallow growing, in contrast with 140 Ruggeri (*V. cinerea var. helleri 'Resseguier#2'* x *V. rupestris)*, which has a root system that is deep growing and ramified (Pongrácz 1983). In *V. vinifera* L., two genes involved in root branching in stem cuttings have been identified viz. VvPRP1 and VvPRP2 (Thomas et al 2003). A homologue gene with the putative function of auxin-mediated lateral root development, viz. NAC1, was related to Quantitative trait loci (QTLs) involved in water deficit responses in rootstocks (Marguerit et al 2012). Nevertheless, studies carried out with several rootstocks have found that the rooting depth does not differ much between rootstocks, although they can have different root densities (Swanepoel and Southey 1989, Southey 1992, Smart et al. 2006), which can explain differences in scion growth performance. Even though the relevance of the genetic origin of the rootstock on the root system development cannot be discarded, it is not possible to understand the role of the rootstock on the plant adaptation to drought without considering the exogenous factors and the genotype-environment interaction.

2.3.1 *Drivers of root system development*

Due to the heterogeneity of the soil structure, water and nutrients will be located irregularly. It has been shown, however, that during periods of minimal transpiration, water movement within a single plant can occur from roots located in wet soil to roots in dry soil patches (Smart et al. 2005, Bauerle et al. 2008a). Despite the general belief that the rooting pattern is mainly due to the genetics of the rootstock (Pouget 1987), experiments have shown that the main driver for root development is soil water content (Morlat and Jacquet 1993, Conradie et al. 2002, Comas et al. 2005), which explains why it is possible to modify the rooting pattern through irrigation (Myburgh 1996, 2007, 2011, Soar and Loveys 2007). Soil structure and texture, which influence the nutrient retention and water-holding capacity of the soil and the air-to-water ratio (Figure 2.3), can affect root growth (Nagarajah 1987). Soil physical limitations, e.g. layers with a bulk density in excess of 1.4 kg/m^3, can also limit root penetration and development in deeper layers (Van Huyssteen 1983). Grapevine roots cannot grow readily into soil if the penetration resistance exceeds about. 2 MPa (Van Huyssteen 1988). A survey showed that this critical penetration resistance limited root system development in a wide range of Australian vineyard soils (Myburgh et al. 1996). In young, grafted grapevines, scion genotype can determine root development (Tandonnet et al. 2010). Limited soil nitrogen (N) content could enhance root growth in order to improve the acquisition of this particular nutrient (Grechi et al. 2007). Lateral root formation can be initiated by the presence of a high soil nitrate concentration, even when root N concentration is adequate (Dodd 2005). This suggests that nitrate could be considered as an N resource, as well as a signal that influences root system development. The grapevine root system responds to available nitrogen in soil with production of new roots which have a high capacity for nitrogen uptake (Volder et al. 2005).

In general, soil properties have a greater influence on root distribution than rootstock genotype (Southey and Archer 1988, Smart et al. 2006). Nevertheless, under similar soil conditions, rootstocks that differ in their ability to confer vigour and drought tolerance to the scion can give rise to differences in root development, which could be related to different strategies to tolerate a water deficit. Under periods of water constraint, rootstocks that tend to induce more vigour and drought tolerance may exhibit more rapid root growth later in the season in wetter soil regimes (Bauerle et al. 2008b). In contrast, rootstocks that induce lower vigour and less drought tolerance could form more roots in deeper soil layers early in the growing season, no matter what soil moisture conditions prevail (Bauerle et al. 2008b). Such grapevines with deep root systems will be better buffered against drought conditions, particularly during the latter part of the season. Furthermore, it was found that roots located deeper in the soil have a longer

lifespan in comparison with shallow roots (Anderson et al. 2003). In a similar way, it was found that drought tolerant grapevine rootstocks formed more new roots in the soil profile during a dry, hot season, thereby increasing the uptake of water, compared to that of drought sensitive rootstocks (Alsina et al. 2011). Since root tips are highly active in absorbing water (Zwieniecki et al. 2003), the formation of new roots could improve water supply to the plant (Alsina et al. 2011).

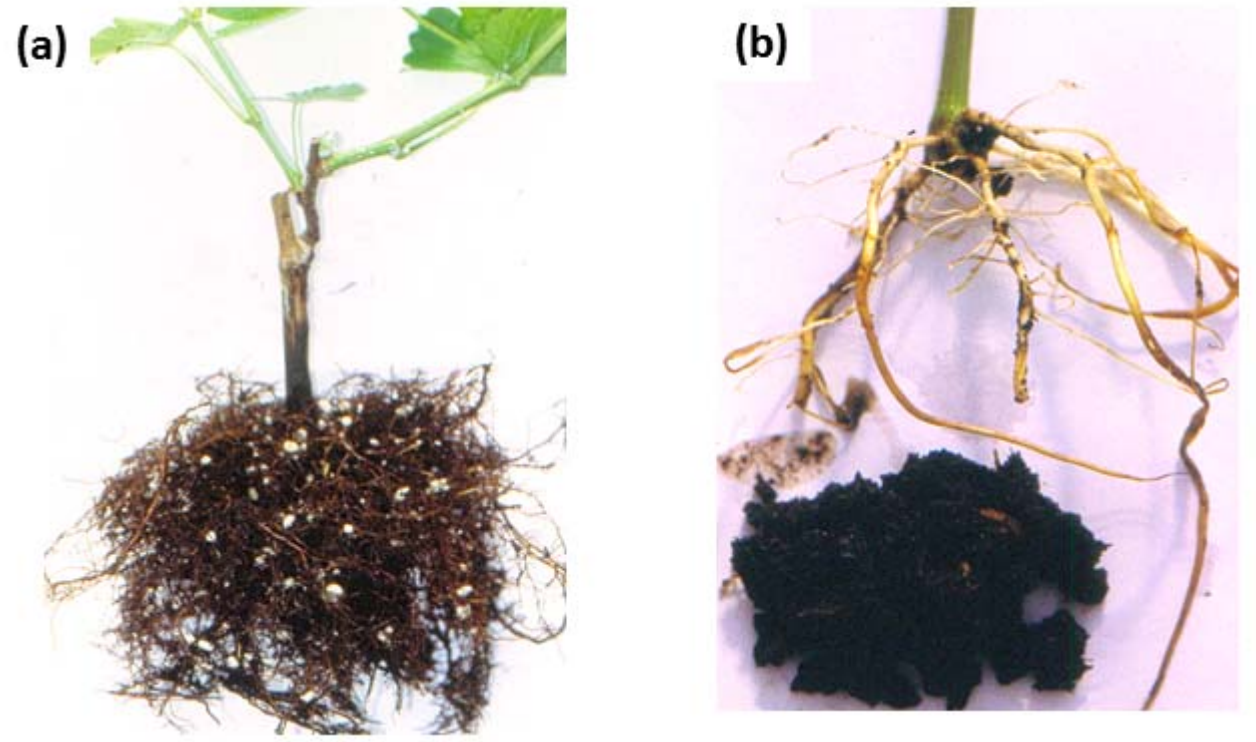

Figure 2.3 Example of the influence of the ratio O_2/water on the development of *V. cinerea var. helleri 'Resseguier#2'* roots in (a) soil with an air-to-water ratio of 30 to 70 and (b) soil with an air-to-water ratio of 10 to 90 (Professor Alain Deloire, unpublished results).

Various studies have been carried out to understand how grapevine canopy size and irrigation can affect root growth and lifespan dynamics (Anderson et al. 2003, Comas et al. 2005), as well as root metabolic activity (Comas et al. 2000). It is still not clear, however, whether different rootstock genotypes have a better tolerance for soil water deficits due to a longer root lifespan and/or different root metabolic activities, which allow improved water uptake and/or soil water deficit sensing via the roots. Several studies have found that rootstock genotypes differ in their nutrient acquisition capacity (Ruhl 1989, Grant and Matthews 1996, Keller et al. 2001, Mpelasoka et al. 2003) and that root physiology and age influence the rate of nutrient uptake (Volder et al. 2005). In a similar manner, rootstock genotypes have different mechanisms that involve root functioning and root tissue differentiation in response to soil water deficits. Differences in root life span between balance pruned grapevines, i.e. 44 buds left per kg of cane pruning from the previous winter, and minimally pruned ones, i.e. only cutting the

hanging stems to 1 m above the ground where canopy pruning decreased root life span, suggest that it might be due to differences in root composition related to carbon concentration (Comas et al. 2000).

2.4 Root functioning

2.4.1 *Water uptake and transport*

It has been proposed that water moves passively into roots as a result of a water potential controlled by transpiration (Steudle and Peterson 1998). Initially, water flows radially through the different tissues into the xylem vessels. This is followed by axial conductance, which depends on the size and number of xylem vessels (Tyerman et al. 2009). The composite transport model explains how the water flows through individual cells and various tissues (Steudle and Frensch 1996), involving apoplastic as well as cell-to-cell, i.e. symplastic and transcellular, pathways operating in parallel (Tyerman et al. 2009).

More drought tolerant rootstocks have higher hydraulic conductance, which could be related to improved xylem development and lower vessel embolisation (Lovisolo et al. 2008b). One aspect that could explain these differences is the presence in plants of aquaporins (Maurel et al. 1993), which are special proteins that act as water conduits (Tyerman et al. 2009). Aquaporins are involved in the regulation of water movement across plasma membranes in the cell-to-cell pathway (Tyerman et al. 1999), and in the recovery from xylem embolism (Lovisolo and Schubert 2006). Eight putative aquaporins were identified that enabled a series of studies at the molecular level in 110 Richter (Baiges et al. 2001), which is considered to be a drought tolerant rootstock (Keller 2010). Furthermore, it was found that the expression of the aquaporin genes in 110 Richter differed between the leaves and the roots (Galmés et al. 2007). In this study it appeared that the expression of the aquaporin genes in the leaves decreased to limit water loss via transpiration, whereas the expression of the same aquaporin gene increased in the roots to enhance water uptake to avoid plant water constraints when water deficits occurred. This particular study also showed a negative correlation between stomatal conductance and abscisic acid (ABA), but not with leaf water potential and hydraulic conductivity in the plant. The latter is attributed in part to the expression of aquaporins, which means that 110 Richter on own roots is able to maintain the same leaf water status, irrespective of soil water deficits. During drought conditions, the intensity of aquaporin regulation in the roots of different *V. vinifera* L. cultivars determines their ability to tolerate soil water deficits (Vandeleur et al. 2009). Differences in aquaporin expression and activity between rootstocks have been detected mainly in the root tip (apical 2 cm of the fine root) in comparison with the mature root zone (10–20 cm behind the tip) (Gambetta et al. 2012).

The graft union, which can play a key role in water transport, is also an important aspect. A successful graft union has to differentiate functional phloem and xylem connections across the graft surface (Keller 2010) in order to allow the transport of water, nutrients and photo assimilates. It has been shown that grafting can have a negative effect on the hydraulic conductivity (Bavaresco and Lovisolo 2000) and therefore on the development and lifespan of the scion. In general, the most important requirement for grafting is the formation of a normal vascular connection across the grafting area and, secondly the maintenance of rootstock-to-scion communication (Aloni et al. 2010).

2.4.2 *Nutrient uptake*

In addition to water uptake, the absorption of nutrients can have a significant impact on the vigour of grapevine vegetative growth. The apical regions of the root exhibit the greatest rates of nutrient uptake and a rapid decline in this capacity with age (Wells and Eissenstat 2003). A similar trend is shown with phosphate uptake in apple and citrus trees (Bouma et al. 2001). In grapevine, the rate of nitrate uptake declines to 50% of the starting rate in fine lateral roots after a single day (Volder et al. 2005). Differences in nutrient uptake among grapevine rootstocks have been described mainly in relation to nitrogen (Keller et al. 2001), phosphorus (Grant and Matthews 1996) and potassium (Ruhl 1989, Mpelasoka et al. 2003). Therefore the capacity of the rootstock to generate new roots will have a positive impact on the capacity of nutrient uptake.

2.5 Assessment of drought tolerance of different rootstocks

Drought tolerance varies among *Vitis* species and is related to the vines' adaptation to their natural habitats (Whiting 2005). Several drought tolerance rankings for grapevine rootstocks have been proposed (Pongrácz 1983, Padget-Johnson et al. 2003, Dry 2007, Keller 2010), but there is no standardised methodology for the classification of rootstocks based on their drought tolerance. Different rankings for the same rootstock can be due to differences in the soil properties and climate where the trial was carried out, as well as the intensity and duration of water deficits imposed on the plants and the choice of drought-related parameters that were studied. For example, early evaluations of drought tolerance induced by rootstocks were based primarily on vegetative vigour (trunk circumference), fruit quality (berry size, berry colour estimate, total soluble solids and total acids) and yield (Lider 1957), but the latter has been the more important measure of rootstock adaptation in the past (May 1994). More recent studies have incorporated physiological indicators, such as stomatal conductance (Carbonneau 1985), leaf water potential (Ezzahouani and Williams 1995, Choné et al. 2001, Deloire et al. 2004, Williams 2010), ABA in the xylem, stomatal conductance (Iacono and Peterlunger 2000) and the

chlorophyll content index (ratio of transmission at 931 nm to 653 nm through a leaf) in rootstocks (Pavlousek 2011). Nevertheless, a classification has been proposed by several authors based on field observations (Samson and Casteran 1971, Fregoni 1977) and evaluations in pots involving different levels of water deficit (Carbonneau 1985) (Table 2.1). It is important to note that the assessment of drought tolerance should consider the ability of a specific scion/rootstock combination to produce an acceptable yield under conditions of water deficit. The early detection of drought tolerance using parameters that correlate with yield is desirable. Nevertheless, some parameters measured, such as leaf water potential and instantaneous leaf water-use efficiency, are not always reflected in yield results (Whiting 2005).

Table 2.1 Rootstock classification based on adaptation to drought, as proposed by Samson and Castéran (1971), Fregoni (1977) and Carbonneau (1985) (adapted from Ollat, INRA Bordeaux, France, personal communication).

Name	Crossing	Samson and Castéran	Fregoni	Carbonneau
110R	*V. rupestris* V. cinerea var. helleri 'Resseguier#2'*	Good	High resistance	High resistance
140Ru	*V. rupestris* V. cinerea var. helleri 'Resseguier#2'*	Average	High resistance	High resistance
44-53M	*V. riparia*V. cordifolia-V. rupestris*	Good	High resistance	High resistance
1103P	*V. rupestris* V. cinerea var. helleri 'Resseguier#2'*	Good	High resistance	Resistance
SO4	*V. riparia* V. cinerea var. helleri 'Resseguier#2'*	Weak	Weak resistance	Resistance
99R	*V. rupestris* V. cinerea var. helleri 'Resseguier#2'*	Average	Average resistance	Resistance
3309C	*Vitis riparia*V. rupestris*	Good	Weak resistance	Sensitive
420A MGt	*V. riparia* V. cinerea var. helleri 'Resseguier#2'*	Weak	Weak resistance	Sensitive
Fercal	*V. cinerea var. helleri 'Resseguier#2' *Vinifera*	Average		Sensitive
5BB	*V. riparia* V. cinerea var. helleri 'Resseguier#2'*	Bad	Weak resistance	Sensitive
161-49C	*V. riparia* V. cinerea var. helleri 'Resseguier#2'*	Weak	Mid resistance	Sensitive
41B MGt	*V. cinerea var. helleri 'Resseguier#2' *V. vinifera*	Average	High resistance	Sensitive
Rupestris du Lot	*V. rupestris*	Bad	Weak resistance	Sensitive
101-14 Mt	*V. riparia*V. rupestris*	Bad	Weak resistance	Very sensitive
Riparia Gloire de Montpellier	*V. riparia*	Bad	Weak resistance	Very sensitive
333EM	*V. cinerea var. helleri 'Resseguier#2' *V. vinifera*	Good	Mid resistance	Very sensitive

2.6 Mechanisms of drought tolerance in rootstocks

Drought escape involves the ability of the plant to complete the whole life cycle before severe water constraint occurs. Drought tolerance with low plant water potential involves desiccation tolerance and the maintenance of turgor, mainly by osmotic adjustment. Drought tolerance with high plant water potential involves a reduction of water loss and an increase in water uptake, which is a way to avoid drought (Chaves and Oliveira 2004). Grapevines do not fall under the drought escape mechanism. Most of the grapevines cultivated around the world are located in a Mediterranean type of climate, meaning that most of the vegetative and reproductive growth occurs under moderate to severe water constraints if irrigation is not applied. Grapevine roots and rootstocks present drought tolerance mechanisms related to low and high plant water potential (Figure 2.4, Tables 2.2, 2.3 and 2.4) involving drought responses, such as stomatal closure, decrease of cell growth and photosynthesis, activation of respiration, accumulation of osmolytes and proteins (Shinozaki and Yamaguchi-Shinozaki 2007). In addition, grapevine rootstocks can affect leaf area and root development depending on the vigour inducing capacity (Gambetta *et al.* 2012) affecting the canopy water demand and supply. During dry hot seasons, higher vigour rootstocks can explore root zones to a greater extent than low vigour rootstocks (Bauerle et al. 2008b) and as a consequence can access water from deeper soil layers (a drought avoidance strategy). This has implications for water availability later in the season. Gambetta et al. *(*2012) found that the higher canopy water demand due to the effect of rootstocks that promote scion vigour appears to be balanced by adjustments in root hydraulic conductivity through fine roots and higher root surface area. The mechanisms involved can develop in different time scales, from minutes to months. For example, an adjustment to stomatal conductance can occur within minutes or less, whereas osmotic adjustment and the response to ABA can occur in hours, and adaptations in terms of root system development can take several days or weeks (Passioura 1996).

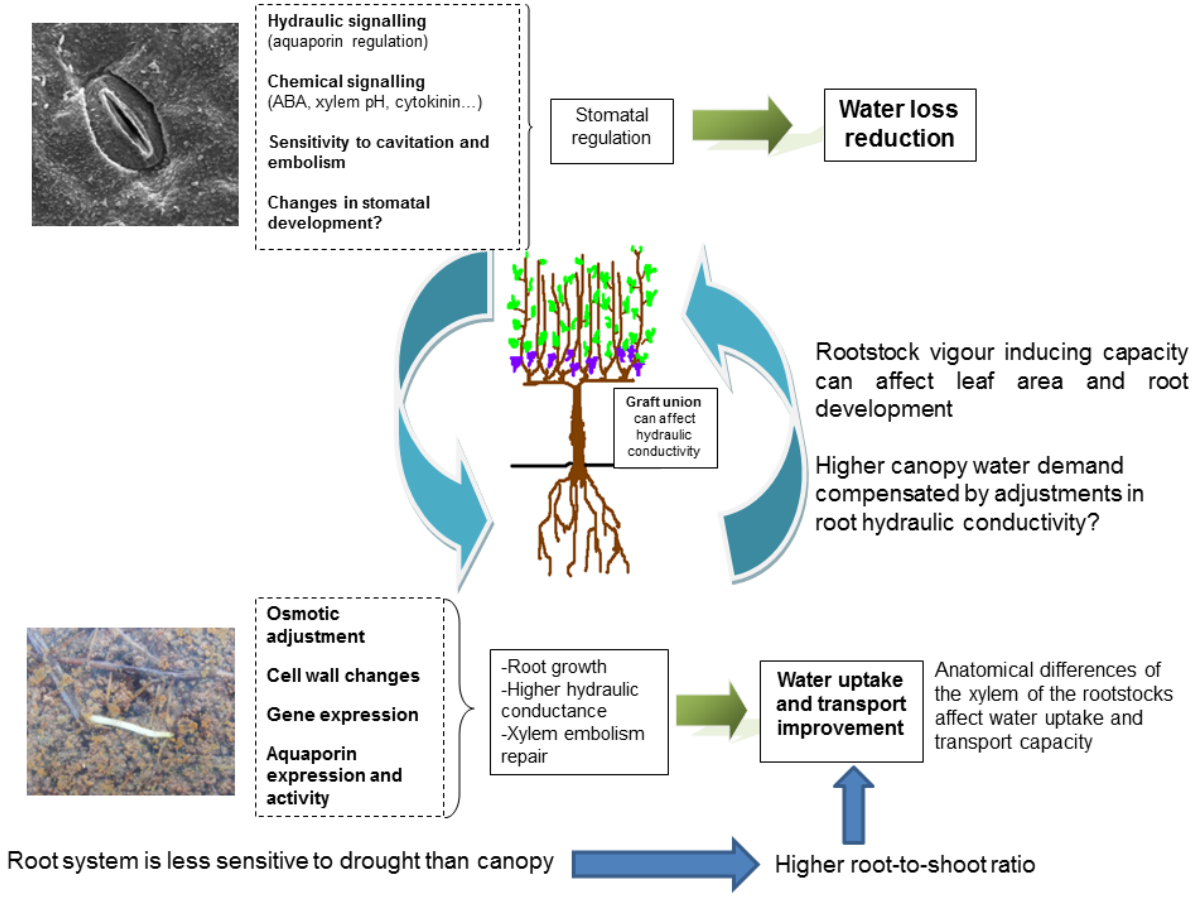

Figure 2.4 Drought tolerance mechanisms induced by rootstocks.

Although many genes related to drought response have been identified, their physiological relevance is not always known (Chaves et al. 2003). Drought tolerance characteristics are controlled by many genes, known as quantitative traits (Bartels and Sunkar 2005), which will complicate the understanding of the plant response to water deficits at a molecular level. QTLs are regions within genomes that contain genes associated with a particular quantitative trait (Jones et al. 1997). Recently, a study carried out on quantitative traits identified one genomic region of the grapevine rootstock that was related to water extraction capacity and scion transpiration and acclimation (Marguerit et al. 2012). This finding supports previous hypotheses that rootstocks differ in their ability to provide water to the scion, and that chemical signalling, primarily ABA, and hydraulic signalling via aquaporins regulate stomatal conductance.

Table 2.2 Proposed drought tolerance mechanism via stomatal regulation based on chemical signalling originating either from scions or rootstocks from field or pots experiments.

Criteria used to measure stomatal regulation by chemical signalling	**Scion/rootstock**	**Genetic origin of the root system**	**Set up**	**Reference**
Root, stem and leaf ABA	Pinot Noir grafted onto 5BB	*V. cinerea var. helleri 'Resseguier#2'* x *V. riparia*	P	Lovisolo et al. (2002)
Leaf xylem ABA	Monastrell grafted onto 1103 P	*V. cinerea var. helleri 'Resseguier#2'* x *V. rupestris*	F	Romero et al. (2012)
Leaf xylem ABA	Shiraz grafted onto 5C SO4 140 Ru, Ramsey, K51-40, 420A MGt, Schwarzmann, Shiraz own roots	 *V. cinerea var. helleri 'Resseguier#2'* x *V. riparia* *V. cinerea var. helleri 'Resseguier#2'* x *V. riparia* *V. cinerea var. helleri 'Resseguier#2'* x *V. rupestris* *V. champini* *V. champini* x *V. riparia* *V. riparia* x *V. cinerea var. helleri 'Resseguier#2'* *V. riparia* x *V. rupestris* *V. vinifera* L.	F	Soar et al. (2006)
Leaf xylem ABA, xylem pH and exogenous ABA with different pH buffers	*V. riparia* x *V. labrusca*	*V. riparia* x *V. labrusca*	P	Li et al. (2011)
Foliar ABA and phaseic acid	Cabernet Sauvignon own roots	*V. vinifera* L.	P	Loveys and Kriedemann (1974)
Endogenous ABA, exogenous ABA and benzyladenine	Bacchus own roots Forta own roots Müller-Thurgau own roots Riesling own roots	*V. vinifera* L. *V. vinifera* L. *V. vinifera* L. *V. vinifera* L.	P	Düring and Broquedis (1980)
Exogenous benzyladenine, leaf xylem sap ABA, xylem sap pH, ABA and cytokinins (zeatine + zeatine riboside) from roots	Cabernet Sauvignon own roots Chardonnay own roots Sultana own roots	*V. vinifera* L. *V. vinifera* L. *V. vinifera* L.	F and P	Stoll et al. (2000)

Table 2.2 (cont.)

Transcript abundance of genes (ABA and cytokinin) of plants under water and salinity constraints in comparison with plants with no constraint state	Cabernet Sauvignon own roots	*V. vinifera* L.	P	Cramer et al. (2007)
Bulk leaf ABA, leaf xylem ABA, root ABA and xylem pH	Mavrodafni own roots Sabatiano own roots	*V. vinifera* L. *V. vinifera* L.	P	Beis and Patakas (2010)

ABA, abscisic acid; F, field; P, pots.

Table 2.3 Proposed drought tolerance mechanism via stomatal regulation in combination with various other regulating or signalling mechanisms from field or pots experiments.

Stomatal regulation mechanism	**Criteria used to measure stomatal regulation by non-chemical signaling**	**Criteria used to measure stomatal regulation by chemical signaling**	**Scion/rootstock**	**Genetic origin of the root system**	**Set up**	**Reference**
Chemical signalling and regulation of homeostasis by aquaporins	Expression of aquaporins genes in roots and leaves	Leaf xylem ABA	110 R on own roots	*V. cinerea var. helleri 'Resseguier#2'* x *V. rupestris*	P	Galmés et al. (2007)
Chemical signalling and embolism repair by aquaporins	Hydraulic conductivity recovery of root, shoot and leaf petiole	Foliar ABA	Grenache grafted onto 420A MGt	*V. riparia* x *V. cinerea var. helleri 'Resseguier#2'*	P	Lovisolo et al. (2008a)
Hydraulic signalling	Leaf specific hydraulic conductance	None	Grenache and Syrah grafted onto *V. rupestris* x *V. cinerea var. helleri 'Resseguier#2'*	*V. rupestris* x *V. cinerea var. helleri 'Resseguier#2'*	F	Schultz (2003)
	Root hydraulic conductance	None	Chardonnay own roots Grenache own roots	*V. vinifera* L. *V. vinifera* L.	P	Vandeleur et al. (2009)

Table 2.3 (cont.)

Chemical and hydraulic signalling	Plant water status	Foliar ABA	Concord own roots	*V. labruscana*	P	Liu et al. (1978)
	Plant hydraulic conductivity	Leaf xylem ABA	110 R on own roots	*V. cinerea var. helleri 'Resseguier#2'* x *V. rupestris*	P	Pou et al. (2008)
	Plant water status	Leaf xylem ABA and xylem sap pH	Castelão and Muscat of Alexandria grafted onto 1103 P	*V. cinerea var. helleri 'Resseguier#2'* x *V. rupestris*	F	Rodrigues et al. (2008)
	QTLs identification with genes associated to hydraulic regulation	QTLs identification with genes associated to ABA regulation	Cabernet Sauvignon grafted onto *V. vinifera* cv. Cabernet Sauvignon X *V. riparia* cv. Gloire de Montpellier	*V. vinifera* X *V.* riparia	P	Marguerit et al. (2012)
	Leaf water potential	Leaf xylem ABA and exogenous ABA application to roots	Semillon own roots	*V. vinifera* L.	F	Rogiers et al. (2012)

ABA, abscisic acid; QTL, quantitative traits loci; F, field; P, pots.

Table 2.4 Proposed grapevine tolerance to drought via osmotic adjustment, aquaporins and root foraging on its own or in combination with different levels of plant water status regulation from field or pots experiments.

Mechanism	**Criteria used to measure plant water status regulation**	**Scion/rootstock**	**Genetic origin of the root system**	**Set up**	**Reference**
Osmotic adjustment in roots	Osmotic potential of roots	Silvaner own roots Riesling own roots	*V. vinifera* L. *V. vinifera* L.	P	Düring (1984)
	Osmotic potential of roots	5BB on own roots	*V. cinerea var. helleri 'Resseguier#2'* x *V. riparia*	P	Düring and Dry (1995)
Presence of aquaporins	Aquaporin genes identification and expression in roots	110 R on own roots	*V. cinerea var. helleri 'Resseguier#2'* x *V. rupestris*	P	Baiges et al. (2001)
Root foraging	Root growth dynamics in response to soil moisture availability	Merlot grafted onto 1103 P and 101-14 MGt	*V. cinerea var. helleri 'Resseguier#2'* x *V. rupestris* *V. riparia* x *V. rupestris*	F	Bauerle et al. (2008b)
Root foraging and different degree of stomatal conductance control	Root growth dynamics and whole root system hydraulic conductance	Merlot grafted onto 1103 P and 101-14 MGt	*V. cinerea var. helleri 'Resseguier#2'* x *V. rupestris* *V. riparia* x *V. rupestris*	F	Alsina et al. (2011)

F, field; P, pots.

2.6.1 *Osmotic adjustment in roots*

Osmotic adjustment, i.e. the active accumulation of solutes involving inorganic solutes taken up from the substrate and organic solutes synthesised by the plant, is a response to drought that enables maintained water absorption and cell turgor pressure (Cattivelli et al. 2008). Evidence for a decrease in osmotic potential in grapevine roots in response to drought was reported by Düring (1984). Furthermore, osmotic adjustment in roots and the maintenance of a positive root water status in grapevines subjected to soil water deficits were shown to have a positive influence on leaf gas exchange (Düring and Dry 1995). It was also speculated that osmotic adjustment may reduce the sensitivity of roots as sensors, and therefore restrict the production of root signals such as ABA (Düring and Dry 1995).

