INTERACTIVE EFFECTS OF DEFICIT IRRIGATION TECHNIQUES AND BOTH ELEVATED CO_2 CONCENTRATION UNDER CONTROLLED CONDITIONS AND TRANSPARENT PLASTIC COVER IN THE FIELD ON PHYSIOLOGICAL ASPECTS OF GRAPEVINES (*Vitis labrusca*)

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CAMPOS DOS GOYTACAZES – RJ
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CONTENTS

ABBREVIATION LIST

Γ^* - CO_2 compensation point in the absence of respiration in the light;

[CO_2] – CO_2 concentration;

Ψ – leaf water potential;

Ψ_{pd} – leaf water potential at predawn;

Ψ_{md} – leaf water potential at midday;

$\delta^{13}C$ – C-isotope composition;

ABA – abscisic acid;

A_{net} – net photosynthetic rates;

A_{net}/g_s – intrinsic water use efficiency;

A_{net}/E – water use efficiency;

C_i/C_a – ratio of the internal to ambient

CO_2 concentration;

CWSI – crop water stress index;

DI_0/CS – dissipation per exited cross section;

E – transpiration rates;

ET_0/ABS – quantum yield of electron transport;

ET_0/TR_0 – efficiency with which a trapped excitation energy can move an electron transport chain further than the Quinone A;

ET_c – crop evapotranspiration;

FI – full irrigated;

FRF – far red band;

F_v/F_m ratio – maximum quantum yield of primary photochemistry;

g_s – stomatal conductance;

iWUE – intrinsic water use efficiency

J – photosynthetic electron transport;

K_c – Michaelis constants for carboxylation;

K_o – Michaelis constants for oxygenation

LCB – Leaf Carbon Balance;

LDW – leaf dry weight;

NI – non-irrigated;

PI – performance index;

PQ – plastoquinone;

PRD – partial rootzone drying;

PSII – photosystem II;

QA – quinone A;

R – leaf respiration rates;

RC/CS_0 – fraction of active reaction centers per excited cross-section of leaf;

R_{dark} - leaf respiration rates in the dark;

RDI – regulated deficit irrigation;

RF – red band;

R_{light} - leaf respiration rates in the light;

Rubisco – ribulose -1,5-bisphosphate carboxylase/oxygenase;

S_m – energy needed to close all reaction centers expressed by the normalized area;

SLW – specific leaf weight

T_{dry} – temperature of a leaf covered with liquid paraffin;

T_{leaf} – leaf temperature;

TPC – transparent plastic cover;

T_{wet} – temperature of a leaf sprayed with water;

V_c – rates of carboxylation of Rubisco;

$V_{c\ 500}$ – rates of carboxylation of Rubisco at 500 µmol $m^{-2}\ s^{-1}$;

$V_{c\ 1500}$ – rates of carboxylation of Rubisco at 1500 µmol $m^{-2}\ s^{-1}$;

$V_{c\ max}$ – maximum rates of carboxylation of Rubisco;

V_o – rates of oxygenation of Rubisco;

$V_{o\ 500}$ – rates of oxygenation of Rubisco at 500 µmol $m^{-2}\ s^{-1}$

$V_{o\ 1500}$ – rates of oxygenation of Rubisco at 1500 µmol $m^{-2}\ s^{-1}$;

$V_{o\ max}$ – maximum rates of oxygenation of Rubisco;

VPD – air vapor pressure deficit.

WUE - water use efficiency.

ABSTRACT

SILVA, Jefferson Rangel da; D.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro; November 2017; **Interactive effects of deficit irrigation techniques and both elevated CO_2 concentration under controlled conditions and transparent plastic cover in the field on physiological aspects of grapevines (*Vitis labrusca*)**; Advisor: Prof. Eliemar Campostrini; Co-Advisor: Prof. Kevin Lee Griffin.

Two experiments were designed to study important growth and physiological traits in grapevines (*Vitis labrusca*) cultivated under both controlled and field conditions. We aimed to investigate the interactive effects of deficit irrigation and both elevated CO_2 concentration [CO_2] under controlled conditions and transparent plastic covering (TPC) in the tropics. In the first experiment (chapter 1), three water management treatments were applied [full-irrigated (FI) - both sides of the rootzone were irrigated to saturation; partial root-zone drying (PRD) - only one side of the rootzone was irrigated to saturation; and non-irrigated - irrigation was suspended on both sides of the rootzone] in each of two 1.4 m^2 growth chambers, each one with different [CO_2] (400 ppm or 800 ppm). Likewise, in the second experiment (chapter 2), three water management techniques were applied in tropically grown grapevines [FI - 100% of the crop evapotranspiration (ET_c) was supplied to both sides of the root system; regulated deficit irrigation (RDI) - 50% of the ET_c was supplied to both sides of the root system; and PRD - 50% of ET_c was alternately supplied to only one side of the root system, whereas the other side of the rootzone remained without water]. These irrigation treatments were replicated such that the two plots were either covered with a polyethylene plastic structure

(TPC) or remained uncovered. Overall, our work demonstrated that: the use of deficit irrigation techniques is a consistent alternative to save water in grapevines cultivated either under elevated [CO_2] or in the tropics, with or without TPC; TPC can improve water use efficiency and Rubisco carboxylation rates in tropically grown grapevines; Elevated [CO_2] delays drought negative effects in grapevines; Although significant effects on water use efficiency were not observed, the use of deficit irrigation techniques is favored in the tropics by the anisohydric behavior since metabolic and photosynthetic limitations are avoided under such conditions; Under elevated [CO_2], *down-regulation* of net photosynthetic rates is observed due N dilution within thicker leaves; Despite leaf respiration presents different responses depending on drought level, leaf respiration rates are not affected by deficit irrigation techniques, regardless growth conditions ([CO_2] or tropics), so that the balance between photosynthesis and leaf respiration is not impaired. Therefore, yield and fruit quality are not damaged when deficit irrigation techniques are used; In addition, light inhibition of respiration (R_{light}:R_{dark}) is highly correlated with Rubisco oxygenation rates so that R_{light}:R_{dark} is intensified under conditions of reduced photorespiration.

Keywords: chlorophyll a fluorescence; leaf carbon balance; leaf respiration rates; net photosynthetic rate; nitrogen concentration; rubisco oxygenation/carboxylation rates.

RESUMO

SILVA, Jefferson Rangel da; D.Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro; dezembro 2017; **Efeitos interativos entre técnicas déficit de irrigação e elevada concentração de CO_2 em condições controladas e cobertura plástica transparente em condições de campo sobre aspectos fisiológicos de videiras (*Vitis labrusca*)**; Orientador: Prof. Eliemar Campostrini; Co-orientador: Prof. Kevin Lee Griffin.

Dois experimentos foram desenvolvidos para estudar importantes parâmetros fisiológicos e de crescimento em videiras (*Vitis labrusca*), tanto em condições controladas como em condições de campo. Nosso objetivo foi averiguar os efeitos interativos entre déficit de irrigação e elevada concentração de CO_2 [CO_2] ou uso de cobertura plástica sob condições tropicais. No primeiro experimento (capítulo 1), três tratamentos de irrigação foram implementados [completamente irrigado (FI) – ambos os lados do sistema radicular eram irrigados até a saturação; irrigação parcial do sistema radicular (PRD) – apenas um dos lados do sistema radicular era irrigado até a saturação; e não-irrigado – a irrigação foi suspensa em ambos os lados do sistema radicular] em duas câmaras de crescimento, cada uma com 1.4 m^2 de área e com diferentes [CO_2] (400 ppm ou 800 ppm). De maneira semelhante, no segundo experimento (capítulo 2), três manejos de irrigação foram aplicados em videiras crescidas em condições tropicais [FI – 100% da evapotranspiração da cultura (ET_c) era fornecida em ambos os lados do sistema radicular; déficit de irrigação regulado (RDI) – 50% da ET_c era fornecido em ambos os lados do sistema radicular; PRD – 50% da ET_c era fornecido em apenas um dos lados do sistema radicular, enquanto o outro lado permanecia sem irrigação]. Tais tratamentos de irrigação foram replicados tanto em uma área coberta por estrutura plástica de polietileno (TPC) como em uma área que não apresentava tal cobertura. De maneira geral, nosso trabalho demonstrou que: o uso das técnicas de déficit de irrigação é uma estratégia útil para economizar água em videiras cultivadas tanto sob elevadas [CO_2], como nos trópicos, com ou sem o uso de TPC; O uso de TPC apresenta grande potencial para melhorar a eficiência no uso da água e as taxas de carboxilação da Rubisco de videiras cultivadas nos trópicos; A elevada [CO_2] retarda os efeitos negativos do estresse hídrico em videiras; Apesar de efeitos significativos na eficiência no uso da água não terem sido observados, o uso de ambas as técnicas de déficit de irrigação é favorecido em videiras anisohídricas cultivadas em condições tropicais, uma vez que, limitações metabólicas e na capacidade fotossintética são evitadas sob tais condições; Sob elevadas [CO_2], o processo de *down*-regulação da fotossíntese é observado devido a diluição da concentração de N em folhas mais espessas; Apesar das taxas de respiração foliar dependerem do nível de estresse hídrico, o uso das técnicas de déficit de irrigação não afeta negativamente tal processo, de forma que, o balanço entre as taxas fotossintéticas líquidas e de respiração foliar não é alterado independente das condições de cultivo ([CO_2] e trópicos). Dessa forma, a produtividade e qualidade de frutos não são prejudicadas quando técnicas de déficit de irrigação são utilizadas; Além do mais, observamos que a inibição da respiração pela luz ($R_{light}:R_{dark}$) é altamente correlacionada com as taxas de oxigenação da Rubisco, de forma que $R_{light}:R_{dark}$ é intensificada em condições de reduzida fotorrespiração.

Palavras-chave: balanço de carbono foliar; fluorescência da clorofila a; taxas de carboxilação/oxigenação da Rubisco; taxas de respiração foliar; taxa fotossintética líquida.

1. INTRODUCTION

According to IPCC (2014), the atmospheric CO_2 concentration ($[CO_2]$) is estimated to reach values between 750 and 1300 ppm by the end of the century, what is likely to be followed by global warming, changes in precipitation patterns and reduction in soil moisture content up to 70% (Schultz 2000; Kimball et al., 2001; Reich et al., 2016). Considering that grapevines (*Vitis* spp.) are grown on nearly every continent (FAOSTAT 2014), climate change is likely to cause profound management modifications in such important crop (Flexas et al., 2010). Thereby, it is imperative to study the effects of climate change on important physiological traits in grapevines, and the consequences on yield and fruit quality.

Increased $[CO_2]$ can improve carbon assimilation in C3 plants, such as grapevines, (Salazar-Parra et al., 2012; Salazar-Parra et al., 2015) because the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the primary carboxylating enzyme of photosynthesis in C3 plants, is CO_2-limited under current atmospheric conditions (Ainsworth and Rogers 2007). Therefore, any increase in $[CO_2]$ could up-regulate grapevines' photosynthetic capacity. However, several aspects can influence this up-regulation of photosynthesis under elevated $[CO_2]$, such as exposure duration, growth conditions and species (Long et al., 2004; Aranjuelo et al., 2005; Erice et al., 2006; Ainsworth and Rogers, 2007; Aranjuelo et al., 2009a; Sanz-Sáez et al., 2010; Salazar-Parra et al., 2015; Rodrigues et al., 2016) so that the maximum photosynthetic potential is not always maintained or achieved (Luo et al., 1994; Griffin and Seemann 1996) and contradictory responses of photosynthesis under elevated $[CO_2]$ have been reported (Herrick and Thomas

2001; Long et al., 2004; Springer et al., 2005; Aranjuelo et al., 2005; Erice et al., 2006; Springer and Thomas, 2007; Ainsworth and Rogers 2007; Aranjuelo et al. 2009a; Albert et al. 2011; Salazar-Parra et al., 2015).

Since climate change is likely to reduce water available for agriculture (Schultz 2000), the future of the sustainable grapevine cultivation requires a major reduction in water use (Flexas et al., 2016). Thereby, a promising alternative to be explored is the use of deficit irrigation techniques, such as Regulated Deficit Irrigation (RDI) and Partial Root-zone Drying (PRD). Under RDI, the water is supplied at levels below that required for full crop evapotranspiration (ET_c) during specific phenological stages, particularly after fruit set, when fruit growth is less sensitive to reductions in water supply (Chaves et al., 2010; Tarara and Peña 2015). Under PRD, on the other hand, the two sides of grapevines' root system are alternately irrigated, providing a spatial reduction in water availability. Although deficit irrigation can improve water use efficiency in plants, the physiological responses in grapevines depend on both specific ambient growth conditions and plants' particularities, such as hydraulic resistances within the soil-plant system, plant age and climate (Schultz 2003; Lovisolo et al., 2010; Chaves et al., 2010; Hochberg et al., 2013). Thus, there is a demand for knowledge about the ecophysiological responses of grapevine cultivars deficit irrigated (Chaves et al., 2010; Flexas et al., 2010; Flexas et al., 2016; Lavoie-Lamoureux et al., 2017), especially in both elevated [CO_2] and tropical conditions.

The future of grapevine cultivation is also likely to require an expansion to unusual sites (Flexas et al., 2016), such as the tropics. Protected cultivation using transparent plastic covering (TPC) could be a good option for extending the range of grapevine cultivation, since this technique can protect plants from adverse meteorological conditions, such as wind and heavy rains (Roberto et al., 2011; Du et al. 2015; Permanhani et al., 2016). However, advantages and disadvantages of TPC use are still under debate in the literature, so that both negative and positive impacts on grapevines' photosynthetic capacity, fruit yield and quality have been observed when TCP is used (Stanghellini 2014; Permanhani et al., 2016).

A complete understanding of the effects of elevated [CO_2], reduced water availability and TPC in grapevines is only possible if the balance between photosynthesis and leaf respiration (*R*) is assessed (Flexas et al.; 2006; Poni et al., 2009). Despite the importance of *R* to plant life, (Amthor 2000, Sharma-Natu and

Ghildiyal 2005), much less attention has been given to this metabolic process (Flexas et al., 2006; Schultz and Stoll 2010; Morales et al., 2016). It is important to consider that *R* occurs continuously throughout the day and the night, so that approximately 30% to 80% of daily carbon fixed by photosynthesis is respired back into the atmosphere (Loveys et al., 2002). Therefore, even small changes in *R* can result in substantial variation in the plant's carbon balance (Poni et al., 2009; Flexas et al., 2010; Griffin and Heskel 2013; Tomás et al., 2014). In addition, maintaining the balance between photosynthesis and leaf respiration could be advantageous to grapevine's growth and development, especially because the starch stored within grapevines' tissues at the end of the current season is crucial to early growth on the following season (Greer and Sicard 2009). Therefore, our work aimed to investigate the interactive effects of deficit irrigation and both elevated CO_2 concentration [CO_2] and TPC in the tropics on important physiological traits in grapevines (*Vitis labrusca*), such as photosynthetic capacity, leaf respiration and leaf carbon balance.

2. LITERATURE REVIEW

2.1- Grapevines

2.1.1- Grapevines' botanical aspects and the cultivars 'Concord' and 'Niagara Rosada'

Grapevines belong to the Cormophytes group (autotrophic organisms differentiated into roots, shoots and leaves), Spermatophyte super-division (plants that produce flowers and seeds), Magnoliophyte division (fruits with seeds), Dicotyledonous class (plants with two cotyledons that originate the first leaves), Rhamnales order (woody plants with a circle of stamens positioned inside the petals) and Vitaceae family (flowers with petals corolla welded on the upper part, valvar pre-flowering, poorly developed chalice, bicarpellary and bilocular gynoecium and fruit berry type) (Hidalgo 1993; Alvarenga et al., 1998). Grapevine is a type of liana (woody climbing vines) that use other plants or structures as support (Creasy et al., 2017). The genera *Vitis, Muscadinia, Amelcissus, Parthenocissus, Landukia, Ampelopsis, Clematicissus* and *Rhoicissus* are all within Vitaceae family. The genus *Vitis* presents remarkable economic importance since it is used to produce table grapes as well as grapes used to produce juice, jams and wine (Sousa 1996; Mullins et al., 2000). Such genus is divided in 2 sub-genera: *Muscadinea* and *Euvitis* (Winkler et al., 1974). Within the *Euvitis*, *Vitis labrusca and Vitis vinifera* are the 2 species with the largest agricultural importance. The *Vitis vinifera* has its center of origin in the southern area of the Caucasus Mountains, currently occupied by Turkey, Iran, Azerbaijan, Georgia, and Armenia. Most researches with grapevines use *V. vinifera* as plant material, since this species is the most widely cultivated in the world (Creasy 2017). On the other hand, the *Vitis labrusca* is originated from North America and is mostly cultivated to produce table grapes for *in natura* consumption as well as for juice and jams manufacturing (Giovannini 1999; Creasy 2017).

The 'Concord' grapevine, generally considered as a pure cultivar of *V. labrusca* species, is native to northeastern North America, where wet and cool climates are typical (Ahmedullah and Watson 1985). However, nowadays such cultivar is present in a wide climate conditions range (Hall et al., 2012; Borges et al., 2013). In Brazil, 'Concord' is frequently grown in Southern regions, such as in Northern Paraná State (Borges et al., 2013). 'Concord' grapevine is both vigorous and productive, producing medium-sized clusters, bearing large blue-black berries with a tough skin, which separates readily from the pulpy flesh (slip-skin) (Zabadal et al., 1988). 'Concord' grapes' juice has been known for the good organoleptic quality (Borges et al., 2013), which is a rich phenolic compounds source (Düsman et al., 2014). For this reason, grape juices made of 'Concord' berries are very appreciated by consumers, whether it is produced as monovarietal or in blends (Pinto et al., 2016). In addition, 'Concord' grapes are used in jellies and preserves, as well as in quantity for wine production and in fresh market sales for *in natura* consumption (Hall et al., 2012; Borges et al., 2013). Therefore, 'Concord' grapes present several purposes and a large market potential (Zabadal et al., 1988).

'Niagara Rosada' is a *V. labrusca* grapevine originated from 'Niagara Branca' natural somatic mutation, what took place in 1933 in the city of Jundiai, metropolitan region of São Paulo, Brazil (Sousa 1996). The 'Niagara Branca' is another North American cultivar obtained from the crossing between two American cultivars, 'Concord' and 'Cassidy'. 'Niagara Rosada' plants present a medium vigor, with relatively small clusters that weigh *ca.* 200 – 300 g. The productive season is short, good yields are achieved and the grapes are well accepted by the consumers due to their sweet flavor and attractive red color (Camargo 1998; Roberto et al., 2002; Ribeiro et al., 2009). Considered as a rustic table grape cultivar, 'Niagara Rosada' is largely produced in Brazil and has a great importance to new planting areas formation, such as in Northern Rio de Janeiro State, since production costs are reduced, and less agricultural traits are required compared to other grapevines cultivars (Protas et al., 2002).

2.1.2- Grapevine cultivation around the world

Grapevine is considered the most widely cultivated fruit in the world. In 2014, it was estimated that 74,489,859 t of grapes were produced in 7,124,512 ha (FAOSTAT 2014). Such expressive number is due to the fate of grapevines are cultivated in nearly every continent of the world. Although Europe produces 38.5% of total grapes commercialized in the world, the most important countries that produce grapes are China (12,545,800 t) and the USA (7,152,063 t) (FAOSTAT 2014). Italy (6,930,794 t) and Spain (6,222,584) are the third and the fourth biggest producers, respectively (FAOSTAT 2014). In South America, Argentina (2,635,109 t), Chile (2,456,269 t) and Brazil (1,454,183 t) have the most expressive production (FAOSTAT 2014).

Although this species is historically associated to dry and warm Mediterranean climates, nowadays grapevines cultivation is spread around the whole globe (Permanhani et al., 2016). *V. vinifera* is typically cultivated under temperate conditions and the harvest is mostly intended for wine production (Creasy 2017). Otherwise, in the tropics, most vineyards have been used for *V. labrusca* cultivation (Tonietto and Pereira 2012), where two harvests are obtained per year (Camargo et al., 2012; Jones et al., 2012; Seccia et al., 2015). Among the tropical countries, India is currently the major grapevine producer, with a production of more than 2.5 million tons, followed by Peru (0.5 million tons) (FAOSTAT 2014) and Brazilians' tropical regions (0.3 million tons) (IBGE 2016). Such data related to Brazilian tropical regions' production excludes the wet subtropical sites located in the Southern region of the country (Köppen 1918). Other tropical grapevine producers are Yemen (151.268 tons), Thailand (77.370 tons), Colombia (27,519 tons) and Venezuela (17.338 tons) (FAOSTAT 2014). It is estimated that *V. labrusca* occupies less than 20% of the total area used for grapevine cultivation (Permanhani et al., 2016). In 2014, worldwide production of table grape (*V. labrusca*) was estimated in *ca.* 21 million tons (Seccia et al., 2015). Brazil is the third largest table grapes producer in America, producing *ca.* 0.76 million tons of fruits within a cultivated area of *ca.* 24,900 ha (FAOSTAT, 2014). Chile (1.2 million tons) and the USA (0.9 million tons) are the first and second most important table grapes producers of America, respectively (OIV 2015).

2.1.3- Favorable growth conditions

Grapevine cultivation sites are currently present between the latitudes 30°N and 50°N, as well as between 30°S and 40°S. In Brazil, vineyards are found under several climate conditions, from Rio Grande do Sul State, where the weather is classified as wet subtropical, to the tropical Northern States (IBGE 2016). Such wide cultivation range is possible because grapevines present a great acclimation capacity to several environments, including sites where very low temperatures are predominant during winter (Creasy 2017). Since grapevines evolved in temperate sites, they tend to grow in regions where the average annual temperature is between 10 and 20 ºC (Creasy 2017). However, careful grapevine cultivar selection and thoughtful management can result in successful commercial production outside temperate sites, so that the cultivation is characterized by agricultural traits defined by each climatic condition (Protas et al., 2002). Regardless this wide acclimation capacity, grapevines present standard growth condition requirements to reach the maximum yield and the best fruit quality (Sentelhas 1998). Overall, the highest yields and the best quality are achieved when the following climatic conditions are present: air temperature between 15 – 30 ºC, sunlight exposure between 1,200 – 1,400 hours throughout the season (Sentelhas 1998), precipitation rates between 400 – 1,000 mm, depending on both climatic conditions and season length (Giovannini 1999).

Grapevines' growing season is characterized by a succession of vegetative cycles alternated with dormancy periods. Overall, the vegetative cycle encompasses the following stages: vegetative growth, between bud break and shoot growth; reproductive stage, between flowering and fruit ripening and; shoot lignification. Between two vegetative cycles there is always a dormancy period. Thus, the vegetative cycle and the dormancy period interdependently intercalate each other (Pedro Júnior et al., 1993; Creasy 2017). The vegetative cycle begins with pruning so that climatic conditions after pruning can change grapevines' growth stages, yield and fruit quality (Creasy 2017). Once inflorescences are formed, warm (*ca.* 30 ºC) and sunny days can increase growth, and therefore, clusters' size (Creasy 2017). Plants exposed to ideal sunlight exposure present better bud break and fertility (Santos, 2006), as well as fruits with higher sugar concentration (Mullins et al., 2000). However, supra-optimal temperatures (over 40 ºC) under drought conditions may lead to massive leaf shedding, causing source-sink imbalance and incomplete berry maturation due to insufficient carbohydrates availability (Chaves et al., 2010). Furthermore, although small water supplements may increase yield and maintain or even improve fruit quality (Matthews and Anderson 1989; dos Santos et al., 2003), excessive water supply may over-stimulate vegetative growth, which causes a negative impact on berry pigments (color) and sugar content (Bravdo et al., 1985; Dokoozlian and Kliewer 1996; Chaves et al., 2010). Moreover, large canopies tend to frequently present fungal diseases (Dry and Loveys 1998). Therefore, environmental conditions strongly influence grapevines' development so that if the weather is favorable during the current season, the following season's yield potential is also improved (Creasy 2017).

2.2. Ecophysiological aspects of grapevines grown under elevated [CO_2]

The anthropogenic action combined with natural processes on Earth have increased [CO_2] in a range of 120 ppm since pre-industrial period, reaching the values of *ca.* 400 ppm observed nowadays (IPCC 2014). It is estimated that the [CO_2] has increased at rates of *ca.* 2 ppm per year so that by 2050, [CO_2] is predicted to reach *ca* 450 and 600 ppm, outpacing 700 ppm on the second half of this century (Collins et al., 2013; Ramalho et al., 2013). Under current atmospheric conditions, Rubisco activity in grapevines is CO_2-limited, as in other C3 plants, whereas the oxygenation activity is not inhibited (Ainsworth and Rogers 2007). Thus, CO_2 fixation rates of grapevines exposed to twice ambient [CO_2] could increase more than 50% in the short-term (minutes to hours) (Salazar-Parra et al., 2012; Salazar-Parra et al., 2015) so that elevated [CO_2] could affect plant's metabolism, physiological functioning, growth and productivity (Drake et al., 1997; Oliveira et al., 2010).

Indeed, greater net photosynthetic rates under elevated [CO_2] can increase leaf area, leaf dry weight (Kizildeniz et al., 2015) and yield without negative effects on fruit quality (Bindi et al., 2001; Kizildeniz et al., 2015). It has also been shown that under elevated [CO_2], both grapevine's stomatal density and stomatal conductance decrease, reducing water losses through transpiration (Moutinho-Pereira et al., 2009; Holtum and Winter 2010; Rogiers et al., 2011). Therefore, plant's water use efficiency (*WUE*) could increase since more carbon is assimilated through photosynthesis at a given transpiration rate (Holtum and Winter 2010).

However, effects of elevated [CO_2] on plant's photosynthesis can depend on exposure duration. In long-term, it has been reported that the initial stimulation of photosynthesis following CO_2 application often does not persist and net photosynthetic rates declines below its maximum potential in an acclimation process described as photosynthetic acclimation or photosynthetic down-regulation (Jifon and Wolfe 2002; Long et al., 2004; Erice et al., 2006; Salazar-Parra et al., 2015). This down-regulation process may be a result of: (1) stomatal limitations, such as those resulting from lower leaf conductance under elevated [CO_2], and the corresponding decrease in the sub-stomatal CO_2 concentration (C_i) (Sánchez-Díaz et al., 2004); or (2) metabolic limitation, usually attributable to reduced carboxylation activity (Aranjuelo et al., 2005; Erice et al., 2006) and/or a reduced rubisco amount at elevated [CO_2] (Urban 2003; Aranjuelo et al., 2005). In addition, the inability of plants to fully utilize the extra photosynthates produced under elevated [CO_2] (Bunce 2001) can also contribute to down-regulation process. Indeed, one of the parameters that can affect photosynthetic down-regulation is the change on source-sink ratio (Urban 2003), and the ability of plants to develop new sinks or expand existing ones (Lewis et al., 2002; Aranjuelo et al., 2009a). Therefore, when plants exposed to elevated [CO_2] have limitations to increase C sink strength, a decrease on their photosynthetic rates to balance C source activity and sink capacity is observed (Thomas and Strain 1991). When C sink strength is not increased, the down-regulation of photosynthesis may cause photoinhibition due to reductions in energy equivalents (ATP and NADPH) consumption in the Calvin-Benson cycle, which increases the probability of overexcitation in photosystem II (PSII) (Lambreva et a., 2005; Lawlor and Tezara 2009; Wilhelm and Selmar 2011). Although many studies have shown that photosynthesis down-regulates to elevated CO_2 over long-term experiments (Long et al., 2004; Aranjuelo et al., 2005; Erice et al., 2006; Ainsworth and Rogers 2007; Aranjuelo et al., 2009a; Albert et al. 2011; Salazar-Parra et al., 2015), some works indicate that down-regulation symptoms does not occur, so that the responses of photosynthesis to long-term (up to 7 years of treatment under elevated [CO_2]) do not differ from those of short-term (Herrick and Thomas 2001; Springer et al., 2005; Springer and Thomas 2007), showing the contradiction among different studies.

Similar to photosynthesis, leaf respiration (R) is a physiological process known to respond to climate change and to elevated [CO_2] (Griffin and Seemann

1996; Flexas et al., 2006; Crous et al., 2012). Functionality of *R* is fundamental to plant life, sustaining growth and maintenance, ion transport and compartmentalization, protein turnover and tissue acclimation to environmental change (Amthor 2000; Sharma-Natu and Ghildiyal 2005). Moreover, *R* plays an important role in the carbon balance of plants (Gonzales-Meler et al., 2004), since respiratory CO_2 efflux from plants is a large fraction of the total gross CO_2 uptake through photosynthesis, and proceeds in daylight as well as during the night (Tcherkez et al., 2012; Griffin and Heskel 2013). For this reason, it is required an intense effort focused on understanding the balance between photosynthesis and *R* in response to several climatic and atmospheric conditions, such as elevated [CO_2] (Cavaleri et al., 2008; Drake et al. 2008; Kirschbaum 2010; Mahecha et al., 2010).

Although initially it was thought that *R* was similar throughout day and night (Graham 1980), it is now accepted that R is strongly suppressed by daylight (Brooks and Farquhar 1985; Krömer 1995; Atkin et al., 2000; Shapiro et al., 2004; Tcherkez et al., 2005; Nunes-Nesi et al., 2007; Tcherkez et al., 2008). As a result, most leaf-level studies show that respiration in the light (R_{light}) is lower than mitochondrial CO_2 release in the dark (R_{dark}) (Hurry et al. 2005; Tcherkez et al. 2010). Several studies have reported that reduction in R_{light} in illuminated leaves can be a result of the deactivation of pyruvate dehydrogenase (Gemel and Randall 1992; Tovar-Mendez et al., 2003), the inhibition of isocitrate dehydrogenase due to higher ATP levels during the day (Igamberdiev and Gardeström 2003; Kasimova et al. 2006) and the inhibition of pyruvate kinase (Lin et al., 1989; Tcherkez et al., 2009). Moreover, studies have shown that the TCA cycle functions in a noncyclical manner in day-light, producing both glutamate and fumarate to provide organic acids and C-skeletons for nitrate assimilation, while also responding to the feedback inhibition by NADH and ATP on the TCA cycle in illuminated leaves (Tcherkez et al., 2009). Changes in the demand for other respiratory products in the light (compared with in darkness) may also contribute to variability in degree of light inhibition of *R* (Flexas et al., 2005; Atkin and Macherel 2009).

Regarding elevated [CO_2], there are studies reporting that *R* is stimulated (Davey et al., 2004; Shapiro et al., 2004; Robredo et al., 2010; Crous et al., 2012) since the number of mitochondria (the organelles that supply energy to the cell in the form of ATP) is higher in plants grown under elevated [CO_2] (Griffin et al., 2001;

Griffin et al., 2004; Wang et al., 2014). In addition, lower light inhibition of *R* (and higher rates of R_{light} per se) in plants grown under elevated [CO_2] has been previously reported in several studies (Wang et al., 2001; Shapiro et al., 2004) and attributed to a greater demand for energy and C-skeletons, and increased availability of respiratory substrates under elevated [CO_2] (Dewar et al., 1999; Atkin et al., 2000; Wang et al., 2001; Tcherkez et al., 2008; Ayub et al., 2011; Crous et al., 2012). However, some reports have shown that *R* can be inhibited under elevated [CO_2] (Reuveni et al., 1995; Gonzàlez-Meler et al., 1996) due to changes in the cytochrome and/or alternative pathway ratios, reduced energy demand for carbohydrate translocation (González-Meler et al., 2004), as well as reduced tissue N content in leaves grown under elevated [CO_2] (Bouma et al., 1994; Drake et al., 1997; Curtis and Wang 1998). Moreover, decreased R_{light}:R_{dark} has been reported under conditions that suppress light-saturated photorespiration rates, such as elevated [CO_2] (Wang et al., 2001; Shapiro et al., 2004; Ayub et al., 2011; Crous et al., 2012; Griffin and Turnbull 2013), since photorespiration regulates key enzymes of the TCA cycle, such as pyruvate decarboxylase, isocitrate dehydrogenase and pyruvate kinase (Griffin and Turnbull 2013). Such contradictory results reported in the literature reinforce the importance of better understanding the effects of elevated [CO_2] on leaf respiration, and the consequeces on leaf carbon balance between photosynthesis and respiration.

2.3. Ecophysiological aspects of grapevines grown under reduced water availability

Water scarcity is one of the major worldwide limitations for viticulture (Chaves et al., 2007) since grapevines are predominantly located in regions where the growing season coincides with the highest annual temperatures and dry months (Flexas et al., 2010). This particular climatic condition and its exacerbation predicted by climate change (IPCC 2014) demand an advance on the knowledge about the ecophysiological responses of grapevines cultivars to low water availability (Collins et al., 2013), especially in tropical areas, such as in Brazil, where information is scarce and precipitation patterns are irregular (Permanhani et al., 2016). It is known that grapevines have the capacity to perceive and respond to alterations in water availability through physiological, cellular, and molecular events developing simultaneously (Chaves et al., 2009). Such responses are modulated by imposed drought intensity, duration, and rate of progression (Pinheiro and Chaves 2011) as well as by specific stress 'tolerance' or 'avoidance' mechanisms presented by grapevine plants (Schultz 2003).

Grapevines have been addressed as the only known species to possess both isohydric and anisohydric behaviors in response to changes in air vapor pressure deficit (VPD) and/or water availability in the soil (Schultz 2003; Soar et al., 2006; Chaves et al., 2010; Tomás et al., 2014; Lavoie-Lamoureux et al., 2017). These mechanisms are stomatal conductance (g_s) sourced (Hocheberg et al., 2013) and for this reason, many works comparing grapevine cultivars in terms of water use efficiency (the amount of carbon gained per unit water used) have presented contrasting results (Bota et al., 2001; Schultz, 2003; Rogiers et al., 2012; Tomás et al., 2014; Zhang et al., 2012; Pou et al., 2012; Tramontini et al., 2014). Indeed, in isohydric plants, ABA accumulation in leaves promotes stomata closure early in response to either decreased water content in the soil or increased VPD, so that leaf water potential (Ψ) remains unchanged or slightly decreases (Schultz 2003; Poni et al., 2007; Lovisolo et al., 2010; Pou et al., 2012; Flexas 2016; Lavoie-Lamoureux et al., 2017). However, foliar ABA can also limit leaf gas exchange over the long-term, by preventing stomatal recovery upon rewatering. However, isohydry may favor embolism repair and preserves water under conditions of fluctuating water availability and repeated drought (Tombesi et al., 2015). On the other hand, anisohydric plants have a high tolerance to reduced water availability and do not significantly modify stomatal apertures as leaf water potential varies (Rogiers et al., 2012; Palliotti et al., 2015; Lavoie-Lamoureux et al., 2017), which in turn results in the maintenance of higher photosynthetic capacity, favoring faster recovery upon re-watering (Pou et al., 2012). Therefore, responses to environment constrains, such as reduced water availability, vary quite a lot within grapevine genotypes (Costa et al., 2012; Tomás et al., 2012) due to intrinsic efficiency of stomatal regulation, photosynthetic capacity and water use efficiency (Chaves et al., 2007; Costa et al., 2012; Tomás et al., 2014; Vaz et al., 2016).

Although restricted CO_2 diffusion across leaves is likely to be the most usual cause for decreased photosynthesis rates under water stress, especially when isohydric behavior is detected, other non-stomatal effects and metabolic impairments may also occur, particularly under severe water stress (Ribas-carbó et al., 2005). When drought progresses, the photochemistry and biochemistry of the photosynthesis can be affected, reducing grapevine's photosynthetic capacity (Flexas and Medrano 2002; Morales et al., 2006). Restrained PSII performance by increasing energy dissipation (Robredo et al., 2010; Yuan et al., 2013), decreased CO_2 concentration in the chloroplasts (Flexas et al., 2002) due to mesophyll conductance limitations (Flexas et al., 2010), oxidative stress (Vincent et al., 2007; Zhou et al., 2007), reduced carboxylation efficiency or reduced amount/activity of Rubisco (Long et al., 2004; Flexas et al., 2009; Salazar-Parra et al., 2015) as well as reduced capacity of regeneration of Rubisco (Medrano et al., 2003) have been reported as the main non-stomatal effects of reduced water availability in grapevines. In addition, differences in photoprotection mechanisms have been observed in grapevines, such as photorespiration and photoprotective state associated with xanthophyll de-epoxidation, both necessary to avoid further damage to photosynthesis under drought (Medrano et al., 2002).

Contrary to photosynthesis, and despite the importance of R to plant production, the effects of water stress on R are largely unknown since there is a limited number of studies available, which report conflicting results (Flexas et al., 2005; Schultz and Stoll 2010). The most probable causes of such contradictions can be related to species and technique used to measure R, interactions with environmental factors and water stress intensity, so that current results report increases, decreases or even no effect on R in response to reduced water availability (Flexas et al., 2005). In addition, leaf respiration rates have been reported to remain within narrow ranges during water stress (Flexas et al., 2006) since two respiratory pathways are present (cytochrome pathway and alternative pathway) (Lambers et al., 2005). Therefore, water stress induces a sharp decrease in the cytochrome respiration rate concomitant with a similar increase in the alternative respiration rate and hence, total respiration rate could remain quite constant (Ribas-Carbó et al., 2005). Furthermore, drought has been associated to substantially increase the degree of light inhibition of R, reflecting greater drought-mediated reductions in rates of R_{light} compared with R_{dark} (Ayub et al., 2011; Crous et al., 2012).

Since the ratio between net photosynthetic rates and leaf respiration can decrease markedly under drought (Flexas et al., 2005; Flexas et al., 2006; Atkin and Macherel, 2009; Ayub et al., 2011), grapevine's yield and quality can be influenced by water availability (Myburgh 2003). Indeed, negative effects of water stress on reproductive growth in grapevines have been widely reported (McCarthy, 1997; Petrie et al., 2004; Santesteban et al., 2011) and yield reduction is one of the most common effect caused by water stress in grapevines (Intrigliolo and Castel, 2010; Santesteban et al., 2011). During berry growth, drought restricts cell division, reducing berry size (Matthews and Anderson 1989; McCarthy 1997; Ojeda et al. 2002). At the beginning of berry developmental stage, berry growth is so sensitive to water deficit that such negative effects cannot be reversed even by supplemental irrigation during the following stages (Kizildeniz et al., 2015). In addition, berry size can indirectly affect phenolic content, including anthocyanins, possibly due to a modified skin surface-to-berry volume ratio (Ojeda et al., 2002; Roby and Matthews 2004; Koundouras et al., 2006).

2.4. Combined ecophysiological effects of reduced water availability and elevated [CO_2]

It has been predicted that increased atmospheric [CO_2] may cause global warming as well as changes in precipitation patterns (Kimball et al., 2001) so that drought occurrence will be more frequent, intense and erratic and will possibly affect regions not currently exposed to drought (Allen, 1994; Centrito et al., 1999). It is predicted that doubling atmospheric [CO_2] will result in decreases in soil moisture content up to 70% (Schultz 2000), what may force viticulturists to change agricultural practices currently applied, such as irrigation (Chaves et al., 2007). Studies considering the combination of two main components, such as elevated CO_2 concentration and water availability are scarce, because such studies are complex, difficult and expensive to run (Salazar Parra et al., 2010). However, since it is impossible to extrapolate plants’ responses to combined environmental conditions from the response derived from a single condition (Rampino et al., 2012), the interactive effects of reduced water availability and increased [CO_2] need to be studied.

Higher yield potentials of water-limited plants grown at elevated [CO_2] may be observed due to a better water status, resulted from lower stomatal conductance, which improves both the *WUE* and the intercellular CO_2 concentration (Chaves and Pereira 1992). Such reduced stomatal conductance and the consequent reduction in transpiration rates may increase soil moisture content (Salazar-Parra 2012). In line with this, Robredo et al. (2007, 2010) reported that the water deficit-mediated decreases of photosynthesis (CO_2 uptake and photochemical capacity) in grapevines (*Vitis vinifera)* were delayed under elevated [CO_2]. In addition, enhanced energy fluxes (absorbed photon flux, trapping flux, and electron transport flux) per active PSII have been reported in barley exposed to both elevated [CO_2] and drought (Zong et al., 2014). Therefore, it has been proposed that elevated [CO_2] ameliorates, mitigates or compensates for the negative impact of drought on plant growth (Wullschleger et al., 2002) and enables plants to remain turgid and functional for a longer period (Centrito et al., 1999). However, studies about the interactive effects of elevated CO_2 and reduced water availability have received less attention and most works have focused on absorption of extra C under increasing [CO_2] (Holtum and Winter 2010). Likewise, relatively few studies have quantified the degree of light inhibition of *R* in plants grown under such conditions (Crous et al., 2012), although leaf *R* can vary in response to both water availability and elevated [CO_2], as stated before in this review.

2.5. Alternative agricultural practices to improve grapevine cultivation

2.5.1. Deficit irrigation in grapevines

Agriculture uses *ca* 70% of global freshwater resources, implying that the supply of freshwater is an essential and limiting factor to crop productivity worldwide (Morison et al., 2008). In this sense, agriculture is facing a dual problem so that on one hand, higher yields are required, what is generally associated with using more water (Blum 2009; Molden et al., 2010), whereas reducing water supply is desirable to maintain the sustainability of present agriculture in many regions (Flexas et al., 2016). Therefore, improving the yield to water consumption ratio has become a major priority (Costa et al., 2007; Morison et al. 2008) and the use deficit irrigation (DI) techniques should be explored based on the ecophysiological knowledge of plants responses to such techniques (Chaves et al., 2007; Blum, 2009).

DI is a method of irrigation where the amount of water used is kept below the maximum level and the minor stress that develops is supposed to cause minimal effects on yield (English and Raja 1996). Moreover, DI does not require specific technical control (Chaves et al., 2010). Regulated Deficit Irrigation (RDI) and Partial

Rootzone Drying (PRD), in turn, are two specific DI techniques that tune water availability temporally (specific timing of the application - RDI) or spatially (alternating dry–wet zones - PRD) (Chaves et al., 2010). Under RDI, the water is supplied at levels below that required for full crop evapotranspiration (ET_c) during specific phenological stages, particularly after fruit set when fruit growth is less sensitive to reductions in water supply (Chaves et al. 2010, Tarara and Peña 2015). By contrast, under PRD, the two sides of grapevines' root system are alternately irrigated providing a spatial reduction in water availability, so that while one rootzone side is being wetted, the other half of the root system is allowed to dry (Chaves et al., 2010).

A major characteristic of DI strategies is to provide enough water to stabilize yield, but enabling imposition of some degree of water deficit that permits to save water while controlling vegetative vigor and positively influencing fruit quality (Fereres and Soriano 2007; Chaves et al., 2007; Costa et al., 2007; Geerts and Raes 2009). The use PRD, for example, is expected to induce abscisic acid (ABA) synthesis in the dried half of the rootzone, and lead to partial stomatal closure without reducing overall leaf water status (Dry and Loveys, 1998; Stoll et al., 2000; Antolín et al., 2006; Antolín et al., 2008; Collins et al., 2010). Such reductions in stomatal conductance and hence, in transpiration, are typically larger than the decrease in net photosynthesis and thus, increased water use efficiency without impairing plant development, yield or fruit quality can be observed (de Souza et al., 2005; Chaves et al., 2007; Pou et al., 2012; Kizildeniz et al., 2015). Indeed, PRD can promote sugar accumulation either as a result of inhibiting lateral shoot growth, which induces a reallocation of carbohydrates to fruits, or as a direct effect of ABA signaling on fruit ripening (Coombe 1989; Deluc et al., 2009). Likewise, RDI use is associated to ABA production and increased stomatal sensitivity to conditions driving water loss, and has been applied as an alternative to the use of plant growth regulators to improve berry color in grapevines (Balint and Reynolds 2013; Fuentes et al., 2014; Niculcea et al., 2014). In addition, RDI can increase the content of soluble solids and reduce the levels of titratable acidity, promoting early ripening (Gelly et al., 2004; Pérez-Pastor et al., 2007). However, such responses of both PRD and RDI are not always observed in the field (dos Santos et al., 2003; Gu et al., 2004; Intrigliolo and Castel, 2009), especially in grapevine plants that can behave as either isohydric or anisohydric in response to water availability in the soil

(Schultz 2003, Soar et al., 2006; Chaves et al., 2010; Tomás et al., 2014; Lavoie-Lamoureux et al., 2017). Indeed, maintaining similar Ψ values through ABA accumulation in response to reduced water availability is typically observed in isohydric plants (Poni et al., 2007; Lovisolo et al., 2010; Pou et al., 2012; Flexas 2016; Lavoie-Lamoureux et al., 2017), whereas anisohydric plants do not respond to exogenous ABA (Coupel-Ledru et al., 2017), and may present totally different responses, closing their stomata at water potentials well below those of well-watered or isohydric plants (Soar et al., 2006; Lavoie-Lamoureux et al., 2017). Therefore, there is a demand for knowledge about the ecophysiological responses of grapevine cultivars to DI strategies (Chaves et al. 2010, Flexas et al. 2010, 2016, Lavoie-Lamoureux et al. 2017), especially under tropical conditions, where studies are scarce.

