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Water to Feed the World

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Preface

This book contains the most relevant communications presented at the *XII Portuguese-Spanish Symposium on Plant Water Relations* which was held at the University of Évora, Portugal, organized by the Portuguese Society of Plant Physiology, the Spanish Society of Plant Physiology and the "Instituto de Ciências Agrárias e Ambientais Mediterrânicas" (ICAAM - University of Évora).

The motto of the Symposium — Water to Feed the World — shows the clear purpose of investigators and academics in the area of plant physiology of giving-back their knowledge and work to the society who supports them, helping to promote a more efficient use of water in agriculture and thus diminishing environmental disturbances.

The papers in this book of proceedings were reviewed by a team of investigators. The editors want to thank to the authors and to the referees.

Renato Coelho

Margarida Vaz

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Water Use in *Montado* Ecosystems

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ABSTRACT

Montado ecosystems are defined and configured by the climate, rapidly changing, and are highly dependent on human management. Over the last two decades our team has been undergoing research on the processes controlling vegetation water use and on the water-use strategies of *montado* vegetation to cope with seasonal drought. The main tree strategies rely on maximizing root water uptake and controlling excessive transpiration losses through tight stomatal regulation. These processes smartly operate on the two exchange surfaces: roots for water uptake and leaves for water release. The dimorphic root system, with superficial roots linked to sinker roots, maximized the exploitation of local resources. Whenever possible, the deep roots accessed the groundwater table. Under these conditions, trees used surface water during the wet season (autumn, winter and spring) and groundwater in summer when the surface soil dried out. The roots also performed hydraulic lift in summer. When root access to groundwater was unrestricted, trees did not show signs of summer water stress (high predawn leaf water potentials), contrary to what occurred when the access was somehow limited. Even in well-watered sites, when the atmospheric demand exceeds tree maximum water uptake capacity (hydraulically limited), stomatal control prevents leaf water potential from falling below cavitation thresholds. When trees coexisted with pasture, ecosystem summer transpiration equaled tree transpiration, as pasture, coupled with near surface soil moisture, stopped transpiring in early summer.

INTRODUCTION

Montado ecosystems are characterized by a two-layered structure, with a sparse tree cover dominated by Mediterranean evergreen oaks - *Quercus suber* and *Quercus ilex* - and an understory of crops, pasture or shrubs. They are defined and configured by the climate, rapidly changing, and are highly dependent on human management. Over the last two decades our team has been undergoing research on the processes controlling vegetation water use and on the water-use strategies of *montado* vegetation (mainly the tree component) to cope with seasonal drought, under different climatic and edaphic conditions. We report on the main achievements on the understanding of ecosystem water use relevant for *montado* management.

METHODS

Study sites and mesurements

The work was carried out at three sites: Mitra I (38º32'N, 8º01'W; *Q. ilex*); Mitra II (38º32'N, 8º00'W; *Q. ilex* and *Q. suber*) and Lezírias (38°50'N; 8°49'W; *Q. suber*). Ecosystem water use was analyzed based on measurements of: meteorological variables; soil water dynamics; water table fluctuations; sap flow (stem and roots); leaf and xylem water potentials; stable isotopic composition in xylem water, soil water and groundwater; xylem properties related to tree hydraulic architecture (vulnerability to drought-induced embolism, hydraulic connectivity from roots to branches, anatomical traits); and rooting patterns.

RESULTS

Tree transpiration patterns and stomatal behavior

The relationships between daily tree transpiration (relative to maximum, estimated from stem sap flow) and *D* (vapour pressure deficit) (Fig. 1) showed a unique maximum plateau, irrespective of season or year, at Mitra I and Lezírias (Fig. 1a), but different seasonal maximum plateaus at Mitra II (Fig. 1b), the lowest one usually observed during summer (August).

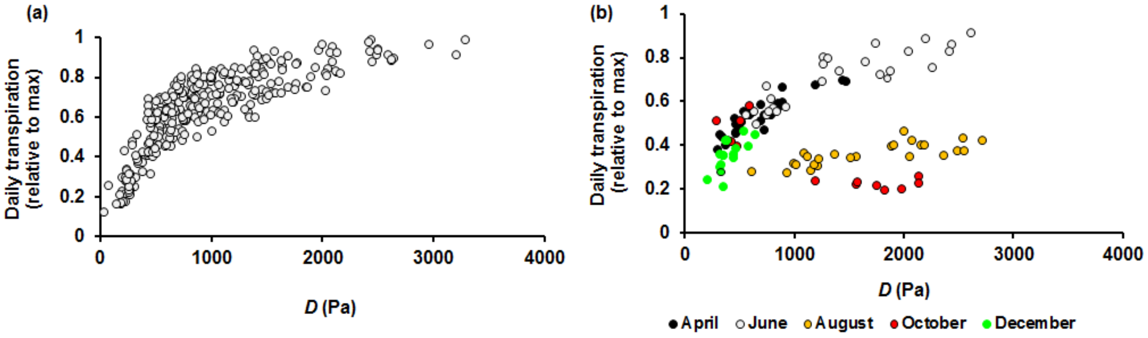

Fig. 1. Relationships between daily tree transpiration (relative to maximum) and *D* observed at Mitra and Lezírias (a) and Mitra II (b).

In very dry years, when the onset of the autumn rains was delayed, the period of water stress lasted longer postponing the occurrence of the minimum transpiration rates (October). The observed transpiration patterns reflected the stomatal behavior (Fig. 2) evidenced by the relationships between midday canopy conductance (g_{cm}, estimated from the Jarvis and McNaughton approach (1986)) and *D*. A unique, seasonally invariable, minimum g_{cm} plateau was observed at Mitra I and Lezírias (Fig. 2a). At Mitra II, g_{cm} decreased seasonally, reaching an absolute minimum plateau during summer (higher partial stomatal closure) or early autumn, depending on the rainfall pattern (Fig. 2b).

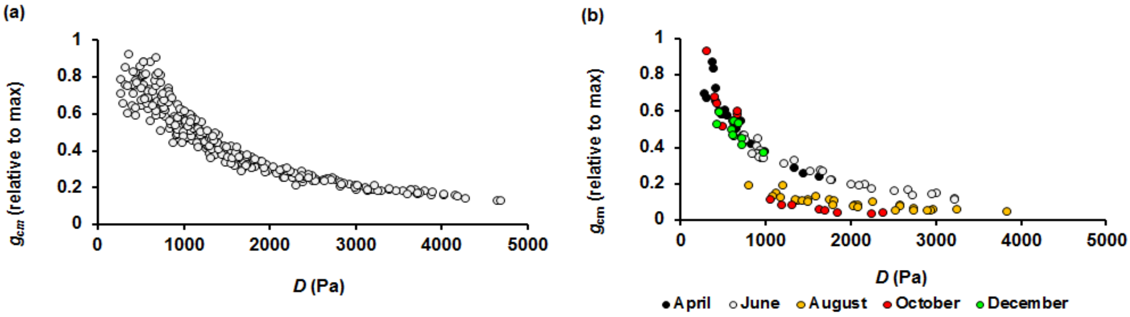

Fig 2 . Relationships between midday canopy conductance (g_{cm}, relative to maximum) and *D* at Mitra I and Lezírias (a) and Mitra II (b) (adapted from David *et al*. (2004) and Paço *et al*. (2009)).

Predawn leaf water potentials were almost invariable during the year at Mitra I and Lezírias, never decreasing below -0.8 MPa and -0.5 MPa, respectively. At Mitra II values decreased to -1.7 MPa in *Q. ilex* and -2.3 MPa in *Q. sube*r at the end of summer (David *et al*. 2004; David *et al*. 2007; Pinto *et al*. 2012). Trees remained well watered at Mitra I and Lezírias but suffered some degree of summer water stress at Mitra II. Due to the low soil water storage capacity of soils at all sites, the maintenance of transpiration in the dry summer periods could only be due to root access to deep water sources, namely groundwater.

Sources of water uptake

The comparison between the isotopic composition (δD and δ^{18}O) of twig xylem water, soil water and groundwater, during summer, showed that the isotopic signature of twig xylem water was similar to that of groundwater and differed from that of soil water (David *et al*. 2007, 2013), supporting the hypothesis of a direct access of tree roots to groundwater when the surface soil dries out. The lower root water uptake at Mitra II, reflected in lower predawn leaf water potentials in summer, might be due to the harder nature of rock (granite) in relation to Mitra I (fractured gneiss) and Lezírias (sand sediment). The water table depth at the end of summer was at -13 m depth at Mitra I (*Q. ilex*), -5 m depth at Mitra II and -4.5 m depth at Lezírias (*Q. suber*).

Tree root system

The in-depth analysis of the rooting pattern and root functioning was only possible at the Lezírias site (*Q. suber*), due to the soil type (Haplic Arenosol). The dimorphic root system evidenced a dense and extensive network of superficial roots linked to sinkers and a taproot diverting into tangles of fine filaments, reaching groundwater. Some of the deep roots survived temporary waterlogging due to the presence of parenchyma aerenchyma.

Root sap flow measurements, through the Heat Field Deformation method (Nadezhdina *et al.* 2008), showed the occurrence of roots connected to soil and groundwater. From root sap flow data, tree stem sap flow was modeled with a high accuracy (David *et al*. 2013). Based on this model we estimated the monthly contribution of soil and groundwater to stem sap flow and quantified the magnitude of hydraulic lift and hydraulic descent (Fig. 3).

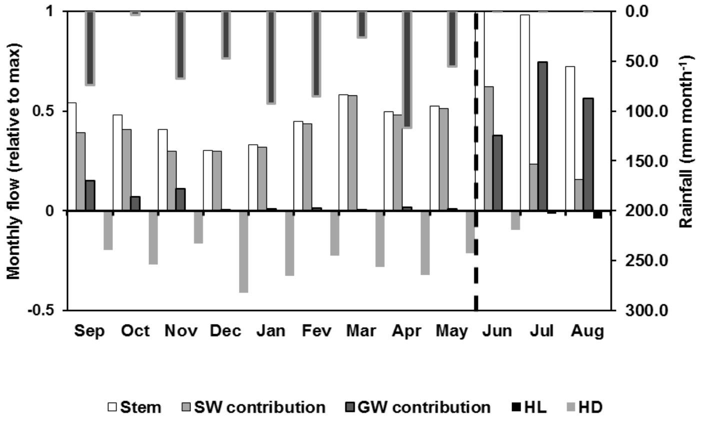

Fig. 3. Monthly flow (relative to maximum) of soil water and groundwater contributions to stem sap flow and relative magnitude of hydraulic lift and hydraulic descent (adapted from David *et al*. (2013)).

Data showed that the trees used surface soil water during the wet season (autumn, winter and spring) and groundwater in summer when the surface soil dried out. The roots performed hydraulic lift in summer and hydraulic descent in the rainy winter.

Overstory and understory transpiration - trees and pasture

The partitioning of ecosystem transpiration (rainless days, measured by the eddy covariance) into its components - trees (measured by sap flow) and pasture (the difference) was analyzed at Mitra II during an extremely dry year (2005) (Fig. 4). Pasture transpiration ceased in summer, when surface soil dried, whereas tree transpiration was maintained throughout summer. On an annual basis, although pasture represented ca. 80% of soil cover and trees only 20%, tree transpiration was about 56% of total ecosystem transpiration and pasture transpiration only 44%. Ecosystem summer transpiration was only due to the tree component (Paço *et al*. 2009).

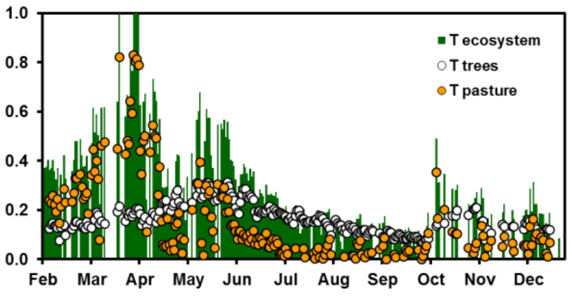

Fig. 4. Partitioning of ecosystem transpiration (relative to maximum) into its components at Mitra II (adapted from Paço *et al*. (2009)).

DISCUSSION AND CONCLUSIONS

The study of *montado* water use at three sites, under different climatic and edaphic conditions, showed that mature evergreen oaks, *Q.ilex* and *Q. suber*, cope with summer drought relying on: (a) root access to deep water sources to maximize water uptake; (b) efficient control (tight stomatal regulation) of water losses by transpiration. These processes smartly operate on the two exchange surfaces: roots for water uptake and leaves for water release. The xylem structure that transports water from roots to leaves, also played a role in the functioning and survival of these oaks, determining the hydraulic conductance, embolism resistance and the degree of embolism spread (David *et al.* 2012; Pinto *et al*. 2012). The xylem was found to be rather resistant to drought-induced embolism (when compared to temperate oaks) and the main water conducting pathway (stem and taproot) showed a high degree of hydraulic modularity (sectoriality), allowing damages to be confined to localized sectors.

The dimorphic root system of trees, with superficial roots linked to sinker roots, maximized the exploitation of environmental resources. Topsoil water was used most of the year and groundwater together with hydraulic lift (also facilitating nutrient absorption from upper soil) in the dry summer. When root access to groundwater was unrestricted, trees did not show signs of summer water stress (high predawn leaf water potentials), contrary to what occurred when the access was somehow limited. Even in well-watered sites stomatal control played an important role. During summer, under high vapour pressure deficit (*D*), the atmospheric demand exceeds tree maximum water uptake capacity (hydraulically limited). Stomatal closure, by reducing transpiration, prevented leaf water potential from falling below the critical xylem embolism thresholds (David *et al*. 2007; Pinto *et al*. 2013). Under limited or nonexistent access to groundwater, the maximum plateau of the relationship between transpiration and vapour pressure deficit decreased, as well as the g_{cm} values for the same *D*, as summer drought progressed due to tighter stomatal closure. Even at Mitra II, where a mild water stress occurred, root water uptake and stomatal control were efficient, maintaining comfortable safety margins above the xylem water potential causing 50% loss in hydraulic conductivity, both in *Q. ilex* and *Q. suber* (Pinto *et al*. 2012). However, in poorer site conditions and under severe drought the hydraulic safety limits might be surpassed predisposing these species to decline.

When trees coexisted with pasture, ecosystem summer transpiration equaled tree transpiration as pasture, mainly composed of annual herbs, was coupled with near surface soil moisture (dependent on the occurrence of rainfall) and stopped transpiring in early summer.

The expected increase in the length and severity of seasonal summer drought may result, in the short-term, in a decrease in the uptake of soil water and an increased reliance on groundwater uptake. In the long-term, the lowering of the water table level might threaten these ecosystems and conflicts may arise between their sustainability and other water uses.

Silvicultural treatments may alter leaf area, canopy conductance, water potential gradients between soil and crowns and the amount of water transpired; also, ploughing may damage superficial roots, connected to sinkers, and decouple trees from the sources of water and nutrient uptake (soil and groundwater).

ACKNOWLEDGEMENTS

This review is a summary of works already published (reference list) in collaboration with a large team to whom the authors wish to express their gratitude.

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Genetic Control of Functional Traits Related to Photosynthesis and Water Use Efficiency in Pinus Pinaster Ait. Drought Response: Integration of Genome Annotation, Allele Association and QTL Detection for Candidate Gene Identification.

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ABSTRACT

Understanding molecular mechanisms that control photosynthesis and water use efficiency in response to drought is crucial for plant species from dry areas. Complex functional trait dissection can be achieved through the analysis of Quantitative Trait Loci (QTL) mapping. QTL mapping allows to locate genomic regions underpinning the expression of quantitative traits and estimate their effect and interactions. This study aimed to identify QTL for different functional traits, including water use efficiency, in a Mediterranean conifer (*Pinus pinaster* Ait.) at three points during a water stress cycle to test their specificity or stability in response to drought. High density genetic maps were used in the detection of QTL for the analyzed traits. A total of 28 significant and 27 suggestive QTL were found. QTL detected for photochemical traits accounted for the higher percentage of phenotypic variance. Functional annotation of mapped genes was performed through the analysis of sequence homology with genes from other species with known function. Functional annotation of genes within the QTL suggested 58 candidate genes for the analyzed traits. Besides, allele association analysis in selected candidate genes showed three SNPs located in a MYB transcription factor that were significantly associated with efficiency of energy capture by open PSII reaction centers and specific leaf area. The integration of QTL mapping of functional traits, genome annotation and allele association yielded several candidate genes involved with molecular control of photosynthesis and water use efficiency in response to drought in a conifer species. The results obtained highlight the importance of maintaining the integrity of the photochemical machinery in *P. pinaster* drought response.

INTRODUCTION

Mediterranean species are particularly threatened by drought, especially in the context of climatic change predictions (Sarris et al., 2007). Unravelling molecular mechanisms that control functional traits, such as those related to carbon fixing or water loss control in response to drought, is especially relevant for these species in view of its implication in survival, growth and biomass production. Genetic control of these complex functional traits can be studied through the analysis of Quantitative Trait Loci (QTL) mapping. Through QTL mapping it is possible to locate in the genome those regions controlling the expression of quantitative traits, estimate their effect and interactions. QTL studies involve development of a segregating progeny for target traits, phenotypic and molecular characterization of the progeny and construction of genetic maps (Paterson et al., 1988). The combination of high density gene based maps with functional annotation allows to identify positional candidate genes within QTL intervals (Sehgal et al., 2012).

QTL studies on functional drought response have been largely implemented for non-forest model species (e.g. Whan et al., 2010; Yin et al., 2010). However, fewer studies have been performed for forest tree species (Thavamanikumar et al., 2013). The main objective of this work was to unravel the genetic basis of different functional parameters related to carbon uptake and water use efficiency in response to drought through QTL mapping, for *Pinus pinaster*, an important Mediterranean conifer.

MATERIAL AND METHODS

162 seedlings from a F_1 full-sib family of *P. pinaster* obtained from a controlled cross between a male parent (Oria6) from Oria, a natural population from South-East Spain and a female parent (Gal1056) from a breeding program established in Pontevedra, North-West Spain, were vegetatively replicated and established in an incomplete block design in a greenhouse. Phenotypic evaluation of gas exchange, chlorophyll fluorescence, Specific Leaf Area (SLA) and water use efficiency (WUE_i and $\delta^{13}C$) was conducted on the 103 clones for which at least 3 replicates were obtained. Three time-points of measurement were carried out: 1st time-point, plants were watered close to full holding capacity; 2nd time-point of measurement, plants were left 7 days without watering; 3rd time-point of measurements was carried out after plants have been 14 days without watering (de Miguel et al., 2012).

The mapping progeny was genotyped with nuclear microsatellites (simple sequence repeats, nSSR), selective amplification of microsatellite polymorphic loci (SAMPL) and single nucleotide polymorphism (SNP) markers, for the construction of two genetic maps, one for each progenitor (Gal1056 and Oria6) and the consensus map for the cross (GxO). Linkage analyses and map estimations were performed using a recombination fraction < 0.35, a LOD > 3 and Kosambi mapping function as mapping parameters. QTL detection was carried out using the regression algorithm. Interval mapping was applied followed by multiple QTL mapping (MQM) when more than one QTL was found for a trait. The thresholds for QTL significance were determined using a chromosome and genome wide permutation test with 10,000 iterations. For those genes within the significant QTL confidence intervals (± 2 LOD), functional annotations were queried to identify functional relationship between the positional candidate genes and each analyzed trait. Association between phenotypes and alleles at candidate loci was further studied by ANOVA using the traits as dependent variables and the SNP genotypes as factors.

RESULTS AND DISCUSSION

For all the analyzed traits several QTL were identified with moderate effects rather than a single or few QTL with large effect (fig. 1, obtained from de Miguel et al., 2014), as expected for complex functional traits.

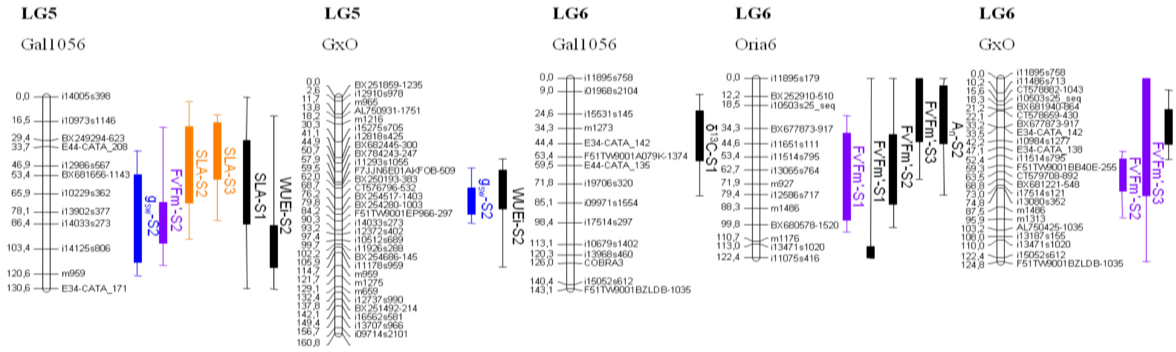

Fig. 1. Representation of QTL identified on the parental (Gal1056 and Oria6) and consensus (GxO) linkage maps (LGs 5 and 6). Confidence intervals are indicated by squares (±1LOD) and lines (±2LOD). Colored QTL are the significant QTL (significant at 99% at chromosome level or 95% at genome wide level) and black QTL are the suggestive QTL (significant at 95% confidence at chromosome level). A_n= net photosynthesis; g_{sw} = stomatal conductance to water vapour; WUE_i = Water Use Efficiency; $\delta^{13}C$ = isotopic composition of ^{13}C; SLA = Specific Leaf Area; $F_v'F_m'$= maximum efficiency of PSII under light conditions; Φ_{PSII} = quantum yield. S1, S2 and S3 stand for 1st, 2nd and 3rd time-point of measurement, respectively.

For gas exchange parameters, percentage of observed phenotypic variance explained when taking into account all significant QTL detected in a single trait at each sampling time-point of measurement, was in average 20%. However for maximum efficiency of PSII under light conditions ($F_v'F_m'$) up to 44 % of the observed phenotypic variance was explained, which points towards a tight genetic control of photochemical traits, as previously described in crop species (Guo et al., 2008; Yin et al., 2010). Suggestive QTL for water use efficiency estimated from different approaches have been reported. For $\delta^{13}C$ only one QTL was detected in LG 6 (fig. 1), in agreement with Brendel et al. (2002) who also found a QTL in the same region of LG 6 for *P. pinaster*. This co-localization between QTL for $\delta^{13}C$ identified in two genetically unrelated progenies from wide geographic origins and growing under different environmental conditions supports its stability. In general, most of the detected QTL were environment-specific, suggesting that genes are differentially activated during maritime pine drought response (Watkinson et al., 2003). Nevertheless, several QTL for photochemical traits and SLA were less sensitive to environmental conditions and maintained the same location with drought imposition, confirming the stability of these QTL across different levels of water stress. Several clusters of QTL were identified, which could be related with pleiotropic effects. QTL co-localization of SLA with WUE_i and $F_v'F_m'$ was found in several linkage groups (fig.1). The coincidence of QTL for two traits is strong evidence that they could be functionally related (Thumma et al., 2001) pointing towards a strong inter-relationship between SLA, WUE_i and $F_v'F_m'$.

Genes co-localizing with QTL, with known function in other species, potentially involved in processes related with this QTL, were selected as candidate genes. A total of 58 candidate genes involved in different mechanisms of drought tolerance were selected. Allele association analysis in selected candidate genes showed a significant association between three SNPs located in a MYB transcription factor with $F_v'F_m'$ and SLA.

CONCLUSIONS

Several genomic regions implicated in the genetic control of drought resistance traits in *P. pinaster* have been identified. The identification of candidate genes leads this project a step beyond the simple detection of QTL. Nonetheless, further association studies with proposed candidate genes are needed in order to validate detected SNP marker-trait associations.

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Effect of Deficit Irrigation and Elaboration Process of Spanish-Style Green Table Olives on Phytoprostanes Content in Manzanilla de Sevilla Olive Flesh

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ABSTRACT

Manzanilla de Sevilla olive trees were summited to three different irrigation treatments. Control (T0) trees were over-irrigated (125 % ETc) and T1 and T2 trees were submitted to regulated deficit irrigation (RDI) according to trunk diameter fluctuations data with a pronounced irrigation water restriction during pit hardening. Seasonal water amounts received by each treatment, without considering precipitation, were 412, 130 and 111 mm in 2012 and 369, 207 and 106 mm in 2013 for T0, T1 and T2 treatments, respectively. Olive yield and fruit size were not affected by deficit irrigation during olive pit hardening. However, this phenological period was critical for olive flesh composition in just harvested and processed olives. Stress integral was better indicator than minimum midday stem water potential for predicting the phytoprostanes (PhytoPs) content, pointing to the idea that the increase in PhytoPs depends in a greater extension of the length of water stress instead of maximum stress achieved. The results suggested that the olive fruit processing enhance the accumulation of total PhytoPs, but decrease the number of these chemical compounds. Consequently, olive table tree culture under deficit irrigation conditions during pit hardening and the processing of its fruits to obtain Spanish-style olives can be considered as complementary actions to enhancing the PhytoP content and hence their potential beneficial effects on human health.

INTRODUCTION

PhytoPs are prostaglandin analogs which belong to a novel family of plant effectors. Several classes of these bioactive lipids are constitutively present in higher plants and are formed from α-linolenic acid via a non enzymatic free radical-catalyzed pathway. It is known that these bioactive compounds act as endogenous mediators capable of protecting cells from damage under various conditions related to oxidative stress and they play a role in regulation of immune function (Minghetti et al., 2014). According to Durand et al. (2009) PhytoPs may contribute to the beneficial effects of the Mediterranean diet, since they display potent anti-inflammatory and apoptosis-inducing activities similar to other prostanoids.

However, to the best of our knowledge, neither exist previous information on the PhytoPs present in olive fruit flesh, nor on the effect of the deficit irrigation during pit hardening on these compounds. Moreover, there is not information on the effect of the deficit irrigation on the PhytoPs present in olive flesh after the Spanish-style green table olives process. For these reasons, the present study aimed to look at the effect of different regulated deficit irrigation treatments and the elaboration process of Manzanilla de Sevilla Spanish style green table olives on the PhytoPs contents in the fruit flesh.