2.6.2 *Control of water loss*

Many studies have shown that rootstocks can modify their leaf gas exchange capabilities in response to water deficit conditions (Candolfi-Vasconcelos et al. 1994, Düring 1994, Bica et al. 2000, Padgett-Johnson et al. 2000). Such responses, however, could vary according to different rootstock/scion combinations (Keller et al. 2012), as well as the level of water deficit experienced (Soar et al. 2006). The effect of rootstock on the photosynthetic capacity of the scion appears to increase under higher water constraint conditions (Soar et al. 2006). Under well-watered conditions it has been reported that the scion genotype predominates the determination of transpiration efficiency, i.e. the CO_2 assimilation to H_2O transpiration ratio compared to the rootstock (Gibberd et al. 2001, Virgona et al. 2003). In the absence of root-to-shoot signals, differences in the leaf anatomy of the scion might play a more relevant role in the regulation of photosynthesis, since they can present different mesophyll conductance to CO_2 (g_m), i.e. the capacity for CO_2 diffusion inside leaves (Flexas et al. 2008). It has been shown that differences in leaf anatomical properties associated with differences in g_m explained the differences in photosynthesis between two Pine species (Peguero-Pina et al. 2012). In relation to grapevines it has been suggested that the level of g_m could be related to the carboxylation efficiency of the specific genotype (Düring 2003). Furthermore, it has been shown that grapevine shoots have some ability to regulate ABA concentration under conditions of low water constraints, independent of root-to-shoot signalling (Soar et al. 2004).

Water losses could also be reduced by limiting transpiration through the regulation of stomatal conductance. Under conditions of water constraint, drought sensitive rootstocks induce a lower stomatal conductance of the scion, leading to a higher reduction in photosynthetic carbon assimilation rates compared to that of drought tolerant rootstocks (Alsina et al. 2011). Stomatal density and stomatal size determine the possible maximum stomatal conductance (Franks and

Beerling 2009). The control of stomatal movement is mediated by changes in guard cell turgor, cytoskeleton organisation, membrane transport and gene expression (Hetherington 2001). Many mechanisms for stomatal regulation have been postulated, such as changes in hydraulic conductivity (Schultz 2003, Christmann et al. 2007), abscisic acid synthesis (Davies et al. 2005, Dodd 2005, Jiang and Hartung 2008) and alkalinisation of the xylem pH (Davies et al. 2002, Davies et al. 2005). Grapevine roots are responsible for sensing the soil water deficit and sending a signal to the shoots, thereby primarily regulating shoot growth and water use (Lovisolo et al. 2010).

Chemical signalling is based on evidence that stomatal closure is well correlated with soil water deficits, whereas it only correlates weakly with leaf water potential (Comstock 2002). Abscisic acid is one of the most studied hormones and is considered to be the most important in root-to-shoot water deficit signalling (Davies et al. 2005, Schachtman and Goodger 2008). This does not, however, rule out the possibility that other compounds are involved (Schachtman and Goodger 2008). It has been confirmed that ABA is synthesised in the roots in response to drought (Lovisolo et al. 2002). Following this, ABA is transported via the xylem to the aerial parts of the plant, where it regulates stomatal functioning and the activity of shoot meristems (Jiang and Hartung 2008). In *V. vinifera* there are two genes, viz. *VvNCED* and *VvZEP*, that have been described putatively to be involved in the ABA biosynthetic pathway (Soar et al. 2004) in response to soil water deficit in the roots (Seo and Koshiba 2002). Soar et al. (2006) have suggested that a difference in concentration in xylem ABA among rootstocks is not due to their ability to synthesise ABA, but primarily due to a difference in water constraints experienced by the rootstock genotypes caused by variable water uptake capacity. The intensity of the root-to-shoot ABA signal is regulated at four anatomical levels: (i) the rhizosphere; (ii) the root cortex; (iii) the stem; and (iv) the leaves (Jiang and Hartung 2008). In *V. riparia* x *V. labrusca*, the intensity of the root-sourced ABA signal is intensified along its way, due in part to a higher xylem pH at higher node positions, resulting in a lower stomatal conductance of leaves at higher nodes compared to lower nodes on the stem (Li et al. 2011). Consequently, the stomatal conductance of leaves at higher nodes along the stem is lower compared to that of leaves at lower nodes. Cytokinins (CKs), which are synthesised mainly in the roots (Aloni et al. 2005), have been described as an antagonist to ABA in stomatal closure (Dodd 2005). In *V. vinifera*, zeatin and zeatin riboside have been found to be reduced by partial root zone drying (PRD) (Stoll et al. 2000). Nevertheless, there still are many questions concerning the role of CKs in stomatal behaviour, since it is not clear which CKs will be affected by drought stress and, more so, which transport forms should be measured in the xylem (Davies et al. 2005, Schachtman and Goodger 2008).

Hydraulic signalling is based on the fact that plants would probably not survive in the absence of root-to-shoot signalling, which responds to changes in hydraulic conductivity and the failure of water transport due to cavitation and embolism (Comstock 2002). Furthermore, it is argued that, within the hydraulic continuum of the root system, the information concerning water availability can be transmitted to the leaves to control stomatal functioning (Christmann et al. 2007). Nevertheless, the mechanisms involved are still under debate (Buckley 2005). Using *Arabidopsis* mutants that are deficient in ABA biosynthesis and defective in ABA signalling, it was demonstrated that water constraint-induced stomatal closure requires hydraulic as well as ABA signals (Christmann et al. 2007). It was concluded that the generation of the hydraulic signal is not dependent on ABA biosynthesis and/or ABA signalling, which proves that the hydraulic signal precedes the ABA signal. It was found that own rooted grapevine cultivars that differ in their response to soil water deficits via differences in the regulation of the leaf water potential also vary in their root response to water soil deficits in terms of aquaporin expression (Vandeleur et al. 2009). This finding suggests a close relationship between root water transport and shoot transpiration. Domec and Johnson (2012) suggested that whole-plant hydraulic conductance is driven by leaf hydraulic conductance under no water deficit and by root hydraulic conductance under water deficit.

The relative importance of chemical and hydraulic signalling in the control of stomatal functioning is debatable (Chaves et al. 2010). Some grapevine studies have concluded that hydraulic signals play a dominant role when water deficits occur (Rodrigues et al. 2008), whereas others have shown that the control is primarily due to ABA signalling and that hydraulic signalling plays a secondary role (Pou et al. 2008). However, only hydraulic signalling is involved during recovery from water deficits (Pou et al. 2008). Hydraulic and chemical signalling are considered to be the most important mechanisms in the regulation of stomatal conductance, and these signals probably function in an integrated way (Comstock 2002, Rodrigues et al. 2008).

Our results showed that stomatal density and size, i.e. number of stomata per unit area, are affected by water constraints and light, and that the same scion grafted onto different rootstock cultivars can have different stomatal densities and sizes (Figures 2.5 and 2.6). Soil water deficit induced a response in the stomatal development that resulted in a reduction of the pore diameter (Figure 2.5). Leaves growing in an environment with a lower light intensity, i.e. lower R/FR ratio, had a lower stomatal density but bigger pore diameter than leaves growing under full sun exposure (Figure 2.6). These results might have implications in the interaction of vigour induced by the rootstock (canopy microclimate) and canopy water demand. Significant differences in stomatal density and size were observed on Pinotage leaves grafted onto different

rootstocks, where plants grafted onto 140 Ruggeri presented lower stomatal density but bigger pore diameter than those grafted onto 110 Richter and 1103 Paulsen (Figure 6). Scienza and Boselli (1981) found that rootstocks considered drought tolerant have lower stomatal density in their leaves in comparison with rootstocks considered drought sensitive. The mechanisms involved in stomatal development, as affected by rootstock, cannot be explained at this stage. It is hypothesised, however, that differences in hydraulic conductance between rootstocks affect the plant water status, thereby affecting leaf growth, and that they consequently cause variability in stomatal density and size that is closely related to leaf gas exchange and water use efficiency (Xu and Zhou 2008).

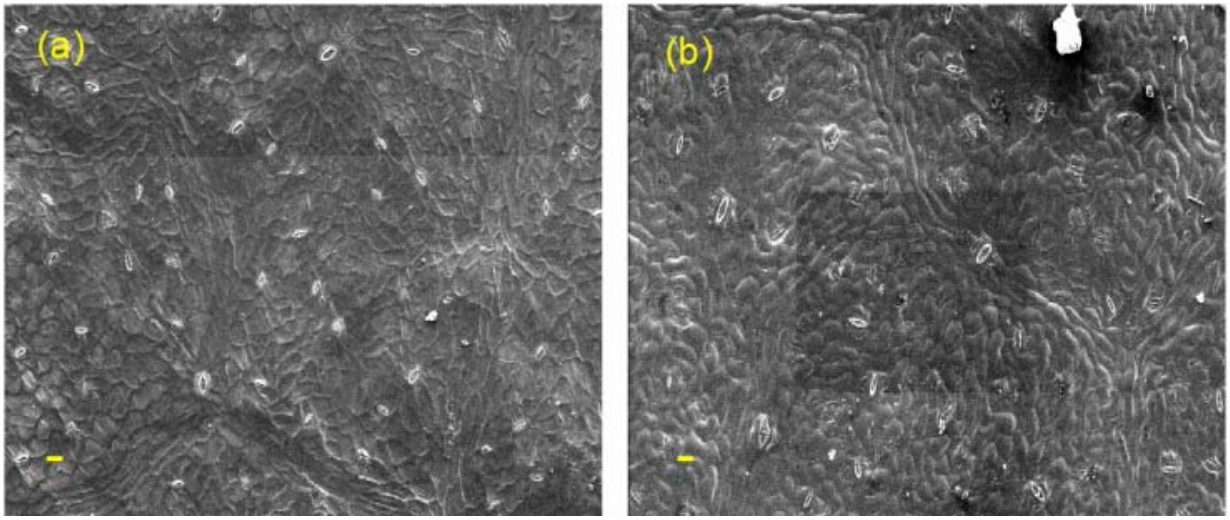

Figure 2.5 Stomata on the abaxial leaf surface of cv. Pinotage (*Vitis vinifera* L.), growing under greenhouse conditions, grafted onto 99 Richter (a) subjected to water constraints and (b) without water constraints. Average stomatal density (pores/mm^2) of 109.5±6.2 and 95.7±6.2 for water constraints and without water constraints, respectively. Average guard cell length (µm) of 13.5±0.24 and 12.6±0.24 for water constraints and without water constraints, respectively (150X magnification, panels a and b; scale bar represents 20 µm) (refer to Chapter 4 for more details).

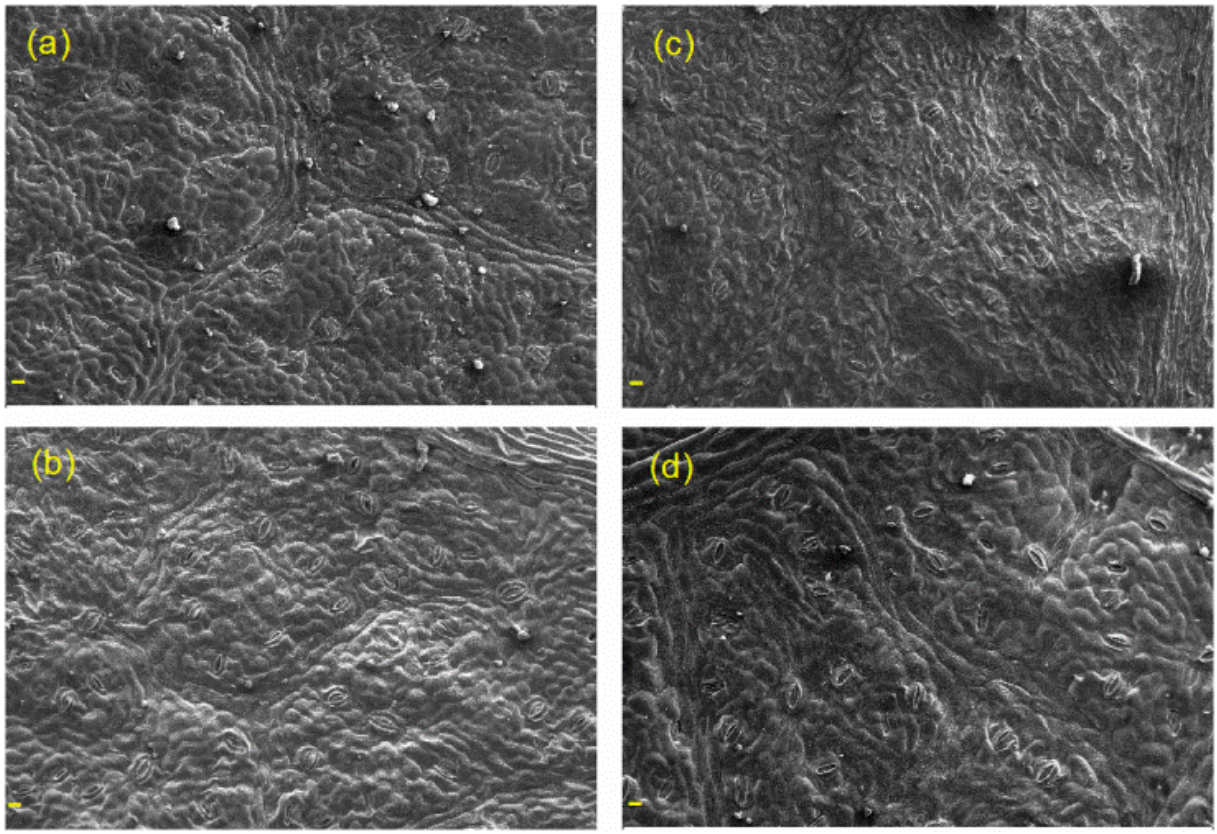

Figure 2.6 Stomata on the abaxial leaf surface of cv. Pinotage, growing under field conditions, grafted onto 1103 Paulsen (a) subjected to water constraints and fully exposed to sunlight, (b) subjected to water constraints and shaded; compared to the same scion grafted onto 140 Ruggeri, (c) subjected to water constraints and fully exposed to sunlight and (d) subjected to water constraints and shaded. Average stomatal density (pores/mm^2) of 119.1±6.3, 91.0±6.3 (Pinotage/1103 P), 113.8 ±6.3 and 96.3±6.3 (Pinotage/140 Ru), fully exposed to sunlight and shaded, respectively. Average guard cell length (µm) of 13.2±0.31, 20.0±0.31 (Pinotage/1103 P), 16.0±0.31 and 17.2±0.31 3 (Pinotage/140 Ru), fully exposed to sunlight and shaded, respectively (150X magnification, panels a, b, c and d; scale bar represents 20 µm) (refer to Chapter 4 for more details).

2.7 Scion and rootstock interaction

There is a differential response of roots and shoots to water deficits. Under drought conditions, vegetative growth, e.g. internode elongation, leaf expansion and tendril extension, as well as transpiration will be reduced (Lovisolo et al. 2010). Nevertheless, the root system is less sensitive to drought. Grapevines can rehydrate 'dry' roots with water moved through the root system at night (Bauerle et al. 2008a). It has been shown that grapevine root growth is enhanced under moderate water constraints, but decreased under severe water constraints (Van Zyl 1984). It has been postulated that a higher root-to-shoot ratio could improve water supply to the grapevine. There is probably ABA involvement in the regulation of some cell wall-modifying proteins that allows growth although water constraints occur (Sasidharan et al. 2011). Furthermore, under soil water deficits, the cytokinin concentration in the roots is reduced,

resulting in a lower concentration in the shoots, which causes a reduction in vegetative growth (Stoll et al. 2000). The relatively higher drought tolerance of roots compared to shoots probably involves osmotic adjustment and changes in the cell wall (Wu and Cosgrove 2000). These adaptations differ between the apical and basal part of the root, involving mechanisms such as an increase in cell wall proteins (viz. expansins), changes in cell wall polysaccharide composition and gene expression, which could protect the root apex and allow root growth at low soil water contents (Wu and Cosgrove 2000).

The scion/rootstock interaction will have a major influence on drought tolerance by affecting canopy structure and size, and therefore affecting grapevine water use (Whiting 2005). Furthermore, ungrafted rootstocks have a determined strategy to tolerate water constraints, e.g. 110 Richter is known to tightly regulate stomatal conductance in response to soil water deficits, maintaining homeostasis through a complex mechanism involving predominantly ABA signalling during water constraint and hydraulic conductivity predominantly during recovery from water deficit (Galmés et al. 2007, Pou et al. 2008). In contrast, studies done on scion cultivars that are own rooted have shown that strategies particular to certain cultivars (e.g. Grenache) have a more tight regulation of stomatal conductance, maintaining homeostasis by hydraulic signalling; Chardonnay, in contrast, has a decreasing leaf water potential under increasing water constraint (Vandeleur et al. 2009). Therefore, grafting a cultivar onto a rootstock implies an interaction of two individual genotypes: the rootstock, which can have a certain type of strategy at the root level, affecting water uptake and transport, and a strategy related to the interaction at the leaf level by root-to-shoot signalling, affecting stomatal conductance. In addition, the rootstock will affect the scion vigour by affecting the uptake of water and nutrients. A study that included all possible combinations of grafting between three *Vitis* genotypes showed that the rootstock affects scion vigour, but also that the scion genotype influences rootstock growth (Tandonnet et al. 2010). In contrast, the scion can act at the stem and leaf level, modifying the intensity of the root-to-shoot signalling, and also at the leaf level, where it determines leaf area, which affects the demand for water. In some cases, certain scion-rootstock combinations have shown improved tolerance to drought in terms of carboxylation efficiency, in comparison to scion own rooted or homografted (Düring 1994, Iacono et al. 1998). Nevertheless, considering drought tolerance in terms of yield, own-rooted Shiraz can be as drought tolerant as when grafted onto rootstocks considered to have a high tolerance to drought (McCarthy et al. 1997).

2.8 Conclusions

Grapevines react at the physiological, biochemical and genetic levels to tolerate water constraints. How the rootstock can improve the drought tolerance of the scion is still not fully understood. It appears that the root system can improve water uptake and transport, but can also detect soil water deficits and send signals that regulate stomatal functioning and/or stomatal development to reduce water losses. Nevertheless, stomatal closure comes at a cost, since it reduces carbon gain. The concept of drought tolerance has to be understood as a compromise between plant survival and yield production. Choosing a rootstock is an important decision because of the potential benefit that viticulturists expect, such as resistance to pests (phylloxera and nematodes), pathogens in the soil, drought tolerance, but also because establishing a vineyard is a long-term investment. The rootstock influence on vigour and the effect on drought tolerance need to be clarified; aspects such as differences in root growth, root hydraulic capacity and stomatal development should be taken into consideration. Most of the previous studies have focused on root system development and structure, but little is known about the genetic regulation of root branching and root mineral uptake. Molecular studies of grapevine drought tolerance are limited, and even more so studies considering rootstocks, compared to research carried out on cereals, for example. Understanding the mechanisms of drought tolerance induced and regulated by rootstocks might be helpful for breeding programs in order to develop more drought tolerant rootstocks.

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Chapter 3

Research results

Leaf water potential and gas exchange responses to drought modulated by rootstocks in grapevine (*Vitis vinifera* L., cv. Pinotage): suggesting possible vine water status thresholds

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Leaf water potential and gas exchange responses to drought modulated by rootstocks in grapevine (*Vitis vinifera* L., cv. Pinotage): suggesting possible vine water status thresholds

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Leaf water potential and gas exchange in response to drought

ABSTRACT

Water scarcity is a key limiting factor for viticulture in dry regions. Drought sensitive varieties have the potential to grow in dry areas with limited water supplies, however mainly through the use of rootstocks. The aim of this study was to evaluate the vine water status and the leaf stomatal conductance of the cv. Pinotage grafted onto several different rootstocks. Irrigated field vines without any water constraint were compared to rain-fed grapevines subjected to a moderate water constraint for 140 Ruggeri, 1103 Paulsen and 110 Richter, known for having different levels of drought tolerance. Vines grafted onto 140 Ruggeri and 110 Richter had lower stomatal conductance in both irrigated and non-irrigated vines and also less negative stem water potentials compared to vines grafted on 1103 Paulsen. Greenhouse trials were also carried out where plants grafted onto 140 Ruggeri, 1103 Paulsen, 99 Richter, 110 Richter and Ramsey were well-watered or subjected to progressive water constraints. Ramsey, known for its drought sensitivity, had the most negative midday stem water potential and the lowest stomatal conductance. Our data

suggested that rootstock regulation occurs up to a point until plant water status becomes the main driver of g_s and A_N. This shift occurred early during the water constraint at a plant water status threshold of around ψ_{stem} values of -0.6 to -0.8 MPa, which could correspond to the limit between no vine water constraint and vine water constraint or water stress for ψ_{stem} values -1.4 MPa. More drought tolerant rootstocks are able to delay the reach of these plant water status thresholds up to a point where plant water status become the main driver of g_s and A_N. These data indicate that rootstocks can have an influence on scion water status and adult leaf gas exchange, but this control is dependent on factors such as the extent of water constraint or stress and how rapidly it is imposed. Regardless of rootstock, however, stomatal conductance was curtailed in vines with stem water potential less than -0.6 to -0.8 MPa, possibly indicating a threshold value that could be useful for irrigation management.

Key words: Pinotage, *Vitis vinifera* L., 99 Richter, 110 Richter, 140 Ruggeri, 1103 Paulsen, Ramsey, stomatal conductance, photosynthesis, drought, plant water status.

3.1 Introduction

Several grapevine varieties are considered to be relatively tolerant to drought and can develop different mechanisms to cope with water deficit. For instance, previous studies have demonstrated reduced stomatal conductance and photosynthesis (Iacono et al. 1998, Koundouras et al. 2008); decreased leaf expansion and internode extension (Schultz and Mathews 1988, Cramer et al. 2007, Lovisolo et al. 2010) and senescence of older leaves (Jackson 1997) in response to water stress. The extent of these responses are, however, variety dependent with those cultivars originating from dry and warm regions, such as Grenache and Carignan, usually better equipped to tolerate such conditions. Drought tolerant rootstocks can offer a practical solution for those sensitive varieties grown in warm climates such as Shiraz and Semillon, especially in light of climate change scenarios predicting increased temperatures and decreased rainfall (IPCC 2007) in some viticultural regions.

The scion-rootstock interaction is complex and depends on the scion characteristics (leaf anatomical characteristics, canopy size related to vigour, capacity to modulate root-to-shoot signalling at the shoot level, etc.) as well as the properties of the rootstock (root anatomy, morphology and growth, efficiency of water and mineral absorption, and root-to-shoot signalling related to hormone biosynthesis) (Serra et al. 2014). The quality of the graft union is also critical and is dependent on the anatomical connection between the vascular systems and the activity of the cambium. While some scion-rootstock combinations may not alter photosynthetic carbon assimilation efficiency under well-watered conditions (Gibberd et al. 2001), under drought there

may be a significant improvement as compared with own rooted vines (Düring 1994, Iacono et al. 1998).

Previous studies have determined that rootstocks can help a cultivar to adapt to drought by increasing water uptake from the soil (Carbonneau 1985, Soar et al. 2006, Marguerit et al. 2012). In order to rank rootstocks in term of drought tolerance, the ratio between total active leaf area and the stomatal conductance of these active leaves were considered under progressing increase of water constraints during the day and during the season (Carbonneau 1985). Rootstocks such as 110 Richter and 140 Ruggeri are classified as highly drought tolerant (Carbonneau 1985). Ramsey, however, exhibits poor drought tolerance while 99 Richter and 1103 Paulsen are intermediate (Keller 2010).

Pinotage was bred in South Africa by A.I. Perold in 1925 (Burger et al. 2009). Currently it is the fourth most planted red cultivar in South Africa (SAWIS 2010). Pinotage is the hybrid from Pinot Noir and Cinsaut. Pinot Noir is a cultivar originating in Burgundy, which has an oceanic type of climate with cool summers, while Cinsaut is a cultivar originating from Provence, characterised by a Mediterranean type of climate with hotter and drier summers. Most of the grapevines for wine production in South Africa are located in the Western Cape which is characterised by a type of Mediterranean climate. Pinotage is known to be drought tolerant and is often cultivated in dry land, grafted onto drought tolerant rootstocks and trained as Goblet (bush vine). To increase the productivity of Pinotage without compromising the fruit and wine quality, this cultivar is cultivated under irrigation and trained in a Vertical Shoot Positioning (VSP) canopy. Few physiological studies have been carried out on Pinotage water use efficiency and leaf functioning in relation to rootstocks. This study was therefore conducted to characterize the role of particular rootstocks on Pinotage (*Vitis vinifera* L.) leaf functioning. Vine water status and leaf gas exchange were assessed in field and potted vines under a range of soil moistures regimes.

3.2 Materials and Methods

3.2.1 Plant material

Plants of cv. Pinotage (*Vitis vinifera* L.) grafted onto five rootstocks having different drought tolerance, were used in field and greenhouse experiments (Table 3.1). Two clones of Pinotage were used: PI 48 and PI 50. Both clones were originated at the "Co-operative Winemakers' Society of South Africa" (KWV) and present average yield and vigour, nevertheless, some differences in the wine's fruity aromas have been reported (Pinotage Association 2012). The rootstocks that have been chosen are the most commonly used rootstocks by the nurseries in South Africa for grafting, including Pinotage. Two classes of rootstocks have been considered: a)

drought tolerant rootstocks, which include 99 Richter, 110 Richter, 140 Ruggeri, and 1103 Paulsen, all with slight differences in drought tolerance, and b) the drought sensitive rootstock, Ramsey, which was chosen as a reference and conferring vigour to the scion under optimally watered conditions.

Field experiments on productive vines were carried out to assess the possible effects of rootstocks on Pinotage leaf functioning and vine water status. In the field, only Pinotage grafted onto three rootstocks were available. Meanwhile greenhouse experiments were used to study the responses of Pinotage leaf functioning and vine water status under climate controlled conditions. The greenhouse experiments allowed us to compare more rootstocks and soil moisture was adjusted in order to induce a progressive water constraint and stress.

3.2.2 Field study

The field trial was carried out at the Welgevallen experimental vineyard (Stellenbosch University; 33°56'S, 18°52'E, altitude: 157 m) during 2010/2011 season. Pinotage grapevines (*Vitis vinifera* L., clone 48A) grafted onto 110 Richter (clone RQ28B), Pinotage (clone 50A) grafted onto 140 Ruggeri (clone RU354B) and Pinotage (clone 48A) grafted onto 1103 Paulsen (clone PS28A) rootstocks were used. Grapevines were 19, 16 and 14 years old for those grafted onto 110 Richter, 140 Ruggeri and 1103 Paulsen, respectively. The vines were trained onto a vertical shoot positioning system, and pruned as a single cordon, with two buds per spur and five spurs per vine. The vine spacing was 1.4 m. The soil type is a red- to yellow-coloured Oakleaf with an orthic A and a neocutanic B horizon (Fey 2010).

Prior to the onset of the experiment the plants were grown without supplementary irrigation. A drip irrigation system was subsequently installed using 2.3 L/h drippers spaced 600 mm apart. Two irrigation strategies were applied in a randomized block design with ten replicates per treatment (twenty replicates per rootstock): (1) irrigation to avoid any water constraint and (2) rain-fed without supplementary irrigation to induce a moderate water constraint (Figure 3.1). Irrigations to avoid water constraint were scheduled to prevent stem water potential (ψ_{stem}) levels lower than -0.6 MPa, whereas the water constraint grapevines received no irrigation. Based on these parameters, vines were irrigated five times (21th December 2010, 11th January, 24th January, 7th February and 22th February 2011).

Table 3.1 Rootstocks used for field and pot trials in greenhouses with automatic temperature control (ATC) and without automatic temperature control (NoATC).