2.5.2. Transparent plastic covering

Protected cultivation, using transparent plastic cover (TPC), has become a common practice in different climates, and can contribute for extending the range of grapevine cultivation around the world, from the traditional mediterranean regions to new tropical sites, such as in Brazil (Permanhani et al., 2017). TPC changes the microclimate of the cultivated area, which has major and sometimes contradictory effects on crop’s growth cycle, WUE, berry quality, yield and diseases incidence (Deluc et al., 2009; Roberto et al., 2011; Permanhani et al., 2017). It has been reported that TPC can either minimizes the damaging effects of high temperature in grapevines (Chavarria et al., 2010) or increase air temperature (Liu et al., 2012; Novello and de Palma 2008; Suvočarev et al., 2013). High air temperatures (over 40^{o} C) have been associated to either earlier bud break and harvest (Kamiloğlu et al., 2011; Roberto et al., 2011; Comiran et al., 2012) or negative impacts on ripening (Ferreira et al., 2004).

Although TPC may increase shading and delay harvest (Ferreira et al., 2004; Novello and de Palma 2008; Roberto et al., 2011) because of reduced sugar accumulation and anthocyanin content (Deluc et al., 2009), there are no doubts about the benefits of protecting grapevines’ canopy from adverse meteorological conditions, such as wind (which causes mechanical damages in both leaves and clusters) and rain (Roberto et al., 2011; Du et al., 2015; Permanhani et al., 2016).

While rainfall increases grapevine vegetative growth, it can reduce fruit quality due to imbalances in the sink/source ratio (Monteiro and Lopes 2007; Chaves et al., 2010). Moreover, leaf wetness caused by rainfall can trigger disease development, such as mildew and botrytis, increasing the use of fungicides and other chemicals (Botelho et al., 2011; Pedro et al., 2011; de Souza et al., 2015; Permanhani et al., 2016). In addition, TPC can increase water use efficiency in grapevines by creating higher humidity and lowering transpiration as compared to open field conditions (Stanghellini 2014, Permanhani et al., 2016). Indeed, lower evapotranspiration in response to TPC use in grapevine cultivation have been reported (Stanghellini 2014). Finally, higher chlorophyll contents can be also observed in grapevines grown under TPC (de Souza et al., 2015), what could improve the photosynthetic capacity of such plants.

3. CHAPTER 1

PHOTOSYNTHETIC ACCLIMATION TO ELEVATED CO_2 COMBINED WITH PARTIAL ROOTZONE DRYING RESULTS IN IMPROVED WATER USE EFFICIENCY, DROUGHT TOLERANCE AND LEAF CARBON BALANCE OF GRAPEVINES (*Vitis labrusca*)[1]

ABSTRACT

We considered the interactive effects of elevated CO_2 concentration [CO_2] and reduced water availability (Partial Rootzone Drying - PRD and drought) on a variety of important physiological and growth traits in grapevine (*Vitis labrusca*). The following questions were addressed: (i) Will there be a down-regulation of photosynthesis at elevated [CO_2] and what role do leaf nitrogen (N) concentration and specific leaf weight (SLW) have in this process? (ii) What are the effects of PRD under elevated [CO_2]? (iii) Can elevated [CO_2] delay the negative effects of drought in grapevines? and (iv) What is the impact of leaf respiration in the light (R_{light}) and in the dark (R_{dark}) on leaf carbon balance (LCB) of grapevines? Three water

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management treatments (*WMT*) were applied [full-irrigated - both sides of the rootzone were irrigated to saturation; PRD - only one side of the rootzone was irrigated to saturation; and non-irrigated - irrigation was suspended on both sides of the rootzone] in each of two 1.4 m^2 growth chambers, each one with different [CO$_2$] (400 ppm or 800 ppm). We found that: (i) net photosynthetic rate (A_{net}) is down-regulated under elevated [CO$_2$], which may be caused by reductions in Rubisco content and/or total activity and by a reduction in the efficiency of the photochemical apparatus due to N dilution within leaves with higher SLW; (ii) Under elevated [CO$_2$], Rubisco carboxylation rate is increased when PRD is applied, leading to increased A_{net}, thereby leading to greater water use efficiency (*WUE*) and intrinsic WUE (*iWUE*). However, these responses were not linked to photochemical or stomatal effects. (iii) Elevated [CO$_2$] delayed drought effects on both A_{net} and Rubisco activity for four days, by reducing stomatal conductance, transpiration and stomatal density; (iv) Leaf respiration responses depend on drought level; and (v) Conditions that reduce N concentration, such as elevated [CO$_2$], also reduce R_{dark} and R_{light}. In addition, elevated [CO$_2$] intensifies light inhibition of respiration and reduces Rubisco oxygenation, as well as improves LCB and contributes for mitigating deleterious effects of drought on LCB in grapevines.

Keywords: light inhibition of respiration; net photosynthetic rate; nitrogen concentration; rubisco oxygenation/carboxylation rates.

RESUMO

Nós consideramos os efeitos interativos da elevada concentração de CO$_2$ [CO$_2$] e da reduzida disponibilidade hídrica (Irrigação parcial do sistema radicular – PRD and estresse hídrico) sobre uma variedade de importantes parâmetros fisiológicos e de crescimento em videira (*Vitis labrusca*). As seguintes questões foram levantadas: (i) Ocorrerá o processo de *down*-regulação da fotossíntese sob elevada concentração de [CO$_2$] e qual o papel da concentração de nitrogênio foliar (N) e da massa foliar específica (SLW) nesse processo? (ii) Quais são os efeitos do PRD sob elevada [CO$_2$]? (iii) A elevada concentração [CO$_2$] pode retardar os efeitos negativos do estresse hídrico em videiras? e (iv) Qual é o impacto da respiração foliar na luz (R_{light}) e no escuro (R_{dark}) sobre o balanço de carbono foliar (LCB) de videiras? Três tratamentos de irrigação foram implementados [completamente irrigado (FI) – ambos os lados do sistema radicular eram irrigados até a saturação; irrigação parcial do sistema radicular (PRD) – apenas um dos lados do sistema radicular era irrigado até a saturação; e não-irrigado – a irrigação foi suspensa em ambos os lados do sistema radicular] em duas câmaras de crescimento, cada uma com 1.4 m^2 de área e com diferentes [CO_2] (400 ppm ou 800 ppm). Nosso trabalho mostrou que: (i) a taxa fotossintética líquida (A_{net}) é *down*-regulada sob elevadas [CO_2], o que pode ser causado tanto por reduções no quantidade e/ou atividade total da Rubisco, como por diminuições na eficiência fotoquímica, devido a um efeito de diluição do N em folhas que apresentam maiores SLW; (ii) Sob elevadas [CO_2], as taxas de carboxilação da Rubisco são elevadas quando PRD é utilizado, levando a um aumento em A_{net} e, consequentemente, a uma melhor eficiência no uso da água (*WUE*) e eficiência intrínseca no uso da água (*iWUE*). Entretanto, tais respostas não foram relacionadas a capacidade fotoquímica ou efeitos estomáticos. (iii) A elevada [CO_2] retarda em quatro dias os efeitos deletérios do estresse hídrico tanto sobre A_{net} como sobre a atividade da Rubisco, por meio de reduções na condutância estomática, na transpiração e na densidade estomática; (iv) a respiração foliar depende do nível de estresse hídrico; e (v) condições que reduzem a concentração de N, tais como elevada [CO_2], também reduzem R_{dark} e R_{light}. Além disso, a elevada [CO_2] intensifica a taxa de inibição da respiração pela luz, diminuem as taxas de oxigenação da Rubisco, melhoram o LCB e contribuem para mitigar os efeitos deletérios da seca sobre LCB em videiras.

Palavras-chave: concentração de nitrogênio; inibição da respiração pela luz; taxas de carboxilação/oxigenação da Rubisco; taxa fotossintética líquida.

INTRODUCTION

Global change and the associated impacts on crop production hold the attention of researchers worldwide. According to the IPCC report (2014), atmospheric CO_2 concentration [CO_2] is estimated to reach values between 750 and

1300 ppm by the end of the century. These increases in atmospheric [CO_2] are likely to be accompanied by global warming and changes in precipitation patterns (Kimball et al., 2001, Reich et al, 2016). According to Schultz (2000), an enhanced atmospheric [CO_2] could also result in a decrease in soil moisture content up to 70% imposing limitations to the cultivation of important crops, such as grapevines. Grapevine is considered to be the most widely cultivated fruit in the world, is grown on nearly every continent (FAOSTAT, 2014), and is likely to undergo a period of profound management changes (Flexas et al., 2010). Given the economic importance of grapevine cultivation, it is imperative to study the effects of predicted global change on the physiological underpinnings of production, as well as possible management alternatives that may be used to maintain this production.

The elevation of atmospheric [CO_2] can positively affect important physiological processes such as photosynthesis and respiration in grapevines and other C3 plants. The enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the primary carboxylating enzyme of photosynthesis, is known to be CO_2-limited under current atmospheric conditions while its oxygenation activity is not (Ainsworth and Rogers, 2007). For these reasons, grapevines' net photosynthetic rates (A_{net}) at twice the ambient [CO_2] concentrations could increase (Salazar-Parra et al., 2012; Salazar-Parra et al., 2015). However, when [CO_2] is increased, the maximum photosynthetic potential is not always maintained, a process known as down-regulation (Luo et al., 1994, Griffin and Seemann, 1996), and this process is known to vary with growth conditions and by species (Long et al., 2004; Aranjuelo et al., 2005, 2009a; Erice et al., 2006; Ainsworth and Rogers, 2007; Sanz-Sáez et al., 2010; Salazar-Parra et al., 2015, Rodrigues et al., 2016). Quantifying the complex photosynthetic responses to elevated [CO_2] is time consuming and difficult, requiring access to expensive instrumentation. Thus, proxies and/or indicators of photosynthetic acclimation based on simpler leaf traits would be useful for agronomist and land managers. Luo et al. (1994) proposed a simplifying model based on the specific leaf weight (SLW – g cm^{-2}) and Nitrogen (N) concentration (gN g^{-1}leaf) to predict the response of net photosynthetic rates to elevated [CO_2]. In this model photosynthesis is up-regulated if changes in SLW are larger than the decrease in N concentration, and photosynthesis is down-regulated if the decrease in N concentration is larger than the increase in SLW. However, the precise physiological mechanism behind Luo's et al. (1994) model remains unknown.

One possible response to the potentially negative effects of global change would be the use of water-saving irrigation techniques, such as Partial Root-zone Drying (PRD). The PRD technique alternates the irrigation of the two sides of the grapevines' root system. This in turn induces root abscisic acid (ABA) synthesis in the dried half of the rootzone, and results in partial stomatal closure without reducing overall leaf water status in isohydric species (Dry and Loveys 1998, Antolín et al. 2006, 2008). In addition, the observed reductions in stomatal conductance and transpiration are typically larger than the decrease in A_{net}. Thus, PRD results in increased water use efficiency without impairing plant development, yield or fruit quality (Stoll et al., 2000; Gaudillère et al., 2002; Chaves e Oliveira, 2004; Chaves et al., 2007; de Souza et al., 2004, 2005; Pou et al., 2012). Since water scarcity resulting from global change could impose limitations to grapevine production and force viticulturists to rely on more efficient irrigation (Chaves et al. 2007; Flexas et al., 2010), PRD technique may be more routinely used in the future. However, there are still no studies of how this technique will interact with elevated [CO_2] or the resulting combined effects on grapevine physiology.

Elevated [CO_2] induces other physiological responses such as reduced stomatal conductance and transpiration rates (Leakey et al., 2009; Salazar-Parra et al., 2012 and 2015; Flexas et al., 2014) as well as morphological changes like reduced stomatal density (Moutinho-Pereira et al. 2009; Rogiers et al., 2011). Such changes could further impact water use efficiency and result in greater drought tolerance (Wullschleger et al., 2002). Thus, quantifying the stomatal and non-stomatal responses (*e.g*. Rubisco carboxylation/oxygenation rates and/or the integrity of the photochemical apparatus) behind the interactive effects of drought and elevated [CO_2], could provide valuable information relevant to the cultivation of economically important crops like grapevines.

Leaf respiration (*R*) is a second key component to the leaf carbon balance and therefore plant growth. Like photosynthesis, *R* is also a physiological process known to respond to global change (Griffin and Seemann, 1996; Flexas et al., 2006; Crous et al., 2012). Functionally leaf respiration is fundamental to plant life, sustaining growth and maintenance, ion transport and compartmentalization, protein turnover and tissue acclimation to environmental change (Amthor 2000, Sharma-Natu and Ghildiyal 2005). Furthermore, although leaf respiration continues 24 hours a day for as long as the plant is alive, the biochemistry of respiration is reorganized in the light as is the assumed function and use of the respiratory products (Tcherkez et al., 2012; Griffin and Heskel, 2013). Thus, it is important to quantify both the rates in the dark (R_{dark}) and in the light (R_{light}). Reports investigating the response R to elevated CO_2 and drought are varied and/or limited, and therefore further empirical testing is required to elucidate these responses and quantify the impact on grape production. Regarding elevated [CO_2], there are studies reporting R is stimulated (Davey et al. 2004; Shapiro et al. 2004; Robredo et al., 2010; Crous et al., 2012) or inhibited (Reuveni et al., 1995; Gonzàlez-Meler et al., 1996). The same is true for the response to drought conditions, where results report increases, decreases or even no effect on R (Flexas et al., 2005). Furthermore, it is known that R_{light} is usually lower than R_{dark}, with the degree of light inhibition responding differently to environmental signals (Tcherkez et al. 2010), such as water availability (Ayub et al., 2011; Crous et al., 2012) and CO_2 concentration (Shapiro et al. 2004; Ayub et al., 2011 and 2014; Crous et al., 2012; Griffin and Turnbull, 2013). Lastly, the rates of both R_{light} and R_{dark} have important consequences for plant and ecosystem carbon balance as they are a variable proportion of the CO_2 assimilated by photosynthesis that goes back to the atmosphere (Ayub et al., 2014). Therefore, changes in R_{light} and R_{dark} in response to drought and elevated [CO_2] must be carefully studied to better understand this important physiological process, which can influence future grapevine production.

We consider the interactive effects of elevated [CO_2] and reduced water availability (PRD application and drought induction) on a variety of important physiological and growth traits in grapevine. We stress that it is not possible to extrapolate plant responses to combined environmental conditions from the response derived from a single condition (Rampino et al., 2012). Thus, we undertook an investigation to explicitly answer the following questions: (i) Considering the model proposed by Luo et al. (1994), will there be a down-regulation of photosynthesis at elevated [CO_2] and what role do leaf nitrogen (N) concentration and specific leaf weight (SLW) have in this process? (ii) What are the effects of PRD under elevated [CO_2]? (iii) Can elevated [CO_2] delay the negative effects drought on grapevines? (iv) and how do R_{light} and R_{dark} respond to drought and elevated [CO_2] and how much does this impact leaf carbon balance in grapevines?

MATERIAL AND METHODS

Plant material, growth conditions and PRD treatments

Rooted ‘Concord’ (*Vitis labrusca*) grapevine cuttings were obtained from a commercial nursery (Kelly Wholesale Nurseries INC., Phelps, NY, US) and planted in two 8.4 L pots, joined one to the other so that the root system could be divided equally to prevent moisture exchange between pots. A commercial substrate (Miraclo-Gro® Potting Mix) composed of peat moss, forest humus, coconut coir, perlite, compost, worm casting and fertilizer (Ammoniacal Nitrogen – 0.12%; Nitrate Nitrogen – 0.09%; Available Phosphate, P_2O_5 – 0.07%; Soluble Potash, K_2O – 0.14% and water soluble iron, Fe – 0.10%) was used. Plants were thinned during the experiment to allow for two branches to grow per plant. Thirty-six plants were grown, equally divided, and placed into one of two 1.4 m^2 growth chambers equipped with the CO_2 enrichment package (Percival® model PGC-15, ETA Process Instrumentation, Peabody, MA, US) at the Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY, USA. Maximum plants’ height allowed inside the chambers was 1.2 m. Chambers interior walls were painted with highly reflective white powder coating and lighting was provided by a fluorescent lamp bank, located *ca.* 0.7 m above the top of the plants so that light irradiance throughout the chambers was uniform. Conditioned air could move in uniform upward direction inside the chambers, that were also fitted with hot gas bypass refrigeration system to provide heating and cooling, as well as with ultrasonic humidifiers to regulate the relative humidity. Environmental characteristics of the two growth chambers were automatically and precisely set using the IntellusUltra^{tm} Controller (Percival®, Intellus Control System, ETA Process Instrumentation, Peabody, MA, US). This software allowed the effective control and monitoring of the environmental features such as temperature, relative humidity (RH), lighting and [CO_2]. Air temperatures were maintained at 22 ± 1°C/11 ± 1 °C (day/night) and relative humidity (RH) at 67 ± 1 %/88 ± 3 % (day/night). The photon flux density of photosynthetically active radiation was 500 $\mu mol.m^{-2}.s^{-1}$ at the top of the plant canopy during the entire 13 h photoperiod. During the first six weeks, all plants were grown at 400 ppm of [CO_2]. Thereon, two [CO_2] conditions were applied in the two chambers, 400 ppm and 800 ppm, by adding CO_2 from a high-pressure cylinder, or scrubbing with soda lime as needed.

All pots were watered until saturation, 3 days a week, for 6 weeks. Thereafter, the plants were exposed to one of three water management treatments: (1) full-irrigated - FI: both sides of the rootzone were irrigated 3 days a week to saturation; (2) partial rootzone drying - PRD: only one side of the rootzone was irrigated to saturation 3 days a week, for 10 days (four irrigation events), whereas the other side of the rootzone remained without water. After this period the treatment was reversed twice, and the alternate side of the root system received water; (3) and non-irrigated - NI: irrigation was suspended for 24 days after initiating treatments (DAIT) on both sides of the rootzone. Thus, six replicate plants of each water management treatment were grown in each of the two growth chambers with different [CO_2] (400 ppm and 800 ppm). All analyses (outlined later) were performed on one fully expanded leaf from the first and oldest branch, in each replicate.

To determine the amount of water required to saturate the substrate, both pots of one model plant from the same treatment was irrigated with a known volume of water to saturation. Outflow from the pot was collected in a graduated cylinder and watering was stopped when water was observed to be leaking from the bottom of the pots. The difference between the volume of this leaked water and the amount water applied was then applied to each pot of the other plants in this treatment. Over the first six weeks, this amount of water was applied in both pots for every plant. After this initial period the PRD treatments were initiated and irrigation proceeded as described above.

The 10 day duration of the PRD treatment prior to alternating the side of the root system irrigated corresponded to the time required for the substrate to dry only by evaporative process. This was determined experimentally prior to the initiation of the treatments, using a pot full of substrate with no plant, which was irrigated until saturation and repeatedly weighed until it reaches a constant mass (Data not shown).

Nitrogen concentration, specific leaf weight, and stomatal density

At 24 DAIT, the leaves were collected and their area (cm^2) was measured using a portable area meter (LI-3000C, Li-Cor, Lincoln, NE, USA). These same leaves were used for determination of abaxial stomatal density. A thick layer of clear nail polish was applied to the abaxial side of each leaf. After the nail polish was dry, clear tape was used to carefully peel away the varnish from the leaf. The impression was placed on a slide and viewed under 400X magnification in a light microscope (Zeiss, Jena South, Thuringia, GE). A representative section of the leaf was chosen and the stomata densities were calculated. The same leaves were then dried in an air forced ventilation oven at 70°C for 72 h and weighed to determine the leaf dry weight (LDW in grams) and then, the specific leaf weight (SLW) (leaf dry mass/leaf area). Thereafter, dried leaves were grounded in a ball mill (8000 Mixer Mill, Spex samplePrep, Metuchen, NJ, USA) and a range of 2 – 4 mg weighed out in tin capsules was analyzed in a CHN analyzer (Elemental Analyzer FlashEA 1112 series, Thermo Fisher Scientific Inc, Waltham, MA, USA) for total Nitrogen.

Gas exchange

Base line gas exchange parameters were obtained seven days before initiating treatments (DBIT) and then two days a week throughout the experiment, from the 3rd to the 24th DAIT. Light response curves of net photosynthetic rate (A_{net}) using a Li-Cor 6400xt portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) were made as described in Shapiro et al. (2004), and taking into consideration the precautions of Pons and Welschen (2002). The system incorporated a CO_2 controller which was used to set the [CO_2] inside the Li-Cor cuvette so that during light response curve measurements, the [CO_2] inside the Li-Cor cuvette matched the growth [CO_2] within each chamber (400 or 800 ppm). The 6 cm^2 cuvette was fitted with a red–blue light source (6400-02B). The net photosynthetic rate (A_{net}), stomatal conductance (g_s) and transpiration rate (E), all at 500 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) were determined from the light response curves with 24 levels of PPFD: 1500, 1200, 800, 500, 200, 100, 90, 80, 70 and every 5 PPFD between 70 and 0 µmol m^{-2} s^{-1}. The 500 µmol m^{-2} s^{-1} light level was previously determined to be the light saturation point and was also the light intensity inside the chamber (data not shown). The rate of respiration in the dark (R_{dark}) was taken as the value of A_{net} at 0 µmol m^{-2} s^{-1} PPFD.

Rate of respiration in the light (R_{light}) was estimated using the method originally described in Kok (1948), as the y-axis intercept of a first-order linear regression fitted to A_{net}–irradiance plots to measurements made over the 25–65 μmol m^{-2} s^{-1} irradiance range. All gas exchange data were corrected for the increase in intercellular CO_2 concentrations (C_i) with decreasing irradiance, which can result in reduced rates of photorespiration and increased rates of carboxylation (Villar et al. 1994). The correction was applied by adjusting the R_{light} through iteration to minimize the intercept of photosynthetic electron transport (J) as a function of irradiance (Kirschbaum and Farquhar 1987). J was calculated according to Farquhar and von Caemmerer (1982):

$$J = \frac{[\left(4\left(A_{net} + R_{light}\right)\right)(C_i + 2\Gamma^*)]}{(C_i - \Gamma_*)} \qquad (1)$$

where Γ* is the CO_2 compensation point in the absence of R_{light} (von Caemmerer and Farquhar 1981). Rates of oxygenation and carboxylation by Rubisco (V_o and V_c, respectively) at saturating irradiance were also calculated according to Farquhar and von Caemmerer (1982):

$$V_c = \frac{1}{3}\left[\left(\frac{J}{4}\right) + 2\left(A_{net} + R_{light}\right)\right] \qquad (2)$$

and

$$V_o = \frac{2}{3}\left[\left(\frac{J}{4}\right) - \left(A_{net} + R_{light}\right)\right] \qquad (3)$$

The effects of varying atmospheric $[O_2]$ or $[CO_2]$ on oxygenation (V_o) and carboxylation (V_c) at saturating light were similarly calculated according to Farquhar and von Caemmerer (1982):

$$V_c = \frac{[CO_2]V_{cmax}}{[CO_2] + K_c\left(1 + \frac{[O_2]}{K_o}\right)} \qquad (4)$$

and

$$V_c = \frac{[O_2]V_{omax}}{[O_2] + K_o\left(1 + \frac{[CO_2]}{K_c}\right)} \qquad (5)$$

Equations 4 and 5 used the Rubisco kinetic constants (K_c, K_o and V_{omax}/V_{cmax}) previously determined by Bernacchi, et al. (2002), and calculated V_{cmax} as:

$$V_{cmax} = \frac{A_{net} - R_{light}}{\frac{[CO_2] - \Gamma^*}{[CO_2] + K_c\left(1 + \frac{[O_2]}{K_c}\right)}} \qquad (6)$$

Γ * (the CO_2 compensation point in the absence of R_{light}), depends on the Rubisco specificity factor, O_2 partial pressure and is calculated according to von Caemmerer and Farquhar (1981):

$$\Gamma^* = \frac{0.5V_{omax}K_c[O_2]}{V_{cmax}K_o} \qquad (7)$$

Here we use the specificity presented in Cousins et al. (2010), the ambient O_2 concentration and the temperature response function of Brooks and Farquhar (1985) to determine Γ*. All rates of V_o and V_c reported here were calculated at ambient CO_2 and O_2 concentration and PPFD of 500 µmol m^{-2} s^{-1} ($V_{o\,500}$ and $V_{c\,500}$).

The degree of light inhibition of respiration was determined by R_{light}/R_{dark} ratio. The A_{net}, g_s and E values were used to calculate the water use efficiency (*WUE*) and the intrinsic water use efficiency (*iWUE*) as the slope of the linear relationship between A_{net} and E or A_{net} and g_s, respectively. In addition, leaf carbon balance (*LCB*) for the experiment was estimated using the following equation:

$$LCB = \frac{A_{net}}{(R_{dark} + R_{light})} \qquad (8)$$

All gas exchange measurements were taken at a relative humidity of approximately 65-75% by manipulating the amount of air passing through a drying column prior to entering the leaf cuvette. The Li-Cor cuvette block temperature was set to 22°C for all measurements to account for the influences of leaf temperature on gas exchange variables. In addition, the flow rate was kept at 300 µmol m^{-2} s^{-1}. Since we aimed to evaluate whether there was *down-regulation* due to the elevated [CO_2] (800 ppm) or not, additional gas exchange measurements were performed in plants grown under 800 ppm of CO_2. Therefore, after obtaining the light response curves, A_{net} for plants grown under 800 ppm of CO_2 was determined at a single light level of 500 µmol m^{-2} s^{-1} and the [CO_2] inside the Li-Cor cuvette was set at 400 ppm. Thus, we obtained two different A_{net} values for plants grown under elevated [CO_2]: one from the light response curves with the [CO_2] inside Li-Cor cuvette matching the growth [CO_2] (800 ppm) and another from the measurements taken with the [CO_2] inside the Li-Cor cuvette set at 400 ppm.

Chlorophyll a fluorescence

Maximum quantum yield of primary photochemistry (F_v/F_m ratio); the quantum yield of electron transport (ET_0/ABS); the efficiency (ET_0/TR_0) with which a trapped excitation energy can move an electron into the electron transport chain further than the Quinone A (QA) and the dissipation per excited cross-section (DI_0/CS) were measured using a modulated fluorometer model OS30p+ (Opti-Sciences, Hudson, NH, US) on the same days when gas exchange was measured. The leaves were dark-adapted for *ca.* 30 min using leaf clips (Opti-Sciences, Hudson, NH, US), so that all reaction centers of photosystem II (PSII) acquired an ‘open’ status, and heat loss was minimal (Strasser et al, 2000) and then, a “JIP” Test protocol (Strasser et al., 2004) was performed using a single strong pulse of light 3 s^{-1} (3500 μmol m^{-2} s^{-1}) applied with the aid of the array of red LEDs (650 nm). These analyses were performed seven DBIT and then two days a week throughout the experiment, from the 3rd to the 24th DAIT.

Statistical analysis

A complete randomized design in a split plot scheme was used with 2 [CO_2] x 3 water management x 6 replications, totaling 36 plots. Nitrogen concentration, stomatal density, SLW, A_{net}, E, g_s, $V_{c\ 500}$, V_{o500}, R_{light}, R_{dark}, R_{light}:R_{dark} ratio and LCB were subjected to analysis of variance tests and the mean pairwise comparisons made using the Tukey HSD test at 5% probability. Liner regression slopes for *WUE* and *iWUE* were calculated. Statistical analysis was made using the software ASSISTAT 7.0 BETA.

RESULTS

Overall, the plants presented the following features at seven DBIT: A_{net} = 4.57 ± 0.51 μmol CO_2 m^{-2} s^{-1}; E = 1.51 ± 1.29 mmol H_2O m^{-2} s^{-1}; g_s = 0.19 ± 0.02 mol H_2O m^{-2} s^{-1}; F_v/F_m ratio = 0.64 ± 0.01; ET_0/AB_S = 0.35 ± 0.06; ET_0/TR_0 = 0.54 ± 0.09; DI_0/CS = 110.09 ± 18.34. When these data were divided into the respective

[CO_2] concentration *versus* water management treatments and subjected to variance analysis, no significant differences were found (data not shown), indicating that the plants were homogeneous before initiating the treatments.

Nitrogen concentration, specific leaf weight, and stomatal density:

The increase in [CO_2] from 400 ppm to 800 ppm influenced leaf N concentration. By the end of the experiment, the plants grown under 800 ppm of CO_2 had a statistically lower N concentration than the plants grown under 400 ppm of CO_2 (Fig. 1 – A). This 18% reduction in N concentration was larger than the observed increase in SLW, which was only 16% higher in plants grown under elevated compared to ambient CO_2 and was not statistically significant (Fig. 1 – B). Stomatal Density was significantly different between the two [CO_2] treatments, where the leaves of the plants grown under 800 ppm had 25% fewer stomata than the plants grown under 400 ppm of CO_2 (Fig. 1 - C). Overall, the parameters outlined above were not affected by water management treatments (data not shown).

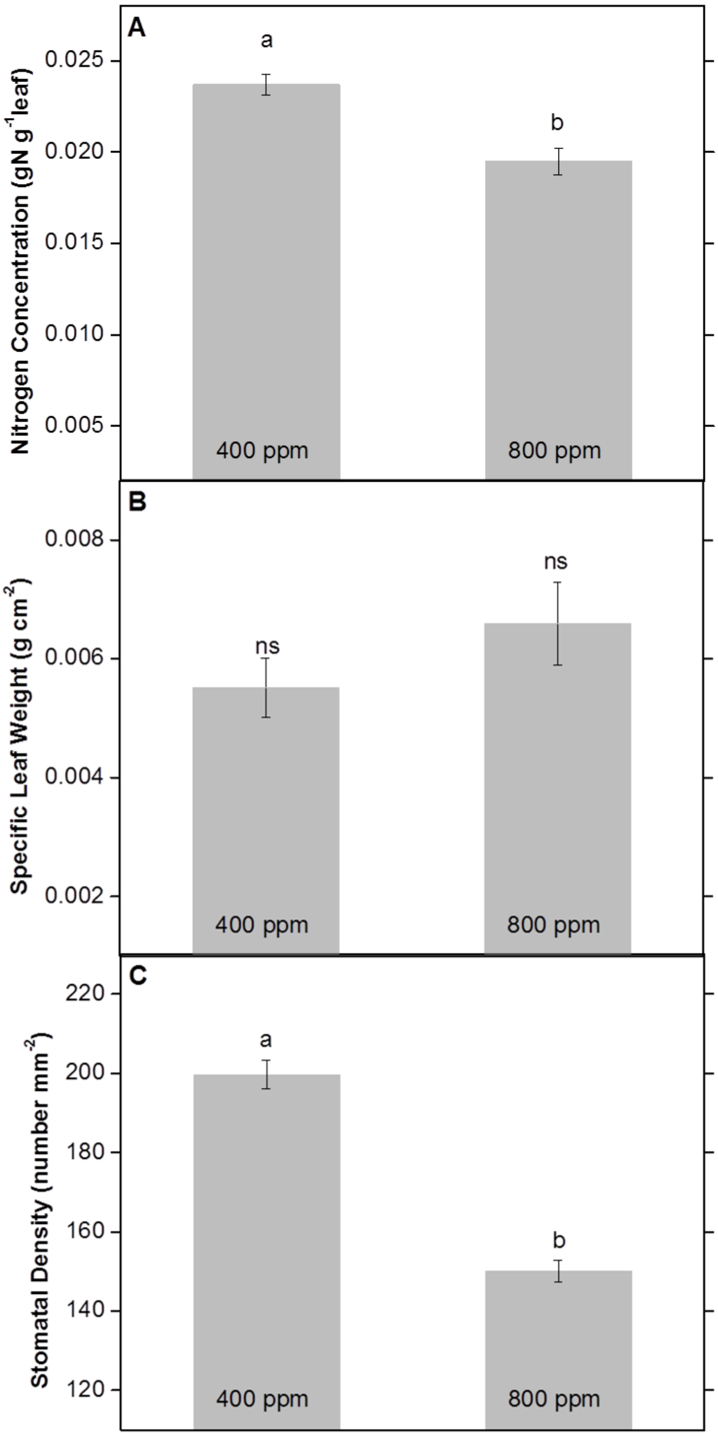

Figure 1. Leaf nitrogen concentration (A), specific leaf weight (B) and stomatal density (C) of grapevine at 24 DAIT grown under either 400 ppm or 800 ppm [CO_2]. Each bar represents the mean of 18 replicates. Means followed by different letters differ statistically by Tukey's test at 5 % probability. *ns* indicates no statistical difference.

Impacts of elevated [CO_2] and different water management on A_{net}, E, g_s and water use efficiency

Overall, PRD did not reduce A_{net}, g_s or E, under either 400 ppm or 800 ppm of CO_2 (Fig. 2 – A, D and G). However, from 13 DAIT onward, PRD plants grown under 800 ppm of CO_2 did have a significantly higher A_{net} than the FI plants under the same [CO_2] (Fig. 2 – B). Water shortage resulted in a decrease in A_{net} in the NI treatments, starting at 13 DAIT for the NI plants grown under 400 ppm, and at 17 DAIT for the NI plants grown under 800 ppm (Fig. 2 – A and B). By 20 DAIT and 24 DAIT, respectively, A_{net} was close to zero in these plants. The decrease in photosynthesis was associated with stomata closure, which occurred from 13 DAIT for the NI plants grown under 400 ppm, and from 20 DAIT for the NI plants grown under 800 ppm (Fig. 2 – D and E). Similarly, there were significant reductions in E, starting at 20 DAIT for both CO_2 concentrations (Fig. 2 - G and H).

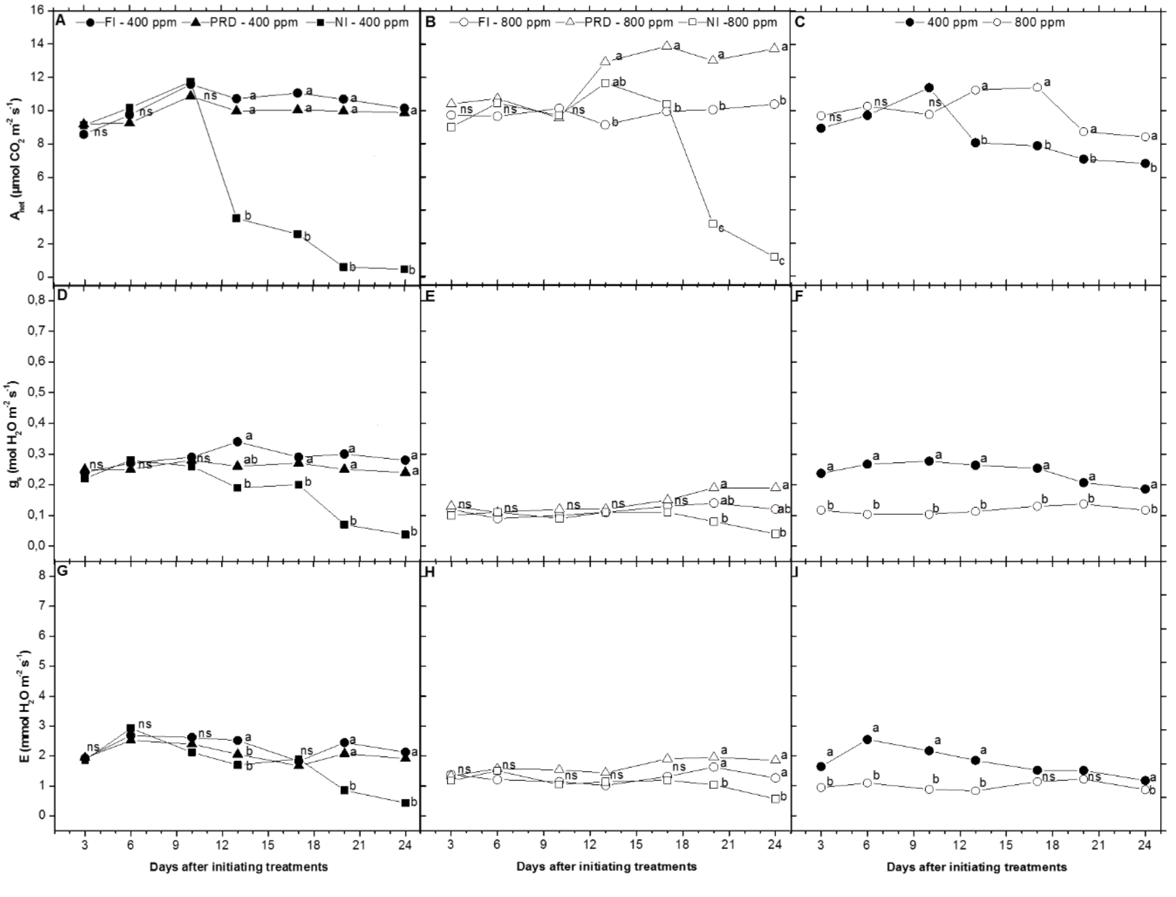

Figure 2. Net photosynthetic rate (A_{net}) (A, B and C), Stomatal Conductance (g_s) (D, E and F) and Transpiration (G, H and I) of grapevine grown under three water management treatments - Full-irrigated (FI); partial rootzone drying (PRD) and non-irrigated (NI) - and either 400 ppm (A, D and G) or 800 ppm [CO_2] (B, E and G) - and the mean CO_2 effect regardless water management treatment (C, F and I). Each point represents the mean of 6 (A, B, D, E, G and H) or 18 (C, F and I) replicates. Means followed by different letters differ statistically by Tukey’s test at 5 % probability within the same day. *ns* indicates no statistical difference.

Regardless of water management treatments, from 13 DAIT onward, plants under 800 ppm of CO_2 had significantly higher A_{net} than the plants grown under 400 ppm of CO_2. However, these increases in A_{net} with to a doubling of [CO_2] were only *ca.* 24% higher (Fig. 2 – C). Additionally, the increase on A_{net} was observed only when the measurement [CO_2] matched the growth [CO_2] (Fig. 2 - C and Fig. 3 - A and B). Plants grown under 800 ppm of CO_2 had lower A_{net} when measured at 400 ppm of CO_2 than at 800 ppm and this rate was lower than that in plants grown and measured at 400 ppm of CO_2. These reductions were *ca.* 43% and 52%, respectively, throughout the experiment, indicating a *down-regulation* of A_{net}. The plants grown under 800 ppm of CO_2 showed significantly lower g_s throughout all the experiment, and often significantly lower *E*, compared to the plants grown under 400 ppm of CO_2 (Fig. 2 – C, F and I).

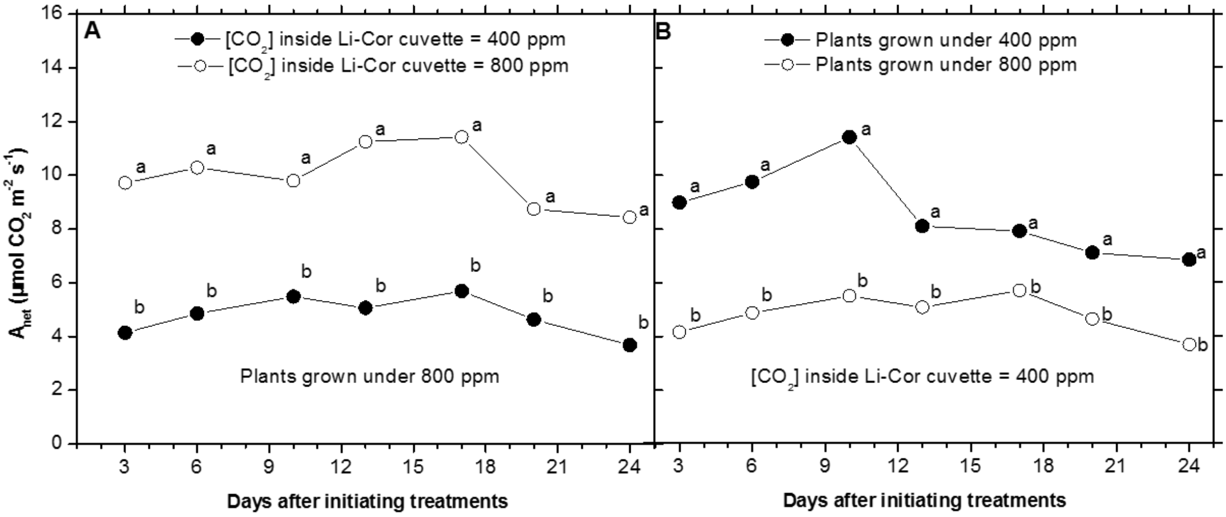

Figure 3. Down-regulation effects: Net photosynthetic rate (A_{net}) of grapevine measured at either 400 or 800 ppm of CO_2, for the plants grown under 800 ppm of CO_2 (A) and measured at 400 ppm for plants grown under either 400 or 800 ppm of CO_2 (B). Each point represents the mean of 18 replicates. Means followed by different letters differ statistically by Tukey's test at 5 % probability within the same day. *ns* indicates no statistical difference.

Within the same CO_2 concentration, both, *WUE* and *iWUE* were higher for the PRD treatments (*WUE* = 2.08 and 4.27 µmol CO_2/mmol H_2O^{-1} and *iWUE* = 18.45

and 33.95 µmol CO_2/mol H_2O^{-1}, for plants grown under 400 and 800 ppm of CO_2, respectively) and for the NI treatments (*WUE* = 3.71 and 6.05 µmol CO_2/mmol H_2O^{-1} and *iWUE* = 25.02 and 60.51 µmol CO_2 / mol H_2O^{-1} for plants grown under 400 and 800 ppm of CO_2, respectively), when compared to FI treatments (*WUE* = 1.01 and 3.04 µmol CO_2/ mmol H_2O^{-1} and iWUE = 7.72 and 30.45 µmol CO_2/ mmol H_2O^{-1} for plants grown under 400 and 800 ppm of CO_2, respectively) (Fig. 4). Plants grown under 800 ppm of CO_2 had higher values of *WUE* and *iWUE* than the correspondent 400 ppm CO_2 treatments with the same irrigation treatment (Fig. 4).