MATERIALS AND METHODS

The plant material consisted of adult olive trees (Olea europaea L., cv Manzanilla de Sevilla) spaced at 7 m x 5 m. Crop water requirements were estimated as described by Fernández et al. (2006) (ETc = ETo x Kc x Kr), without considering precipitation (73 and 50 mm in 2012 and 2013, respectively). During the 2012 and 2013 growing seasons, plants were irrigated using a drip irrigation system. Control treatment (T0) trees were over-irrigated (125% ETc) and T1 and T2 trees were submitted to regulated deficit irrigation (RDI) according to trunk diameter fluctuations data with a pronounced irrigation water restriction during pit hardening (Moriana et al., 2013).

Trunk diameter fluctuations were measured in four trees per treatment, using LVDT transducers (Moriana et al., 2013). Midday stem water potential (Ψstem) was measured in fully developed leaves near to the main trunk from two trees of each replicate (Galindo et al., 2014). The water stress integral (SI) was calculated using the expression

$$\sum\left(\left(\overline{\square}\text{stem} - (-1.4)\right) \times \text{n}\right|\,|$$

where $\overline{(}$stem is the average Ψstem values for any interval and n is the number of the days in the interval. Olive fleshes were milled with a solution of MeOH and butylated hydroxyanisole (*BHA*). PhytoPs in olive flesh and olive covering liquid were extracted using a dilution followed by a solid-phase extraction (SPE) and analyzed by UHPLC–QqQ-MS/MS.

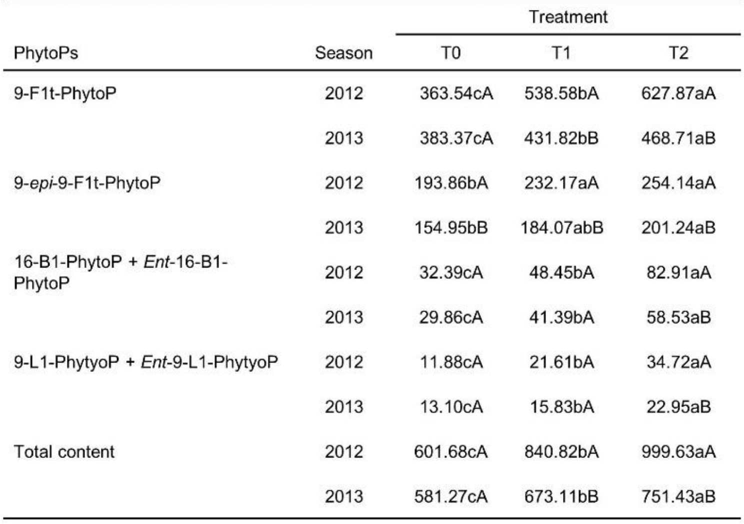
RESULTS AND DISCUSSION

Both years SI values showed a significant tendency to increase as a result of decreasing irrigation volumes (data not shown). Considering that olive fruit size is a key feature for table olives, the fact that yield and average olive fruit weight were not affected by deficit irrigation treatments (data not shown) is of top importance because can maximize farmer incomes.

Taking into account that the only requirement for PhytoP formation are the presence of linolenic acid and molecular oxygen, suggesting that PhytoP formation not necessarily requires metabolic activity of living cells (Loeffler et al., 2005), the fact that 9-F1t-PhytoP and 9-epi-9-F1t-PhytoP contents in the flesh of treated olive fruits increased respect to that observed in non-treated fruits (Tables 1 and 2) could be due to the olive processing conditions, which could favour the formation of these PhytoPs.

The fact that 16-B1-PhytoP + Ent-16-B1-PhytoP and 9-L1-PhytyoP + Ent-9-L1-PhytyoP were not detected in the flesh of treated olives (Table 2) and their covering liquid (Table 4) suggested that the absence of these PhytoPs was not due to an extraction process effect during processing and/or storage. On the contrary, considering that these PhytoP are terminal compounds in the non enzymatic lipid peroxidation from α-linolenic acid (Thoma et al., 2003), the absence of these PhytoPs suggests that 16-B1-PhytoP + Ent-16-B1-PhytoP and 9-L1-PhytyoP + Ent-9-L1-PhytyoP were submitted to degradation reactions during the olive processing (Tables 1, 2 and 4).

Table 1. Effect of irrigation treatments (T0, T1 and T2) on phytoprostanes (PhytoPs) content (ng/100g FW) in the flesh of just harvested Manzanilla de Sevilla table olive fruits in 2012 and 2013 seasons. Means within a row for each PhytoP and season followed by different small letter, and within a column for each PhytoP and treatment followed by different capital letter are significantly different at P = 0.05 by Tukey's test.



		Treatment		
PhytoPs	Season	T0	T1	T2
9-F1t-PhytoP	2012	363.54cA	538.58bA	627.87aA
	2013	383.37cA	431.82bB	468.71aB
9-*epi*-9-F1t-PhytoP	2012	193.86bA	232.17aA	254.14aA
	2013	154.95bB	184.07abB	201.24aB
16-B1-PhytoP + *Ent*-16-B1-PhytoP	2012	32.39cA	48.45bA	82.91aA
	2013	29.86cA	41.39bA	58.53aB
9-L1-PhytyoP + *Ent*-9-L1-PhytyoP	2012	11.88cA	21.61bA	34.72aA
	2013	13.10cA	15.83bA	22.95aB
Total content	2012	601.68cA	840.82bA	999.63aA
	2013	581.27cA	673.11bB	751.43aB

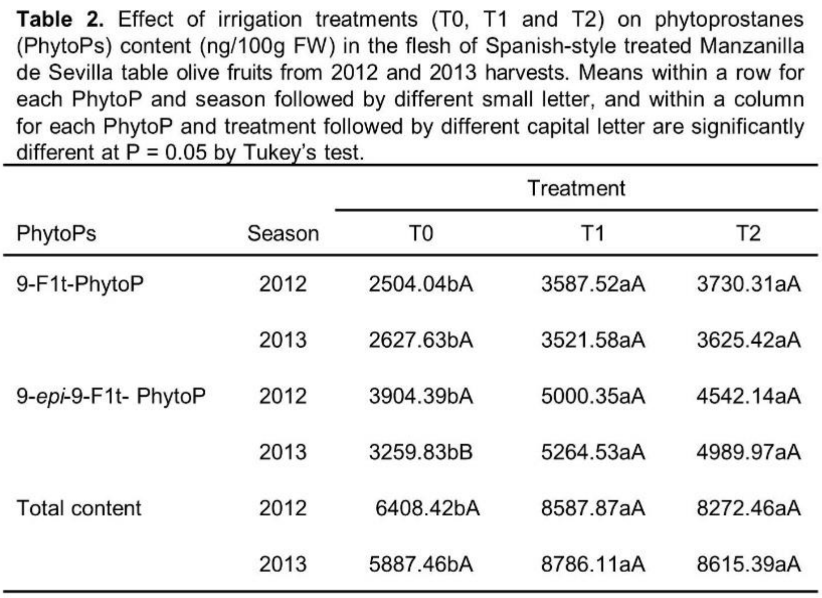


Table 2. Effect of irrigation treatments (T0, T1 and T2) on phytoprostanes (PhytoPs) content (ng/100g FW) in the flesh of Spanish-style treated Manzanilla de Sevilla table olive fruits from 2012 and 2013 harvests. Means within a row for each PhytoP and season followed by different small letter, and within a column for each PhytoP and treatment followed by different capital letter are significantly different at P = 0.05 by Tukey's test.

		Treatment		
PhytoPs	Season	T0	T1	T2
9-F1t-PhytoP	2012	2504.04bA	3587.52aA	3730.31aA
	2013	2627.63bA	3521.58aA	3625.42aA
9-*epi*-9-F1t- PhytoP	2012	3904.39bA	5000.35aA	4542.14aA
	2013	3259.83bB	5264.53aA	4989.97aA
Total content	2012	6408.42bA	8587.87aA	8272.46aA
	2013	5887.46bA	8786.11aA	8615.39aA

The increase of PhytoPs content by water deficit effect (Tables 1, 2 and 3) could be related to the enhance of reactive oxygen species (ROS) production under drought stress which induce the formation of an array of lipid peroxidation products, including structural congeners of jasmonates, the PhytoPs (Thoma et al., 2003). As a consequence, the phase II of fruit growth phenological period can be non-critical considering fruit yield or fruit size (Moriana et al., 2013) but is clearly critical for PhytoPs content in treated and non-treated olive fleshes (Tables 1 and 2). In non-treated olive flesh, the fact that the first-order linear relations between total PhytoPs and each PhytoP content and SI values in all cases showed significant coefficients of determination and higher than those obtained with min Ψstem (Table 3) indicated that SI is a better predictor for PhytoPs content and that the length of water stress instead of maximum stress is a key factor in the content of these compound.

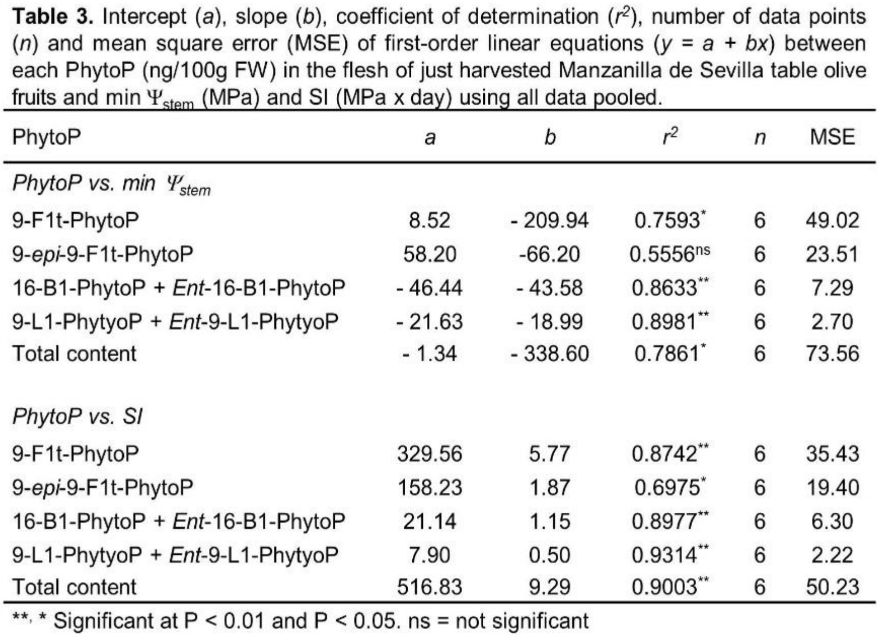


Table 3. Intercept (*a*), slope (*b*), coefficient of determination (r^2), number of data points (*n*) and mean square error (MSE) of first-order linear equations ($y = a + bx$) between each PhytoP (ng/100g FW) in the flesh of just harvested Manzanilla de Sevilla table olive fruits and min Ψ_{stem} (MPa) and SI (MPa x day) using all data pooled.

PhytoP	*a*	*b*	r^2	*n*	MSE
PhytoP vs. min $\mathit{\Psi}_{stem}$					
9-F1t-PhytoP	8.52	- 209.94	0.7593^{*}	6	49.02
9-*epi*-9-F1t-PhytoP	58.20	-66.20	0.5556^{ns}	6	23.51
16-B1-PhytoP + *Ent*-16-B1-PhytoP	- 46.44	- 43.58	0.8633^{**}	6	7.29
9-L1-PhytyoP + *Ent*-9-L1-PhytyoP	- 21.63	- 18.99	0.8981^{**}	6	2.70
Total content	- 1.34	- 338.60	0.7861^{*}	6	73.56
PhytoP vs. SI					
9-F1t-PhytoP	329.56	5.77	0.8742^{**}	6	35.43
9-*epi*-9-F1t-PhytoP	158.23	1.87	0.6975^{*}	6	19.40
16-B1-PhytoP + *Ent*-16-B1-PhytoP	21.14	1.15	0.8977^{**}	6	6.30
9-L1-PhytyoP + *Ent*-9-L1-PhytyoP	7.90	0.50	0.9314^{**}	6	2.22
Total content	516.83	9.29	0.9003^{**}	6	50.23

$^{**, *}$ Significant at $P < 0.01$ and $P < 0.05$. ns = not significant

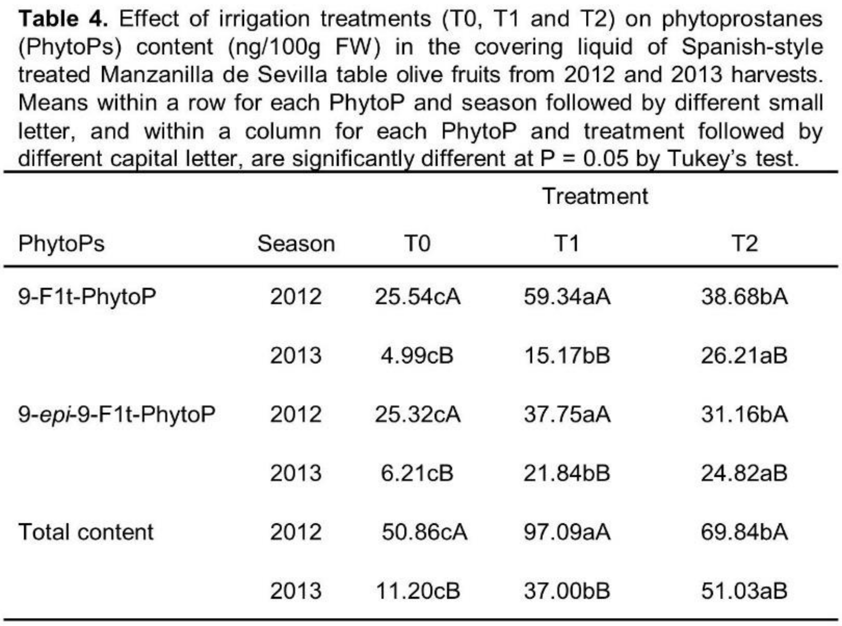


Table 4. Effect of irrigation treatments (T0, T1 and T2) on phytoprostanes (PhytoPs) content (ng/100g FW) in the covering liquid of Spanish-style treated Manzanilla de Sevilla table olive fruits from 2012 and 2013 harvests. Means within a row for each PhytoP and season followed by different small letter, and within a column for each PhytoP and treatment followed by different capital letter, are significantly different at P = 0.05 by Tukey's test.

		Treatment		
PhytoPs	Season	T0	T1	T2
9-F1t-PhytoP	2012	25.54cA	59.34aA	38.68bA
	2013	4.99cB	15.17bB	26.21aB
9-*epi*-9-F1t-PhytoP	2012	25.32cA	37.75aA	31.16bA
	2013	6.21cB	21.84bB	24.82aB
Total content	2012	50.86cA	97.09aA	69.84bA
	2013	11.20cB	37.00bB	51.03aB

Consequently, table olive tree culture under RDI conditions and the processing of its fruits to obtain Spanish-style olives can be considered as complementary actions to enhancing the PhytoP content and hence their potential beneficial effects on human health.

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Differences on the Decline of Leaf Hydraulic Conductance with Dehydration in Olive and Almond: Effects on Stomatal Conductance Regulation and Methodological Considerations.

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ABSTRACT

Leaf hydraulic conductance (K_{leaf}) may be important in regulating stomatal conductance (g_{s}) as it is a major resistance component of the plant hydraulic system, but their relation is still poorly understood. New approaches providing fast K_{leaf} measurements are available and would be really helpful, but they still present some uncertainties. We conducted an experiment to quantify the dehydration response of K_{leaf} in olive and almond young trees. Data obtained with two methods were compared and the effect of air entry into xylem conduits upon cutting the petioles under water was assessed. Vein density (*VD*) was also determined in both species to explain possible differences between them. Results showed that the vulnerability curves for K_{leaf} determined by the two different methods were indistinguishable. However, attaching the leaves out of the water (even if the petioles were cut under water) resulted in a K_{leaf} decrease due to the experimental artifact of air entry into the leaf xylem conduits. K_{leaf} was significantly higher in almond than in olive for decreasing leaf water potential (Ψ_{leaf}), in accordance with higher *VD* in almond than in olive and with the fact that almond is able to keep higher g_{s} than olive for lower Ψ_{leaf}. However, K_{leaf} slightly varied for Ψ_{leaf} values that are normally obtained during the day in trees growing in areas of Mediterranean climate, which is opposite to previously observed g_{s} dynamics for both species. Thus, a high K_{leaf} may favor a high g_{s} in almond and olive but, according to our results, K_{leaf} would not determine the stomatal closure occurring at low Ψ_{leaf} values.

INTRODUCTION

A major resistance component of the plant hydraulic system has been found to be in the leaf (Sack and Holbrook, 2006). Consequently, leaf hydraulic conductance (K_{leaf}) may play an important role in the regulation of stomata. However, the effect of K_{leaf} on stomatal conductance (g_{s}) is still poorly understood. Reliable and fast K_{leaf} measurements would be really helpful for studies relating K_{leaf} and g_{s}. Although there are new approaches providing K_{leaf} measurements within minutes, such as the “Dynamic rehydration kinetics method” (DRKM, Blackman and Brodribb, 2011), some uncertainties are still unresolved (Flexas et al., 2013) such as the possible embolism caused by cutting the xylem under tension before K_{leaf} measurement. Moreover, the extent of the K_{leaf} decline with water stress and its recovery varies from species to species, even within the same habitat (Scoffoni et al., 2012). In addition, the knowledge of its relations to leaf structure and ecological strategy remains incomplete (Blackman et al., 2010). Vein density (*VD*) is one of the major structural features contributing to multiple key functions such as enabling higher K_{leaf} and greater g_{s} (Sack and Scoffoni, 2013). We conducted an experiment to quantify the dehydration response of K_{leaf} in well irrigated young individuals of two Mediterranean fruit tree species with different ecology (olive-*Olea europaea* and almond-*Prunus dulcis*). Data obtained with DRKM and Evaporative flux method (EFM) were compared and the effect of air entry into xylem conduits upon cutting the petioles under water was assessed. *VD* was also determined in both species to explain possible differences between them.

MATERIALS AND METHODS

Olive (*Olea europaea* L., cv Arbequina) and almond (*Prunus dulcis* (Mill.) *D*.A. Webb cv. Guara) shoots were sampled from well irrigated 4-year-old potted trees. The trees were in an orchard nearby Seville (Spain) (37º 15’ N, -5º 48’ W). The area has a Mediterranean climate with hot and dry weather from May to September, being mild and wet for the rest of the year. Measurements were taken in the summers of 2013 and 2014.

K_{leaf} was measured in fully developed, sun exposed leaves of the current year. The shoots were cut under water and then allowed to dehydrate to obtain a wide range of leaf water potential values (Ψ_{leaf}) and allowed to equilibrate for at least 30 min in dark plastic bags with wet paper towel. The leaves were then cut under distilled water and rapidly attached to the flowmeter. A group of leaves was taken out of the water before being attached to the flowmeter and another group was attached under water. The tubing connected each sampled leaf to a pressure transducer (Omega Engineering Ltd., UK), which was, in turn, connected to a CR1000 data logger (Campbell, Campbell Scientific Ltd., UK). This recorded readings every 1 s to calculate the flow rate through the leaf (mmol s^{-1} m^{-2}). K_{leaf} was estimated in 106 olive and 104 almond leaves using DRKM, being almost half of them connected to the flowmeter under water. Prior to the flow rate measurement, initial leaf water potential (Ψ_{o}) was measured in neighboring leaves. K_{leaf} was also determined in 29 leaves of each species using EFM, after Scoffoni et al., (2012). For direct comparisons K_{leaf} was determined in 10 leaves of each species using both methods. To build the vulnerability curve for each species, K_{leaf} was then related to the lowest Ψ_{leaf} which is Ψ_{o} in DRKM and Ψ_{o} or final leaf water potential (Ψ_{final}) in EFM (Scoffoni et al., 2012). Values of Ψ_{leaf} were measured with a Scholander-type pressure chamber (PMS Instrument Company, Albany, Oregon, USA).

For both species, 10 leaves of the same type than the leaves where K_{leaf} was determined were used to quantify vein density (*VD*) (mm mm^{-2}), not considering the major 1st vein. Images of cleared and stained leaves were captured using a Canon Powershot A620 camera mounted on a stereoscope (Zeiss Stereo Discovery V8). Images of 4 mm^2 in almond and 11 mm^2 in olive were taken close to the central nerve and in the top, middle and bottom thirds of the leaves and the ImageJ image processing program (Schneider et al., 2012) was used to quantify the vein length.

The fitted functions to our Ψ_{leaf} – K_{leaf} dataset were selected using Maximum likelihood as described in Scoffoni et al., (2012) were compared using Linear Models (Ψ_{leaf} was previously Ln-transformed) where in one of the models the covariates "species identity and methodology" were added. Significant differences due to the covariates were reported when differences between the models were <0.05. R package was used for the analyses (R version 2.14.0).

RESULTS AND DISCUSSION

Results showed that the vulnerability curves for K_{leaf} determined both by DRKM and EFM were statistically indistinguishable (*p* values of 0.87 and 0.12 for olive and almond, respectively). Similar K_{leaf} values were also obtained with both methods, although DRKM values were slightly lower than EFM values (r^2 = 0.79 and slope = 0.82). However, a statistically significant decrease occurred $p<0.05$) in the vulnerability curves for K_{leaf} of both species due to the experimental artifact of air entry into xylem conduits in the leaves cut under water but connected to the flowmeter out of it (Fig. 1). This effect was more pronounced in leaves with low Ψ_{leaf} values.

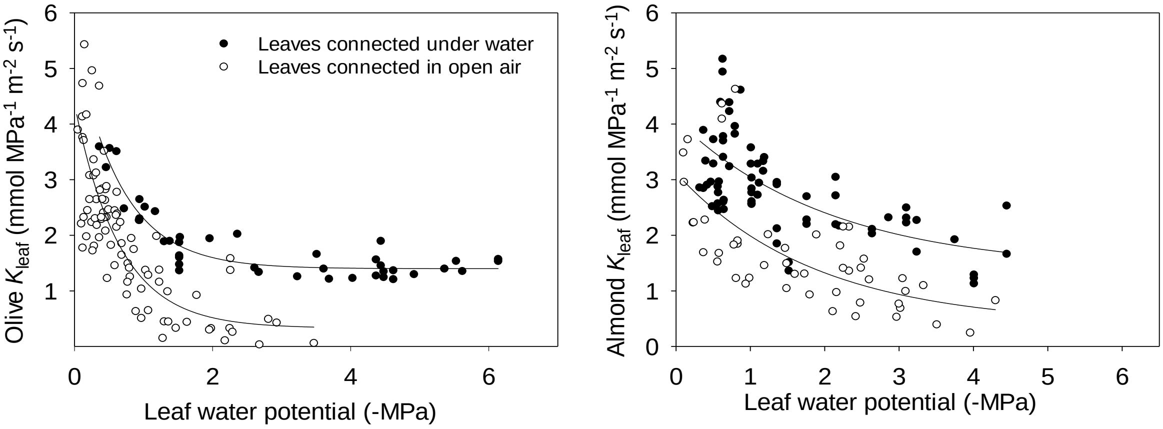

Fig. 1. Vulnerability curves for K_{leaf} for (A) olive and (B) almond determined using two approaches (petioles cut under water-black circles or in air-white circles) to test the effect of air entry into xylem conduits upon cutting the petioles.

For both species, vulnerability curves for K_{leaf} were best described by exponential functions. However, K_{leaf} was significantly higher in almond than in olive (Fig. 2) for decreasing Ψ_{leaf} which is in accordance with higher *VD* in almond (11.33±0.28 mm mm^{-2}) than in olive (6.74±0.19 mm mm^{-2}). Likewise these results also agree with the fact that almond shows higher g_s than olive at low Ψ_{leaf} values (Fernández et al., 1997; Rodriguez-Dominguez et al., *in preparation*).

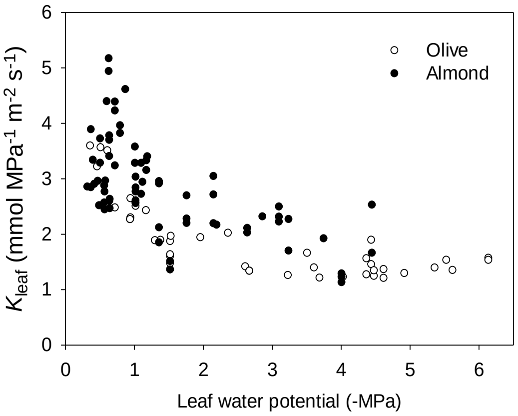

Fig. 2. Comparison of vulnerability curves for K_{leaf} for olive (white circles) and almond (black circles) estimated using DRKM.

High *VD* would allow almond leaves to achieve greater K_{leaf} favoring higher g_s. However, K_{leaf} hardly varied for Ψ_{leaf} values that are normally achieved during the day in the field (from around -1 MPa to -3 MPa), contrary to the marked daily changes on g_{s} observed for both species. Stomatal conductance has been observed to be highly variable on a daily basis, especially in olive, having a maximum early in the morning and decreasing for the rest of the day (Fernández et al., 1997; Rodriguez-Dominguez et al., *in preparation*). Thus, a high K_{leaf} may favor greater g_{s} in almond and olive. However, according to our results, K_{leaf} would not determine the stomatal closure observed at low Ψ_{leaf} values, because both variables usually show different trends in the short-term. Further work is needed to assess these hypotheses.

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More Fruit per Drop: Optimizing Irrigation in “Rocha” Pear Orchards in Central Portugal

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ABSTRACT

Water consumption in irrigated fruit orchards is an important determinant of economic viability. Physiological parameters related with the primary productivity are being periodically measured in representative trees from four orchards, in central Portugal, where water economy is expected to be fully characterized. Assessed physiological parameters include photosynthetic performance, photosynthetic pigments, photochemical efficiency and cell membrane stability. Preliminary results show photosynthetic and photochemical differences between orchards, probably reflecting local microclimate conditions and different agronomic procedures.

INTRODUCTION

Water availability is the main limiting factor for agricultural production worldwide. Water deficit affects plants at several levels. Decrease of turgor potential, cell expansion and growth are among the first processes to decline under water stress (Beck et al., 2007). With progressive water deficit, photosynthesis is adversely affected, initially due to diminished inorganic supply resulting from stomatal closure and in a later stage due to mesophyll limitations (Marques da Silva and Arrabaça, 2004). “Rocha” is a Portuguese pear variety with high economic importance. This work aims to optimize water use in irrigated orchards of “Rocha” pear in Central Portugal.