Year	Type of experiment	Rootstocks	Treatments
2010/2011	Field experiment	1103 Paulsen	Well-watered
			Water deficit
		110 Richter	Well-watered
			Water deficit
		140 Ruggeri	Well-watered
			Water deficit
2011	ATC greenhouse experiment	99 Richter	Well-watered
			Water deficit
		110 Richter	Well-watered
			Water deficit
2012	NoATC greenhouse experiment	1103 Paulsen	Well-watered
			Water deficit
		110 Richter	Well-watered
			Water deficit
		140 Ruggeri	Well-watered
			Water deficit
		99 Richter	Water deficit
		Ramsey	Water deficit

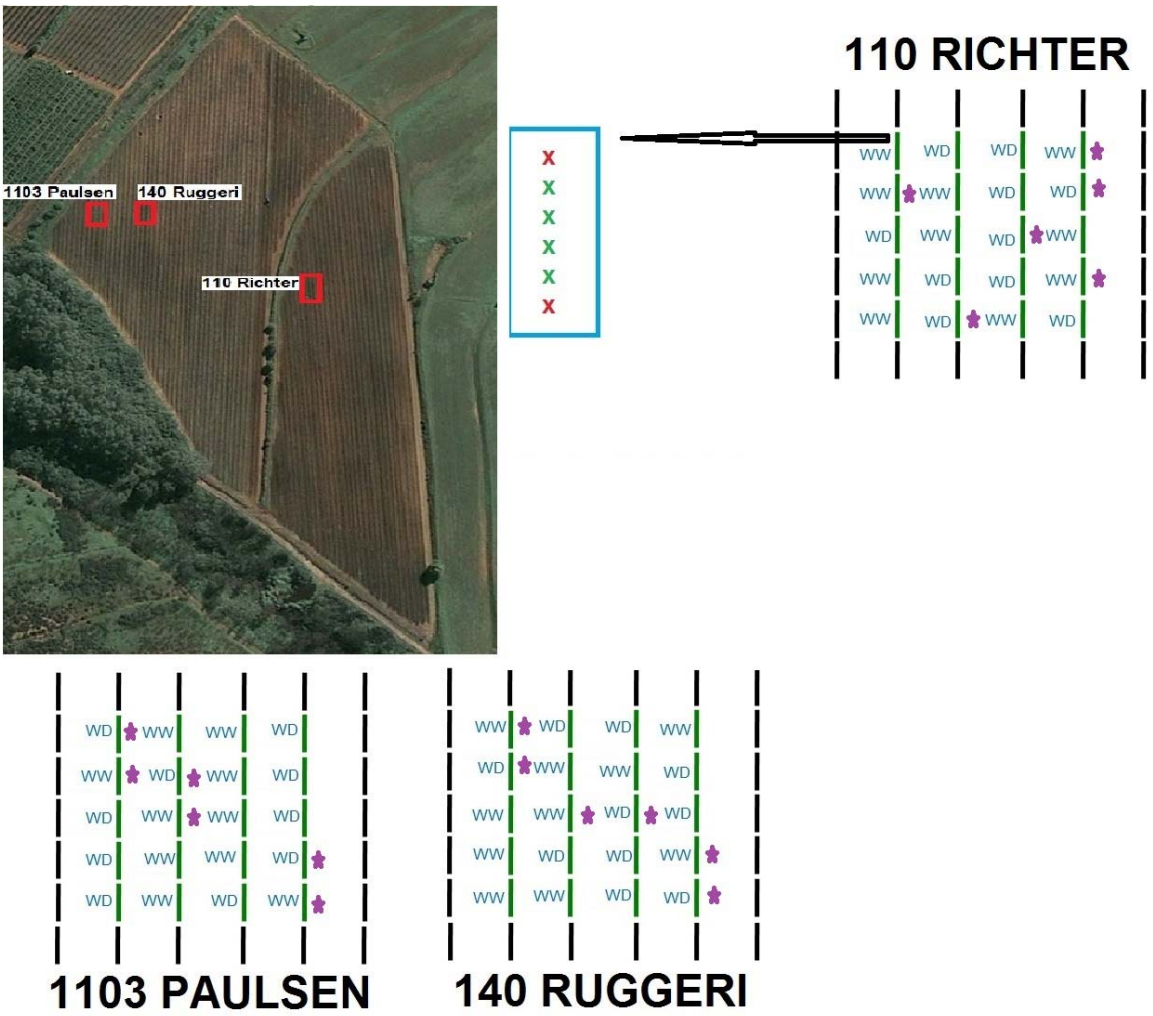

Figure 3.1 Aerial picture of the field experiment where red rectangles show locations of the experiments. Layout diagram of the field experiment where WW and WD represent panels under well watered and water deficit conditions respectively. Each panel consisted in 6 vines, in red are represented buffer vines and in green the vines used for the measurements and purple stars represent the locations where neutron probe measurements took place.

3.2.3 Potted grapevine studies

Two experiments were carried out in two separate greenhouses. The first experiment was located in a greenhouse fitted with automatic temperature control (ATC) and the second in a greenhouse without automatic temperature control (NoATC). One year old grapevines, grafted the previous season, growing in 21 L pots, filled with sandy soil, to allow a better control of the soil moisture, were used for both experiments. All the plants had one single primary shoot per vine, the laterals and inflorescences were removed.

3.2.3.1 Pot Experiment A (ATC)

In 2011, twenty grapevines cv. Pinotage (clone PI48) grafted onto 99 Richter (clone RY25IM) and 110 Richter (clone RQ28C) were used for this experiment. Five replicates were used in a fully randomized design. Once all the grapevines selected for the study attained at least 15

primary leaves per shoot (including adult leaves with more than 50% of the final leaf size and young leaves at the shoot apex with less than 50% of the final leaf size), half of the vines were subjected to water constraint by terminating the irrigation for 12 days (5th to 17th October 2011) and then were re-watered and submitted to a second water constraint period for 5 days (2nd to 7th November 2011) in order to evaluate a possible recovery after the imposed drought period. The other half of the vines was irrigated to field capacity on alternate days.

3.2.3.2 Pot Experiment B (NoATC)

A second experiment was performed in a different greenhouse in order to evaluate the previous rootstocks in addition to rootstocks with different adaptation to drought stress under a semi-controlled environment. In 2012, twenty four grapevines each of cv. Pinotage (clone PI48) grafted onto 99 Richter (clone RY25IM), 110 Richter (RQ28C), 140 Ruggeri (clone RU354E), 1103 Paulsen (clone PS281) and Ramsey (clone 5C18AB) were used. Three replicates were employed in a fully randomized design. Once all the grapevines developed at least 15 primary leaves per shoot, half of the vines were subjected to water stress by withholding irrigation over a 10 day period (from 14th to 24th February 2012). The other half was watered to field capacity on alternate days. In the case of the rootstocks 99 Richter and Ramsey, only data for those grapevines subjected to water stress are included due to the limited number of grapevines with at least 15 primary adult leaves per shoot. The grapevine characteristics used in the experiments and the duration of the water constraints are described in Table 3.2.

Table 3.2 Characteristics of the plants used in the greenhouse at the onset of the experiments and the duration of the water constraint treatments.

Rootstock	Shoot length (cm)	Total leaf area (m^2)	Duration of the water constraint (days)
Greenhouse with automatic temperature control (ATC)			
99 Richter	92.3±3.8	0.16±0.01	12 (first water constraint) 5 (second water constraint)
110 Richter	91.7±3.8	0.17±0.01	12 (first water constraint) 5 (second water constraint)
Greenhouse without automatic temperature control (NoATC)			
99 Richter	130.2±7.8	0.22±0.02	10
110 Richter	159.0±7.8	0.27±0.02	10
140 Ruggeri	157 .2±7.8	0.30±0.02	10
1103 Paulsen	116.2±7.8	0.23±0.02	10
Ramsey	179.3±10.0	0.27±0.02	10

3.2.4 Soil water content

In the field trial, soil water content was determined by means of the neutron scattering technique at 0-300 mm, 300-600 mm and 600-900 mm in three replications of each treatment. Under field conditions, irrigation commenced late December and ended late February. The measurements were carried out approximately every four days from 5th November 2010 to 9th March 2011. The probe count ratios were calibrated against gravimetric soil water content. The volume of irrigation applied was about 163, 181 and 213 mm for 110 Richter, 140 Ruggeri and 1103 Paulsen respectively divided among five irrigations.

In the greenhouse experiment, soil water content was determined by means of gravimetric soil water content, where soil samples from three pots per treatment were weighed and dried at 105ºC for 16 hours. Soil water content was calculated as (wet-dry/dry) x 100. At the temperature controlled greenhouse (ATC), the measurements were carried out approximately every four days during the experimentation while at the greenhouse without the accurate control of temperature (NoATC) the measurements were carried out only at the beginning and at the end of the experimentation.

3.2.5 Air temperature and relative humidity

Air temperature and relative humidity were recorded using a data logger (Gemini Tiny Tag TGP-4500, Gemini Dataloggers SA (PTY) Ltd) placed in a Gill screen above the canopy. The data were recorded from budburst to harvest in the field experiment, while in the greenhouses the data were recorded for approximately two months during the treatment duration. Midday VPD was calculated over the treatment period. Air VPD was used.

3.2.6 Light conditions

In the ATC greenhouse, the solar radiation at noon (clear sky) was measured with a light sensor (Davis Vantage Pro solar radiation sensor, Davis Instruments, Hayward, California, USA), connected to a logger (DataTaker DT82E data logger, Thermo Fisher Scientific Australia Pty Ltd, Scoresby, Victoria, Australia). Solar radiation averaged at 750 (W/m^2) over the treatment period.

3.2.7 Gas exchange measurements

Net photosynthesis (A_N) and stomatal conductance (g_s) were measured using three to five mature and fully-exposed leaves from primary shoots at nodes 9 to 11for field experiments, from nodes 8 to 12 for greenhouse with controlled environment and from nodes 13 to 16 for greenhouse with semi controlled environment per treatment and per date, using an open gas exchange analyser

(Li-6400; Li-Cor, Inc., Lincoln, NE). Each healthy sun-exposed leaf was selected from a single grapevine. The measurements were carried out approximately once a week in the field experiment, every four days in the ATC greenhouse and every three days in the NoATC greenhouse. All measurements were performed at a quantum flux of 1600 µmol m^{-2} s^{-1}, which was determined to be above the light saturation level, with a CO_2 concentration in the cuvette of 400 µmol CO_2 mol^{-1} air and a flow rate of 350 µmol s^{-1}. Leaf temperature was set at 25ºC.

3.2.8 Leaf water potential

Following the gas exchange measurements, midday grapevine stem water potential (ψ_{stem}) (for field and greenhouse experiments) and predawn leaf water potential ($\psi_{predawn}$) (only for field experiment) was determined using a Scholander pressure chamber (Choné et al. 2001). For ψ_{stem}, each leaf was wrapped in a bag prior to excision using a razor blade and the measurements were carried out on three to five leaves per treatment and per date. Each fully-expanded leaf was selected from a single grapevine.

3.2.9 Leaf area

Leaf area was determined by measuring the length of the leaf main vein of the primary (potted and field vines) and secondary shoots (field vines only). Five and ten shoots per grafted combination (Pinotage x rootstock) were selected for leaf area measurements for potted and field grapevines respectively. Equations based on field grapevines were established for the correlation between 'Pinotage' leaf area and leaf main vein length, viz. $y = -34.2857+13.163*x$ ($r^2 = 0.94$) for primary shoots and $y = -36.4218+12.8845*x$ ($r^2 = 0.84$) for secondary shoots, where "y" is leaf area (cm^2) and "x" is the length of the leaf main vein (cm). Total leaf area per grapevine was calculated by multiplying the total primary and secondary (only for field experimentation) shoot leaf area by the number of primary and secondary (only for field experimentation) shoots per grapevine. This method was used for field and potted grapevines. In the case of field grapevines, 15 shoots per treatment on the same position on the cordon were selected to determine the vine leaf area whereas in potted grapevines all the plants were assessed for leaf area.

3.2.10 Statistical analysis

Data were analysed using a repeated measures analysis of variance (ANOVA) and means were separated by Fisher's least significant difference (LSD) test ($P < 0.05$). All analyses were done with Statistica version 11.0.

3.3 Results

3.3.1 Field experiment

3.3.1.1 Climatic conditions

Temperature and air humidity data are provided as examples of a characteristic day during which vine water status was recorded between berries at the pea-size and at the harvest-ripe stages of development (from December 2010 to February 2011) (Figure 3.2). Mean temperatures are presented in Table 3.3. Under field conditions the maximum temperatures were above 30º C but > to 33° for at least 3 hours for the specific day. For the field conditions, the growing degree day (GDD), i.e. the summation of temperature above 10 °C (Winkler et al. 1974), was 2261 for the period September to March. According to the Winkler heliothermic index, this corresponds to a warm Mediterranean climate (Class IV-V).

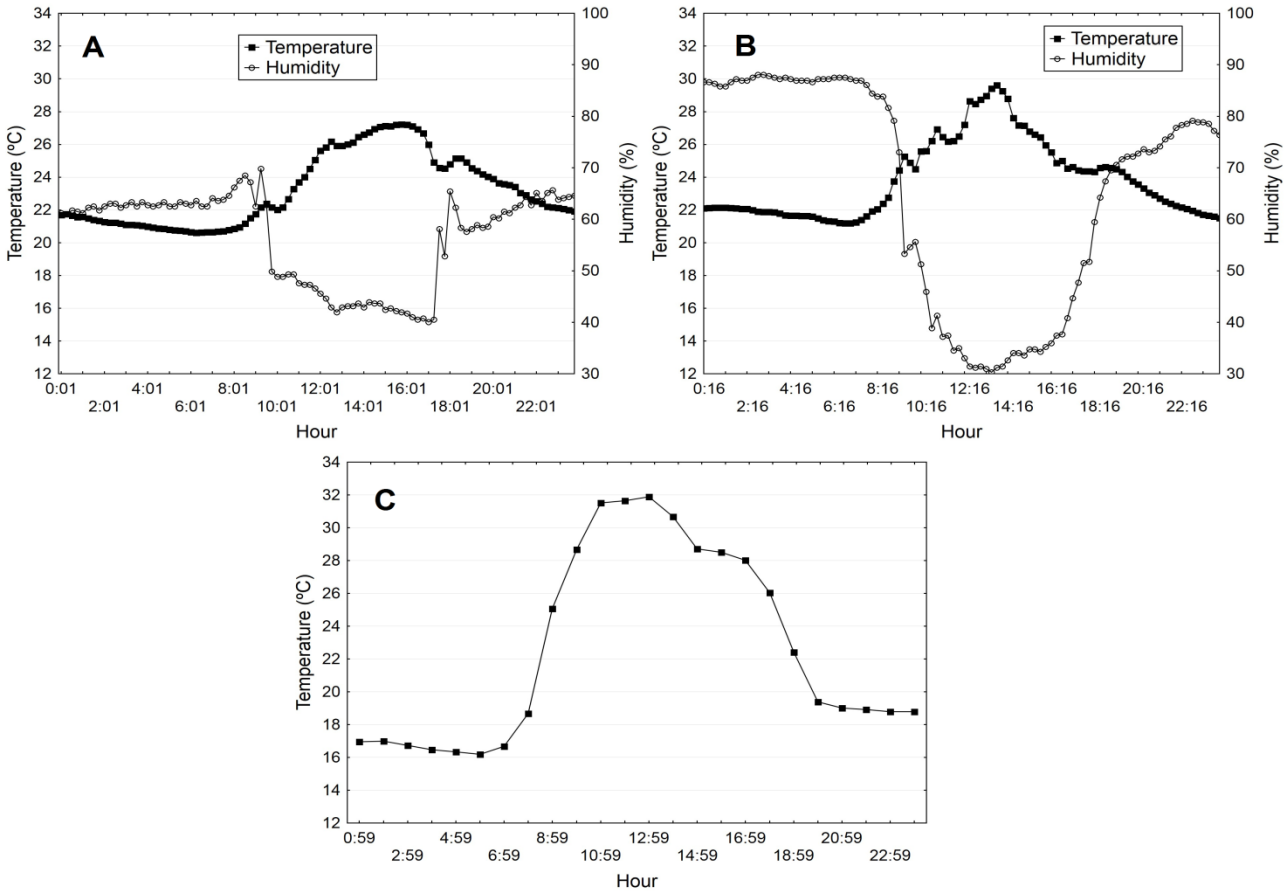

Figure 3.2 Temperature and air humidity data, recorded every 15 minutes, as examples of a characteristic day during the experimentation where the physiological measurements took place. A: ATC greenhouse experiment; B: NoATC greenhouse experiment; C: Field experiment 2010/2011.

Table 3.3 Temperatures and vapour pressure difference (VPD) during experimentation in field (from budburst to harvest) and in greenhouses with automatic temperature control (ATC) (from 5th October to 7th November 2011) and without automatic temperature control (NoATC) (from 14th to 24th February 2012).

Experiments	Mean temp max	Mean temp min	VPD
ATC greenhouse experiment	28,1 °C ± 0,5	20,9 °C ± 0,3	1,58 ± 0,06
NoATC greenhouse experiment	30,0 °C ± 1,0	19,7 °C ± 0,5	2.39 ± 0,10
Field experiment	32,1 °C ± 0,6	17,0 °C ± 0,3	ND*

*No data.

3.3.1.2 Soil moisture

Soil water content is presented in Figure 3.3A. Under field conditions, the water deficit treatments reached values of soil water content of 140 (mm/m), whereas in well-watered treatments the irrigations were carried out when the soil water content reached 160 (mm/m).

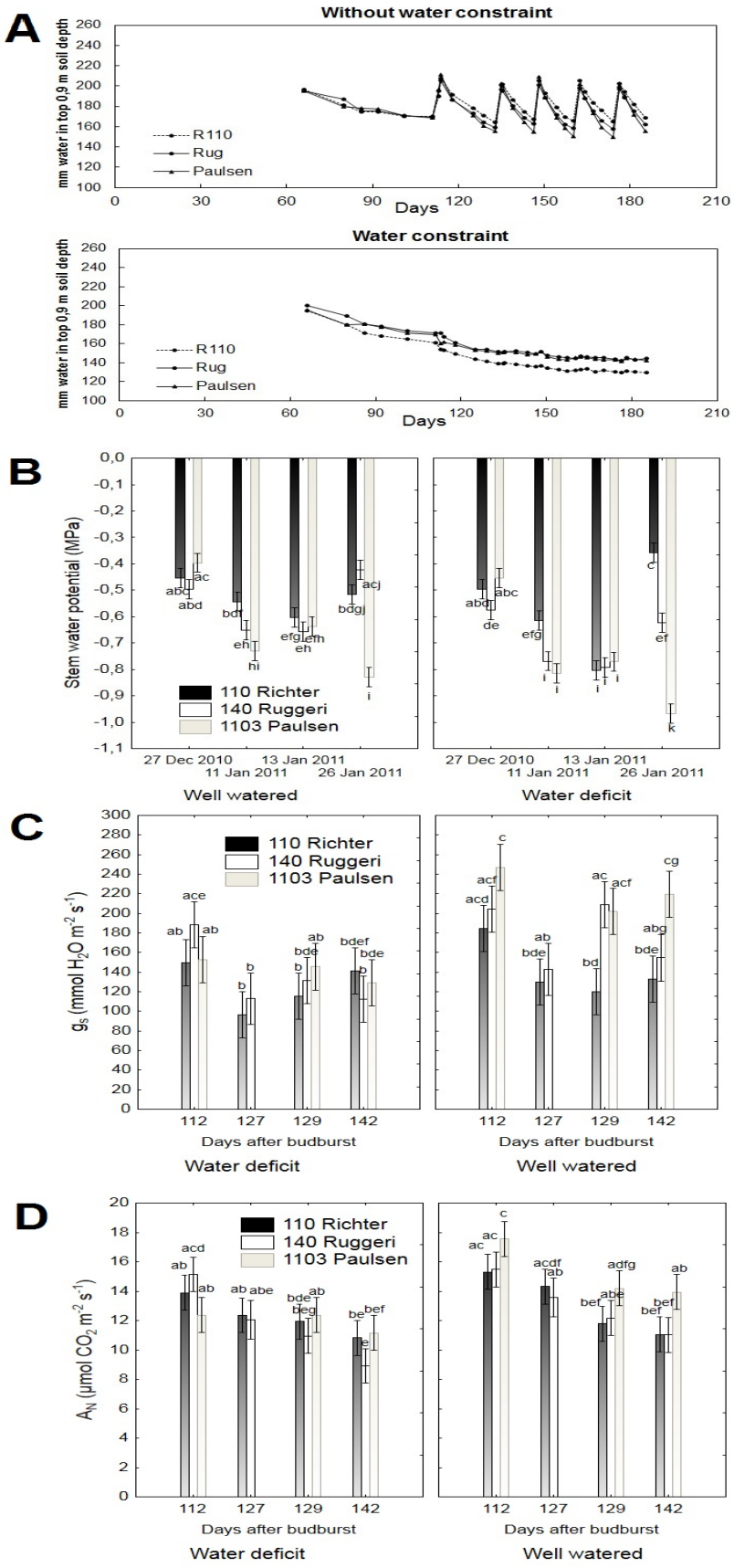

Figure 3.3 Soil water content (A), stem water potential, ψ_{stem} (B), stomatal conductance, g_s (C) and net photosynthesis, A_N (D) during field experiments of cv. Pinotage grafted onto 110 Richter, 140 Ruggeri and 1103 Paulsen subjected to water constraint and without water constraint. Vertical bars denote +/- standard errors.

3.3.1.3 Leaf area

There were no significant differences in total leaf area for Pinotage grafted onto the different rootstocks (2.5, 2.2 and 2.0 m^2/grapevine for 110 Richter, 140 Ruggeri and 1103 Paulsen respectively) and among irrigation treatments (2.2 m^2/grapevine for both no constraint and moderate water constraint treatments).

3.3.1.4 Stem water potential and predawn leaf water potential

Under field conditions, only moderate water constraints were obtained, in comparison with well-watered plants (Figure 3.3B). ψ_{stem} ranged from -0.39 to -0.83 MPa during the well-watered treatment and -0.36 to -0.97 MPa in the water deficit treatment. Predawn leaf water potentials confirm a moderate water constraint in the water deficit treatments of the field experiments, ranging from -0.21 to -0.40 MPa (Figure 3.4) (Deloire and Heyns 2011). Previous soil profiles in the study site revealed a deep root system with access to a water table and provide a likely explanation for the lack of a severe water constraint. Plant water status was, however, affected by rootstock under water deficit conditions ($p < 0.001$) (Figure 3.3B). Those plants grafted onto 110 Richter had on average less negative values of ψ_{stem} (-0.55 MPa) than those grafted onto 140 Ruggeri and 1103 Paulsen (-0.63 and -0.70 MPa, respectively). The soil moisture values for the water deficit treatment at the time the ψ_{stem} were taken were: 149.3, 161.3 and 158.5 (mm/m) for 110 Richter, 140 Ruggeri and 1103 Paulsen respectively for the first measurement of ψ_{stem}; 139.3, 151.9 and 150.1 (mm/m) for 110 Richter, 140 Ruggeri and 1103 Paulsen respectively for the second measurement of ψ_{stem}; 139.8, 151.7 and 151.4 (mm/m) for 110 Richter, 140 Ruggeri and 1103 Paulsen respectively for the third measurement of ψ_{stem}; 136.8, 151.9 and 151.5 (mm/m) for 110 Richter, 140 Ruggeri and 1103 Paulsen respectively for the fourth measurement of ψ_{stem}.

3.3.1.5 Stomatal conductance and photosynthesis

Only small differences in g_s and A_N were detected between the well-watered and moderate water deficit treatments and between the different rootstocks (Figures 3.3C and 3.3D). Stomatal conductance ranged from 120.0±23.7 to 246.8±23.7 (mmol H_2O m^{-2} s^{-1}) during the well-watered treatment and 96.4±23.7 to 188.4±23.7 (mmol H_2O m^{-2} s^{-1}) in the water deficit treatment. Leaves of grapevines grafted onto 110 Richter, 140 Ruggeri and 1103 Paulsen had average values of 138.9±16.2, 160.1±16.1 and 181.7±16.2 (mmol H_2O m^{-2} s^{-1}) respectively. Net photosynthesis ranged from 11.0±1.2 to 17.6±1.2 (µmol CO_2 m^{-2} s^{-1}) during the well-watered treatment and 8.9±1.2 to 15.1±1.2 (µmol CO_2 m^{-2} s^{-1}) in the water deficit treatment. Leaves of grapevines grafted onto 110 Richter, 140 Ruggeri and 1103 Paulsen had average values of

12.5±0.8, 12.1±0.8 and 13.7±0.8 (μmol CO_2 m^{-2} s^{-1}) respectively. Stomatal conductance did not respond to ψ_{stem} since most of the vines did not undergo the same level of water stress as in the glasshouse trials (data not shown). The relationships between A_N and g_s during field treatments is presented in Figure 3.5. As expected a tight curvilinear correlation was found between g_s and A_N (Figure 3.5).

3.3.2 ATC Glasshouse experiment

3.3.2.1 Climatic conditions

Temperature and air humidity data are provided as examples of a characteristic day during which the vine water status was recorded (from October to November 2011) (Figure 3.2). Mean temperatures and VPD are presented in Table 3.3. The maximum temperature during the day did not usually exceed 28º C. As expected the climate controlled greenhouse (ATC) presented the lowest fluctuation of temperature and humidity, in comparison with the greenhouse without the climate control (NoATC) and field conditions.

3.3.2.2 Soil moisture

During the physiological measurements in the ATC greenhouse the soil water content was 14.7% ± 0.57 on a dry-mass basis for the well-watered treatments. At the end of the water deficit treatments (3 November 2011), the soil water content dropped to 1.3% ± 1.61 on a dry-mass basis for the water deficit treatments (Figure 3.6A).

3.3.2.3 Stem water potential

The water constraint treatments carried out in the greenhouses produced clear differences in terms of plant water status, in comparison to well-watered treatments ($p < 0.001$) (Figure 3.6B). Stem water potential ranged from -0.28 to -0.53 MPa during the well-watered treatment and -0.32 to -1.39 MPa in the water deficit treatment. In the ATC greenhouse, severe water constraint resulted in visible effects after approximately 10 days such as the senescence of basal leaves and wilting of the leaves near the apex (Figure 3.7). The well-watered plants maintained a constant plant water status during the experiment. Grapevines grafted onto 99 Richter and 110 Richter did not differ significantly in plant water status, -0.63 and -0.60 MPa respectively (Figure 3.6B).

3.3.2.4 Stomatal conductance and photosynthesis

As expected, water constraints resulted in a reduction in g_s ($p < 0.01$) and A_N ($p < 0.001$) (Figures 3.6C, 3.6D). Stomatal conductance ranged from 46.1±38.8 to 287.6±38.8 (mmol H_2O m^{-2} s^{-1}) during the well-watered treatment and 7.7±38.8 to 159.1±38.8 (mmol H_2O m^{-2} s^{-1}) in the

water deficit treatment. Net photosynthesis ranged from 3.5±1.2 to 15.5±1.2 (µmol CO_2 m^{-2} s^{-1}) during the well-watered treatment and 1.6±1.2 to 11.8±1.2 (µmol CO_2 m^{-2} s^{-1}) in the water deficit treatment. Rootstock had no effect on g_s or A_N (Figures 3.6C, 3.6D). Grapevines grafted onto 99 Richter and 110 Richter, had average values of g_s at 102.6±10.4 and 85.0±10.4 (mmol H_2O m^{-2} s^{-1}) and average values of A_N of 7.8±0.3 and 7.2±0.3 (µmol CO_2 m^{-2} s^{-1}) respectively. The relationships between g_s and ψ_{stem}, A_N and g_s are presented in Figure 3.8. In general, g_s responded to ψ_{stem} at less than -0.6 to -0.7 MPa across the rootstocks in the glasshouse experiments. Irrespective of rootstock, under values of -0.6 MPa in stem water potential, g_s became responsive, as in agreement with Lovisolo et al. (2010). These results validated to certain extend the thresholds of stem water potential and their potential practical application (Deloire and Heyns 2011). As expected a tight curvilinear correlation was found between g_s and A_N (Figure 3.8B).

3.3.3 Glasshouse experiment NoATC

3.3.3.1 Climatic conditions

Temperature and air humidity data are provided as examples of a characteristic day during which the vine water status was recorded (February 2012) (Figure 3.2). Mean temperatures and VPD are presented in Table 3.3. The maximum temperature during the day did not exceed 30º C in general.

3.3.3.2 Soil moisture

Well-watered treatments had a soil water content of 14.5% ± 1.72 while the water deficit treatment dropped to 1.5% ± 2.44 on a dry-mass basis (Figure 3.9A).

3.3.3.3 Stem water potential

The water constraint treatments carried out in the greenhouses produced clear differences in terms of plant water status, in comparison to well-watered treatments ($p < 0.001$) (Figure 3.9B). The well-watered plants maintained a constant plant water status during the experiment. Stem water potential ranged from -0.38 to -0.63 MPa during the well-watered treatment and -0.36 to -1.5 MPa in the water deficit treatment. Plant water status was affected by rootstocks only under water deficit conditions ($p < 0.05$). Those plants grafted onto 99 Richer and 110 Richter had 16% and 34% less negative ψ_{stem}, respectively than those grafted onto Ramsey (Figure 3.10A). The differences in ψ_{stem} were apparent throughout the progression of increasing water stress. At the end of the water constraint period, when plants were near the permanent wilting point (Figure 3.9A), similar values of plant water status between the rootstocks were observed probably due to

the inability of the root system to take up enough water to counterbalance the loss of water by transpiration from leaves.

3.3.3.4 Stomatal conductance and photosynthesis

As expected, water constraints induced a reduction in g_s ($p < 0.001$) and A_N ($p < 0.001$). Clear differences in g_s and A_N between the well-watered and water constraint treatments were found in the experiments carried out in greenhouses (Figures 3.9C and 3.9D). Stomatal conductance ranged from 64.2±54.4 to 323.7±54.4 (mmol H_2O m^{-2} s^{-1}) during the well-watered treatment and 24.1±54.4 to 243.7±54.4 (mmol H_2O m^{-2} s^{-1}) in the water deficit treatment. Net photosynthesis ranged from 7.3±2.1 to 16.1±2.1 (µmol CO_2 m^{-2} s^{-1}) during the well-watered treatment and 2.7±2.1 to 11.2±2.1 (µmol CO_2 m^{-2} s^{-1}) in the water deficit treatment. When all the rootstocks were compared under water deficit conditions, 110 Richter and 99 Richter tended to have higher A_N ($p \leq 0.05$) and significantly higher g_s ($p \leq 0.01$) in comparison with Ramsey (Figures 3.10B and 3.10C), but these differences were not reflected in differences in intrinsic leaf water use efficiency (A_N/ g_s) among rootstocks (data not shown). Stomatal conductance in grapevines grafted onto 99 Richter, 110 Richter, 140 Ruggeri, 1103 Paulsen and Ramsey had average values of 116.6±19.4, 150.1±19.4, 76.7±19.4, 64.1±19.4 and 59.0±19.4 (mmol H_2O m^{-2} s^{-1}) respectively. Net photosynthesis in grapevines grafted onto 99 Richter, 110 Richter, 140 Ruggeri, 1103 Paulsen and Ramsey had average values of 8.4±0.9, 8.6±0.9, 7.4±0.9, 6.1±0.9 and 5.2±0.9 (µmol CO_2 m^{-2} s^{-1}) respectively. These differences between 99 Richter and Ramsey were only apparent during well-watered conditions, while the differences in A_N between 110 Richter and Ramsey lasted for a few days during the increased water constraint. When the water constraint treatment was terminated, all the rootstocks regained similar values of g_s below 50 (mmol H_2O m^{-2} s^{-1}) verifying that a severe water constraint treatment had been applied (Cifre et al. 2005, Lovisolo et al. 2010).