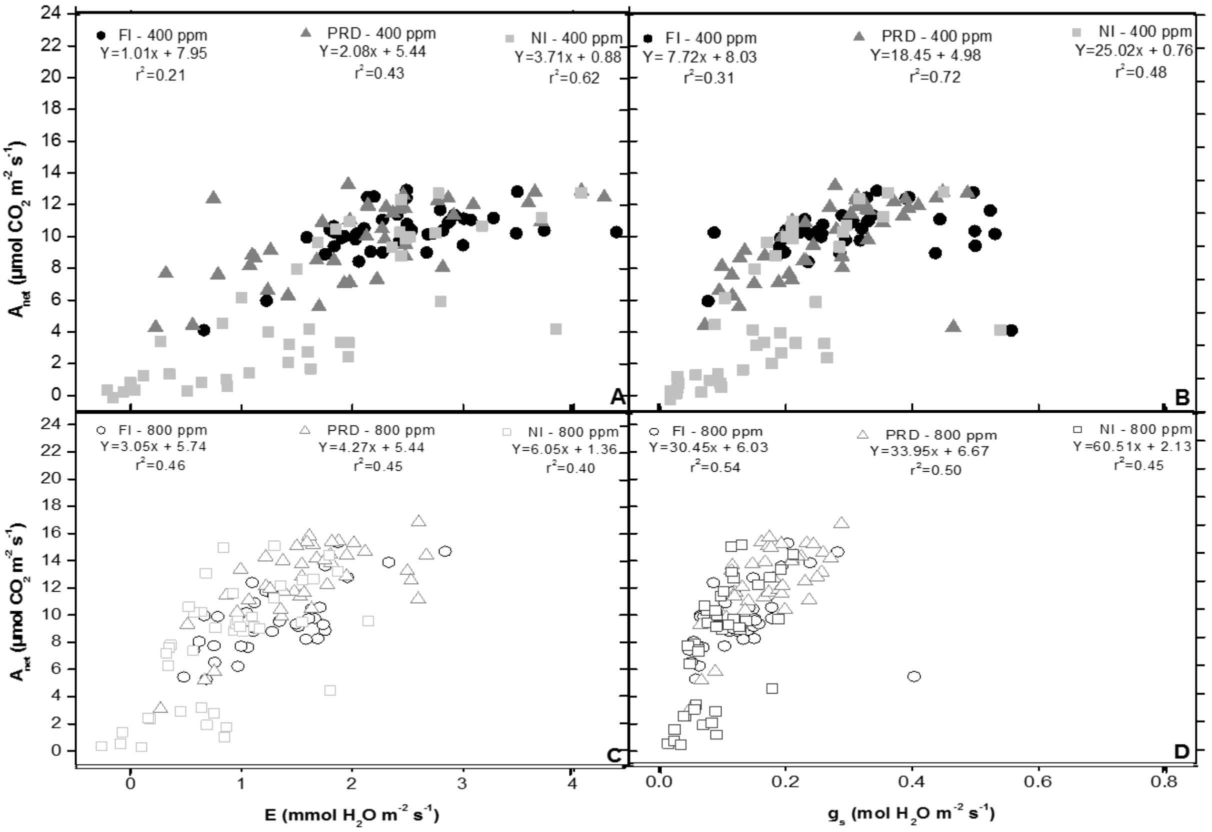

Figure 4. Water Use Efficiency (*WUE*) (A and C) and Intrinsic Water Use Efficiency (*iWUE*) (B and D) of grapevine grown under either – 400 (A and B) or 800 ppm [CO_2] (C and D) - and three water management treatments - Full-irrigated (FI); partial rootzone drying (PRD) and non-irrigated (NI). Each point represents one unit of data collected over the experimental period.

Rubisco carboxylation/oxygenation rates

Rubisco carboxylation rate at 500 µmol m^{-2} s^{-1} PPFD ($V_{c\ 500}$) did not differ between the FI and PRD treated plants grown under 400 ppm of CO_2. However, at 800 ppm of CO_2, the PRD plants had significantly higher $V_{c\ 500}$ than the FI plants from 17 to 24 DAIT. As expected, there was a reduction in $V_{c\ 500}$ of NI plants compared to plants from the FI and PRD treatments. These reductions began at 13 and 17 DAIT for the NI plants grown under 400 and 800 ppm of CO_2, respectively, reaching the lowest values at 24 DAIT (0.5 and 1.5 µmol.m^{-2}.s^{-1}, respectively) (Fig. 5 – A and B). The reduction in $V_{c\ 500}$ in NI plants grown under 400 ppm of CO_2 reduced the overall mean $V_{c\ 500}$ of all plants grown under 400 ppm of CO_2 at 13 and 17 DAIT regardless of water management (*ca.* 14.5% reduction, Fig. 6 - A). However, when only FI plants were compared, from 17 DAIT onward, $V_{c\ 500}$ was *ca.* 19 ± 3% lower in the plants grown under 800 ppm of CO_2 (Fig. 6 – B).

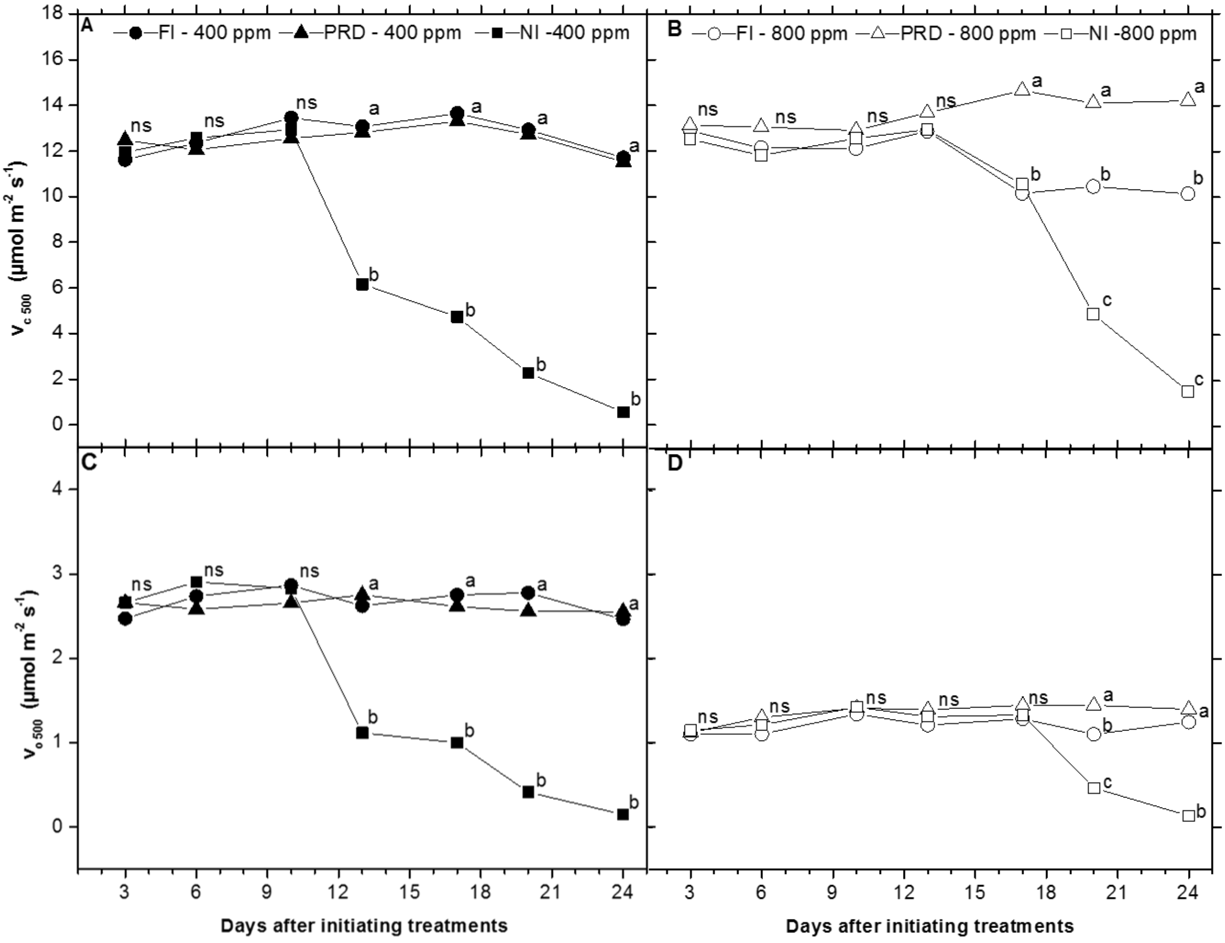

Figure 5. Rubisco carboxylation ($V_{c\ 500}$) (A and B) and oxygenation ($V_{o\ 500}$) (C and D) of grapevine grown under three water management treatments - Full-irrigated (FI); partial rootzone drying (PRD) and non-irrigated (NI) - and either 400 ppm (A and C) or 800 ppm [CO_2] (B and D). Each point represents the mean of 5 replicates. Means followed by different letters differ statistically by Tukey test at 5 % probability within the same day. *ns* indicates no statistical difference.

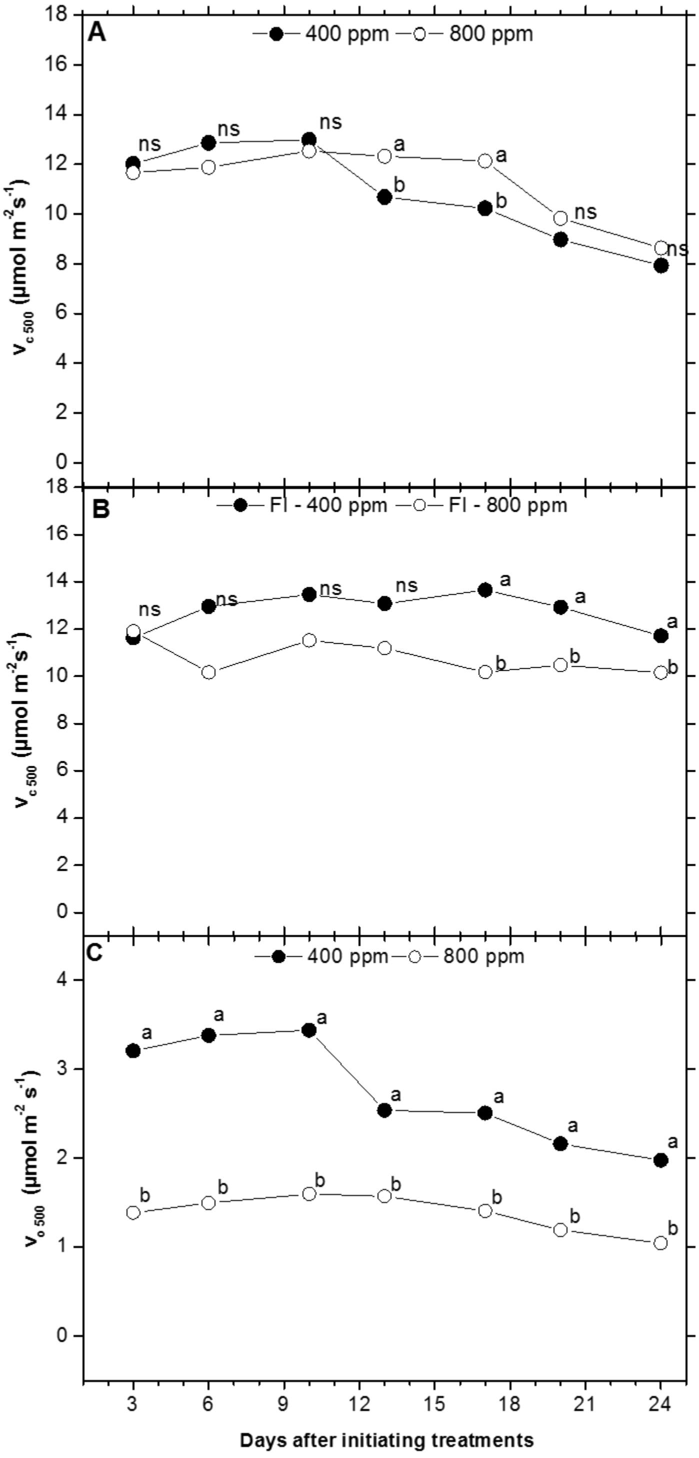

Figure 6. Rubisco carboxylation ($V_{c\ 500}$) for all water management treatments (A) and Full-irrigated (FI)(B) and oxygenation ($V_{o\ 500}$) for all water management treatments (C) of grapevine grown under either 400 ppm or 800 ppm [CO_2]. Each point represents the mean of 15 (A and C) or 5 (B) replicates. Means followed by different letters differ statistically by Tukey's test at 5 % probability within the same day. *ns* indicates no statistical difference.

Overall, the calculated rate of Rubisco oxygenation at 500 µmol m^{-2} s^{-1}PPFD ($V_{o\ 500}$) did not differ between the FI and PRD treated plants grown under, either 400 or 800 ppm CO_2 (Fig. 5 - C and D). In the NI plants, $V_{o\ 500}$ was significantly reduced relative to the FI and PRD treated plants (under the same CO_2 concentration) (Fig. 5 - C and D). These effects appeared on the same days as the reduction in $V_{c\ 500}$, reaching values close to zero at 24 DAIT (Fig. 5 – C and D). Throughout the experiment, regardless of water management treatments, $V_{c\ 500}$ in plants grown under 800 ppm of CO_2 was 49 ± 3% lower than in plants grown under 400 ppm CO_2 (Fig 6 – C).

Chlorophyll fluorescence responses

Generally, there were no significant interactions between irrigation treatments and growth CO_2 concentrations on chlorophyll *a* fluorescence. Similarly, there were no overall differences in fluorescence between FI and PRD treatments (data not shown). Effects of the growth CO_2 concentration on the biophysical parameters of the Chlorophyll a Fluorescence (JIP_{test} - Strasser et al., 2000) are shown in Fig. 7. Beginning 6 DAIT, the F_v/F_m of all treatments were low and remained so throughout the duration of the experiment, probably due to the relatively low light intensity inside the growth chambers (500 µmol m^{-2} s^{-1}). F_v/F_m averaged 0.71 ± 0.003 for plants grown under 400 ppm of CO_2 and 0.69 ± 0.008 for plants grown under 800 ppm of CO_2. At 10, 13, 17 and 20 DAIT, the plants grown under 800 ppm of CO_2 had *ca.* 4.2% lower F_v/F_m compared to the plants grown under 400 ppm of CO_2 (Fig. 7 - A). Similarly, at 6, 10, 13, 17 DAIT, plants grown under 800 ppm of CO_2 had *ca.* 17.0% lower ET_0/ABS and *ca.* 12.7% lower ET_0/TR_0 than the plants grown under 400 ppm of CO_2 (Fig. 7 – B and C). Concomitantly, the plants grown under 800 ppm had a significantly higher DI_0/CS_0 at 6, 10, 13, 20, 24 DAIT (*ca.* 24.70%) (Fig. 7 - D). Thus, regardless of irrigation treatment, there is a trend in all parameters for the plants grown under 800 ppm of CO_2 having less efficiency in the flow of energy through PSII.

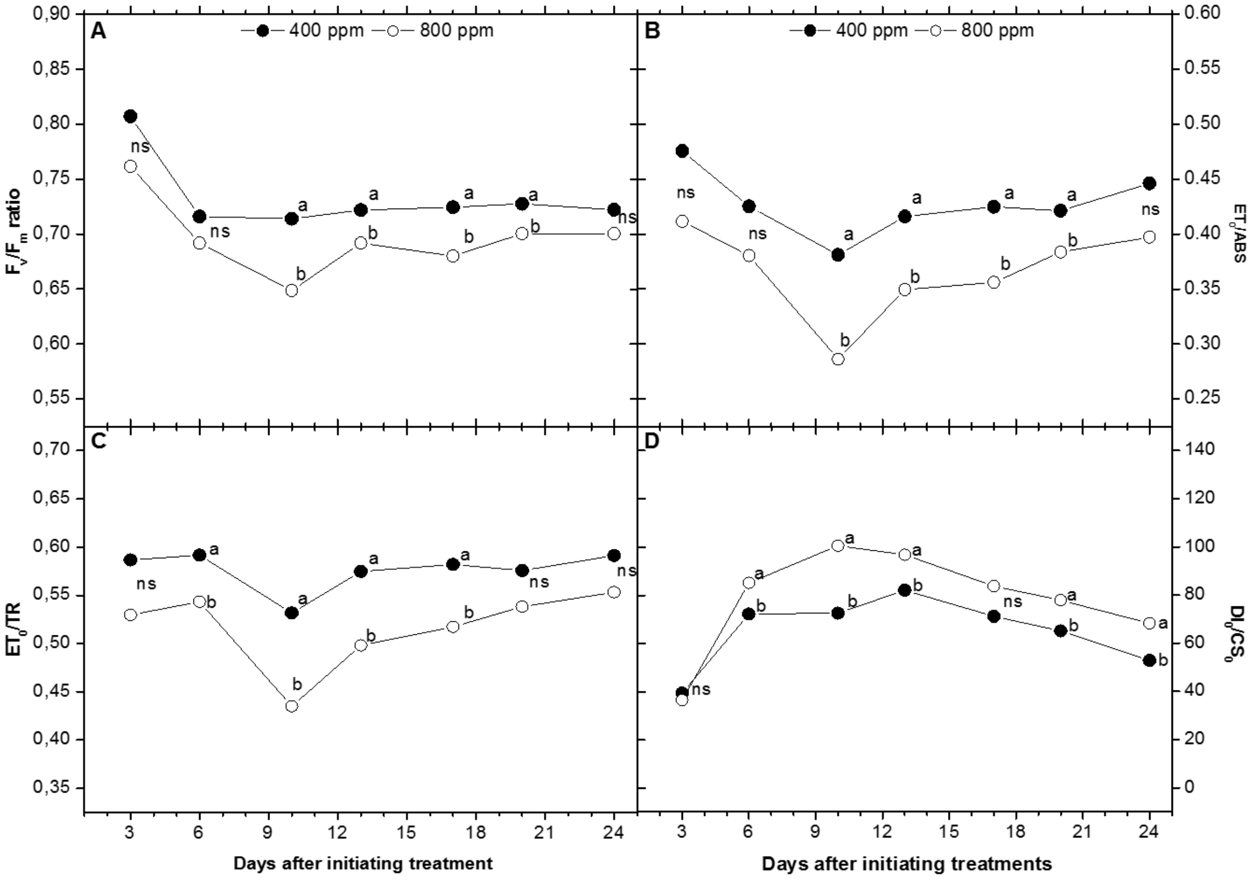

Figure 7. Maximum quantum yield of primary photochemistry (F_v/F_m ratio) (A); the quantum yield of electron transport (ET_0/ABS) (B); efficiency (ET_0/TR_0) with which a trapped exciton can move an electron into the electron transport chain further than the Quinone A (QA) (C); dissipation per excited cross-section (DI_0/CS) (D) of grapevine grown under either 400 ppm or 800 ppm [CO_2]. Each point represents the mean of 18 replicates. Means followed by different letters differ statistically by Tukey's test at 5 % probability within the same day. *ns* indicates no statistical difference.

Leaf respiration and carbon balance responses to elevated [CO_2] and different water management

No significant differences were found between FI and PRD treatments for R_{dark} or R_{light} throughout the experiment, at either [CO_2]. However, full water restriction (NI) caused a distinct change in R_{dark} and R_{light} values. NI plants grown under 400 ppm of CO_2, at 13, 17 and 20 DAIT, showed significantly higher values of R_{dark} and R_{light} than FI and PRD treatments under the same CO_2 concentration, reaching values of R_{dark} and R_{light} at 17 DAIT that were 62 and 69% higher, respectively. However, no difference was found among treatments at 24 DAIT. Similarly, NI plants grown under 800 ppm of CO_2, at 20 DAIT, presented values of R_{dark} and R_{light} 64 and 63% higher than FI and PRD treatments, respectively, under the same CO_2 concentration. By 24 DAIT, these differences were 45%, showing the same trend observed in the plants grown under 400 ppm of CO_2 (Fig. 8 – A, B, D and E).

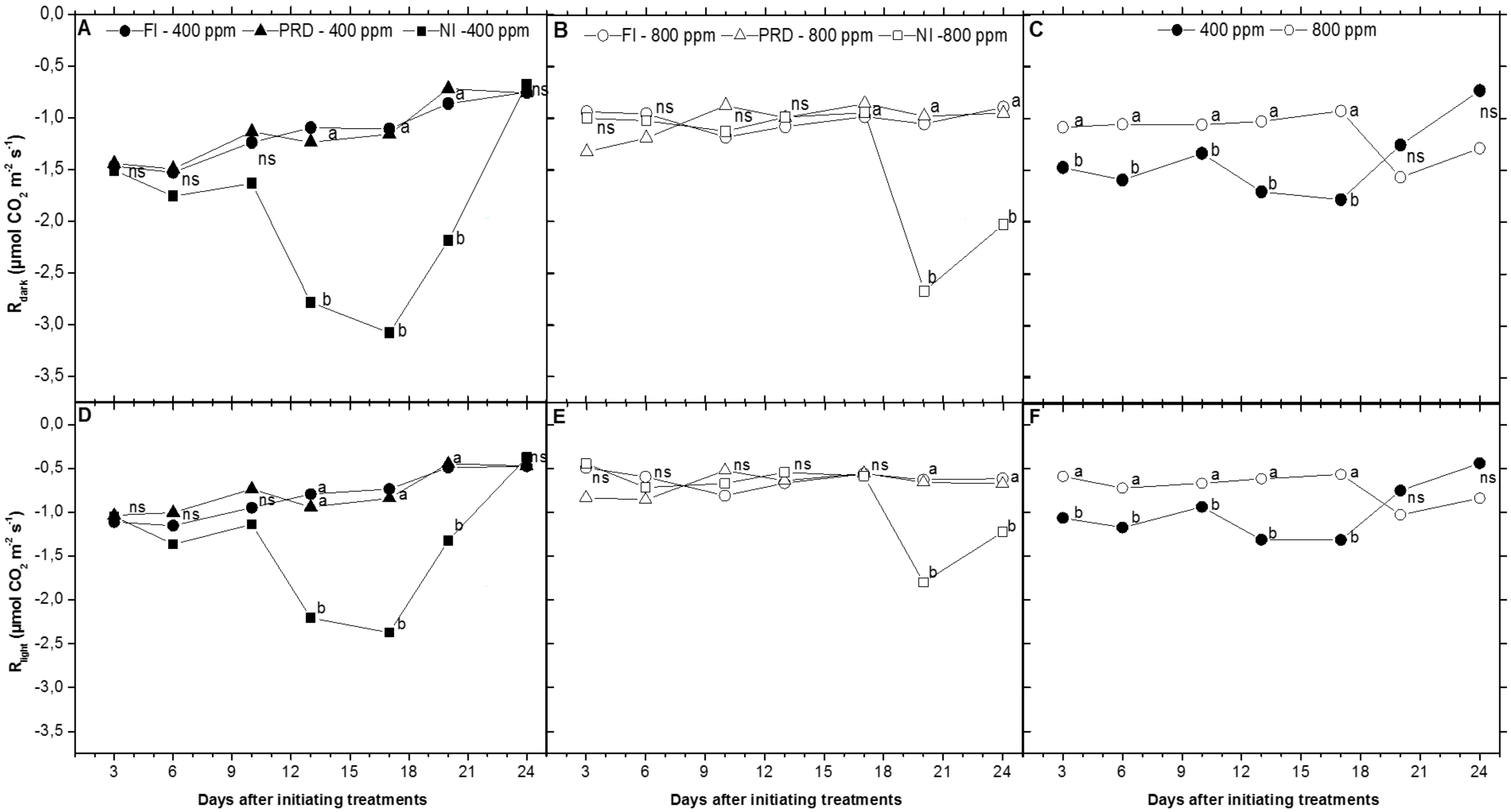

Figure 8. Leaf respiration in the dark (R_{dark}) (A, B and C) and in the light (R_{light}) (D, E and F) of grapevine grown under three water management treatments - Full-irrigated (FI); partial rootzone drying (PRD) and non-irrigated (NI) - and either 400 ppm (A and D) or 800 ppm [CO_2] (B and E) – and comparison of the CO_2 effect regardless of water management treatment (C and F). Each point represents the mean of 5 (A, B, D and E) or 15 (C and F) replicates. Means followed by different letters differ statistically by Tukey’s test at 5 % probability within the same day. *ns* indicates no statistical difference.

Regardless of the irrigation treatment, at 3, 6, 13 and 17 DAIT, R_{dark} was significant lower (*ca.* 37%) in plants grown under 800 ppm CO_2. This same pattern was observed for R_{light} from 3 to 17 DAIT (*ca.* 44% lower) (Fig. 8 – C and F). No differences among water management treatments were found for R_{light}:R_{dark}, at both [CO_2] (data not shown). However, from 3 to 17 DAIT, the degree of light inhibition of *R* increased significantly (*ca.* 18%) in the plants grown under 800 ppm of CO_2, compared to the plants grown under 400 ppm of CO_2 (Fig. 9).

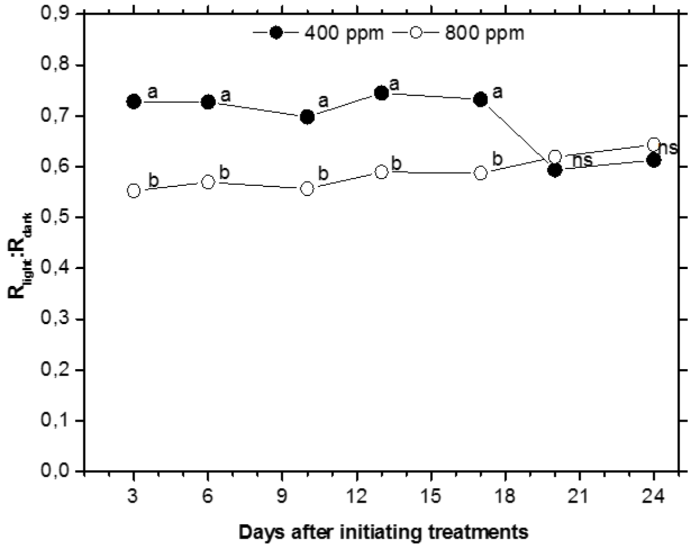

Figure 9. Light inhibition of respiration (R_{light}:R_{dark}) of grapevine grown under either 400 ppm or 800 ppm [CO_2]. Each point represents the mean of 10 replicates. Means followed by different letters differ statistically by Tukey's test at 5 % probability within the same day. *ns* indicates no statistical difference.

Water shortage resulted in a decrease in LCB in the NI treatments, starting at 13 DAIT for the NI plants grown under 400 ppm, and at 20 DAIT for the NI plants grown under 800 ppm (Fig. 10 – A and B). At both 17 and 24 DAIT, PRD plants grown under 800 ppm of CO_2 had a significantly higher LCB than the FI plants under the same [CO_2] (Fig. 10 – B). Regardless of water management treatments, from 3 to17 DAIT, plants under 800 ppm of CO_2 presented significantly higher LCB (*ca.* 39%) than the plants grown under 400 ppm of CO_2 (Fig. 10 – C).

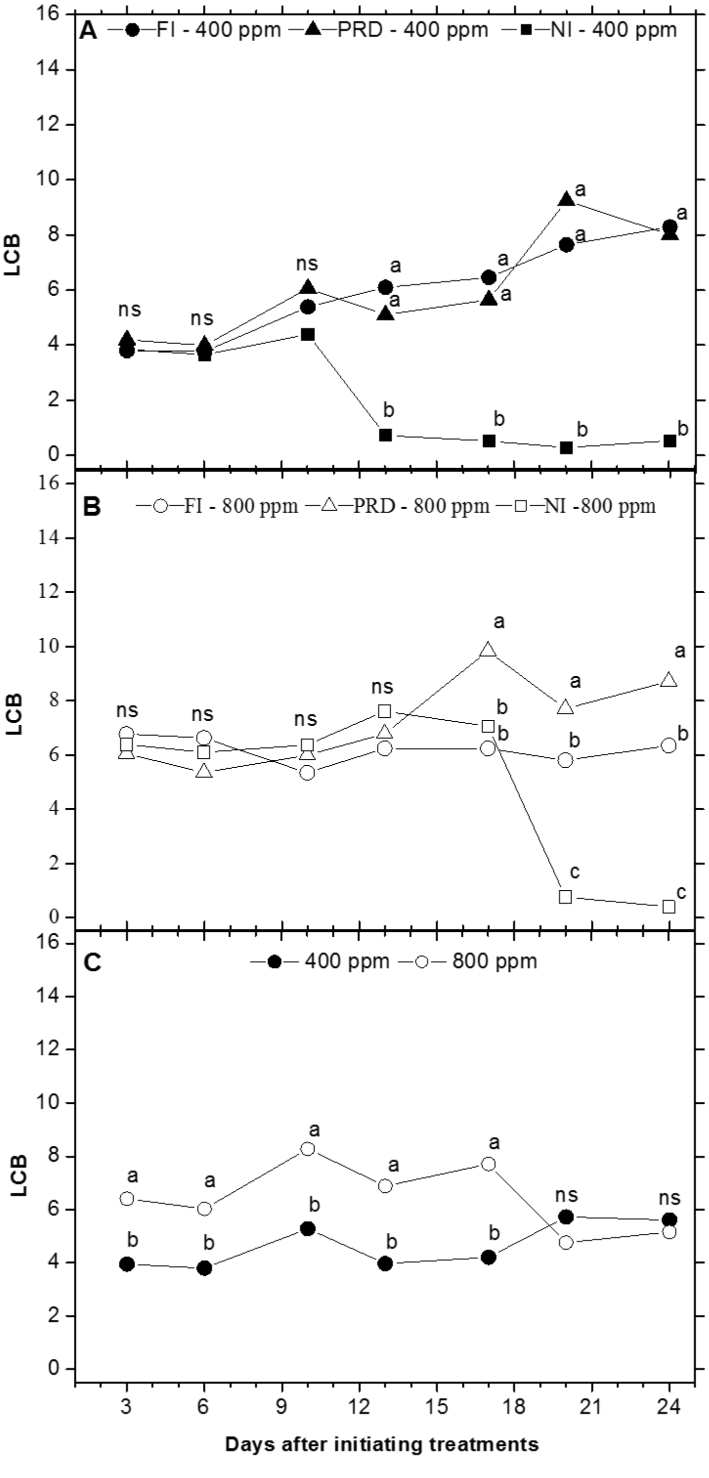

Figure 10. Leaf Carbon Balance (LCB – $A_{net}/(R_{dark} + R_{light})$) of grapevine grown under three water management treatments - Full-irrigated (FI); partial rootzone drying (PRD) and non-irrigated (NI) - and either 400 ppm (A) or 800 ppm [CO_2] (B) - and the mean CO_2 effect regardless water management treatment (C). Each point represents the mean of 5 (A and B) or 15 (C) replicates. Means followed by different letters differ statistically by Tukey's test at 5 % probability within the same day. *ns* indicates no statistical difference.

DISCUSSION

Down-regulation of A_{net} is predicted by leaf N concentration and SLW

As a C3 plant, the net photosynthesis of grapevines grown under twice ambient [CO_2] might be expected to increase (Salazar-Parra et al., 2012; Salazar-Parra et al., 2015). However, these increases are not always as large as expected, and instead A_{net} is considered *down-regulated* (Luo et al., 1994, Griffin and Seemann, 1996). To more readily predict photosynthetic acclimation to growth in elevated [CO_2], Luo et al., (1994) proposed a model that postulates when elevated [CO_2] results in changes in SLW that are larger than the decreases in leaf N concentration, photosynthesis will be *up-regulated*, whereas when the decrease in the leaf N concentration is larger than the increase in SLW, photosynthesis is predicted to be *down-regulated*. Our results are broadly supportive of this model. We observed larger reduction in N concentration than the increase in SLW (Fig. 1 – A and B) and this resulted in an increment in A_{net} of only *ca.* 24% when [CO_2] was doubled (Fig. 2 – C). This increase in A_{net} does not match the maximum potential increase (~50%), and thus can be considered *down-regulated* (Long, 1991; Drake et al., 1997; Moore et al., 1999; Long et al., 2004, Salazar-Parra et al., 2012). In addition, observed lower A_{net} of grapevines grown under elevated CO_2, but measured at 400 ppm of CO_2 (Fig. 3) also suggests an apparent *down-regulation* of the photosynthetic apparatus (Epron et al, 1995). Thus, leaf N concentration can be considered a central point to the *down-regulation* of grapevine A_{net} in response to growth in elevated [CO_2].

The most reasonable explanation of the importance of leaf N concentration in predicting A_{net} *down-regulation* is the large fraction of leaf N in the photosynthetic apparatus. Chlorophyll molecules and Rubisco, are the most substantial nitrogen investments in the plant (Griffin and Seemann, 1996; Zhang et al. 2008, Sage, 2013). Any compromise in leaf N concentration can affect the functions performed by Rubisco and chlorophyll molecules, affecting the photosynthetic apparatus and the rate of carbon fixation. In the present work, plant growth under increased [CO_2] not only decreased leaf N concentrations, but also led to reductions in enzyme activity. Both $V_{o\ 500}$ (Fig. 6 - C) and in $V_{c\ 500}$ in FI grapevines (Fig. 6 – B) were significantly reduced. In addition, plant growth in elevated [CO_2] resulted in a trend in chlorophyll *a* fluorescence that indicates a lower photochemical efficiency. We found that PSII efficiency was reduced, due to an increase in energy dissipation (DI_0/CS_0) and declines in both quantum yield of electron transport (ET_0/ABS) and maximum quantum yield of primary photochemistry (F_v/F_m), as well as reductions in plastoquinone (PQ) pool and the inhibition of QA^- reoxidation (ET_0/TR_0) (Fig. 7) (Strasser et al., 2000; Force et al., 2003; Strauss et al., 2006). These results further support a key role for leaf N concentration in explaining why A_{net} did not reach its maximum potential with a doubling of the growth [CO_2]. Thus, the reduction in leaf N concentration when grapevines are grown under elevated [CO_2] can be responsible for the metabolic limitations to carboxylation efficiency due to reduced carboxylation activity and/or Rubisco amount, as previously reported (Escalona et al. 1997; Urban, 2003; Aranjuelo et al., 2005; Lambreva et al., 2005; Erice et al., 2006), as well as for decreasing photochemical energy production, that is essential to carboxylation reactions (Hymus et al., 2001) and hence, for the occurrence of the *down-regulation* of A_{net}.

The reduction in leaf N concentration observed in the present work, may be a consequence of the trend in increasing the SLW (Fig. 1 – B). It is likely that the increase in SLW in response to elevated [CO_2], is the result of greater cell enlargement and carbohydrate accumulation (Maroco et al. 2002; Ellsworth et al., 2004; Moutinho-Pereira et al., 2009). These two factors in turn may have caused N dilution within the leaves. Thus, this relationship between leaf N concentration and SLW holds with, and explains the effectiveness of the model proposed by Luo et al. (1994) when applied to grapevine cultivation. In summary, A_{net} *down-regulation* under elevated [CO_2] was a result of reductions in Rubisco content and/or total Rubisco activity, and of changes in the photochemical apparatus leading to reduced efficiency, especially in PSII. These physiological responses are linked to, and predicted by, N dilution within the leaves and a trend toward higher SLW.

Potential use of PRD under elevated atmospheric [CO_2]

Atmospheric [CO_2] is predicted to increase dramatically until the end of the century (IPCC, 2014) causing changes in precipitation patterns (Kimball et al., 2001) and decreases in soil moisture content of up to 70% (Schultz, 2000). For these reasons, water-saving irrigation techniques, such as PRD, will become very valuable to maintain crop production in the future. Thus, one aim of our study was to analyze the potential benefits of the use of PRD techniques under elevated atmospheric [CO_2].

Our results are very promising, demonstrating PRD treated plants grown under 800 ppm of CO_2 can achieve higher A_{net} (Fig. 2 – B), $V_{c\ 500}$ (Fig. 5 - B) and LCB (Fig. 10 – B) than FI plants at the same [CO_2]. In addition, no difference was found between PRD and FI treatments for $V_{o\ 500}$ (Fig. 5 - D). Thus, under 800 ppm of CO_2, Rubisco carboxylation rate efficiency is increased with PRD (compared to FI and NI at the same [CO_2]). As a consequence, both A_{net} and LCB are increased. By contrast, there was no evidence of either photochemical or stomatal effects, with no significant differences between FI and PRD treatments in Chlorophyll *a* Fluorescence parameters (data not shown) or in g_s (Fig. 2 - E).

This increase in A_{net} in the PRD plants grown under 800 ppm of CO_2 led to a positive effect on *WUE* and *iWUE* (Fig. 4), despite the lack of significant differences between FI and PRD treated plants for g_s and *E* (Fig. 2 – E and H). Curiously, PRD in grapevines is usually associated to larger reduction in g_s and *E* than in A_{net}, resulting in increased *iWUE* (Gaudillère et al., 2002; Chaves et al., 2004, 2007; de Souza et al., 2004, 2005; Pou et al., 2012) and *WUE* (Stoll et al., 2000; de Souza et al., 2005; Chaves et al., 2007). However, this trend was not observed under either [CO_2] in our work. In addition, A_{net} of PRD treated plants was maintained at 400 ppm of CO_2 (Fig. 2–B), as well as greater *WUE* and *iWUE* were also observed for PRD treated plants in relation to FI under the same [CO_2] (Fig. 4). Thus, our results highlight the potential of the PRD technique to save water under both ambient and elevated [CO_2]. This is particularly true under 800 ppm of CO_2, where PRD use seems to contribute to an improved overall carbon gain. For these reasons we urge the further evaluation and use of PRD treatments in grapevine cultivation.

Elevated [CO_2] and drought tolerance

Elevated [CO_2] dramatically increased drought tolerance of grapevines. The reduction in A_{net} in NI plants (Fig.2 – A and B), that is associated with decreases in g_s and *E* (Fig. 2 – D, E, G and H), was delayed for 4 days when plants were grown in elevated [CO_2]. This delay in the reduction of A_{net} can be explained by the fact that plants grown under 800 ppm of CO_2 had lower values of g_s and E than the plants grown under 400 ppm of CO_2 (Fig. 2. – F and J), and thus, water depletion in the root zone developed at a slower rate (Grossman-Clarke et al., 2001; Robredo et al., 2007). As a result, the eventual decrease of A_{net} due to drought, was delayed (Long et al. 2004; Ainsworth and Rogers 2007, Albert et al. 2011). In addition, we found that growth in elevated CO_2 also resulted in a significant reduction in stomatal density (Fig. 1 - C), which further contributed to the reduction in water loss. When combined, these effects of elevated CO_2 on the various stomatal responses resulted in higher values of *WUE* and *iWUE* compared to the plants grown under 400 ppm of CO_2 and with the same irrigation treatments (Fig. 4). The highest A_{net} and the lowest g_s *and E* values (Fig. 2) resulted in higher *WUE* and *iWUE* of the plants grown under 800 ppm of CO_2, as observed by Salazar-Parra et al. (2012 and 2015) in grapevine. However, we also find that drought tolerance due to elevated [CO_2] in grapevines is not solely related to stomatal responses but includes changes in photosynthetic and respiratory physiology.

Withholding water resulted in reductions in both $V_{c\ 500}$ and $V_{o\ 500}$ (Fig. 5) throughout the experiment, and both reductions were delayed in plants grown in elevated [CO_2]. It is likely that, in the present work, as a consequence of drought, there was a decrease in Rubisco regeneration (Giménez et al. 1992; Lawlor and Tezara, 2009) and/or the accumulation of Rubisco degradation products (Vincent et al., 2007), leading to the observed reductions in both $V_{c\ 500}$ and $V_{o\ 500}$ (Fig 5– A, B, D and E). However, the slower rate of water depletion in the rootzone resulting from fewer and more closed stomata at 800 ppm of CO_2, may have reduced the negative effects of slowed Rubisco regeneration and/or accumulation of Rubisco degradation products that normally occur with drought, and thereby delaying the onset of negative effects.

Changes in Leaf Respiration in response to drought and elevated [CO_2]

Our study further evaluated how R_{light} and R_{dark} responded to drought and elevated [CO_2] in grapevines to gain a more complete understanding of the physiological control of leaf carbon balance. We found that water stress caused significant changes in both R_{dark} and R_{light}. NI plants initially showed an increase in R_{dark} and R_{light} relative to FI and PRD treatments, followed by a reduction at the 24th

DAIT (Fig. 8 A, B, D and E). Increased leaf respiration under drought is likely to have been caused by hormonal changes, senescence, accumulation of proline and other compatible solutes, as well as a general change in plant metabolism (Flexas et al., 2005). In addition, leaf respiration is strongly enhanced by increased leaf temperature (Zufferey et al. 2000), a condition occurring under drought due to stomatal closure, as observed in the present work (Fig. 2 – E and F). Moreover, decreases in leaf respiration, as drought became more intense, was probably the result of reductions in cell expansion, cell wall synthesis, protein synthesis and, as observed in the present work, photosynthesis (Flexas et al., 2005; Gimeno et al., 2010) and stomatal conductance. Thus, regardless of [CO_2], drought likely lead to both a reduction in demand for the products of leaf respiration and the production of Tricarboxylic acid cycle (TCA) substrates, such as glucose, by reduced A_{net} (Fig. 2 – A and B).

Plants grown in elevated CO_2 also differed from 400 ppm CO_2 grown grapevines in the rates of carbon flux through leaf respiration. In general, under 800 ppm of CO_2, both R_{dark} and R_{light} were lower than the plants grown under 400 ppm of CO_2 (Fig. 8 – C and F). Reductions in R_{dark} and R_{light} under elevated [CO_2] are usually attributed to changes in the cytochrome and/or alternative pathway ratios as well as reduced energy demand for carbohydrate translocation (Gonzáles-Meler et al., 2004). In addition, according to Crous et al. (2012), both R_{dark} and R_{light} are positively related to N concentration so that, in our work, the R_{dark} and R_{light} decreases matched the lowest leaf N concentration under 800 ppm of CO_2 (Fig. 1 – A). The degree of light inhibition of respiration of the plants grown under 800 ppm of CO_2 was also higher than the plants grown under 400 ppm of CO_2 (Fig. 9). It is important to highlight that under 800 ppm [CO_2] $V_{\text{o 500}}$ was also reduced (Fig. 6 – C) and, as previously reported by Tcherkez et al. (2008) and Griffin and Turnbull (2013), conditions which suppress $V_{o\ 500}$ can also increase the degree of light inhibition of respiration, due to a decrease in the demand for TCA cycle substrates associated with the recovery of photorespiratory cycle intermediates in the peroxisome. However, such relationships between $V_{o\ 500}$ and R_{light}:R_{dark} seems to solely be related to elevated [CO_2], since the reductions in $V_{o\ 500}$ in response to drought (Fig. 5 – C and D) did not result in such consequences in R_{light}:R_{dark} in either [CO_2] (data not shown).

Studying the physiological control of leaf carbon balance through the estimation of the amount CO_2 assimilated by photosynthesis that is released to the atmosphere by respiration (LCB) is important, since higher LCB values typically represent more carbon available for plant growth (Flexas et al., 2006, Escalona et al., 2012, Ayub et al., 2014). Net leaf carbon gain estimated from leaf photosynthesis alone typically overestimates grapevine responses to a range of growth conditions (Escalona et al., 2012). Thus, the higher LCB observed in plants grown under elevated [CO_2] (Fig. 10 – C) in the present work is relevant to grapevine cultivation. Improved LCB could be advantageous to grapevine growth and development, since starch stored within grapevines' tissues at the end of the current season are crucial to early season growth the following season (Greer and Sicard, 2009). Moreover, under water shortage, respiration rates rarely decrease up to 100%, whereas photosynthesis does, resulting in a progressively decreased carbon balance (Flexas et al. 2006). We found the decrease in LCB was delayed under elevated [CO_2] for 7 days (Fig. 10 - A and B). Reductions in the LCB can be a main cause of reduced plant growth under drought conditions (Flexas et al., 2006). Importantly growth in elevated [CO_2] appears to mitigate or delay the deleterious effects of drought on grapevines through the maintenance of the LCB.