MATERIALS AND METHODS

Four “Rocha” pear orchards [Brejo (39,477; -9,114) Rol (39,218; -9,258), Ruiva (39,496; -9,023) and Junqueira (39,502; -9,045)] in four homogeneous microclimatic and edaphic areas (Alfeizerão, Lourinhã, Alcobaça and Cela, respectively) were selected, and soil probes were set up in order to monitor irrigation by Smart Irrigation Management - Hidrovolution™ (Hidrosoph, Portugal). Three representative trees were selected in each orchard, and measurements were performed monthly since the appearing of fully expanded leaves (May to July). Light response (A/I) curves were performed in 2 non-detached leaves per tree with an infrared gas analyzer (LCpro+ Adc BioScientific Ltd, UK), under controlled environment (50 ± 5 % relative humidity, 390 ppm CO^2, 25 ± 2 ºC), according to Nunes et al. (2009). A/I curves were analyzed and the corresponding parameters were calculated as in Lambers et al. (1998). Intrinsic water use efficiency (WUEi) was calculated as the ratio photosynthesis/stomatal conductance at 200 and 1200 µmol m^{-2} s^{-1}. Chlorophyll a fluorescence was measured with a Handy Plant Efficiency Analyzer – Chlorophyll Fluorimeter (Hansatech Instruments, England) and JIP test parameters calculated as in Silvestre et al. (2014). Pigment content was assessed according to Lichtenthaler (1987). Membrane stability was assessed by the electrolyte leakage method (Bajii et al. 2002). Conductivity (Cond) of the solution containing the leaked electrolytes was measured with a conductimeter Eutech Ecoscan Con 5 (EUTECH INSTRUMENTS, Singapore). Total conductivity (CondT) was measured after heating samples for 90 min at 90 ºC and relative conductivity (CondRel) calculated as (Cond/CondT)*100. Data were analyzed by two-way ANOVA with GraphPad Prism 6.01 (GraphPad Software, USA).

RESULTS AND DISCUSSION

In May, the photosynthetic capacity (Amax, Fig. 1A) and the photosynthetic apparent quantum yield (Φ, Fig. 1B) at Junqueira were significantly lower than at the other locations. At Junqueira and Brejo the photosynthetic capacity in May was significantly lower than at June and July (Fig. 1A). The quantum yield was also lower in May than in June and July at Junqueira and Rol. On the contrary, the light compensation point to CO_2 was, in May, much higher in Junqueira than in the other locations and a decrease from May to July was observed therein, as well as in Brejo (Fig. 1C). This is, at least in part, due to the higher dark respiration rates observed in May in Junqueira and Brejo (Fig. 1D), that also contribute to the decrease of Φ. Brejo and Junqueira showed the higher WUEi in May, but this parameter decreased sharply in Junqueira, whereas it stayed steady in Brejo (Fig. 1E, F).

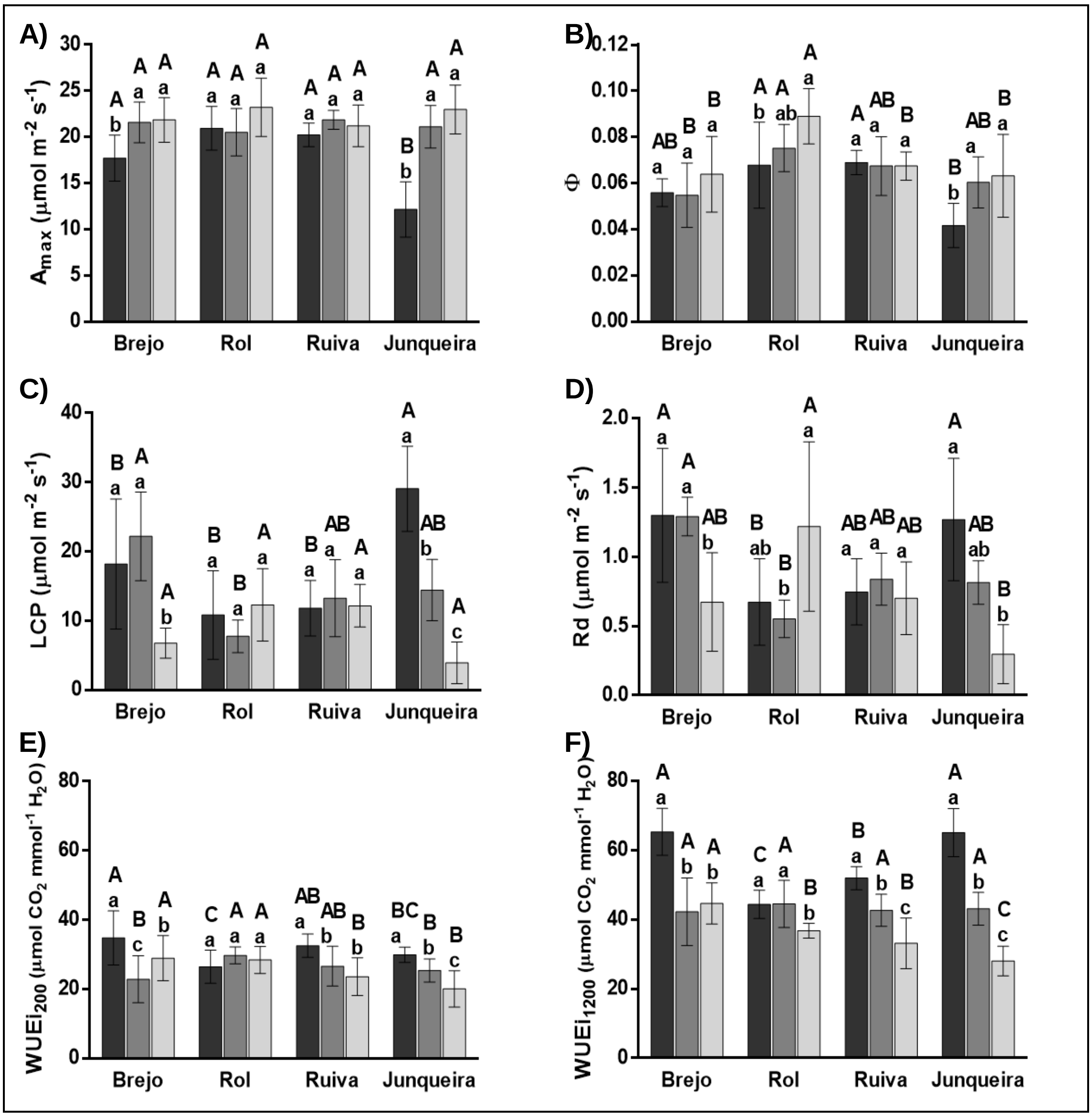

Fig. 1. A) Maximum photosynthesis (A_{max}), B) photosynthetic apparent quantum yield (ϕ), C) apparent light compensation point (LCP), D) dark respiration point (Rd), and intrinsic water use efficiency (WUEi) at E) 200 and F) 1200 □mol m^{-2} s^{-1} for the four orchards Brejo, Rol, Ruiva and Junqueira during ■ May, ■ June, and ■ July. Different letters indicate significant differences between orchards (uppercase) and months (lowercase). Values are mean ± SD.

In May, the Performance Index of Junqueira was lower than in the other locations, which is in accordance with the data from photosynthesis. In all locations, an increase in PIABS was observed from May to July (Fig. 2A). Also Fv/Fm of Junqueira and Brejo were lower in May, although Junqueira, but not Brejo, recovered in July (Fig. 2B).

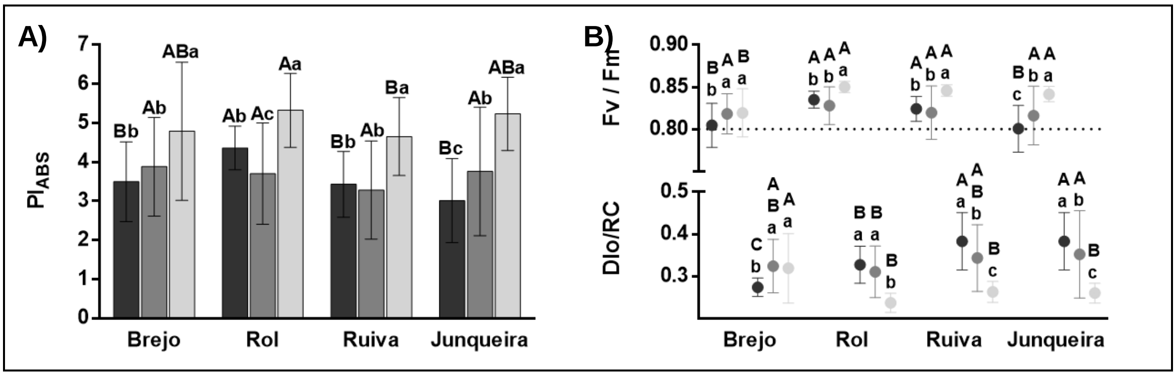

Fig. 2. A) Performance index on absorption basis (PI$_{ABS}$), B) maximum quantum yield of PSII (Fv/Fm), and energy flux of dissipation energy per reaction center (DIo/RC) for the four orchards Brejo, Rol, Ruiva and Junqueira during ■ May, ■ June, and ■ July. Different letters indicate significant differences between orchards (uppercase) and months (lowercase). Values are mean ± SD.

Furthermore, while Brejo showed a low non-photochemical energy dissipation in May but an increase in July, Junqueira showed a high dissipation in May but a much lower value in July (Fig. 2B). No differences between sites were found in the total chlorophyll content per area in May, but Brejo showed the higher and Rol the lower content in July (Fig. 3A).

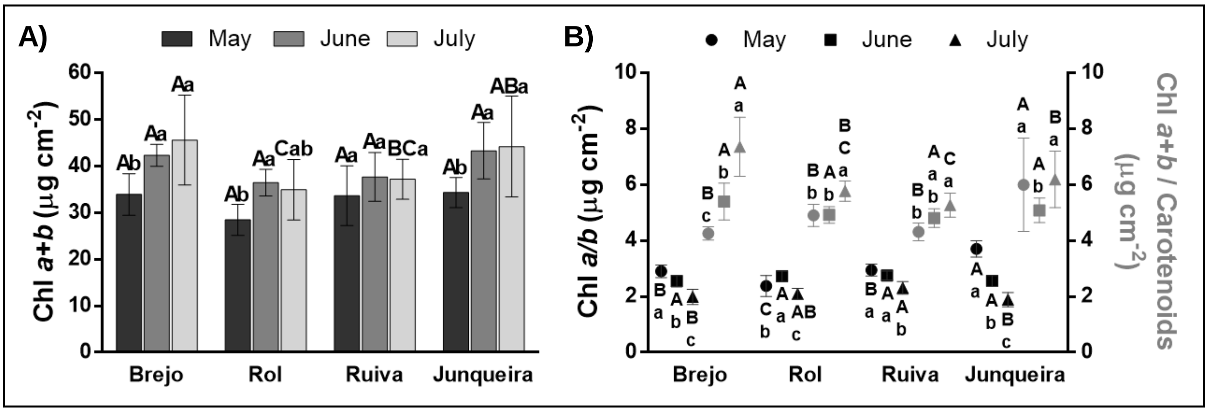

Fig. 3. A) Total chl content (Chl *a*+*b*), B) chl a/b ratio (Chl a/b), and the ratio between total chl content and carotenoids (Chl *a*+*b* / Carotenoids) for the four orchards Brejo, Rol, Ruiva and Junqueira during May, June, and July. Different letters indicate significant differences between orchards (uppercase) and months (lowercase). Values are mean ± SD.

Brejo and Junqueira showed the highest Chl a/b ratio in May but the lower in July (Fig. 3B). Junqueira showed the higher Chl (a+b) / Carotenoids ratio in May and Brejo in July (Fig. 3B). Junqueira and Brejo showed the lower membrane stability in July. In all sites except Brejo membrane stability decreased in July, as shown by the increased relative conductivity of the solution containing the leaked electrolytes (Fig. 4).

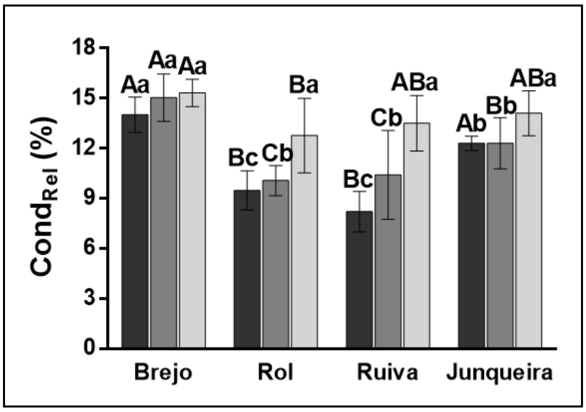

Fig. 4. Relative conductivity (Cond$_{Rel}$) for the four orchards Brejo, Rol, Ruiva and Junqueira during ■ May, ■ June, and ■ July. Different letters indicate significant differences between orchards (uppercase) and months (lowercase). Values are mean ± SD.

Taken together, these results point to a lower photosynthetic performance of Junqueira in May, which was overcome in the following months. Brejo also performed poorly in May, but did not fully recover in July. Rol, and mainly Ruiva, showed a more balanced behaviour during the period studied. A correlation analysis comprising microclimatic and cultural parameters is on course, aiming to unravel the factor supporting the observed physiological differences between sites.

ACKNOWLEDGEMENTS

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Short-Term Responses of Water and Carbon Fluxes to Cork Stripping

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ABSTRACT

There is a knowledge gap regarding cork stripping effects in tree water and carbon balance. Reliable and appropriate methods are mandatory to evaluate tree water losses and its partition inside tree-compartments. In the early summer of 2014 we have selected two similar pairwise sets of stripped and unstripped control trees and monitored water and carbon fluxes throughout the summer. Daily and seasonal measurements comprised: 1) Integrated branch carbon uptake, respiration and transpiration (Licor 840); 2) Stem respiration and transpiration (LCPro+, Bioscience); 3) Tree water status (predawn and midday xylem leaf water potentials). We observed that under mild summer conditions, cork harvest had no significantly effect in leaf carbon uptake which was mainly driven by soil water availability and vapour pressure deficit (VPD). Stem water losses and respiration reached a steady-state condition approximately two weeks after cork harvesting. In the short-term, carbon and water fluxes at the stem tree level responded strongly to microclimate conditions (e.g. VPD). We conclude that under soil water availabilities and usual summer evaporative demands cork stripping does not represent an additional stress factor to the tree.

INTRODUCTION

There is a general consensus regarding cork-oak decline all over the Mediterranean basin (Camilo-Alves 2013). Cork stripping is commonly referred as a cause of tree stress and its practice is generally avoided during unfavourable climatic years. However, only a few studies have addressed the effects of cork stripping in the cork oak ecophisiology (Oliveira 2012). Cork harvesting implies two immediate consequences for the tree: carbon and water losses with concomitant changes in photosynthate allocation and in water balance. Since bark stripping takes place in summer, a period of water scarcity, and can enhance water deficits, intensive monitoring of tree water fluxes in response to stripping is of particular importance.

In this study we assessed cork stripping effects on water and carbon fluxes at the tree level. We aim to evaluate the time needed for trees to regain carbon and water balance, therefore providing valuable experimental knowledge to improve cork harvesting management practices.

MATERIAL AND METHODS

The experimental site is a 50-yr-old cork oak open woodland (*Quercus suber*) located in Herdade da Machoqueira do Grou in Coruche region (39°08′20.9″ N, 8°19′57.7″ W). The average annual precipitation is 680 mm with a mean annual temperature of 15.9 °C. This site is part of the research Integrated Carbon Observation System (ICOS), and is measuring continuously since 2009 the water and carbon fluxes at the ecosystem level using the eddy covariance method. In the early summer of 2014 two similar pairwise sets of stripped and unstripped control trees (n=6) were selected and monitored for water and carbon fluxes throughout the summer. Days of measurements comprised the harvesting day (7July 2014) and then 1, 4, 8, 17 and 28 days after cork removal. A control period was also considered for branch carbon uptake before cork harvesting. Periodical measurements included: 1) Integrated branch carbon uptake, respiration and transpiration, measured with a Licor 840a coupled with closed dark and light chambers (n=4); 2) Stem respiration and transpiration using a LCPro+ (Bioscience) coupled with an adapted soil hood (n=6); 3) Continuously sap flow monitoring through Granier and heat pulse methodology (n=6); 4) Predawn and midday xylem leaf water potentials (n=6). Here we present preliminary results from the following variables: branch carbon uptake (fNEE in µmol CO_2 m^{-2} s^{-1}), stem respiration (µmol CO_2 m^{-2} s^{-1}) and stem water loss (mmol H_2O m^{-2} s^{-1}).

RESULTS AND DISCUSSION

In addition to an abundant rainfall in the previous winter and spring (435 mm), June and July 2014 months were relatively wet and cooler when compared with the 6 year average for the same site. The temperatures were 1ºC below the average and precipitation was 10 mm above the 6 years average. Accordingly, average daylight vapour pressure deficit (VPD) was 2 hPa below the average. In result, trees were not subjected to water deficit as confirmed by predawn leaf water potentials in mid-July (ca. -0.3 MPa). Therefore, the summer presented less climatic stress conditions than the average.

Carbon uptake

Cork harvesting did not significantly affect branch carbon uptake (Fig. 1-b). Although control trees presented slightly higher carbon sink strength during the study period, the differences were not significant compared with cork stripped trees.

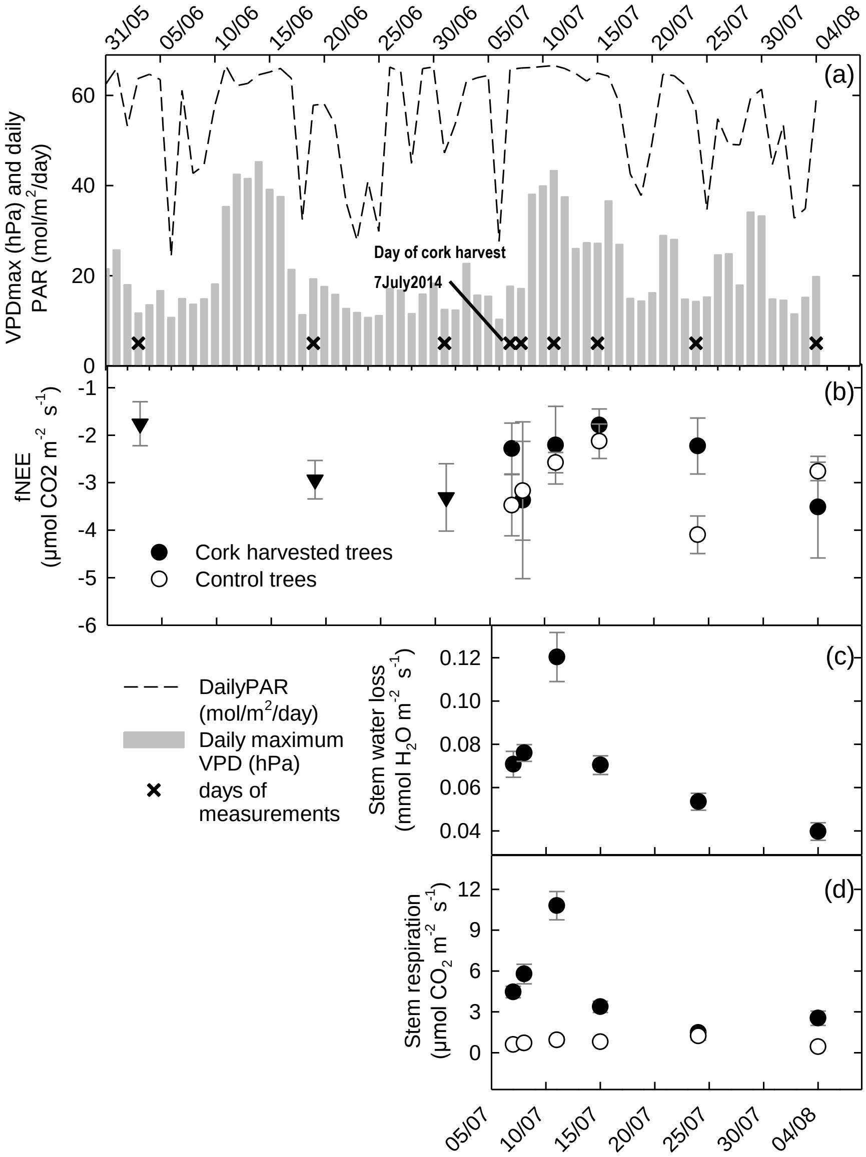

Fig. 1. (a) Meteorological data during June and July 2014 daily average of total incident photosynthetically active radiation (PAR, mol m^{-2} d^{-1}) and maximum daily vapour pressure deficit (VPDmax, hPa), (X) represent the days of measurements (b) Net carbon uptake measured in the light chamber (µmol m^{-2} d^{-1}) for the days of measurements, (c) Stem water loss (mmol H_2O m^{-2} d^{-1}) for the days of measurements and (d) Stem respiration for the days of measurements (µmol CO_2 m^{-2} d^{-1}). Erros bars represent standard errors.

However, 17 days after cork harvesting control trees showed a significant higher carbon uptake which is due to a combination of high photosynthesis rates and low branch respiration rates. After cork harvesting a carbon uptake decrease was observed in both treatments and can be attributed to an increase in VPD leading to an high evaporative demand and lower stomatal conductances (Fig. 1-a and b).

Stem gas exchanges

Although stem water after cork harvest was clearly perceptible by the humidity at the stem surface, this was not reflected in a pronounced amount of water loss. In fact, maximum stem water loss was recorded 4 days after cork harvesting coincident with maximum VPD, dropping linearly thereafter until a minimum 28 days after cork harvesting (Fig. 1-c). Thus, the atmospheric evaporative demand is the main driver for stem water loss in the first days after cork removal limiting the rate of CO_2 and H_2O diffusion from the stem to the air. Noticeably, our values are 30 times lower than the stem water loss estimates presented by Oliveira (2012). Stem respiration presented a temporal variability similar to stem water loss with maximum values coincident with higher VPD (Fig.1-d). Stem gas exchanges decreased with time after cork removal which is probably related to cell tissue reconstruction of phellogen limiting CO_2 and H_2O efflux.

CONCLUSIONS

Cork harvesting do not significantly affect carbon uptake under mild summer conditions.

Stem water and carbon losses reach a steady-state condition approximately 15 days after cork stripping.

The atmospheric evaporative demand is a key driving factor of carbon and water fluxes both at leaf and stem level. Further analysis is needed to consubstantiate the results obtained.

ACKNOWLEDGEMENTS

We wish to thank Herdade da Machoqueira for the facilities and help in the establishment of this field trial.

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Effects of an Extreme Dry Winter on Cork Oak Woodland: Net Ecosystem Exchange and Phenology Adjustments

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ABSTRACT

The current study is based on the monitoring of an extremely dry winter in a cork oak woodland under the Mediterranean climate of central Portugal. Results are centred on net ecosystem exchange (NEE), phenology and tree growth measurements during two contrasting years: 2011, a wet year with a standard summer drought pattern and 2012, with an extreme dry winter (only 10 mm of total rainfall) that exacerbated the following summer drought effects. The main aims of this study were to assess: 1) effects of winter drought in annual and seasonal NEE; 2) interactions between cork oak phenological events and NEE.

The dry year 2012 was marked by a 45% decrease in carbon sequestration (-388 vs. -214 gC m^{-2} $year^{-1}$), a 63% reduction in annual tree diameter growth but only a 9% reduction in leaf area index compared to the wet year 2011. On the contrary to male flower production fruit setting was severely depressed by water stress showing a reduction of 54% in the dry year.

Our results suggest that leaf growth and leaf area maintenance are ecophysiological traits preserved under drought winter and are a sink priority for photoassimilates contrarily to tree diameter growth. Thus, carbon sequestration reductions under low water availabilities in cork oak woodland should be ascribed to stomatal regulation or photosynthetic limitations and in a much less extent to leaf area reductions.

INTRODUCTION

In seasonally dry climates, such as the Mediterranean, lack of rainfall in the normally wet winter season may originate severe droughts and a great variability in annual precipitation. Droughts, in turn, are a main source of inter-annual variation in carbon sequestration when winter rainfall diminishes. This may alter the seasonal pattern of photosynthetic uptake, which is determined by leaf phenology and gas exchange limitations.

Phenological patterns in Mediterranean regions are strongly influenced by a marked climatic seasonality and species evolved to synchronise maximum vegetative activity to the most favourable periods of the year (Misson et al., 2011; Pinto et al., 2011). Timing of budburst and growing season length can directly impact on annual net ecosystem carbon uptake (Richardson et al., 2010) and leaf age on canopy carbon uptake. However, an earlier spring onset can be associated with either enhanced or decreased productivity later in the growing season (e.g. depending on interactions with water availability), and thus early-season gains being offset by sustained late-season reductions in physiological activity (Richardson et al., 2010). Furthermore, even if early leaf development is generally advantageous in terms of carbon uptake, there are trade-offs between increasing growing season length versus increases in the probability of early-spring frost damages. Therefore, the evaluation of interactions between climate change effects, phenological events and net ecosystem exchange requires a species-specific ecosystem scale analysis.

MATERIAL AND METHODS

The experimental site is located at Herdade da Machoqueira in Central Portugal (39°08’18.29’ N, 8°19’57.68’ W). Vegetation consists of a ca. 50-yr-old cork oak (*Quercus suber*) open woodland with an understory of shrub species (e.g. *Cistus* sp., Ulex sp.) and native grassland. The climate is Mediterranean, where average annual precipitation is 680±210 mm and mean annual temperature is 15.9 ºC (period 1955–2007). The infrastructures in the site consist on an eddyflux tower with an ultrasonic anemometer (R3, Gill Instruments) and a closed-path gas analyzer for CO_2 and water vapour concentrations (Li-7000 IRGA, Licor). An automatic weather station is also present measuring meteorological data (e.g. air temperature, air relative humidity, PAR, rainfall, soil water content).

Eddy flux data was treated using the eddy-covariance data acquisition and processing software package EddySoft (Meteotool). Further details on flux data processing, computation and data quality control are described in Correia et al. (2014).

Litter fall was collected by 16 baskets of 1 m^2 placed in two transects across the site and sampled every 15 days throughout 2011 and 2012, with separation of leaves, branches, male flowers (catkins) and acorns. Budburst time and individual leaf dimension were registered in a sampled branch in six trees to determine the start and duration of the leaf growth period. Tree leaf area index (LAI) was calculated using leaf biomass from litter fall and specific leaf area following Limousin et al. (2009). Tree-stem diameter growth was measured with dendrometer bands (± 0.1 mm) installed in 12 trees at breast height and registered every 15 days throughout 2011 and 2012. Leaf xylem water potential was measured at predawn and midday with a Scholander-type pressure chamber in six trees throughout the summer for both years.