The relationships between g_s and ψ_{stem}, A_N and g_s during the NoATC greenhouse treatments are presented in Figure 3.11. In general, g_s responded to ψ_{stem} at values less than -0.6 to -0.7 MPa across the rootstocks in the glasshouse experiments. 99 Richter and 110 Richter had particularly high g_s at high ψ_{stem} but under water restraints, g_s declined to similar values as that of the other rootstocks (Figure 3.11C). As expected a tight curvilinear correlation was found between g_s and A_N (Figures 3.11B and 3.11D).

In summary, the field results did not result in significant treatment differences due to only moderate water deficits, suggesting a deep foraging root system capable of extracting water. This was later confirmed by soil pits. Nevertheless, when the three rootstocks with similar tolerance to drought were compared (110 Richter and 140 Ruggeri are classified as highly tolerant to

drought while 1103 Paulsen is classified as tolerant to drought (Carbonneau 1985)), some differences in terms of plant water status and stomatal conductance between rootstocks were present which might be explained by vineyard heterogeneity and other abiotic factors which were not under control. Alternatively it could suggest that a rootstock-cultivar is able to induce different leaf functioning for the same the scion-cultivar (*Vitis vinifera* L.) genetics. No differences were found in A_N.

Greenhouse experimentations allowed the study of more rootstocks introducing higher genetic variability between rootstocks and, therefore, more differences in terms of possible scion-cultivar responses to drought (99 Richter and Ramsey were introduced in addition to the rootstocks used in field experiments; they are respectively classified as drought tolerant and drought sensitive (Keller 2010)). In addition, we were able to study rootstocks under an increasing water constraint and severe stress using potted vines and sandy soil. When all rootstocks were assessed under increasing water deficit in the greenhouse, compared to 140 Ruggeri, 110 Richter, 99 Richter and 1103 Paulsen, Pinotage grafted onto Ramsey had the most negative midday stem water potential and the lowest stomatal conductance indicating lower control of Ramsey over the leaf functioning of the scion in response to drought or less ability to pump water suggested by an early reach of 0.6 (MPa) in stem water potential (Figure 3.10A) or a higher production of ABA.

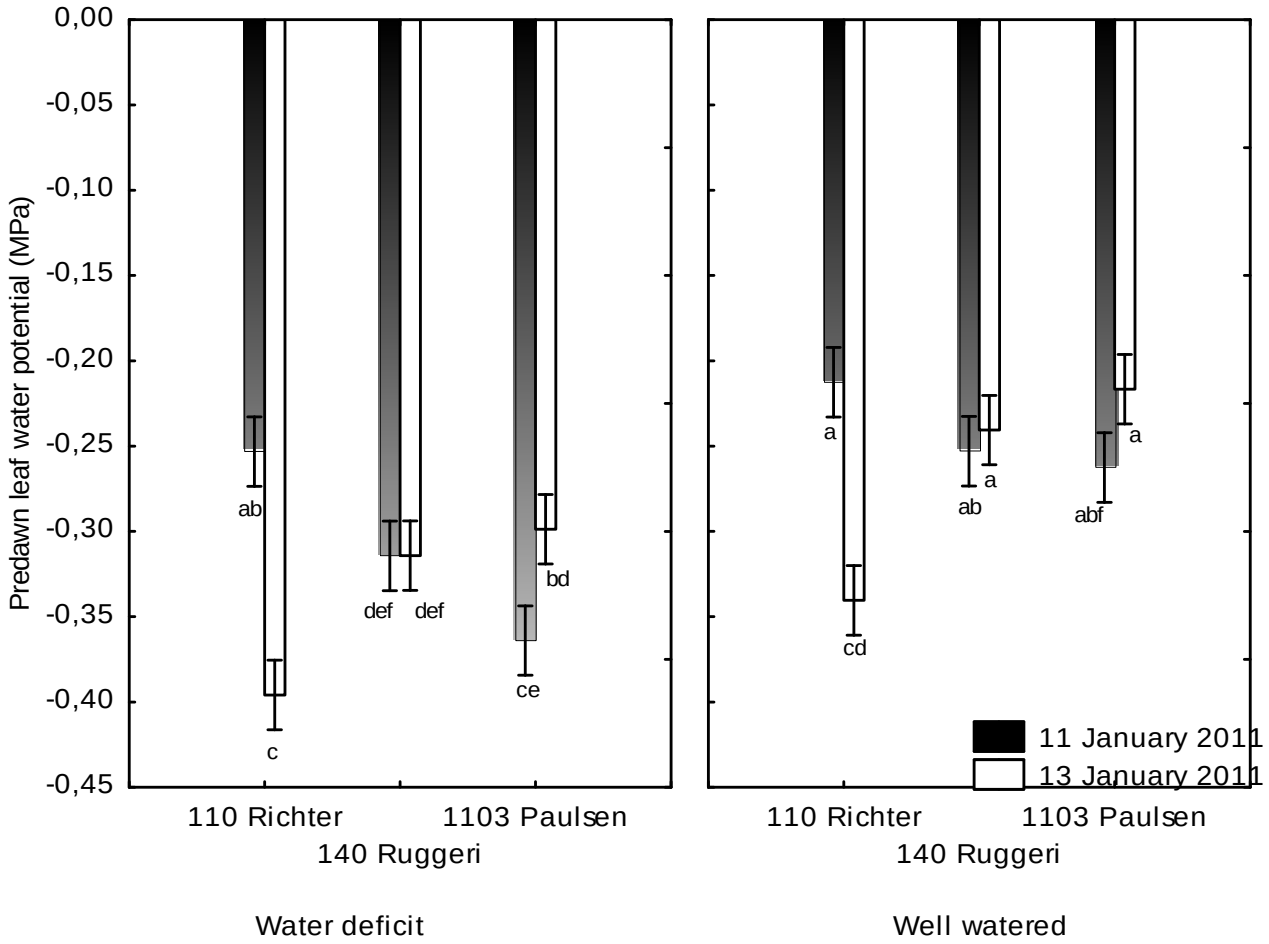

Figure 3.4 Predawn leaf water potentials of field experiments carried out before and after the second irrigation. Vertical bars denote +/- standard errors.

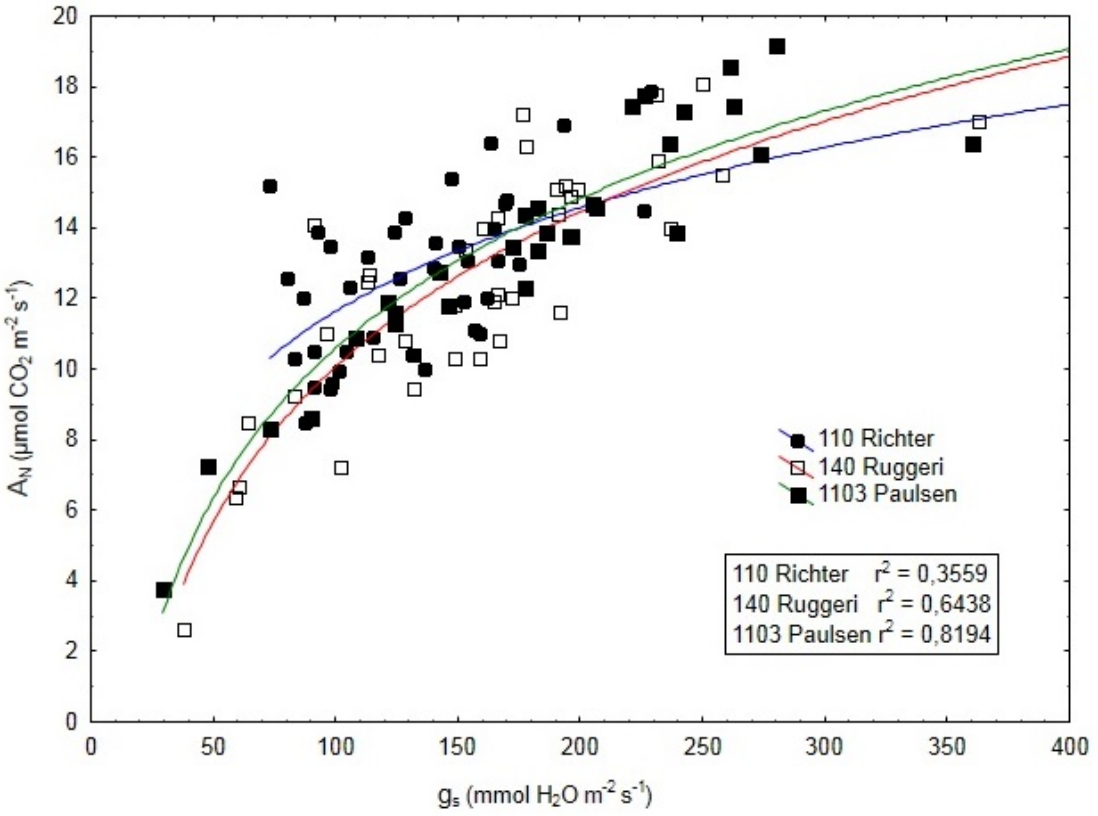

Figure 3.5 The relationships between net photosynthesis and stomatal conductance during field experiments of cv. Pinotage grafted onto 110 Richter, 140 Ruggeri and 1103 Paulsen.

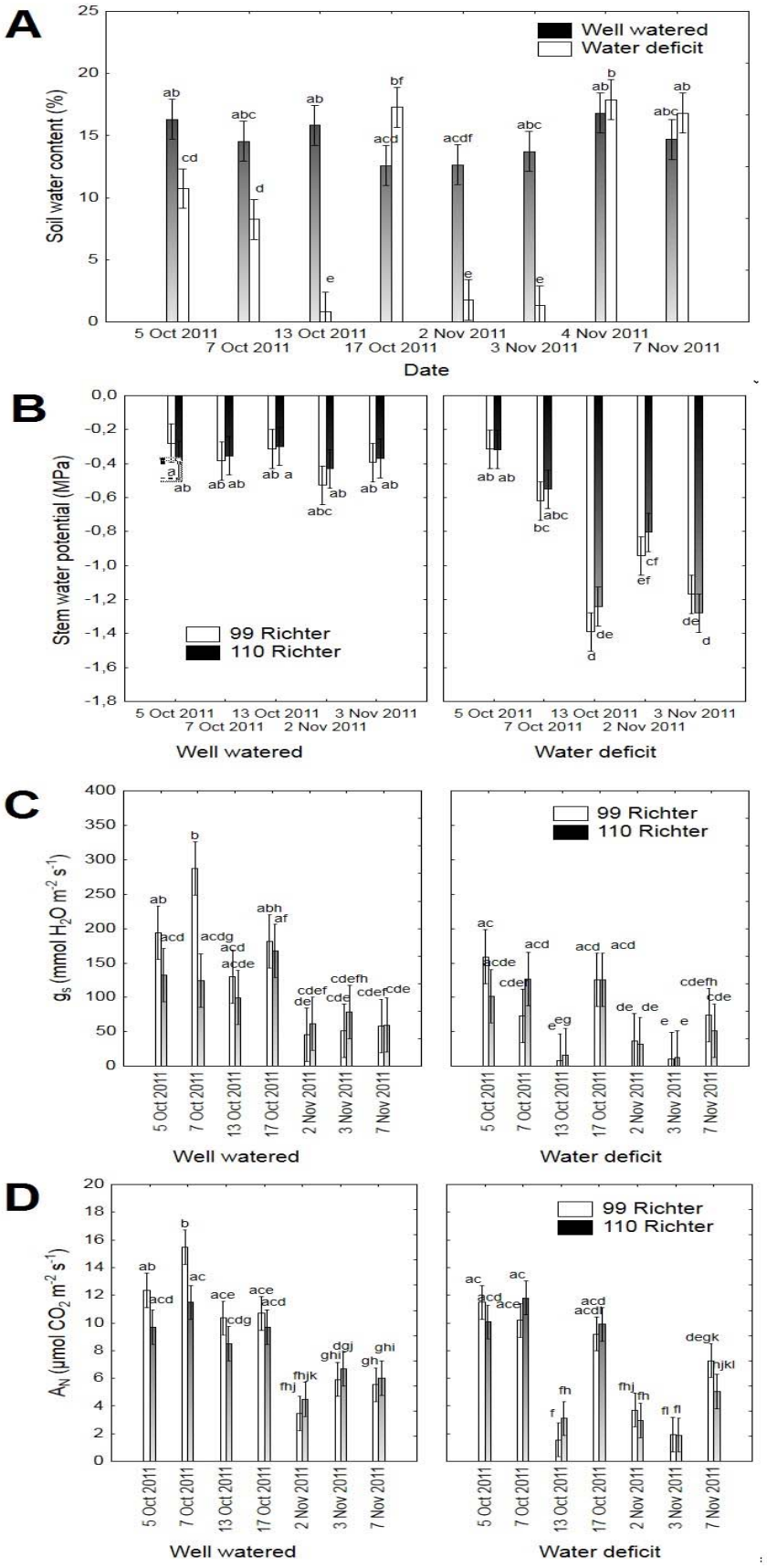

Figure 3.6 Soil water content (A), stem water potential (B), stomatal conductance (C) and net photosynthesis (D) during controlled temperature greenhouse experiments of cv. Pinotage grafted onto 99 Richter and 110 Richter subjected to water constraint and without water constraint. Vertical bars denote +/- standard errors.

Figure 3.7 Senescence of older basal leaves (left) and leaves wilting at the top part of the primary shoot (right) in vines under water deficit treatments in ATC greenhouse.

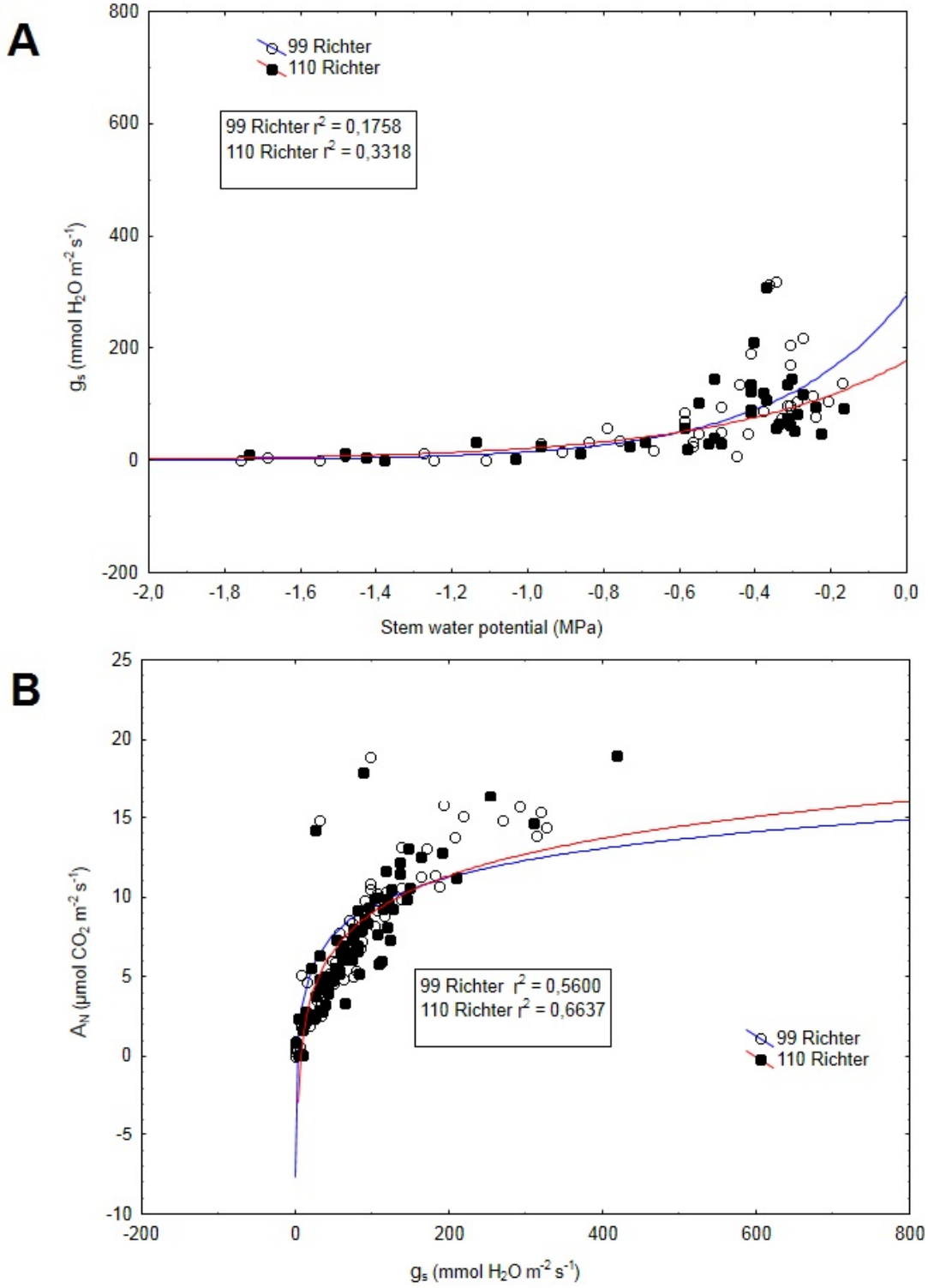

Figure 3.8 The relationships between stomatal conductance and stem water potential (A), net photosynthesis and stomatal conductance (B) during temperature controlled greenhouse experiments of cv. Pinotage grafted onto 99 Richter and 110 Richter. The figure clearly shows how -0.6 MPa is a limit from no water constraint to water constraint.

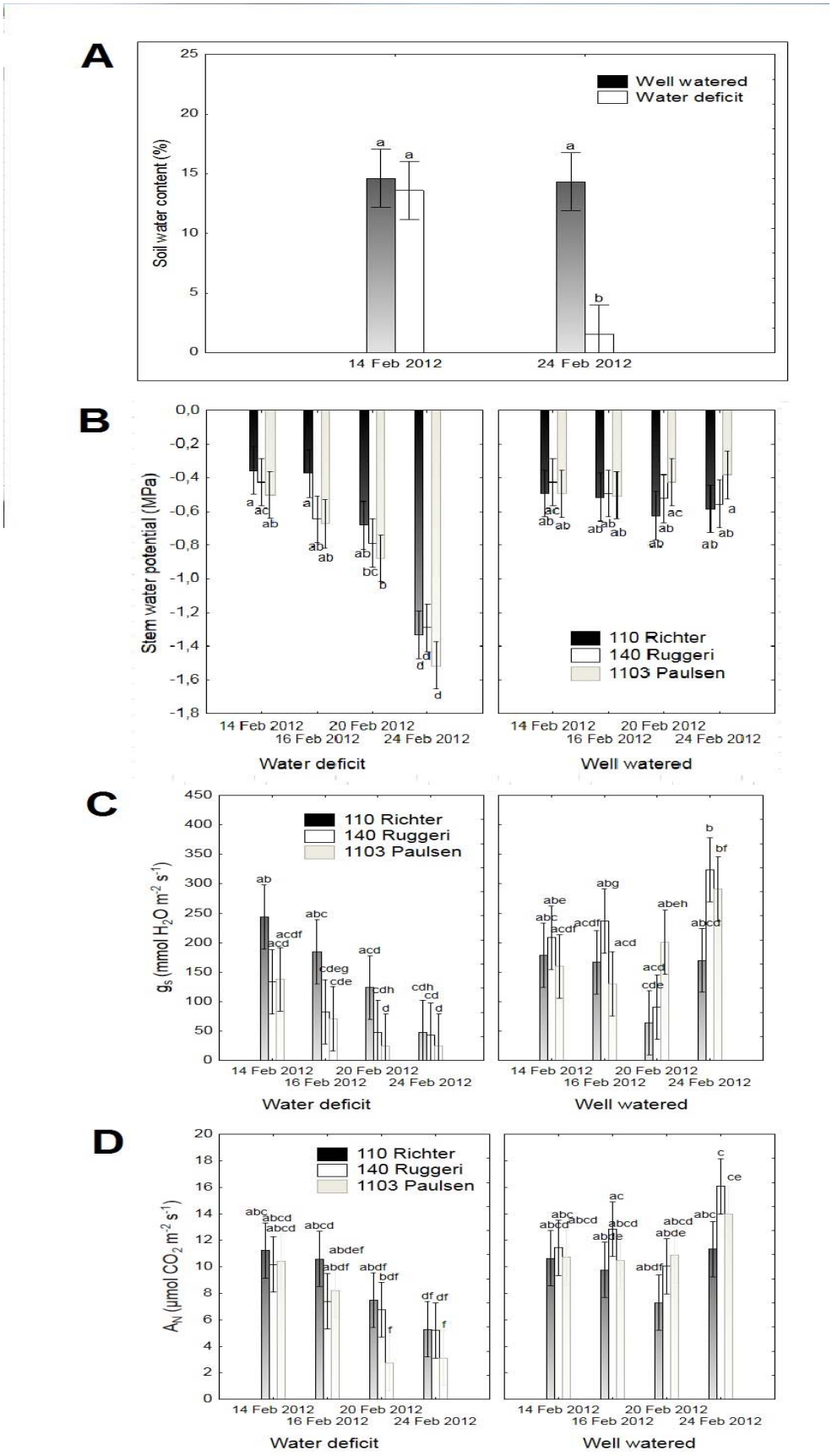

Figure 3.9 Soil water content (A), stem water potential (B), stomatal conductance (C) and net photosynthesis (D) during NoATC greenhouse experiments of cv. Pinotage grafted onto 110 Richter, 140 Ruggeri and 1103 Paulsen subjected to water constraint and without water constraint. Vertical bars denote +/- standard errors.

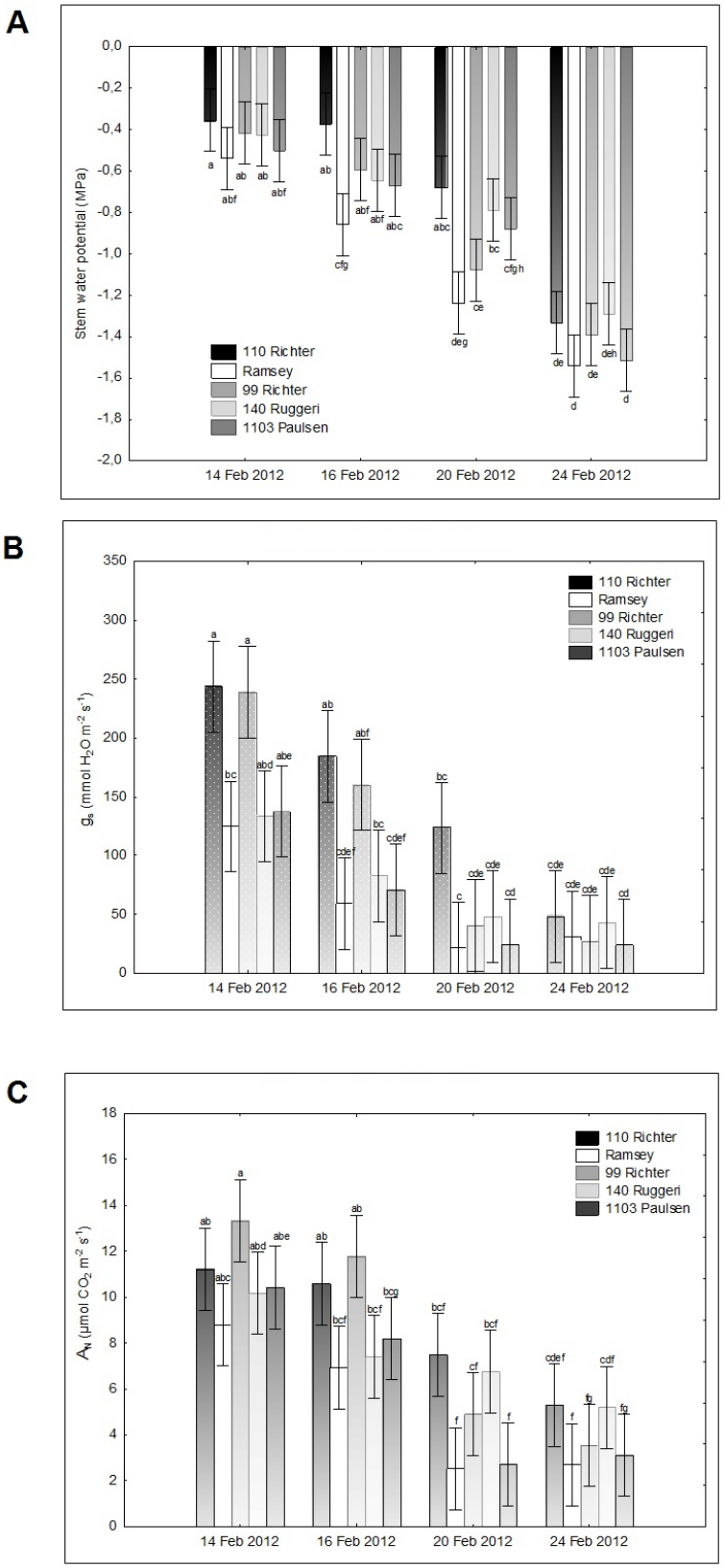

Figure 3.10 Stem water potential (A), stomatal conductance (B) and net photosynthesis (C) during NoATC greenhouse experiments of cv. Pinotage grafted onto 110 Richter, Ramsey, 99

Richter, 140 Ruggeri and 1103 Paulsen subjected to an increasing water constraint. Vertical bars denote +/- standard errors.

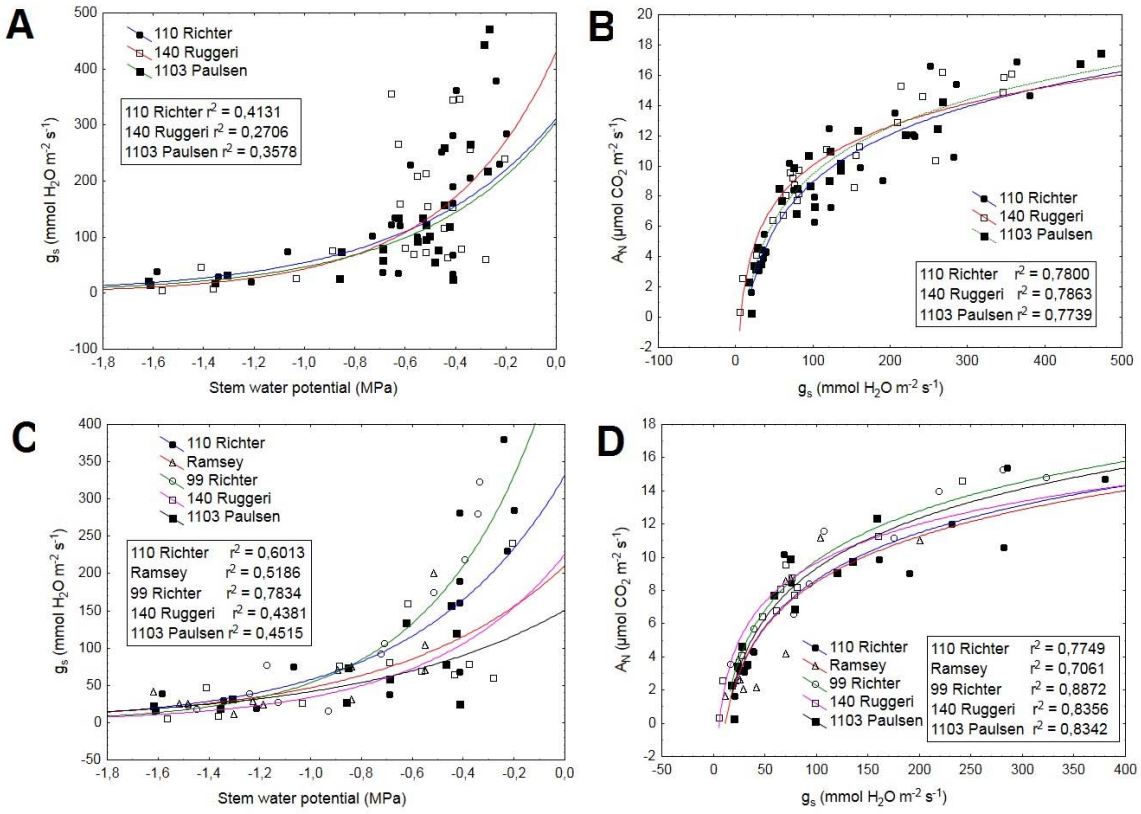

Figure 3.11 The relationships between stomatal conductance and stem water potential (A), net photosynthesis and stomatal conductance (B) during NoATC greenhouse experiments of cv. Pinotage grafted onto 110 Richter, 140 Ruggeri and 1103 Paulsen subjected to water constraint and without water constraint. Stomatal conductance and stem water potential (C), net photosynthesis and stomatal conductance (D) during NoATC greenhouse experiments of cv. Pinotage grafted onto 110 Richter, Ramsey, 99 Richter, 140 Ruggeri and 1103 Paulsen subjected to an increasing water constraint.