CONCLUSIONS

Our work demonstrates that: (i) the *down-regulation* of A_{net} under elevated [CO_2] is caused by reductions in Rubisco content and/or total activity, and by reductions in the efficiency of the photochemical apparatus due to N dilution within leaves with a higher SLW. These findings suggest a physiological explanation of the Luo et al. (1994) model and demonstrate the models' effectiveness; (ii) Under 800 ppm of CO_2, efficiency of Rubisco carboxylation is increased by PRD, leading to an increase in A_{net} and hence, greater *WUE* and *iWUE*. These responses do not appear to be related to photochemical or stomatal effects; (iii) Elevated [CO_2] can delay drought effects on A_{net} and Rubisco activity by reducing g_s, *E* and stomatal density; (iv) Leaf respiration responses depend on drought level and (v) Conditions that reduce N concentration, such as elevated [CO_2], also reduce R_{dark} and R_{light}.

R_{light}:R_{dark} is intensified under elevated [CO_2], a condition that also reduced V_{o500}. Overall, elevated [CO_2] improves the LCB and this mitigates the deleterious effects of drought on grapevines. The effects of elevated CO_2 levels on grapevine physiology with different water management strategies needs our continued attention as the effective the cultivation of grapevines is likely to be affected by current and future global change.

REFERENCES

Amthor, J.S., 2000. The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. Ann Bot-London. 86, 1–20.

Ainsworth, E.A., Rogers, A., 2007. The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ. 30, 258- 270.

Albert, K.R., Mikkelsen, T.N., Michelsen, A., Ro-Poulsen, H., van der Linden, L. 2011. Interactive effects of drought, elevated CO_2 and warming on photosynthetic capacity and photosystem performance in temperate heath plants. J Plant Physiol. 168, 1550-1561.

Antolín, M.C., Ayari, M., Sánchez-Díaz, M., 2006. Effects of partial rootzone drying on yield, ripening and berry ABA in potted Tempranillo grapevines with split roots. Aust J Grape Wine Res. 12, 13–20.

Antolín, M.C., Santesteban, H., Santamaría, E., Aguirreolea, J. and Sánchez-Díaz, M., 2008. Involvement of abscisic acid and polyamines in berry ripening of Vitis vinifera (L.) subjected to water deficit irrigation. Aust J Grape Wine Res. 14, 123–133.

Aranjuelo, I., Pérez, P., Hernández, L., Irigoyen, J.J., Zita, G., Martínez-Carrasco, R., Sánchez-Díaz, M., 2005. The response of nodulated alfalfa to water supply, temperature and elevated CO_2: photosynthetic down-regulation. Physiol Plant. 123, 348–58.

Aranjuelo, I., Pardo, A., Biel, C., Savé, R., Azcón-Bieto, J., Nogués, S., 2009a. Leaf carbon man-agement in slow-growing plants exposed to elevated CO_2. Glob Change Biol. 15, 97–109.

Ayub, G., Smith, R. A., Tissue, D.T., Atkin, O.K., 2011. Impacts of drought on leaf respiration in darkness and light in Eucalyptus saligna exposed to industrial-age atmospheric CO_2 and growth temperature. New Phytol. 190, 1003–1018.

Ayub, G., Zaragoza-Castells, J., Griffin, K. L., Atkin, O. K., 2014. Leaf respiration in darkness and in the light under pre-industrial, current and elevated atmospheric CO_2 concentrations. Plant Sci., 226, 120-130.

Bernacchi, C. J., Portis, A. R., Nakano, H., Caemmerer, S. Von, and Long, S. P., 2002. Temperature Response of Mesophyll Conductance. Implications for the Determination of Rubisco Enzyme Kinetics and for Limitations to Photosynthesis in Vivo. Plant Physiol. 130, 1992–1998.

Brooks, A., Farquhar, G.D., 1985. Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-biphosphate carboxylase/ oxygenase and the rate of respiration in the light. Estimates from gas exchange measurements on spinach. Planta. 165, 397–406.

von Caemmerer, S., Farquhar, G.D., 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta. 153, 376-387.

Chaves, M.M., Santos, T.P., Souza, C.R., Ortuño, M. F., Rodrigues, M.L., Lopes, C.M., Maroco, J.P., Pereira, J.S., 2007. Deficit irrigation in grapevine improves water-use-efficiency without controlling vigour and production quality. Ann Appl Biol. 150, 237–252.

Cousins, A.B., Ghannoum, O., von Caemmerer S., Badger M.R., 2010. Simultaneous determination of Rubisco carboxylase and oxygenase kinetic parameters in *Triticum aestivum* and *Zea mays* using membrane inlet mass spectrometry. Plant Cell Environ. 33, 444–452.

Crous, K. Y., Zaragoza-Castells, J., Ellsworth, D. S., Duursma, R. A., Tissue, M. D. T., Atkin, O., K., 2012. Light inhibition of leaf respiration in field-grown *Eucalyptus saligna* in whole-tree chambers under elevated atmospheric CO_2 and summer drought. Plant Cell Environ. 35, 966–981.

Davey, P.A., Hunt S., Hymus, G.J., Delucia, E.H., Drake, B.G., Karnosky, D.F., Long, S.P., 2004. Respiratory oxygen uptake is not decreased by an instantaneous elevation of [CO_2], but is increased with long-term growth in the field at elevated [CO_2]. Plant Physiol. 134, 520–527.

Drake, B.G., González-Meler, M.A., Long, S.P., 1997. More efficient plants: A consequence of rising atmospheric CO_2? Annu. Rev. Plant Phys. 48, 609–639.

Dry, P.R., Loveys, B.R., 1998. Factors influencing grapevine vigour and the potential for control with partial rootzone drying. Aust J Grape Wine Res. 4, 140–148.

Ellsworth, D. S., Reich, P. B., Naumburg, E. S., Koch, G. W., Kubiske, M. E., Smith, S. D., 2004. Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. Global Change Biol. 10, 2121–38.

Epron, D., Godard, D., Cornic, G., Genty, B., 1995. Limitation of net CO_2 assimilation rate by internal resis- tances to CO_2 transfer in the leaves of two tree species (*Fagus sylvatica* L and *Castanea sativa* Mill). Plant Cell Environ. 18,43–51.

Erice, G., Irigoyen, J.J., Pérez, P., Martínez-Carrasco, R., Sánchez-Díaz, M., 2006. Effect of elevated CO_2, temperature and drought on photosynthesis of nodulated alfalfa during a cutting regrowth cycle. Physiol Plant. 126, 458–468.

Escalona, J., Delgado, E., Medrano, H., 1997. Irrigation effects on grapevine photosynthesis. Acta Hort. 449, 449–455.

Escalona, J.M., Tomàs, M., Martorell, S., Medrano, H., Ribas-Carbo, M., Flexas, J., 2012. Carbon balance in grapevines under different soil water supply: importance of whole plant respiration. Aust J Grape and Wine Res. 1 – 11.

FAOSTAT., 2014 FAO data for agriculture: statistics database: <http://faostat.fao.org/faostat/collections?version=extandhasbulk=0andsubset =agricul-ture> Access in: 25 de ago. 2015.

Farquhar, G.D., von Caemmerer, S., 1982. Modelling of photosynthetic response to environmental conditions. In: Lange O.L., Nobel P.S., Osmond C.B., Ziegler H. (Eds). Encyclopedia of plant physiology. Physiological plant ecology II. Water relations and carbon assimilation. Vol. 12 B, Springer, Berlin, Germany. pp 551–587.

Flexas, J., Bota, J., Galmés, J., Medrano, H., Ribas-Carbo, M., 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiol Plantarum. 127, 343–352.

Flexas, J., Carriquí, M., Coopman, R. E., Gago, J. Galmés, J. Martorell, S., Morales, F., Diaz-Espejo, A., 2014. Stomatal and mesophyll conductances to CO_2 in different plant groups: Underrated factors for predicting leaf photosynthesis responses to climate change? Plant Sci. 226, 41–48.

Flexas, J., Galmés J., Gallé, A., Gulías, J., Pou, A., Ribas-Carbó, M., Tomás, M., Medrano H., 2010. Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. Aust J Grape Wine Res. 16, 106–121.

Flexas, J., Galmés, J., Ribas-Carbo, M., Medrano, H., 2005. The effects of drought in plant respiration. In: Lambers, H., Ribas-Carbo, M. (eds) Advances in Photosynthesis and Respiration. Plant Respiration: from Cell to Ecosystem. Kluwer Academic Publishers, Dordrecht. pp 85–94.

Force, L., Critchley, C., van Rensen, J. J. S., 2003. New fluorescence parameters for monitoring photosynthesis in plants. Photosynth Res. 78: 17–33.

Gaudillère, .JP., Van Leeuwen, C., Ollat, N., 2002. Carbon isotope composition of sugars in grapevine, an integrated indicator of vineyard water status. J Exp Bot. 53: 757–763.

Giménez, C., Mitchell, V.J., Lawlor, D., 1992. Regulation of photosynthesis rate of two sunflower hybrids under water stress. Plant Physiol. 98, 516-524.

Gimeno, T.E., Sommerville, K.E., Valladares, F., Atkin, O.K., 2010. Homeostasis of respiration under drought and its important consequences for foliar carbon balance in a drier climate: insights from two contrasting Acacia species. Funct Plant Biol. 37, 323–333.

Gonzàlez-Meler, M. A., Drake, B. G., Azcón-Bieto J., 1996. Rising atmospheric carbon dioxide and plant respiration. In: Breymeyer, A. I., Hall, D. O., Melillo, J. M., A. Gren GI (Eds). Global change: effects on coniferous forests and grasslands. SCOPE, Vol. 56, UK: John Wiley and Sons. 161–181.

González-Meler, M.A., Taneva, L., Trueman, R.J., 2004. Plant respiration and elevated atmospheric CO_2 concentration: cellular responses and global significance. Ann Bot-London. 94, 647–656.

Greer, D.H., Sicard, S.M., 2009. The net carbon balance in relation to growth and biomass accumulation of grapevines (*Vitis vinifera* cv. Semillon) grown in a controlled environment. Funct Plant Biol. 36, 645–653.

Griffin, K.L., Heskel, M., 2013. Breaking the cycle: how light, CO_2 and O_2 affect plant respiration. Plant Cell Environ. 36, 498–500.

Griffin, K. L., Seemann, J. R., 1996. Plants, CO_2 and photosynthesis in the 21st. Chem Biol. 3 (4), 245-254.

Griffin, K. L., Turnbul, M. H., 2013. Light saturated RuBP oxygenation by Rubisco is a robust predictor of light inhibition of respiration in *Triticum aestivum* L. Plant Biol. 15, 769–775.

Grossman-Clarke, S., Pinter Jr., P.J., Kartschall, T., Kimball, B.A., Hunsaker, D.J.,Wall, G.W., García, R.L., LaMorte, R.L., 2001. Modelling a spring wheat crop under elevated CO_2 and drought. New Phytol. 150, 315–335.

Hymus, G.J., Dijkstra, P., Baker, N.R., Drake, B.G., Long, S.P., 2001. Will rising CO_2 protect plants from the midday sun? A study of photoinhibition of *Quercus myrtifolia* in a scrub-oak community in two seasons. Plant Cell Environ. 24, 1361–1368.

IPCC, 2014. Intergovernmental panel on climate change. Summary for policymakers. In: Edenhofer O, Pichs-Madruga R, Sokona Y, Farahani E, Kadner S, Seyboth K, AdlerA, Baum I, Brunner S, Eickemeier P, Kriemann B, Savolainen J, Schlömer S, von Ste-chow C, Zwickel T, Minx JC, editors. Climate change 2014. Mitigation of climate change. Contribution of working group III to the fifth assessment report of the intergovernmental panel on climate change. Cambridge, United Kingdom/NewYork, NY, USA: Cambridge University Press.

Kimball, B.A., Morris, C.F., Pinter Jr., P.J., Wall, G.W., Hunsaker, D.J., Adamsen, F.J., LaMorte, R.L., Leavitt, S.W., Thompson, T.L., Matthias, A.D., Brooks, T.J., 2001. Elevated CO_2, drought and soil nitrogen effects on wheat grain quality. New Phytol. 150, 295–303.

Kirschbaum M.U.F., Farquhar G.D., 1987. Investigation of the CO_2 dependence of quantum yield and respiration in *Eucalyptus pauciflora*. Plant Physiol, 83. 1032–1036.

Kok B., 1948. A critical consideration of the quantum yield of *Chlorella*-photosynthesis. Enzymologial. 13, 1–56.

Lambreva, M., Stoyanova-Kolevab, D., Baldjievb, G., Tsonev, T., 2005. Early acclimation changes in the photosynthetic apparatus of bean plants during short-term exposure to elevated CO_2 concentration under high temperature and light intensity. Agr Ecosyst Environ. 106: 219–232.

Lawlor, D.W., Tezara, W., 2009. Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. Ann Bot-London. 103: 561-579.

Leakey, A.D.B., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P., Ort, D.R., 2009. Elevated CO_2 effects on plant carbon, nitrogen and water relations: six important lessons from FACE. J Exp Bot. 60, 2859–2876.

Long, S.P., Ainsworth, E.A., Rogers, A., Ort, D.R., 2004. Rising atmospheric carbon dioxide: plants face the future. Ann Rev Plant Biol. 55:591–628.

Long, S.P., 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? Plant Cell Environ. 14, 729–739.

Luo, Y., Field, C. B., Mooney, H. A., 1994. Predicting responses of photosynthesis and root fraction to elevated [CO_2]: interactions among carbon, nitrogen and growth. Plant Cell Environ. 17,1195-1204.

Maroco, J.P., Rodrígues, M.L., Lopes, C., Chaves, M.M., 2002. Limitations to leaf photosynthesis in field-grown grapevine under drought – metabolic and modelling approaches. Funct Plant Biol. 29, 451–459.

Moore, B.D., Cheng, S. H., Sims, D., Seemann, J.R., 1999. The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. Plant Cell Environ. 22, 567–582.

Moutinho-Pereira, J.M., Gonçalves, B., Bacelar, E., Boaventura Cunha, J., Coutinho, J., Correia, C.M., 2009. Effects of elevated CO_2 on grapevine (*Vitis vinifera* L.): physiological and yield attributes. Vitis. 48, 159–165.

Pons, T.L., Welschen, R.A.M., 2002. Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. Plant Cell Environ. 25, 1367–1372.

Pou, A., Medrano, H., Tomàs, M., Martorell, S., Ribas-Carbó, M., Flexas, J., 2012. Anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric behavior. Plant Soil. 359:335–349.

Rampino, P., Mita, G., Fasanoa, P., Borrelli, G. M., Aprilea, A., Dalessandro, G., De Bellis, L., Perrotta, C., 2012. Novel durum wheat genes up-regulated in response to a combination of heat and drought stress. Plant Physiol Biochem. 56:72–8.

Reich, P. B., Sendall1, K. M., Stefanski1, A., Wei1, X., Rich, R. L., Montgomery, R. A., 2016. Boreal and temperate trees show strong acclimation of respiration to warming. Nature. 531: 633-636.

Reuveni, J., Gale, J., Mayer, A. M., 1995. High ambient carbon dioxide does not affect respiration by suppressing the alternative cyanide-resistant respiration. Ann Bot-London. 76: 291–295.

Robredo, A., Pérez-López, U., de la Maza, H.S., González-Moro, B., Lacuesta, M., Mena-Petite, A., Munõz-Rueda, A., 2007. Elevated CO_2 alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. Environ Exp Bot. 59, 252–263.

Robredo A., Pérez-López, U., Lacuesta, M., Mena-Petite A., Munõz-Rueda A., 2010. Influence of water stress on photosynthetic characteristics in barley plants under ambient and elevated CO_2 concentrations. Biol Plantarum. 54, 285–292.

Rodrigues, W.P., Martins, M.Q., Fortunato, A.S., Rodrigues, A.P., Semedo, J.N., Simões-Costa, M.C., Pais, I.P., Leitão, A.E., Colwell, F., Goulao, L., Máguas, C., Maia, R., Partelli, F.L., Campostrini, E., Scotti-Campos, P., Ribeiro-Barros, A.I., Lidon, F.C., DaMatta, F.M., Ramalho, J.C., 2016. Long-term elevated air [CO_2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical *Coffea arabica* and *C. canephora* species. Glob Change Biol. 22, 415 - 431.

Rogers, H. H., Dahlman, R.D., 1993. Crop response to CO_2 enrichment. Vegetatio. 104/105, 117-131.

Rogiers, S. Y., Hardie, W. J., Smith, J. P., 2011. Stomatal density of grapevine leaves (*Vitis vinifera* L.) responds to soil temperature and atmospheric carbon dioxide. Aust J Grape Wine Res. 17, 147–152.

Sage, R.F., 2013. Photorespiratory compensation: a driver for biological diversity. Plant Biol. 15, 624–638.

Salazar-Parra, C., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J., Morales, F., 2012. Photosynthetic response of Tempranillo grapevine to climate change scenarios. Ann Appl Biol. 161, 277–292.

Salazar-Parra, C., Aranjuelo, I., Pascual, I., Erice, G., Sanz-Sáez, A., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J., Araus, J.L., Morales, F., 2015. Carbon balance, partitioning and photosynthetic acclimation in fruit-bearing grapevine (*Vitis vinifera* L. cv. Tempranillo) grown under simulated climate change (elevated CO_2, elevated temperature and moderate drought) scenarios in temperature gradient greenhouses. J Plant Physiol. 174, 97–109.

Sanz-Sáez, A., Erice, G., Aranjuelo, I., Nogués, S., Irigoyen, J.J., Sánchez-Díaz, M., 2010. Photosynthetic down-regulation under elevated CO_2 exposure can be prevented by nitrogen supply in nodulated alfalfa. J Plant Physiol. 167: 1558–1565.

Sharma-Natu, P., Ghildiyal, M.C., 2005. Potential targets for improving photosynthesis and crop yield. Curr Sci India. 88, 1918–1928.

Shapiro, J.B., Griffin, K.L., Lewis, J.D., Tissue D.T., 2004. Response of Xanthium strumarium leaf respiration in the light to elevated CO_2 concentration, nitrogen availability and temperature. New Phytol. 162, 377–386.

Schultz, H.R., 2000. Climate change and viticulture: European perspective on climatology, carbon dioxide and UV-B effects. Aust J Grape Wine Res. 6, 2–12.

Shapiro, J.B., Griffin, K.L., Lewis, J.D., Tissue D.T., 2004. Response of Xanthium strumarium leaf respiration in the light to elevated CO_2 concentration, nitrogen availability and temperature. New Phytol. 162, 377–386.

de Souza, C. R.; Maroco, J. P.; Santos, T.; Rodrigues, M. L.; Lopes, C.; Pereira, J. S.; Chaves, M. M.; 2004. Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. Agric Ecosyst Environ. 106(2–3), 261–274.

de Souza, C.R., Maroco, J.P., Dos Santos, T.P., Rodrigues, M.L., Lopes, C.M., Pereira, J.S., Chaves, M.M., 2005. Grape berry metabolism in field-grown grapevines exposed to different irrigation strategies. Vitis. 44:103–109.

Stoll, M., Loveys, B. and Dry, P., 2000. Hormonal changes induced by partial rootzone drying of irrigated grapevine. J Exp Bot. 51, 1627–1634.

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus, M., Pathre, U., Mohanty, P. (Eds), Probing Photosynthesis: Mechanisms, Regulation and Adaptation. Taylor and Francis, London. pp 445–483.

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M., 2004. Analysis of the chlorophyll a fluorescence transient. In: Papageorgiou, C.G., Govindjee, (Eds), Chlorophyll fluorescence: a signature of photosynthesis. Advances in photosynthesis and respiration. Kluwer Academic Publishers, Netherlands. pp 321–362.

Strauss, A. J., Krugera, J. H. J., Strasser, R. J., Van Heerden, P.D.R., 2006. Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll a fluorescence transient O-J-I-P. Environ Exp Bot. 56, 147–157.

Tcherkez, G., Bligny, R., Gout, E., Mahe, A., Hodges M., Cornic G., 2008. Respiratory metabolism of illuminated leaves depends on CO_2 and O_2 conditions. P Natl Acad Sci Usa. 105, 797–802.

Tcherkez, G., Mahé, A., Guérard, F., Boex-Fontvieille, E.R.A., Gout, E., Lamothe, M., Barbour, M.M., Bligny, R., 2012. Short-term effects of CO2 and O2 on citrate metabolism in illuminated leaves. Plant Cell Environ. 35, 2208–2220.

Tcherkez, G., Schaufele, R., Nogues, Piel, C., Boom, A., Lanigan, G., Barbaroux, C., Mata, C., Elhani, S., Hemming, D., Maguas, C., Yakir, D., Badeck, F. W., Griffiths, H., Schnyder, H., Ghashghaie, J., 2010. On the $^{13}C/^{12}C$ isotopic signal of day and night respiration at the mesocosm level. Plant Cell Environ. 33, 900–913.

Urban, O., 2003. Physiological impacts of elevated CO_2 concentration ranging from molecular to whole plant responses. Photosynthetica. 41:9–20.

Villar, R., Held, A. A., Merino, J., 1994. Comparison of Methods to Estimate Dark Respiration in the Light in Leaves of Two Woody Species. Plant Physiol. 105: 167-172.

Vincent, D., Ergül, A., Bohlman, M.C., Tattersall, E.A.R., Tillett, R.L., Wheatley, M.D., Woolsey, R., Quilici, D.R., Joets, J., Schlauch, K., Schooley, D.A., Cushman, J.C. and Cramer, G.R., 2007. Proteomic analysis reveals differences between *Vitis vinifera* L. cv. Chardonnay and cv. Cabernet Sauvignon and their responses to water deficit and salinity. J Exp Bot. 58(7):1873-92.

Wullschleger, S.D., Tschaplinski, T.J., Norby, R.J., 2002. Plant water relations at elevated CO_2 – implications forwater-limited environment. Plant Cell Environ. 25, 319–331.

Zhang, X., Wollenweber, B., Jiang, D., Liu, F., Zhao, J., 2008. Water deficits and heat shock effects on photosynthesis of a trans- genic Arabidopsis thaliana constitutively expressing ABP9, a bZIP transcription factor. J Exp Bot. 59, 839–848.

Zufferey, V., Murisier, F., Schultz, H.R., 2000. A model analysis of the photosynthetic response of *Vitis vinifera* L. cvs Riesling and Chasselas leaves in the field: I. Interactions of age, light and temperature. Vitis. 39, 19–26.

4. CHAPTER 2

DEFICIT IRRIGATION AND TRANSPARENT PLASTIC COVERS CAN SAVE WATER AND IMPROVE GRAPEVINE CULTIVATION IN THE TROPICS

ABSTRACT

This study examines the interactive effects of deficit irrigation and transparent plastic covering (TPC) on key physiological traits in tropically grown grapevines. ‘Niagara Rosada’ grapevine (*Vitis labrusca*) were subjected to both Regulated Deficit Irrigation (RDI) and Partial Rootzone Drying (PRD) while being grown under a TPC to address the following questions: (i) Does the grapevine present anisohydric or isohydric behavior? (ii) How does deficit irrigation affect leaf water potential (Ψ)? (iii) Can RDI and PRD improve whole plant water use efficiency? (iv) How does deficit irrigation affect leaf photochemical and biochemical capacity? (v) What are the effects of deficit irrigation on leaf respiration and leaf carbon balance? (vi) Is it possible to save water without affecting yield and fruit quality? (vii) Is TPC a good strategy to cultivate grapevine in Tropical conditions? Three water management techniques were applied: full-irrigated (FI): 100% of the crop evapotranspiration (ET_c) was supplied to both sides of the root system; RDI: 50% of the ET_c was supplied to both sides of the root system; and PRD: 50% of ET_c

was alternately supplied to only one side of the root system, whereas the other side of the rootzone remained without water. These irrigation treatments were replicated such that the two plots were either covered by a polyethylene plastic structure or remained uncovered. We found that: (i) ‘Niagara Rosada’ grapevine presented anisohydric behavior, (ii) deficit irrigation did not affect leaf Ψ; (iii) Neither RDI nor PRD had a significant effect on intrinsic (A_{net}/g_s) or water use efficiency (A_{net}/E) (iv) no limitations by the carboxylation reactions of photosynthesis or Rubisco oxygenation ($V_{o\ 1500}$) were observed, and photochemical capacity was not inhibited; (v) Leaf respiration rates in the light or in the dark were not affected by either RDI or PRD and therefore deficit irrigation did not damage the leaf carbon balance in ‘Niagara Rosada’; (vi) a considerable volume of water was saved when either RDI or PRD was used, without affecting yield or fruit quality; (vii) the use of TPC can be an effective strategy for growing ‘Niagara Rosada’ grapevine in Tropical conditions.

Keywords: chlorophyll a fluorescence; leaf carbon balance; light inhibition of respiration; rubisco oxygenation/carboxylation rates; photosynthetic capacity; water use efficiency;

RESUMO

Este estudo avaliou os efeitos interativos do uso do deficit de irrigação e de cobertura plástica transparente (TPC) em importantes parâmetros fisiológicos de videira cultivada em condições tropicais. A videira ‘Niagara Rosada’ (*Vitis labrusca*) foi submetida a deficit de irrigação regulado (RDI) e a irrigação parcial do sistema radicular (PRD) sob TPC para responder as seguintes questões: (i) Tal videira apresenta um comportamento anisohídrico ou isohídrico? (ii) Como o deficit de irrigação afeta o potencial hídrico foliar (Ψ)? (iii) RDI e PRD aumentam a eficiência no uso da água? (iv) Como o deficit de irrigação afeta a capacidade fitoquímica e bioquímica? (v) Quais são os efeitos do deficit de irrigação sobre a repiração foliar e o balanço de carbono? (vi) É possível economizer água sem afetar a produtividade e qualidade de frutos? (vii) A TPC é uma boa estratégia para o cultivo de videira em condições tropicais? Três manejos de irrigação foram aplicados em videiras crescidas em condições tropicais [totalmente irrigados (FI) – 100% da evapotranspiração da cultura (ET_c) era fornecida em ambos os lados do sistema radicular; RDI – 50% da ET_c era fornecido em ambos os lados do sistema radicular; PRD – 50% da ET_c era fornecido em apenas um dos lados do sistema radicular, enquanto que o outro lado permanecia sem ser irrigado]. Tais tratamentos de irrigação foram replicados tanto em uma área coberta por estrutura plástica de polietileno (TPC) como em uma área que não apresentava tal cobertura. Nós encontramos que: (i) a videira 'Niagara Rosada' apresentou um comportamento anisohídrico; (ii) o déficit de irrigação não afetou Ψ; (iii) RDI e PRD não tiveram efeitos significativos tanto na eficiência intrínseca (A_{net}/g_s) como na eficiência no uso da água (A_{net}/E); (iv) Limitações nas reações de carboxilação da fotossíntese ou nas taxas de oxigenação da Rubisco ($V_{o\ 1500}$) não foram observadas e a capacidade fotoquímica não foi inibida; (v) as taxas de respiração foliar na luz e no escuro não foram afetadas tanto pelo RDI como pelo PRD e, consequentemente, o uso de déficit de irrigação não contribuem negativamente para o balanço de carbono foliar em 'Niagara Rosada'; (vi) um volume considerável de água foi economizado quando tanto RDI como PRD foram utilizados sem afetar negativamente a produtividade e qualidade de frutos; (vii) o uso da TPC porde ser uma estratégia efetiva para cultivar a videira 'Niagara Rosada' em condições tropicais.

Palavras-chave: balanço de carbono foliar; capacidade fotossintética; eficiência no uso da água; fluorescência da clorofila a; inibição da respiração pela luz; taxas de oxigenação/carbolixilação da rubisco.

INTRODUCTION

Grapevines are the most widely cultivated fruit in the world and are grown on nearly every continent (FAOSTAT, 2014). However, grapevine production is water intensive and water shortage is the most significant limiting factor of crop production worldwide (FAOSTAT 2014). The future of sustainable grapevine cultivation is likely to require expansion to more favorable locations, or a major reduction in water use (Flexas et al., 2016). While grapevines can be grown in tropical areas, such as in Brazil the climatic conditions can be even more problematic (Permanhani et al., 2016) due to the intense irregularity of precipitation patters. Given the requisite climatic conditions and predicted effects of global warming (IPCC 2014), the future of grapevine cultivation is uncertain, and the use of alternative irrigation techniques such as Regulated Deficit Irrigation (RDI) and Partial Root-zone Drying (PRD) should be explored.

RDI and PRD are two specific deficit irrigation techniques that tune water availability temporally (specific timing of the application - RDI) or spatially (alternating dry–wet zones - PRD) (Chaves et al., 2010). Under RDI, the water is supplied at levels below that required for full crop evapotranspiration (ET_c) during specific phenological stages, particularly after fruit set when fruit growth is less sensitive to reductions in water supply (Chaves et al. 2010, Tarara and Peña 2015). This technique was designed to optimize fruit number, size and quality by balancing vegetative growth and potential for production (Poni et al. 2009, Chaves et al. 2010). By contrast, under PRD, the two sides of the grapevines' root system are alternately irrigated providing a spatial reduction in water availability. PRD can induce the synthesis of abscisic acid (ABA) by roots in the dried half of the rootzone, and lead to partial stomatal closure without reducing overall leaf water status (Stoll et al. 2000, Dry and Loveys 1998, Antolín et al. 2006, 2008). Such reductions in stomatal conductance and transpiration are typically larger than the decrease in net photosynthesis, and thus increase water use efficiency without impairing plant development, yield or fruit quality (de Souza et al. 2005, Chaves et al. 2007, Pou et al. 2012).

Although both RDI and PRD systems can improve water use in plants, deficit irrigation responses in grapevines are dependent on specific stress 'tolerance' or 'avoidance' mechanisms (Schultz 2003). Grapevines can have either isohydric or anisohydric behavior depending on the stomatal control strategy presented in response to changes in air vapor pressure deficit (VPD) and/or water availability in the soil (Schultz 2003, Soar et al. 2006, Chaves et al. 2010, Tomás et al. 2014 Lavoie-Lamoureux et al. 2017). In isohydric plants, ABA accumulation in leaves promotes stomata closure early in response to either decreased water content in the soil or increased VPD, so that leaf water potential (Ψ) remains unchanged or slightly decreases (Schultz 2003, Poni et al. 2007, Lovisolo et al.

2010, Pou et al. 2012, Flexas 2016, Lavoie-Lamoureux et al. 2017). However, foliar ABA can also limit leaf gas exchange over the long-term, by preventing stomatal recovery upon rewatering and resulting in the down-regulation of transpiration. Isohydry may favor embolism repair and preserves water under conditions of fluctuating water availability and repeated drought (Tombesi et al. 2015). On the other hand, anisohydric plants have a high tolerance to reduced water availability and do not significantly modify stomatal apertures as Ψ varies (Rogiers et al. 2012, Palliotti et al. 2015, Lavoie-Lamoureux et al. 2017). It has been reported that such iso/anisohydric behavior is influenced by the specific ambient growth conditions, such as hydraulic resistances within the soil-plant system, plant age and climate (Schultz 2003, Lovisolo et al. 2010, Chaves et al. 2010, Hochberg et al. 2013). Currently there is a demand for knowledge about the ecophysiological responses of grapevine cultivars to a reduction in water availability (Chaves et al. 2010, Flexas et al. 2010, 2016, Lavoie-Lamoureux et al. 2017), especially under tropical conditions.

Limitations to plant growth and crop yield imposed by water availability are mainly due to reductions in the carbon balance, and therefore dependent on the equilibrium between photosynthesis and respiration (Poni et al. 2009, Flexas et al. 2006). Viticultural methods for reducing water losses through stomata closure without resulting in concomitant reductions in CO_2 uptake are desired. More specifically, improving the metabolic efficiency of photosynthesis by increasing Rubisco carboxylation capacity, could improve the growth grapevines under limited water conditions (Flexas et al. 2016). Much less attention has been given to respiration (Flexas et al. 2006, Schultz and Stoll 2010, Morales et al. 2016), despite the fact that leaf respiration occurs continuously and even small changes in this process can result in substantial variation in the plant carbon balance (Poni et al. 2009, Flexas et al. 2010, Griffin and Heskel 2013, Tomás et al. 2014). It has been previously reported that grapevine leaf respiration can increase under moderate water shortage (Silva et al. 2017), and deficit irrigation could result in significant carbon losses through leaf respiration (Flexas et al. 2005, Flexas et al. 2010, Salazar-Parra et al. 2015). Moreover, it is known that leaf respiration in the light (R_{light}) is usually lower than in the dark (R_{dark}), with the degree of light inhibition responding differently to environmental signals (Tcherkez et al. 2010), such as water availability (Ayub et al. 2011, Crous et al. 2012). A full understanding of the leaf carbon balance requires that the rate of respiration in both the dark (R_{dark}) and in the light (R_{light}) be quantified.

Parallel to deficit irrigation use, protected cultivation, using transparent plastic covering (TPC), is now becoming a common practice in table grapes (Permanhani et al. 2016). TPC has been reported to increase the water use efficiency in grapevines by creating higher humidity's and lowering transpiration as compared to open field conditions (Stanghellini 2014, Permanhani et al. 2016). Higher shoot growth rates, leaf areas and chlorophyll contents have been observed in grapevines grown under TPC (de Souza et al. 2015). TPC can also protect a grapevine canopy from adverse meteorological conditions, such as wind and rain (Roberto et al. 2011, Du et al. 2015, Permanhani et al. 2016). While rainfall increases grapevine vegetative growth, it can reduce fruit quality due to imbalances in the sink/source ratio (Monteiro and Lopes 2007, Chaves et al. 2010). Moreover, leaf wetness caused by rainfall can trigger disease development, such as mildew and botrytis, leading to use of fungicides and other chemicals (Botelho et al. 2011, Pedro et al. 2011, de Souza et al. 2015, Permanhani et al. 2016). However, the use of specific TPC methodologies (type of plastic used and distance of canopy) can present disadvantages as well such as excessive overheating due to inadequate air circulation (Liu et al. 2012). High temperatures can impair leaf function and alter grape cluster microclimates with a likely negative impact on yield and grape composition (Permanhani et al. 2016). The effects of TPC on grapevines in tropical settings has not been studied, but could provide more in-depth knowledge about the potential use of this technique for extending the range of grapevine cultivation.

To the best of our knowledge, this is the first work designed to analyze the effects of deficit irrigation on table grapes cultivated in tropical conditions under TPC. Studies about protected cultivation in grapevines under tropical conditions are scarce. Moreover, the table grapevine cultivar used in the present work, 'Niagara Rosada', is poorly researched, even though it is widely accepted by consumers and therefore widely cultivated in Brazil. Thus, we undertook an investigation to answer the following set of questions regarding the 'Niagara Rosada' grapevine grown under tropical conditions: (i) Does this genotype present an anisohydric or an isohydric behavior? (ii) How does deficit irrigation affect leaf Ψ? (iii) Can RDI and PRD increase the ratio of A_{net}:g_s and improve water use efficiency (A_{net}/E)? (iv) How does deficit irrigation affect the photochemical and biochemical capacity of the grapevine; (v) What are the effects of deficit irrigation on leaf respiration rates and leaf carbon balance? (vi) Is it possible to save water without affecting yield and fruit quality? (vii) Is polyethylene plastic covering an effective strategy to cultivate ‘Niagara Rosada’ grapevine in Tropical conditions?

MATERIAL AND METHODS

Field conditions and plant material

The research was conducted in a commercial vineyard, located in São Fidelis city, northern Rio de Janeiro state, Brazil (21° 30’ 58” S and 41° 42’ 49.6” W). The climate of the region is wet tropical, with a rainy summer and a dry winter. According to Esteves (2015), the soil is classified as a Red-Yellow Argisol and at a depth of 0 – 40 cm, the soil presents 639.0 g kg^{-1} of sand, 73.5 g kg^{-1} of silt, 288.5 g kg^{-1} of clay and total porosity of 0.4 m^3 m^{-3}. The study was carried out during two seasons, from December 2015 to March 2016 (summer, first season) and from August 2016 to December 2016 (spring, second season). ‘Niagara Rosada’ (*Vitis labrusca*) grapevines grafted on IAC 766 rootstock were used. The 7 years old vineyard has a planting density of 1,850 plants per hectare with grapevines spaced 2.0 m within and 2.7 m between rows (5.4 m^2 $plant^{-1}$). Plants have been trained on a bilateral cordon system using a horizontal shoot positioning with two pairs of wires (one pair on each side of the cordons) set perpendicularly to shoot’s growth and parallel to cordons’ growth. Mixed pruning was performed so that the mean bud number retained per cane was two on the first season (summer) (short spurs) and between six to eight on the second season (spring) (long spurs). Shoots were trimmed at 0.30 m away from the outermost wire *ca*. 35 days after the first production pruning. Standard cultural practices in the region were applied to all plants. Thirty days before pruning, 10 – 15 L of cattle manure were applied per plant on both seasons. After pruning, six soil fertilizations were made, corresponding to a total of 230 kg of N, 170 kg of P_2O_5 and 140 Kg of K_2O ha^{-1} $season^{-1}$.

Climate variables, polyethylene plastic cover and water management treatments

The experimental site was equally divided in two so that one-half was covered with two TPC, measuring 375.0 m^2. Each structure had an arch-shaped ceiling with UV-protection provided by a 150.0 µm thick polyethylene plastic positioned 4.0 m from the ground, and 2 m from the canopy. The sides were closed with anti-bird screen and galvanized gutters were used to prevent rainwater entrance under the cover structure. Plants cultivated on the other one-half of the experimental site (uncovered) were all protected with black anti-bird screen (0.5 m from canopy).

Climate variables were monitored in both the covered and uncovered site. Air temperature and relative humidity were recorded using a data logger (model 1000 Series, Spectrum Technologies, Plainfield, Illinois, USA). Photosynthetic photon flux density (PPFD) was monitored with a quantum light sensor (model LightScout, Spectrum Technologies, Plainfield, Illinois, USA) and recorded with a datalogger (Watch Dog Model 1000 Series, Spectrum Technologies, Plainfield, Illinois, USA), both positioned at the top of grapevine canopies within each site (data was collected each 30 min). Air VPD was calculated according to Jones (1992).

Water was supplied according to the crop evapotranspiration (ET_c) calculated with the equation proposed by Hargreaves and Samani (1985) and corrected with the crop coefficients estimated by Esteves (2015) for different phenological stages. Two irrigation systems were used throughout the experiment. The first one was used for 71 and 64 days, on the first (summer) and second (spring) season, respectively, between pruning and fruit set [bunch closure, stage number 33 according to the grapevine development classification of Eichhorn and Lorenz (1977)] so that all plants were equally irrigated with drip emitters, two per plant, operating at 4.0 L h^{-1} and positioned 0.5 m from the vine trunk, distributed on both sides of the root system. Thereafter, using the second irrigation system, both covered and uncovered plants were exposed to one of three water management treatments: (1) full-irrigated – FI: 100% of the ET_c was supplied simultaneously to both sides of the root system with four drip emitters (two drippers on each side of the root system) operating at 3.8 L h^{-1}; (2) regulated deficit irrigation – RDI: 50% of the ET_c was supplied simultaneously to both sides of the root system with two drip emitters (one dripper on each side of the root system) operation at 3.8 L h^{-1}; and (3) partial rootzone drying – PRD: 50% of ET_c was supplied to only one side of the root system with 2 drip emitters operating at 3.8 L h^{-1}, whereas the other side of the rootzone remained without water. The treatment was reversed every 15 days, and the alternate side of the root system received water. Thus, six plants of each water management treatment were grown in each of the two sites (covered and uncovered). Such water management treatments were applied from fruit set to harvest, for 42 and 54 days (days after initiating treatments – DAIT) on the first and second season, respectively, and total water amount applied during each period were recorded. Overall, plants were irrigated three times a week throughout both seasons.

The rainfall over the uncovered site was monitored using a pluviometer. Cumulative rainfall after initiating treatments was 110 and 240 L m^{-2} during the first and second seasons, respectively. During the first season, volumetric soil moisture was monitored every 15 DAIT at a depth of 0.2 m, 0.5 m from the vine trunk, using a soil moisture probe (Extech Instruments USA, model number MO750). During the second season, soil water potential was recorded using a soil matric potential sensor (model MPS-6 Decagon Devices, Pullman WA, USA and Watermark 200SS, Irrometer Co., Riverside, CA, USA) placed at a depth of 0.4 m, 0.5 m from the vine trunk. The sensors were coupled to a Em50 data collection system (Decagon Devices, Pullman WA, USA and Watermark 200SS, Irrometer Co., Riverside, CA, USA) so that the data were stored every 30 min. Overall, both volumetric soil moisture and soil water potential were measured in one randomly selected plant per treatment, while for PRD, measurements were taken on both sides of the root system (PRD 1 – side that received the first irrigation shift; and PRD 2 – side that first remained without water).

Gas exchange

Overall, the gas exchange measurements and other analyses outlined below were made on one fully expanded well-exposed leaf opposite to the first cluster of the first cane, in each plant. Gas exchange parameters were obtained on the 0, 15th and 30th DAIT of the first season (summer) and on the 15th, 30th and 45th DAIT of the second season (spring), between 08:00 and 12:00 h. Light response curves of net photosynthetic rate (A_{net}) were made using a Li-Cor 6400xt portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA) as described in Shapiro et al. (2004), and taking into consideration the precautions of Pons and Welschen (2002). The system incorporated a CO_2 controller which was used to set the CO_2 concentration inside the Li-Cor cuvette at 400 µL L^{-1}. The 6 cm^2 cuvette was fitted with a red–blue light source (6400-02B). The net photosynthetic rate (A_{net}), stomatal conductance (g_s), transpiration rate (E) and the internal to ambient CO_2 concentration ratio (C_i/C_a) all at 1500 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) were determined from the light response curves with 24 levels of PPFD: 1500, 1200, 800, 500, 200, 100, 90, 80, 70 and every 5 PPFD between 70 and 0 µmol m^{-2} s^{-1}. The rate of respiration in the dark (R_{dark}) was taken as the value of A_{net} at 0 µmol m^{-2} s^{-1} PPFD.

Rate of respiration in the light (R_{light}) was estimated using the method originally described in Kok (1948), as the y-axis intercept of a first-order linear regression fitted to A_{net}–irradiance plots to measurements made over the 25–65 µmol m^{-2} s^{-1} irradiance range. All gas exchange data were corrected for the increase in intercellular CO_2 concentrations (C_{i}) with decreasing irradiance, which can result in reduced rates of photorespiration and increased rates of carboxylation (Villar et al. 1994). The correction was applied by adjusting the R_{light} through iteration to minimize the intercept of photosynthetic electron transport (J) as a function of irradiance (Kirschbaum and Farquhar 1987). J was calculated according to Farquhar and von Caemmerer (1982):

$$J = \frac{[\left(4\left(A_{net} + R_{light}\right)\right)(C_i + 2\Gamma^{*})]}{(C_i - \Gamma_{*})} \qquad (1)$$

where Γ^* is the CO_2 compensation point in the absence of R_{light} (von Caemmerer and Farquhar 1981). Rates of oxygenation and carboxylation by Rubisco (V_{o} and V_{c}, respectively) at saturating irradiance were also calculated according to Farquhar and von Caemmerer (1982):

$$V_c = \frac{1}{3}\left[\left(\frac{J}{4}\right) + 2\left(A_{net} + R_{light}\right)\right] \qquad (2)$$

and

$$V_o = \frac{2}{3}\left[\left(\frac{J}{4}\right) - \left(A_{net} + R_{light}\right)\right] \qquad (3)$$

The effects of varying atmospheric [O_2] or [CO_2] on oxygenation (V_o) and carboxylation (V_c) at saturating light were similarly calculated according to Farquhar and von Caemmerer (1982):

$$V_c = \frac{[CO_2]V_{cmax}}{[CO_2] + K_c\left(1 + \frac{[O_2]}{K_o}\right)} \qquad (4)$$

and

$$V_o = \frac{[O_2]V_{omax}}{[O_2] + K_o\left(1 + \frac{[CO_2]}{K_c}\right)} \qquad (5)$$

Equations 4 and 5 used the Rubisco kinetic constants (K_c, K_o and V_{omax}/V_{cmax}) previously determined by Bernacchi, et al. (2002), and calculated V_{cmax} as:

$$V_{cmax} = \frac{A_{net} - R_{light}}{\frac{[CO_2]-\Gamma^*}{[CO_2]+K_c\left(1+\frac{[O_2]}{K_c}\right)}} \qquad (6)$$

Γ * (the CO_2 compensation point in the absence of R_{light}), depends on the Rubisco specificity factor, O_2 partial pressure and is calculated according to von Caemmerer and Farquhar (1981):

$$\Gamma^* = \frac{0.5V_{omax}K_c[O_2]}{V_{cmax}K_o} \qquad (7)$$

Here we use the specificity presented in Cousins et al. (2010), the ambient O_2 concentration and the temperature response function of Brooks and Farquhar (1985) to determine Γ*. All rates of V_o and V_c reported here were calculated at ambient CO_2 and O_2 concentration and PPFD of 1500 μmol m^{-2} s^{-1} ($V_{o\,1500}$ and $V_{c\,1500}$).