RESULTS AND DISCUSSION

Effects of extreme dry winter on carbon fluxes:

The effect of a severe dry winter in 2012 led to a 45% decrease in annual carbon uptake in relation to 2011, from -388 to -214 g C m^{-2} $year^{-1}$, respectively (Fig. 1).

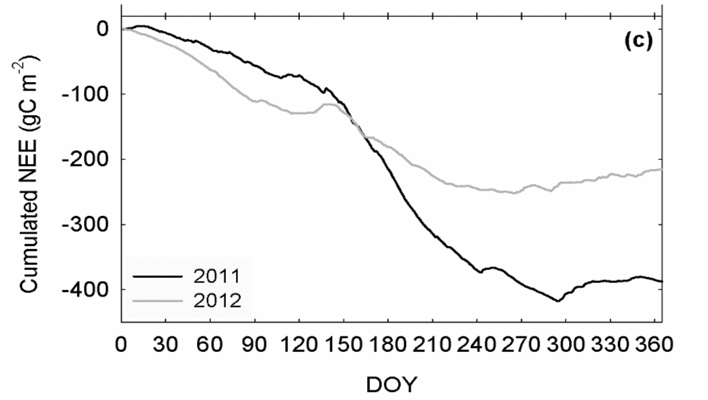

Fig. 1. Cumulated values of net ecosystem exchange (NEE, g C m^{-2}) during 2011 and 2012. Negative values represent carbon sequestration in the ecosystem.

However, in 2012, carbon sequestration in winter was significantly higher than in winter 2011 in response to a 57% higher incident PAR. Thus, tree productivity in winter seems to be highly dependent on available incident radiation suggesting an advantage of evergreeness allowing a significant ecosystem productivity to be achieved outside of the most favourable growing period – spring to early summer – through an adjustment of growth to environmental resources availability. Nevertheless, it was in summer that the ecosystem showed to be more affected by the low water availability with a reduction of 66% in carbon sequestration in relation to summer 2011 (Fig. 1).

Extreme dry winter effects in tree growth and phenology:

Annual stem diameter growth decreased 63% in 2012 compared to 2011 (Fig. 2).

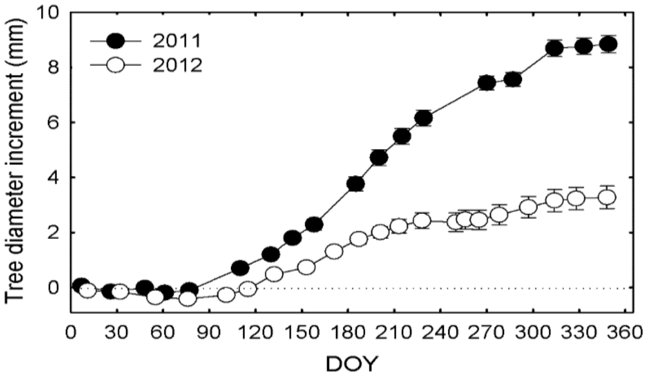

Fig. 2. Tree diameter increment (mm) during 2011 and 2012. Values are means ± *se* (*n*=9).

Diameter growth reductions in 2012 relatively to 2011 were especially higher during summer (76%), where growth impairment can be directly linked to the observed low soil water availabilities and photosynthetic limitations. Relative to 2011 a higher intensity of leaf shedding was observed in 2012 in the early spring. As a result, the minimum LAI in 2012 was 65% lower than in 2011 (Fig. 3). This was reflected both in a steeper NEE increase (Fig. 1) and in a concomitant stem diameter growth stop in the same period (doy 127 to 156, Fig. 2) in opposition to 2011 where stem diameter growth rates were sustained.

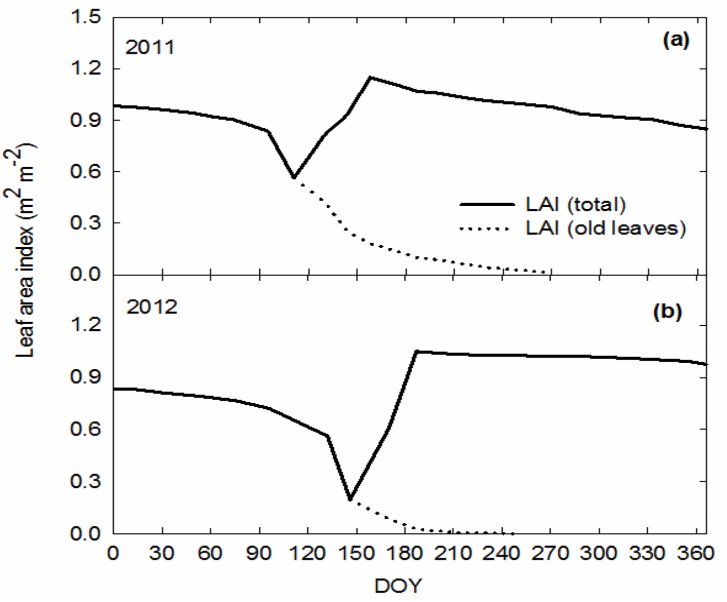

Fig. 3. Tree leaf area index (LAI) during 2011 (a) and 2012 (b). The dash line represents LAI of old leaves matured in the previous spring.

This strongly suggests that stored and current carbon uptake were insufficient in spring 2012 for meeting growth sink demands and that new leaf expansion was a priority sink in opposition to diameter stem growth, in accordance to its much greater reduction when compared to LAI (9%). Thus, assuring tree canopy renewal and maintaining a relatively stable LAI seems to be an ecological trait preserved even under extreme low winter precipitation and reflects Q. suber resilience to drought.

ACKNOLEDGEMENTS

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The invasion of *Oenothera drummondii* on Huelva coastal dunes, previsions of short-time success

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ABSTRACT

Biological invasions occur when natural communities are threatened by an increase in distribution and abundance of exotic species, causing significant changes in the ecosystem composition, function and structure. *Oenothera drummondii* is an alien invasive species of coastal dune ecosystem, which is causing changes in biodiversity, and stability of ecological processes. The species is native to coastal sands of Mexican Gulf and it is recently invading coastal dunes of Cadiz Gulf.

For the whole year 2013-2014, we have compared several ecophysiological traits in this alien species with a native species *Otanthus broteri* in the field. Both species exhibit similar structural characteristics and they share the same habitat. 15 individuals of each species, growing in the same area have been marked and monitored with a seasonal periodicity, during 2013-14 annual cycle.

O. drumondii showed a lower photochemical efficiency than the native species, but its assimilation rates were always higher than *O. broteri*, with the exception of the winter period. The invasive species also exhibited a better water performance than the native species, probably linked to a better water use efficiency and higher root growth.

These first results evidence that *O. drumondii* is perfectly adapted to coastal dunes under Mediterranean climate.

INTRODUCTION

Invasive species present structural and functional traits that enable a high competition capacity by limiting resources, such as water and nutrients, and can also cause significant changes in soil composition and in nutrient cycles (Kourtev et al. 2002; Levine et al. 2003). The invasive success of some introduced plants and their ecological impacts on both native communities and ecosystem functioning depend on plant traits, environmental characteristics of the receptive area and biological interactions with native organisms (Ehrenfeld 2010; Bottollier-Curtet et al. 2013), as competition, plant-herbivore interaction namely that related with seed dispersion, and establishment of new population. *Oenothera drummondii* is an alien invasive species of coastal dune ecosystem, which is causing changes in biodiversity, and stability of ecological processes. The species is native to coastal sands of Mexican Gulf and it is recently invading coastal dunes of Cadiz Gulf.

Our starting hypothesis was the following; *Oenothera drummondii* is an alien invasive species of coastal dune ecosystem, with an extremely high adaptation capacity to environmental conditions of invaded dune systems, as it may exhibit a water use strategy more efficient and competitive than native dune species. To answer this question we have compared several ecophysiological traits in this alien species, with the native species *Otanthus broteri* in the field.

MATERIAL AND METHODS

The study site was a new dune area; originated by fast sand deposition to the West of the breakwater of Huelva harbour (this breakdown was built to protect the harbour from sand accretion in 1970s). We have chosen 15 individuals of *O. drumondii* and 15 of *O. broteri* growing in the same area; all the plants were marked and monitored with a seasonal periodicity, during 2013-14 annual cycle. Measurements of gas-exchange, PSII fluorescence and leaf water potential were taken in the field and leaf samples were collected for leaf mass area, relative water use efficiency, proline and photosynthetic pigment analysis.

Measurement of net photosynthetic rates (A), stomatal conductance (g_s) and transpiration rates (E) were made using a portable compact CO_2/H_2O system (LCi-portable photosynthesis ADC system, UK). Intrinsic water use efficiency (WUEi) was calculated as the ratio of net photosynthesis to stomatal conductance (WUEi=A/g_s). Leaf surface was computed in the laboratory (Midebmp, Ordiales, 2000), and gas exchange measurements were recalculated for leaf surfaces. Leaf Mass Area was calculated as the ratio between leaf dry weight and leaf surface (g/m^2). Gas-exchange measurements were made between 08.30 and 10.30 hours solar time, to avoid possible midday depression of leaf conductance.

Midday leaf water potential (Ψ_{md}) was determined in the field with a Scholander type pressure chamber (Manofrigido, Lisbon, Portugal) using terminal shoots.

Chlorophyll fluorescence kinetics of leaves was determined in situ, at midday, by means of the pulse-amplitude modulation technique using a portable fluorometer (mini-PAM). Maximum quantum yield of PSII was determined from the ratio of variable to maximal fluorescence, i.e. $F_v/F_m = (F_m-F_0)/Fm$, where F_0 and F_m are initial and maximal fluorescence of dark-adapted leaves during 15 minutes, a period found to be sufficient to allow complete reoxidation of the PSII reaction centres. The effective quantum yield (Φ_{PSII}), or photochemical efficiency of PSII in a light adapted state, was estimated on light adapted leaves with the leaf-clip-holder, as $\Phi_{PSII}= (F'_m-F)/F'_m$ (F'_m maximal and F steady-state fluorescence under actinic irradiance).

Photosynthetic pigments were extracted from frozen-kept leaves with 100% acetone and determined spectrophotometrically following Lichtenthaler (1987). Free proline content, as a measure of stress, or an osmotic regulator in plants, was determined colorimetrically by the nihydrine acid method of Bates et al. (1973).

One-way ANOVAs were applied to compare significant differences between species for any of the studied variables. Bivariate correlation tests were made between the different variables. All statistical tests were made with SPSS for Windows.

RESULTS AND DISCUSSION

Leaf water potentials (Ψ) of both species were higher during the mild winter and spring, figures decline in summer, reaching the lowest values in July and September (Fig. 1). *O. drumondii* always exhibited a less negative water potential than *O. broteri* which evidenced a better water performance in the exotic species

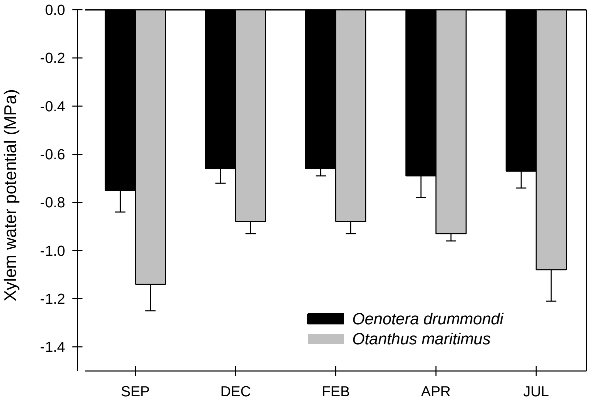

Fig. 1. Average (±sd) midday leaf water potential, in the two study species, over the 2013-14 annual cycle.

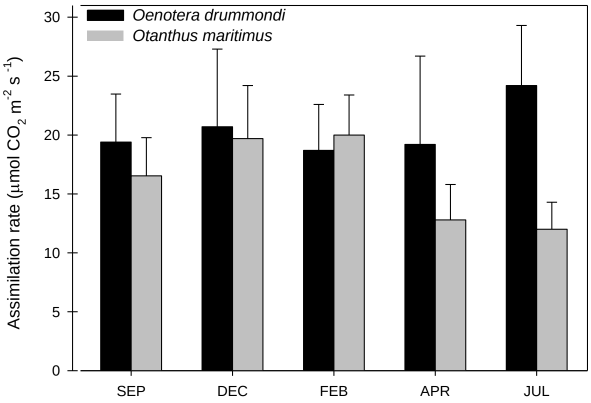

Fig. 2. Average (±sd) net assimilation rate in the two study species, over the 2013-14 annual cycle.

O. drumondii assimilation rates were always higher than *O. broteri*,, with the exception of the winter period, but these differences were especially evident in July 2014 (P<0.01). In the alien species, assimilation rates range around 20 µmol.m^{-2}.s^{-1} the whole year, while in the native species figures exhibit a seasonal fluctuation (Fig. 2).

Optimal and Effective quantum yield were higher in the native than in the exotic species, being these differences significant in some months (Fig. 3 and 4). These differences might be related to the tropical origin of *O. drumondii*, as it has been shown in other species (Zunzunegui et al 2005).

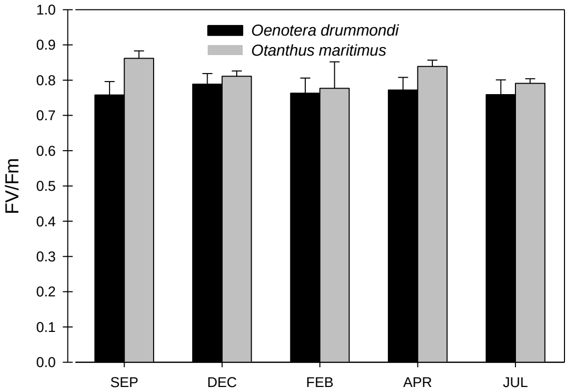

Fig. 3. Average (±sd) midday F_v/F_m of dark adapted leaves, in the two study species over the 2013-14 annual cycle.

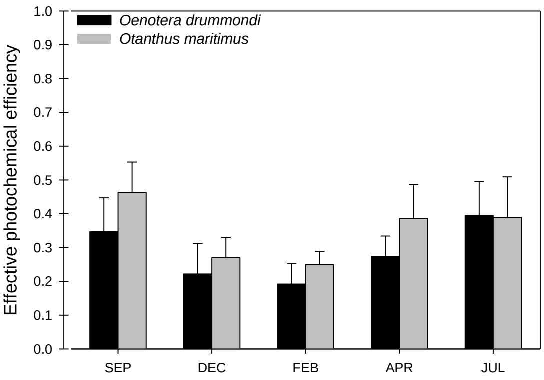

Fig. 4. Average (±sd) midday effective quantum yield, in the two study species over the 2013-14 annual cycle.

CONCLUSIONS

These first results show that O. drumondii is perfectly adapted to coastal dunes under Mediterranean climate, exhibiting a better water status and higher carbon assimilation than the study native species, probably linked to a better water use efficiency and higher root growth. But regarding photochemical efficiency, the lower values found in the alien species in comparison with *O. broteri* might be related to the tropical origin of O. drumondii that makes this species more cold temperatures sensitive.

Our study suggests that plasticity in water uptake may be the underlying mechanism that explains the competition capacity in *O. drumondii*, which transform this species in a dangerous alien invasive; only cold temperatures might be a limiting factor to control the invasion.

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Combining a Process-Based Model of Stomatal Conductance with Leaf Turgor Pressure Related Probe Measurements to Study the Regulation of Plant Water Status and Stomatal Conductance under Drought

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ABSTRACT

The recently developed plant sensor of relative changes in leaf turgor pressure (LPCP probe) was compared with the turgor pressure output simulated with a process-based stomatal model (BMF model). Our results confirm the good agreement between the simulated turgor pressures and those derived from LPCP readings. The combined use of the BMF model and LPCP probes raised new insights into the regulation of hydraulic conductivity and osmotic pressure.

INTRODUCTION

The use of plant-based sensors is in many cases the recommended option for precision irrigation in horticultural crops since plants are an integral component of the soil-plant-atmosphere continuum (Jones, 2004). The output of the recently developed leaf patch clamp pressure probe (LPCP probe) targets on turgor pressure (Rüger *et al.*, 2010), one of the physiological variables recognized to be among the most sensitive to water stress (Jones, 2004). Process-based models have been also suggested as powerful tools to improve our understanding of plant physiological mechanisms involved in the response to water stress (Buckley & Mott, 2013). Our main objectives were (i) to assess whether the diurnal leaf turgor pressure changes derived from LPCP outputs agrees with absolute changes in leaf turgor pressures modeled by the BMF model (Buckley *et al.*, 2003) and (ii) to understand mechanisms of stomatal conductance regulation under water stress.

MATERIAL AND METHODS

The experiment was conducted in June 25 and August 3 of 2012 in a commercial hedgerow olive orchard (1667 tree ha^{-1}) near Seville, Spain. Sunny and shaded leaves were used to explain the physiological basis in the response of LPCP probes observed in a wide range of values in two irrigation treatments (one tree per treatment): well watered (WW), with daily irrigation to replace 100 % of the maximum potential crop evapotranspiration (ET$_c$); and water stressed (WS), with a total of 30 % ET$_c$ (details are given in Fernández *et al.* 2013). Leaf water potential (Ψ_{leaf}) and stomatal conductance (g_s) were measured diurnally every 1.5

hours with a Scholander-type pressure chamber (PMS Instrument Company, Albany, Oregon, USA) and a Li-6400 (LI-COR, Lincoln, NE, USA) with a 2 × 3 cm standard chamber, respectively. Relative changes in leaf turgor pressure were derived from *in situ* measurements with the non-invasive, online-monitoring LPCP probe (ZIM Plant Technology GmbH, Hennigsdorf, Germany). Relative leaf turgor pressure is determined by measuring the output leaf patch pressure, P_{p}, upon application of a constantly kept external magnetic pressure (P_{clamp}). The attenuation of the applied external pressure and thus P_{p} depends on the magnitude of the turgor pressure of the leaf (P_{c}) which is opposed to P_{clamp} (details are given in Rüger *et al.* −2010− and Ehrenberger *et al.* 2012). Three LPCP probes per canopy position were installed (clamping procedure in Fernández *et al.* 2011). To allow average the three LPCP probes of each position and comparison among them, a normalizing procedure of P_{p} (P'_{p}) was carried out:

(1) $P'_{\mathrm{p}} = \dfrac{P_{\mathrm{p}} - P_{\mathrm{min,1}}}{P_{\mathrm{max,1}} - P_{\mathrm{min,1}}}$, where $P_{\mathrm{min,1}}$ is the minimum value (maximum turgor pressure) reached at pre-dawn of the decided experimental day (1 = June 25; 2 = August 3) and $P_{\mathrm{max,1}}$ is the maximum value (minimum turgor pressure) reached during the same day.

We used a simplified version of the stomatal conductance model originally presented by Buckley *et al*. (2003) (BMF model) to derive leaf turgor pressure for assessing LPCP probes measurements:

(2) $g_{\mathrm{s,model}} = \dfrac{\chi\beta\tau K(\Psi_{\mathrm{s}} + \pi)}{K + \chi\beta\tau \mathrm{VPD}}$, where K is leaf-specific hydraulic conductance, χ is a proportionality factor that includes effects of stomatal density, β is a parameter that describes sensitivity to epidermal turgor and ATP concentration, τ is ATP concentration in photosynthesising cells, Ψ_{s} is soil water potential, π is bulk leaf osmotic pressure and VPD is air vapour pressure deficit. The model assumes that the resistance from epidermal to guard cells is negligible compared to the resistance from the soil to the epidermis, and epidermal and bulk leaf osmotic pressure are similar. Variable hydraulic conductance (K_{var}) was obtained by fitting g_{s} data to the BMF model at single measuring time solving Equation (2) as:

(3) $K_{\mathrm{var}} = \dfrac{g_{\mathrm{s}} \mathrm{VPD}}{\Psi_{\mathrm{s}} + \pi - \dfrac{g_{\mathrm{s}}}{\chi\beta\tau}}$.

The modeled leaf turgor pressure (P_{model}) was derived from: $P_{\mathrm{model}} = \Psi_{\mathrm{l,model}} + \pi$, where $\Psi_{\mathrm{l,model}}$ is the modeled leaf water potential estimated as:

(4) $\Psi_{\mathrm{l,model}} = -\left(\dfrac{g_{\mathrm{s,model}} \mathrm{VPD}}{K} - \Psi_{\mathrm{s}}\right)$, and π is the maximum value estimated as:

(5) $\pi = \dfrac{g_{\mathrm{s}}}{\chi\beta\tau} - \Psi_{\mathrm{leaf}}$.

RESULTS AND DISCUSSION

In this study, we have found a good agreement between the output leaf patch pressure (P_p) and the turgor pressure estimated by the BMF model. This relationship was explained by a power function (Fig. 1) due to losses of the external clamped pressure applied to the leaf, which are theoretically embedded in the term leaf-specific attenuation factor (Ehrenberger *et al.*, 2012).

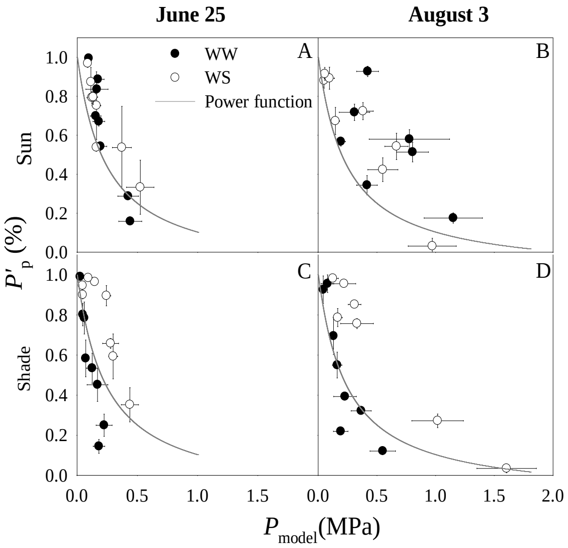

Fig. 1. Relationships between the normalized output leaf patch pressure (P'_p, %) and the absolute leaf turgor pressure modeled with the BMF model (P_{model}) on the two experimental dates in sunny and shaded leaves and for the two water treatments (WW and WS). Points are mean and error bars are standard errors for $n = 3$. Power function (gray line) is according to Ehrenberger *et al.* (2012).

We found a shift toward a more positive value of turgor for leaves of the WS treatment. We think that the most likely reason for explaining the difference between both treatments is related to the model output. Modeled turgor for the WS leaves was higher than that of the WW (Fig. 1D), which makes not much sense. The origin of the wrong performance of the BMF model might be due to the assumption of a constant π along the day. The existence of an active osmotic adjustment was evident not only in a diurnal basis. Seasonally our results suggest an increase in π leading to the shift toward more negative values of Ψ_{leaf} in its relationship with P'_p (Fig. 2). The increase in π allows leaves to maintain turgor pressure at lower Ψ_{leaf}. If this hypothesis is correct, it would suggest as well that the hysteresis found in August in the WS leaves (Fig. 2B, D) was a consequence of the likely diurnal adjustment of π previously mentioned.

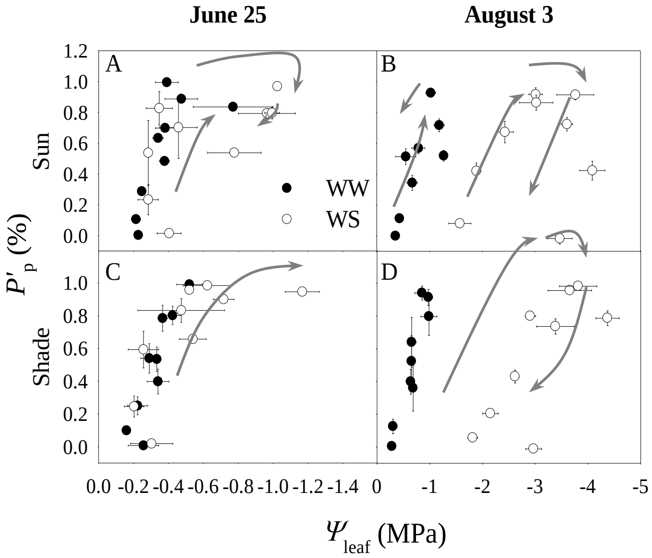

Fig. 2. Relationships between the normalized output leaf patch pressure (*P'*$_p$, %) and the leaf water potential (□$_{leaf}$, −MPa). Gray arrows indicate the diurnal evolution of the measurements. Rest as in Fig. 1.

The results of this study have awarded us not only on the need of using a dynamic parameter π in the BMF model, especially under water stress. Despite of the satisfactorily simulation by the BMF model of g_s (Fig. 3), the model was not able to interpret some points at the onset and end of the day (e.g. Fig. 3A).

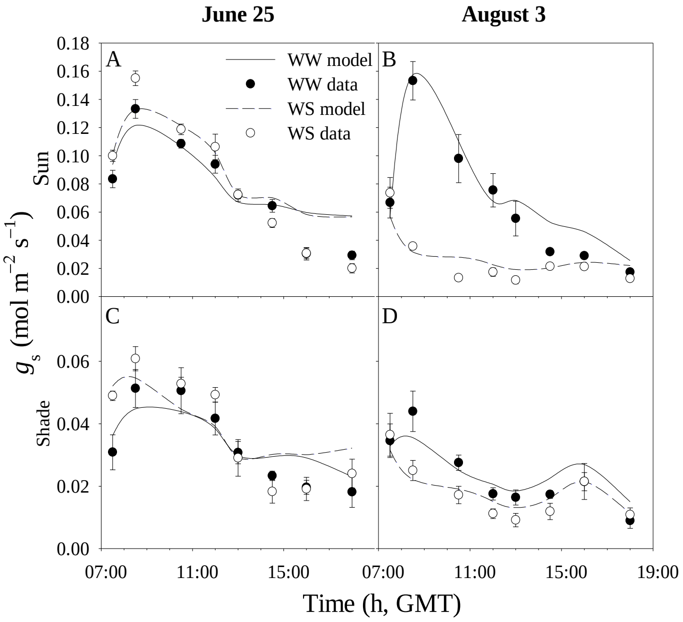

Fig. 3 (left). Evolution of gs data (points) and gs fitted with the BMF model (lines). Rest as in Fig. 1 n = 4 for gs data. GMT = Greenwich Mean Time.