3.4 Discussion

Stomatal closure is one of the first responses to drought (Chaves et al. 2003). Many studies have shown that rootstocks are able to limit transpiration through stomatal regulation (Candolfi-Vasconcelos et al. 1994, Düring 1994, Lovisolo et al. 2002, de Souza et al. 2003, Soar et al. 2006, Marguerit et al. 2012). In agreement with the results from the literature, our study suggests that g_s can be altered by rootstock. Under an increasing water deficit, Pinotage grafted either onto 110 Richter or 99 Richter maintained higher g_s than when grafted onto Ramsey. In addition, plants grafted onto 99 Richter and 110 Richter presented a less negative ψ_{stem} under water constraints in comparison with Ramsey. In a study of potted 110 Richter grapevines on own roots, Pou et al. (2008) also found that this rootstock can exert tight regulation over stomatal conductance in response to water deficit while maintaining almost constant leaf water potentials.

The responses in g_s and plant water status appeared to be related, where midday ψ_{stem}, which was measured on a non-transpiring leaf, is highly correlated to the capacity of the grapevine to conduct water from the soil to the atmosphere (Choné et al. 2001). However, Lovisolo et al. (2002) showed that stomatal closure induced by ABA root-to-shoot signals and plant hydraulic conductance act independently. Grapevines under partial rootzone drying (PRD) synthesize chemical compounds in drying roots which act as long distance signals inducing leaf stomatal closure without significant changes in plant water status (Chaves et al. 2010). Vegetative growth, vigour and canopy density is reduced in vines under PRD (dos Santos et al. 2003). Lack of differences in plant water status under PRD might be explained by reduced water loss through stomatal closure or improved water uptake by the remaining hydrated roots (Stoll et al. 2000).

Under the greenhouse conditions of this study a wide range in soil water content was attained, whereas the irrigation treatments of the field conditions did not result in significant differences in plant water status. It is important to note that the pot experiment induced a rapid and severe water stress while in the field experiment there was a gradual build-up of moderate water constraints. This situation can be linked to the soil matrix, which affects soil moisture availability and root growth (Serra-Stepke 2010), and to root morphology, distribution and functioning, which are key factors affecting water uptake capacity of the root system (Tyerman et al. 2009, Vandeleur et al. 2009). Also the field vines were much older than the potted vines and their larger and deeper root systems likely helped maintain a moderate vine water constraint. Under these conditions, rootstocks affected the grapevine water status moderately where grapevines grafted onto 110 Richter had higher ψ_{stem} compared to those on 140 Ruggeri and 1103 Paulsen, but no differences in g_s were detected. Furthermore, inherent microclimate differences and leaf age differences between field and greenhouse canopies can influence the response of the vines to water deficit. Field experimentation presented in our case a drawback for this kind of water relations study. The natural heterogeneity and variability of a vineyard at various levels (soil structure and depth, meso and micro climate, cultural practices and vine age) are strong limiting factors for research trying to understand and explain plant mechanisms.

Our data from the greenhouses supported the hypothesis that the rootstock effect on vine water status is heightened under increasing water deficit (Soar et al. 2006). Physiological responses to drought induced by rootstocks (water uptake and transport, stomatal regulation) are triggered by soil water deficits so it is hypothesized that the intensity of the water constraint can have a direct impact on the intensity of the physiological response (up to a point), where genetic differences among rootstocks can make a difference in scion response to drought. Potential differences between rootstocks involve differences in root morphology (xylem vessels size and

density), growth and distribution (generation of new fine roots) and functioning (aquaporin expression and activity which influence hydraulic conductance and xylem vessel embolism repair).

Leaf wilting is the result of a loss in turgor due to the inability of the cell to obtain sufficient water to counterbalance the loss by transpiration or translocation (Knight 1922). Leaf senescence, on the other hand, includes chlorophyll degradation and in conjunction with leaf abscission involves a plant strategy in response to drought that leads to the decrease of canopy size (Rivero et al. 2007), therefore reducing whole vine transpiration. Water stress results in a reduction in photosynthesis due to non stomatal limitations (biochemical limitations such as Rubisco impairment and decreased rate of electron transport) and stomatal closure (diffusional limitations such as reduced CO_2 availability) (Cifre et al. 2005, Lovisolo et al. 2010). In general, the differences detected in plant water status and g_s among rootstocks were not reflected in differences in A_N between rootstocks. Previous studies have found an effect of rootstock on A_N under field grown conditions involving different levels of water constraints (Candolfi-Vasconcelos et al. 1994, Düring 1994) but in some studies no effect was found under well-watered conditions (Gibberd et al. 2001). These differences might be related to differences in scion/rootstock combinations and intensity of the water constraint. During mild water constraints, A_N is mainly limited by stomatal closure whereas at moderate water constraints stomatal and non-stomatal limitations occur. Ultimately at severe water stress, non-stomatal limitations (metabolic and/or restricted internal CO_2 diffusion) to photosynthesis are predominant, especially during simultaneous high temperature and irradiance conditions (Lovisolo et al. 2010).

Recently, a link has been established between rootstock-cultivar and differential expression of hormone signalling related genes at the scion-cultivar shoot apex level, suggesting an alteration of the scion defense response (Cookson and Ollat 2013). Quantitative trait loci (QTLs) analyses have been used to identify genomic regions involved in water deficit responses by rootstocks. It seems that a genomic region in the rootstock is able to control transpiration rate and its acclimation to soil water deficit through hormonal (particularly ABA) and hydraulic (aquaporins) signalling (Marguerit et al. 2012), and this regulation occurs up to a point until plant water status becomes the main driver of A_N and g_s. Our data suggested that this shift occurred early during the water constraint at a plant water status threshold of around ψ_{stem} values of -0.6 to -0.8 MPa, which could correspond to the limit between no vine water constraint and vine water constraint or water stress for ψ_{stem} values of -1.4 MPa. This confirms, to a point, the thresholds of predawn leaf water potential and stem water potential which are used for research

or practically to assess vine water status and manage irrigation (Carbonneau 1998, Williams and Araujo 2002, Carbonneau et al. 2007, Deloire and Heyns, 2011).

It seems that at least under the conditions of this study, more drought tolerant rootstocks (99 Richter and 110 Richter in comparison with Ramsey, in this case) are able to delay the reach of plant water status threshold where stomatal conductance and photosynthesis present a more prominent decline. This data show that when the vine reach plant water status threshold of around ψ_{stem} values of -0.6 to -0.8 MPa, g_s is reduced to values of approximately 100 (mmol H_2O m^{-2} s^{-1}) which correspond to moderate water constraint or transition stage as defined by Cifre et al. (2005) and Lovisolo et al. (2010). At this point A_N is further reduced. From this point onwards, plant water status is the main driver of g_s and A_N irrespective of rootstocks.

3.5 Conclusions

Our study suggest that rootstocks grafted on Pinotage scions had an effect on scion-cultivar water status and leaf gas exchange, implying an influence on water uptake and transport and regulation of leaf stomatal conductance. Rootstock influence on scion response to drought occurred from the first stages of water constraint up to a point where plant water status became the main driver of the photosynthesis and stomatal conductance, irrespective of the rootstock/scion cultivar. The level of rootstock regulation on scion stomatal conductance is dependent on the ability of the rootstock to cope with drought. The *V. rupestris* x *V. cinerea* var. helleri resseguier#2 family (99 Richter, 110 Richter) are drought tolerant and confer a better stomatal control of the scion. The understanding of the ability of a rootstock to regulate the transpiration of the scion-cultivar requires more study at the genetic level. This will be useful for the selection of new rootstocks tolerant to drought in a context of water scarcity, providing sustainable solutions to the wine industry.

Acknowledgements

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Chapter 4

Research results

Stomatal development of grapevine leaves (*Vitis vinifera* L., cv. Pinotage) in response to the combined effect of light, plant water status and rootstocks

Stomatal development of grapevine leaves (*Vitis vinifera* L., cv. Pinotage) in response to the combined effect of light, plant water status and rootstocks

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Stomatal development in response to the combined effect of light, plant water status and rootstocks

Abstract

Drought-tolerant rootstocks are expected to improve grapevine response to water deficit by improving the water uptake and transport and by reducing the water loss in leaves by root-to-shoot signalling. Nevertheless, the mechanisms of rootstocks' tolerance to drought are not yet fully understood. The aims of this study were to evaluate the adaptation of cv. Pinotage (*Vitis vinifera* L.) leaf stomata (density and size) to soil water deficit and light intensity in interaction with different rootstocks known for their different drought tolerance. The field trial was carried out at the Welgevallen experimental vineyard (Stellenbosch University) during two seasons. Two irrigation strategies were studied: irrigated grapevines to avoid any water constraint and rain-fed grapevines subjected to moderate water constraint. Stomatal density and size were determined on sun exposed and shaded adult leaves. Greenhouse trials were carried out with automatic temperature control (ATC) and without automatic temperature control (NoATC). Half of the grapevines were subjected to severe water constraints. The other half were maintained well watered. The results suggest that stomatal development is affected by light and water deficit and probably by the rootstocks which might regulate the cultivar's stomatal size (anatomical changes during leaf growth) in addition to the effect on stomatal functioning (stomatal regulation) through a complex signalling process. Thus one mechanism of Pinotage leaf adaptation to water constraints was structural during leaf growth, with a reduction in pore size to possibly reduce plant water loss.

Key words: Pinotage, *Vitis vinifera* L., 99 Richter, 110 Richter, 140 Ruggeri, 1103 Paulsen, Ramsey, stomatal density, stomatal size, drought, plant water status.

4.1 Introduction

Stomata are pores surrounded by two guard cells that control the gas exchange between the leaf and the atmosphere (Hetherington and Woodward 2003), which is necessary for transpiration and photosynthesis. Stomatal density and size determine the maximum stomatal conductance (g_s) (Franks and Beerling 2009) which, in conjunction with mesophyll conductance to CO_2 (g_m), plays a key role in leaf photosynthetic capacity (Flexas et al. 2008). During the development of the leaf, a continuous production of stomata occurs and the number and distribution of the pores are genetically regulated (Nadeau and Sack 2002). Stomatal development is controlled and closely related to cell growth and division (Pillitteri and Torii 2012). Stomata are always present with at least one non stomatal cell in between (Peterson et al. 2010) and the density is determined by the area of the non-stomatal cells and the number of stomata formed. The adaptation of plant's stomatal development to environmental conditions is still not totally elucidated (Casson and Hetherington 2010).

In grapevine, the experimental data showed that stomatal development is under genetic control with differences between grapevine cultivars (Rogiers et al. 2009). Stomatal density and size is under the influence of environmental conditions such as CO_2 concentration (Moutinho-Pereira et al. 2009, Rogiers et al. 2011) and light (Palliotti et al. 2000). Recently Rogiers et al. (2011) showed a response in epidermal cell expansion to soil temperature resulting in a lower stomatal density and larger epidermal cells and leaves of plants with warmed roots and an inverse correlation between trunk and root carbohydrate reserves and the number of stomata per leaf area. Factors that influence the signalling for stomatal differentiation and/or cell growth might influence the number of stomata per area (Wang et al. 2007). In addition to this, signalling from mature leaves to developing leaves has been postulated (Lake et al. 2001). In poplar it has been shown that a change in stomatal conductance in mature leaves will affect the stomatal development in new leaves (Miyazawa et al. 2006), involving a long distance signalling mechanism (Casson and Gray 2008). During leaf development, stomatal density can be influenced by environmental factors, however once it is fully differentiated no changes are expected (Schlüter et al. 2003).

From an evolutionary point of view, the morphology (due to the type of guard cells that shape the stomata: dumb-bell-shaped typical of grasses and kidney-shaped typical of other species such *Vitis sp.*), distribution, number and size of the stomata allowed plants to colonize

different habitats (Hetherington y Woodward 2003, Haworth et al. 2011). Due to stomata plasticity response to environmental factors, it has been proposed that the study of stomata adaptation to a changing environment is crucial to understand how plants will adapt to new climatic conditions (Hetherington y Woodward 2003, Nicotra et al. 2010). The aims of this study were to evaluate the adaptation of cv. Pinotage (*Vitis vinifera* L.) leaf stomata (density and size) to soil water deficit and light intensity in interaction with different rootstocks known for their different drought tolerance characteristics.

4.2 Materials and Methods

4.2.1 Plant material

Pinotage (*Vitis vinifera* L.) plants grafted onto five rootstocks having different drought tolerance were used in field and greenhouse experiments (Table 4.1). The same greenhouse experiments used in Chapter 3 were used for this Chapter. For the field experiment during season 2010/2011, only Pinotage grafted onto 110 Richter was assessed due to time constraints. In addition, another field experiment was performed during season 2011/2012 using only plants of Pinotage grafted onto 1103 Paulsen and 140 Ruggeri due to the proximity of the experimental plots (located less than 25 meters apart).

4.2.2 Field grown grapevines: growth conditions and treatments

The field trial was carried out at the Welgevallen experimental vineyard (Stellenbosch University; 33°56'S, 18°52'E, altitude: 157 m) during two seasons (2010/2011 and 2011/2012). For season 2010/2011 see Chapter 3 for details. For field experiment during season 2011/2012 three irrigations were applied (21th December 2011, 19th January and 30th January 2012) for the well watered grapevines' treatment. In order to evaluate the light influence, full sun exposed and shaded adult leaves (growing inside the canopy) were sampled at harvest. 1103 Paulsen and 140 Ruggeri experiments were located less than 25 meters apart in the same block with similar topography (refer to Chapter 3 for more details).

Table 4.1 Rootstocks used for field and pot trials in greenhouses with automatic temperature control (ATC) and without automatic temperature control (NoATC).

Year	Type of experiment	Rootstocks used in the experiments	Treatments	
2010/2011	Field experiment	110 Richter	Well watered	
			Water deficit	
2011	ATC greenhouse experiment	99 Richter	Well watered	
			Water deficit	
		110 Richter	Well watered	
			Water deficit	
2011/2012	Field experiment	1103 Paulsen	Well watered	leaves full sun exposure
				Leaves in the shade
			Water deficit	leaves full sun exposure
				leaves in the shade
		140 Ruggeri	Well watered	leaves full sun exposure
				leaves in the shade
			Water deficit	leaves full sun exposure
				leaves in the shade
2012	NoATC greenhouse experiment	1103 Paulsen	Well watered	
			Water deficit	
		110 Richter	Well watered	
			Water deficit	
		140 Ruggeri	Well watered	
			Water deficit	
		99 Richter	Water deficit	
		Ramsey	Water deficit	

4.2.3 Potted grapevines: growth conditions and treatments

Two experiments were carried out in two separate greenhouses. See Chapter 3 for details.

4.2.4 Leaf stomatal density and size

Adult leaves were sampled according to the type of experiment (Table 4.2) from primary shoots at nodes 11 for field experiments, at nodes 11 and 12 for greenhouse with automatic temperature control (ATC) and at node 16 for the greenhouse without automatic temperature control (NoATC). The sampling dates were 3rd and 4th March 2011 for field experiment (2010/2011), 7th February 2012 for field experiment (2011/2012), 2nd and 3rd November 2011 for ATC greenhouse and 29th March 2012 for NoATC greenhouse. The water deficit treatments were imposed when adult leaves (100% of the final leaf area) were present from the base until at approximately node 6 and node 8 in ATC and NoATC greenhouses respectively.

Table 4.2 Leaf and stomata sampling

Type of experiment	Number of leaves per treatment and per date	Timing of the sampling	Number of stomata randomly selected for stomatal size
Field (2010/2011)	8	At harvest	96
Field (2011/2012)	5	At harvest	400
ATC greenhouse experiment	4	At the end of the water constraint (two different dates)	320
NoATC greenhouse experiment	3	10 days after water constraint	150

A sample of fresh leaf section of approximate 100 mm^2 between the main leaf vein and the first right lateral vein was taken from each leaf. Prior to imaging, the samples were mounted on a stub with double sided carbon tape. Specimens were coated with a thin gold layer and examined using a scanning electron microscope (SEM) (LEO® 1430VP, LEO Co. LTD.). Beam conditions during surface analysis were 7 KV and approximately 1.5 nA, with a working distance of 13 mm and a spot size of 150. All photomicrographs were taken under the same conditions of magnification. The stomata counting and size were analyzed using ImageJ software (National Institutes of Health, Bethesda, Maryland). The stomatal pore was measured considering the length in micrometres between the junctions of the guard cells at each end of the stoma. To determine the stomatal size, six stomata were randomly selected for each sample for the field experiment (2010/2011) and 10 stomata for the rest of the experiments (Table 4.2).

4.2.5 Leaf water potential

Grapevine ψ_{stem} was determined using a Scholander pressure chamber (Choné et al. 2001). See Chapter 3 for details.

4.26 Air temperature and relative humidity

Air temperature and relative humidity were recorded using a data logger (Gemini Tiny Tag TGP-4500, Gemini Dataloggers SA (PTY) Ltd) placed in a gill screen above the canopy. See Chapter 3 for details.

4.2.7 Light conditions

In order to characterise the leaf growing conditions, in terms of the ratio of red light (660 nm) to far-red light (730 nm) (R:FR), readings were performed for full sun exposure and for leaves growing inside the canopy, under permanent shade, during field experiment season 2011/2012.

The readings were carried out using a point sensor (Skye instruments, Powys, UK). For ATC greenhouse light growing conditions refer to Chapter 3.

4.2.8 Statistical analysis

Data were analysed using analysis of variance (ANOVA) and means were separated by Fisher's least significant difference (LSD) test ($P < 0.05$). All analyses were done with Statistica version 11.0.

4.3 Results

4.3.1 Experimental conditions and plant water status

Temperature and air humidity data are provided as examples of a characteristic day during which the grapevine water status was recorded (December 2011 to February 2012 for field experiment 2011/2012) (Figure 4.1). Under field conditions during season 2011/2012 the maximum temperatures were above 30º C with maximum temperatures higher than 32° C for at least 3 hours for the specific day. For ATC greenhouse experiment, NoATC greenhouse experiment and field experiment 2010/2011 refer to Chapter 3.

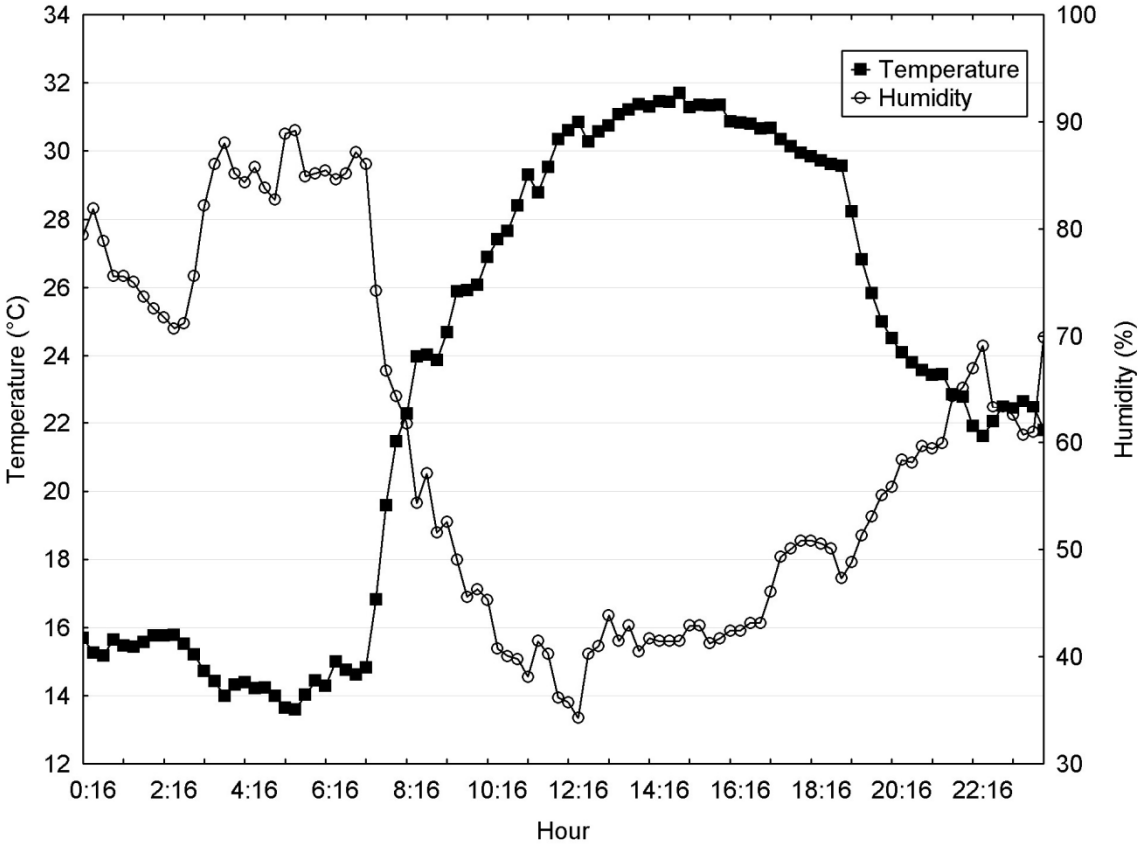

Figure 4.1 Temperature and humidity of field experiment 2011/2012.

The water constraint treatments carried out in the greenhouses induced clear differences in terms of plant water status, in comparison to well-watered treatments (refer to Chapter 3). In the greenhouse with ATC greenhouse experiment, severe water constraint resulting in visible effects such as senescence of older leaves and leaves wilting was observed approximately after 10 days of stopping the irrigation, for the treatments without any irrigation (refer to Chapter 3). The well watered plants presented a constant plant water status during the experiment. A similar situation was observed in the NoATC greenhouse experiment. Under field conditions 2011/2012, and for the water constraint treatments, only moderate water constraints (ψ_{stem}= -0.8 MPa) were obtained, in comparison with well-watered plants (ψ_{stem}= -0.5 MPa) (Figure 4.2). A deep root system and available water from a water table might explain these results. For field experiment 2010/2011 refers to Chapter 3.

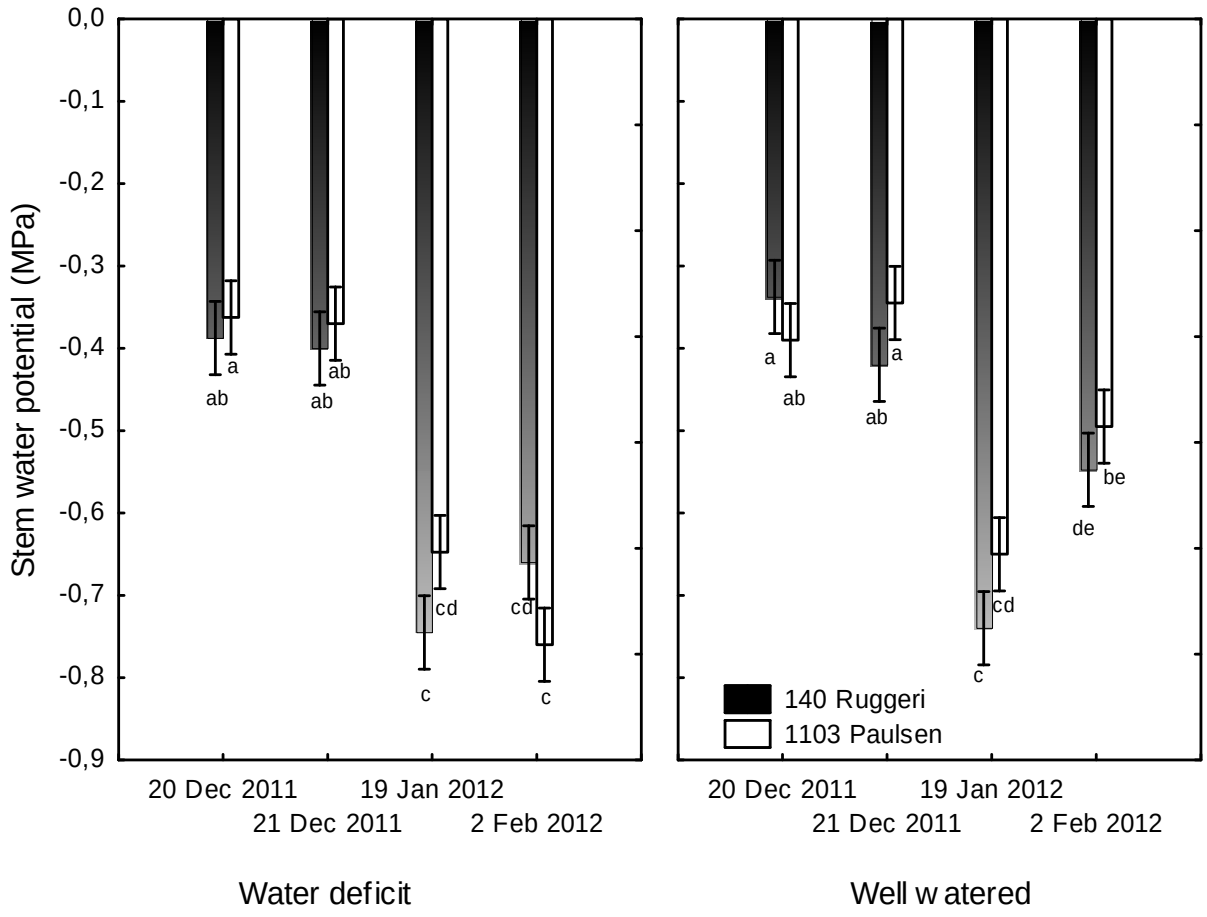

Figure 4.2 Plant water status during the experimentations on stomatal density and size in field experiment 2011/2012. Vertical bars denote +/- standard errors.

Overall, the plant water status of the grapevines in response to an increase in soil water deficit was not different among the different rootstocks (refer to Chapter 3). Nevertheless, under NoATC greenhouse experiment, plants grafted onto 99 Richter and 110 Richter presented a less

negative plant water status under water constraint in comparison with Ramsey (refer to Chapter 3). The differences in terms of stem water potential were found at the beginning and during most part of the soil water deficit evolution. At the end of the water constraint period similar values of plant water status were observed.

As expected, leaves under full sun exposure were exposed to higher R/FR ratio, therefore more likely experiencing higher light intensities, in comparison to leaves growing inside the canopy under permanent shade. Under shaded conditions, vines grafted onto 1103 Paulsen presented differences between well watered and water deficit treatments in terms of the ratio R/RF received. Plants under water deficit were likely exposed to a higher light intensity in comparison with the leaves growing in the plants under well watered conditions probably due to less dense canopies (Figure 4.3). Lack of measurements in photosynthetically active radiation are a clear drawback for this study.

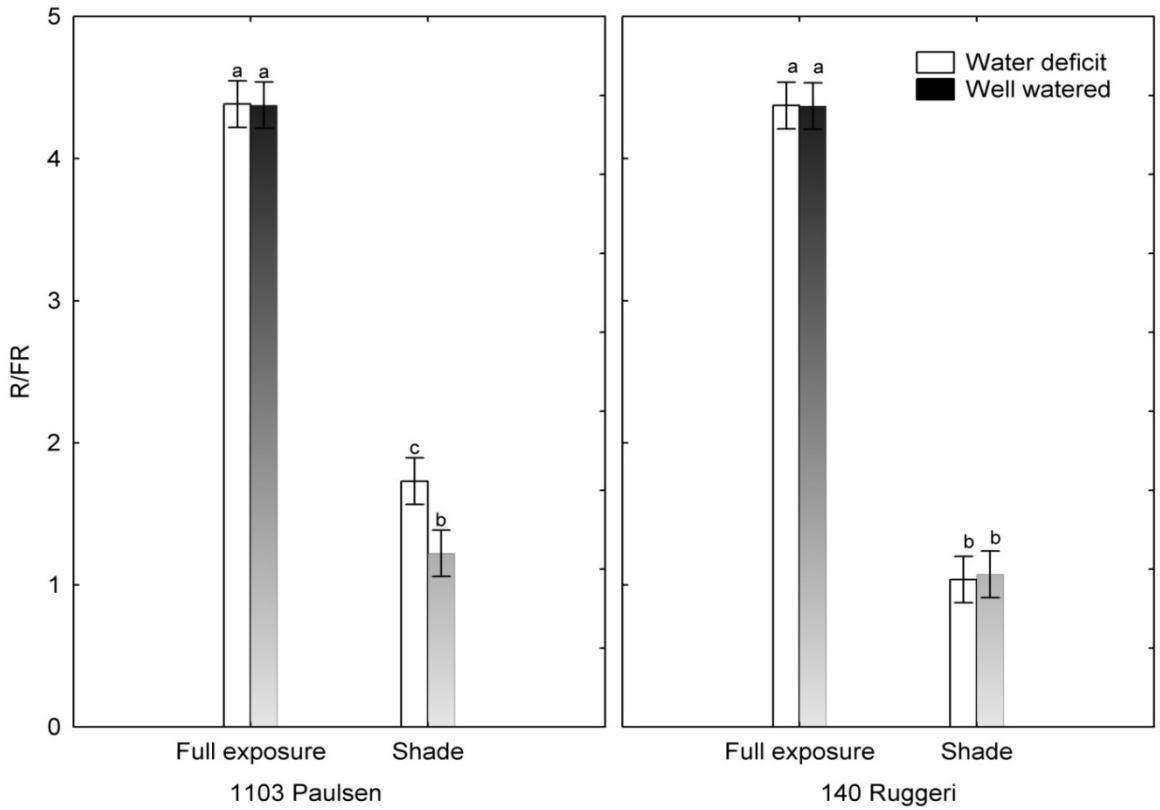

Figure 4.3 Red: Far red ratio. Field experiment 2011/2012. Vertical bars denote +/- standard errors.