The A_{net}, g_s and E values at 1500 μmol m^{-2} s^{-}1 were used to calculate the water use efficiency (A_{net}/E) and the intrinsic water use efficiency (A_{net}/g_s). In addition, leaf carbon balance (LCB) for the experiment was estimated using the following equation:

$$LCB = \frac{A_{net}}{(R_{dark} + R_{light})} \qquad (8)$$

All gas exchange measurements were taken at a relative humidity of approximately 65-75% by manipulating the amount of air passing through a drying column prior to entering the leaf cuvette. The Li-Cor cuvette block temperature was set to 30°C for all measurements to account for the influences of leaf temperature on gas exchange variables. In addition, the flow rate was kept at 300 µmol m^{-2} s^{-1}.

SPAD values and Chlorophyll a fluorescence

SPAD values and Chlorophyll *a* fluorescence were measured on the same intact leaves used for gas exchange measurements and on the same days, between 08:00 and 10:00 h. Five *SPAD* values were averaged using the SPAD-502 Chlorophyll Meter (Minolta Co. Ltd., Osaka, Japan). Maximum quantum yield of primary photochemistry (F_v/F_m) and the performance index [*PI* - energy cascade processes from the first light absorption events until plastoquinone reduction (Strasser et al., 2004)] were measured using a non-modulated fluorimeter model Pocket PEA (Plant Efficiency Analyser, Hansatech, King's Lynn, Norfolk, UK). The leaves were dark-adapted for *ca.* 30 min. using leaf clips (Plant Efficiency Analyser, Hansatech, King's Lynn, Norfolk, UK) so that all reaction centers of photosystem II (PSII) acquired an 'open' status, and heat loss was minimal (Strasser et al. 2000).

The JIP-test equations (Strasser and Strasser 1995, Strasser et al. 1995, Strasser and Tsimilli-Michael 2001, Strasser et al. 2004) were applied to calculate: the energy needed to close all reaction centers expressed by the normalized area – S_m [Area/(F_m / F_v)]; fraction of active reaction centers per excited cross-section of leaf – RC/CS_0; the efficiency with which a trapped excitation energy can move an electron into the electron transport chain further than the Quinone A (*QA*) - ET_0/TR_0; the quantum yield of electron transport – ET_0/ABS; and dissipation energy flux at the antenna chlorophylls level per excited cross-section - DI/CS_0.

Leaf temperature

Leaf temperature (T_{leaf}) was measured with a ThermaCAM^{TM} Flir i50 (Flir Systems Inc., Goleta, CA, USA). The emissivity was set at 0.96 and the camera was placed *ca.* 0.5 m from the same leaves used for gas-exchange measurements. Data were collected between 11:00–12:00 h at 0, 15 and 30 DAIT on the first season, as well as at 15, 30 and 45 DAIT on the second season. Thermal images were analyzed with FLIR QuickReport 1.2 (FLIR Systems, Inc., Goleta, CA, USA). The crop water stress index (*CWSI*) at leaf level was calculated according to Grant et al., (2007):

$$CWSI = \frac{T_{dry} - T_{leaf}}{T_{dry} - T_{wet}} \qquad (9)$$

where T_{dry} is the temperature of a leaf covered with liquid paraffin applied below the leaf, which avoided transpiration (increased leaf temperature), while T_{wet} is the temperature of a leaf sprayed with fresh water (reduced leaf temperature). T_{leaf} values that are close to T_{wet} will result in high *CWSI* values, which are indicative of intense transpiration. When a plant is transpiring fully the leaf temperature is 1 to 4 degrees below air temperature and the CWSI is 1. As the transpiration decreases, the leaf temperature rises and can reach to 4 to 6 degrees above air temperature. When the plant is no longer transpiring the CWSI is 0.

Leaf water potential

Leaf water potential (Ψ) was measured immediately after leaf excision at pre-dawn (Ψ_{pd}) and midday (Ψ_{md}) according to Schölander et al. (1965), using a pressure chamber (Model 1000, PMS Instrument Co., Albany, OR, USA). The drop (%) of Ψ_{md} in relation to Ψ_{pd} was also calculated (Ψ_{drop}). Measurements were performed on leaves adjacent to those used for the analyses previously outlined (well-exposed fully expanded leaf opposed to the first cluster of the first cane) and on the 0, 15th and 30th DAIT of the first season, as well as on the 15th, 30th and 45th DAIT of the second season.

C-isotope composition ($\delta^{13}C$)

On the 54th DAIT of the second season, tips of shoot (*ca.* 0.01 m, where leaves used for previous analyses were inserted) were dried in an air forced ventilation oven at 70°C for 72 h, grounded in a mill grinder (Willy–MSSL-031) and a range of *ca.* 2.00 mg weighed out in tin capsules. Thereafter, the carbon ($^{13}C/^{12}C$) isotope ratio of the samples were analyzed in an Isoprime isotope ratio mass spectrometer (Micromass, UK) coupled to an elemental analyzer (EuroVector, Italy). The isotopic C-ratio was calculated using the following standard δ notation:

$$\delta^{13}\mathrm{C} = \left(\left(\frac{\mathrm{Rsample}}{\mathrm{Rreference}}\right) - 1\right) * 1000(‰) \qquad (10)$$

where R = $^{13}C/^{12}C$ for carbon. The isotope ratios were calibrated against the international standards IAEA CH6 and IAEA CH7. $\delta^{13}C$ results were referenced against PeeDee Belemnite (PDB). Precision (the standard deviation of the set of standards analyzed in each batch) was 0.06‰.

Fruit quality and yield

On the 42nd and 54th DAIT of the first and second seasons, respectively, the clusters opposite to the leaves used for the analyses previously outlined were collected to count total berry number, cluster length and diameter of clusters using a digital caliper (± 0.01 mm precision) and cluster weight using a digital balance (± 0.001 g precision). Anthocyanins index was estimated using the Multiplex 2 (Mx) sensor (Force-A, Orsay, France). Three measurements were averaged per cluster. This instrument has four light-emitting diode sources in the UV-A (370 nm), blue (460 nm), green (515 nm) and red (637 nm) spectral regions (Betemps et al. 2012). The excitation light is sequentially pulsed at 476 Hz with 20 µs per flash and synchronized with the photodiode detectors to record the fluorescence signals. Chlorophyll fluorescence, both in the red (680–690 nm - RF) and far-red (730–780 nm - FRF) bands, was acquired sequentially at all the excitation wavelengths. The combination of RF and FRF fluorescence signals obtained with the different excitation bands provided the variable:

$$\text{Anthocyanins (ANTH RG)} = \log\left(\frac{FRF_{red}}{FRF_{green}}\right) \qquad (11)(\text{Tuccio et al. } 2011)$$

Thereafter, 10 berries of each cluster were randomly selected for measurement of diameter, length and weight. The same 10 berries were used to estimate the total soluble solids (◦Brix) with a refractometer (model ATAGO N1 E, Atago CO., LTD, Itabashi-ku, Tokyo, Japan). These same 10 berries were then manually macerated and 5 mL of juice were added to 45 mL of deionized water in order to measure the pH, using a digital pH meter (model QA400SE, Quimis, Diadema, São Paulo, Brazil). Total acidity was obtained by titration with NaOH as recommended by OIV (1990) and the relation between total soluble solids/total titratable acidity was calculated. Moreover, at harvest, yield components were assessed, following manual harvesting and weighing the production on-site. Cluster number and yield per vine were recorded for all plants on each site.

Statistical analysis

A randomized block design in a split plot scheme was used with two growth conditions (covered and uncovered) x three water management x six replicates, totaling 36 plots. Data were subjected to analysis of variance tests and the mean pairwise comparisons made using the Tukey HSD test at 5% probability. Linear regression slopes for the relationship between Ψ and VPD was calculated. Statistical analysis was made using the software ASSISTAT 7.0 BETA.

RESULTS

During the first season (summer), the seasonal average air temperature was 27.9 ºC in both the covered and uncovered sites (Figure 1 – A). Both the maximum and average relative humidity were *ca.* 12% higher in the covered site, reaching values close to 100% on most days (Figure 1 – B). Similarly, the average VPD was *ca.* 15% higher in the covered site (Figure 1 – D). The average, maximum and minimum PPFD were *ca.* 14% higher in the covered site, indicating that during the first season, more PPFD was intercepted by the anti-bird screen of the uncovered site than by the TPC (Figure 1 – C). Climate variables widely varied throughout the second season (Figure 2). The thermal amplitude of the uncovered site was higher, with lower minimum (*ca.* 5%) and higher maximum (*ca.* 5%) air temperatures being recorded throughout the season (Figure 2 – A). On the other hand, the highest (96.7%) and the lowest (37.2%) relative humidity values were observed in the covered site (Figure 2 – B). Due to dust accumulation over the TPC, PPFD in the covered site was *ca.* 10 % lower than the uncovered site during the second season (Figure 2 – C). The highest VPD values observed in the covered and uncovered sites were 6.0 and 5.5 kPa, respectively (Figure 2 – D).

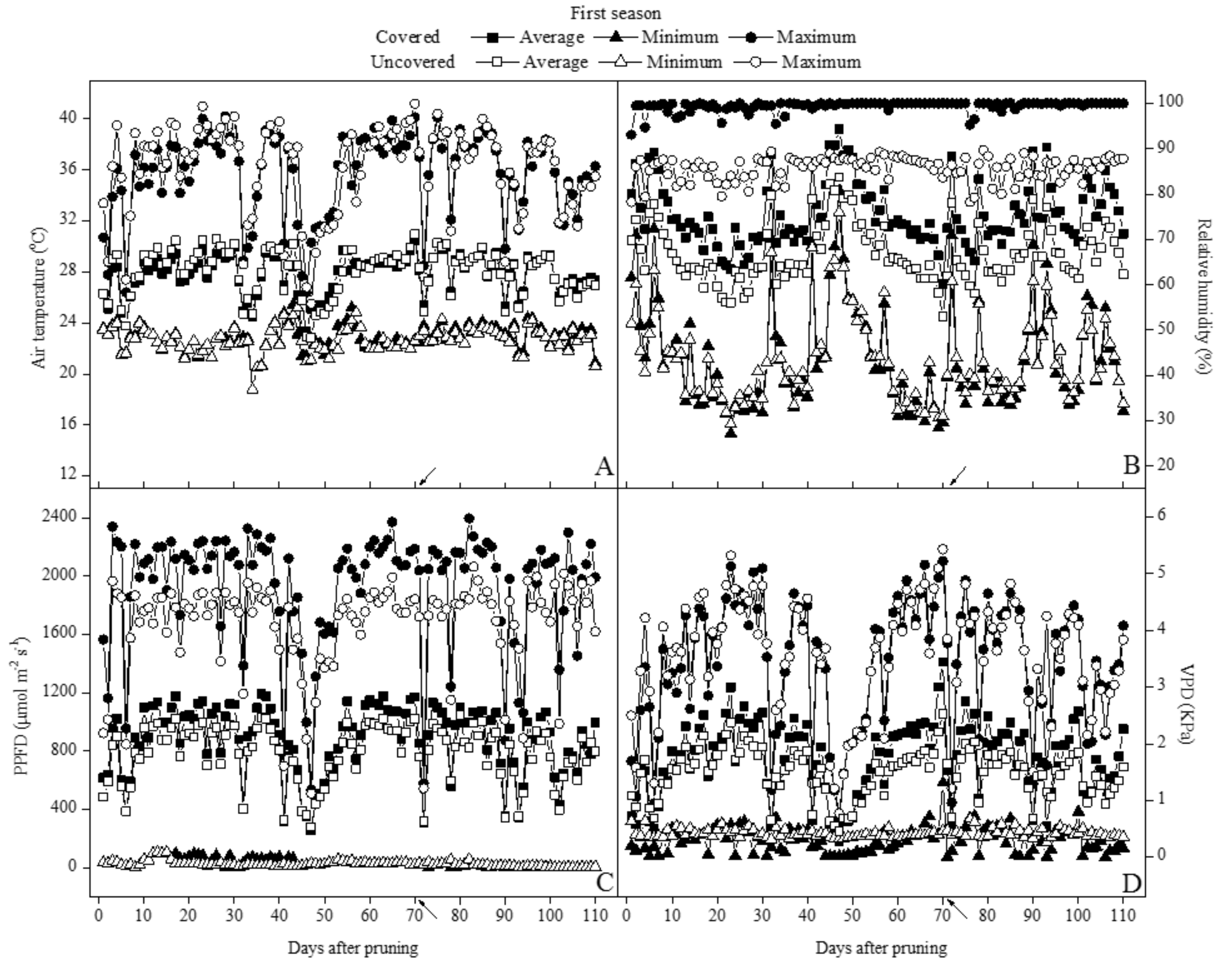

Figure 1. Average, minimum and maximum air temperature (A), relative humidity (B), photosynthetic photon flux density (PPFD) (C) and air vapor pressure deficit (VPD) (D) throughout the first season of the experiment. The arrows indicate water management treatments start (71 days after pruning).

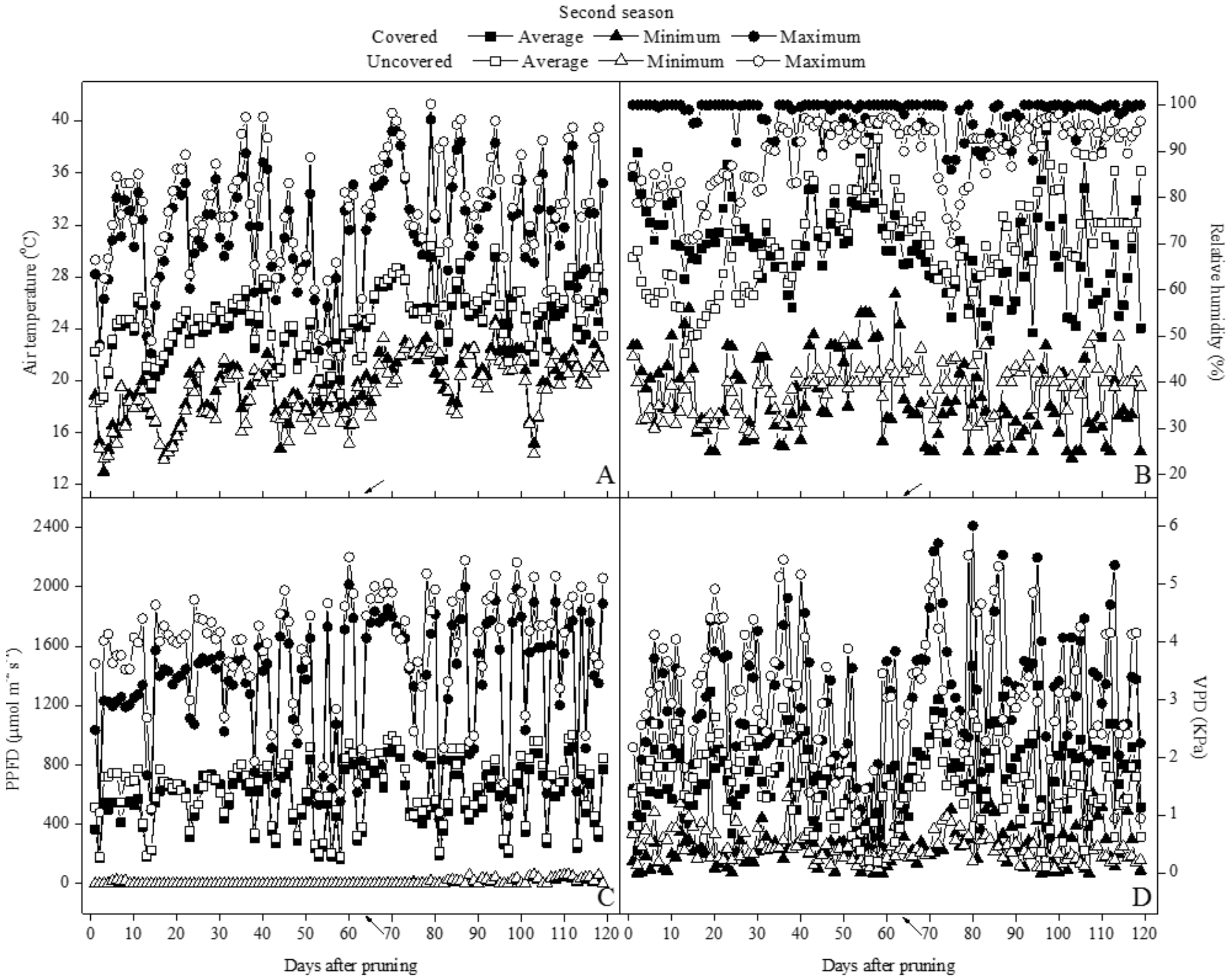

Figure 2. Average, minimum and maximum air temperature (A), relative humidity (B), photosynthetic photon flux density (PPFD) (C) and air vapor pressure deficit (VPD) (D) throughout the second season of the experiment. The arrows indicate water management treatments start (64 days after pruning).

Volumetric soil moisture was maintained at *ca.* 15% in FI plants grown in the covered site and at *ca.* 16% in the uncovered site during the first season (Figure 3). The lowest values of soil moisture in RDI plants were 6.7 and 9.1% in covered and uncovered sites, respectively (Figure 3). The non-irrigated side of the PRD treatment approached the lowest volumetric soil moisture values at 28 DAIT in the covered site (5.7%) and at 42 DAIT in the uncovered site (8.7%) (Figure 3). Soil water potential measured during the second season can be observed in Figure 4. In covered RDI and PRD (non-irrigated side) plots, soil water potential decreased as much as −112.2 and −160.2 kPa, respectively, while FI plants were maintained at *ca.* -21 kPa (Figure 4 – A). Due to the rainfalls during the second season, differences in soil water potential among water management treatments were not observed in the uncovered site (Figure 4 – B).

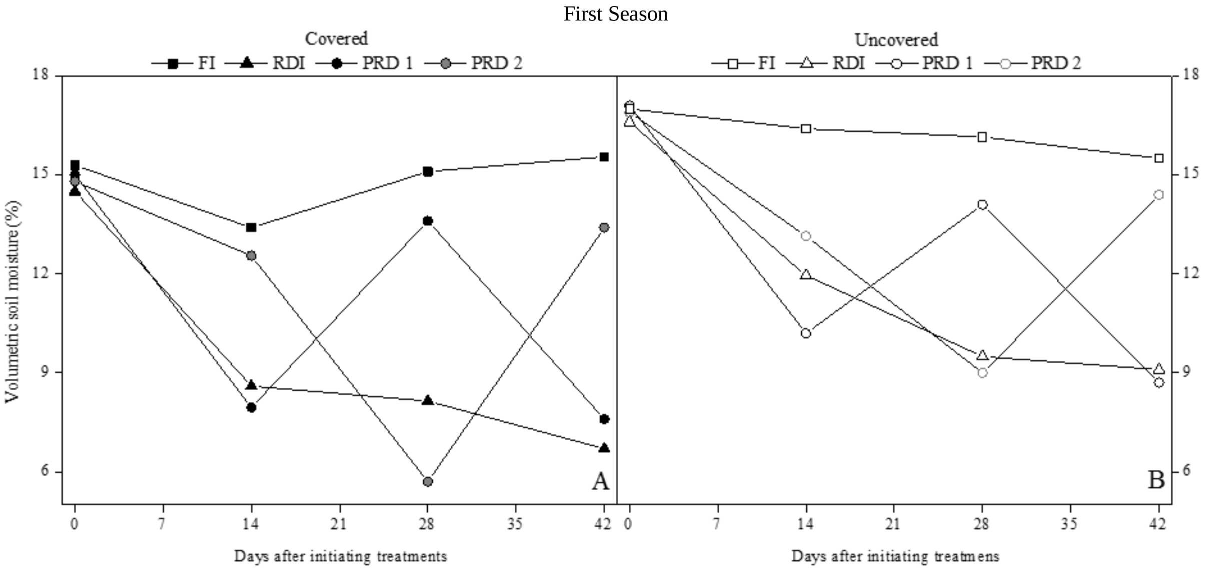

Figure 3. Volumetric soil moisture (%) during the first season of the experiment of three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered (A) or uncovered sites (B).

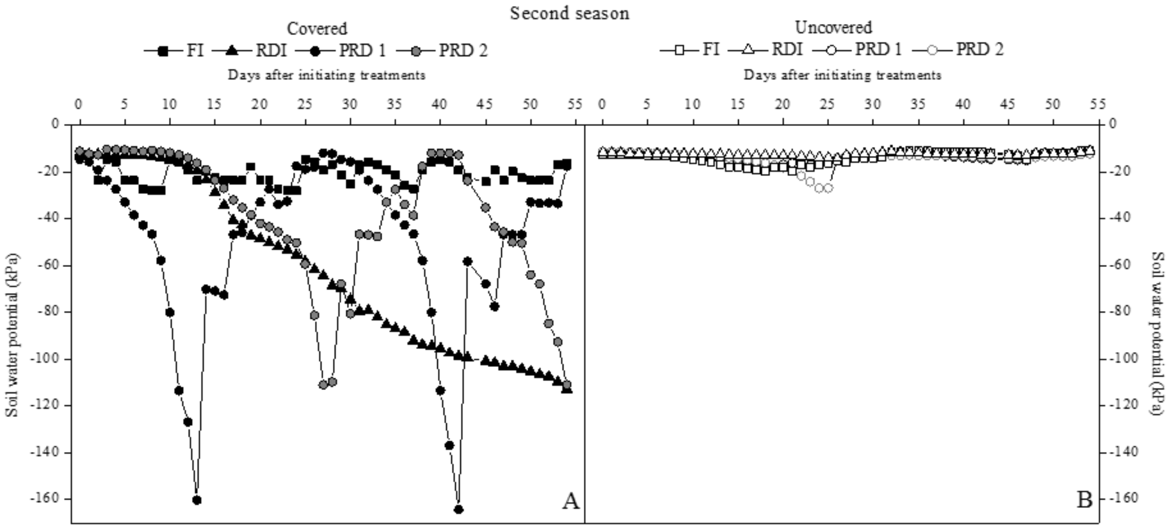

Figure 4. Soil water potential (kPa) during the second season of the experiment of three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered (A) or uncovered sites (B).

Since differences in soil water potential among water management treatments were not observed in the uncovered site during the second season (spring) (Figure 4 - B), only mean values regarding such site and season (regardless of water management) are noted in most Figures and Tables presented. Results regarding *SPAD* values, chlorophyll a fluorescence parameters, *CWSI*, leaf water potential, A_{net}, E, g_s, C_i/C_a, $V_{c\,1500}$ and $V_{o\,1500}$, measured at 0 and 15 DAIT on the first season and at 15 and 30 DAIT during the second season, are not shown (no significant differences). Moreover, no significant differences were observed between RDI and PRD treatments.

Under the experimental conditions, ‘Niagara Rosada’ grapevines presented an anisohydric behavior in response to increase in the ambient VPD during the day (Figure 5). In both sites and seasons, plants of all water management treatments, including FI, showed at midday (Ψ_{md}), when air VPD was higher than 2.92 kPa, a leaf water potential value that was *ca.* 81% lower than Ψ_{pd} recorded at air VPD lower than 0.57 kPa (Table 1). As a consequence, a close negative linear relationship between Ψ and air VPD occurred (Figure 5). However, no effects of differential water supply and/or TPC treatments were observed on either Ψ_{pd} or Ψ_{md} values (Table 1). Furthermore, there were no stomatal effects in response to water management treatments, and A_{net}, E and g_s did not significantly change in either seasons (Table 2). Although the covered PRD plants presented a non-significant tendency to present lower E and g_s values than both RDI and FI at 30 and 45 DAIT of the first and second season, respectively (Table 2), this trend was not observed on the other days (data not shown). The lowest g_s value observed was 0.17 mol H_2O m^{-2} s^{-1} in the covered PRD plants at both seasons (Table 2). Although without significant differences, regardless of water management treatments, a tendency towards lower g_s and E values (22.76% and 14.1%, respectively) were observed in the covered site throughout the entire experiment, whereas A_{net} was slightly higher (*ca.* 1.4%) (Table 2). Water management treatments did not show significant differences for calculated rates of Rubisco carboxylation and oxygenation at 1500 µmol m^{-2} s^{-1} ($V_{c\ 1500}$ and $V_{o\ 1500}$) as well as C_i/C_a and $\delta^{13}C$, in both sites (Table 2). However, regardless of water management treatments, plants grown in the covered site presented non-significant trends, throughout both seasons, to lower C_i/C_a (*ca.* 10%), higher $V_{c\ 1500}$ (*ca*. 4%) and $V_{o\ 1500}$ (*ca.* 5%), as well as slightly less negative $\delta^{13}C$ (*ca.* 3%) than uncovered plants at the end of the second season (Table 2).

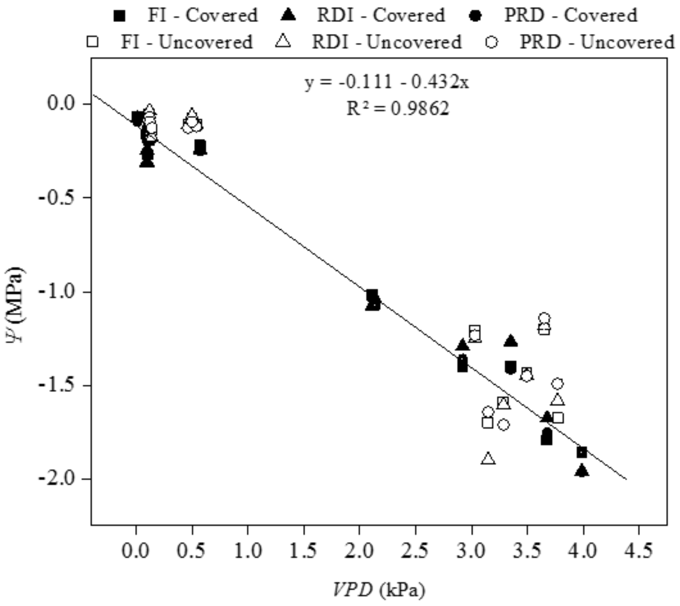

Figure 5 - Relationship between *Ψ* and VPD of grapevine during the first and the second season of the experiment under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered or uncovered sites. Each point represents the mean of six replicates (n = 72).

Table 1. Leaf water potential at predawn (Ψ_{pd}), midday (Ψ_{md}), drop (%) of Ψ_{md} in relation to Ψ_{pd} (Ψ_{drop}) and air vapor pressure deficit (*VPD*) at predawn and midday of grapevine grown for two seasons under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered or uncovered sites. Each treatment mean represents the average of 6 replicates

	First Season - 30 DAIT*						Second Season - 45 DAIT*					
	Covered			Uncovered			Covered			Uncovered		
	ψ_{pd} (MPa)	ψ_{md} (MPa)	ψ_{drop} (%)	ψ_{pd} (MPa)	ψ_{md} (MPa)	ψ drop (%)	ψ_{pd} (MPa)	ψ_{md} (MPa)	ψ_{drop} (%)	ψ_{pd} (MPa)	ψ_{md} (MPa)	ψ_{drop} (%)
FI	-0.22	-1.40	84.29	-0.18	-1.21	85.12	-0.27	-1.40	80.71	-	-	-
RDI	-0.24	-1.29	81.40	-0.15	-1.21	87.60	-0.29	-1.39	79.14	-	-	-
PRD	-0.25	-1.32	81.06	-0.19	-1.23	84.55	-0.26	-1.43	81.82	-	-	-
Mean	-0.24	-1.34	82.09	-0.17	-1.22	86.07	-0.27	-1.41	80.56	-0.31	-1.26	75.40
VPD (kPa)	0.57	2.92	-	0.50	3.03	-	0.18	3.35	-	0.14	3.65	-
CV (%)	39.55	14.37	-	39.55	14.37	-	49.03	17.66	-	49.03	17.66	-

* No statistical differences were observed according to analysis of variances tests.

Table 2. Net photosynthetic rate (A_{net}), Stomatal Conductance (g_s), Transpiration (E), ratio of internal to external CO_2 concentration (C_i/C_a), Rubisco carboxylation ($V_{c\ 1500}$) and oxygenation ($V_{o\ 1500}$) rates and C-isotope composition ($\delta^{13}C$) of grapevine grown for two seasons under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered or uncovered sites. Each treatment mean represents the average of six replicates

	First Season - 30 DAIT*									Second season - 45 DAIT*					
	Covered				Uncovered					Covered				Uncovered	
	FI	RDI	PRD	Mean	FI	RDI	PRD	Mean	CV (%)	FI	RDI	PRD	Mean	Mean	CV (%)
A_{net} (μmol CO_2 m^{-2} s^{-1})	11.86	12.59	10.23	11.56	12.13	11.02	11.21	11.45	24.87	12.48	14.20	11.69	12.79	12.55	28.56
E (mmol H_2O m^{-2} s^{-1})	3.27	3.44	3.06	3.25	3.97	3.58	3.85	3.80	19.56	3.12	3.45	2.63	3.07	3.81	25.34
g_s (mol H_2O m^{-2} s^{-1})	0.20	0.21	0.17	0.19	0.25	0.21	0.22	0.23	25.19	0.19	0.20	0.17	0.19	0.22	25.11
C_i/C_a	0.72	0.71	0.71	0.71	0.80	0.76	0.71	0.78	7.85	0.73	0.67	0.65	0.68	0.76	4.09
$V_{c\ 1500}$ (μmol m^{-2} s^{-1})	16.40	17.49	15.85	16.58	17.26	15.87	15.44	16.19	19.73	15.34	18.87	14.03	16.08	15.67	20.44
$V_{o\ 1500}$ (μmol m^{-2} s^{-1})	5.74	6.11	5.83	5.89	6.05	5.68	5.25	5.66	22.20	5.62	5.81	5.83	5.75	5.40	28.93
$\delta^{13}C$	-	-	-	- -	-	-	-	- -	-	-25.68	-25.55	-25.18	-25.47	-26.11	2.10

* No statistical differences were observed according to analysis of variances tests.

No significant differences were found for either season in A_{net}/g_s and A_{net}/E among water management treatments in covered (Figure - 6 A and D; Figure 7 – A and C) and uncovered (Figure 6 – B and E) sites, throughout the experiment. Regardless of water management treatments, there were non-statistical trends for higher A_{net}/g_s (*ca.* 12.7%, Figure 6 – C and Figure 7 - B) and A_{net}/E ratios (*ca.* 14.8%, Figure 6 – F and Figure 7 – D) in covered plants than the uncovered plants throughout both seasons and measurement days.

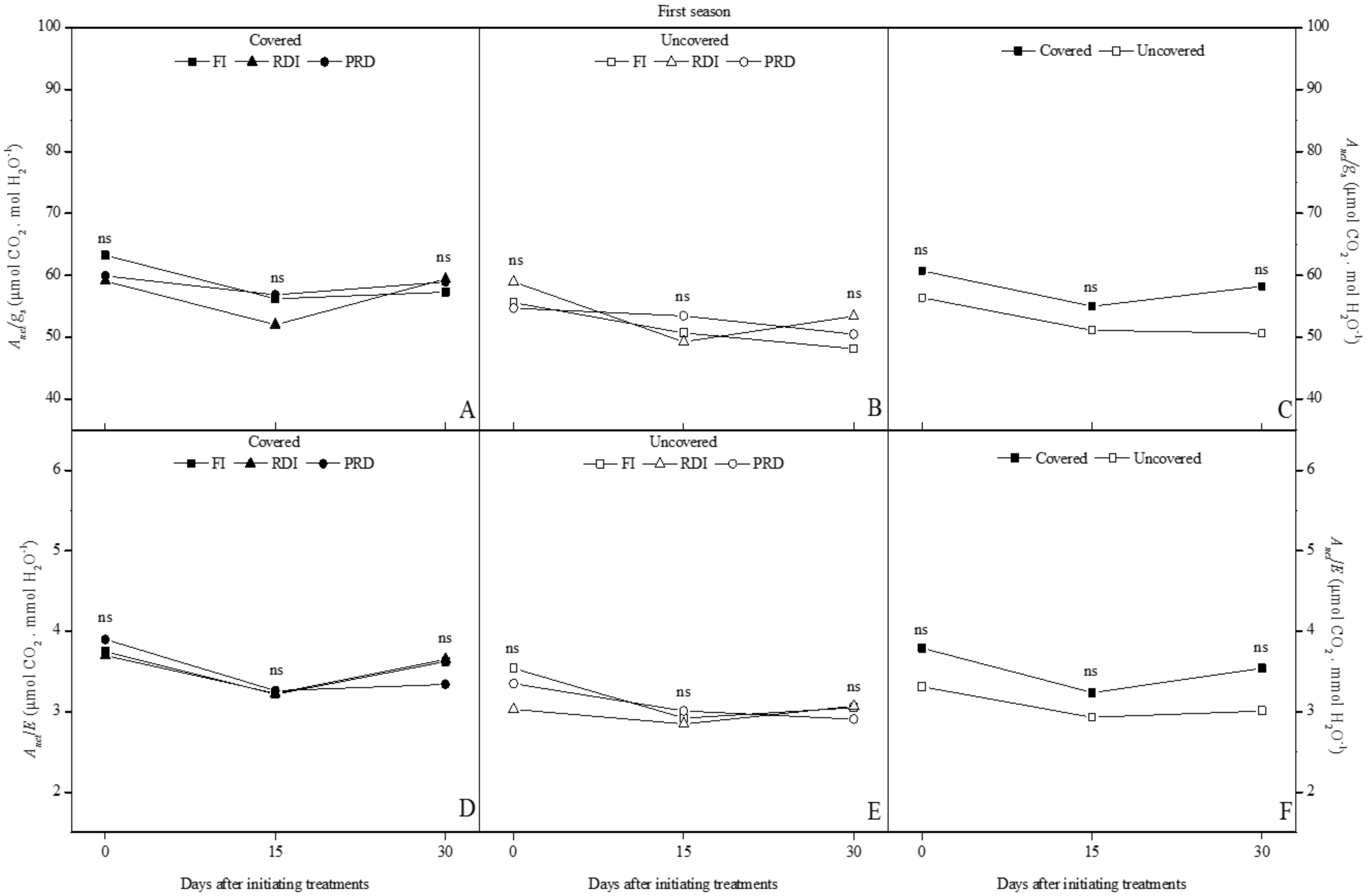

Figure 6 - Intrinsic Water Use Efficiency (*A_{net}/g_s*) (A, B and C) and Water Use Efficiency (*A_{net}/E*) (D, E and F) of grapevine during the first season of the experiment under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered (A and D) or uncovered (B and E) sites. Each treatment mean represents the average of 6 (A, B, D and E) or 18 replicates (C and F). *ns* indicates no statistical difference according to analysis of variances tests.

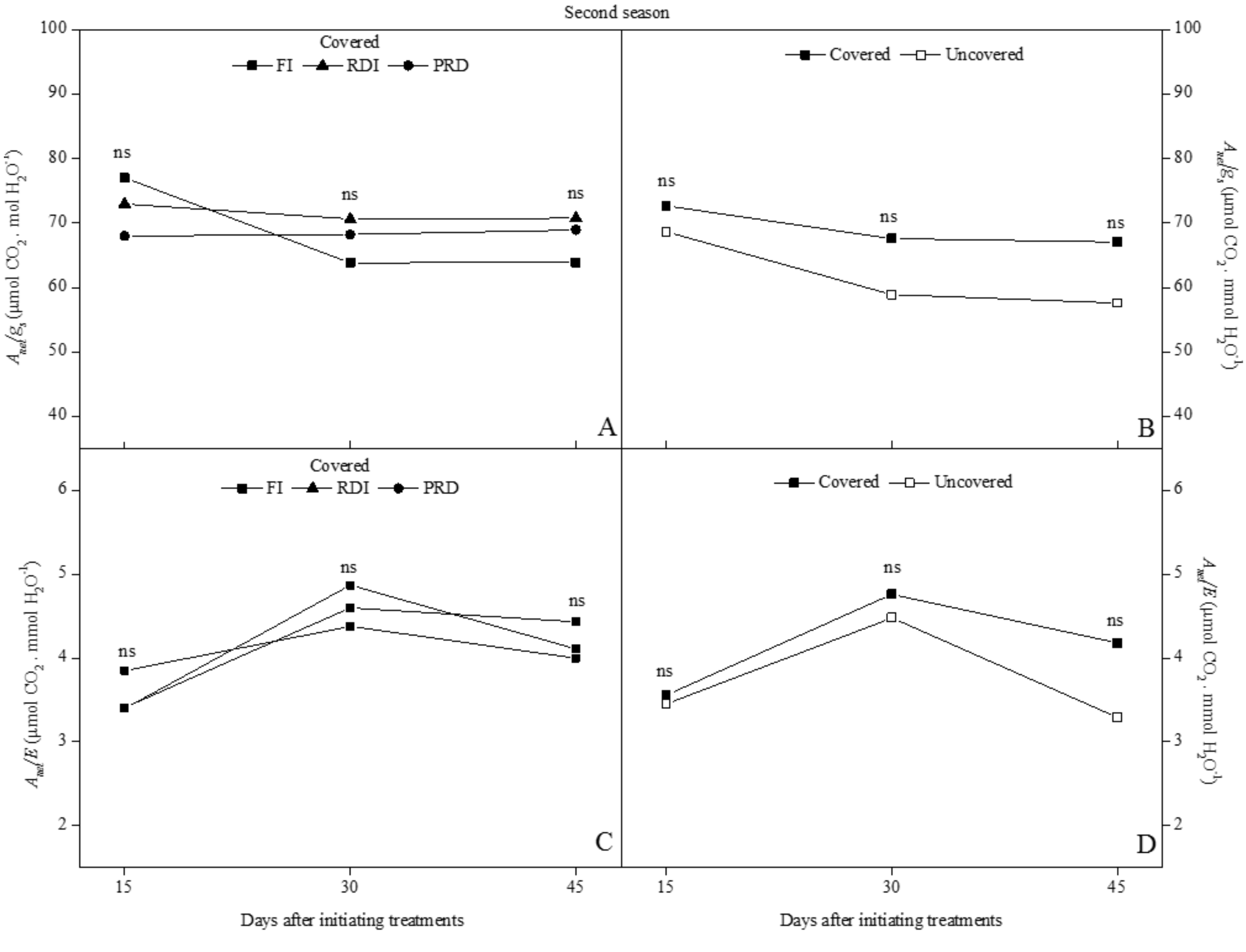

Figure 7 - Intrinsic Water Use Efficiency (A_{net}/g_s) (A and B) and Water Use Efficiency (A_{net}/E) (C and D) of grapevine during the second season of the experiment under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered (A and C) or uncovered sites (comparison regardless water management treatments). Each treatment mean represents the average of 6 (A and C) or 18 replicates (B and D). *ns* indicates no statistical difference according to analysis of variances tests.

SPAD values were not affected by the TPC, or by reducing water supply through either RDI or PRD, (Table 3). Similarly, photochemical capacity was not influenced by either water management treatments or TPC use, and thus significant differences in chlorophyll fluorescence variables were not observed and F_v/F_m values remained higher than 0.81 at the end of each season for all treatments (Table 3). When either RDI or PRD was used, T_{leaf} did not differ from those in the FI treatment (Table 3) in either the covered or uncovered sites and hence, *CWSI* was not affected (Table 3). However, regardless water management treatments, there were non-statistical trends towards higher T_{leaf} (*ca.* 6%) and lower *CWSI* values (*ca.* 11%) in covered plants relative to uncovered plants throughout both seasons (Table 3).

Table 3. *SPAD* values, maximum quantum yield of primary photochemistry (F_v/F_m ratio) (A), performance index (PI), energy needed to close all reaction centers expressed by the normalized area (S_m), fraction of active reaction centers per excited cross-section of leaf (RC/CS_0), efficiency with which a trapped excitation energy can move an electron into the electron transport chain further than the Quinone A (ET_0/TR_0), quantum yield of electron transport (ET_0/ABS), dissipation energy flux at the antenna chlorophylls level per excited cross-section (DI/CS_0) leaf temperature (T_{leaf}) and crop water stress index (*CWSI*) of grapevine grown for two seasons under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered or uncovered sites. Each treatment mean represents the average of six replicates

	First Season - 30 DAIT[*]									Second season - 45 DAIT[*]					
	Covered				Uncovered					Covered				Uncovered	
	FI	RDI	PRD	Mean	FI	RDI	PRD	Mean	CV (%)	FI	RDI	PRD	Mean	Mean	CV (%)
SPAD values	53.47	54.20	50.55	52.74	54.95	53.20	53.90	54.02	2.49	51.55	50.38	49.97	50.63	49.55	4.06
F_v/F_m ratio	0.82	0.83	0.82	0.82	0.82	0.82	0.82	0.82	1.23	0.84	0.83	0.81	0.83	0.83	1.75
PI	5.24	5.88	5.07	5.40	5.05	4.86	4.95	4.95	17.84	6.87	6.67	6.04	6.53	5.91	21.78
S_m	24.94	23.38	25.77	24.70	20.61	20.69	23.24	21.51	14.30	19.11	20.89	21.04	20.35	19.91	17.37
RC/CS_0	45.08	45.59	44.42	45.03	42.98	40.86	46.64	43.49	6.40	50.96	51.83	49.03	50.61	52.30	10.20
ET_0/TR_0	0.64	0.64	0.60	0.63	0.63	0.63	0.63	0.63	3.73	0.65	0.64	0.60	0.63	0.61	5.51
ET_0/ABS	0.51	0.52	0.48	0.50	0.51	0.50	0.51	0.51	5.97	0.51	0.52	0.48	0.50	0.53	6.67
DI_0/CS_0	1147.64	1141.07	1146.01	1144.91	1201.17	1141.07	1262.49	1201.58	10.72	1075.82	1144.72	1184.92	1135.15	1190.13	6.27
T_{leaf} (ºC)	34.31	34.45	34.43	34.39	33.21	32.26	33.55	33.01	3.40	32.25	32.15	32.19	32.19	29.82	3.49
CWSI	0.55	0.54	0.49	0.53	0.58	0.69	0.60	0.62	26.56	0.58	0.56	0.59	0.58	0.63	14.64

[*] No statistical differences were observed according to analysis of variances tests.

Reducing water supply through either RDI or PRD in both sites, as well as using TPC (regardless water management treatments), did not significantly change R_{dark}, R_{light} and R_{light}:R_{dark} in relation to FI and uncovered conditions respectively, throughout both seasons (Figures 8 and 9). Likewise, LCB did not significantly vary in response to treatments applied during the entire experiment (Figures 10 and 11).