To account for these discrepancies, we evaluated a dynamic diurnal variation in *K* (*K*$_{var}$) as a possible physiological mechanism not considered in the model at its previous version. When the results were plotted as a function of Ψ_{leaf} (Fig. 4), we obtained a relation similar to that reported recently for olive leaves (Torres-Ruiz *et al.*, 2013). This suggests that *K* is also a highly dynamic parameter during the day and should be considered so for an adequate use and interpretation of model outputs.

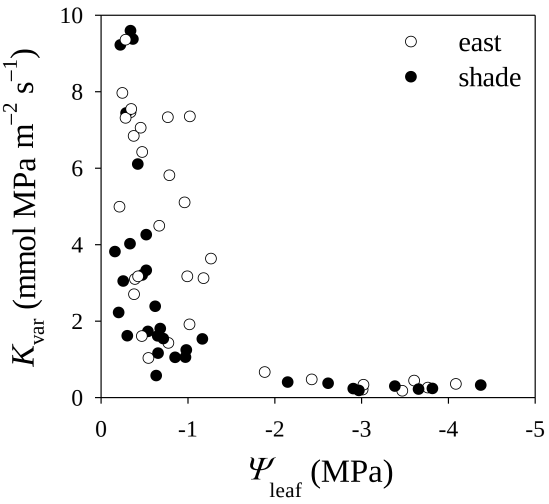

Fig. 4. Diurnal variation of soil-to-leaf hydraulic conductance (K_{var}) plotted against leaf water potential (Ψ_{leaf}) measured in sunny and shaded leaves and mixing the two water treatments and the two experimental dates. Each point represents the average of $n = 4$ (K_{var}) and $n = 3$ (Ψ_{leaf}). Error bars are not presented for a clearer view of the figure.

CONCLUSIONS

The present research has confirmed the potential of the LPCP probes as tools for an automatic monitoring of leaf turgor pressure under field conditions. The combined use with the BMF model highlights the importance of considering in the future the dynamics of leaf osmotic potential and hydraulic conductance, both at a seasonal and daily scale.

ACKNOWLEDGEMENTS

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Phytohormone Profile of Selected Provenances of Pinus Pinaster Aiton.

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ABSTRACT

Pinus pinaster is a forestry species with great economic importance. In order to characterize different genotypes and provide insight into plastic responses, hormonal profile and ecophysiological parameters, clones from contrasting provenances were analyzed during two years of growth. Major changes in ecophysiological parameters were found between seasons, reflecting differences in responses depending on water availability. In contrast, phytohormones showed significant trends depending on the provenance of the materials assayed.

INTRODUCTION

Pinus pinaster is a forestry species with great economic importance mainly due to its facility to adapt to different ecological conditions. However, its adaptation ability is considered to be genotype dependent. Characterization and analysis of the various patterns of adaptation in the early growth stages of different genotypes is essential to determine which individuals are the most suitable for a specific environment.

Phytohormones play a crucial role in plant development and control biotic and abiotic plant responses. While they are structurally diverse compounds, each one having characteristic biological effects, their action mechanisms are interconnected through cross-talk. Thus, monitoring the balance of several phytohormones in different plant developmental stages, as well as in different plant materials, is clearly of great importance.

The objective of this work was to study the phytohormone profiles of three contrasting provenances of *P. pinaster* during two years of growth in order to provide insight into the reasons behind phenotypic diversity. It was thus necessary to develop an analytical method for quantifying the greatest number of plant growth regulators with the least plant material possible.

MATERIAL AND METHODS

A total of 10 provenances representing the natural variation of *Pinus pinaster* were established in Grado, Asturias (Finca Experimental La Mata, 43° 32' N 7° 00 W, 65m). A randomized complete block design (RCB) with 8 blocks and single-tree plots was considered as the baseline experimental design. Clones were planted in 2009 with square spacing (2.4 m x 0.7 m) resulting in 1,632 trees. On the basis of earlier studies where it was observed that certain traits enable the provenance of origin to be discerned, three provenances from different and contrasting climate conditions - Cadavedo (Spanish Atlantic), Oria (Spanish Mediterranean) and Tamrabta (Moroccan Mediterranean) - were sampled three times a year (spring, summer and autumn) during their second and third growing seasons using needles from branches of the most recently grown whorl.

Needle fresh weight (FW), hydrated weight (HW) after immersion in distilled water during 24 h at 4 ºC and dry weight (DW) after one week at 60º C were obtained. Relative leaf water content (RWC) was calculated as (FW-DW)/(HW-DW)*100.

Leaf water potential at predawn (Ψ_{pd}) and midday (Ψ_{md}) were obtained using a Scholander chamber following the methodology described by Scholander et al (1965) and Turner (1988).

Leaf osmotic potential (Ψ_{π}) was measured with a psychrometer using a slight modification of the protocol described by Ngyuen-Querens et al (2002). Leaf osmotic potential at full turgor ($\Psi_{\pi100}$) was also obtained, by rehydrating needles in distilled water during 24 hours at 4 °C in darkness.

Gas exchange parameters; net photosynthesis (A), transpiration rate (E), stomatal conductance (g_s) and intracellular CO_2 concentration (Ci), were measured using an infrared gas analyzer at midday. Intrinsic water use efficiency (WUEi) and carboxylation efficiency (CE) were also calculated as the ratio of A/g_s and A/Ci respectively.

The analysis of different plant growth regulators (epibrasinolide, 24EB; abscisic acid, ABA; indolacetic acid, AIA; benziladenine, BA; castasterone, BK; dihydrozeatin, DHZ; dihydrozeatinriboside, DHRZ; gibberellins GA_1, GA_3, GA_4, GA_7 and GA_9; homobrasinolide, HBL; isopentenyl adenine,iP; isopentenyl adenosine, iPR; jasmonic acid, JA; salicylic acid, SA; zeatin, Z; and zeatinriboside, RZ) was carried out by a modified protocol based on Pan et al (2008): 60 mg of lyophilized plant needles were ground into powder. Then 500 µl of 2-propanol/H_2O/concentrated HCl (2:1:0.002, v/v/v) with internal standards (d_7-BA (10 ng); d_6-ABA, d_5-AIA, DHJA, d_3-DHZ, d_6-SA (20 ng); d_5-BK y d_2-GA1 (40 ng)) were added. Next the mixture was agitated for 30 min at 4 °C after which CH_2Cl_2 (1 mL) was added and agitated again for a further 30 min at 4 °C. This resulted in two phases being formed with the plant debris between them. The lower layer was collected, concentrated in 2 ml glass vials with nitrogen flow and stored until analysis at -20 °C. The cycle of agitations and extraction was then repeated.

Samples were re-suspended in 150 μL of 100% methanol and filtered through a 0.2 μm regenerated cellulose filter. All compounds were separated out and quantified by ultra-high performance liquid chromatography (UHPLC) in a 6460 Triple Quad LC/MS (Agilent Technologies) using the protocol described by Novak et al (2008) for cytokinins, but here performed for each of the plant growth regulators to be analyzed. Chromatographic separation was made using a reverse phase column (Zorbax SB-C18 2.1 x 50 mm column) kept at 40 °C. As mobile phase two solvents were used, MeOH and ultra-purified water, both of them buffered at pH4 with ammonium formate (10 mM). A linear gradient of MeOH from 10% to 50% in 7 minutes and then reaching 100% in 2 minutes was used for analyte elution at a flow rate of 0.45 mL min^{-1}. Plant growth regulators were quantified using ion spray source (ESI), working in both positive and negative mode and acquired by dynamic multireaction monitoring (MRM) of their $[M+H]^+$ and the appropriate product ions, using optimized cone voltages and collision energies for diagnosis of each compound analyzed. Data acquisition and processing were performed using Masshunter Workstation software (Agilent technologies).

Quantitative data were analyzed by repeated measures ANOVA with nested factors (considering year of growth and season as random factors) with a confidence interval set at 95%. All analyses were performed by R Statistical Environment.

RESULTS

Of the hydric potential parameters, Ψ_{pd} presented significant differences ($P<0.05$) between seasons, while Ψ_{md} presented differences between both seasons and provenances. Significant differences in $\Psi_{\pi100}$ were found between seasons, spring being the season which presented the lowest values. In contrast, there were no significant differences in RWC.

Differences in gas parameters were found in A between seasons (P=0.0341), reaching the highest values during summer, while g_s and E presented significant differences only between provenances. There were no significant changes observed in WUEi or Ksp.

Significant differences were, though, found between the different phytohormones analyzed during the periods sampled. Four compounds from structurally different families –AIA, BK, iPR and GA_3- presented differences between provenances while 24EB showed significant differences between seasons.

DISCUSSION

Drought conditions imposed by changing climate conditions represent a signal to which plants must adapt, generally comprising changes such as osmotic adjustment (Nguyen 2002) and photosynthesis adjustment (Ocheltree et al. 2013), commonly mediated by hormonal balance (Granda et al. 2011).

As was to be expected, the major limitations in water availability take places during the summer season, when all the hydric potential measures showed the lowest scores. These values were consistent with the data recorded by the humidity sensors (data not shown) and Ψ_{pd} values. Changes in $\Psi_{\pi 100}$ may indicate an osmotic adjustment, since there is a decline from -1,18MPa in spring to -1,62 and -1,65 MPa in summer and autumn, respectively. At the same time, global content of 24-epibrassinolide, which is closely associated with antioxidation and osmoregulation responses (Xi et al. 2013), tended to increase in summer and autumn, from 0.199 to 0,375 and 0.364 nmol/gDW respectively.

During photosynthesis, plants must confront the challenge of maximizing photosynthesis while minimizing transpiration through their ability to regulate photosynthetic rate and stomatal conductance (Jaime et al. 2014). Photosynthetic rate fell during drought seasons, as did transpiration and stomatal conductance, but the major differences in these two latter parameters were more strongly related to the provenance of origin. It was noted that, although it would be expected that the Atlantic provenance Cadavedo would have the highest photosynthetic rate due to the climatic similarities between this provenance and the study site, this was in fact only the case in the second year of study. A possible explanation is that in the first study year, rooting was not sufficiently well established for the difference to become apparent.

Tamrabta and Oria also differed from Cadavedo provenance in terms of global content of AIA, BK, iPR and GA_3, the former presenting the lowest levels of GA_3 and the highest levels of AIA and BK, both essential regulators of physiological and developmental processes which have been suggested to act inter-dependently and synergistically (Sakamoto et al. 2013). Meanwhile, Oria differed from Cadavedo in iPR content, presenting on average 30% higher levels, consistent with the higher number of growth cycles per year (2.23) observed in this provenance.

Significant differences in global content of phytohormones were found between provenances. These differences were not found between seasons, probably due to large within-season differences in the years of the study at the location where the plants were established.

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Drought induced changes in membrane fatty acids and physiological responses of Arabidopsis plants altered in the expression of the phospholipase A gene *pPLAIIα*

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ABSTRACT

Cell membranes are one of the targets of drought, and early changes in their lipid composition can contribute to tolerance, whereas in latter stages mainly reflect damage. We have identified a drought-induced patatin-like gene in Arabidopsis, *pPLAIIα*, encoding an enzyme that releases fatty acids from membrane lipids. Here we have used plants overexpressing (OE) or silenced (AS) for *pPLAIIα*, to investigate the role of this enzyme in total fatty acid composition under control and drought conditions. To evaluate the physiological impact of membrane lipids changes we calculated photochemical parameters based on OJIP fast fluorescence kinetics. Results show that well watered plant leaves from the three lines display very similar fatty acid composition, except for a higher content of oleic acid (C18:1) in OE. In response to progressive drought OE and wild-type plants are able to increase the content of linolenic acid (C18:3), whereas AS plants do not display a significant C18:3 increase. The photochemical performance also indicates that AS plants are more affected by drought, namely the maximum quantum efficiency of photosystem II (Fv/Fm) and the performance index (PI_{ABS}). The specific energy fluxes per reaction center particularly show limited electron transport (ETo/RC), without changes in absorption (ABS/RC) and trapping (TRo/RC). Our results indicate that pPLAIIα impacts membrane fatty acid composition and improves the photochemical performance, contributing to drought tolerance.

INTRODUCTION

The maintenance of cell membranes function homeostasis is crucial for survival, when plants are subjected to water deficit. Alterations in membrane fatty acid composition may reflect remodeling processes, leading to adaptation, or result from degradation processes (Gigon et al., 2004). Although changes in lipid composition have been described in several plants, the identity of the enzymes involved in such processes remains largely unknown. Patatins belong to a family of lipid hydrolyzing enzymes, firstly described as the major storage proteins in potato tubers (see Scherer et al., 2010). These enzymes are also present in other plants and tissues and release fatty acids from phospho- and galactolipids, the major plastidial lipids. Patatin-like genes are up-regulated by stress, including drought (Matos et al., 2001; Matos et al., 2008; Matos and Pham-Thi, 2009). We have previously observed that the lack of the major drought-induced patatin-like gene in Arabidopsis, pPLAIIα, compromises drought tolerance and negatively impacts photosynthesis under water deficit (Vieira da Silva et al., 2012). However, the composition of the free fatty acid pool was not altered in antisense (AS) or over-expressing (OE) plants. To get further insights on the biochemical and physiological roles of pPLAIIα we analyzed here the total fatty acid composition of wild type (WT) and transformed plants and investigated possible changes in the chlorophyll fluorescence parameters, likely related to drought-induced changes in chloroplast membranes properties.

MATERIALS AND METHODS

Six lines of *Arabidopsis thaliana* were used: two wild type (WT3 and WT8), two RNAi silenced line (AS10 and AS14) and two overexpressing lines (OE10 and OE14), kindly provided by Dr. T. Heitz (La Camera et al., 2005). Plants were grown in 200 cm^3 pots in a mixture of commercial soil (Combo Sana) and vermiculite (1:1) in a growth chamber with a photoperiod of 10h, light intensity of 170 $\mu mol\ m^{-2}\ s^{-1}$, temperature of 23/18°C (day/night) and relative humidity of 70 %. Plants were watered with distilled water and water deficit was imposed by withholding watering for 12 days, to 30-34 days old plants. Lipids were extracted from fully expanded control and drought-stressed leaves accordingly to Bligh and Dyer (1959) and fatty acids methyl esters (FAME) were prepared by acidic trans-esterification as previously described (Matos et al., 2008). FAME were separated in a gas chromatograph (3900 Gas Chromatograph, Varian) equipped with a hydrogen flame-ionization detector using a fused silica 0.25 mm i.d. × 50 m capillary column (WCOT Fused Silica, CP-Sil 88 for FAME, Varian). Margaric acid (C17:0) was used as an internal standard. Chlorophyll *a* fluorescence was measured with a Handy Plant Efficiency Analyzer – Chlorophyll Fluorimeter (Hansatech Instruments, England) as in Silvestre *et al.* (2014).

RESULTS AND DISCUSSION

Under control conditions, the lines did not differ in the relative fatty acid composition, except for oleic acid (C18:1), that was significantly higher in OE (Fig. 1). No differences between lines and treatments were found in C16:1, C16:2 and C18:0. A drought-induced decrease was found in C16:3 and C18:1 in all lines, the decrease of C18:1 in AS being particularly notorious. C18:2 and C16:0 increased with water stress in all lines, but whereas the C18:2 response was similar for all lines, the C16:0 increase was most notorious for AS. OE and wild-type plants were able to increase the content of linolenic acid (C18:3) in response to drought, in contrast with AS plants (Fig. 1).

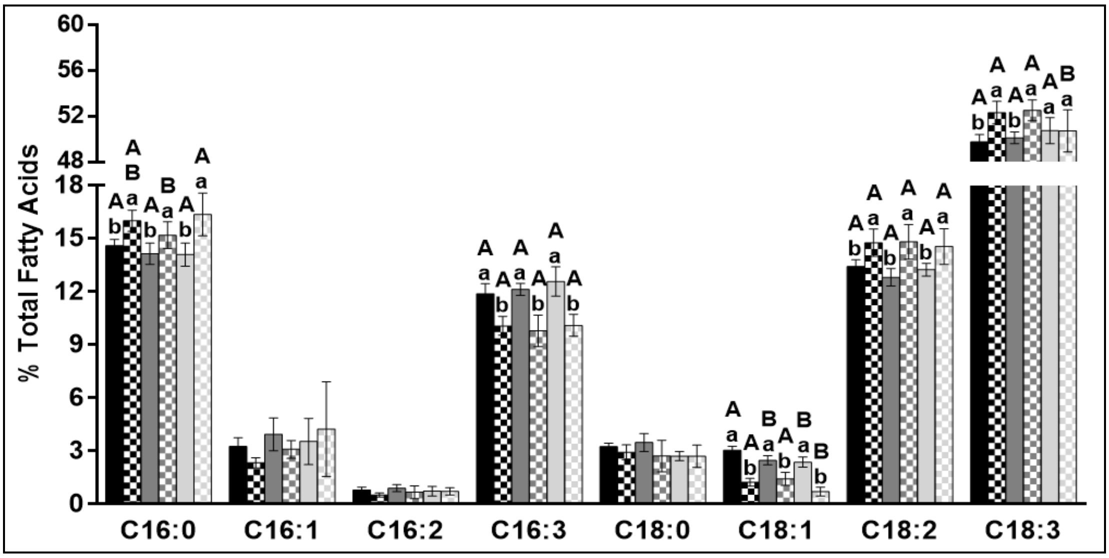

Fig. 1. Fatty acid composition of total lipids from fully expanded leaves of *pPLAIIα* overexpressing (■OE), wild type (■WT), and antisense (■AS) lines under control (no pattern) and drought (pattern) conditions. Values are mean ±SD. Different letters indicate significant differences between lines (uppercase) and treatment (lowercase); no differences were found in C16:1, C16:2 and C18:0.

Linolenic acid is the major polyunsaturated fatty acid present in leaves, comprising the bulk of fatty acids in galactolipids, which are exclusively located in chloroplasts. C18:3 has a fundamental role in maintaining membrane fluidity and decreases in its content are generally associated to membrane damage, while increases might be related to stress tolerance (Gigon et al., 2004). Under control conditions no differences in photochemical parameters between the lines were found (Fig. 2 and 4) except for PI_{ABS} which was significantly higher in OE (Fig. 3).

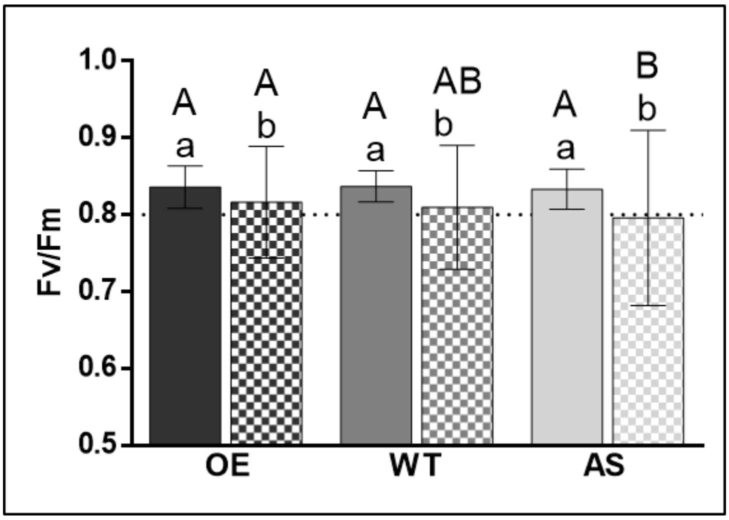

Fig. 2. Maximum quantum efficiency of PSII (Fv/Fm) for *pPLAIIα* overexpressing (■OE), wild type (■WT), and antisense (■AS) lines under control (no pattern) and drought (pattern). Values are mean ± SD. Different letters indicate significant differences between lines (upper) and treatment (lowercase).

It is worth to note that OE showed the only difference on fatty acid composition under control conditions – an increase in C18:1 – suggesting a possible connection between high C18:1 content and the increased photochemical performance. Drought induced a decrease of Fv/Fm in all lines, which however was of lower magnitude in OE and higher in AS (Fig. 2). Also PI_{ABS} had a drought-induced decrease in all lines, but OE was still able to keep a higher value under stress (Fig. 3).The specific energy flux per RC for absorption (ABS/RC) increased similarly in all lines in drought conditions (Fig. 4A).

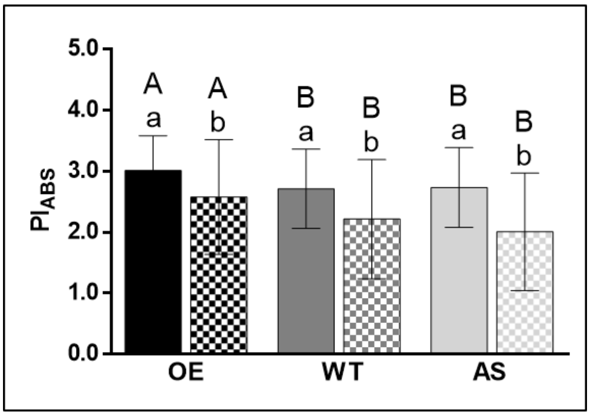

Fig. 3. Performance index on absorption basis (PI_{ABS}) for *pPLAIIα* overexpressing (■OE), wild type (■WT), and antisense (■AS) lines under control (no pattern) and drought (pattern). Values are mean ± SD. Different letters indicate significant differences between lines (upper) and treatment (lowercase).

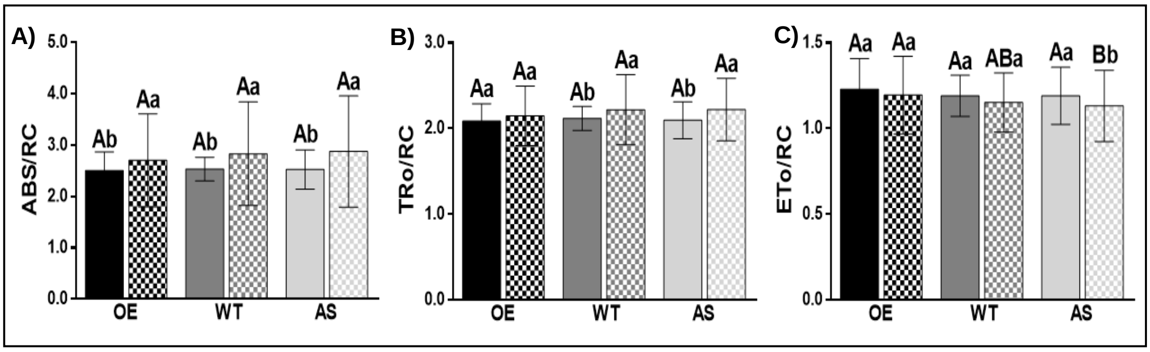

Fig. 4. Energy fluxes per reaction center for absorption A) (ABS/RC), B) trapping (TRo/RC), and C) electron transport (ETo/RC) for *pPLAIIα* overexpressing (■OE), wild type (■WT), and antisense (■AS) lines under control (no pattern) and drought (pattern) conditions. Values are mean ± SD. Different letters indicate significant differences between lines (upper) and treatment (lowercase).

Also the specific energy flux for trapping (TRo/RC) increased with drought in all lines but OE. On the contrary, the specific energy flux for electron transport (ETo/RC) did not respond to drought except in AS, where a decrease was observed (Fig. 4C). Physiological assessment shows that OE line has higher photochemical resilience to drought, contrasting with the increased sensitivity of AS. Taken together, results suggest that pPLAIIα is required for drought-induced membrane adjustments and the lack of this protein negatively impacts photochemical performance.

ACKNOWLEDGMENTS

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Membrane Tolerance in Coffea Species in Response to Environmental Constraints

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ABSTRACTS

Drought is one of the major climate factors that limit coffee production in tropical regions. The aim of this work is to evaluate the effects of drought in photosynthetic capacity (A_{max}) and chloroplast membrane tolerance in two *Coffea* genotypes. Under moderate drought Icatu presented a better photosynthetic performance (A_{max}) than Apoatã. Drought enhanced chloroplast lipid biosynthesis in Icatu, and such newly synthesized lipids presented higher unsaturation (higher DBI), mainly due to higher C18:3 percentage and decrease of the more saturated fatty acids. Higher unsaturation in chloroplasts suggests Icatu has a higher ability to cope with oxidative stress and lipid peroxidation under drought.

INTRODUCTION

Coffee is a tropical crop and one of the most agricultural commodities traded worldwide, constituting the social and economic basis of many developing countries (Ramalho et al., 2013). In the context of climate changes and global warming, low water availability episodes are expected to occur more often, affecting plant physiology and yield. Drought is one of the major climatic limitations in coffee production regions, particularly in marginal lands where coffee cultivation has recently spread (DaMatta and Ramalho, 2006). Water stress causes rapid stomatal closure, reducing transpiration water loss but also the internal [CO_2] and, thus leaf photosynthetic rate. Concomitantly, inhibition or damage in the primary photochemical and biochemical processes may occur. Mesophyll impairments are reflected in the photosynthetic capacity (A_{max}), which allows the evaluation of non-stomatal limitations of photosynthesis and drought tolerance of the photosynthetic machinery (Campos et al., 1999). Drought induced oxidative stress may damage cell structures, further affecting leaf photosynthesis (Chaves et al., 2009). The aim of this work is to evaluate drought responses of 2 *Coffea* genotypes as regards A_{max}, membrane cellular damage (MDA) and chloroplast lipids (Total Fatty Acids, TFA; Double Bond Index, DBI).

MATERIAL AND METHODS

The experiments were carried out using 1.5-year-old potted (10 L) plants of *C. arabica* L. cv. Icatu and *C. canephora* Pierre Ex A. Froehner cv. Apoatã, after transfer into walk-in growth chambers (10000 EHHF, ARALAB, Portugal), under controlled conditions of temperature (25/20 ºC, day/night), photoperiod (12 h), RH (65–70%), irradiance (750–850 □molm^{-2} s^{-1} at the upper part of the canopy) and CO_2 (380 µL L^{-1}). Drought was imposed by gradually reducing irrigation and three treatments were established: well irrigated (Ctr), moderate drought (MD) and severe drought (SD), registering midday relative water contents (RWC) close to 86, 76 and 69%, respectively. These values were kept for 2 weeks before measurements were carried out in the 2 top pairs of recent mature leaves from each branch, from 8 to 10 plants per genotype.