4.3.2 Stomatal density and size in response to water deficit

In general stomatal density was not affected by soil water deficit, except for the field experiment where leaves under water constraint treatment presented a higher stomatal density with no changes in stomatal size in comparison to leaves of the well-watered treatment (Figures 4.4, 4.5

and 4.6). The pore diameter was affected by water constraint treatments in most of the experiments that reached severe water constraint (Figure 4.6A and 4.6B). Severe water constraints (a plant water status threshold of around -1.0 to -1.4 (MPa) in stem water potential) induced a reduction in the pore diameter size (Figures 4.6 and 4.7). Under moderate water constraint conditions (a plant water status threshold of around -0.6 to -0.8 (MPa) in stem water potential), which was the case in field experiments, the stomatal size was mostly not affected (Figure 4.6C and 4.6D). The only exception was observed on shaded leaves from vines grafted onto 140 Ruggeri where the water deficit induced a reduction in the stomatal size (Figure 4.6D). A significant interaction between rootstock and irrigation treatments ($p<0.05$) and between irrigation treatments and light exposure ($p<0.05$) were found affecting stomatal size. No significant interaction on stomatal density was found.

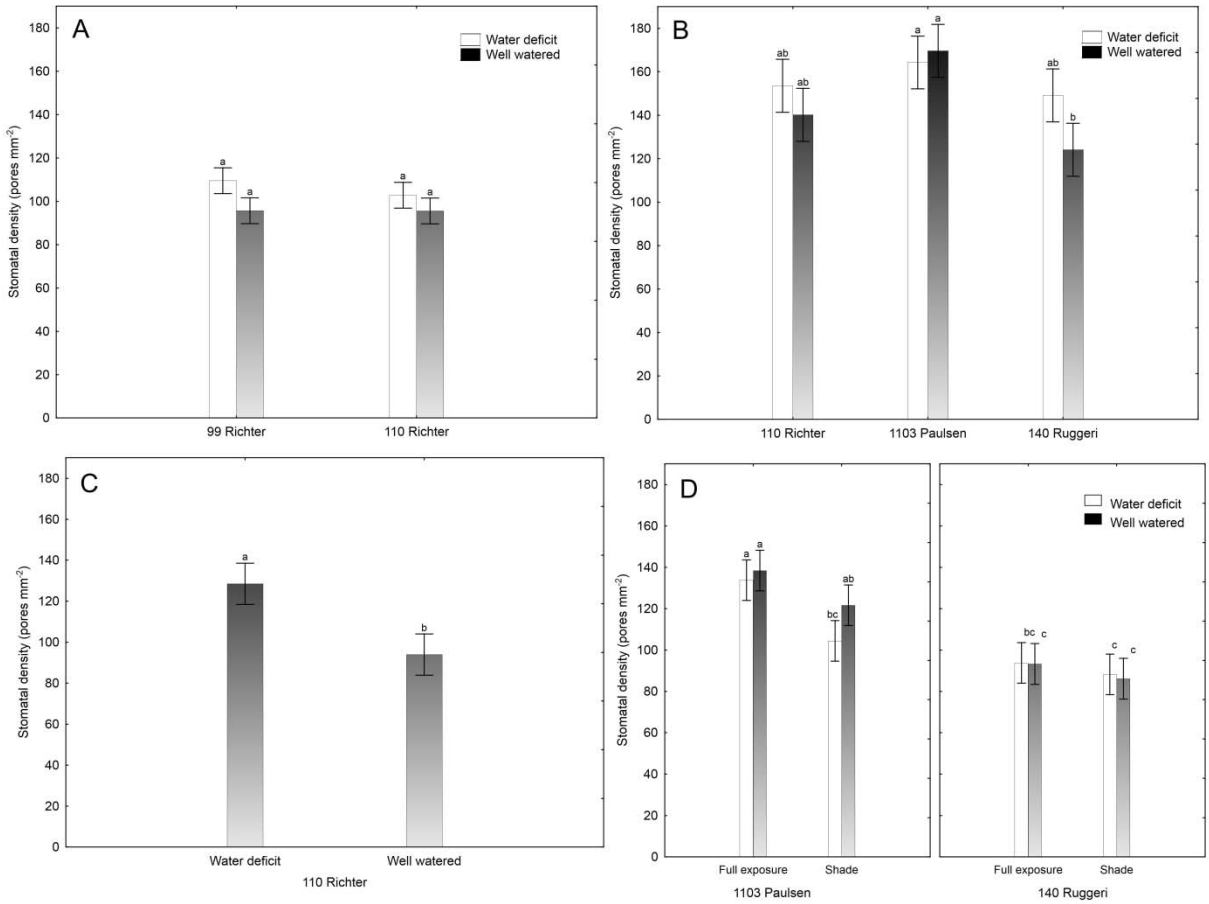

Figure 4.4 Stomatal density. A: ATC greenhouse experiment; B: NoATC greenhouse experiment; C: Field experiment 2010/2011; D: Field experiment 2011/2012. Vertical bars denote +/- standard errors.

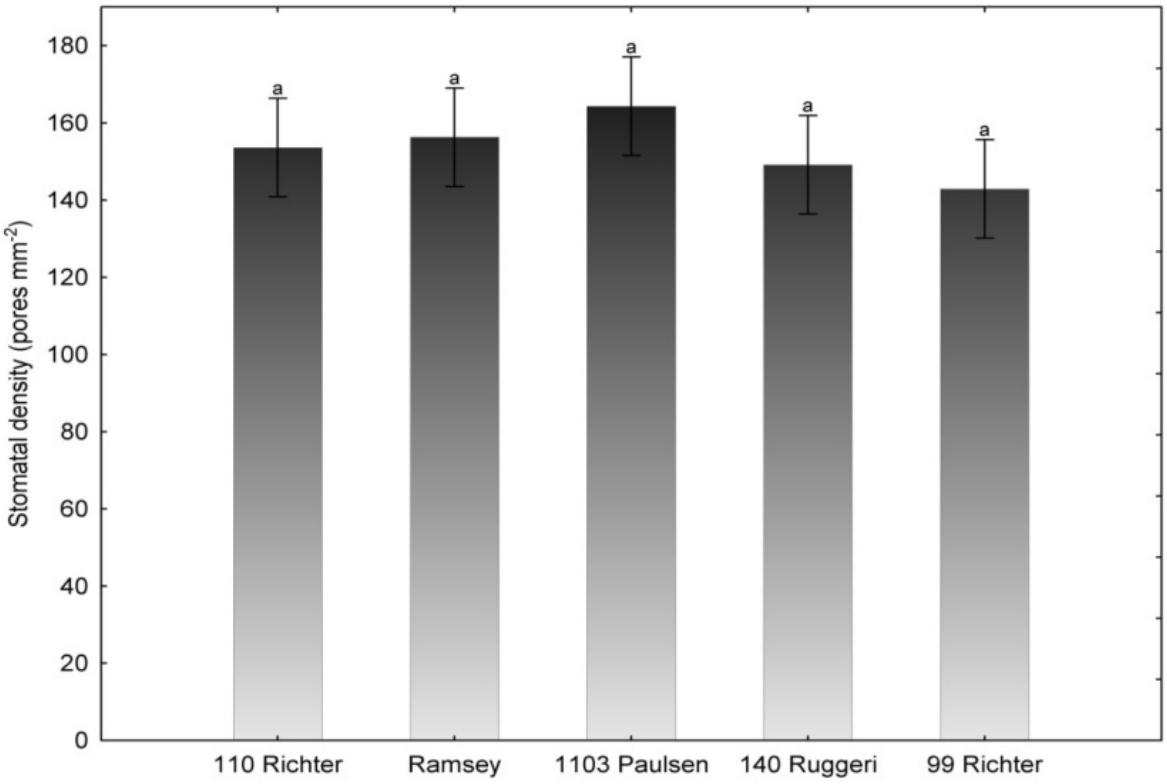

Figure 4.5 Stomatal density. NoATC greenhouse experiment under increasing water deficit. Vertical bars denote +/- standard errors.

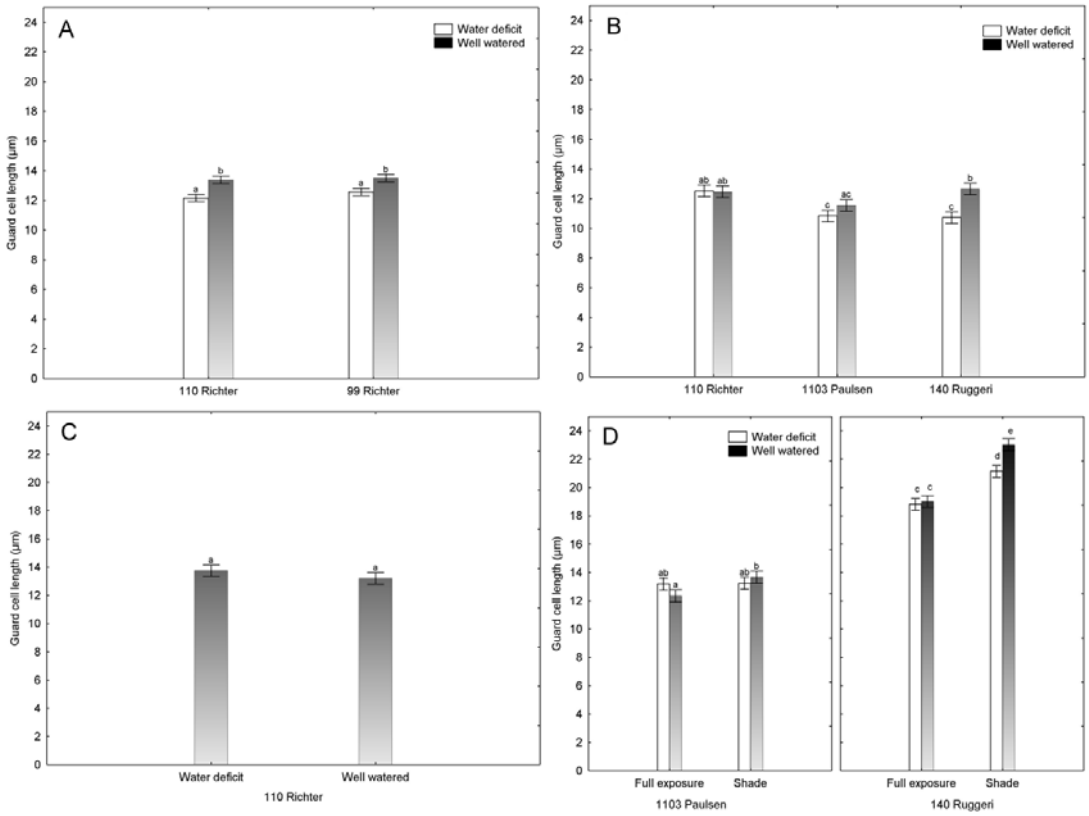

Figure 4.6 Stomatal size. A: ATC greenhouse experiment; B: NoATC greenhouse experiment; C: Field experiment 2010/2011; D: Field experiment 2011/2012. Vertical bars denote +/- standard errors.

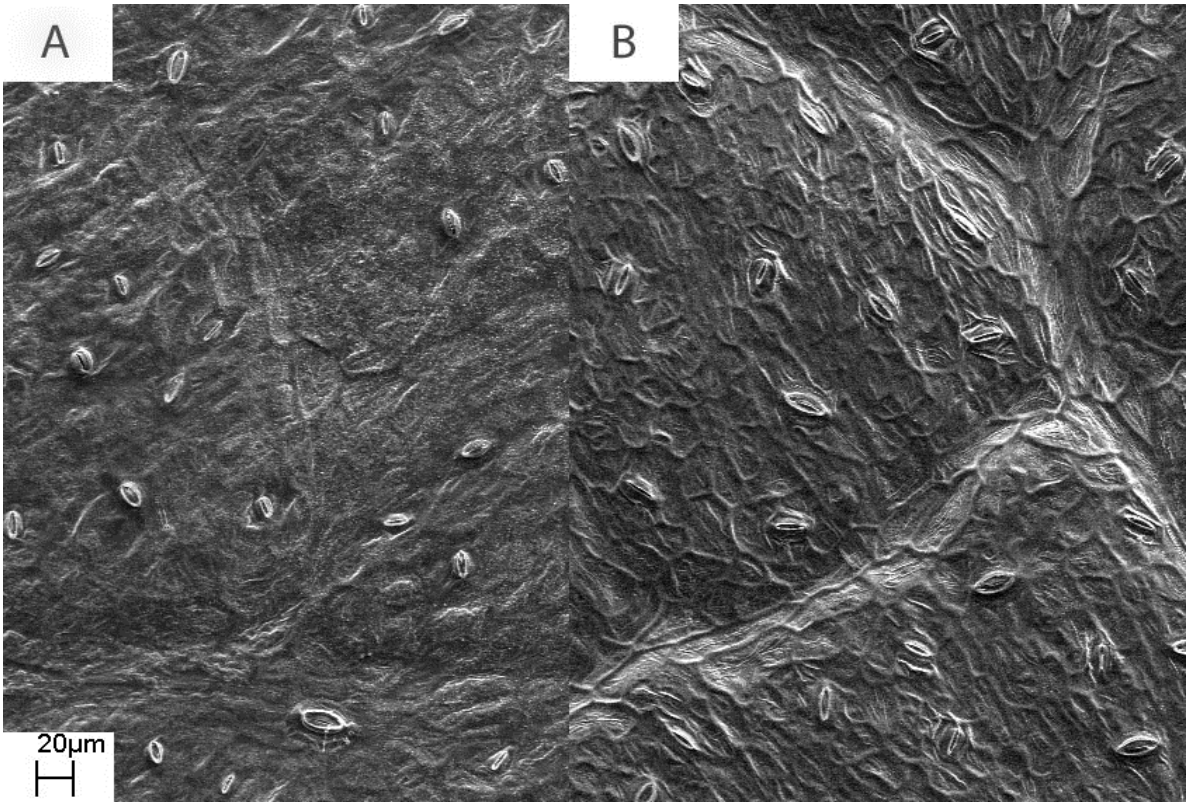

Figure 4.7 Stomata on the abaxial surface of cv. Pinotage grafted onto 1103 Paulsen. A: water constraint. B: no water constraint. The figure clearly shows how the soil water deficit induces a response in the stomatal development that results in a reduction in pore size. Average stomatal density (pores/mm2) of 164.3 ± 13.7 and 169.7 ± 13.7 for water constraints and without water constraints, respectively. Average guard cell length (µm) of 10.8 ± 0.38 and 11.6 ± 0.38 for water constraints and without water constraints, respectively (150 × magnification, panels a and b).

4.3.3 Stomatal density and size in response to light exposure

Leaves growing in an environment with lower R/FR ratios, and most likely being shaded for most of the day, tended to have lower stomatal densities but bigger pore diameters in comparison with leaves growing in full sun exposure (Figures 4.4, 4.6 and 4.8). A significant interaction for stomatal size (but not for stomatal density) between rootstock and light exposure ($p<0.001$) and between irrigation treatments and light exposure ($p<0.05$) were observed.

4.3.4 Stomatal density and size in response to rootstocks

Differences in stomatal density and size were observed on Pinotage leaves grafted onto different rootstocks, where plants grafted onto 140 Ruggeri presented lower stomatal density but bigger pore diameter than plants grafted onto110 Richter and 1103 Paulsen (Figures 4.4C, 4.4D, 4.6C, 4.6D, 4.8 and 4.9). Interestingly, under conditions of increasing water constraint, the rootstocks that tended to experience the lower water constraint (less negative plant water status; stem water

potential values of -0.6 (MPa) for 99 Richter and -0.4 (MPa) for 110 Richter versus -0.9 (MPa) for Ramsey after two days from stopping the irrigation and values of -1.0 (MPa) for 99 Richter and -0.7 (MPa) for 110 Richter versus -1.2 (MPa) for Ramsey after six days from stopping the irrigation) are those that present a higher pore diameter (Figures 4.4 and 4.9). As mentioned before, significant interactions between rootstock and vine water status and between rootstock and light intensity have shown an effect on leaf stomatal size. No significant interaction between rootstock and grapevine water status and between rootstock and light intensity was found for stomatal density. Rogiers et al. (2009) found that transpiration rate seems dependant on stomatal size rather than density. These results clearly showed the potential importance of rootstock on the stomatal development mainly affecting stomatal size.

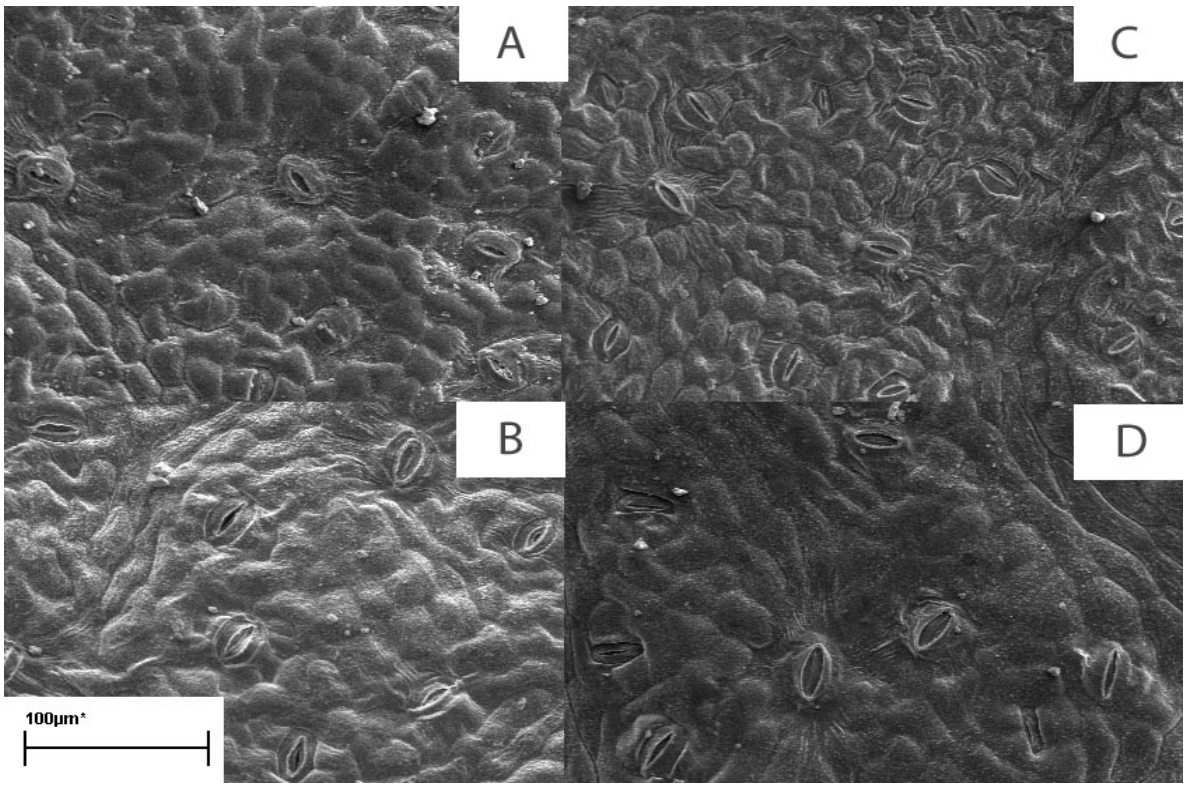

Figure 4.8 Stomata on the abaxial surface of cv. Pinotage grafted onto 1103 Paulsen and 140 Ruggeri. A: 1103 Paulsen, interaction water constraint and full exposure. B: 1103 Paulsen, interaction water constraint and shade. C: 140 Ruggeri, interaction water constraint and full exposure. D: 140 Ruggeri, interaction water constraint and shade (150 × magnification, panels a, b, c and d). The figures clearly show that leaves growing in an environment with a lower R/FR ratio had a lower stomatal density but bigger pore diameter. Differences in stomatal density and size were observed on Pinotage leaves grafted onto different rootstocks, where plants grafted onto 140 Ruggeri presented lower stomatal density but bigger pore diameter than plants grafted onto 1103 Paulsen.

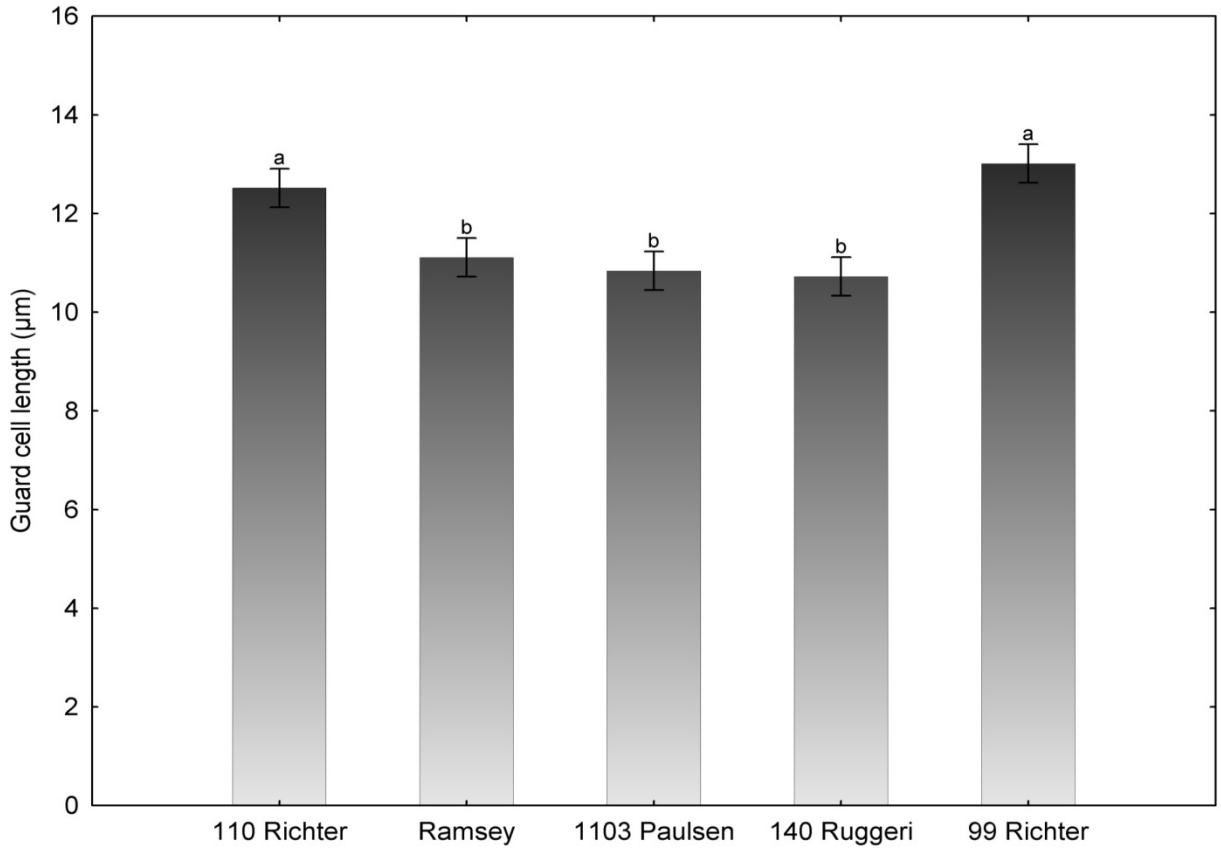

Figure 4.9 Stomatal size. NoATC greenhouse experiment under increasing soil water deficit. Vertical bars denote +/- standard errors.

As a summary, Table 4.3 shows the most relevant data found in the experiments.

Table 4.3 Effect of rootstock, water status and light exposure on stomatal size and density.

Treatments	Stomatal size (µm)	Stomatal density (pores mm^{-2})
FIELD 2010/2011		
Rootstock		
110 Richter	13.5	111.2
Water status		
No water constraint (ψ_{stem}= -0.5 MPa)	13.2 a	94.0 b
Moderate water constraint (ψ_{stem}= -0.8 MPa)	13.8 a	128.5 a
ATC GREENHOUSE		
Rootstocks		
99 Richter	13.0 a	102.6 a
110 Richter	12.8 a	99.2 a
Water status		
No water constraint (ψ_{stem}= -0.4 MPa)	13.4 a	95.6 a
Severe water constraint (ψ_{stem}= -1.4 MPa)	12.4 b	106.1 a
Interaction[1]		
Rootstock X water status	ns	ns
NoATC GREENHOUSE		
110 Richter	12.5 ab	146.9 ab
140 Ruggeri	11.7 b	136.6 ab
1103 Paulsen	11.2 ac	167.0 a
Water status		
No water constraint (ψ_{stem}= -0.51MPa)	12.2 ab	144.7 ab
Water constraint (ψ_{stem}= -0.91 MPa)	11.4 ab	155.7 ab
Interaction[1]		
Rootstock X water status	*	ns
Under increasing water deficit		
99 Richter	13.0 a	142.9 a
110 Richter	12.5 a	153.6 a
140 Ruggeri	10.7 b	149.1 a
1103 Paulsen	10.8 b	164.3 a
Ramsey	11.1 b	156.3 a
FIELD 2011/2012		
Rootstocks		
1103 Paulsen	13.1 b	124.6 a
140 Ruggeri	20.5 a	90.4 b
Water status		
No water constraint (ψ_{stem}= -0.5 MPa)	17.0 a	109.9 a
Moderate water constraint (ψ_{stem}= -0.8 MPa)	16.6 a	105.1 a
Light exposure		
Full sun exposure	15.8 b	114.8 a
Shade	17.8 a	100.1 b
Interaction[1]		
Rootstock X water status	*	ns
Rootstock X light exposure	***	ns
Water status X light exposure	*	ns
Rootstock X water status X light exposure	ns	ns

[1]ns, *, **, ***, not significant and significant at $P \leq 0.05$, 0.01, and 0.001, respectively. Numbers with different letters differ significantly at the 0.05 level by Fisher's significant difference.

4.4 Discussion

In order to overcome abiotic constraints, the plants have developed complex strategies to adapt to the changing environment. Drought induced a reduction of the photosynthesis via processes non related to stomatal limitations (biochemical limitations such as Rubisco impairment and decreased rate of electron transport) or related to stomatal limitations (stomatal closure causing diffusional limitations such as reduced CO2 availability) (Cifre et al. 2005, Lovisolo et al. 2010). Stomata play a key role in the exchange of water and carbon dioxide between the leaf and the atmosphere. The regulation of the stomatal development by modifications in stomatal density, distribution and size and stomatal conductance by opening and closing of the pores, presents a relevant way for the plant to adapt to soil water deficit by trade-offs between water losses and carbon gains. The stomatal responses to drought can take from minutes in the case of stomatal conductance, to several days in the case of changes in stomatal density, distribution and size during leaf formation.

Several publications have shown contrasting results of stomatal density in response to water deficit. Some species such as *Ricinus communis* presented an increase in stomatal density (Heckenberger et al. 1998), no differences were found in deciduous trees (Aasamaa et al. 2001) and, in grass, an increase of stomatal density were observed with moderate water deficit and a reduction of stomatal density under severe water constraint (Xu and Zhou 2008). It is probable that these apparent contradictions are related to different water constraint conditions from one situation to another and different plant species responses to drought at the genetic, epigenetic and physiological levels. In general our results showed no effect of plant water status on stomatal density. Nevertheless, an increase in stomatal density was found under moderate water constraint (Figures 4.4 and 4.5). Recently, it was found in *Arabidopsis* that the transcription factor GTL1, a molecule that regulate the gene expression of SDD1 (STOMATAL DENSITY AND DISTRIBUTION1), was down regulated under water constraint (Yoo et al. 2010). The study showed that in the case of *Arabidopsis*, drought tolerance was regulated by modulating the stomatal density. In a similar way, another study in poplar shows that GTL1 is down regulated by water deficits. The study showed that lower GTL1 activity leads to an increase in stomatal density through reduced trans-repression of SDD1 (Weng et al. 2012).

Factors that control stomatal density also affect stomatal size (Doheny-Adams et al. 2012) which can explain the close relationship between stomatal density and size which is general for all type of plants (Hetherington and Woodward 2003). The present study showed that the influence of water deficit on stomatal size depended on the intensity of the grapevine water constraint, where a moderate water constraint has almost no effect (Figure 4.6C and 4.6D); a

severe water constraint induced a reduction in the stomatal size (Figures 4.6A, 4.6B and 4.7). Similar results were found in grass where a positive correlation between stomatal size and plant water status was found (Xu and Zhou 2008). In deciduous trees, stomatal conductance correlated positively with the length of the stomatal pore. It was determined that the length of the stomatal pore is the anatomical characteristic that plays the most important role in the determination of the variability of the stomatal conductance in deciduous trees (Aasamaa et al. 2001). In grass, the changes in stomatal density and size due to water constraint were closely related to changes in leaf gas exchange and water use efficiency, implying a positive balance between carbon and water exchange. Higher stomatal conductance under water constraint occurs simultaneously with higher stomatal density and smaller guard cells sizes (Xu and Zhou 2008).

The development of a leaf in specific microclimatic conditions will induce structural and biochemical changes in stomatal density and size. Leaves which develop in shaded conditions are thinner, have smaller palisade parenchyma cells and have fewer cells in the spongy parenchyma (Iland et al. 2011). Our data suggested that modifications in the stomatal development occured as well. Leaves growing in shaded conditions tended to have lower stomatal density but bigger pore diameter, in comparison with leaves growing in full sun exposure (Figures 4.4, 4.6 and 4.8). The data are consistent with results found in cv. Cabernet Franc and Trebbiano Toscano (Palliotti et al. 2000) and in *Arabidopsis* where stomatal density increased with the increase of light supply during leaf growth (Schlüter et al. 2003). A higher light intensity enhanced the number of stomata (Casson and Hetherington 2010, Pillitteri and Torii 2012). Studies in *Arabidopsis* allowed determining that the stomatal development influenced by light quality and intensity was mediated by cumulative function of the genes CRYPTOCHROME (CRY1 and CRY2) and PHYTOCHROME (PHYB and PHYA) which are blue-light photoreceptor and red/far-red photoreceptor respectively (Casson et al. 2009, Casson and Hetherington 2010, Kang et al. 2009, Pillitteri and Torii 2012).