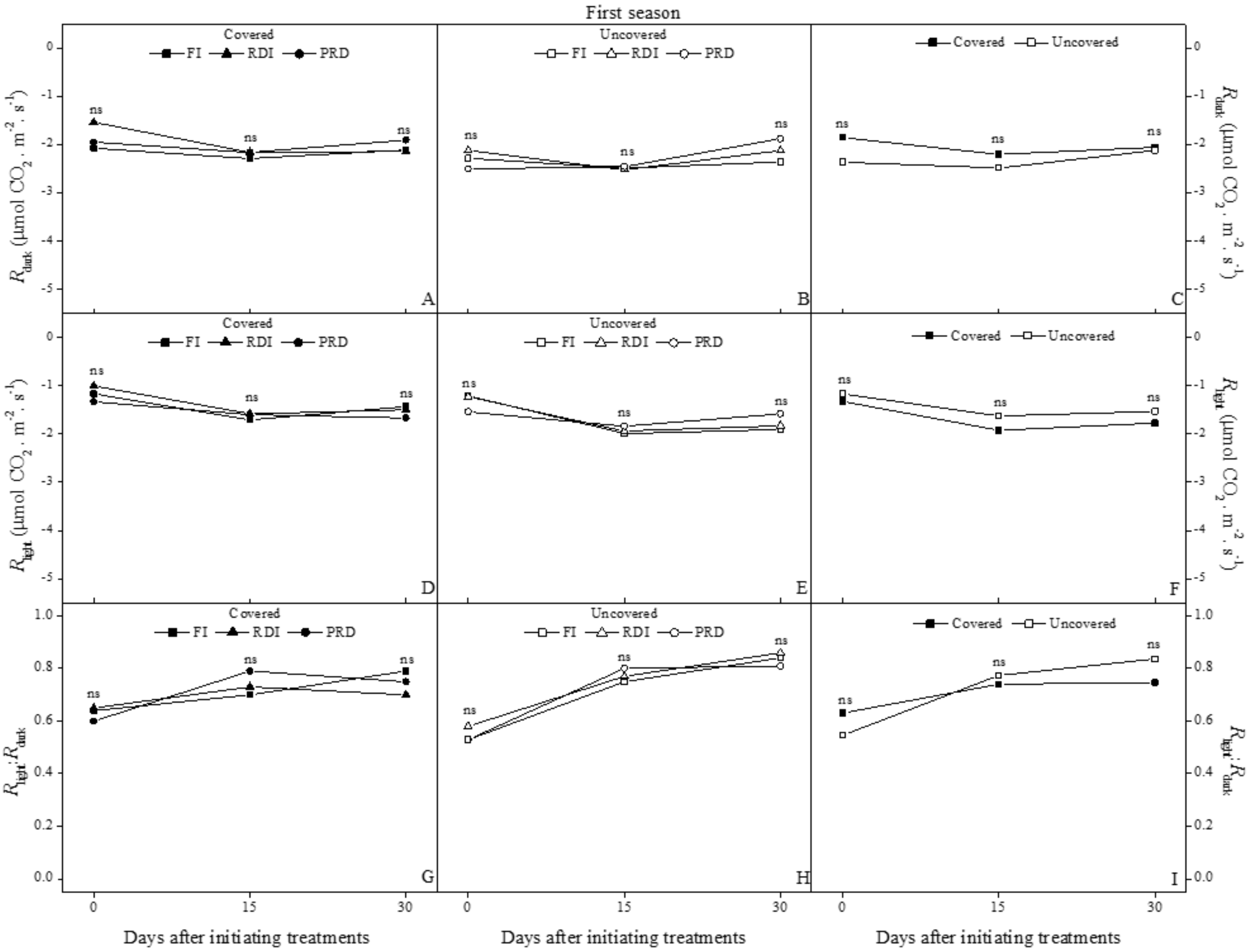

Figure 8. Leaf respiration in the dark (R_{dark}) (A, B and C) and in the light (R_{light}) (D, E and F) and light inhibition of respiration (R_{light}:R_{dark}) (G, H and I) of grapevine during the first season of the experiment under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered (A, D and G) or uncovered (B, E and H) sites. Each treatment mean represents the average of 6 (A, B, D, E, G and H) or 18 replicates (C, F and I). *ns* indicates no statistical difference according to analysis of variances tests.

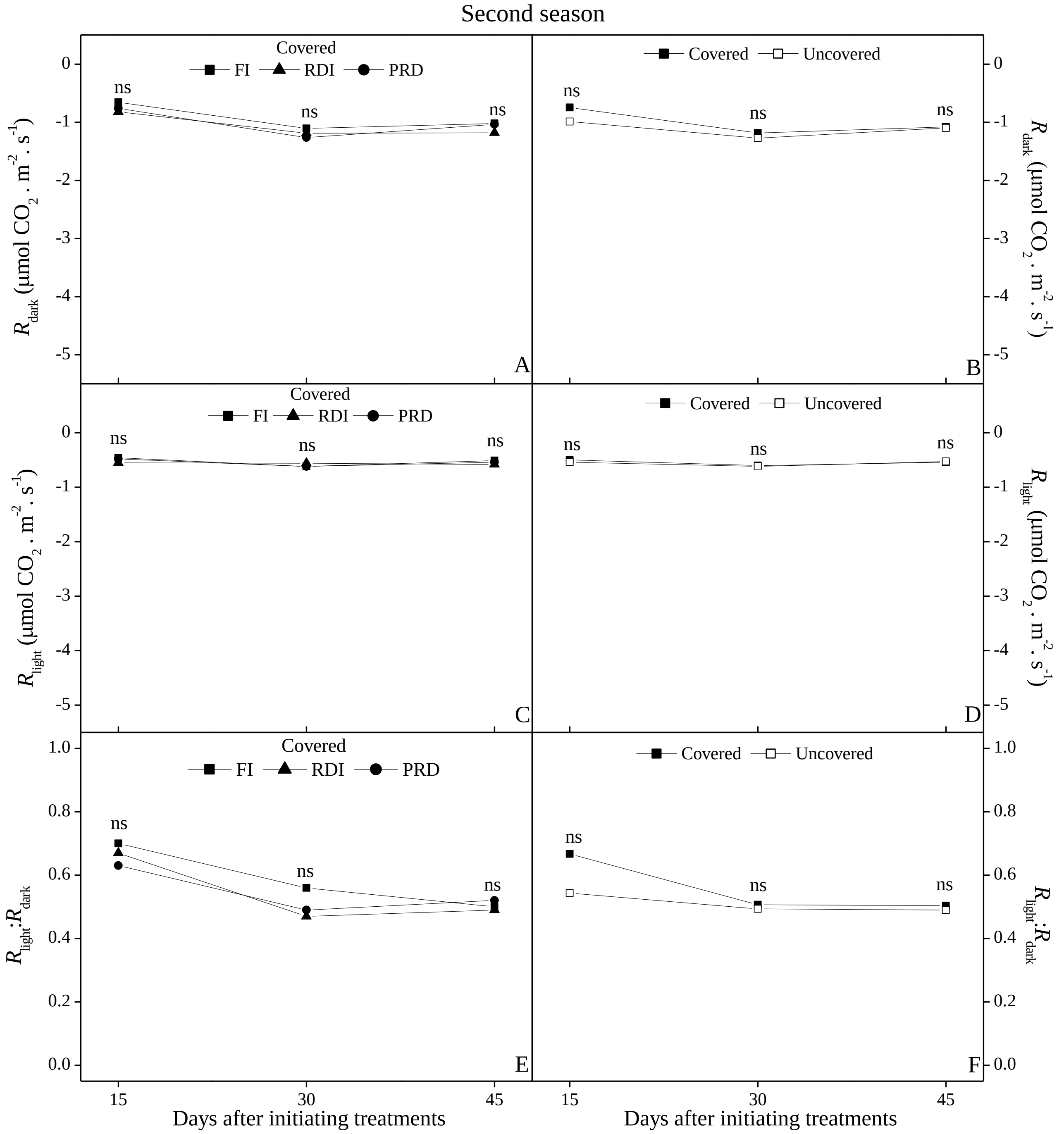

Figure 9. Leaf respiration in the dark (R_{dark}) (A and B) and in the light (R_{light}) (C and D) and light inhibition of respiration (R_{light}:R_{dark}) (E and F) of grapevine during the second season of the experiment under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered (A, C and E) or uncovered (comparison regardless water management treatments) sites. Each treatment mean represents the average of 6 (A, C and E) or 18 replicates (B, D and F). *ns* indicates no statistical difference according to analysis of variances tests.

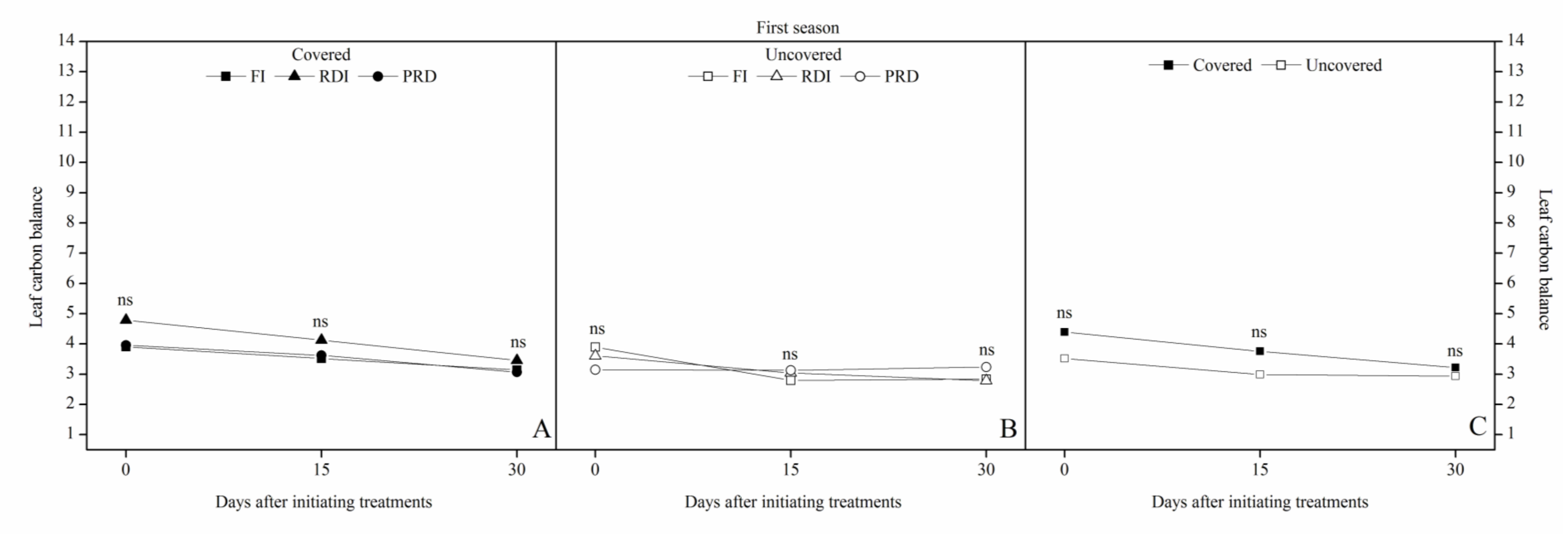

Figure 10. Leaf carbon balance of grapevine during the first seasons of the experiment under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered (A) or uncovered (B) sites. Each treatment mean represents the average of 6 (A and B) or 18 replicates (C). *ns* indicates no statistical difference according to analysis of variances tests.

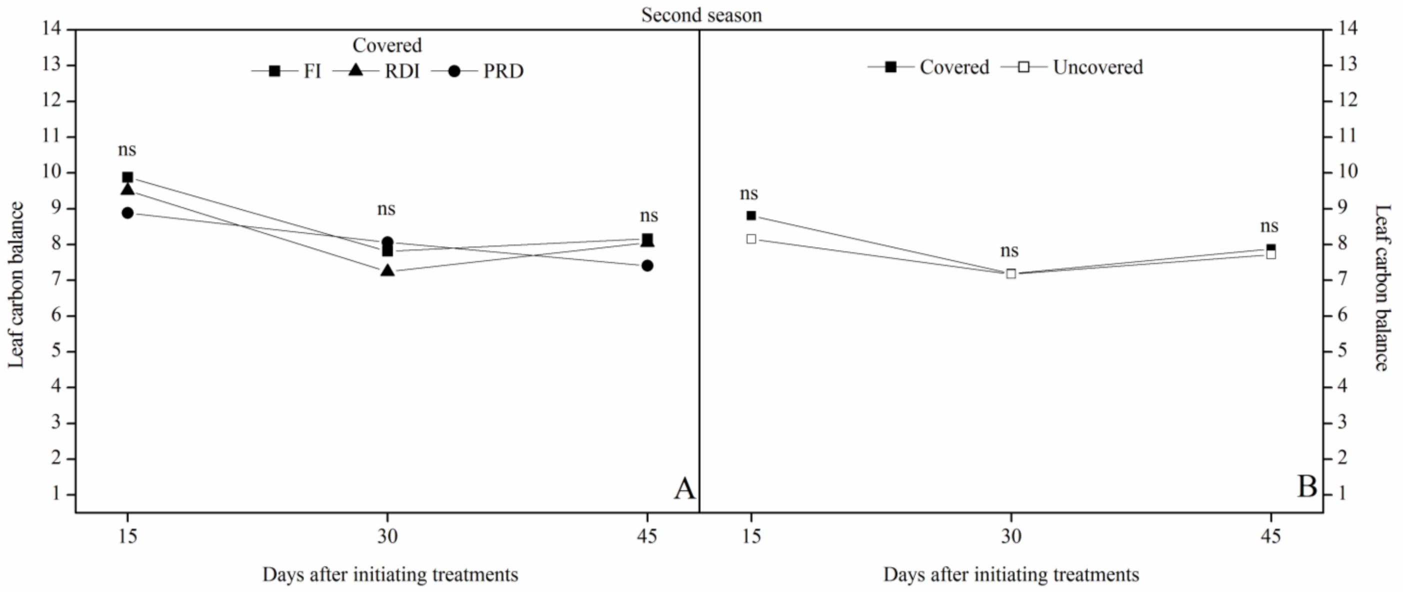

Figure 11. Leaf carbon balance of grapevine during the second seasons of the experiment under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered (A) or uncovered (comparison regardless water management treatments) sites. Each treatment mean represents the average of 6 (A) or 18 replicates (B). *ns* indicates no statistical difference according to analysis of variances tests.

During the 42 DAIT of the first season, 154.3 and 150.4 L of water were saved per plant in the covered and uncovered sites, respectively, when either RDI or PRD was applied (Figure 12 – A and C). Likewise, during the second season, these water management treatments (RDI and PRD) reduced the amount of water applied per plant by 195.4 and 184.5 L in the covered and uncovered sites, respectively, throughout the 54 DAIT period (Figure 12 – B and D). Considering a planting density of 1,850 grapevines per hectare, 323 ± 38 and 310 ± 31 m^3 of water per hectare (covered and uncovered sites, respectively) can be saved each season when either RDI or PRD is used from fruit set to harvest of ‘Niagara Rosada’ grapevine cultivated under the experimental conditions used in this work. The reductions in water applied through the use of either RDI or PRD did not affect any of the fruit quality parameters analyzed, in either the covered or uncovered sites in either season (Table 4). Moreover, there were no negative effects of TPC on fruit quality or yield (Table 4). Since the number of buds retained per cane was higher on the second season (six to eight - long spurs) than on the first season (two - short spurs), higher yields were obtained on the second season (Table 4).

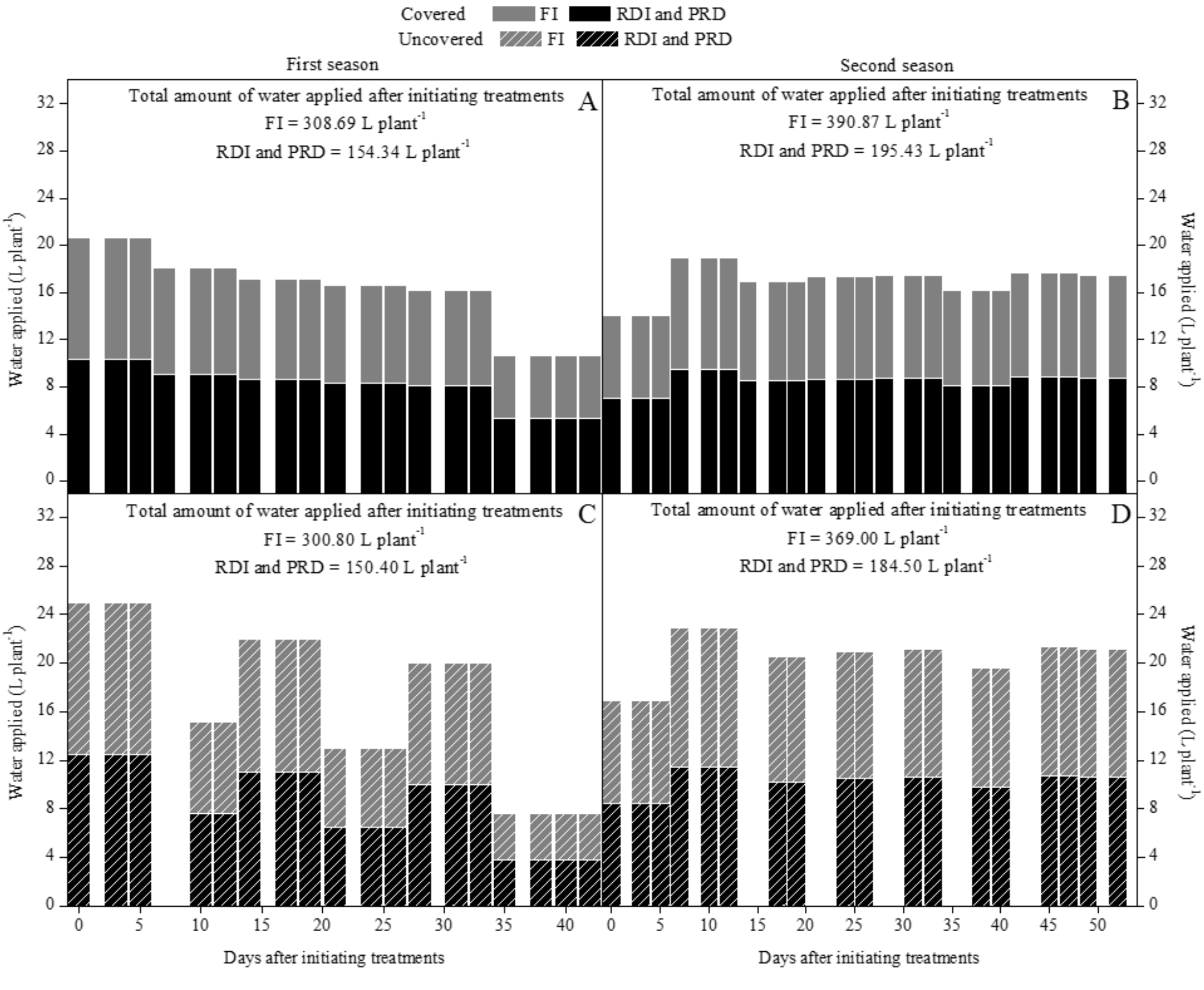

Figure 12. Amount of water applied after initiating treatments during the first (A and C) and the second (B and D) season of the experiment of three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered (A and B) or uncovered (C and D) sites.

Table 4. Yield and quality components at harvest of grapevine grown for two seasons under three water management treatments - Full-irrigated (FI); regulated deficit irrigation (RDI) and partial rootzone drying (PRD) - and in either covered or uncovered sites. Each treatment mean represents the average of six replicates

	First Season*									Second season*					
	Covered				Uncovered					Covered				Uncovered	
	FI	RDI	PRD	Mean	FI	RDI	PRD	Mean	CV (%)	FI	RDI	PRD	Mean	Mean	CV (%)
Nº of grape barries per cluster	48.83	47.16	48.17	48.05	40.50	47.83	47.17	45.17	12.57	64.17	63.33	65.65	64.38	62.39	14.24
Length of clusters (mm)	113.33	110.83	111.67	111.67	95.83	103.33	114.17	104.44	7.29	145.00	134.17	152.50	143.89	143.33	11.50
Diameter of clusters (mm)	57.17	55.83	56.67	56.55	59.17	63.33	60.00	60.83	10.38	56.67	59.17	59.17	58.34	60.00	9.05
Weight of clusters (g)	194.81	189.14	202.23	192.41	187.42	194.36	194.83	192.20	11.12	237.49	220.27	245.98	234.58	237.03	9.27
Diameter of 10 grape barries (mm)	183.67	182.50	182.33	182.83	179.00	181.00	180.50	180.17	2.56	182.17	181.00	180.83	181.33	188.56	2.31
Length of 10 grape barries (mm)	217.17	214.83	218.00	225.67	202.00	208.17	218.33	209.50	4.21	207.50	201.83	204.67	204.67	210.94	1.73
Weight of 10 barries (g)	43.12	42.40	38.66	41.06	39.13	40.03	39.92	39.69	9.48	39.22	39.77	39.28	39.42	42.60	8.10
pH	3.74	3.77	3.76	3.76	3.79	3.82	3.69	3.77	2.56	3.61	3.63	3.58	3.61	3.60	2.69
Total soluble solids (°Brix)	17.10	17.91	17.48	17.49	17.71	17.85	17.09	17.55	6.55	14.97	14.97	15.49	15.14	15.60	6.93
Total titratable acidity (% tartaric acid)	0.34	0.33	0.31	0.33	0.34	0.31	0.33	0.33	12.39	0.30	0.32	0.31	0.31	0.32	20.68
Total soluble solids/total tritable acidity	50.29	54.87	55.09	53.42	52.09	57.56	51.79	53.81	14.69	49.90	46.78	49.967	48.88	48.26	17.99
Anthocyanins	1.00	1.05	1.07	1.04	1.03	0.93	1.04	1.00	5.76	1.08	1.09	1.15	1.11	1.14	5.66
Nº of clusters per plant	27.33	38.50	33.33	33.05	32.00	29.00	42.00	34.33	26.30	66.67	70.50	66.83	68.00	63.81	18.14
Yeild per plant (Kg)	4.59	7.07	5.66	5.77	6.05	5.31	5.97	5.78	21.19	13.36	12.16	12.73	12.75	12.02	22.01

* No statistical differences were observed according to analysis of variances tests.

DISCUSSION

Anisohydric behavior of ‘Niagara Rosada’ grapevine

Grapevines show various strategies, known as stress ‘tolerance’ or ‘avoidance’ mechanisms, so that different cultivars have been classified as either anisohydric or isohydric in response to changes in air VDP and/or water availability (Schultz 2003, Soar et al. 2006, Chaves et al. 2010, Tomás et al. 2014, Lavoie-Lamoureux et al. 2017). Under the tropical experimental conditions studied, ‘Niagara Rosada’ grapevine presented anisohydric behavior. Plants from all water management treatments, including FI plants, showed reduced Ψ as air VPD increased at midday (Ψ_{md}) in both sites, resulting in a considerably high Ψ_{drop} (Table 1) and a strong negative linear relationship between Ψ and air VPD (Figure 5). Indeed, anisohydric plants tend to show variable Ψ values in response to increased evaporative demand, so that Ψ_{md} is substantially lower than Ψ_{pd} (Schultz 2003, Chaumont and Tyerman 2014).

Despite such anisohydric behavior, no effects on Ψ_{pd}, Ψ_{md} and Ψ_{drop} were observed when either RDI or PRD was used in both sites (Table 1). Maintaining similar Ψ values regardless of water availability is typically achieved by reducing g_s in isohydric plants (Poni et al. 2007, Lovisolo et al. 2010, Pou et al. 2012, Flexas 2016, Lavoie-Lamoureux et al. 2017). ABA accumulation in leaves can promote early stomatal closure in response to decreased soil water content and, thereby, reduce water loss and preserve Ψ (Schultz 2003, Pou et al. 2012, Tombesi et al. 2015). However, this mechanism was not observed in the present study and neither g_s nor E were reduced when deficit irrigation treatments were applied (Table 2). Instead, our results indicate that although the soil water content of both RDI and PRD decreased considerably in relation to FI (Figures 3 and 4) in TPC area, Ψ did not change (Table 1) and the resulting water availability was sufficient to maintain the plants’ internal water status. Green *et al.* (1997) observed in mature apple trees that previously dehydrated roots responded to irrigation by exhibiting higher sap flow rates than occurs when the entire root zone is watered. This increase in root hydraulic conductivity seems to be mediated by aquaporin activity (Lovisolo and Schubert 2006). Thus, we speculate that factors other than the stomatal control are likely to be involved in the observed response of Ψ among water management treatments in the anisohydric ‘Niagara Rosada’ grapevine (Lavoie-Lamoureux et al. 2017), such as up-regulation of aquaporin expression (Vandeleur et al. 2009, Vitali et al. 2016), water redistribution within the plant *via* lateral flow between vessels in both radial and tangential directions (Halis et al. 2012) and/or osmotic adjustment (Patakas and Noitsakis 1999).

Water use efficiency and intrinsic water use efficiency in grapevine exposed to deficit irrigation in tropical conditions

Anisohydric plants show high tolerance to reduced water availability and close their stomata at water potentials well below those of well-watered plants (Soar et al. 2006, Lavoie-Lamoureux et al. 2017). For this reason, anisohydric grapevines can maintain photosynthetic rates at high levels under conditions of low water availability in the soil (Flexas et al. 2006, Palliotti et al 2015). In the present work conducted under tropical conditions, A_{net} did not change when both RDI and PRD were used, regardless of site (Table 2). Since both g_s and E did not vary among water management treatments, no significant differences in A_{net}/g_s (Figure 6 – A and B; Figure 7 – A) or in A_{net}/E (Figure 6 – D and E; Figure 7 – C) were observed in either site. Curiously, in grapevines RDI and PRD are usually associated with a larger reduction in g_s and E than in A_{net}, resulting in increased A_{net}/g_s (de Souza et al. 2005, Chaves et al. 2007, Pou et al. 2012, Hocheberg et al. 2013) and A_{net}/E (de Souza et al. 2005, Chaves et al. 2007). However, this trend was not observed in the present work, or in our previous growth chamber study (Silva et al. 2017). In general, these effects are not as pronounced in anisohydric as they are in isohydric plants, since the stomatal responses of isohydric plants to reduced water availability are typically stronger (Poni et al. 2007, Lovisolo et al. 2010, Pou et al. 2012, Flexas 2016, Lavoie-Lamoureux et al. 2017).

Our observed responses of both A_{net}/g_s and A_{net}/E corroborate with our $\delta^{13}C$ results, since significant variation was not observed among water management treatments at the end of the second season in either sites (Table 2). Previous work found that plants grown under reduced water availability had a less negative $\delta^{13}C$ when g_s was lower (Souza et al. 2005, Chaves et al. 2007) and therefore, both A_{net}/g_s and A_{net}/E were higher (Condon et al. 2004), an affect not observed in the present work (Table 2; Figure 6 – A, B, D and E; Figure 7 – A and C). Moreover, considering that $\delta^{13}C$ integrates photosynthetic activity throughout the period over which the tissue was synthesized (Salazar-Parra et al. 2015) and reflects the interplay among all aspects of plant carbon and water relations (Aranjuelo et al. 2009b), we can assume that neither RDI or PRD cause damage to the capacity for CO_2 assimilation during water status in ‘Niagara Rosada’ grapevine grown under tropical conditions.

Effects of deficit irrigation on biochemical and photochemical traits of grapevine grown in tropical conditions

Although a restricted CO_2 supply is considered an important cause for decreased photosynthetic rates under reduced water availability (Flexas et al. 2006), the amount of carbon assimilated is strongly driven by the leaf biochemical potential (Flexas et al. 2016). In the present work, $V_{c\ 1500}$, as well as $V_{o\ 1500}$, did not vary among the water management treatments, regardless of site or seasons (Table 2). By closing stomata later in response to reduced water availability, anisohydric plants delay metabolic limitations (Flexas et al. 2006, Pou et al. 2012, Palliotti et al. 2015) and maintain the activity of Calvin Cycle enzymes (Souza et al., 2005), as observed in the present work through $V_{c\ 1500}$ and $V_{o\ 1500}$ values (Table 2). These results demonstrate that the affinity of Rubisco to CO_2 was not affected by either RDI and PRD (Gago et al. 2014, Galmés et al. 2014, Galmés et al. 2015, Flexa et al. 2016) under tropical conditions, a desirable feature that matches photoprotection with a positive carbon assimilation (Hochberg et al. 2013). The non-variable C_i/C_a values (Table 2) demonstrates that CO_2 accumulation in the mesophyll did not occur in either RDI or PRD treatments and thus the overall activity of the Calvin Cycle enzymes was stable regardless of water availability (de Souza et al. 2005). Therefore, both RDI and PRD can be used for ‘Niagara Rosada’ grapevine cultivation under the experimental conditions of this work, without harming the biochemical potential for photosynthesis.

Leaf photochemistry is co-regulated with Rubisco content and/or activity through feed-back or feed-forward changes in redox balance homeostasis *via* ‘‘photosynthetic control’’ (Foyer et al. 1990, Galmès et al. 2013). Since neither RDI or PRD caused biochemical impairments on ‘Niagara Rosada’ photosynthesis, no damage to chlorophyll biosynthesis or breakdown was observed, as indicated by the *SPAD* values not being affected by water management treatments (Table 3). Hence, regardless of site, no effects of either RDI or PRD on *PI*, S_m, RC/CS_0, ET_0/TR, ET_0/ABS or DI/CS_0 were shown in both seasons (Table 3). Moreover, the F_v/F_m ratio, an important variable which provides a measure of the rate of linear electron transport (Jamil et al. 2007, Tang et al. 2007, Balouchi 2010), was higher than 0.81 at the end of both seasons in all treatments (Table 3). Thus, the PSII reaction centers of 'Niagara Rosada' grapevine show no indication of damage or inactivation (Zlatev 2009, Vaz and Sharma 2011) and there was no apparent blockage of electron transfer between the acceptors (Shu et al. 2012) as the result of the applied reduction in water availability in Tropical conditions studied. Indeed, metabolic impairment on biochemical and photochemical capacity of photosynthesis has been shown to occur only when stomatal conductance drops below 0.05–0.10 mol H_2O m^{-2} s^{-1} (Bota et al. 2004, Flexas et al. 2006), which did not occur in our experiment (Table 2). Considering that climate change will likely enhance the severity of drought (IPCC 2014), anisohydric grapevines that do not reduce g_s in response to reduced water availability can avoid biochemical or photochemical damage (Pou et al. 2012, Palliotti et al. 2015) and thus should be preferentially cultivated in Tropical regions.

Respiration and leaf carbon balance in grapevines deficit irrigated

Analyzing all the physiological processes involved in C metabolism can be extremely difficult (Morales et al. 2016). However, both photosynthesis and respiration are basic components of plant productivity that should be considered together in grapevines' ecophysiological studies (Schultz and Stoll 2010). It has been previously reported that *R* in grapevines can increase under moderate water shortage (Silva et al. 2017), suggesting that deficit irrigation could result in increased carbon losses (Flexas et al. 2010, Salazar-Parra et al. 2015). Increased respiratory carbon losses were not observed in the present study and reducing water supply with either RDI or PRD did not significantly change R_{dark} or R_{light} in relation to FI in either seasons (Figures 8 – A, B, D and E; Figure 9 – A and C). Indeed, leaf respiration can be strongly enhanced by increases in T_{leaf} caused by reduced transpiration when water availability is reduced (Flexas et al. 2010), a condition not observed in the present work, regardless of site (Tables 2 and 3). Instead, in the anisohydric ‘Niagara Rosada’ grapevines neither g_s or E were reduced by deficit irrigation (RDI and PRD) (Table 2) and T_{leaf} was unaffected by the water management treatments (Table 3).

R_{light}:R_{dark} was not affected by reduced water availability through either RDI or PRD treatments in either site (Figures 8 – G and H; Figure 9 – E). This response is related to the anisohydric behavior as well. In general, R_{light}:R_{dark} is directly related to changes in V_o, so that conditions which increase V_o can also increase R_{light}:R_{dark}, due to high demand for TCA cycle substrates associated with the recovery of photorespiratory cycle intermediates in the peroxisome (Tcherkez et al. 2008; Griffin and Turnbull 2013). Since anisohydric plants delay metabolic limitations by closing the stomata later in response to reduced water availability (Flexas et al. 2006, Pou et al. 2012), both RDI and PRD did not cause significant effects on $V_{o\ 1500}$ (Table 2) and thereby, no effects on R_{light}:R_{dark} were observed during either season in both sites (Figures 8 – G and H; Figure 9 –E).

In order to analyze the effectiveness of deficit irrigation use in grapevines grown in a given region, the LCB must be assessed. The potential limitations imposed by reduced water availability are primarily dependent on the balance between photosynthesis and respiration (Flexas et al. 2006). In the present work, regardless of site, the LCB did not vary significantly in response to either RDI or PRD in relation to FI throughout the entire experiment (Figure 10 – A and B; Figure 11 - A). Again, this response was caused by the anisohydric behavior of ‘Niagara Rosada’, which resulted in unchanged rates of A_{net} (Table 2), R_{light} and R_{dark} (Figures 8 – A, B, D and E; Figure 9 – A and C). Since LCB is directly proportional to carbon available for plant’s growth and yield (Flexas et al. 2006, Escalona et al. 2012, Ayub et al. 2014), we can assume that the use of deficit irrigation techniques in anisohydric grapevines, such as ‘Niagara Rosada’, will not result in a substantial reduction in carbon gain under tropical climates.

Effects of deficit irrigation on grapevine’s fruit quality and yield in tropical conditions

The use of RDI or PRD in tropically grown grapevines saved a considerable amount of water both with and without TPC (Figure 12). Our trials suggest that more than 310 m^3 of water can be saved per hectare each season when either RDI or

PRD is applied from fruit set to harvest of ‘Niagara Rosada’. Importantly, the reduced water supply had no negative effects on ‘Niagara Rosada’s’ yield or fruit quality if the water management treatments are started after fruit set (Table 4). At this developmental stage, the berry’s connectivity to the vine is *via* the phloem (Thomas et al. 2006; Tarara and Peña 2015) and any reduction in berry size or quality would be indirectly related to decreases in photosynthesis (Wang et al. 2003). However, negative effects on photosynthesis were not observed in the present work, and both A_{net} and $V_{c\ 1500}$ values remained unchanged (Table 2). Similarly, photochemical capacity was not affected by either RDI or PRD treatments (Table 3). As a result, it should not be surprising that, yield and fruit quality were unaffected (Table 4). Moreover, reductions in yield and changes in fruit quality, are commonly associated with increased ABA synthesis under deficit irrigation (Deluc et al. 2009, Chaves et al. 2010). ABA limits cell division and consequently grapevines produce small berries and have reduced yields (Chaves et al. 2010). An increase of ABA in the leaves of table grapes has also been associated with the accumulation of leaf hexose (Deluc et al. 2009), as a means of controlling sugar transport to grape berries (Lecourieux et al. 2010). Finally, ABA has also been associated with the regulation of anthocyanin synthesis and accumulation in the skin of the fruit (Peppi et al. 2006, Lund et al. 2008, Deluc et al. 2009, Chaves et al. 2010). However, the anisohydric behavior of ‘Niagara Rosada’ grapevine limited ABA synthesis and accumulation (Poni et al. 2007, Lovisolo et al. 2010, Pou et al. 2012, Flexas 2016, Lavoie-Lamoureux et al. 2017), as indicated by the stable g_s values among the various water management treatments in our trials (Table 2). Therefore, regardless of site, there were no effects of either PRD or RDI on yield and quality parameters of ‘Niagara Rosada’ grapevine under tropical conditions (Table 4).

No differences were found between RDI and PRD treatments for any of traits analyzed in the present work, suggesting that both water management techniques can be useful alternatives to save water in grapevines cultivated in tropical conditions, particularly for anisohydric cultivars. However, as previously reported (dos Santos et al. 2003, Gu et al. 2004, Intrigliolo and Castel 2009, Collins et al. 2010), the total cost of the irrigation system and not just the method of application should be considered. Since PRD requires twice the amount of tubing as RDI, it incurs higher installation and maintenance costs (Permanhani et al. 2016), and thus RDI may economically preferred.

Use of Transparent Plastic Covering (TPC)

Regardless of water management treatments, plants grown in the covered site trended towards higher A_{net}/g_s (Figure 6 – C and Figure 7 - B) and A_{net}/E (Figure 6 – F and Figure 7 – D) during both seasons, as a result of having lower g_s and E values, respectively (Table 2). TPC is known to effect both A_{net}/E and A_{net}/g_s in table grapes (Permanhani et al. 2016), by altering the microclimate (Deluc et al. 2009, Roberto et al. 2011), and increasing leaf surface temperatures (Liu et al. 2012). In the present work, despite significant within and between season variation in climatic conditions (Figures 1 and 2), there was a consistent tendency for covered plants to have slightly higher T_{leaf} (Table 3) and lower *CWSI* (*ca.* 11.2%) (Table 3). *CWSI* is typically related to stomatal regulation and thus can affect plant energy balance (Grant et al. 2007, Vaz et al. 2016). In our experiments, g_s and E were both reduced (Table 2) leading to a positive effect of TPC on A_{net}/g_s (Figure 6 – C and Figure 7 - B) and on A_{net}/E (Figure 6 – F and Figure 7 – D). Concomitantly, discrimination against ^{13}C was reduced, and less negative $\delta^{13}C$ values were observed in covered plants at the end of the second season in response to reduced g_s (Table 2). Such negative $\delta^{13}C$ values are typically associated with higher water use efficiency (Condon et al. 2004), a typical trend observed throughout the entire experiment in the covered site (Table 2; Figure 6 – C and F; Figure 7 – B and D).

Covered plants tended towards lower C_i/C_a ratios (Table 2), indicating that the stomatal limitation of photosynthesis due to the slightly reduced g_s of covered plants (Table 2) was compensated by higher Rubisco carboxylation rates in plants from the covered site, represented by $V_{c\ 1500}$ values (Table 2). Rubisco kinetics and maximum activity are directly affected by leaf temperature (Gago et al. 2014, Galmés et al. 2014, Galmés et al. 2015, Flexas et al. 2016) and the higher T_{leaf} of the covered plants (Table 3) increased both $V_{c\ 1500}$ and $V_{o\ 1500}$ (Table 2). It is important to consider that, although photorespiration can diminish the potential photosynthetic activity of plants (Salazar-Parra et al. 2015), the simultaneous increase of photorespiration and photosynthesis, as observed in the present work in covered plants (Table 2), provides an avoidance mechanism against photoinhibition, maintaining a positive carbon balance (Hochberg et al., 2013). Indeed, photosynthetic activity was positively rather than negatively affected by the TPC, and slightly higher A_{net} values were observed in plants grown under the covering (Table 2).

The use of TPC to cultivate Niagara Rosada' grapevine in a tropical area had no negative effects on photochemical capacity (Table 3) or on leaf respiration rates (Figure 8 – C, F and I; Figure 9 – B, D and F) and LCB (Figure 10 – C; Figure 11 – B). Furthermore, fruit quality and yield were maintained during both seasons (Table 4). Although excessive rainfall can damage grape production in this region, particularly during summer, this did not occur over the duration of our experiments. However, TPC can minimize negative effects of heavy rains on fruit quality (Monteiro and Lopes 2007, Chaves et al. 2010) and reduce fungicide use by preventing the development of disease triggered by leaf wetness, such as mildew and botrytis (Botelho et al. 2011, Pedro et al. 2011). In the present work, the plants grown in uncovered area received 3 times more pesticides than the TPC plants (data not showed). Therefore, the use of TPC can be a beneficial strategy for 'Niagara Rosada' grapevines grown in wet Tropical regions. Clearly, further studies focused on phytosanitary and its economic impacts are needed to give more details about the effectiveness of TPC use in Tropical areas.

CONCLUSIONS

Our work growing Niagara Rosada' grapevine in tropical conditions demonstrates that: (i) Although the grapevine presents an anisohydric behavior, (ii) deficit irrigation treatments do not affect Ψ (although leaf water potential was affected by air VPD); (iii) Neither RDI nor PRD had significant effects on either A_{net}/g_s or A_{net}/E in anisohydric grapevine; (iv) by not closing stomata in response to reduced water availability, limitations on both carboxylation reactions of photosynthesis and Rubisco oxygenation rates are not observed, and the photochemical capacity is not inhibited; (v) Leaf respiration rates are not affected by either RDI of PRD. Since deficit irrigation in anisohydric grapevine does not limit $V_{o\ 1500}$, R_{light}:R_{dark} is not changed. The use of either RDI or PRD does not reduce carbon gain, and LCB is not affected; (vi) considerable amounts of water are saved when either RDI or PRD is used from fruit set to harvest, without affecting yield and fruit quality. RDI is the preferable treatment method for practical reasons; (vii) the use of TPC can be an effective strategy for growing ‘Niagara Rosada’ grapevines in wet tropical areas, since it avoids the negative effects of heavy rains on fruit quality, and limits the development of disease caused by leaf wetness while showing the potential to improve A_{net}/E, A_{net}/g_s and $V_{c\ 1500}$.

REFERENCES

Antolín, M.C., Ayari, M., Sánchez-Díaz, M., 2006. Effects of partial rootzone drying on yield, ripening and berry ABA in potted Tempranillo grapevines with split roots. Aust. J. Grape Wine R. 12, 13–20.

Antolín, M.C., Santesteban, H., Santamaría, E., Aguirreolea, J., Sánchez-Díaz, M., 2008. Involvement of abscisic acid and polyamines in berry ripening of *Vitis vinifera* (L.) subjected to water deficit irrigation. Aust. J. Grape Wine R. 14, 123–133.

Aranjuelo. I., Cabrera-Bosquet, L., Mottaleb, S.A., Araus, J.L., Nogues, S., 2009b. $^{13}C/^{12}C$ isotope labeling to study carbon partitioning and dark respiration in cereals subjected to water stress. Rapid Commun Mass SP. 23, 2819–2828.

Ayub, G., Smith, R.A., Tissue, D.T., Atkin, O.K., 2011. Impacts of drought on leaf respiration in darkness and light in *Eucalyptus saligna* exposed to industrial-age atmospheric CO_2 and growth temperature. New Phytol. 190, 1003–1018.

Ayub, G., Zaragoza-Castells, J., Griffin, K.L., Atkin, O.K., 2014. Leaf respiration in darkness and in the light under pre-industrial: current and elevated atmospheric CO_2 concentrations. Plant Sci. 226, 120–130.

Balouchi, H.R., 2010. Screening wheat parents of mapping population for heat and drought tolerance, detection of wheat genetic variation. Internat. J. Biol. Life Sci. 6, 56–66.

Bernacchi, C.J., Portis, A.R., Nakano, H., Caemmerer, S., Von Long, S.P., 2002. Temperature response of mesophyll conductance. implications for the determination of rubisco enzyme kinetics and for limitations to photosynthesis in vivo. Plant Physiol. 130, 1992–1998.

Betemps, D.L., Fachinello, J.C., Galarça, S.P., Portela, N.M., Remorini, D., Massai, R., Agati, G., 2012. Non-destructive evaluation of ripening and quality traits in apples using a multiparametric fluorescence sensor. J. Sci. Food Agric. 92(9), 1855-1864.

Bota, J., Medrano, H., Flexas, J., 2004. Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? New Phytol. 162, 671–681.

Botelho, R.V., Pavanello, A.P., Pires, E.J.P., Terra, M.M., Müller, M.M.L., 2011. Produção orgânica de uvas para vinho tinto sob cobertura plástica em região subtropical do Brasil. Ciênc. Agrotec. 35, 1186-1195.

von Caemmerer, S., Farquhar, G.D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta. 153, 376– 387.

Carbonneau, A., 2011. tropical viticulture: specificities and challenges for a quality viticulture. Acta Hortic. 910, 15-34.

Chaumont, F., Tyerman, S.D., 2014. Aquaporins: highly regulated channels controlling plant water relations. Plant Physiol. 164, 1600–1618.

Chaves, M.M., Zarrouk, O., Francisco, R., Costa, J.M., Santos, T., Regalado, A.P., Rodrigues, M.L., Lopes, C.M., 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. Ann. Bot-London. 105, 661–676.