Photosynthetic capacity

Measurements of O_2 evolution expressing photosynthetic capacity, A_{max}, were performed in leaf discs (1.86 cm^2) under irradiance (PPFD 800 µmol m^{-2} s^{-1}) and CO_2 (*ca.* 7%) saturating conditions, at 25ºC, in a Clark-type leaf-disc O_2 electrode (LD2/2, Hansatech, UK). Saturating PPFD was provided by a Björkman lamp (Hansatech), which enabled to study the non-stomatal limitation of photosynthesis under water stress.

Membrane cellular damage (Lipid Peroxidation)

Malonyldialdehyde (MDA) measurements were performed in 500 mg $_{fw}$ of frozen leaf samples using the thiobarbituric acid (TBA) protocol (Cakmak and Horst, 1991). The amount of MDA-TBA complex was calculated from the absorbance at 532 nm, after subtracting the non-specific absorption at 600 nm, using the MDA molar extinction coefficient of 155 mM cm^{-1}.

Chloroplast lipid composition

Chloroplast membranes were obtained from 3-4 g_{fw} of leaf tissue. Lipids were extracted for fatty acids (FA) analysis as described earlier (Scotti-Campos et al., 2014). Fatty acid methyl esters were separated and quantified by gas-liquid chromatography (Unicam 610 Series Gas Chromatograph, Unicam Ltd., U.K.), using a DB-Wax column (J & W Scientific, USA). Total fatty acids (TFA) correspond to the sum of individual FAs. Double bond index (DBI), was calculated as DBI = [(%monoenes + 2 x %dienes + 3 x %trienes) / % saturated FAs].

RESULTS AND DISCUSSION

Under MD, the potential photosynthetic activity (A_{max}) was affected (by 21%) only in Apoatã, whereas under SD both genotypes were significantly impaired (25-30%), still reflecting a high potential (Fig. 1). Well watered plants of both genotypes presented similar TFA amounts, but opposed responses were observed under drought (Fig. 1). TFA reductions were observed under MD (13%) and SD (40%) in Apoatã, whereas increases occurred in Icatu, close to 60% in MD and SD. As regards DBI (Fig. 2), no changes were observed in Apoatã plants under drought, but a strong DBI rise (64%) was found in Icatu under MD, being the increased values maintained under SD (Fig.2). Such rise was linked to changes in major FAs (Tab.1). In fact, palmitic (C16:0) and stearic (C18:0) acids decreased 27 and 31%, respectively, while highly unsaturated linolenic acid (C18:3) increased 22%. Concomitantly to stable DBI, Apoatã depicted unaltered MDA values (Fig. 2).

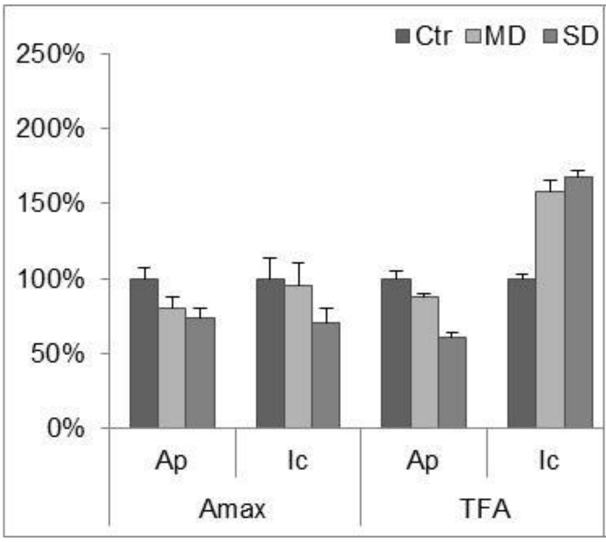

Fig. 1. Effect of moderate (MD) and severe (SD) drought in photosynthetic capacity (A_{max}) and chloroplast total fatty acids (TFA) in two *Coffea* genotypes. Results as % of control, representing the mean ± SE (n=3-6)

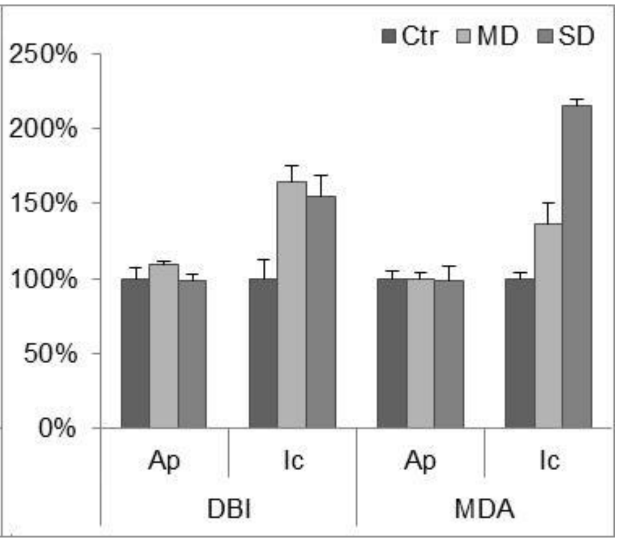

Fig. 2. Effect of moderate (MD) and severe (SD) drought in chloroplast double bond index (DBI) and malonyldialdehyde (MDA) in two *Coffea* genotypes. Results as % of control, representing the mean ± SE (n=3-4).

However, in Icatu MDA increased under MD (36%) and SD (115%). It is generally accepted that drought-tolerant plants maintain their membrane lipid composition as a result of efficient antioxidant mechanisms and control of hydrolytic activities (Torres-Franklin et al., 2009). Although in Apoatã no changes occurred in bulk lipids unsaturation (DBI) or in MDA, a gradual TFA decrease denoted some lipid degradation, and may have contributed for lower A_{max} already under MD. In the case of Icatu, enhanced lipid biosynthesis occurred with drought, as inferred from higher TFA in MD, and their maintenance under SD. Increased lipid amount was previously observed in *Coffea* genotypes responses to photoinhibition, high temperature and chilling (Ramalho et al., 1998; 2013; Campos et al., 2003). As regards drought, increased lipid amounts were reported for several legume species, being considered an adaptation mechanism that allows plants to cope with dehydration induced membrane injury (Scotti-Campos et al., 2013). Furthermore, in Icatu newly synthesized lipids presented higher unsaturation (higher DBI), mainly due to higher linolenic acid (C18:3) percentage and to a decrease in the more saturated fatty acids. Increased unsaturation may confer a higher fluidity to the membranes and contribute to sustain their functionalities (Campos et al., 2003), particularly as regards chloroplasts (Ramalho et al., 1998). However it can also stimulate MDA production, one of the final products of stress-induced lipid peroxidation of polyunsaturated FAs (Campos et al., 2003), what was observed in Icatu. Still, this genotype denoted a better photosynthetic performance (A_{max}) than Apoatã, indicating that MDA rise could reflect a substitution lipid dynamics more than membrane lipid damages. Altogether results suggest Icatu has a higher ability to cope with oxidative stress and lipid peroxidation processes mainly under moderate drought.

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Physiological Evaluation of Drought Tolerance in *Triticum Durum* Genetic Resources

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ABSTRACT

In Mediterranean regions, drought is a major cause for yield reduction of many important food crops, such as wheat. Plants of 7 durum wheat (*Triticum durum* L.) varieties from different breeding groups were grown in a greenhouse under semi-controlled conditions. Drought was imposed by withholding irrigation, during grain filling. Measurements of water potential (ψ_w), gas exchange, instantaneous water use efficiency (iWUE), membrane integrity and pigments were performed. Drought reduced leaf net photosynthesis (P_n) in all varieties, but different responses were observed as regards stomatal conductance (g_s). As regards yield parameters, traditional genotype Cocorit was the less affected, what agrees with its higher iWUE under water stress and unaltered membrane leakage. Advanced Line 2 showed unaltered g_s and an increased yield under drought.

INTRODUCTION

Durum wheat (*Triticum durum*) is considered a minor cereal crop (8-10% of cultivated wheat around the world), but it is an economically important crop due to its unique features related to grain end-use products, namely for pasta industry (Pinheiro et al., 2013). Under Mediterranean conditions, drought is a major cause for yield reduction. The use of more adapted varieties can improve the best use of limited natural resources such as water (Dodd et al., 2011), and to tackle predictable effects of climate changes. Stomatal conductance is considered a useful trait in drought tolerance evaluation (Munns et al., 2010). Under moderate water deficit gradual stomatal closure frequently occurs avoiding transpiration water loss, but leading to CO_2 starvation at the carboxylation sites (Chaves and Oliveira, 2004). Water shortage may induce membrane damage through oxidative stress, which results in cell decompartmentation and death. Therefore, membrane injury can be evaluated through increased electrolyte leakage (Scotti-Campos et al., 2014). The aim of this work was to compare the effects of drought on physiological responses and yield of 7 durum wheat varieties, as a contribute to evaluate genetic variability and adaptability to dry environments, in context of Portuguese wheat breeding programs.

MATERIALS AND METHODS

Durum wheat (*Triticum durum* L.) varieties were selected from different breeding groups: traditional varieties (Castiço, Cocorit), modern currently used varieties (Celta) and four advanced lines: Line 1 (Gediz/Fgo//…), Line 2 (Minimus_6/Plata_16//Immer/3/Sora/…), Line 3 (Ajaia-12/F3Local(Sel.Ethio.135.85)//…) and Line 4 (Arment//2*Sooty_9/…). Seeds were sown in 60 L containers, filled with clay loam soil and maintained in a greenhouse (mean daily values: temperature 23-26ºC; relative humidity 50-60%). Drought was imposed by withholding irrigation for ten days, after visual assessment of anthesis (*ca*. 75-89 DAS). After that, plants were kept under controlled irrigation (droughted plants: 50% of the water given to fully irrigated controls) until harvest, to quantify yield. Leaf water potential, gas exchanges, electrolyte leakage and pigments content were measured in the flag leaf and in the leaf immediately below. Three replicates were performed per treatment. ANOVA ($P < 0.05$) was applied followed by Tukey test for mean comparison.

Water potential

Midday leaf water potential (Ψ_w) was obtained using a PMS1000 (Instruments & Co, USA) pressure chamber (Schölander et al. 1965).

Gas exchange measurements

Leaf gas exchange (net photosynthetic rate, P_n; stomatal conductance, g_s; transpiration, E) were measured in the morning (10:00-12:00h) using a portable CO_2/H_2O infrared gas analyzer (LI-6400, LI-Cor Inc., Lincoln, U.S.A.), according to Matos et al. (1998). Instantaneous water use efficiency (iWUE) was estimated as P_n/E.

Membrane electrolyte leakage

Electrolyte leakage in leaf sections using a conductimeter (GLP 31, Crison Instruments, Spain), and expressed as a percentage of total conductivity, as described in Scotti-Campos et al. (2014).

Leaf pigments

Total leaf chlorophylls and carotenoids were extracted and determined spectrophotometrically (Shimadzu UV160A, Japan) according to Lichtenthaler (1987).

Yield

The number of kernels per spike, the grain weight per spike, the thousand-kernel weight (TKW) and test weight (kg/hl) were obtained after oven drying at 35^{o}C for 72 h.

RESULTS AND DISCUSSION

Gas exchange was compared in well watered ($\psi_w \approx -0.65$ MPa) and drought ($\psi_w \approx -1.3$ MPa) plants (Tab. 1). P_n decreased in all varieties, being Line 4 and Line 3 the less and the most affected (*ca*. 10% and 28% reductions, respectively).

Table 1. Leaf water potential (ψ_w), net photosynthesis (P_n), stomatal conductance (g_s), transpiration (E), instantaneous water use efficiency (iWUE), chlorophyll (Chl), carotenoid (Car) and internal CO_2 (C_i) values, in *T. durum* varieties, under control (Ctr) or water stress (WS), imposed after anthesis. Means ± SE (n=3). Different letters for significant differences between genotypes (a,b,c,d) or between treatments in the same genotype (r,s).

		ψ_w (-MPa)	P_n (μmol $m^{-2}s^{-1}$)	g_s (mmol $m^{-2}s^{-1}$)	E (mmol $m^{-2}s^{-1}$)	iWUE (μmolCO_2 mmolH_2O^{-1})	Chl (mg g^{-1}_{dw})	Car. (mg g^{-1}_{dw})	C_i (μmol mol^{-1})
Castiço	Ctr.	$0.77\pm0.03^{a\ s}$	$16.3\pm0.4^{a\ r}$	$337.2\pm13.3^{ab\ r}$	$6.3\pm0.3^{a\ r}$	$2.8\pm0.2^{a\ r}$	$8.4\pm0.2^{b\ r}$	$1.8\pm0.1^{ab\ r}$	$277.9\pm2.6^{bc\ r}$
	WS	$1.25\pm0.05^{c\ r}$	$12.1\pm0.6^{ab\ s}$	$282.5\pm18.6^{b\ s}$	$5.0\pm0.2^{b\ s}$	$2.4\pm0.1^{c\ s}$	$7.9\pm0.2^{ab\ r}$	$1.9\pm0.1^{a\ r}$	$305.8\pm3.2^{ab\ s}$
Cocorit	Ctr.	$0.70\pm0.06^{a\ s}$	$16.4\pm0.5^{a\ r}$	$424.0\pm21.2^{a\ r}$	$6.3\pm0.3^{a\ r}$	$2.6\pm0.2^{a\ s}$	$6.7\pm0.2^{bc\ r}$	$1.6\pm0.1^{ab\ r}$	$291.2\pm4.9^{ab\ r}$
	WS	$1.33\pm0.03^{bc\ r}$	$12.6\pm0.6^{ab\ s}$	$190.6\pm11.5^{d\ s}$	$2.7\pm0.1^{c\ s}$	$4.9\pm0.1^{a\ r}$	$6.8\pm0.4^{abc\ r}$	$1.7\pm0.1^{ab\ r}$	$256.3\pm4.1^{c\ s}$
Celta	Ctr.	$0.67\pm0.03^{a\ s}$	$14.8\pm0.2^{bc\ r}$	$371.6\pm33.2^{ab\ r}$	$5.7\pm0.3^{a\ r}$	$2.8\pm0.2^{a\ r}$	$8.5\pm0.6^{b\ r}$	$1.9\pm0.1^{ab\ r}$	$297.2\pm8.4^{ab\ r}$
	WS	$1.25\pm0.03^{c\ r}$	$11.0\pm0.8^{ab\ s}$	$431.8\pm40.7^{a\ r}$	$5.0\pm0.3^{b\ r}$	$2.2\pm0.1^{c\ s}$	$8.5\pm0.4^{a\ r}$	$2.1\pm0.1^{a\ r}$	$334.0\pm2.8^{a\ s}$
Line 1	Ctr.	$0.57\pm0.04^{a\ r}$	$14.2\pm0.1^{c\ r}$	$336.0\pm8.3^{ab\ r}$	$6.0\pm0.2^{a\ r}$	$2.4\pm0.1^{a\ r}$	$11.3\pm0.8^{a\ r}$	$2.3\pm0.2^{a\ r}$	$293.7\pm2.6^{ab\ r}$
	WS	$1.45\pm0.05^{a\ r}$	$10.7\pm0.2^{b\ s}$	$265.1\pm12.8^{bc\ s}$	$5.3\pm0.2^{b\ s}$	$2.0\pm0.1^{c\ s}$	$6.2\pm0.3^{bc\ s}$	$1.5\pm0.0^{ab\ s}$	$313.2\pm2.5^{ab\ s}$
Line 2	Ctr.	$0.63\pm0.03^{a\ s}$	$14.0\pm0.2^{c\ r}$	$278.2\pm23.6^{b\ r}$	$5.6\pm0.4^{a\ r}$	$2.8\pm0.2^{a\ r}$	$5.0\pm0.3^{c\ r}$	$1.2\pm0.1^{c\ r}$	$269.0\pm6.2^{c\ r}$
	WS	$1.20\pm0.00^{cd\ r}$	$11.8\pm0.2^{ab\ s}$	$268.7\pm9.5^{bc\ r}$	$5.6\pm0.1^{b\ r}$	$2.1\pm0.0^{c\ s}$	$5.5\pm0.5^{c\ r}$	$1.3\pm0.1^{b\ r}$	$305.9\pm1.8^{ab\ s}$
Line 3	Ctr.	$0.57\pm0.03^{a\ s}$	$14.8\pm0.4^{bc\ r}$	$292.9\pm24.5^{b\ r}$	$6.6\pm0.3^{a\ r}$	$2.3\pm0.1^{a\ s}$	$8.1\pm0.8^{b\ r}$	$1.9\pm0.2^{ab\ r}$	$266.9\pm4.9^{c\ r}$
	WS	$1.42\pm0.04^{ab\ r}$	$10.7\pm0.5^{b\ s}$	$192.7\pm7.5^{cd\ s}$	$3.3\pm0.1^{c\ s}$	$3.2\pm0.1^{b\ r}$	$7.7\pm0.8^{abc\ r}$	$1.9\pm0.2^{a\ r}$	$291.5\pm1.6^{b\ s}$
Line 4	Ctr.	$0.67\pm0.03^{a\ s}$	$15.2\pm0.3^{b\ r}$	$394.9\pm15.6^{a\ s}$	$6.6\pm0.3^{a\ r}$	$2.4\pm0.1^{a\ r}$	$7.8\pm0.6^{b\ r}$	$1.7\pm0.1^{bc\ r}$	$300.3\pm2.3^{a\ r}$
	WS	$1.12\pm0.04^{d\ r}$	$13.7\pm0.1^{a\ s}$	$487.2\pm10.5^{a\ r}$	$7.0\pm0.0^{a\ r}$	$2.0\pm0.0^{c\ r}$	$8.2\pm0.4^{ab\ r}$	$2.0\pm0.1^{a\ r}$	$329.2\pm1.4^{a\ s}$

As regards g_s, no differences were found in Line 2. Celta and Line 4 showed 16% a 23% rises, respectively, while the remaining presented g_s decreases, particularly Cocorit (55%) and Line 3 (34%). Such g_s reductions reflect a strong stomatal closure that lowers CO_2 availability at the mesophyll and chloroplast levels, being responsible for P_n reductions (Matos et al., 1998). This seems to be the case in Cocorit, where a decrease in internal CO_2 (C_i) was observed concomitantly to the highest g_s decrease. The other varieties depicted higher C_i (*ca*. 10%), that may reveal non-stomatal effects of water stress on photosynthetic metabolism, as previously described in bread wheat varieties subjected to heat (Scotti-Campos et al., 2014). Stronger g_s decreases in Cocorit and Line 3 were reflected in greater E reductions (*ca*. 50%). According to Munns et al. (2010), a large stomatal response could be useful for long-term drought, while tolerant genotypes, presenting a small stomatal response, could be useful for irrigation in arid conditions. Cocorit and Line 3 also presented an increased iWUE (90 and 38%, respectively), what is considered a desirable trait for the preservation of plants production (Coutinho et al., 2013). In the remaining genotypes, iWUE showed reductions (12-26%) under drought (Tab. 1). Chlorophylls and carotenoids (Tab. 1) remained unaltered except in Line 1 (decreases of 45 and 34%, respectively), which also presented a high electrolyte leakage (45% increase) under stress (Fig. 1),

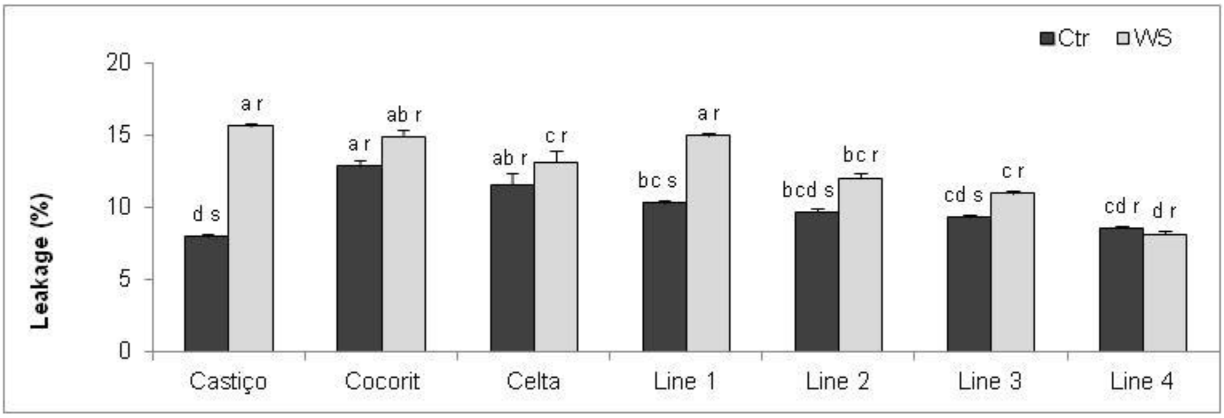

Fig.1. Changes in electrolyte leakage values in leaf sections of seven *T. durum* varieties, under control (Ctr) or water stress (WS) conditions imposed after anthesis. Results are means ±SE (n=3). Different letters express significant differences between genotypes (a,b,c,d) or between control and water stress in the same genotype (r,s).

suggesting the occurrence of membrane lipoperoxidative processes that may lead to senescence (Scotti-Campos et al., 2013). Castiço presented the highest leakage increase (96%), denoting a low protoplasmic tolerance to drought. As regards yield, the negative effect of drought on Line 1 was observed through decreased kernel weight per spike that resulted from a lower number of kernels per spike (Tab. 2).

Table 2 - Changes induced by drought in 1000 kernel weight (TKW), grain yield (number of kernels and kernel weight per spike) and test weight in seven *T. durum* varieties, expressed as variation (%) in relation to control.

	Castiço	Cocorit	Celta	Line 1	Line 2	Line 3	Line 4
TKW (g)	0	-2.1	-6.5	-9.3	-7.5	-6.8	-12.5
Number of kernels/spike	-10.0	-1.1	-17.0	-23.6	31.0	-3.0	-3.7
Kernel weight/spike (g)	-10.3	-2.5	-21.3	-30.3	22.7	-9.2	-14.6
Test weight (kg/hl)	-7.0	0	0	0	0	-2.5	0

In Celta and Lines 1, 2, 3 and 4 (Tab. 2), TKW reduced significantly and test weight (commercial yield indicator giving the mass in kg of 1 hl grain) remained unaltered, indicating that well shaped kernels were formed and grain filling was preserved, in spite of the smaller grain size reflected by lower TKW. Konopka et al. (2007) reported that water-stressed wheat kernels were smaller in size due to a reduction in their thickness and width. During early seed development, cell proliferation process is controlled by seed size regulatory genes which are regulated by abiotic stresses, among other factors (Kesavan et al., 2013). As regards yield parameters, Cocorit was the less affected (Tab. 2), what agrees with the highest iWUE observed under drought (Tab. 1). Advanced Line 2 showed an increased performance under drought (rises in number of kernels and kernel weight per spike), probably linked to the ability to maintain unaltered g_s values and a moderate 16% reduction in P_n.

ACKNOWLEDGMENTS

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Microclimatic Conditions and Gas Exchange in a Vineyard Under Plastic Cover

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ABSTRACT

Plastic covers are being used in an increasing number of vineyards to avoid problems derived from high radiation and rainfall levels. The effects of this new management system on microclimatic, gas exchange and crop water demand are not well known. In this context, the objective of this study was to analyse possible changes in soil moisture, microclimatic variables and gas exchange in a vineyard (cv. Niágara Rosada, for table consumption) under transparent plastic cover. The experiments were performed in São Fidelis, Rio de Janeiro State, Brazil, on a growing cycle (March to June 2013). The crop was covered at the top with a 160 µm thick low density polyethylene film. Within a commercial vineyard (1 ha) we had two plots (covered and uncovered, 0.035 ha each), each one instrumented with a meteorological station. Soil humidity was determined by gravimetry. Stomatal conductance (g_s) and net photosynthesis (A_N) measurements were made at 08.00-10.00 am, in 5 plants of each treatment and on days 42, 56, 68, 91 and 106 after pruning. The plastic cover permitted greater soil humidity, reduced the photosynthetic active radiation, and increased the maximum and medium temperature and the vapor pressure deficit. The greater soil water availability, however, may not lead to greater plant water use efficiency.

INTRODUCTION

Plastic covers are being used in an increasing number of vineyards to avoid problems derived from high radiation and rainfall levels. The effects of this new management system on microclimatic, gas exchange and crop water demand are not well known, although there are evidences for the use of plastic covers having a major influence on microclimatic conditions (Chavarria et al., 2009).

According to Barradas et al. (2005) a transparent plastic cover upon the grape canopy may decrease solar radiation and atmospheric demand. In addition, the plastic cover would affect the photosynthetic rate by means of the reduction of light incidence.

In fact, quantifying the effects of covers of crops over a variety of plants has risen considerable interest in the scientific community (Tanny, 2013). In this context, the objective of this study was to analyse possible changes in soil moisture, microclimatic variables and gas exchange in a vineyard (cv. Niágara Rosada, for table consumption) under transparent plastic cover.

MATERIALS AND METHODS

The experiments were performed in São Fidelis, Rio de Janeiro State, Brazil, on a growing cycle (March to June 2013). The experimental area is between latitude 21º 30' 58" S and longitude 41º 42' 49.6" W. The cultivar was Niágara Rosada grafted on the IAC-766 root stock, in trellising system, spacing 2.0 m x 2.7 m, drip irrigated.

The crop was covered at the top with a 160 μm thick low density polyethylene film (Fig. 1). Within a commercial vineyard (1 ha) we had two plots (with plastic cover and without plastic cover, 0.035 ha each). Each plot was instrumented with a meteorological station for collecting data on temperature (ºC), relative humidity of the air (%) and photosynthetic active radiation (μmol m^{-2} s^{-1}). The vapor pressure deficit (VPD, kPa) was calculated according to Allen et al. (1998). For the assessment of soil humidity by means of the gravimetric method, soil samples were collected every 15 days, down to a depth of 0.6 m, and at about 0.5 m from the stem of three plants per plot.

Fig. 1. Detail of plastic cover over the canopy of Niagara Rosada.

The stomatal conductance and net photosynthesis was performed with a Licor Li-6400 portable infrared analyzer (LI-COR, Lincoln, NE, UA). We measured on 5 plants per treatment, from 08.00 to 10.00 am on days 42, 56, 68, 91 and 106 after pruning. Light intensity within the chamber was fixed to 1500 μmol m^{-2} s^{-1}, to evaluate maximum gas exchange under non-restrictive light conditions.

RESULTS AND DISCUSSION

All environmental variables, except minimum air temperature (T_{min}), were affected by the cover (Table 1). The lack of effect on T_{min} could be explained by major effects of the plastic cover on air temperature occurring on daytime, thus affecting mainly maximum temperature (Cardoso et al., 2008).