Interestingly, stomatal size was affected by a significant interaction between water constraint and light, suggesting a possible connection in the pathways that regulate stomatal development under water constraint and shaded conditions. No interaction between water constraint and light was found affecting stomatal density. A study in *Arabidopsis* proved that SDD1 was not required for the light response (Schlüter et al. 2003), nevertheless the SDD1 expression was affected in developing leaves of shaded plants (Coupe et al. 2006) which was associated with a reduction of the stomatal density, suggesting and alternative pathway for stomatal development in terms of density (Pillitteri and Torii 2012). Under the experimental conditions of this study, stomatal size was more sensitive to light intensity and water constraint than stomatal density. It has been observed that for genetically manipulated *Arabidopsis*, the

maximal stomatal conductance was adjusted by reduction in stomatal size, rather than density, under restriction of water availability (Rogiers et al. 2009, Doheny-Adams et al. 2012).

In the present study, the influence of rootstocks in the stomatal development, specifically stomatal size, is suggested (Figures 4.8 and 4.9). Similar results were found by Scienza and Boselli (1981) who showed that rootstocks considered drought tolerant have lower stomatal density in their leaves in comparison with rootstocks considered drought sensitive. In cherry citrus and peach, rootstocks that have larger xylem vessels tend to induce more vigour (Rodríguez-Gamir et al. 2010, Tombesi et al. 2010, Zorić et al. 2012) which can affect leaf growth and stomatal development therefore. It is hypothesised that the possible mechanism involved is related to the effect of rootstock on plant water content (Figures 4.4 and 4.9). This is in agreement with the work of Marguerit et al. (2012) which suggested a genetic control of the vine stomatal functioning under the control of the rootstock.

4.5 Conclusions

In summary, we have demonstrated that stomatal development is affected by light, drought and possibly by rootstocks. Further research is still needed to elucidate the ways in which rootstocks can affect the stomatal size and conductance and therefore potentially the photosynthetic capacity in response to drought. The results suggested that the rootstock is regulating the cultivar's stomatal size (anatomical changes during leaf growth). In addition, rootstocks are able to regulate stomatal functioning through a complex signalling process. This regulation occurred up to a point where the plant water status, linked to the level of drought, seemed to become the main driver of stomatal conductance and consequently photosynthesis (Lovisolo et al. 2010). Thus one possible mechanism of Pinotage leaf adaptation to water constraints was structural during leaf growth, with a reduction in pore size to reduce plant water loss. The effect of light is interesting in the context of canopy microclimate and canopy manipulation. Under the context of climate change (increase in drought and water scarcity) the choice of the vine architecture become more relevant. The interaction/signalling of the combination rootstock-cultivar is important for the understanding of grapevine adaptation to sites (soil x climate) where drought and water scarcity will be the main concerns.

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Chapter 5

General discussion and perspectives

Chapter 5: General conclusions and perspectives

5.1 General conclusions and perspectives

The interaction between rootstock and scion cultivar in response to water deficit is quite complex considering the effects on canopy size, yield, plant water status and leaf gas exchange due to rootstock vigour inducing capacity, root-to-shoot signalling, rootstock absorption capacity of water and minerals and compatibility between rootstock/scion. This thesis focused whether rootstocks can influence scion cultivar leaf water status and gas exchange and influence stomatal development.

Regarding the effect on plant water status, under field conditions, differences between rootstocks were found; where Pinotage grafted onto 110 Richter showed on average less negative values of ψ_{stem} than vines grafted onto 140 Ruggeri and 1103 Paulsen. Under greenhouse conditions, Pinotage grafted onto 99 Richer and 110 Richter had less negative ψ_{stem} than vines grafted onto Ramsey.

Concerning the effect on leaf gas exchange and under greenhouse conditions, the differences were less clear where under water deficit conditions 110 Richter and 99 Richter seemed to have higher A_N and significantly higher g_s in comparison with Ramsey. These differences between 99 Richter and Ramsey were only apparent during well-watered conditions, while the differences in A_N between 110 Richter and Ramsey lasted for a few days during the increased water constraint. These findings confirm the influence of rootstocks on scion cultivar water status and leaf gas exchange, suggesting an influence on water uptake and transport and a tight regulation of the stomatal conductance. It is important to consider that rootstock influence on scion response to drought occurred from the first stages of water constraint up to a point where plant water status became the main driver of the photosynthesis and stomatal conductance, irrespective of the rootstock/scion cultivar combinations. Field experimentation presented clear limitations for this type of study due to inherent heterogeneity at different levels and in our case to the difficulty to generate a strong vine water deficit.

In addition, this study suggested that stomatal development is affected by light, drought and possibly by rootstock cultivar. As already demonstrated in other plant species and grapevine, leaves under shaded condition tended to have lower stomatal density but bigger pore diameter in comparison with leaves growing in full sun exposure. It should be mentioned that the lack of measurements in photosynthetically active radiation are a clear drawback. Severe water stress induced a reduction in pore size under greenhouse conditions. Differences in stomatal density

and size were observed on Pinotage leaves grafted onto different rootstocks, where plants grafted onto 140 Ruggeri presented lower stomatal density but bigger pore diameter than plants grafted onto110 Richter and 1103 Paulsen. A previous study showed that rootstocks considered drought tolerant have lower stomatal density in their leaves in comparison with rootstocks considered drought sensitive (Scienza and Boselli 1981). Nevertheless, it is still not clear how the rootstock affects stomatal development and the possible influence on scion water use. A previous study has showed that the transpiration rate of leaves is more related to stomatal size than density (Rogiers et al 2009). Thus this study suggested that one possible mechanism of Pinotage leaf adaptation to water constraints is structural during leaf growth, with a reduction in pore size to reduce plant water loss. The results confirmed that the rootstock is regulating the cultivar's stomatal size (anatomical changes during leaf growth) and functioning (stomatal regulation) through a complex signalling process. The effect of light on stomatal development is interesting in the context of canopy density and leaf microclimate, including canopy manipulation. The choice of the vine architecture vs training and trellising systems and canopy manipulations which will impact on canopy size and the choice of yield per vine are important cultural practice decisions in the context of climate change versus increase in drought and water scarcity.

The use of rootstocks is a long term investment which aimed to provide resistance to soil pest and pathogens and to confer drought and salt tolerance to the scion cultivar. The results of this study improved the understanding on the interaction of rootstock/scion cultivar to water deficit, particularly in relation to stomata responses, but further studies are required to understand the signalling processes between the rootstock and the scion (root-shoot interaction). Such results could be relevant to the choice of appropriate rootstocks tolerant to drought in a context of water scarcity, providing a sustainable solution to the wine industry using grafted varieties. This type of research could assist the selection of new rootstocks adapted to dry viticulture regions.

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Addendum

Research note

Preliminary results on the responses of Pinotage (*Vitis vinifera* L.) fruit growth and composition affected by abiotic factors (light and temperature)

Research note. Preliminary results on the responses of Pinotage (*Vitis vinifera* L.) fruit growth and composition affected by abiotic factors (light and temperature)

Introduction

Several factors, including grapevine cultivar and clones, rootstocks, climate (meso and microclimate) and crop load could influence berry growth and composition and the ripening process. Plant water status is one of the main abiotic factor affecting berry growth and composition (Ojeda et al. 2001, Chaves et al. 2010, Lovisolo et al. 2010) and is an important factor impacting on source-sink relationships, indirectly regulating stomatal conductance and photosynthesis. Furthermore, rootstocks are known to alter vigour or biomass allocation affecting leaf and shoot growth and root/shoot biomass partitioning (Jones 2012, Cookson and Ollat 2013) producing changes in source sink relationships (Miller et al. 1996). Leaf and/or lateral removal can modify the source-sink ratio and consequently affect berry growth and composition (Keller 2010). These cultural practices can influence the leaf age pattern (percentage of young leaves versus mature leaves) at the primary shoots (Vasconcelos and Castagnoli 2000) that can affect leaf photosynthetic capacity (Candolfi-Vasconcelos et al. 1994). Modifications of exposed leaf area to yield ratio could affect the tempo of berry ripening, and modify fruit and wine composition and wine sensorial properties in association with changing the fruit zone microclimate (Šuklje et al. 2013). Canopy manipulation practices in the grapevine fruit zone will modify the microclimate, which will alter the fruit zone aeration, relative humidity, temperature and light conditions (quantity and quality) which can change (improve or alter berry growth and composition, according to the site, the row orientation and the cultivar (Smart 1985, Reynolds et al. 1986, Bergqvist et el. 2001) and which can reduce disease pressure (English et al. 1989, Gubler et al. 1987). Leaf removal can have various effects: i) reduction of titratable acidity, mainly due to higher berry temperature; ii) a decrease in the concentration of malic acid (Smart and Robinson 1991); iii) an increase in the total anthocyanin content (Price et al. 1995) and iv) a reduction in methoxypyrazines concentration due to an increase in fruit light exposure and/or temperature (Šuklje et al. 2012). The presence of laterals shoots can protect the bunches, accelerate fruit maturation and improve berry colour (Hirano et al. 1994, Vasconcelos and Castagnoli 2000).

Pinotage (Pinot Noir x Cinsaut) is cultivated almost exclusively in South Africa and was bred by A.I. Perold in 1925 (Burger et al. 2009). Currently it is the fourth most planted red

cultivar in this country (SAWIS 2010). Regardless of the research done on this cultivar, some questions are still pending regarding to the effect of abiotic factors on the fruit growth and composition. The aim of this work was to study the influence of abiotic factors (temperature and light), modifying the fruit zone microclimate, monitoring the vine water status, on the evolution of berry growth and composition during ripening on cv. Pinotage (*Vitis vinifera* L.). In terms of berry composition, only some of the classical parameters used by the wine industry to take a decision on fruit quality and harvest date were considered.

Materials and Methods

Plant material, growth conditions and treatments

The field trial was carried out at the Welgevallen experimental vineyard (Stellenbosch University) during 2009/2010 season. This locality is at 33°56'S, 18°52'E at an altitude of 157 m. Adult Pinotage (*Vitis vinifera* L.) (clone 48A) grapevine plants grafted onto 110 Richter (clone RQ28B) rootstocks were used in North-South orientated rows. Grapevines were trained as a vertical shoot positioning training system and spur pruned with two buds/shoots per spur, five spurs per vine and using a single cordon. Grapevines were only rain-fed and exposed to moderate water constraints. Four levels of canopy manipulation were studied: Control (leaves and lateral shoots undisturbed), FLatR (full lateral shoot removal), LeafR-east (leaf removal only in the bunch zone on the east side of the canopy) and LeafR-west (leaf removal only in the bunch zone on the west side of the canopy) (Figures 1 and 2). The treatments were applied on the 18th December 2009, five weeks after full bloom (around berry pea size). After that, laterals were removed weekly as they grew.

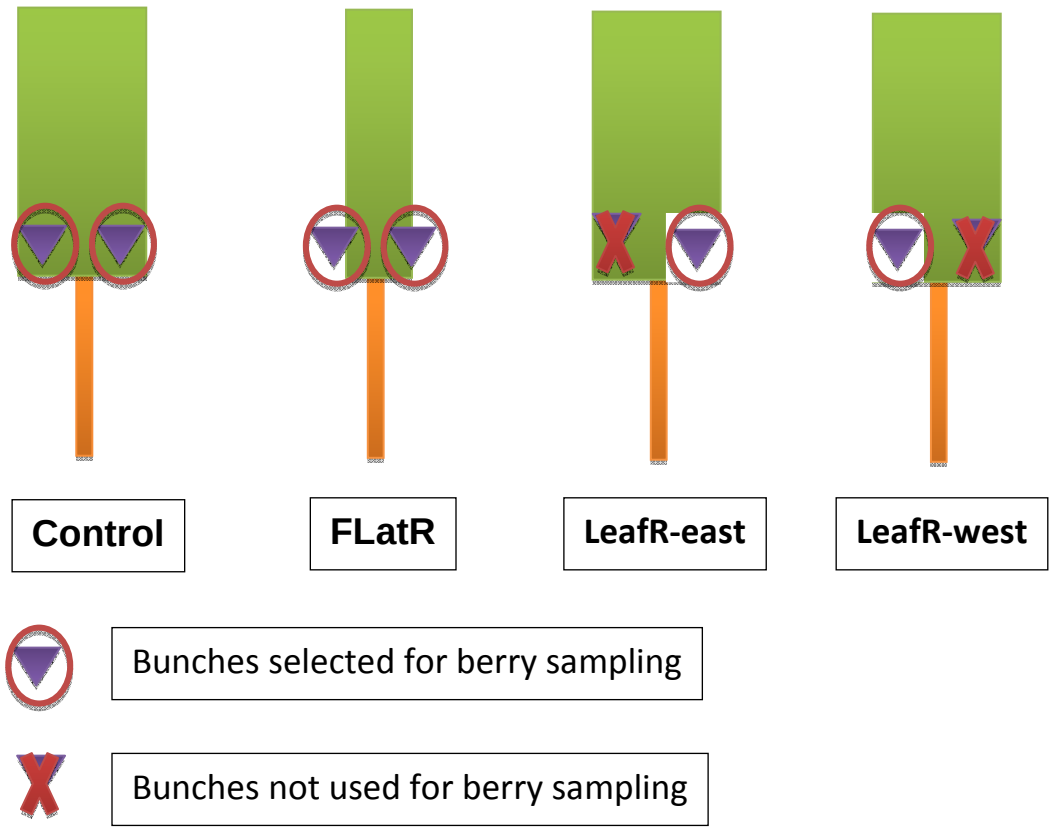

Figure 1 Representation of the canopy manipulation treatments and bunches selected for berry sampling.

Figure 2 Canopy manipulation treatments. A: FLatR (full lateral shoot removal); B: Control (leaf and lateral shoots undisturbed).

Air temperature, relative humidity and berry temperature

Air temperature and relative humidity were recorded continuously in a gill screen positioned above the canopy in the experimental block, using one data logger (Gemini Tiny Tag TGP-4510, Gemini Dataloggers SA (PTY) Ltd) from 18th December 2009 to 17th March 2010 (Figure 3). Temperature measurements were recorded every 15 minutes. At the berry level, two data loggers (TK-4023, Chichester, UK) were used, one located inside the control canopy and another located alternative in the leaf removal treatment on the east side of the canopy (from 23th to 26th January 2010 and then again from 5th February to 15th February 2010 and from 1st to 10th March 2010) and in the leaf removal treatment on the west side of the canopy (from 19th to 23th January 2010 and then again from 26th January to 5th February 2010 and from 15th February to 1st March 2010). For berry temperature measurements, the data logger had a probe which was introduced into the fruit (therefore in contact with the flesh) for about 5 days and then introduced into another berry in order to avoid berry shrivelling. Temperature measurements were recorded every 15 minutes.

Figure 3 Temperature and humidity measurements at the mesoclimatic level.

Leaf area measurements

Twelve shoots per treatment were sampled on 13th January 2010 (at veraison). Measurements included the following: primary and secondary shoot length (cm), number of primary and secondary leaves per shoot, and leaf area (cm^2). Leaf area was measured using a leaf area meter (Delta-T devices Ltd, Cambridge, UK). Equations based on field grapevines were established for the correlation between 'Pinotage' leaf area and leaf main vein length, viz. $y = -34.2857+13.163*x$ ($r^2 = 0.94$) for primary shoots and $y = -36.4218+12.8845*x$ ($r^2 = 0.84$) for secondary shoots, where "y" is leaf area (cm^2) and "x" is the leaf main vein's length (cm). Total leaf area per grapevine was calculated by multiplying the total primary and secondary shoot leaf area by the number of primary and secondary shoots per vine.

Leaf water potential

Grapevine predawn leaf water potential ($\psi_{predawn}$) and stem water potential (ψ_{stem}) were determined using a Scholander pressure chamber on mature leaves on primary shoots. $\psi_{predawn}$ was measured at the end of the night according to Choné et al. (2001). ψ_{stem} was measured at

around midday according to Deloire and Heyns (2011). Values of $\psi_{predawn}$ and ψ_{stem} are the mean of six measurements collected on six grapevines.

Berry measurements

From véraison onwards, two sets of 150-berries were sampled at random in the targeted bunches by cutting the pedicel and were collected from each treatment every week. The first set of berry samples was used to determine berry fresh and dry masses and the concentration of total soluble solids (°Brix), using a digital temperature compensating refractometer (Atago PAL-1, Tokyo, Japan), in order to calculate the amount of sugar per berry over time (Deloire, 2011, 2013). For the dry mass, berries were dried at 60ºC for 9 days in average until constant weight. The second set of berry samples was used to measure the pH and titratable acidity (TA) using a 785 DMP Metrohm Titrino automatic titration instrument. Approximately 10 berries were sampled per vine per date. Six sampling dates were performed.

Experimental layout and statistical analysis

The four canopy management treatments were applied in a randomized block design. Six blocks with six vines for each canopy management treatments were used. All measurements were taken on four vines, having one buffer vine at each side. Data were analysed using a repeated measures analysis of variance (ANOVA) and means were separated by Fisher's least significant difference (LSD) test ($P < 0.05$). All analyses were done with Statistica version 11.0.

Results

Experimental conditions and leaf area

A representative ripening day is shown (Figure 4). Maximum temperatures were above 30º C and the relative humidity under 15% for at least 6 hours. The minimum temperature was registered around 6 a.m. and the highest temperatures from around 12 to 6 p.m. which corresponded with the lowest humidity values.

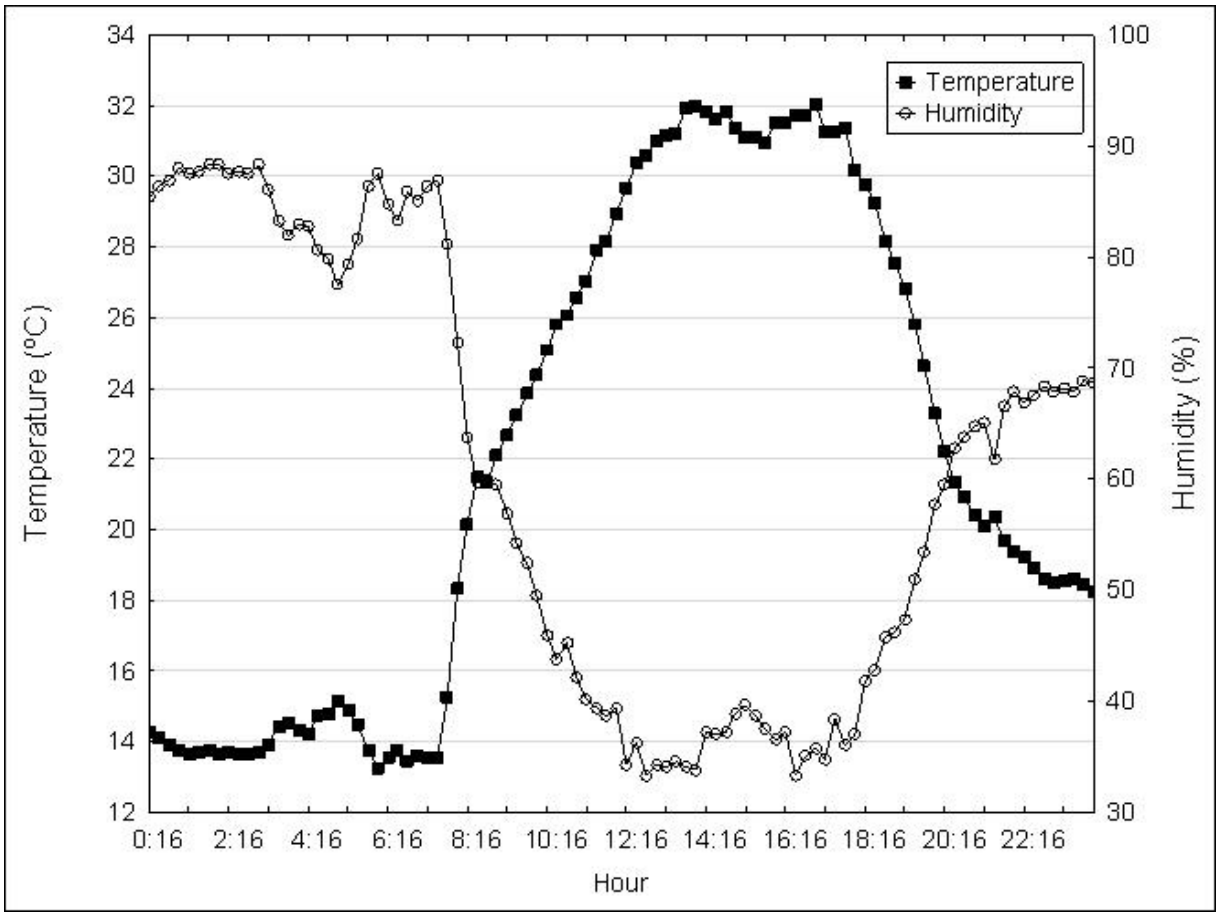

Figure 4 Mesoclimatic data: mean hour temperature (ºC) and relative humidity (%) over the berry ripening period.

A drawback of this study is that temperature measurements were done on different days for leaf removal east side versus west side, therefore the analysis focused on the comparison between the leaf removal treatments and the control. It is interesting to observe that the difference between control and LeafR-west treatments, for the considered period, is not relevant for day and night (Figures 5 and 6). The same percentage of daily temperatures (from 19 to 23 January 2010) above 35ºC was found in treatments leaf removal west side and control (20% of the day temperatures above 35ºC in the treatment leaf removal west side versus 20% in the control). As expected, the LeafR-east showed differences in temperature at the fruit level during day (Figure 7). Differences in the percentage of daily temperature above 35ºC were found in treatment leaf removal east side versus control (40% of the day temperatures above 35ºC in the treatment leaf removal east side versus 20% in the control), with no differences during the night in comparison with control (Figure 8).

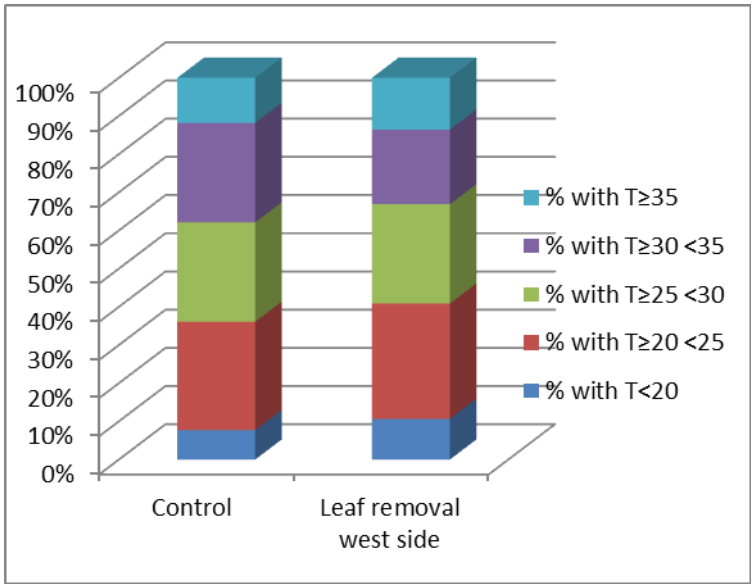

Figure 5 Example of day berry temperature data, from 19 to 23 January 2010. The temperature thresholds have been chosen according to physiological plant functioning temperature thresholds, for control and West side leaf removal treatments. The average is calculated from 08:00 to 18:00 hours.

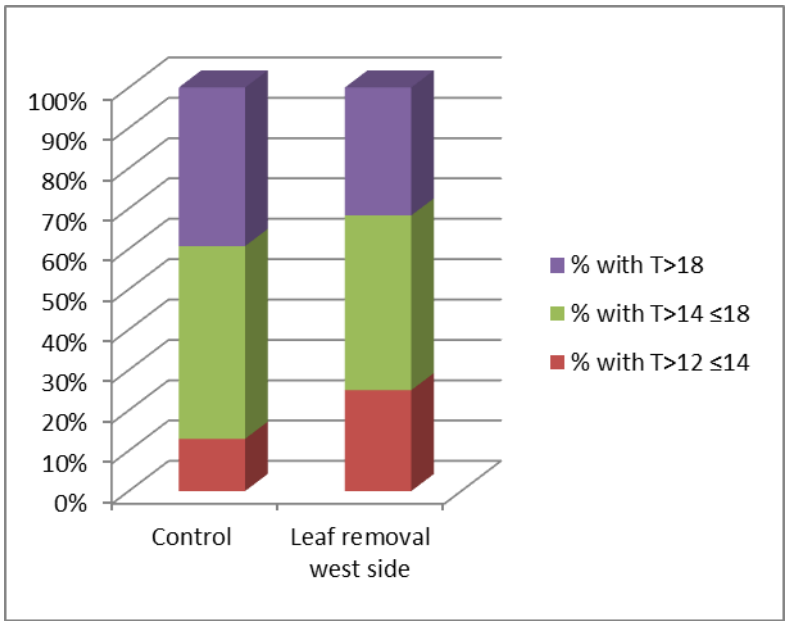

Figure 6 Example of night berry temperature data from 19 to 23 January 2010. The temperature thresholds have been chosen according to physiological plant functioning temperature thresholds, for control and West side leaf removal treatments. The average is calculated from 18:00 to 07:59 hours.

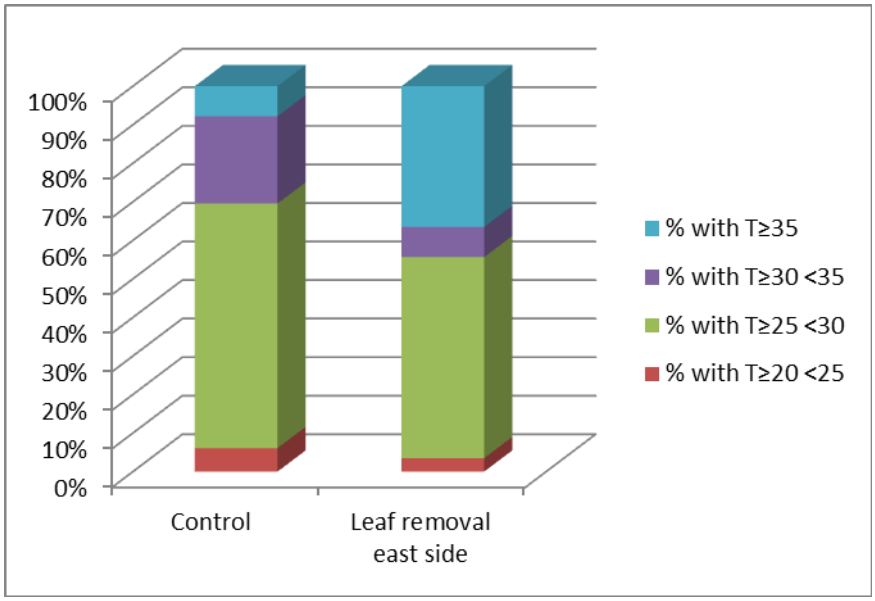

Figure 7 Example of day berry temperature data, from 23 to 26 January 2010. The temperature thresholds have been chosen according to physiological plant functioning temperature thresholds for control and East side leaf removal treatments. The average is calculated from 08:00 to 18:00 hours.

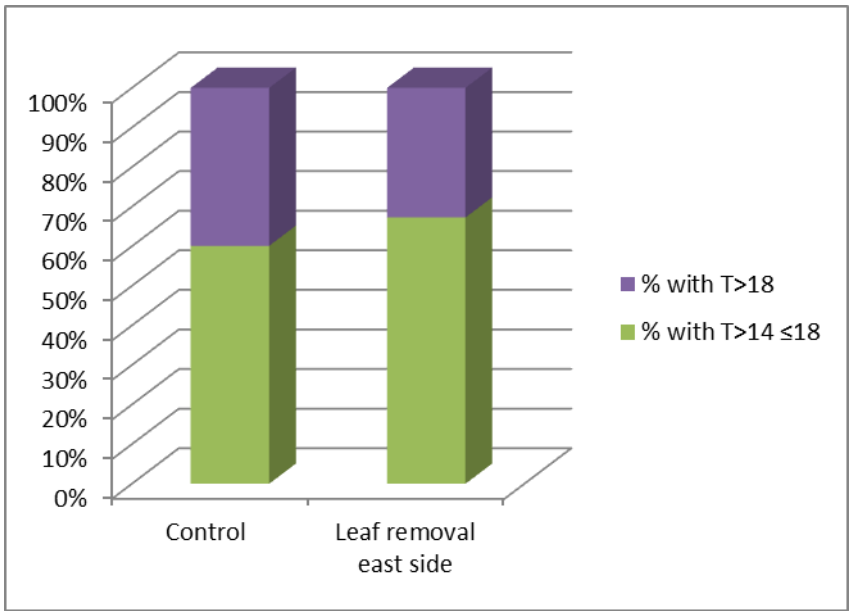

Figure 8 Example of night berry temperature data from 23 to 26 January 2010. The temperature thresholds have been chosen according to physiological plant functioning temperature thresholds for control and East side leaf removal treatments. The average is from 18:00 to 07:59 hours.