Chaves, M.M., Santos, T.P., Souza, C.R., Ortuño, M.F., Rodrigues, M.L., Lopes, C.M., Maroco, J.P., Pereira, J.S., 2007. Deficit irrigation in grapevine improves water- use efficiency while controlling vigour and production quality. Ann. Appl. Biol. 150 (2), 237–252.

Collins, M.J., Fuentes, S., Barlow, E.W.R., 2010. Partial root zone drying and deficit irrigation increase stomatal sensitivity to vapour pressure deficit in anisohydric grapevines. Funct. Plant Biol. 37, 128–138.

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D., 2004. Breeding for high water-use efficiency. J. Exp. Bot. 55, 2447-2460.

Cousins, A.B., Ghannoum, O., von Caemmerer, S., Badger, M.R., 2010. Simultaneous determination of Rubisco carboxylase and oxygenase kinetic parameters in Triticum aestivum and Zea mays using membrane inlet mass spectrometry. Plant Cell Environ. 33, 444–452.

Crous, K.Y., Zaragoza-Castells, J., Ellsworth, D.S., Duursma, R.A., Tissue, M.D.T., Atkin, O.K., 2012. Light inhibition of leaf respiration in field-grown Eucalyptus saligna in whole-tree chambers under elevated atmospheric CO_2 and summer drought. Plant Cell Environ. 35, 966–981.

Deluc, L.G., Quilici, D.R., Decendit, A., Grimplet, J., Wheatley, M.D., Schlauch, K.A., Merillon, J.M., Cushman, J.C., Cramer, G.R., 2009. Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. BMC Genomics 10, 212–245.

Dry, P.R., Loveys, B.R., 1998. Factors influencing grapevine vigour and the potential for control with partial rootzone drying. Aust. J. Grape Wine R. 4, 140–148.

Du, F., Deng, W., Yang, M., Wang, H., Mao, R., Shao, J., Fan, J., Chen, Y., Fu, Y., Li, C., He, X., Zhu, Y., Zhu, S., 2015. Protecting grapevines from rainfall in rainy conditions reduces disease severity and enhances profitability. Crop Prot. 67, 261–268.

Eichhorn, K.W., Lorenz, D.H., 1977. Phänologische Entwicklungsstadien der Rebe. Nachrichtenbl. Deut. Pflanzenschutzd. 29, 119-120.

Escalona, J.M., Tomàs, M., Martorell, S., Medrano, H., Ribas-Carbo, M., Flexas, J., 2012. Carbon balance in grapevines under different soil water supply: importance of whole plant respiration. Aust. J. Grape Wine R. 1–11.

Esteves, B.S., 2015. Fluxo de seiva, consumo hídrico e manejos diferenciados de irrigação na videira Niagara Rosada (*Vitis labrusca* L.). PhD thesis, UENF, Campos dos Goytacazes, Brazil. 119p.

FAOSTAT (2014) FAO data for agriculture: statistics database: http://faostat.fao.org/faostat/collections?version=extandhasbulk=0andsubset =agricul-ture (01 May 2017 date last accessed).

Farquhar, G.D., von Caemmerer, S., 1982. Modelling of photosynthetic response to environmental conditions. In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (Eds.), Encyclopedia of Plant Physiology. Physiological Plant Ecology II. Water Relations and Carbon Assimilation, 12. Springer, Berlin, Germany, pp. 551– 587.

Flexas, J., Bota, J., Galmés, J., Medrano, H., Ribas-Carbó, M., 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiol. Plantarum. 127, 343–352.

Flexas, J., Díaz-Espejo, A., Conesa, M.A., Coopman, R.A., Douthe, C., Gago, J., Gallé, A., Galmés, J., Medrano, H., Ribas-Carbo, M., Tomàs, M., Niinemets, Ü., 2016. Mesophyll conductance to CO_2 and Rubisco as targets for improving intrinsic water use efficiency in C3 plants. Plant Cell Environ. 39, 965–982.

Flexas, J., Galmés, J., Gallé, A., Gulías, J., Pou, A., Ribas-Carbó, M., Medrano, H., 2010. Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. Aust. J. Grape Wine R., 16, 106–121.

Flexas J., 2016. Genetic improvement of leaf photosynthesis and intrinsic water use efficiency in C3 plants: Why so much little success? Plant Sci. 9407, 1-7.

Foyer, C., Furbank, R., Harbinson, J., Horton, P., 1990. The mechanisms contributing to photosynthetic control of electron transport by assimilation in leaves. Photosynth. Res. 25, 83–100

Gago, J., Douthe, C., Flórez-Sarasa, I., Escalona, J., Galmés, J., Fernie, A.R., Flexas, J., Medrano, H., 2014. Opportunities for improving leaf water use efficiency under climate change conditions. Plant Sci. 226, 108–119.

Galmés, J., Conesa, M.À., Díaz-Espejo, A., Mir, A., Perdomo, J.A., Niinemets, Ü., Flexas, J., 2014. Rubisco catalytic properties optimised for present and future climatic conditions. Plant Sci. 226, 61–70.

Galmés, J., Kapralov, M.V., Copolovici. O., Hermida-Carrera, C., Niinemets, Ü., 2015. Temperature responses of the Rubisco maximum carboxylase activity across domains of life: phylogenetic signals, trade-offs, and importance for carbon gain. Photosynth. Res. 123, 183–201.

Grant, O.M., Tronina, L., Jones, H.G., Chaves, M.M., 2007. Exploring thermal imaging variables for the detection of stress responses in grapevine under different irrigation regimes. J. Exp. Bot. 58, 815–825.

Grenn, S.R., Clothier B.E., McLoud, D.J., 1997. The response of sap flow in apple roots to localized irrigation. Agric. Water Manage. 33, 63-78.

Griffin, K.L., Heskel, M., 2013. Breaking the cycle: how light, CO_2 and O_2 affect plant respiration. Plant Cell Environ. 36, 498–500.

Griffin, K.L., Turnbul, M.H., 2013. Light saturated RuBP oxygenation by Rubisco is a robust predictor of light inhibition of respiration in *Triticum aestivum* L. Plant Biol. 15, 769–775.

Gu, S., Guoqiang, D., Zoldoske, D., Hakim, A., Cochran, R., Fugelsang, K., Jorgensen, G., 2004. Effects of irrigation amount on water relations, vegetative growth, yield and fruit composition of Sauvignon blanc grapevines under partial root-zone drying and conventional irrigation in the San Joaquin Valley of California, USA. The J. Hortic. Sci. Biotech. 79, 26–33.

Hochberg, U., Degu, A., Fait, A., Rachmilevitch, S., 2013. Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. Physiol. Plantarum. 147, 443–452.

Hargreaves, G.H., Samani, Z.A., 1985 Reference crop evapotranspiration from temperature. J. Appl. Eng. Agr., 1(2), 96-99.

Halis, Y., Djehichi, S., Senoussi, M.M., 2012. Vessel development and the importance of lateral flow in water transport within developing bundles of current-year shoots of grapevine (*Vitis vinifera* L.). Trees. 26, 705–714.

Intrigliolo, D.S., Castel, J.R., 2009. Response of *Vitis vinifera* cv. “Tempranillo” to partial root-zone drying in the field: Water relations, growth, yield and fruit and wine quality. Agr. Water Manage. 96, 282–292.

IPCC, 2014. Intergovernmental panel on climate change. Summary for policymakers. In: Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Farahani, E., Kadner, S., Seyboth, K., Adler, A., Baum, I., Brunner, S., Eickemeier, P., Kriemann, B., Savolainen, J., Schlömer, S., von Ste-chow, C., Zwickel, T., Minx, J.C. (Eds.), Climate Change 2014. Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom/NewYork, NY, USA.

Jamil, M., Rehman, S., Rha, E.S., 2007. Salinity effect on plant growth, PSII photochemistry and chlorophyll content in sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleraceacapitata* L.). Pak. J. Bot. 39, 753–760.

Jones, H.G., 1992. Plant and microclimate: a quantitative approach to environmental plant physiology. Cambridge University Press, pp. 428.

Kirschbaum, M.U.F., Farquhar, G.D., 1987. Investigation of the CO_2 dependence of quantum yield and respiration in *Eucalyptus pauciflora*. Plant Physiol., 83, 1032– 1036.

Kok, B., 1948. A critical consideration of the quantum yield of Chlorella-photosynthesis. Enzymologial. 13, 1–56.

Lavoie-Lamoureux, A., Sacco, D., Risse, P.A., Lovisolo, C., 2017. Factors influencing stomatal conductance in response to water availability in grapevine: a meta-analysis. Physiol. Plantarum. 159, 468–482.

Lecourieux, F., Lecourieux, D., Vignault, C., Delrot, S., 2010. A sugar-inducible protein kinase, VvSK1, regulates hexose transport and sugar accumulation in grapevine cells. Plant Physiol. 152(2), 1096-1106.

Liu, G-T., Wang, J-F., Cramer, G., Dai, Z-W., Duan, W., Xu, H-G., Wu, B-H., Fan, P-G., Wang, L-J., Li, S-H., 2012. Transcriptomic analysis of grape (*Vitis vinifera* L.) leaves during and after recovery from heat stress. BMC Plant Biol. 12, 10.

Lovisolo, C., Perrone, I., Carra, A., Ferrandino, A., Flexas, J., Medrano, H., Schubert, A., 2010. Drought-induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. Funct. Plant Biol., 37, 98–116.

Lovisolo, C., Schubert, A., 2006. Mercury, hinders recovery of shoot hydraulic conductivity during rehydration: evidence from a whole-plant approach. New Phytologist. 172, 469–478.

Monteiro, A., Lopes, C.M., 2007. Influence of cover crop on water use and performance of vineyard in Mediterranean Portugal. Agr. Ecosyst. Environ. 121, 336–342.

Morales, F., Antolín, M.C., Aranjuelo, I., Goicoechea, N., Pascual, I., 2016. From vineyards to controlled environments in grapevine research: investigating responses to climate change scenarios using fruit-bearing cuttings. Theor. Exp. Plant Physiol. 28(2), 171-191.

Palliotti, A., Tombesi, S., Frioni, T., Silvestroni, O., Lanari, V., D’Onofrio, C., Matarese, F., Bellincontro, A., Poni, S., 2015. Physiological parameters and protective energy dissipation mechanisms expressed in the leaves of two *Vitis* *vinifera* L. genotypes under multiple summer stress. J. Plant. Physiol., 185, 84-92.

Patakas, A., Noitsakis. B., 1999. Mechanisms involved in diurnal changes of osmotic potential in grapevines under drought conditions. J. Plant Physiol. 154, 767–774.

Pedro, M.J.P. Jr., Hernandes, J.L., Rolim, G.S., 2011. Sistema de condução em Y com e sem cobertura plástica: microclima, produção, qualidade do cacho e ocorrência de doenças fúngicas na videira ‘Niagara Rosada’. Bragantia. 70, 228-233.

Permanhani, M., Miguel Costa, J.M., Conceição, M.A.F., de Souza, R.T., Vasconcellos, M.A.S., Chaves. M.M., 2016. Deficit irrigation in table grape: eco-physiological basis and potential use to save water and improve quality. Theor. Exp. Plant Physiol., 28(1), 85-108.

Peppi, M.C., Fidelibus, M.W., Dokoozlian, N., 2006. Abscisic acid application timing and concentration affect firmness, pigmentation, and color of 'Flame Seedless' grapes. HortScience. 41, 1440–144.

Poni, S., Bernizzoni, F., Civardi, S., 2007. Response of ‘Sangiovese’ grapevines to partial rootzone drying: gas-exchange, growth and grape composition. Sci Hortic 114, 96–103.

Poni, S., Bernizzoni, F., Civardi, S., Gatti, M., Porro, D., Camin, F., 2009. Performance and water-use efficiency (single-leaf vs. whole-canopy) of well-watered and half-stressed split-root Lambrusco grapevines grown in Po Valley (Italy). Agri. Eco. and Environ. 129, 97-106.

Pons, T.L., Welschen, R.A.M., 2002. Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. Plant Cell Environ. 25, 1367–1372.

Pou, A., Medrano, H., Tomàs, M., Martorell, S., Ribas-Carbó, M., Flexas, J., 2012. Anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric behaviour. Plant Soil. 359(1–2), 335–349.

Roberto, S.R., Colombo, L.A., de Assis, A.M., 2011. Review: protect cultivation in viticulture. Cienc. Tec. Vitivinic. 26, 11–16.

Rogiers, S.Y., Greer, D.H., Hatfield, J.M., Hutton, R.J., Clarke, S.J., Hutchinson, P.A., Somers, A., 2012. Stomatal response of an anisohydric grapevine cultivar to evaporative demand, available soil moisture and abscisic acid. Tree Physiol. 32, 249–261.

Salazar-Parra, C., Aranjuelo, I., Pascual, I., Erice, G., Sanz-Sáez, A., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J., Araus, J.L., Morales, F., 2015. Carbon balance, partitioning and photosynthetic acclimation in fruit-bearing grapevine (*Vitis vinifera* L. cv. Tempranillo) grown under simulated climate change (elevated CO_2, elevated temperature and moderate drought) scenarios in temperature gradient greenhouses. J. Plant Physiol. 174, 97–109.

dos Santos, T.P., Lopes, C.M., Rodriguez, M.L., de Souza, C.R., Maroco, J.P., Pereira, J.S., Silva, J.R., Chaves, M.M., 2003. Partial rootzone drying: effects on growth and fruit quality of field- grown grapevines (*Vitis vinifera*). Funct. Plant Biol., 30, 663–671.

Shapiro, J.B., Griffin, K.L., Lewis, J.D., Tissue, D.T., 2004. Response of Xanthium strumarium leaf respiration in the light to elevated CO_2 concentration, nitrogen availability and temperature. New Phytol. 162, 377–386.

Schultz, H.R., 2003. Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. Plant Cell Environ. 26, 1393–1405.

Schultz, H.R., Stoll, M., 2010. Some critical issues in environmental physiology of grapevines: future challenges and current limitations. Aust. J. Grape Wine R. 16, 4–24.

Silva, J.R., Patterson, A.E., Rodrigues, W.P., Campostrini, E., Griffin, K.L., 2017. Photosynthetic acclimation to elevated CO_2 combined with partial rootzone drying results in improved water use efficiency, drought tolerance and leaf carbon balance of grapevines (*Vitis labrusca*). Environ. Exp. Botany. 134, 82–95.

Shu, S., Guo, S.R., Sun, J., Yuan, L.Y., 2012. Effects of salt stress on the structure and function of the photosynthetic apparatus in *Cucumis sativus* and its protection by exogenous putrescine. Physiol. Plant. 146, 285–296.

Soar, C.J., Speirs, J., Maffei, S.M., Penrose, A.B., McCarthy, M.G., Loveys, B.R., 2006. Grape vine varieties Shiraz and Grenache differ in their stomatal response to VPD: apparent links with ABA physiology and gene expression in leaf tissue. Aust. J. Grape Wine R. 12, 2–12.

de Souza, C.R., Maroco, J.P., dos Santos, T.P., Rodrigues, M.L., Lopes, C.M., Pereira, J.S., Chaves, M.M., 2005. Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. Agr. Ecosyst. Environ. 106, 261–274.

de Souza, C.R., Mota, R.V., Dias, F.A.N., Melo, E.T., Pimentel, R.M.A., Souza, L.C., Regina, M.A., 2015. Physiological and agronomical responses of Syrah grapevine under protected cultivation. Bragantia. 74(3), 270-278.

Stanghellini. C., 2014. Horticultural production in greenhouses: efficient use of water. Acta Hortic. 1034, 25–32.

Stoll, M., Loveys, B., Dry, P., 2000. Hormonal changes induced by partial root-zone drying of irrigated grapevine. J. Exp. Bot. 51, 1627–1634.

Strasser, B.J., Strasser, R.J., 1995. Measuring fast fluorescence transients to address environmental questions: The JIP-test. In: Mathis, P. (Ed.), Photosynthesis: from light to biosphere. Kluwer, Dordrecht, pp. 977–980.

Strasser. R.J., Tsimilli-Michael, M., 2001. Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-Test, Chim. Nouvelle (SRC). 75, 3321–3326.

Strasser, R.J., Srivastava, A., Govindjee, 1995. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. Photochem. Photobiol. 61, 32–42.

Strasser. R.J., Srivastava, A., Tsimilli-Michael, M., 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus, M., Pathre, U., Mohanty, P. (Eds.), Probing photosynthesis: mechanisms, regulation and adaptation. Taylor and Francis, London, ENG, pp. 445–483.

Strasser, R.J., Tsimilli-Michael, M., Srivastava, A., 2004. Analysis of the chlorophyll fluorescence transient. In: Papageorgiou, G.C., Govindjee (Eds.), Chlorophyll fluorescence: a signature of photosynthesis, advances in photosynthesis and respiration, 19. Springer, Dordrecht, pp. 321–362.

Tang, D., Shi, S., Li, D., Hu, C., Liu, Y., 2007. Physiological and biochemical responses of *Scytonema javanicum* (cyanobacterium) to salt stress. J. Arid. Environ. 71, 312–320.

Tarara, J.M., Peña, J.E.P., 2015. Moderate water stress from regulated deficit irrigation decreases transpiration similarly to net carbon exchange in grapevine canopies. J Am. Soc. Hortic. Sci. 140, 413–426.

Tcherkez, G., Bligny, R., Gout, E., Mahe, A., Hodges, M., Cornic, G., 2008. Respiratory metabolism of illuminated leaves depends on CO_2 and O_2 conditions. Proc. Natl. Acad. Sci. USA. 105, 797–802.

Tcherkez, G., Schaufele, R., Nogues Piel, C., Boom, A., Lanigan, G., Barbaroux, C., Mata, C., Elhani, S., Hemming, D., Maguas, C., Yakir, D., Badeck, F.W., Griffiths, H., Schnyder, H., Ghashghaie, J., 2010. On the $^{13}C/^{12}C$ isotopic signal of day and night respiration at the mesocosm level. Plant Cell Environ. 33, 900–913.

Thomas, T.R., Matthews, M.A., Shackel, K.A., 2006. Direct in situ measurement of cell turgor in grape (*Vitis vinifera* L.) berries during development and in response to plant water deficits. Plant Cell Environ. 29, 993–1001.

Tomás, M., Medrano, H., Escalona, J.M., Martorell, S., Pou, A., Ribas-Carbó, M., Flexas, J., 2014. Variability of water use efficiency in grapevines. Environ. Exp. Bot. 103, 148–157.

Tombesi S, Nardini A, Frioni T, Soccolini M, Zadra C, Farinelli D, Poni S, Palliotti A (2015) Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. Sci Rep 5: 1-12

Tuccio, L., Remorini, D., Pinelli, P., Fierini, E., Tonutti, P., Scalabrelli, G., Agati, G., 2011. Rapid and non-destructive method to assess in the vineyard grape berry anthocyanins under different seasonal and water conditions. Aust. J. Grape Wine R. 17, 181–189.

Wang, Z., Deloire, A., Carbonneau, A., Federdpiel, B., Lopez, F., 2003. An in vivo experimental system to study sugar phloem unloading in ripening grape berries during water deficiency stress. Ann. Bot. 92, 523–528.

Vandeleur, R.K., Mayo, G., Shelden, M.C., Gilliham, M., Kaiser, B.N., Tyerman, S.D., 2009. The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. Plant Physiol. 149(1), 445–460.

Vaz, M., Coelho, R., Rato, A., Samara-Lima, R., Silva, L.L., Campostrini, E., Mota, J.B., 2016. Adaptive strategies of two Mediterranean grapevines varieties (*Aragonez syn. Tempranillo* and *Trincadeira*) face drought: physiological and structural responses. Theor. Exp. Plant Physiol. 28(2), 205-220.

Vaz, J., Sharma, P.K., 2011. Relationship between xanthophy cycle and non-photochemical quenching in rice (*Oryza sativa* L.) plants in response to light stress. Indian J. Exp. Bot. 49, 60–67.

Villar, R., Held, A.A., Merino, J., 1994. Comparison of methods to estimate dark respiration in the light in leaves of two woody species. Plant Physiol. 105, 167–172.

Vitali, M., Cochard, H., Gambino, G., Ponomarenko, A., Perrone, I., Lovisol, C., 2016. VvPIP2;4N aquaporin involvement in controlling leaf hydraulic capacitance and resistance in grapevine. Physiol. Plant. 158, 284–296

Zlatev, Z., 2009. Drought-induced changes in chlorophyll fluorescence of young wheat plant. Biotechnology. 23, 437–441.

5. CONCLUDING REMARKS

This work demonstrated that deficit irrigation is useful to save water in grapevines cultivated either in the tropics (with or without TPC) or under elevated [CO_2]. Elevated [CO_2] delays drought effects on both A_{net} and Rubisco activity by reducing g_s, E and stomatal density (Chapter 1). Moreover, the efficiency of Rubisco carboxylation is improved by PRD under elevated [CO_2], leading to an increase in A_{net} and hence, greater *WUE* and *iWUE* is observed (Chapter 1). Due to the anisohydric behavior presented by 'Niagara Rosada' grapevine in the tropics, such significant effects on either *WUE* (A_{net}/E) or *iWUE* (A_{net}/g_s) were not observed when deficit irrigation techniques were applied (Chapter 2), indicating that grapevines with isohydric behavior are the ones tending towards improved water use efficiency in response to reduced water availability. However, TPC shows the potential to improve *WUE*, *iWUE* and Rubisco carboxylation rates of grapevines in the tropics (Chapter 2). In addition, deficit irrigation use is favored in the tropics by the anisohydric behavior. By maintaining stomata opened under reduced water availability, limitations on both carboxylation reactions of photosynthesis and Rubisco oxygenation rates are not observed in anysohidric grapevines, and the photochemical capacity is not inhibited (Chapter 2).

Under elevated [CO_2], a *down-regulation* of A_{net} is observed due to reductions in Rubisco content and/or total activity, and by reductions in the efficiency of the photochemical apparatus due to N dilution within leaves with higher SLW (Chapter 1). Although R_{dark} and R_{light} are changed by such conditions that reduce N concentration, as well as by drought level (Chapter 1), leaf respiration rates are not negatively affected by deficit irrigation, so that LCB is not impaired, either under elevated [CO_2] or TPC in the tropics (Chapters 1 and 2). Therefore, yield and fruit quality are not damaged when deficit irrigation is used (Chapter 2). In addition, light inhibition of respiration (R_{light}:R_{dark}) is highly correlated with Rubisco oxygenation rates so that R_{light}:R_{dark} is intensified under conditions of reduced V_o, such as elevated [CO_2] (Chapter 1). Since deficit irrigation does not limit V_o, R_{light}:R_{dark} is not changed (Chapters 1 and 2).

REFERENCES

Ahmedullah, M., Watson, J.W. (1985) *Site selection for grapes in Eastern Washington. Cooperative Extension*, Washington State University. EB 1358, Prosser, 36p.

Amthor, J.S. (2000) The McCree-de Wit-Penning de Vries-Thornley respiration paradigms: 30 years later. *Ann Bot-London*. 86:1–20.

Ainsworth, E.A., Rogers, A. (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. *Plant Cell Environ.* 30:258- 270.

Albert, K.R., Mikkelsen, T.N., Michelsen, A., Ro-Poulsen, H., van der Linden, L. (2011) Interactive effects of drought, elevated CO_2 and warming on photosynthetic capacity and photosystem performance in temperate heath plants. *J Plant Physiol*. 168:1550-1561.

Allen Jr., L.H. (1994) Carbon dioxide increase: direct impacts on crops and indirect effects mediated through anticipated climate changes. *In*: Boote, K.J., Bennett, J.M., Sinclair, T.R., Paulse, G.M. (Eds.) *Physiology and Determination of Crop Yield*. Madison: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. p. 425–459.

Alvarenga, A. A.; Abrahão, E.; Regina, M. A. (1998) Origem e classificação botânica da videira. *Informe Agropecuário* 19 (194):5-8.

Antolín, M.C., Ayari, M., Sánchez-Díaz, M. (2006) Effects of partial rootzone drying on yield, ripening and berry ABA in potted Tempranillo grapevines with split roots. *Aust J Grape Wine Res*. 12:13–20.

Antolín, M.C., Santesteban, H., Santamaría, E., Aguirreolea, J. and Sánchez-Díaz, M. (2008) Involvement of abscisic acid and polyamines in berry ripening of *Vitis vinifera* (L.) subjected to water deficit irrigation. *Aust J Grape Wine Res*. 14:123–133.

Aranjuelo. I., Cabrera-Bosquet, L., Mottaleb, S.A., Araus, J.L., Nogues, S. (2009b) $^{13}C/^{12}C$ isotope labeling to study carbon partitioning and dark respiration in cereals subjected to water stress. *Rapid Commun Mass SP*. 23:2819–2828.

Aranjuelo, I., Pérez, P., Hernández, L., Irigoyen, J.J., Zita, G., Martínez-Carrasco, R., Sánchez-Díaz, M. (2005) The response of nodulated alfalfa to water supply, temperature and elevated CO_2: photosynthetic down-regulation. *Physiol Plant*. 123: 348–58.

Aranjuelo, I., Pardo, A., Biel, C., Savé, R., Azcón-Bieto, J., Nogués, S. (2009ª) Leaf carbon management in slow-growing plants exposed to elevated CO_2. *Glob Change Biol.* 15:97–109.

Ayub, G., Smith, R. A., Tissue, D.T., Atkin, O.K. (2011) Impacts of drought on leaf respiration in darkness and light in Eucalyptus saligna exposed to industrial-age atmospheric CO_2 and growth temperature. *New Phytol*. 190:1003–1018.

Ayub, G., Zaragoza-Castells, J., Griffin, K. L., Atkin, O. K. (2014) Leaf respiration in darkness and in the light under pre-industrial, current and elevated atmospheric CO_2 concentrations. *Plant Sci*., 226:120-130.

Atkin, O.K., Evans J.R., Ball M.C., Lambers H., Pons T.L. (2000) Leaf respiration of snow gum in the light and dark. Interactions between temperature and irradiance. *Plant Physiol*. 122:915– 923.

Atkin, O.K., Macherel, D. (2009) The crucial role of plant mitochondria in orchestrating drought tolerance. *Annals Bot-London.* 103:581–597.

Balint, G., Reynolds, A. G. (2013) Impact of irrigation strategies on abscisic acid and its catabolites profiles in leaves and berries of Baco noir grapes. *J. Plant Growth Regul*. 32:884–900.

Balouchi, H.R. (2010) Screening wheat parents of mapping population for heat and drought tolerance, detection of wheat genetic variation. *Internat. J. Biol. Life Sci.* 6:56–66.

Bernacchi, C. J., Portis, A. R., Nakano, H., Caemmerer, S. Von, and Long, S. P. (2002). Temperature Response of Mesophyll Conductance. Implications for the Determination of Rubisco Enzyme Kinetics and for Limitations to

Photosynthesis in Vivo. *Plant Physiol*. 130:1992–1998.

Betemps, D.L., Fachinello, J.C., Galarça, S.P., Portela, N.M., Remorini, D., Massai, R., Agati, G. (2012) Non-destructive evaluation of ripening and quality traits in apples using a multiparametric fluorescence sensor. *J. Sci. Food Agric*. 92(9):1855-1864.

Bindi, M., Fibbi, L., Miglietta, F. (2001) Free Air CO_2 Enrichment (FACE) of grapevine (*Vitis vinifera* L.): growth and quality of grape and wine in response to elevated CO_2 concentrations. *Eur. J. Agron*. 14:145–155.

Blum, A. (2009). Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crop Res.* 112:119–123.

Borges, R.S., Silva, G.A., Roberto, S.R., Assis, A.M., Yamamoto, L.Y. (2013) Phenolic compounds, favorable oxi-redox activity and juice color of ‘Concord’ grapevine clones. *Sci. Hortic-Amsterdam*. 161:188–192.

Bota, J., Flexas, J. and Medrano, H. (2001) Genetic variability of photosynthesis and water use in Balearic grapevine cultivars. Ann. Appl. Biol. 138:353–361.

Bota, J., Medrano, H., Flexas, J. (2004) Is photosynthesis limited by decreased Rubisco activity and RuBP content under progressive water stress? *New Phytol*. 162:671–681.

Botelho, R.V., Pavanello, A.P., Pires, E.J.P., Terra, M.M., Müller, M.M.L. (2011) Produção orgânica de uvas para vinho tinto sob cobertura plástica em região subtropical do Brasil. *Ciênc. Agrotec*. 35:1186-1195.

Bouma, T.J., Devisser, R., Janssen, J.H.J.A., Dekock, M.J., Vanleeuwen, P.H., Lambers, H. (1994) Respiratory energy requirements and rate of protein turnover in vivo determined by the use of an inhibitor of protein synthesis and a probe to assess its effect. *Physiol. Plantarum*. 92:585–594.

Bravdo, B., Naor, A., Zahavi, T., Gal, Y. (2004) The effects of water stress applied alternatively to part of the wetting zone along the season (PRD – partial rootzone drying) on wine quality, yield, and water relations of reed wine grapes. *Acta Hortic*. 664:101–109.

Brooks, A., Farquhar, G.D. (1985) Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-biphosphate carboxylase/ oxygenase and the rate of respiration in the light. Estimates from gas exchange measurements on spinach. *Planta*. 165, 397–406.

Bunce, J.A. (2001) Effects of prolonged darkness on the sensitivity of leaf respiration to carbon dioxide concentration in C3 and C4 species. *Ann Bot.-London.* 87:463–468.

von Caemmerer, S., Farquhar, G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta*. 153, 376-387.

Camargo, U.A. (1998) Cultivares para a viticultura tropical no Brasil. *Informe Belo Horizonte.* 19(194):15-19.

Camargo, U., Mandelli, F., Conceicão, M.A.F., Tonietto, J. (2012) Grapevine performance and production strategies in tropical climates. *As J Food Agro-Ind*. 5(04):257–269.

Carbonneau, A. (2011) Tropical viticulture: specificities and challenges for a quality viticulture. *Acta Hortic*. 910:15-34.

Cavaleri, M.A., Oberbauer, S.F., Ryan, M.G. (2008) Foliar and ecosystem respiration in an old-growth tropical rain forest. Plant Cell Environ. 31:473–483.

Centrito, M., Magnani, F., Lee, H.S.J., Jarvis, P.J. (1999) Interactive effects of elevated [CO_2] and drought on cherry (*Prunus avium*) seedlings. II: Photosynthetic capacity and water relations. *New Phytol*. 141:141– 153.

Centritto, M., Loreto, F., Chartzoulakis, K. (2003) The use of low [CO_2] to estimate diffusional and non-diffusional limitations of photosynthetic capacity of salt-stressed olive saplings. *Plant Cell Environ.* 26:585–594.

Chaumont, F., Tyerman, S.D. (2014) Aquaporins: highly regulated channels controlling plant water relations. *Plant Physiol*. 164:1600–1618.

Chavarria, G., Santos, H.P., Zanus, M.C., Marodin, G.A.B., Chalaça, M.Z., Zorzan, C. (2010) Grapevine maturation of moscato giallo under plastic cover. *Rev. Bras. Frutic*. 32:151-160.

Chaves, M.M., Flexas, J., Pinheiro, C. (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot-London*. 103:551–560.

Chaves, M.M., Santos, T.P., Souza, C.R., Ortuño, M. F., Rodrigues, M.L., Lopes, C.M., Maroco, J.P., Pereira, J.S. (2007) Deficit irrigation in grapevine improves water-use-efficiency without controlling vigour and production quality. *Ann Appl Biol*. 150:237–252.

Chaves, M.M., Pereira, J.S. (1992) Water stress, CO_2 and climate change. *J. Exp. Bot.* 43 (8):1131–1139.

Chaves, M.M., Zarrouk, O., Francisco, R., Costa, J.M., Santos, T., Regalado, A.P., Rodrigues, M.L., Lopes, C.M. (2010) Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann. Bot-London*. 105:661–676.

Collins, M.J., Fuentes, S., Barlow, E.W.R. (2010) Partial root zone drying and deficit irrigation increase stomatal sensitivity to vapour pressure deficit in anisohydric grapevines. *Funct. Plant Biol*. 37:128–138.

Collins, M.R., Knutti, R., Arblaster, J., Dufresne, J.-L., Fichefet, T., Friedlingstein, P., Gao, X., Gutowski, W.J., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A.J., Wehner, M. (2013) Long-term climate change: projections, commitments and irreversibility. *In*: Stocker, T.F., Qin, D., Plattner, K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), *Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, United Kingdom and New York, NY: Cambridge University Press. p. 1029-1136.

Comiran, F., Bergamaschi, H., Heckler, B.M.M. Santos, H.P., Alba, D., Sarettaet, E. (2012) Microclimate and production of ‘‘Niagara Rosada’’ grapevines in organic cultivation under plastic covering. *Rev Bras Frutic*. 34:152–159.

Coombe, B.G. (1989) The grape berry as a sink. *Acta Hortic.* 239:149–158.

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D. (2004) Breeding for high water-use efficiency. *J. Exp. Bot.* 55:2447-2460.

Costa, J.M., Ortuño, M.F. and Chaves, M.M. (2007) Deficit irrigation as a strategy to save water: physiology and potential appli- cation to horticulture. J. Integrative Plant Biol. 49:1421–1434.

Costa, J.M., Ortunõ, M.F., Lopes, C.M., Chaves, M.M. (2012) Grape-vine varieties exhibiting differences in stomatal response to water deficit. *Funct Plant Biol.* 39(3):179–189.

Coupel-Ledru, A., Tyerman, S., Masclef D., Lebon E., Christophe A., Edwards E.J.,Simonneau T. (2017) Abscisic acid down-regulates hydraulic conductance of grapevine leaves in isohydric genotypes only. *Plant Physiol*. 175(2). 38 p.

Cousins, A.B., Ghannoum, O., von Caemmerer S., Badger M.R. (2010) Simultaneous determination of Rubisco carboxylase and oxygenase kinetic parameters in *Triticum aestivum* and *Zea mays* using membrane inlet mass spectrometry. *Plant Cell Environ*. 33:444–452.

Crous, K.Y., Zaragoza-Castells, J., Ellsworth, D.S., Duursma, R.A., Tissue, M.D.T., Atkin, O.K. (2012) Light inhibition of leaf respiration in field-grown *Eucalyptus saligna* in whole-tree chambers under elevated atmospheric CO_2 and summer drought. *Plant Cell Environ*. 35:966–981.

Creasy, G.L. (2017). Viticulture: Grapevines and Their Management. *Encyclop. Appl Plant Sci*. 2(3):281-288.

Curtis, P.S., Wang, X. (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia* 113:299–313.

Davey, P.A., Hunt S., Hymus, G.J., Delucia, E.H., Drake, B.G., Karnosky, D.F., Long, S.P. (2004) Respiratory oxygen uptake is not decreased by an instantaneous elevation of [CO_2], but is increased with long-term growth in the field at elevated [CO_2]. *Plant Physiol*. 134:520–527.

Deluc, L.G., Quilici, D.R., Decendit, A., Grimplet, J., Wheatley, M.D., Schlauch, K.A., Merillon, J.M., Cushman, J.C., Cramer, G.R. (2009) Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics* 10:212–245.

Dewar, R.C., Medlyn, B.E., Mcmurtrie, R.E. (1999) Acclimation of the respiration/photosynthesis ratio to temperature: insights from a model. *Glob. Change Biol*. 5:615–622.

Dokoozlian, N.K., Kliewer, W.M. (1995) The light environment within grapevine canopies. II. Influence of leaf area density on fruit zone light environment and some canopy assessment parameters. *Am. J. Enol. Viticult*. 46:209–226.

Drake, B.G., González-Meler, M.A., Long, S.P. (1997) More efficient plants: A consequence of rising atmospheric CO_2? *Annu. Rev. Plant Phys*. 48:609–639.

Drake, J.E., Stoy, P.C., Jackson, R.B., Delucia, E.H. (2008) Fine-root respiration in a loblolly pine (*Pinus taeda* L.) forest exposed to elevated CO_2 and N fertilization. *Plant Cell Environ.* 31:1663–1672.

Dry, P.R., Loveys, B.R. (1998). Factors influencing grapevine vigour and the potential for control with partial rootzone drying. *Aust J Grape Wine Res*. 4:140–148.

Du, F., Deng, W., Yang, M., Wang, H., Mao, R., Shao, J., Fan, J., Chen, Y., Fu, Y., Li, C., He, X., Zhu, Y., Zhu, S. (2015) Protecting grapevines from rainfall in rainy conditions reduces disease severity and enhances profitability. *Crop Prot*. 67:261–268.

Düsman, E., Almeida, I.V., Lucchetta, L., Vicentini, V.E.P. (2014) Effect of processing, post-Harvest irradiation, and production system on the cytotoxicity and mutagenicity of Vitis labrusca L. juices in HTC cells. *PLoS One.* 9, e107974.

Eichhorn, K.W., Lorenz, D.H. (1977). Phänologische Entwicklungsstadien der Rebe. *Nachrichtenbl. Deut. Pflanzenschutzd*. 29:19-120.

Ellsworth, D. S., Reich, P. B., Naumburg, E. S., Koch, G. W., Kubiske, M. E., Smith, S. D. (2004) Photosynthesis, carboxylation and leaf nitrogen responses of 16 species to elevated pCO_2 across four free-air CO_2 enrichment experiments in forest, grassland and desert. *Global Change Biol*. 10:2121–38.

English, M., Raja, S.N. (1996) Perspectives on deficit irrigation. *Agr. Water Manag*. 32:1-14.

Epron, D., Godard, D., Cornic, G., Genty, B. (1995) Limitation of net CO_2 assimilation rate by internal resis- tances to CO_2 transfer in the leaves of two tree species (*Fagus sylvatica* L and *Castanea sativa* Mill). *Plant Cell Environ.* 18:43–51.

Erice, G., Irigoyen, J.J., Pérez, P., Martínez-Carrasco, R., Sánchez-Díaz, M. (2006) Effect of elevated CO_2, temperature and drought on photosynthesis of nodulated alfalfa during a cutting regrowth cycle. *Physiol Plant*. 126, 458–468.

Escalona, J., Delgado, E., Medrano, H. (1997) Irrigation effects on grapevine photosynthesis. *Acta Hort*. 449, 449–455.

Escalona, J.M., Tomàs, M., Martorell, S., Medrano, H., Ribas-Carbo, M., Flexas, J. (2012) Carbon balance in grapevines under different soil water supply: importance of whole plant respiration. *Aust J Grape and Wine Res*. 1 – 11.

Esteves, B.S., 2015. *Fluxo de seiva, consumo hídrico e manejos diferenciados de irrigação na videira Niagara Rosada (Vitis labrusca L.)*. (PhD in plant production) – Campos dos Goytacazes – RJ – UENF. 119p.

FAOSTAT (2014) FAO data for agriculture: statistics database: http://faostat.fao.org/faostat/collections?version=extandhasbulk=0andsubset=agricul-ture (01 May 2017 date last accessed).

Farquhar, G.D., von Caemmerer, S. (1982) Modelling of photosynthetic response to environmental conditions. *In*: Lange O.L., Nobel P.S., Osmond C.B., Ziegler H. (Eds). *Encyclopedia of plant physiology. Physiological plant ecology II. Water relations and carbon assimilation*. Berlin: Springer. p. 551–587.

Fereres, E., Soriano, M.A. (2007) Deficit irrigation for reducing agricultural water use. J. Exp. Bot. 58:147–159.

Ferreira, E.A., Regina, M.D.A., Chalfun, N.N.J., Antunes, L.E.C. (2004) Harvest anticipation for ‘‘Niagara Rosada’’ grapes in southern Minas Gerais, Brazil. *Ciência e Agrotecnologia* 28:1221–1227.

Flexas, J., Bota, J., Galmés, J., Medrano, H., Ribas-Carbo, M. (2006) Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiol. Plantarum*. 127:343–352.

Flexas, J., Carriquí, M., Coopman, R. E., Gago, J. Galmés, J. Martorell, S., Morales, F., Diaz-Espejo, A. (2014) Stomatal and mesophyll conductances to CO_2 in different plant groups: Underrated factors for predicting leaf photosynthesis responses to climate change? *Plant Sci*. 226:41–48.

Flexas, J., Díaz-Espejo, A., Conesa, M.A., Coopman, R.A., Douthe, C., Gago, J., Gallé, A., Galmés, J., Medrano, H., Ribas-Carbo, M., Tomàs, M., Niinemets, Ü. (2016) Mesophyll conductance to CO_2 and Rubisco as targets for improving intrinsic water use efficiency in C3 plants. *Plant Cell Environ*. 39:965–982.

Flexas, J., Galmés J., Gallé, A., Gulías, J., Pou, A., Ribas-Carbó, M., Tomás, M., Medrano H. (2010) Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. *Aust J Grape Wine Res*. 16:106–121.

Flexas, J., Galmés, J., Ribas-Carbo, M., Medrano, H. (2005) The effects of drought in plant respiration. *In*: Lambers, H., Ribas-Carbo, M. (Eds*). Advances in Photosynthesis and Respiration. Plant Respiration: from Cell to Ecosystem*. Dordrecht: Kluwer Academic Publishers. p. 85–94.

Flexas J. (2016) Genetic improvement of leaf photosynthesis and intrinsic water use efficiency in C3 plants: Why so much little success? *Plant Sci*. 9407:1-7.

Flexas, J., Medrano, H. (2002) Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Ann. Bot*. 89:183—189.

Flexas, J., Barón, M., Bota, J., Ducruet, J.M., Gallé, A., Galmés, J., Jiménez, M., Pou, A., Ribas-Carbó, M., Sajnani, C., Tomàs, M., and Medrano, H. (2009).

Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted Vitis hybrid Richter-110 (*V. berlandieri* x *V. rupestris*). *J. Exp. Bot.* 60(8):2361-2377.

Flexas, J., J. Bota, J.M. Escalona, B. Sampol and H. Medrano. (2002) Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Funct. Plant Biol*. 29:461-471.

Force, L., Critchley, C., van Rensen, J. J. S. (2003) New fluorescence parameters for monitoring photosynthesis in plants. *Photosynth Res*. 78:17–33.

Foyer, C., Furbank, R., Harbinson, J., Horton, P. (1990) The mechanisms contributing to photosynthetic control of electron transport by assimilation in leaves. *Photosynth. Res.* 25:83–100.

Fuentes, S., De Bei, R., Collins, R.J., Escalona, J.M., Medrano, H., Tyerman. S. (2014) Night-time responses to water supply in grapevines (Vitis vinifera L.) under deficit irrigation and partial root-zone drying. Agr. Water Manage. 138:1–9.

Gago, J., Douthe, C., Flórez-Sarasa, I., Escalona, J., Galmés, J., Fernie, A.R., Flexas, J., Medrano, H. (2014) Opportunities for improving leaf water use efficiency under climate change conditions. *Plant Sci*. 226:108–119.

Galmés, J., Conesa, M.À., Díaz-Espejo, A., Mir, A., Perdomo, J.A., Niinemets, Ü., Flexas, J. (2014) Rubisco catalytic properties optimised for present and future climatic conditions. *Plant Sci*. 226:61–70.

Galmés, J., Kapralov, M.V., Copolovici. O., Hermida-Carrera, C., Niinemets, Ü. (2015) Temperature responses of the Rubisco maximum carboxylase activity across domains of life: phylogenetic signals, trade-offs, and importance for carbon gain. *Photosynth. Res*. 123:183–201.

Gaudillère, J.P., Van Leeuwen, C., Ollat, N. (2002) Carbon isotope composition of sugars in grapevine, an integrated indicator of vineyard water status. *J Exp Bot*. 53:757–763.

Gelly, M.; Recansens, I.; Girana, J.; Mata, M.; Arbones, A.; Rutat, J.; Marsal, J. (2004) Effects of stage II and post-harvest deficit irrigation on peach quality during maturation and after cold storage. *J. Sci. Food Agri.* 84:561–568.