Table 1. Maximum (T_{max}), minimum (T_{min})) and average (T_{avg}) air temperatures, maximum (RH_{max}), minimum (RH_{min}) and average (RH_{avg}) relative humidities, vapor pressure deficit (VPD), active photosynthetic radiation (PAR) and average soil volumetric moisture (SVM). Values are for the studied period.

Variables	Without plastic cover	With plastic cover
T_{max} (°C)	30.68	33.32**
T_{min} (°C)	18.58	18.65
T_{avg} (°C)	24.42	25.98**
RH_{max} (%)	89.32	86.80**
RH_{min} (%)	47.50	43.47**
RH_{avg} (%)	68.41	65.13**
VPD (kPa)	2.42	2.66*
PAR (μmol m^{-2} s^{-1})	708.20	371.50**
SVM (%)	20.58	26.85*

**significant difference at 1% probability, between treatments, according to *F*-test.

*significant difference at 5% probability, between treatments, according to *F*-test.

The lower PAR values registered in the covered plot agree with findings reported in the literature. The availability of solar radiation inside of protected environments is smaller in comparison to open environments due to light reflection and absorption by the plastic material of the cover (Beckmann et al., 2006), reducing soil evaporation. This fact can explain the major soil moisture under plastic cover environment. Potential g_s values were greater in plants under cover, in almost all the measured days. Just on the first measuring day (day 42 after pruning) similar g_s values were observed in the two treatments. Under the plastic cover the water content of the soil was higher during the whole experimental period, which possibly contributed to the highest rates of stomatal opening. Chaves et al. (2002) reported that the stomatal responses are more closely related to soil water content than to plant water status. However, under the conditions of this study, the potential photosynthesis rate did not differ between the treatments.

Table 2. Stomatal conductance (g_s) and net photosynthesis (A_N) values.

	g_s (mol m^{-2} s^{-1})		A_N (µmol CO_2 m^{-2} s^{-1})	
Days after pruning	Without plastic cover	With plastic cover	Without plastic cover	With plastic cover
42	0.29 a	0.41 a	9.19 a	10.60 a
56	0.32 b	0.43 a	13.74 a	13.90 a
68	0.28 b	0.44 a	14.54 a	14.90 a
91	0.28 b	0.42 a	10.09 a	13.33 a
106	0.26 b	0.41 a	9.75 a	12.46 a

Values followed by the same letter are not significantly different (Tukey, $p \leq 0.05$)

Two main findings can be deduced from these results. First, g_s in plants under cover is favored by the greatest soil water availability. Second, the recorded increase in g_s does not increases the plant water use efficiency, likely because of the A_N/g_s relationship for grapevine plants, which shows a reduced impact of any increase in g_s over ca. 0.3 mol H_2O m^{-2} s^{-1} on A_N (Flexas et al., 2002).

CONCLUSIONS

The plastic cover permitted greater soil humidity, reduced the photosynthetic active radiation, and increased the maximum and medium temperature and the VPD. The greater soil water availability, however, may not lead to greater plant water use efficiency.

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Chloride Nutrition Regulates Water Balance in Plants

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ABSTRACT

Cl^- is a strange micronutrient since actual Cl^- concentration in plants is about two orders of magnitude higher than the content required as essential micronutrient. This accumulation requires a high cost of energy, and since Cl^- is a major osmotically active solute in the vacuole, we propose that Cl^- plays a role in the regulation of water balance in plants. We show here that, when accumulated to macronutrient levels, Cl^- specifically regulates leaf cell elongation and water balance parameters, improving water relations at both the leaf tissue and the whole plant levels, increasing drought resistance in higher plants.

INTRODUCTION

Chloride (Cl^-) is one of the 16 elements essential for plant growth. Because it is supposed to be needed in small quantities for healthy growth of higher plants (<50-100 mM in the nutrient media), Cl^- was described as a micronutrient (Johnson *et al.*, 1957; Terry, 1977). Usually non-halophytic plants present critical deficiency contents below 0,2 mg/g shoot dry weight (reviewed in Flowers, 1988; Marschner, 1995; White and Broadley, 2001), under which deficient plants show a significant decrease in leaf area as a result of a reduction in cell division rates (Terry, 1977). However actual Cl^- concentration in plants is typical of the content of a macronutrient (10-60 mg/g, about 50-300 times higher than the content required as essential micronutrient; Marschner, 1995; Brumós *et al.*, 2010). This accumulation requires a high cost of energy (Brumós *et al.,* 2010), and since Cl^- is a major osmotically active solute in the vacuole (Flowers, 1988), we hypothesize that Cl^- may regulate water relations and drought resistance in higher plants when accumulated to macronutrient levels.

MATERIALS AND METHODS

Tobacco plants were grown under well-irrigated semi-hydroponic conditions subjected to different nutritional treatments consisting on a basal nutrient solution (BS) or BS supplemented with different salt combinations: Cl^- salts (CL); NO_3^- salts (N); and SO_4^{2-} +

PO_4^{3-} salts (SP). All treatments (CL, N and SP) contained the same concentration of charge-balancing cations. Plants were subjected to two irrigation treatments: optimal irrigation (Control, 100% of field capacity), and water deficit, in which pots were irrigated every two days to 60% of field capacity. Materials and methods are described in Franco-Navarro *et al.* (2013 a,b). Mean values ± SE, $n = 4 - 6$. Levels of significance (ANOVA and MANOVA test): $P \leq 0.05$ (*); $P \leq 0.01$ (**) and $P \leq 0.001$ (***).

RESULTS AND DISCUSSION

As it was shown before (Franco-Navarro *et al.*, 2013a,b), no differences of leaf cations content (Ca^{2+}, Mg^{2+} and K^+) were observed among CL, N and SP treatments. Cl^- is an essential cofactor in photosynthesis, but the primary factor involved in the reduced growth under a deficient condition was a reduced rate of cell division in the leaves (Terry, 1977). We verified that photosynthesis and leaf cell division rate were not impaired in plants subjected to low Cl^- treatments, demonstrating that BS, SP or N treatments covered essential micronutrient requirements of Cl^- (Fig. 1A-B).

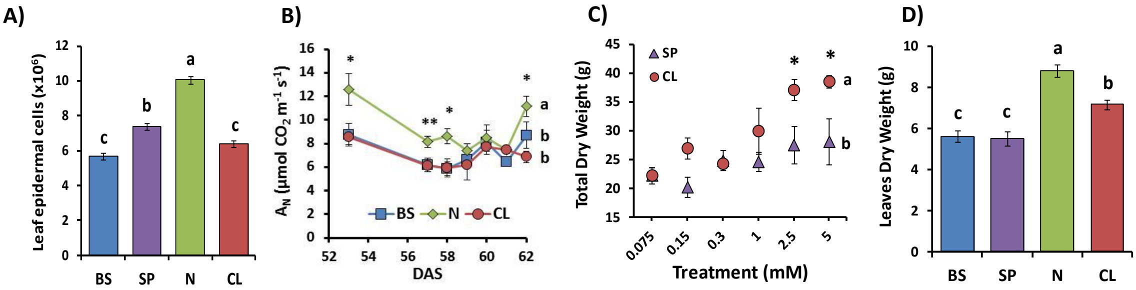

Fig. 1. Effect of Cl^- nutrition on photosynthesis, cell division and growth. (A) Cell division rate, quantified as the number of epidermal cells per leaf. (B) Effect on net Photosynthetic rate (A_N) measured in fully expanded and photosynthetic leaves. (C) Total plant dry weight. (D) Leaves Dry Weight. Days after sowing (DAS).

Plant growth was stimulated with NO_3^- and Cl^- treatments (Fig. 1D). As it was expected, NO_3^--treated plants showed the highest growth and biomass because of the important role of nitrogen in plant growth and development (Marschner, 1995). Cl^--treated plants showed a progressively enhanced growth with increasing concentrations of Cl^- from 1 mM (Fig. 1C). Whereas NO_3^- stimulated cell division (Fig. 1A) and photosynthetic metabolism (Fig. 1B), Cl^- specifically stimulated cell elongation in the leaf (Fig. 2). This was particularly evident in epidermal cells (Fig 2A), significantly more elongated in Cl^--treated plants (Fig 2B), and this effect was progressively enhanced with increasing concentrations of Cl^- (Fig 2C).

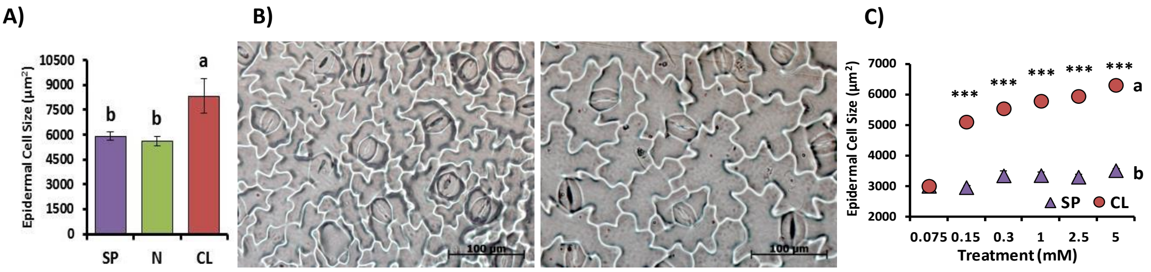

Fig. 2. Effect of Cl^- nutrition on cell elongation. (A,C) Epidermal Cell Size. (B) Epidermal cell size observed in microscopy images of leaf epidermal impressions of SP-treated plants (left) and Cl^--treated plants (right).

Our working hypothesis was that Cl^- nutrition in the millimolar range might specifically improve the hydric state of plant tissues and water parameters at the whole plant level. In the leaf, water content was specifically stimulated by Cl^- over SP in the millimolar range concentration (Fig. 3A).

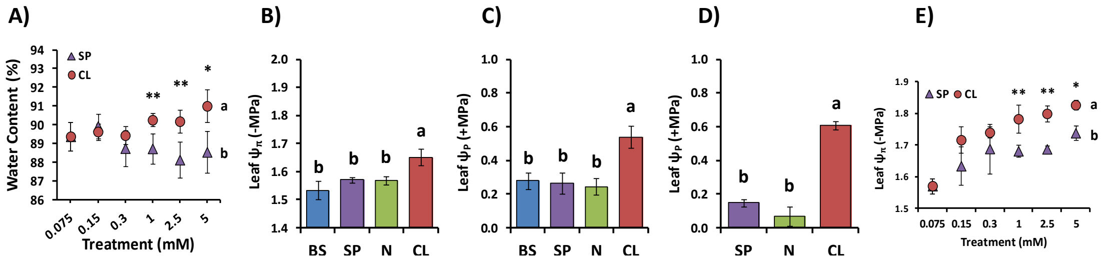

Fig. 3. Effect of Cl^- nutrition on leaf osmotic potential and turgor, and water content. (A) Leaf Water Content. (B,E) Mature Leaf Osmotic potential; (C,D) Mature Leaf Turgor potential; (B,C,E) Pre-dawn values; (D) Midday values.

The higher leaf tissue osmolarity observed in Cl^--treated plants reflects a higher amount of osmotically active solutes per dry weight unit (Franco-Navarro *et al.*, 2013a,b). This correlates with more negative leaf osmotic potential ($\Psi\pi$) in mature leaves of plants treated with Cl^- in the millimolar range (Fig. 3B, 3E). It was observed that pre-dawn leaf turgor (Ψp) from Cl^--treated plants was significantly higher than that from BS, SP and N plants (Fig. 3C), and differences were more evident at mid-day (Fig. 3D). Occasionally, adult plants treated with NO_3^- showed visual symptoms of turgor loss in the greenhouse at times of high evapotranspiration demand (not shown), which correlated with turgor values close to zero measured at mid-day (Fig. 3D). In plants, the most important factor regulating water content is leaf transpiration. Interestingly, CL plants (from 1-5 mM) showed lower transpiration than SP, N and BS plants, quantified as the relative loss of fresh weight measured in detached leaves (Fig. 4A).

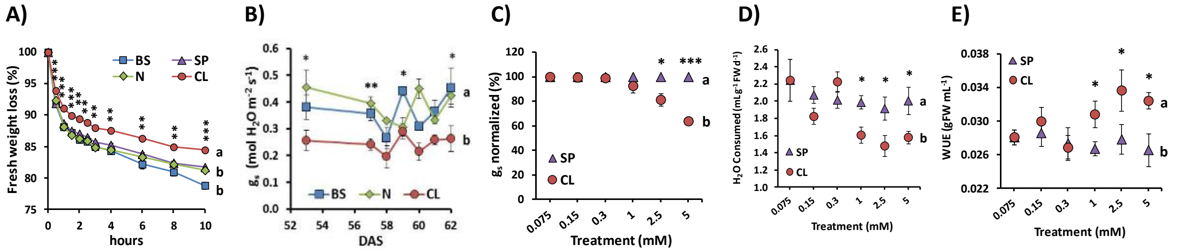

Fig. 4. Effect of Cl⁻ nutrition on leaf transpiration. (A) Fresh weight loss in detached leaves. (B,C) Stomatal conductance; (D) water consumption related to fresh biomass; (E) Integrated Water Use Efficiency;

Reduced transpiration of CL plants was a consequence of the lower stomatal conductance (g_S) observed in Cl⁻-treated plants (Fig. 4B-C), which in turn determined lower consumption of water (Fig. 4D) and higher water use efficiency measured as total plant weight relative to total water consumption (WUE; Fig. 4E).

When subjected to water deficit, Cl⁻ plants were able to keep higher leaf turgor values compared to SP (Fig. 5A). Cl⁻ plants presented significantly higher A_N (Fig. 5B), resulting in a higher photosynthetic water-use efficiency (WUEi; Fig. 5C); As a consequence, Cl⁻-treated plants presented higher PSII quantum yield values along the water deficit treatment (Fig. 5D), indicating a more favorable preservation of PS-II integrity and, therefore, a better ability of plants to withstand water deficit.

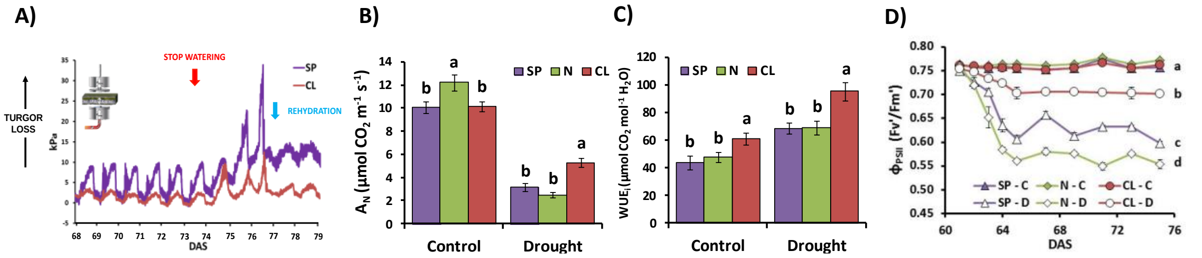

Fig. 5. Effect of Cl- nutrition on drought resistance. (A) Leaf turgor measurement using the magnetic leaf patch clamp pressure probe (Zimmermann et al., 2008). Red arrow indicates stop watering and blue arrow indicates rehydration. (B,C) 3-days averaged data measured from plants subjected to 2 irrigation treatments: (B) Net Photosynthetic rate (AN); (C) Photosyntetic Water Use Efficiency. (D) PSII Efficiency or quantum yield (ɸPSII). Labeled "C" (filled symbols) corresponds to control irrigation, and "D" (empty symbols) corresponds to water-deficit irrigation.

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Physiological Comparison Behaviors between Two Cultivars of *Vitis Vinifera* (Grenache and Tempranillo Cvars.)

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ABSTRACT

Knowledge about regulation of stomatal conductance is necessary to improve grapevine water use efficiency. The rich amount of grapevine varieties let us the opportunity to choose the better enhanced cultivars to global warming changing conditions. A comparison between two reputed cultivars (Tempranillo and Grenache) with contrasted water use efficiency was performed during two years in a field experiment. Water relations, leaf gas exchange and abscisic acid (ABA) were measured in different stages of grapevine growing season. A clear difference in the regulation of leaf water relations parameters under water stress was observed between both cultivars. Results indicated that there is a clear relationship between hydraulic conductance (K_h) and stomatal regulation. ABA can exert a role on the stomatal control during different stages of grapevine growth period. The combination of both signals, ABA and K_h, controls stomatal aperture in a different way in the two varieties leading to differences in water use efficiency between them.

INTRODUCTION

Grapevines are among the most genetically heterogeneous plant species (Mullins *et al.* 1992). Such large genetic variability should allow the opportunity to choose cultivars with the highest water use efficiency (WUE), to counteract potential negative effects of global warming. Many works have compared cultivars in terms of WUE, resulting in contrasting results depending on environmental conditions, namely the intensity and duration of water stress, as well as the diversity of grapevine cultivars (Bota *et al.* 2001; Shultz, 2003; Tomàs *et al.* 2012; Pou *et al.* 2012). Different regulatory mechanisms have been identified in the control of turgor on guard cells and accordingly in g_s, but it is still complex to know exactly how they act, since the response could be mediated by various processes at the same time.

In this study, a field trial was conducted with two cultivars with contrasting reputation in their adaptation to drought. The Grenache cultivar is reputed as more adapted to hot and dry areas than Tempranillo (Shultz 2003; Medrano et al. 2003), and usually presents higher WUE (Tomàs *et al.* 2012; Pou *et al.* 2012). A deeper knowledge on how these two cultivars regulate g_s and water relations in response to water stress is of paramount importance if we intend to understand the fundamental processes of stomatal control on transpiration and its effect on WUE. As a consequence, we have examined in this study which are the physiological characteristics that differentiate Tempranillo and Grenache cultivars responses to water stress. The main objective was to unravel the role played by hydraulic and chemical signals in the regulation of transpiration by stomata in both cultivars.

MATERIAL AND METHODS

Plant material and treatments

The experiment was conducted in the experimental field of the University of Balearic Islands on grapevine plants of Tempranillo and Grenache cultivars during the summer 2011 and 2012. Plants were two years old (planted in 2010) grafted on Richter-110 and planted in rows. They were trained in a bilateral double cordon having between 6-8 canes per plant in 2011 and 10-12 canes per plant in 2012. Two irrigation treatments were applied: well watered plants (WW), which received around 3 liters per plant and day (K_c around 0.3) by 2011 and around 9 liters per day per plant in 2012 (K_c around 0,75). The dosage increase was adjusted because the much higher vegetative growth in 2012 than in 2011 (approximately 50% of difference in total leaf area between both years). The Water Stress (WS) treatment consisted in withholding irrigation during the whole summer in both years.

Leaf water relations

Midday leaf water potential (Ψ_{md}), and predawn water potential (Ψ_{pd}) were measured every week from May to September. Water potentials were measured with a pressure chamber (Soil moisture Equipment, Corp., Santa Barbara, CA, USA). Measurements of Ψ_{pd} were before sunrise and Ψ_{md} was measured at noon. Four replicates per treatment and cultivar were measured.

Pressure volume curves were measured to obtain leaf turgor loss point ($\Psi_{\pi,TLP}$), osmotic potential at full turgor ($\Psi_{\pi,FT}$), relative water content at turgor loss point (RWC_{TLP}) and bulk leaf modulus of elasticity (ε) in five replicates per treatment (Tyree and Hamel 1972). Curves were performed at the initial phase of water stress treatments (later June dates for each season). Five replicates per treatment were done. *Gas exchange measurements*

Leaf gas exchange was measured using a portable open gas exchange system (Li-6400; Li-Cor, Inc., Nebraska, USA) equipped with a light source (Li-6400-02B LED, Li-Cor).

Environmental conditions in the chamber were: 1500 µmol m^{-2} s^{-1} of photosynthetic photon flux density, ambient air temperature and a CO_2 concentration of 400 µmol mol^{-1}. Stomatal conductance (g_s), net photosynthesis (A_N) and transpiration (E) were measured monthly at mid-morning around 11-13 am (local time) in younger fully expanded leaves of five plants per treatment.

Hydraulic conductance measurements

Whole plant hydraulic conductance (K_{hplant}) was calculated considering K_{hplant} from the Ohm's law analogy for the soil–plant–atmosphere continuum (Lovisolo *et al.* 2002):

$E = K_{hplant}$ x ($\Psi_{soil} - \Psi_{leaf}$);

where E, Ψ_{leaf} and Ψ_{soil}, were transpiration rate, leaf water potential and soil water potential, respectively. Ψ_{md} was taken as Ψ_{leaf} and Ψ_{pd} was taken as a proxy for $\Psi_{soil.}$

ABA extraction, purification and quantification

ABA was determined by liquid chromatography–tandem mass spectrometry (UHPLC-MS-MS, Agilent 1290 Infinity, Waldbronn, Germany) following the method described by Gomez-Cadenas *et al.* (2002). Xylem leaf sap was extracted in the field with a Scholander chamber at 10th July 2012 and 23rd and 27th of August 2011 and 2012 respectively at 9 o'clock (local hour). A maximum overpressure of 0.2 MPa was applied until 10-15 µL of sap was obtained, which occured typically after 2-3-min.

RESULTS AND DISCUSSION

Consistent with previous reports in potted plants, Grenache showed slightly higher water use efficiency (WUE) than Tempranillo (Flexas *et al.* 2010; Tomàs *et al.* 2012;). The data presented in this study for field growing plants during two consecutive years confirmed the reputation of Grenache as more recommendable for drought prone areas (Jones, 2006; Van Leeuwen *et al.* 2013) and of increasing interest for foreseeing climatic change conditions. Higher WUE was more evident under water stress even though under irrigation or mild water stress the differences were also present.

The identification of the physiological basis of this difference was attempted by measuring, leaf gas exchange rates, plant hydraulic conductance and ABA in xylem sap (Fig 1 and 2). Grenache and Tempranillo showed differential regulation of water relations and ABA. Tempranillo plants were able to modify osmotic water potential at full turgor and turgor loss point enabling to maintain turgor at lower water potentials (data not shown).

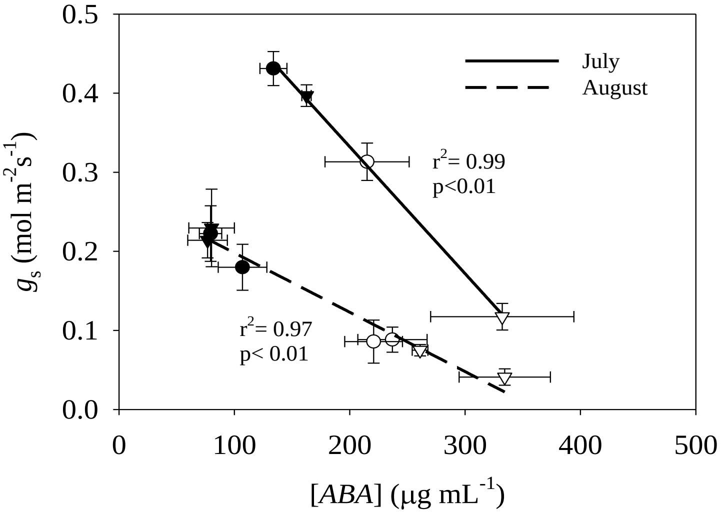

Fig. 1. Stomatal conductance (g_s) versus abscisic acid xylem sap concentration ([ABA]) under well watered conditions (full symbols) and water stress (blank symbols) between both cultivars. Grenache is represented by triangles and Tempranillo by circles. Two sampling dates were differentiated, July solid line and August dashed line. Values are five replicates ± standard errors.

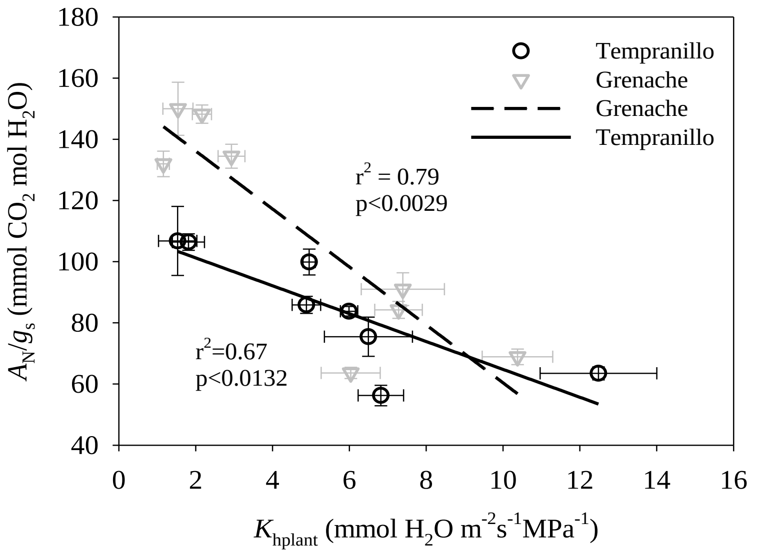

Fig. 2. Relationship between plant hydraulic conductance (K_{hplant}) and water use efficiency (A_N/g_s) only for water stress treatments. Circles represent Tempranillo variety and triangles Grenache. Each regression line represents a different variety including data from 2011 and 2012. Regression coefficients are shown on the graphic and values are five replicates ± standard errors.

Also, a lower ABA concentration was found constitutively in Tempranillo (Fig. 1). In contrast, Grenache showed more constant water relations and displayed higher levels of ABA. Both cultivars were different in terms of WUE and the combination of ABA, K_{hplant} and leaf water relations were the responsible for the distinct behavior between both cultivars (Fig. 1 and 2).

ACKNOWLEDGEMENTS

This work was funded by the Spanish Ministry of Science and innovation (research projects AGL2008-04525-C02-01, AGL2009-11310/AGR and AGL2011-30408-C04-01). S.M. benefited from a FPI grant BES-2009-016906 from the Spanish Ministry of Science and Innovation. Also we are very grateful to the facilitation in the use of the UHPLC-MS-MS for ABA analysis at CITIUS, University of Seville.

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Testing four methods to assess leaf area in young olive trees

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ABSTRACT

Assessment of leaf area is of fundamental importance to calculate diverse physiological parameters such as the total rate of transpiration or photosynthesis of a plant or its leaf area index (LAI).

This paper reports the determination of total leaf area in 3 year-old olive trees of two varieties using four different methods and leaf counting as the control method.