Total leaf area per grapevine varied from 1.3 to 3.4 m^2 (Table 1). The differences in total leaf area were due exclusively to the difference in lateral shoot leaf area, where full lateral removal produced a significant reduction of the total leaf area in comparison with leaf removal treatments (reduction of 61.8% and 50% in comparison with leaf removal in the east side and west side respectively). No differences were obtained with full lateral and leaf removal treatments in comparison with control grapevines. Values of estimated exposed leaf area, using the CELAP

formula (Deloire 2012) were similar among treatments (Table 1). The ratio TLA/CELAP can be a good indicator of shading within the canopy (Smart 1985). The value is 1 when canopies have no interior leaves. The values obtained in control and leaf removal treatments showed little differences in terms of shading and hypothetically low number of interior leaves (Table 1). Full lateral removal treatment resulted in a low value of this ratio (Table 1) leading to a potential higher exposure of bunches to sunlight.

Table 1 Leaf area parameters per treatment analysed at veraison.

	Control	**FLatR**	**LeafR-east**	**LeafR-west**
Main shoots (m^2/grapevine)	1.0 a	1.1 a	1.1 a	0.9 a
Lateral shoots (m^2/grapevine)	1.4 a	0.2 c	2.4 b	1.7 ab
Total leaf area (TLA) (m^2/grapevine)	2.4 ab	1.3 a	3.4 b	2.6 b
CELAP* (m^2/grapevine)	[(1.2x2) + 0.3] x 0.8 = 2.2	[(1.2x2) + 0.15] x 0.8 = 2.0	[(1.2 + 1.0) + 0.3] x 0.8 = 2.0	[(1.2 + 1.0) + 0.3] x 0.8 = 2.0
Ratio TLA/CELAP	1.1	0.7	1.7	1.3

*Values within a column followed by the same letter do not differ significantly at the 0.05 level by Fisher's least significant difference. *Canopy leaf area perimeter (CELAP) was calculated considering an estimated of 20 cm as leaf removal in the bunch zone.*

Plant water status

Although this vineyard is rain-fed without irrigation, at post veraison, only moderate water constraints were reached and no differences in predawn leaf water potential were found between treatments (Figure 9A). A deep root system and available water from a water table might explain these results as previous soil profiles performed in the area of the study showed. None of the canopy manipulation treatments induced significant differences in daily plant water status during berry ripening, measured using the stem water potential (Figure 9B).

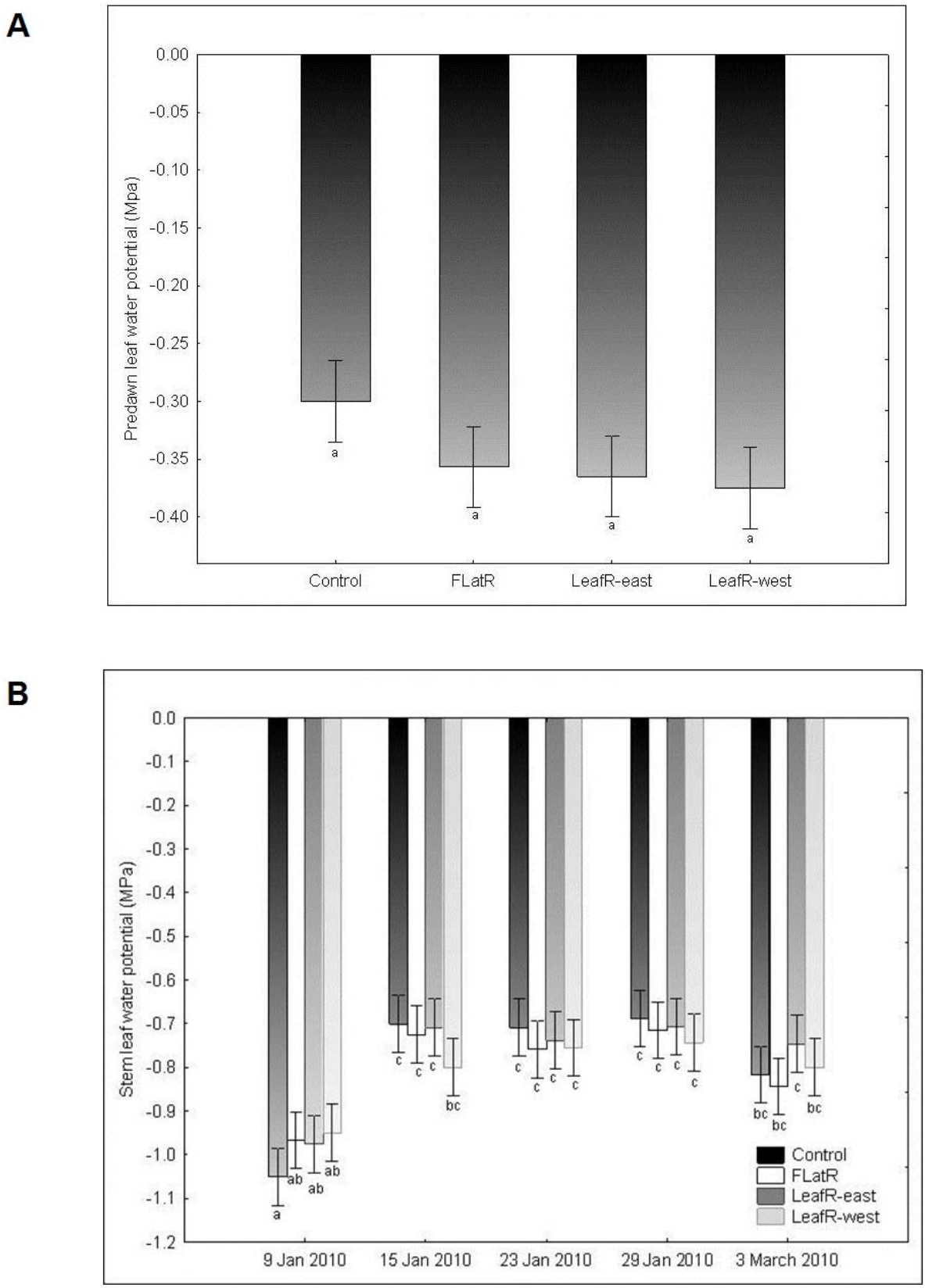

Figure 9 Predawn leaf water potential as affected by canopy manipulation at post veraison (A) and stem water potential as affected by canopy manipulation during the period veraison to harvest (B). Vertical bars denote +/- standard errors. Data are means ± SE of 6 replicates.

Berry growth and composition

Canopy manipulations slightly reduced berry growth ($p < 0.05$) (Figure 10), pH ($p < 0.05$) (Figure 11A), TA ($p < 0.05$) (Figure 11B) and sugar per berry: ($p < 0.05$) (Figures 12A and 12B). In general the changes were very small and were due only to full lateral removal and leaf removal in the east side in comparison with the control. Lateral removal slightly reduced dry mass, pH and the accumulation of sugar per berry but not fresh mass and TA in comparison with the control. Leaf removal in the fruit zone at the east side slightly reduced fresh and dry masses, TA and the accumulation of sugar per berry, but not pH in comparison with the control. Leaf removal in the west side did not affect any of the parameters in comparison with the control.

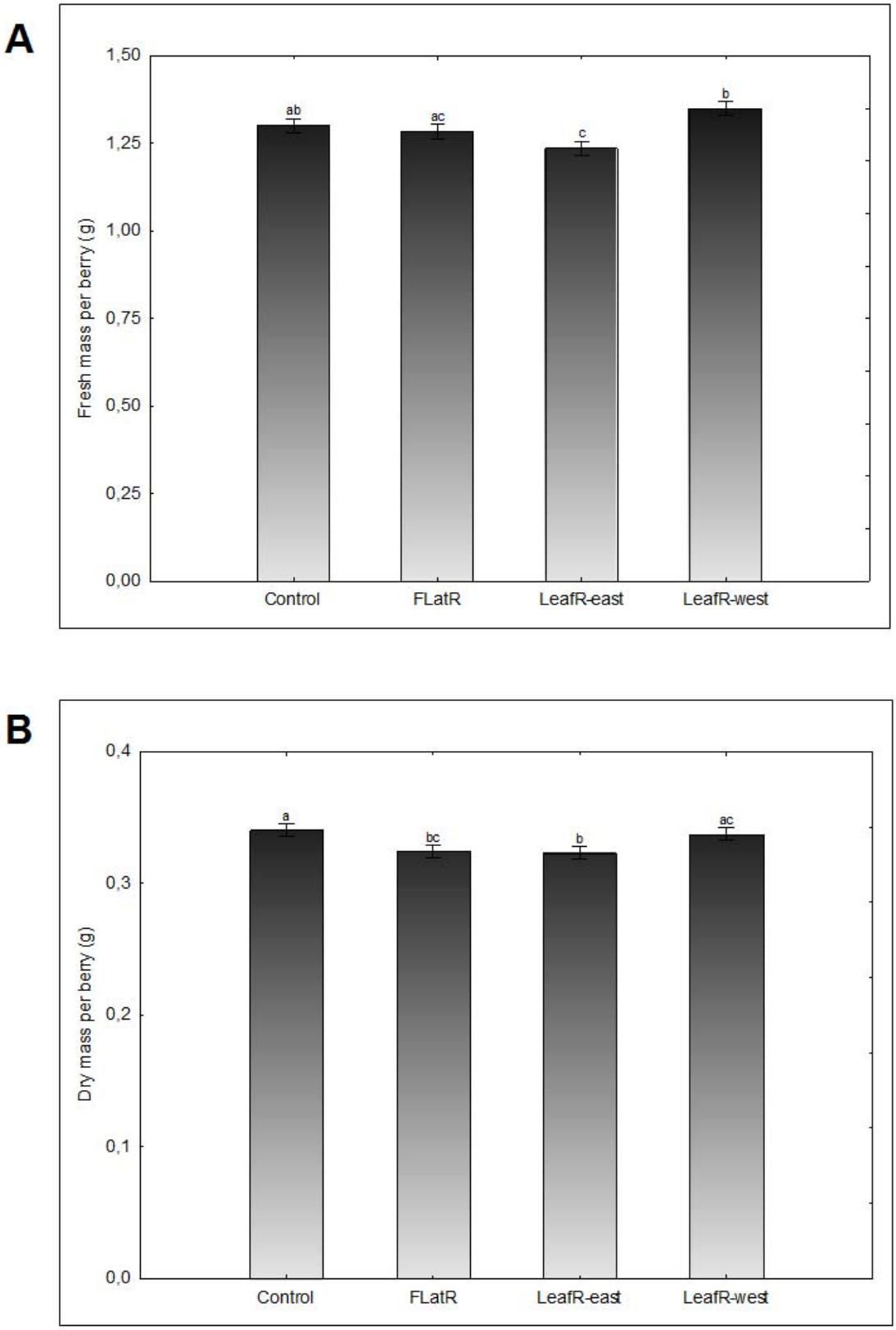

Figure 10 Effect of canopy manipulation on fresh (A) and dry (B) berry masses. Data correspond to values over the whole ripening period (from 5th January to 15th February corresponding to six sampling dates). Vertical bars denote +/- standard errors. Data are means ± SE of 12 replicates.

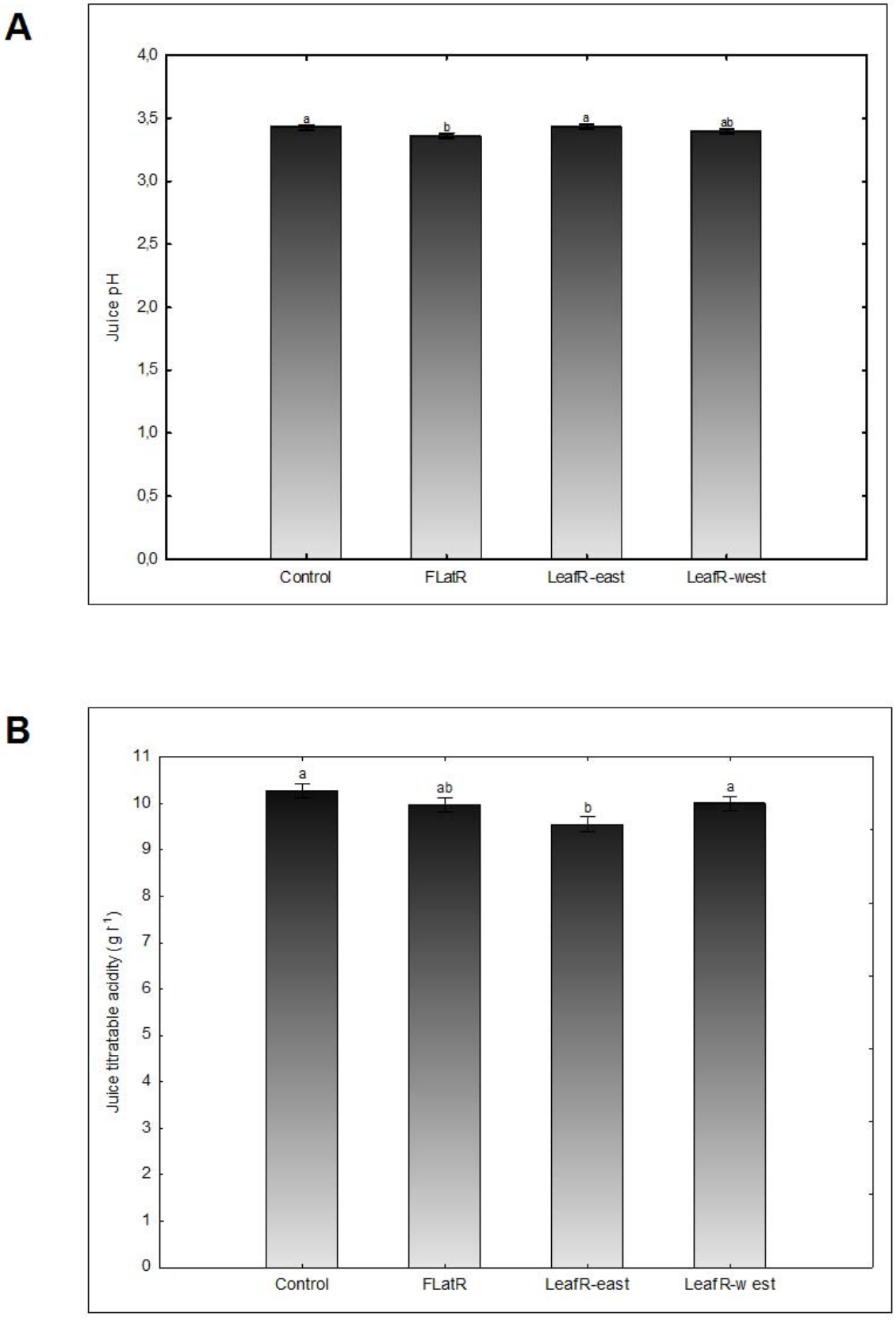

Figure 11 Effect of canopy manipulation on juice pH (A) and on juice titratable acidity (B). Data correspond to values over the whole ripening period (from 5th January to 5th February corresponding to five sampling dates). Vertical bars denote +/- standard errors. Data are means ± SE of 10 replicates.

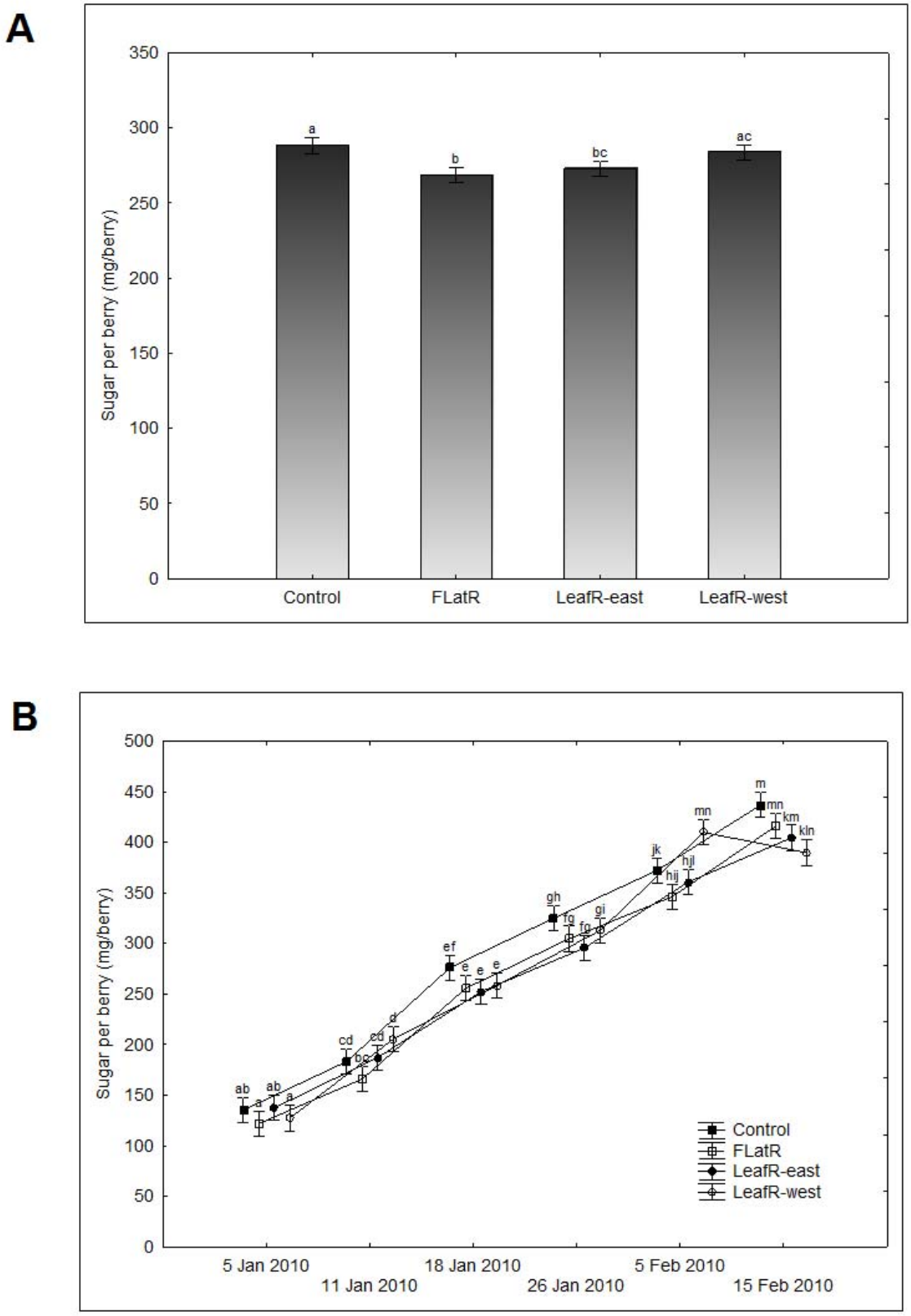

Figure 12 Effect of canopy manipulation on the accumulation of sugar per berry (A) and during the period veraison to harvest (B). For the last sampling date the values of sugar per berry were: control 437 (mg/berry), full lateral removal 416 (mg/berry), leaf removal east-side 404 (mg/berry) and leaf removal west-side 390 (mg/berry). Data correspond to values over the whole ripening period (from 5th January to 15th February corresponding to six sampling dates). Vertical bars denote +/- standard errors.

The sugar accumulation per berry per day seemed to be more active for the control and FlatR, until period 11/18 January. There is a decrease in daily sugar accumulation for leafR-west on the period 26Jan/5Feb which corresponds to the plateau of berry sugar accumulation (Figure 13) (see Deloire, 2011, 2013). The continuous but slow quantity of sugar accumulation per berry per day, including in the control, indicated that there is no major stress or even constraint in terms of vine water status (therefore possibly no strong reduction and/or inhibition of stomatal conductance and photosynthesis (Cifre et al. 2005, Lovisolo et al. 2010), that the vines were in balance in terms of the ratio fruit load to exposed leaf area, and the bunch micro climate was appropriate. This is confirmed by the non-observation of berry sunburn and shrivelling or fruit water loss over the ripening period. A drawback in this study is that crop load per unit of leaf area was not measured in order to determine the influence on source-sink relations from the treatments.

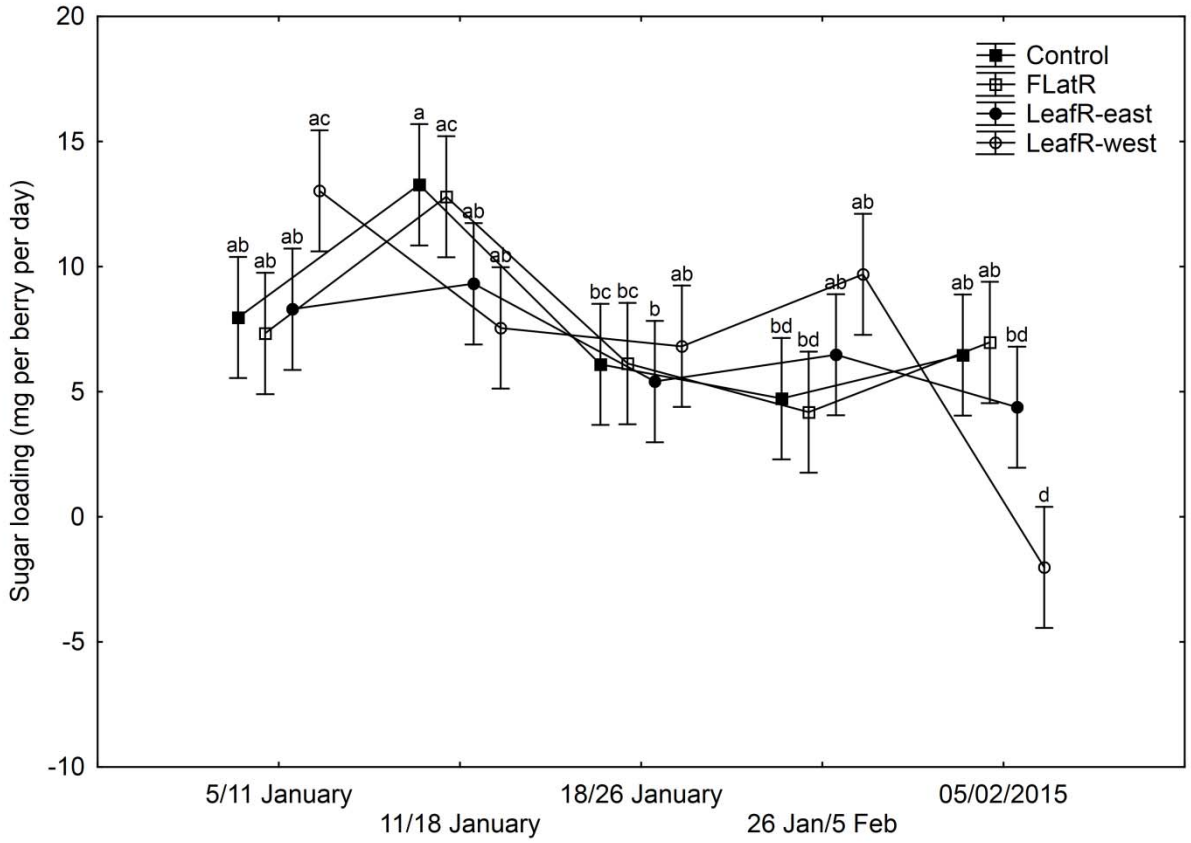

Figure 13 Effect of canopy manipulation on the sugar accumulation per berry per day during the period veraison to harvest. Vertical bars denote +/- standard errors.

Discussion

From veraison (i.e. berry softening), the ripening fruit is a strong sink for photosynthates which could be provided by the leaves and/or the carbohydrate reserve from the permanent structures of

a grapevine, mainly the roots (Williams 1996, Holzapfel and Smith 2012). Several studies have shown that canopy manipulation can affect berry growth and ripening by changing total leaf area, leaf age pattern and bunch microclimate (Jackson and Lombard 1993, Poni et al. 2013). In order to obtain a photosynthesis response to defoliation, the source-sink ratio has to be significantly modified. Candolfi-Vasconcelos et al. (1994) found that removal of main leaves on Pinot Noir grapevines only produced a slightly lower or similar photosynthetic rate in comparison with control grapevines. In another study, Candolfi-Vasconcelos and Koblet (1991) found that main leaf removal produced a compensatory response only during pre-veraison period; on the contrary, lateral shoot removal produced a higher phothosynthetic rate on the remaining main leaves and up to harvest. These authors suggested that leaves from laterals have a primary role as source in comparison with main leaves which could have a limited role during berry ripening, probably due to their physiological age. Leaves from laterals can have photosynthetic rate similar to the main leaves at the top of the canopy (Candolfi-Vasconcelos et al. 1994). Full lateral removal can reduce total leaf area (Poni and Giachino 2000, Serra-Stepke 2010) and consequently increase the percentage of mature leaves in the canopy. The remaining leaves tend to compensate by an increase in their photosynthetic activity (Hunter and Visser 1989, Candolfi-Vasconcelos and Koblet 1991, Poni and Giachino 2000).

Our study involved different canopy management practices (main leaves and full lateral removal) applied to field-grown cv. Pinotage, in order to assess the effect of the altered canopies and bunch microclimate on berry growth and composition. Under the conditions of this study, the little effect of full lateral removal on berry growth, considering the significant reduction of total leaf area in comparison with leaf removal treatments, might be explain by compensation of the remaining leaves in terms of photosynthetic activity. In addition, Candolfi-Vasconcelos and Koblet (1990) reported a delay in leaf senescence and abscission of the main leaves after lateral removal. Furthermore, it has been shown that earlier defoliations had more significant effects on berry weight when compared with a late defoliation (Candolfi-Vasconcelos and Koblet 1990). In cv. Sauvignon Blanc, a late lateral removal (at pea size) was not able to produce changes in the accumulation of sugar per berry (Serra-Stepke 2010), which was the case in this study. In contrast, main leaf removal and partial lateral removal decreased fruit yield mainly due to decrease in berry fresh mass (Koblet et al. 1994, Poni and Giachino 2000).

It is known that soil water availability can influence berry growth (Ojeda et al. 2001). Esteban et al. (1999) found that berry fresh mass is increased by irrigation. Nevertheless, under the conditions of this study, there were no differences in terms of plant water status between treatments and the treatments only showed moderate water constraints. Candolfi-Vasconcelos and Koblet (1991) found that lateral shoot removal in field grown grapevines showed no

response in transpiration rates, at the primary shoot level, in comparison with control grapevines. Young potted grapevines subjected to lateral shoot removal had higher rates of transpiration than grapevines without lateral shoot removal. These authors suggested that potted grapevines have a higher sensitivity of leaf gas exchange due to restricted soil volume available to root growth which could affect plant water status.

Berry sugar and acidity content are highly dependent on environmental conditions and canopy management (Jackson and Lombard 1993). During berry ripening a huge amount of sugars is accumulated in the vacuoles of mesocarp cells. Sugar transport into the berry is mainly apoplastic and via the phloem (Zhang et al. 2006, Agasse et al. 2009). In general, it is considered that bunches exposed to sunlight have a higher content of total soluble solids (probable concentration effect), flavonoid phenolics (positive effect of light on anthocyanins biosynthesis (He et al. 2010) and on flavonols and flavan-3-ols biosynthesis (Matus et al. 2009)), lower content of titratable acidity (perhaps due to the increase in temperature) and a higher juice pH. In addition, carotenoid biosynthesis is affected by light and water status and methoxypyrazines are degraded by light (Dunlevy et al. 2009). The effect of light on berry composition is dependent on the temperature thresholds the berries are subjected to (Bergqvist et al. 2001). In addition, a late leaf removal above the bunch zone can delay ripeness (Poni et al. 2013). An excessive crop load not in balance with the exposed leaf area can slow down berry sugar accumulation (Deloire 2011), which might explain the lower sugar per berry content obtained in the treatment subjected to full lateral removal. Our data showed a small reduction of titratable acidity due to leaf removal, east side, which was the treatment that produce higher berry temperatures in comparison with the control (40% of the day temperatures above 35ºC in the treatment leaf removal east side versus 20% in the control). Reduction of organic acids is affected more by higher temperatures than by light (Spayd et al. 2002, Pereira et al. 2006) and mainly due to malic acid degradation/respiration (Lakso and Kliewer 1975, Parra et al. 2010).

Conclusions

This study showed the following:

- Leaf and or lateral removal could be a useful cultural practices to improve fruit zone microclimate without affecting significantly some primary metabolisms used by the Wine Industry as indicators of fruit quality (including bunch sanitary conditions).
- Leaf and lateral removal, when appropriately applied, does not significantly affect the balance of a vine in terms of fruit to exposed leaf ratio, and could allow improving fruit quality, as it has been shown in many studies.
- The ability of a vine to compensate its photosynthetic activity remains important.

However and for time and practical reasons, this study presents some drawbacks:

- Only a very few primary metabolisms were analysed. It should have been relevant to consider some secondary metabolisms as the anthocyanins and the total phenols.
- This study did not present the results on the wines even if wines were made, mainly using the sugar loading method do a sequential harvest. The wines were not analysed due to time constraint.

It is known that modifying the bunch microclimate and / or the vine water status could affect the fruit composition (for example the amino acid concentration) which will affect the yeast functioning and therefore change the wine ester profile and its sensory characteristics (Garde-Cerdan et al. 2009, Šuklje et al. 2014). The relation between fruit and wine composition is still not obvious despite numerous publications on the topics. Further studies are needed focusing on specific berry compounds linked to yeast functioning as amino acids and nitrogen.

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