Gemel, J., Randall, D.D. (1992) Light regulation of leaf mitochon- drial pyruvate dehydrogenase complex. Role of photorespira- tory carbon metabolism. *Plant Physiol.* 100:908–914.

Giménez, C., Mitchell, V.J., Lawlor, D. (1992). Regulation of photosynthesis rate of two sunflower hybrids under water stress. *Plant Physiol*. 98:516-524.

Gimeno, T.E., Sommerville, K.E., Valladares, F., Atkin, O.K., (2010) Homeostasis of respiration under drought and its important consequences for foliar carbon balance in a drier climate: insights from two contrasting Acacia species. *Funct Plant Biol*. 37:323–333.

Giovannini, E. (1999) *Produção de uvas para vinho, sucos e mesa*. Porto Alegre: Renascença. 364 p.

Gonzàlez-Meler, M. A., Drake, B. G., Azcón-Bieto J. (1996) Rising atmospheric carbon dioxide and plant respiration. *In*: Breymeyer, A. I., Hall, D. O., Melillo, J. M., A. Gren GI (Eds). *Global change: effects on coniferous forests and grasslands*. UK: SCOPE, John Wiley and Sons. p. 161–181.

González-Meler, M.A., Taneva, L., Trueman, R.J. (2004) Plant respiration and elevated atmospheric CO_2 concentration: cellular responses and global significance. *Ann Bot-London*. 94:647–656.

Graham, D. (1980) Effects of light and ‘dark’ respiration. *In*: Davies, D.D (Ed). *The Biochemistry of Plants. A Comprehensive Treatise*. New York: Academic Press. p. 525–579.

Grant, O.M., Tronina, L., Jones, H.G., Chaves, M.M. (2007) Exploring thermal imaging variables for the detection of stress responses in grapevine under different irrigation regimes. *J. Exp. Bot*. 58:815–825.

Greer, D.H., Sicard, S.M. (2009) The net carbon balance in relation to growth and biomass accumulation of grapevines (*Vitis vinifera* cv. Semillon) grown in a controlled environment. *Funct Plant Biol*. 36:645–653.

Geerts, S., Raes, N. (2009) Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. *Agric. Water Manag*. 96:1275–1284.

Grenn, S.R., Clothier B.E., McLoud, D.J. (1997) The response of sap flow in apple roots to localized irrigation. *Agric. Water Manage*. 33:63-78.

Griffin, K.L., Heskel, M. (2013) Breaking the cycle: how light, CO_2 and O_2 affect plant respiration. *Plant Cell Environ*. 36:498–500.

Griffin, K. L., Seemann, J. R. (1996). Plants, CO_2 and photosynthesis in the 21^{st}. *Chem Biol*. 3 (4):245-254.

Griffin K.L., Anderson O.R., Tissue D.T., Turnbull M.H., Whitehead D. (2004) Variations in dark respi- ration and mitochondrial numbers within needles of

Pinus radiata grown in ambient or elevated CO_2 partial pressure. *Tree Physiol*. 24:347–353.

Griffin, K.L., Tissue, D.T., Turnbull, M.H., Schuster, W., Whitehead, D. (2001) Leaf dark respiration as a function of canopy position in Nothofagus fusca trees grown at ambient and elevated CO_2 partial pressures for 5 years. *Funct. Ecol*. 15:497–505.

Griffin, K.L., Turnbul, M.H. (2013) Light saturated RuBP oxygenation by Rubisco is a robust predictor of light inhibition of respiration in *Triticum aestivum* L. *Plant Biol*. 15:769–775.

Grossman-Clarke, S., Pinter Jr., P.J., Kartschall, T., Kimball, B.A., Hunsaker, D.J., Wall, G.W., García, R.L., LaMorte, R.L. (2001) Modelling a spring wheat crop under elevated CO_2 and drought. *New Phytol*. 150:315–335.

Gu, S., Guoqiang, D., Zoldoske, D., Hakim, A., Cochran, R., Fugelsang, K., Jorgensen, G. (2004) Effects of irrigation amount on water relations, vegetative growth, yield and fruit composition of Sauvignon blanc grapevines under partial root-zone drying and conventional irrigation in the San Joaquin Valley of California, USA. *The J. Hortic. Sci. Biotech*. 79:26–33.

Halis, Y., Djehichi, S., Senoussi, M.M. (2012) Vessel development and the importance of lateral flow in water transport within developing bundles of current-year shoots of grapevine (*Vitis vinifera* L.). *Trees*. 26:705–714.

Hall, D., Yuan, X.X., Murata, J., Luca, V. (2012) Molecular cloning and biochemical characterization of the UDP-glucose: Flavonoid 3-O-glucosyltransferase from Concord grape (*Vitis labrusca*). *Phytochemistry* 74:90–99.

Hargreaves, G.H., Samani, Z.A. (1985) Reference crop evapotranspiration from temperature. *J. Appl. Eng. Agr*. 1(2):96-99.

Herrick, J.D., Thomas, R.B. (2001) No photosynthetic down-regulation in sweetgum trees (*Liquidambar styraciflua* L.) after three years of CO_2 enrichment at the Duke Forest FACE experiment. *Plant, Cell & Environment* 24:53–64.

Hidalgo, L. (1993) *Tratado de viticultura general*. Madrid: Mundi-Prensa. 983 p.

Hochberg, U., Degu, A., Fait, A., Rachmilevitch, S. (2013) Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. *Physiol. Plantarum*. 147:443–452.

Holtum J.A.M., Winter K. (2010) Elevated [CO_2] and forest vegetation: more a water issue than a carbon issue? *Funct. Plant Biol.* 37:694–702.

Hymus, G.J., Dijkstra, P., Baker, N.R., Drake, B.G., Long, S.P. (2001) Will rising CO_2 protect plants from the midday sun? A study of photoinhibition of *Quercus myrtifolia* in a scrub-oak community in two seasons. *Plant Cell Environ*. 24:1361–1368.

IBGE (2016) – Sistema IBGE de recuperação automática – SIDRA. Disponível em: <http//www.sidra.ibge.gov.br.>.(01 May 2017 date last accessed)

Igamberdiev, A.U., Gardeström, P. (2003) Regulation of NAD^- and $NADP^-$ dependent isocitrate dehydrogenases by reduction levels of pyridine nucleotides in mitochondria and cytosol of pea leaves. BBA.–Bioenergetics 1606:117– 125.

IPCC (2014) Intergovernmental panel on climate change. Summary for policymakers. *In*: Edenhofer O, Pichs-Madruga R, Sokona Y, Farahani E, Kadner S, Seyboth K, AdlerA, Baum I, Brunner S, Eickemeier P, Kriemann B, Savolainen J, Schlömer S, von Ste-chow C, Zwickel T, Minx JC, editors. Climate change 2014. *Mitigation of climate change. Contribution of working group III to the fifth assessment report of the intergovernmental panel on climate change.* Cambridge, United Kingdom/NewYork, NY, USA: Cambridge University Press.

Intrigliolo, D.S., Castel, J.R. (2009) Response of *Vitis vinifera* cv. “Tempranillo” to partial root-zone drying in the field: water relations, growth, yield and fruit and wine quality. *Agr. Water Manage*. 96:282–292.

Jamil, M., Rehman, S., Rha, E.S. (2007) Salinity effect on plant growth, PSII photochemistry and chlorophyll content in sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleraceacapitata* L.). *Pak. J. Bot*. 39:753–760.

Jifon, J., Wolfe, D. (2002) Photosythetic acclimation to elevated CO_2 in Phaseolus vulgaris L. is altered by growth response to nitrogen supply. Glob. Change Biol. 8:1018–1027.

Jones, G.V., Reid, R., Aleksander, V. (2012) Climate, grapes, and wine: Structure and suitability in a variable and changing climate. *In*: *The geography of wine—regions, terroir and techniques* p. 109–133.

Jones, H.G. (1992) *Plant and microclimate: a quantitative approach to environmental plant physiology*. Cambridge: Cambridge University Press. 428 p.

Kamiloğlu, Ö., Polat, A., Durgac, C. (2011) Comparison of open field and protected cultivation of five early table grape cultivars under Mediterranean conditions. *Turk J Agric For.* 35:491–499.

Kasimova, M.R., Grigiene, J., Krab, K., Hagedorn, P.H., Flyvbjerg, H., Andersen, P.E., Moller, I.M. (2006) The free NADH concentration is kept constant in plant mitochondria under different metabolic conditions. *The Plant Cell.* 18:688–698.

Kimball, B.A., Morris, C.F., Pinter Jr., P.J., Wall, G.W., Hunsaker, D.J., Adamsen, F.J., LaMorte, R.L., Leavitt, S.W., Thompson, T.L., Matthias, A.D., Brooks, T.J. (2001) Elevated CO_2, drought and soil nitrogen effects on wheat grain quality. *New Phytol*. 150:295–303.

Kirschbaum, M.U.F. (2010) The temperature dependence of organic matter decomposition: seasonal temperature variations turn a sharp short-term temperature response into a more mod- erate annually averaged response. Glob. Change Biol. 16:2117–2129.

Kirschbaum, M.U.F., Farquhar G.D. (1987) Investigation of the CO_2 dependence of quantum yield and respiration in *Eucalyptus pauciflora*. *Plant Physiol*. 83:1032–1036.

Kizildeniz, T., Mekni, I., Santesteban, H., Pascual, I., Morales, F., Irigoyen, J.J. (2015). Effects of climate change including elevated CO_2 concentration, temperature and water deficit on growth, water status, and yield quality of grapevine (*Vitis vinifera* L.) cultivars. *Agricul. Water Manage.* 159:155–164.

Kok, B. (1948) A critical consideration of the quantum yield of *Chlorella*-photosynthesis. *Enzymologial*. 13:1–56.

Köppen, W. (1918) Klassifikation der Klimate nach Temperatur, Niederschlag und Jahresablauf (Classification of climates according to temperature, precipitation and seasonal cycle). *Petermanns Geogr. Mitt*. 64: 243-248.

Koundouras, S., Marinos, V., Gkoulioti, A., Kotseridis, Y., Van Leeuwen, C. (2006). Influence of vineyard location and vine water status on fruit maturation of non-irrigated cv. Agiorgitiko (*Vitis vinifera* L.). Effects of wine phenolic and aroma components. *J. Agric. Food Chem*. 54:5077–5086.

Krömer, S. (1995) Respiration during photosynthesis. Annu. Rev. Plant Phys. 46:45–70.

Lambers, H., Robinson, S.A., Ribas-Carbo, M. (2005) Regulation of respiration in vivo. The effects of drought in plant respiration. *In*: Lambers H, Ribas-Carbó M

(Eds) Advances in Photosynthesis and Respiration. Plant Respiration: From Cell to Ecosystem. Dordrecht: Kluwer Academic Publishers. p. 1–15.

Lambreva, M., Stoyanova-Kolevab, D., Baldjievb, G., Tsonev, T. (2005) Early acclimation changes in the photosynthetic apparatus of bean plants during short-term exposure to elevated CO_2 concentration under high temperature and light intensity. *Agr Ecosyst Environ*. 106:219–232.

Lavoie-Lamoureux, A., Sacco, D., Risse, P.A., Lovisolo, C. (2017) Factors influencing stomatal conductance in response to water availability in grapevine: a meta-analysis. *Physiol. Plantarum*. 159:468–482.

Lawlor, D.W., Tezara, W. (2009) Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. *Ann Bot-London*. 103:561-579.

Leakey, A.D.B., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P., Ort, D.R. (2009) Elevated CO_2 effects on plant carbon, nitrogen and water relations: six important lessons from FACE. *J Exp Bot.* 60:2859–2876.

Lecourieux, F., Lecourieux, D., Vignault, C., Delrot, S. (2010) A sugar-inducible protein kinase, VvSK1, regulates hexose transport and sugar accumulation in grapevine cells. *Plant Physiol*. 152(2):1096-1106.

Lewis, J.D., Wang, X.Z., Griffin, K.L., Tissue, D.T. (2002) Effects of age and ontogeny on photosynthetic response of a determinate annual plant to elevated CO2 concentrations. *Plant Cell Environ.* 25:359–368.

Lin M., Turpin D.H., Plaxton W.C. (1989) Pyruvate kinase isozymes from the green alga, Selenastrum minutum. II. Kinetic and regulatory properties. Arch Biochem. Biophys. 269:228–238.

Liu, G-T., Wang, J-F., Cramer, G., Dai, Z-W., Duan, W., Xu, H-G., Wu, B-H., Fan, P-G., Wang, L-J., Li, S-H. (2012) Transcriptomic analysis of grape (*Vitis vinifera* L.) leaves during and after recovery from heat stress. *BMC Plant Biol*. 12-10.

Long, S.P., Ainsworth, E.A., Rogers, A., Ort, D.R. (2004) Rising atmospheric carbon dioxide: plants face the future. *Ann. Rev. Plant Biol*. 55:591–628.

Long, S.P. (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? *Plant Cell Environ*. 14:729–739.

Loveys B.R., Scheurwater I., Pons T.L., Fitter A.H., Atkin O.K. (2002) Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast- and slow- growing plant species. *Plant Cell and Environ*. 25:975–988.

Lovisolo, C., Perrone, I., Carra, A., Ferrandino, A., Flexas, J., Medrano, H., Schubert, A. (2010) Drought-induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. *Funct. Plant Biol*. 37:98–116.

Lovisolo, C., Schubert, A. (2006) Mercury, hinders recovery of shoot hydraulic conductivity during rehydration: evidence from a whole-plant approach. *New Phytol.* 172:469–478

Luo, Y., Field, C. B., Mooney, H. A. (1994) Predicting responses of photosynthesis and root fraction to elevated [CO_2]: interactions among carbon, nitrogen and growth. *Plant Cell Environ*. 17:1195-1204.

Mahecha M.D., Reichstein M., Jung M., Seneviratne, S.I., Zaehle, S., Beer, C., Braakhekke, M.C., Carvalhais, N., Lange, H., Le Maire, G., Moors, E. (2010) Comparing observations and process-based simulations of biosphere-atmosphere exchanges on multiple timescales. J. Geophys. Res. 115:G02003

Maroco, J.P., Rodrígues, M.L., Lopes, C., Chaves, M.M. (2002) Limitations to leaf photosynthesis in field-grown grapevine under drought – metabolic and modelling approaches. *Funct Plant Biol*. 29:451–459.

Matthews, M.A., Anderson, M.M. (1989) Reproductive development in grape (*Vitis vinifera* L.): responses to seasonal water deficits. *Am J Enol Vitic.* 40:52–60.

McCarthy, M.G. (1997) The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 3:102–108

Medrano, H., Escalona, J.M., Bota, J., Gulías, J., Flexas, J. (2002). Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Ann. Bot-London*. 89:895–905.

Medrano, H., Escalona, J.M., Cifre, J., Bota, J., Flexas, J. (2003) A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Funct. Plant Biol*. 30:607–619.

Molden, D., Oweis, T., Steduto, P., Bindraban, P., Hanjra, M.A., Kijne, J. (2010) Improving agricultural water productivity: between optimism and caution. *Agric. Water Manag*e. 97:528–535.

Monteiro, A., Lopes, C.M. (2007) Influence of cover crop on water use and performance of vineyard in Mediterranean Portugal. *Agr. Ecosyst. Environ*. 121:336–342.

Moore, B.D., Cheng, S. H., Sims, D., Seemann, J.R. (1999) The biochemical and molecular basis for photosynthetic acclimation to elevated atmospheric CO_2. *Plant Cell Environ*. 22:567–582.

Morales, F., Abadía, A., Abadía, J. (2006) Photoinhibition and photoprotection under nutrient deficiences, drought and salinity. *In*: Demmig-Adams, B., Adams, W.W.(Eds.) *Photoprotection, Photoinhibition, Gene Regulation and Environment*. Netherlands: Springer. p. 65–85.

Morales, F., Antolín, M.C., Aranjuelo, I., Goicoechea, N., Pascual, I. (2016) From vineyards to controlled environments in grapevine research: investigating responses to climate change scenarios using fruit-bearing cuttings. *Theor. Exp. Plant Physiol*. 28(2):171-191.

Morison, J.I.L., Baker, N.R., Mullineaux, P.M., Davies, W.J. (2008) Improving water use in crop production. Philos.T. R. Soc. A. 363,639–658.

Moutinho-Pereira, J.M., Gonçalves, B., Bacelar, E., Boaventura Cunha, J., Coutinho, J., Correia, C.M. (2009) Effects of elevated CO_2 on grapevine (*Vitis vinifera* L.): physiological and yield attributes. *Vitis*. 48:159–165.

Mullins, M.G., Bouquet, A., Williams, L.E. (2000) *Biology of the grapevine*. Cambridge: University Press, 239 p.

Myburgh, P.A. (2003) Responses of *Vitis vinifera* L. cv. Sultanina to water deficits during various pre- and post-harvest phases under semi-arid conditions. *S. Afr. J. Enol. Vitic.* 24:25–33.

Niculcea, M., López, J., Sánchez-Díaz, M., Antolín, M.C. (2014) Involvement of berry hormonal content in the response to pre- and post-veraison water deficit in different grapevine (*Vitis vinifera* L.) cultivars. *Aust. J. Grape Wine Res*. 20:281–291.

Novello, V., de Palma, L. (2008) Growing grapes under cover. *Acta Hortic.* 785:353–362.

Nunes-Nesi, A., Sweetlove, L.J., Fernie, A.R. (2007) Operation and function of the tricarboxylic acid cycle in the illuminated leaf. *Physiol. Plantarum* 129:45–56.

OIV – International Organization of Vine and Wine http://www.oiv.int/ (last accessed August 19th, 2017)

Ojeda, H., Andary, C., Kraeva, E., Carbonneau, A., Deloire, A. (2002) Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic*. 53:261–267.

Oliveira, V.F., Zaidan, L.B.P., Braga, M.R., Aidar, M.P.M., Carvalho, M.A.M. (2010) Elevated CO_2 atmosphere promotes plant growth and inulin production in the cerrado species Vernonia herbacea. Funct. Plant Biol. 37:223-231.

Palliotti, A., Tombesi, S., Frioni, T., Silvestroni, O., Lanari, V., D’Onofrio, C., Matarese, F., Bellincontro, A., Poni, S. (2015) Physiological parameters and protective energy dissipation mechanisms expressed in the leaves of two *Vitis vinifera* L. genotypes under multiple summer stress. *J. Plant. Physiol*. 185:84-92.

Patakas, A., Noitsakis. B. (1999) Mechanisms involved in diurnal changes of osmotic potential in grapevines under drought conditions. *J. Plant Physiol*. 154:767–774.

Pedro, M.J.P.J., Hernandes, J.L., Rolim, G.S. (2011) Sistema de condução em Y com e sem cobertura plástica: microclima, produção, qualidade do cacho e ocorrência de doenças fúngicas na videira ‘Niagara Rosada’. *Bragantia*. 70:228-233.

Pedro Júnior, M.J., Sentelhas, P.C., Pommer, C.V., Martins, F.P., Gallo, P.B., Dos Santos, R.R., Bovi, V., Sabino, J.C. (1993) Caracterização fenológica da videira ‘Niagara Rosada’ em diferentes regiões paulistas. *Bragantia*, 52 (2): 153-160.

Peppi, M.C., Fidelibus, M.W., Dokoozlian, N. (2006) Abscisic acid application timing and concentration affect firmness, pigmentation, and color of 'Flame Seedless' grapes. *HortScience*. 41:1440–144.

Pérez-Pastor, A., Ruiz-Sánchez, M.C., Martínez, J.A., Nortes, P., Artés, F., Domingo, R. (2007) Effect of deficit irrigation on apricot fruit quality at harvest and during storage. *J. Sci. Food Agric.* 87:2409-2415.

Permanhani, M., Miguel Costa, J.M., Conceição, M.A.F., de Souza, R.T., Vasconcellos, M.A.S., Chaves. M.M. (2016) Deficit irrigation in table grape:

eco-physiological basis and potential use to save water and improve quality. *Theor. Exp. Plant Physiol*. 28(1):85-108.

Petrie, P.R., Cooley, N.M., Clingeleffer, P.R. (2004) The effect of post-veraison water deficit on yield components and maturation of irrigated Shiraz (*Vitis vinifera* L.) in the current and following season. *Aust. J. Grape Wine Res*. 10:203–215.

Pinheiro, C., Chaves, M.M. (2011) Photosynthesis and drought: can we make metabolic connections from available data? *J. Exp. Bot.* 62(3):869–882.

Pinto, E.P., Perin, E.C., Schott, I.B., Rodrigues, R.S., Lucchetta, L., Manfroi, V., Rombaldi, C.V. (2016). The effect of postharvest application of UV-C radiation on the phenolic compounds of conventional and organic grapes (*Vitis labrusca* cv. ‘Concord’). Postharvest Biol.Tech. 120:84–91.

Poni, S., Bernizzoni, F., Civardi, S. (2007) Response of ‘Sangiovese’ grapevines to partial rootzone drying: gas-exchange, growth and grape composition. *Sci. Hortic-Amsterdan.* 114:96–103.

Poni, S., Bernizzoni, F., Civardi, S., Gatti, M., Porro, D., Camin, F. (2009) Performance and water-use efficiency (single-leaf vs. whole-canopy) of well-watered and half-stressed split-root Lambrusco grapevines grown in Po Valley (Italy). *Agri. Eco. and Environ*. 129:97-106.

Pons, T.L., Welschen, R.A.M. (2002) Overestimation of respiration rates in commercially available clamp-on leaf chambers. Complications with measurement of net photosynthesis. *Plant Cell Environ*. 25:1367–1372.

Pou, A., Medrano, H., Tomàs, M., Martorell, S., Ribas-Carbó, M., Flexas, J. (2012) Anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric behavior. *Plant Soil*. 359:335–349.

Protas, J. F. S.; Camargo, U. A.; Melo, L. M. R. A. (2002) Viticultura brasileira: realidade e perspectivas. In: *Simpósio Mineiro de Viticultura e Enologia.* Viticultura e enologia: atualizando conceitos. Caldas – MG, p. 17 -32.

Ramalho, J. C., Rodrigues, A. P., Semedo, J. N., Pais, I. P., Martins, L. D., Simões-Costa, S. C., Leitão, A. E., Fortunato, A. S., Batista-Santos, P., Palos, I. M., Tomaz, M. A., Scotti-Campos, P., Lidon, F. C., DaMatta, F. M. (2013a) Sustained photosynthetic performance of Coffea spp. under long-term enhanced [CO2]. *PLoS One*, 8:e82712.

Rampino, P., Mita, G., Fasanoa, P., Borrelli, G. M., Aprilea, A., Dalessandro, G., De Bellis, L., Perrotta, C. (2012) Novel durum wheat genes up-regulated in response to a combination of heat and drought stress. *Plant Physiol. Biochem.* 56:72–8.

Reich, P. B., Sendall, K. M., Stefanski1, A., Wei, X., Rich, R. L., Montgomery, R. A. (2016) Boreal and temperate trees show strong acclimation of respiration to warming. *Nature*. 531:633-636.

Reuveni, J., Gale, J., Mayer, A. M. (1995) High ambient carbon dioxide does not affect respiration by suppressing the alternative cyanide-resistant respiration. *Ann Bot-London*. 76:291–295.

Ribas-Carbó, M., Taylor, N.L., Giles, L., Busquets, S., Finnegan, P.M., Day, D.A., Lambers, H., Medrano, H., Berry, J.A., Flexas, J. (2005) Effects of water stress on respiration in soybean leaves. *Plant Physiol.* 139:466–473.

Ribeiro, D.P., Corsato, C.E., Lemos, J.P., Filho, J.A.S. (2009) Desenvolvimento e exigência térmica da videira ‘Niagara Rosada’, cultivada no Norte de Minas Gerais. *Rev. Bras. Frutic.* 31(3): 890-895.

Roberto, S.R., Colombo, L.A., de Assis, A.M. (2011) Review: protect cultivation in viticulture. *Cienc. Tec. Vitivinic*. 26:11–16.

Roberto, S. R., Kanai, H. T., Yano, M. Y., Sasano, E. M., Genta, W. (2002) Avaliação da brotação e da fertilidade de gemas da videira ‘Niagara rosada’ submetida a diferentes intensidades de poda de frutificação. *Semina*: *Ciências Agrárias.* 23 (2):185-190.

Robredo, A., Pérez-López, U., de la Maza, H.S., González-Moro, B., Lacuesta, M., Mena-Petite, A., Munõz-Rueda, A. (2007) Elevated CO_2 alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environ Exp Bot*. 59:252–263.

Robredo A., Pérez-López, U., Lacuesta, M., Mena-Petite A., Munõz-Rueda A. (2010) Influence of water stress on photosynthetic characteristics in barley plants under ambient and elevated CO_2 concentrations. *Biol Plantarum*. 54:285–292.

Roby, G. and Matthews, M.A. (2004) Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. Aust. J. Grape Wine Res. 10:74–82.

Rodrigues, W.P., Martins, M.Q., Fortunato, A.S., Rodrigues, A.P., Semedo, J.N., Simões-Costa, M.C., Pais, I.P., Leitão, A.E., Colwell, F., Goulao, L., Máguas, C., Maia, R., Partelli, F.L., Campostrini, E., Scotti-Campos, P., Ribeiro-Barros, A.I., Lidon, F.C., DaMatta, F.M., Ramalho, J.C. (2016) Long-term elevated air [CO_2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical *Coffea arabica* and *C. canephora* species. *Glob Change Biol.* 22:415 - 431.

Rogers, H.H., Dahlman, R.D. (1993). Crop response to CO_2 enrichment. *Vegetatio*. 104/105:117-131.

Rogiers, S.Y., Greer, D.H., Hatfield, J.M., Hutton, R.J., Clarke, S.J., Hutchinson, P.A., Somers, A. (2012) Stomatal response of an anisohydric grapevine cultivar to evaporative demand, available soil moisture and abscisic acid. *Tree Physiol*. 32:249–261.

Rogiers, S.Y., Hardie, W.J., Smith, J.P. (2011) Stomatal density of grapevine leaves (*Vitis vinifera* L.) responds to soil temperature and atmospheric carbon dioxide. *Aust J Grape Wine Res*. 17:147–152.

Sage, R.F. (2013) Photorespiratory compensation: a driver for biological diversity. *Plant Biol*. 15:624–638.

Salazar-Parra, C., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J., Morales, F. (2012) Photosynthetic response of Tempranillo grapevine to climate change scenarios. *Ann Appl Biol*. 161:277–292.

Salazar-Parra, C., Aranjuelo, I., Pascual, I., Erice, G., Sanz-Sáez, A., Aguirreolea, J., Sánchez-Díaz, M., Irigoyen, J.J., Araus, J.L., Morales, F. (2015) Carbon balance, partitioning and photosynthetic acclimation in fruit-bearing grapevine (*Vitis vinifera* L. cv. Tempranillo) grown under simulated climate change (elevated CO_2, elevated temperature and moderate drought) scenarios in temperature gradient greenhouses. *J Plant Physiol*. 174:97–109.

Sánchez-Díaz, M., Irigoyen, J.J., Gómez-Casanovas, N., Pardo, A., Azcón-Bieto, J. (2004) *In*: Reigosa, M., Pedro, N., Sánchez-Moreiras, A. (Eds). *El cambio climático global. Efecto previsible del CO_2 sobre los vegetales. La Ecofisiología Vegetal. Una Ciencia de Síntesis*. Vigo, Spain: Universidad de de Vigo. p. 1111-1140.

Santesteban, L.G., Miranda, C., Royo, J.B. (2011) Regulated deficit irrigation effects on growth yield, grape quality and individual anthocyanin composition in *Vitis vinifera* L. cv. Tempranillo. *Agric. Water Manage*. 98:1171–1179.

dos Santos, T., C.M. Lopes, M.L. Rodrigues, C.R. Souza, J.P. Maroco, J.S. Pereira, J.R. Silva and M.M. Chaves. (2003) Partial rootzone drying: effects on growth, and fruit quality of fieldgrown grapevines (*Vitis vinifera* L.). *Funct. Plant Biol*., 30:663-671.

Santos, H.P. (2006) *Aspectos ecofisiológicos na condução da videira e sua influência na produtividade do vinhedo e na qualidade dos vinhos*. Bento Gonçalves-RS: EMBRAPA-CNPUV, (Comunicado Técnico, 71). 9 p.

Sanz-Sáez, A., Erice, G., Aranjuelo, I., Nogués, S., Irigoyen, J.J., Sánchez-Díaz, M. (2010). Photosynthetic down-regulation under elevated CO_2 exposure can be prevented by nitrogen supply in nodulated alfalfa. *J Plant Physiol*. 167:1558–1565.

Seccia, A., Santeramo, F.G., Nardone, G., 2015. Trade competitiveness in table grapes: a global view. Outlook Agric. 44, 127–134.

Sentelhas, P.C. (1998) Aspectos climáticos para a viticultura tropical. *Informe Agropecuário/Viticultura Tropical.* 19 (194): 9-14.

Sharma-Natu, P., Ghildiyal, M.C., (2005) Potential targets for improving photosynthesis and crop yield. *Curr Sci India*. 88:1918–1928.

Shapiro, J.B., Griffin, K.L., Lewis, J.D., Tissue D.T. (2004) Response of Xanthium strumarium leaf respiration in the light to elevated CO_2 concentration, nitrogen availability and temperature. *New Phytol*. 162:377–386.

Schultz, H.R. (2000) Climate change and viticulture: European perspective on climatology, carbon dioxide and UV-B effects. *Aust J Grape Wine Res*. 6:2–12.

Schultz, H.R. (2003) Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant Cell Environ.* 26:1393–1405.

Schultz, H.R., Stoll, M. (2010) Some critical issues in environmental physiology of grapevines: future challenges and current limitations. *Aust. J. Grape Wine R*. 16:4–24.

Shapiro, J.B., Griffin, K.L., Lewis, J.D., Tissue D.T. (2004) Response of *Xanthium strumarium* leaf respiration in the light to elevated CO_2 concentration, nitrogen availability and temperature. *New Phytol*. 162:377–386.

Silva, J.R., Patterson, A.E., Rodrigues, W.P., Campostrini, E., Griffin, K.L. (2017) Photosynthetic acclimation to elevated CO_2 combined with partial rootzone drying results in improved water use efficiency, drought tolerance and leaf carbon balance of grapevines (*Vitis labrusca*). *Environ. Exp. Botany*. 134:82–95.

Shu, S., Guo, S.R., Sun, J., Yuan, L.Y. (2012) Effects of salt stress on the structure and function of the photosynthetic apparatus in *Cucumis sativus* and its protection by exogenous putrescine. *Physiol. Plant*. 146:285–296.

Soar, C.J., Speirs, J., Maffei, S.M., Penrose, A.B., McCarthy, M.G., Loveys, B.R. (2006) Grape vine varieties Shiraz and Grenache differ in their stomatal response to VPD: apparent links with ABA physiology and gene expression in leaf tissue. *Aust. J. Grape Wine R*es. 12:2–12.

Sousa, J. S. I. (1996) *Uvas para o Brasil*. 2 ed. Piracicaba: FEALQ, 791p.

de Souza, C. R., Maroco, J. P., Santos, T., Rodrigues, M. L., Lopes, C., Pereira, J. S., Chaves, M. M. (2004) Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. *Agric Ecosyst Environ*. 106(2–3):261–274.

de Souza, C.R., Maroco, J.P., dos Santos, T.P., Rodrigues, M.L., Lopes, C.M., Pereira, J.S., Chaves, M.M. (2005) Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. *Agr. Ecosyst. Environ*. 106:261–274.

de Souza, C.R., Maroco, J.P., Dos Santos, T.P., Rodrigues, M.L., Lopes, C.M., Pereira, J.S., Chaves, M.M. (2005) Grape berry metabolism in field-grown grapevines exposed to different irrigation strategies. *Vitis*. 44:103–109.

de Souza, C.R., Mota, R.V., Dias, F.A.N., Melo, E.T., Pimentel, R.M.A., Souza, L.C., Regina, M.A. (2015) Physiological and agronomical responses of Syrah grapevine under protected cultivation. *Bragantia*. 74(3):270-278.

Springer, C.J., Delucia, E.H., Thomas, R.B. (2005) Relation- ships between net photosynthesis and foliar nitrogen concentrations in a loblolly pine forest ecosystem grown in elevated atmospheric carbon dioxide. *Tree Physiology*, 25:385–394.

Springer, C.J., Thomas R.B. (2007) Photosynthetic responses of forest understory tree species to long-term exposure to elevated carbon dioxide concentration at the Duke Forest FACE experiment. *Tree Physiology*. 27:25–32.

Stanghellini. C. (2014) Horticultural production in greenhouses: efficient use of water. *Acta Hortic*. 1034:25–32.

Stoll, M., Loveys, B. and Dry, P. (2000) Hormonal changes induced by partial rootzone drying of irrigated grapevine. *J Exp Bot*. 51:1627–1634.

Strasser, B.J., Strasser, R.J. (1995). Measuring fast fluorescence transients to address environmental questions: The JIP-test. *In*: Mathis, P. (Ed.), *Photosynthesis: from light to biosphere*. Dordrecht: Kluwer. p. 977–980.

Strasser, R.J., Srivastava, A., Govindjee (1995) Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochem. Photobiol*. 61:32–42.

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M. (2000) The fluorescence transient as a tool to characterize and screen photosynthetic samples. *In*: Yunus, M., Pathre, U., Mohanty, P. (Eds). *Probing Photosynthesis: Mechanisms, Regulation and Adaptation*. London: Taylor and Francis. p. 445–483.

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M. (2004) Analysis of the chlorophyll a fluorescence transient. *In*: Papageorgiou, C.G., Govindjee (Eds). *Chlorophyll fluorescence: a signature of photosynthesis. Advances in photosynthesis and respiration*. Netherlands: Kluwer Academic Publishers. p. 321–362.

Strasser. R.J., Tsimilli-Michael, M. (2001). Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-Test, *Chim. Nouvelle (SRC)*. 75:3321–3326.

Strauss, A. J., Krugera, J. H. J., Strasser, R. J., Van Heerden, P.D.R. (2006) Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll a fluorescence transient O-J-I-P. *Environ Exp Bot*. 56:147–157.

Suvočarev, K., Blanco, O., Faci, J.M., Medina, E.T., Martínez-Cob, A. (2013) Transpiration of table grape (Vitis vinifera L.) trained on an overhead trellis system under netting. *Irrig. Sci.* 31:1289–1302.

Tang, D., Shi, S., Li, D., Hu, C., Liu, Y. (2007) Physiological and biochemical responses of *Scytonema javanicum* (cyanobacterium) to salt stress. *J. Arid. Environ*. 71:312–320.

Tarara, J.M., Peña, J.E.P. (2015) Moderate water stress from regulated deficit irrigation decreases transpiration similarly to net carbon exchange in grapevine canopies. *J Am. Soc. Hortic. Sci*. 140:413–426.

Tcherkez, G., Bligny, R., Gout, E., Mahe, A., Hodges M., Cornic G. (2008) Respiratory metabolism of illuminated leaves depends on CO_2 and O_2 conditions. *P. Natl. Acad. Sci. Usa*. 105:797–802.

Tcherkez, G., Cornic, G., Bligny, R., Gout, E., Ghashghaie, J. (2005) *In vivo* respiratory metabolism of illuminated leaves. *Plant Physiology.* 38:1596–1606.

Tcherkez G., Mahe A., Gauthier P., Mauve C., Gout E., Bligny R., Cornic G., Hodges M. (2009) In folio respiratory fluxomics revealed by ^{13}C isotopic labeling and H/D isotope effects high-light the non-cyclic nature of the tricarboxylic acid ‘cycle’ in illuminated leaves. *Plant Physiol.* 151:620–630.

Tcherkez, G., Mahé, A., Guérard, F., Boex-Fontvieille, E.R.A., Gout, E., Lamothe, M., Barbour, M.M., Bligny, R. (2012) Short-term effects of CO_2 and O_2 on citrate metabolism in illuminated leaves. *Plant Cell Environ*. 35:2208–2220.

Tcherkez, G., Schaufele, R., Nogues, Piel, C., Boom, A., Lanigan, G., Barbaroux, C., Mata, C., Elhani, S., Hemming, D., Maguas, C., Yakir, D., Badeck, F. W., Griffiths, H., Schnyder, H., Ghashghaie, J. (2010). On the $^{13}C/^{12}C$ isotopic signal of day and night respiration at the mesocosm level. *Plant Cell Environ*. 33:900–913.

Thomas, T.R., Matthews, M.A., Shackel, K.A. (2006) Direct in situ measurement of cell turgor in grape (*Vitis vinifera* L.) berries during development and in response to plant water deficits. *Plant Cell Environ*. 29:993–1001.

Thomas, R.B., Strain, B.R. (1991) Root restriction as a factor in photosynthetic acclimation of cotton seedling grown in elevated carbon dioxide. *Plant Physiol.* 96:626–34.

Tomás, M., Medran, H., Pou, A., Escalona, J.M., Martorell, S., Ribas-Carbó, M., Flexas, J. (2012) Water use efficiency in grapevine cultivars grown under controlled conditions: effects of water stress at the leaf and whole plant level. *Aust. J. Grape Wine Res.* 18:164–172.

Tomás, M., Medrano, H., Escalona, J.M., Martorell, S., Pou, A., Ribas-Carbó, M., Flexas, J. (2014) Variability of water use efficiency in grapevines. *Environ. Exp. Bot*. 103:148–157.

Tombesi, S., Nardini, A., Frioni, T., Soccolini, M., Zadra, C., Farinelli, D., Poni, S., Palliotti, A. (2015) Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. *Sci Rep.* 5:1-12.

Tonietto, J., Pereira, G.E. (2012) A concept for the viticulture of “tropical wines”. *IXe International Terroirs Congress.* Dijon/Reims, Burgundy/Champagne, France. p. 2-34.

Tovar-Mendez, A., Miernyk, J.A., Randall, D.D. (2003) Regulation of pyruvate dehydrogenase complex activity in plant cells. *Eur. J. Biochem.* 270:1043–1049.

Tramontini, S., J. Döring, M. Vitali, A. Ferrandino, M. Stoll, and C. Lovisolo. (2014) Soil water-holding capacity mediates hydraulic and hormonal signals of near-isohydric and near-anisohydric *Vitis* cultivars in potted grapevines. *Funct. Plant Biol*. 41:1119–1128.

Tuccio, L., Remorini, D., Pinelli, P., Fierini, E., Tonutti, P., Scalabrelli, G., Agati, G. (2011) Rapid and non-destructive method to assess in the vineyard grape berry anthocyanins under different seasonal and water conditions. *Aust. J. Grape Wine R*es. 17:181–189.

Urban, O. (2003) Physiological impacts of elevated CO_2 concentration ranging from molecular to whole plant responses*. Photosynthetica*. 41:9–20.

Vandeleur, R.K., Mayo, G., Shelden, M.C., Gilliham, M., Kaiser, B.N., Tyerman, S.D. (2009) The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. *Plant Physiol*. 149(1):445–460.

Vaz, M., Coelho, R., Rato, A., Samara-Lima, R., Silva, L.L., Campostrini, E., Mota, J.B. (2016) Adaptive strategies of two Mediterranean grapevines varieties (*Aragonez syn. Tempranillo* and *Trincadeira*) face drought: physiological and structural responses. *Theor. Exp. Plant Physiol*. 28(2):205-220.

Vaz, J., Sharma, P.K. (2011) Relationship between xanthophy cycle and non-photochemical quenching in rice (*Oryza sativa* L.) plants in response to light stress. *Indian J. Exp. Bot*. 49:60–67.

Villar, R., Held, A. A., Merino, J. (1994) Comparison of methods to estimate dark respiration in the light in leaves of two woody species. *Plant Physiol*. 105:167-172.

Vincent, D., Ergül, A., Bohlman, M.C., Tattersall, E.A.R., Tillett, R.L., Wheatley, M.D., Woolsey, R., Quilici, D.R., Joets, J., Schlauch, K., Schooley, D.A., Cushman, J.C. and Cramer, G.R. (2007) Proteomic analysis reveals differences between *Vitis vinifera* L. cv. Chardonnay and cv. Cabernet Sauvignon and their responses to water deficit and salinity. *J Exp Bot*. 58(7):1873-92.

Vitali, M., Cochard, H., Gambino, G., Ponomarenko, A., Perrone, I., Lovisol, C. (2016) VvPIP2;4N aquaporin involvement in controlling leaf hydraulic capacitance and resistance in grapevine. *Physiol. Plant*. 158:284–296.

Wang, X., Lewis, J.D., Tissue, D.T., Seemann, J.R., Griffin, K.L. (2001) Effects of elevated atmospheric CO_2 concentration on leaf dark respiration of *Xanthium strumarium* in light and in darkness. *Proc. Natl. Acad. Sci. USA.* 98:2479—2484.

Wang, Y., Noguchi, K., Ono, N., Inoue, S.I., Terashima, I., Kinoshita T. (2014) Overexpression of plasma membrane H^+-ATPase in guard cells promotes light-induced stomatal opening and enhances plant growth. *P. Ntnl. Acad. Sci. USA*. 111:533–538.

Wang, Z., Deloire, A., Carbonneau, A., Federdpiel, B., Lopez, F. (2003) An *in vivo* experimental system to study sugar phloem unloading in ripening grape berries during water deficiency stress. *Ann. Bot*. 92:523–528.

Wilhelm, C., Selmar, D. (2011) Energy dissipation is an essential mechanism to sustain the viability of plants: The physiological limits of improved photosynthesis. *J. Plant Physiol*. 168:79-87.

Winkler, A.J., Cook, J.A., Kliewer, W.M., Lider, L.A. (1974). *General viticulture*. 2ª. Berkley: University of California, 710 p.

Wullschleger, S.D., Tschaplinski, T.J., Norby, R.J. (2002) Plant water relations at elevated CO_2 – implications forwater-limited environment. *Plant Cell Environ.* 25:319–331.

Yuan, J., Xu, M., Duan, W., Fan, P., Li, S. (2013) Effects of whole-root and half-root water stress on gas exchange and chlorophyll fluorescence parameters in apple trees. *J. Am. Soc. Hortic. Sci*. 138:395–402.

Zabadal, T.J., Bartsch, J;A., Blanpied, G.D., Dennehy, T.J., Pearson, R.C., Pool, R.M., Reisch, B.I. (1988). *Concord table grapes: a manual for growers*. Genera, NY: Communications services Agricultural Experiment Station. 78 p.

Zhang, X., Wollenweber, B., Jiang, D., Liu, F., Zhao, J. (2008) Water deficits and heat shock effects on photosynthesis of a transgenic *Arabidopsis thaliana* constitutively expressing ABP9, a bZIP transcription factor. *J. Exp. Bot*. 59:839–848.

Zhang, Y., Oren, R., Kang, S. (2012) Spatiotemporal variation of crown-scale stomatal conductance in an arid *Vitis vinifera* L. cv. Merlot vineyard: direct effects of hydraulic proper- ties and indirect effects of canopy leaf area. *Tree Physiol*. 32:262–279.

Zhou, Y., Lau, H.M., Zhang, J. (2007) Inhibition of photosynthesis and energy dissipation induced by water and high light in rice. *J. Exp. Bot.* 58:1207–1217.

Zlatev, Z. (2009) Drought-induced changes in chlorophyll fluorescence of young wheat plant. *Biotechnology*. 23:437–441.

Zong, Y.Z., Wang, W.F., Xue, Q.W., Shangguan, Z.P. (2014) Interactive effects of elevated CO2 and drought on photosynthetic capacity and PSII performance in maize. *Photosynthetica.* 52 (1):63-70.

Zufferey, V., Murisier, F., Schultz, H.R. (2000) A model analysis of the photosynthetic response of *Vitis vinifera* L. cvs Riesling and Chasselas leaves in the field: Interactions of age, light and temperature. *Vitis*. 39:19–26.