Leaf area was determined on 7 plants of each variety, Arbequina and Cobrançosa, by thoroughly counting the total number of leaves on each plant and then multiplying by the average leaf area for each variety

Leaf area of the same plants was then estimated by (1) the analysis, with software ImageJ, of a photo of each plant from a side or from the top, (2) the crown light scattering measured with a ceptometer (AccuPAR Model LP-80) in the ground at 10 and 20 cm from the trunk, (3) the analysis with software Hemisfer of a photo of the plant from the top, and finally (4) by the determination of the transpiration (mmol s-1) of each plant over a 2 hour period by a weighing method and dividing this value by the mean transpiration rate (E) of each plant, calculated from the measurement of stomatal conductance (g_s) on 10 leaves with a porometer and mean air RH and leaf and air temperature over the same period.

Both the ceptometer and the Hemisfer software give a value of leaf area index (LAI). In order to obtain the leaf area, LAI was divided by the projected area of the crown obtained on photos from the top.

Average leaf area (n = 7 × 30) was $0,238 \times 10^{-3}$ m^2 and $0,264 \times 10^{-3}$ m^2 for Arbequina and Cobrançosa, respectively. Total leaf area of each plant varied from 3,38 m^2 and 4,85 m^2 for Arbequina and 0,86 m^2 and 3,99 m^2 for Cobrançosa. Ceptometer and analysis of photos from a side gave estimates of leaf area under 50% of the actual leaf area. Analysis of photos from the top gave on average 70% the actual values. The most accurate methods were analysis with software Hemisfer and by the ratio of transpiration by E which gave values 5% bellow or 20% above actual values, respectively.

INTRODUCTION

Assessment of leaf area (Jonckheere et al. 2004; Weiss et al. 2004) is of fundamental importance to calculate diverse physiological parameters such as the total transpiration or photosynthesis of a plant or its leaf area index (LAI).

This paper reports the determination of total leaf area in 3 year-old olive trees of two varieties, Arbequina and Cobrançosa, using five different methods, leaf counting (as the control method), photographs from a side or from the top, ceptometer, Hemisfer software and finally by measuring plant transpiration and leaf conductance.

MATERIALS AND METHODS

Leaf area was determined on 7 plants of each variety, Arbequina and Cobrançosa, by thoroughly counting the total number of leaves on each plant and then multiplying this figure by the average leaf area for each variety. Average leaf area was determined by the measurement, on photographs, of the exact leaf area of seven samples of 30 leaves each using ImageJ software. This procedure gave a value for each plant's total leaf area with an estimated error of less than 10%.

Leaf area of the same plants was then estimated by four different methods. On the first method, a ceptometer was used (AccuPAR Ceptometer Model LP-80, Decagon Devices, Inc., U.S.A.) to measure the crown light scattering in the ground at 10 and 20 cm from the trunk. The second method consisted simply on the analysis of plant photos from the top with software Hemisfer (ref). The third method to determine leaf area was done by the analysis, with software ImageJ (Rasband, 1997), of photos of each plant from two sides and from the top, against a white background. Finally, leaf area was estimated by the determination of the transpiration (mmol s^{-1}) of each plant over a 2 hour period (10 to 12 am) by a weighing method and dividing this value by the mean leaf transpiration rate (E, mmol m^{-2} s^{-1}) obtained from the measurements of mean leaf conductance (g_s) measured at 11 am on 10 leaves, 7 sun-exposed and 3 shadowed, with a porometer (AP4, Delta-T devices, U.K.), and the mean difference in the molar fraction of water vapor between the leaf and the air obtained from leaf temperature and air humidity and temperature at 11 am.

Both the ceptometer (AccuPAR Model LP-80) and the Hemisfer software give a value of leaf area index (LAI). In order to obtain the leaf area, it was necessary to get a value for the projection of the crown. This value was obtained analysing the photos from the top with ImageJ and looking at the total area (including blank spaces) of the crown (Fig. 1, C).

RESULTS

Actual leaf area of each plant was initially estimated by counting the number of leaves on each plant and multiplying this value by the average leaf area for each variety. Average area of a leaf (n = 7 × 30) was 0,238 × 10^{-3} m^2 and 0,264 × 10^{-3} m^2 for Arbequina and Cobrançosa, respectively. Total leaf area of each plant varied from 3,38 m^2 and 4,85 m^2 for Arbequina and 0,86 m^2 and 3,99 m^2 for Cobrançosa.

After that, leaf area was indirectly assessed by four other methods. Fig. 1 shows an example of the determination of leaf area by these indirect methods.

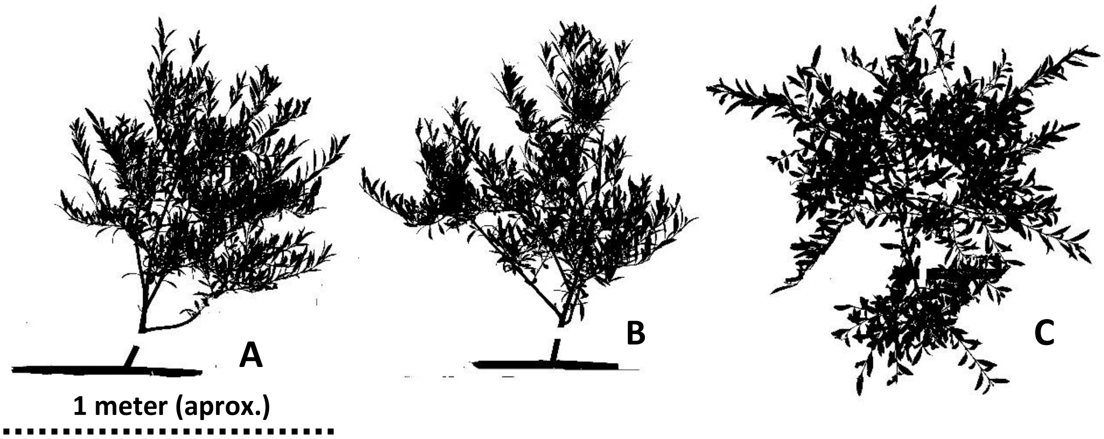

Fig. 1. Example of the determination of the leaf area and leaf area index (LAI) of a young olive tree by four different methods. In this example, figure shows adjusted photos from a side (A and B) and from the top (C) of an Arbequina tree (Arb 3 on Table 1.). Leaf area determined by leaf counting on this tree was estimated to be 0,350 m^2. Leaf area determined with ImageJ on photo A was 0,138 m^2, 0,118 m^2 on photo B and 0,229 m^2 on photo C (from the top), i.e., 34%, 39% or 64% of the actual leaf area, respectively. The projected area of the crown was estimated with ImageJ on photo C to be 0,284 m^2. The mean values of LAI measured on this tree with the ceptometer and Hemisfer software were 0,30 and 1,54, respectively. For the measured projected area, this would give a leaf area of 0,085 m^2 with the ceptometer and 0,438 m^2 with Hemisfer, i.e., about 25% and 125% of the actual leaf area, respectively. Leaf area estimated by the ratio between transpiration (0,762 mmol s^{-1}, measured by the decrease in weight of the pot over a 2 hour period) and transpiration rate (2,40 mmol m^{-2} s^{-1}, calculated from the average stomatal conductance measured with a porometer, 155 mmol m^{-2} s^{-1} , and the difference in molar fraction of water vapor, 0,016) was 0,317 m^2, i.e., 9% below actual leaf area.

Table 1 shows that ceptometer and analysis of photos from a side underestimated leaf area by about 50%. Analysis of photos from the top was, on average, around 70% the actual values. The most accurate methods were analysis with software Hemisfer and the ratio of transpiration by *E* which gave values 5% bellow or 20% above actual values, respectively.

Table 1. Leaf area (m^2) of the seven Arbequina (Arb) and seven Cobrançosa (Cob) young olive trees. Actual leaf area was calculated by leaf counting. Leaf area was also estimated with a ceptometer, Hemisfer software, image analysis of photographs from a side and from the top and finally by dividing the transpiration of each plant by its transpiration rate.

Plant	*Actual leaf area*	*Ceptom.*	*Hemisfer*	*Side photos*	*Top photo*	Transp/*E*
Arb 1	0,485	0,299	0,491	0,184	0,285	0,477
Arb 2	0,413	0,168	0,450	0,153	0,259	0,360
Arb 3	0,350	0,085	0,438	0,128	0,229	0,317
Arb 4	0,408	0,159	0,679	0,206	0,297	0,418
Arb 5	0,430	0,203	0,314	0,171	0,331	0,369
Arb 6	0,404	0,071	0,359	0,162	0,251	0,297
Arb 7	0,338	0,168	0,376	0,144	0,228	0,375
Cob 1	0,221	0,069	0,127	0,121	0,154	0,329
Cob 2	0,399	0,129	0,384	0,144	0,240	0,471
Cob 3	0,260	0,032	0,182	0,095	0,163	0,231
Cob 4	0,095	0,013	0,075	0,064	0,100	0,157
Cob 5	0,224	0,057	0,274	0,117	0,178	0,308
Cob 6	0,064	0,004	0,031	0,034	0,037	0,123
Cob 7	0,086	0,057	0,060	0,037	0,066	0,143

Nevertheless, analyses of photos from a side or from the top were reasonably consistent, i.e., the ratio of measured values *vs* actual values showed little variation. Therefore, using a proper correction factor, this method should also give a good estimate of leaf area.

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Influence of sustained deficit irrigation on the phenolic composition and quality of grape berries (*Vitis vinifera* L.) cv. 'Tempranillo' and 'Graciano'

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ABSTRACT

The aim of this study was to analyze skin phenolic composition during development and ripening of berries by characterizing the response of *Vitis vinifera* L. cv. 'Tempranillo' and 'Graciano' to sustained water-deficit irrigation. The study was carried out using container-grown grapevines grown under controlled conditions. Two irrigation treatments were imposed: control (well-watered) and sustained deficit irrigation (SDI). In 'Tempranillo', water limitation reduced total anthocyanins and flavonols, and increased hydroxycinnamic acids. In 'Graciano', water deficit resulted in increased flavonols and reduced catechins. We conclude that under water-deficit irrigation, 'Graciano' grapes presented a differential composition of phenolic compounds that could result in improved fruit quality.

INTRODUCTION

Grapevine grows well in arid and semi-arid environments because it has relatively high drought tolerance (Chaves et al. 2010) but physiological responses of berries to water deficit appear to be dependent on the cultivar (Niculcea et al. 2014a,b). Spain hosts a large number of native *Vitis vinifera* varieties but most of those genotypes remain uncharacterized, which limits their ability to improve berry quality traits. Therefore, the aim of this study was to analyze phenolic composition during berry development and ripening of berries by characterizing the response of 'Tempranillo' and 'Graciano' to sustained water-deficit irrigation.

MATERIAL AND METHODS

The study was carried out using container-grown 'Tempranillo' and 'Graciano' grapevines grown under controlled conditions in a greenhouse (see more details in Niculcea et al.

2014a,b). Two irrigation treatments were imposed: a control (well watered), and a sustained deficit irrigation (SDI). Berry samples were collected at three stages of development: (1) pea size, corresponding to berries 7 mm in diameter [E-L phenological stage 31] (Coombe 1995); (2) veraison [E-L phenological stage 35]; and (3) harvest, (approximately 22ºBrix) [E-L phenological stage 38]. Anthocyanins, hydroxycinnamic acids, flavonols, and flavan-3-ols (catechins) were determined as described by Guadalupe and Ayestarán (2008). Soluble solids, must pH and titratable acidity were analysed according to the OIV methods (OIV 2014).

RESULTS AND DISCUSSION

Plants subjected to SDI had reduced vegetative growth and berry size and yield (Table 1). In ‘Tempranillo’, SDI induced no changes in must characteristics but in ‘Graciano’ water deficit resulted in decreased juice titratable acidity.

Table 1. Plant and fruit characteristics recorded at harvest from fruiting cuttings of ‘Tempranillo’ and ‘Graciano’ subjected to different irrigation treatments: full irrigation (Control) or sustained deficit irrigation (SDI). Values represent means (n = 5). Within each file and variety, means followed by a different letter are significantly different (P<0.05).

	‘Tempranillo’		‘Graciano’	
Measurement	Control	SDI	Control	SDI
Leaf area (m^2 $plant^{-1}$)	0.37 a	0.11 b	0.23 a	0.09 b
Yield (g $plant^{-1}$)	212.08 a	115.71 b	143,65 a	85.50 b
Leaf area/crop mass (cm^2 g^{-1})	17.5 a	9.5 b	16.1 a	9.5 b
Berry volume (mm^3)	969 a	735 b	667 a	455 b
Total soluble solids (°Brix)	21.6 a	20.1 a	19.0 a	21.2 a
Juice pH	3.7 a	3.8 a	2.7 a	3.0 a
Titratable acidity (g L^{-1})	5.8 a	4.7 a	7.4 a	4.6 b

In Tempranillo, SDI treatment reduced total anthocyanins and flavonols, but increased hydroxycinnamic acids at harvest, but in ‘Graciano’ SDI increased flavonols and reduced catechins (Fig. 1.). Application of different water treatments also resulted in changes of anthocyanin composition in both varieties (Table 2). Thus, in ‘Tempranillo’, the contribution of *3*-monoglucosides to total anthocyanins was decreased, while the contribution of *3-p*-coumaroyl and *3*-acetyl-glucosides increased. In ‘Graciano’, the contribution of *3-p*-

coumaroyl-glucosides to total anthocyanins increased suggesting that under SDI conditions ‘Graciano’ could have high fruit quality (Niculcea et al. 2014b). The reduction in flavonols in SDI ‘Tempranillo’ was due mainly to decreased myricetin *3-O*-glucoside (Table 2).

Table 2. Effect of irrigation treatment (SDI and control) on individual composition of anthocyanins, flavonols and hydroxycinnamic acids determined at harvest in berries of fruiting cuttings of ‘Tempranillo’ and ‘Graciano’. Values represent means (n = 25). Within each file and variety, means followed by a different letter are significantly different (P<0.05). ND: not detected.

		‘Tempranillo’		‘Graciano’	
		Control	SDI	Control	SDI
Anthocyanins	*3-Monoglucosides*	86.7 a	72.2 b	83.4 a	77.9 b
(% of total)	*3-Acetyl-glucosides*	2.5 b	3.4 a	7.0 a	6.9 a
	3 p-Coumaroyl-glucosides	10.8 b	24.4 a	9.6 b	15.2 a
Flavonols	Myricetin 3-*O*-glucoside	20.5 a	12.4 b	ND	ND
(mg kg^{-1})	Quercetin 3-*O*-glucuronide + Quercetin 3-*O*-glucoside	5.4 a	5.3 a	ND	ND
	Quercetin 3-*O*-glucoside	ND	ND	8.8 b	13.1 a
	Myricetin	ND	ND	8.8 b	12.0 a
	Quercetin	ND	ND	1.4 a	1.6 a
	Isorhamnetin	ND	ND	1.0 b	1.5 a
Hydroxycinnamic acids	*c*-caftaric acid	6.3 a	2.6 b	ND	ND
(mg kg^{-1})	*t*-caftaric acid	12.8 b	2.7 a	9.7 a	7.1 b
	c-coutaric acid	ND	ND	2.8 a	1.5 b
	t- coutaric acid	ND	ND	3.6 a	2.4 b
	Caffeic acid	3.8 b	16.8 a	0.5 a	0.5 a
	Coumaric acid	ND	ND	0.4 b	3.5 a
	Ferulic acid	ND	ND	ND	0.5

By contrast in ‘Graciano’, flavonol synthesis was improved under SDI conditions because of increased quercetin-*3-O*-glucoside, myricetin and isorhamnetin. Individual composition of hydroxycinnamic acids of ‘Tempranillo’ showed increased *t*-caftaric and caffeic acids in SDI. However, in ‘Graciano’ SDI reduced concentrations of *t*-caftaric, *c*-coutaric and *t*-coutaric and increased coumaric and ferulic acids. These changes in hydroxycinnamic acid composition could be relevant because are involved in browning reactions, and since they are precursors of volatile phenols in wine (Chatonnet et al. 1993).

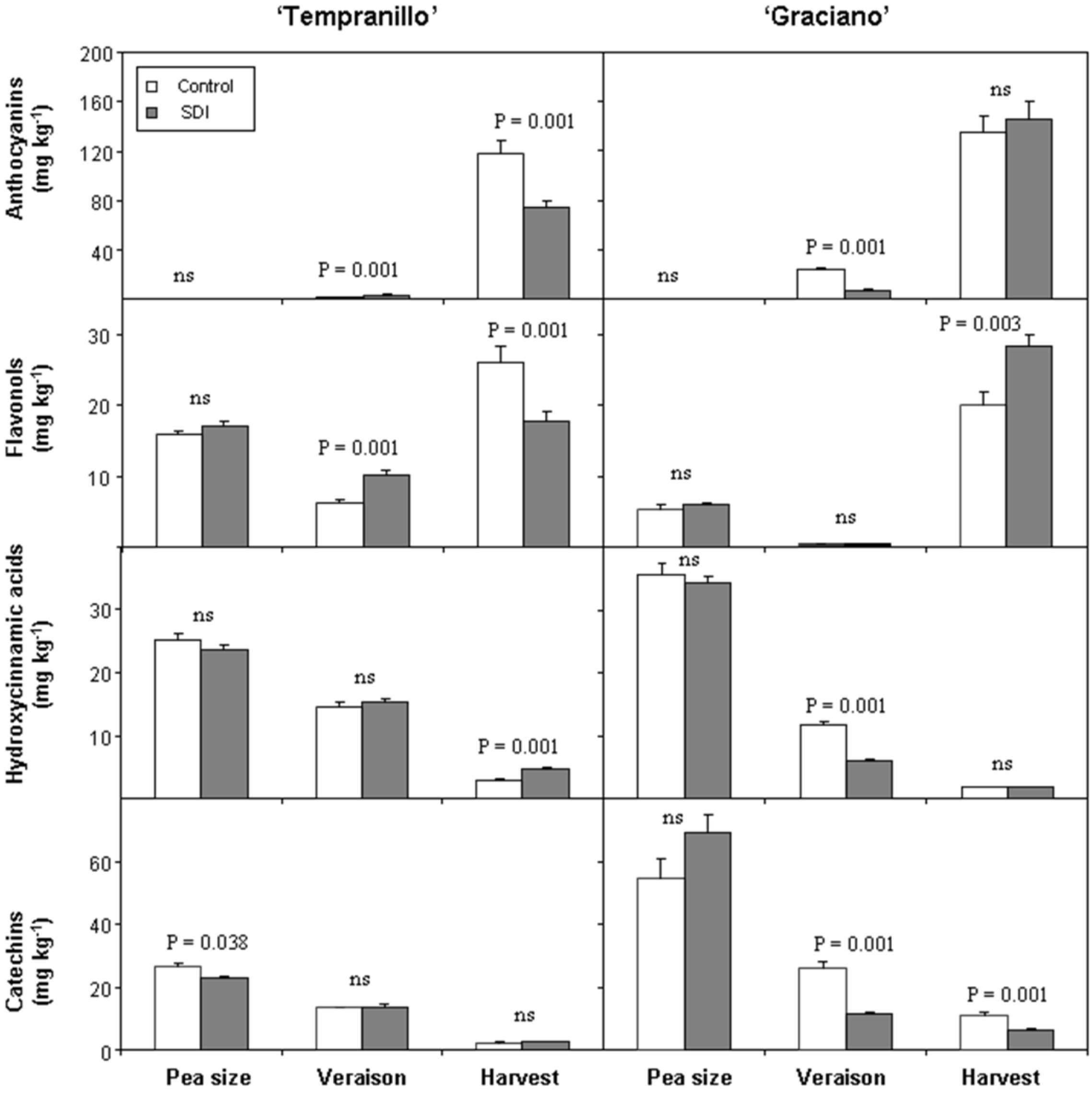

Fig. 1. Phenolic composition at different stages of berry growth and ripening of ‘Tempranillo’ and ‘Graciano’ grapevines subjected to different irrigation treatments: full irrigation (Control), or sustained deficit irrigation (SDI). Values represent means ± SE (n=25). Within each phenological stage and variety means were considered statistically different when $P<0.05$. ns: not significant.

CONCLUSION

Under water deficit irrigation, ‘Graciano’ achieved higher contents of total anthocyanins and flavonols than ‘Tempranillo’. Analyses of monomeric phenolic compounds suggest that under these conditions, ‘Graciano’ could result in improved fruit quality.

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Effects of water availability on water use and aesthetic of five Mediterranean species in terms of suitability for green roof implementation

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ABSTRACT

Green roof implementation has particular benefits and difficulties. Mediterranean zones still represent an important challenge since rainfall distribution is very heterogeneous along the year, leading to frequent drought periods not only in summer but also during spring and autumn. Therefore it is necessary to improve the scientific knowledge on the biology of plant species, in order to increase local industry plant species options, combining aesthetical interest and low maintenance requirements.

Present work aims to study water deficit response of five Mediterranean species (*Asteriscus maritumus, Brachipodium phoenciocides, Crithmum maritimun, Limonium virgatum*, *Sedum sediforme* and *Sporobolus pungens*)**,** evaluating water use, biomass production, and aesthetic appearance as indicators of its potential in green roof implementation. Species were grown under 3 water treatments: well irrigated, mild and severe drought. Gas exchange parameters, relative appearance (RA), biomass production and Relative Water Content (RWC) were determined at the initial and at the end of the experiment. Results show an important reduction of stomatal conductance as a consequence of both severe and mild water deficit. All species but *S. pungens* didn't show significant differences in RWC between predawn and midday values under water deficit conditions. *C. maritimun* and *S. sediforme* showed both the lowest water consumption and biomass accumulation. In most species, RA declined only at the end of the experiment. This decrease was more evident under water deficit conditions. Moreover, *C. maritimun* and *S. sediforme* showed no significant variation of RA along the experiment in any treatment.

INTRODUCTION

Green roofs are considered new green infrastructures, quickly embraced in European cities, and in north American regions because of the synergy of its multiple ecosystem benefits, like retention of storm water, energy conservation, reduction of urban heat island, reduction of atmospheric contamination, improvement of biodiversity in urban areas and psychological and social positive impacts (MacIvor & Lundholm, 2011). Green roofs, like other greening solutions, deal with water availability as a critical factor in Mediterranean Climate areas, characterized by long periods of drought during summer. Moreover, green roof conditions - shallow and low water retention substrates -, lead to short periods of water limiting conditions even during the rainy season. In these areas, vegetation responses to water stress effects is a crucial issue for green roof designers, planners and managers (Farrell, Szota, Williams, & Arndt, 2013). Green roof efficiency largely depends on the characteristics of its components, including plant species performance in this particular environment. Previous research proved that plant traits like biomass production, leaf area index, plant health and plant shape, determine green roof functionality and efficiency (MacIvor & Lundholm 2011; Speak *et al.* 2013). Moreover, since numerous experiments have established that irrigation is needed for an optimal plant establishment and growth (Nagase & Dunnett, 2010)**,** plant performance under water deficit appears to be a key point in green roof plant selection. The general aim of this work is to study the performance of 5 Mediterranean species (*A. Maritimus, B. phoenicoides, C. maritimum, S. pungens*and *S. sediforme*) under extensive green roof conditions. The following specific objectives were addressed: *i)* To study the ability of these species to cope with water stress by determining water consumption, plant growth and survival under three different water regimes and *ii)* to assess the aesthetic value of these species under well-watered and drought conditions.

MATERIALS AND METHODS

The experiment was carried out at the University of the Balearic Islands, Spain (West Mediterranean Basin, 39°38′ N, 2°38′ E, 80 m asl). It was performed during spring and summer 2013, being meteorological data recorded by a meteorological station at the experimental site.

Plant selection: *Five species* from the Balearic Islands (*A. maritumus, B. phoenciocides, C. maritimun, L. virgatum*, *S. sediforme* and *S. pungens*) were selected according to life habitat (characterized by low soil and water availability), perennially and/or self-reseeding ability, and aesthetic interest.

Experimental design: Seeds were germinated in Autumn 2012, and transplanted into 4L plastic pots (14,7x14,7cm and a 20 cm height; 1 plant per pot) at February 2013. In order to mimic extensive green roof conditions, a commercial green roof substrate was used, composed by a recycled clay tiles and 3,6 % of organic material (ZinCoterra, ZincoGmbH).

The experiment lasted 88 days (from 6th May to 2nd August) and18 plants (pots) per species were kept at soil field capacity by restoring 100% of the water consumed every 2-3 days. After 28 days, a progressive water-limited treatment was imposed on 12 plants by restoring only 70% of consumed water until they reached 75% or 50% of field capacity. From 40th day after initiation of the experiment, 6 plants per treatments were kept at 100%, 75% and 50% of field capacity (well-watered (WW), mild drought (MD) and severe drought (SD). Soil water content at field capacity (27,9%) was previously determined by gravimetric methods, 18 substrate-only pots were used to determine evaporation rates in order to adjust water loss estimation.

Biomass, water consume, gas exchange and RWC measurements: Total Dry Biomass (TDB) was determined at the initial and at the end of the experiment on 6 plants per species and treatment in order to estimate plant biomass production, by oven-drying fresh biomass during 72 h at 70°C. Plant water use efficiency (WUE) was estimated for each plant as TDB / consumed water (determined by gravimetric methods every 2-3 days). Net photosynthesis (A) and stomatal conductance (g_s) at leaf level were determined at the end of the experiment (except in *S. sediforme*) (Li-Cor 6400, Li-CorInc, USA). Leaf pre-dawn and midday (PD and MD) (RWC) were determined at the end of the experiment.

For the propose of an integral and accurate quantitative assessment an aesthetic evaluation methodology was adapted. Prior methods were performed by visual estimations, considering: color and health (Monterusso, Rowe, & Rugh, 2005). percentage of leaves and color(Butler & Orians, 2011), health score, stress status(Nagase & Dunnett, 2010). So the methodology used in this survey included four parameters: coloration, shape, lustiness, and flowers absence or presence; coloration was scored in function of color richness, shape accorder to its compactness, lustiness considering hydrated, sign of dehydrated and dehydrated aspect. each parameter was rated on a scale (of 1 to 3, where: 1 (optimal), 2 (tolerable) and 3 (unacceptable). Relative Appearance was performed as a mean of a weekly evaluation. All measurements were done in 6 replicates of each species and treatments.

RESULTS AND DISCUSSIONS

TDB values were lower in SD than in MD and C plants (Table 1). Those differences were larger in grasses. However, drought treatments didn't cause any effect on Senescent Biomass (SB), even though SB varied significantly among species, *S. sediforme* showing the lowest accumulation of SB and *A. maritimus* the highest. Total water consumption differed between species, *B. phoenicoides* and *L. virgatum* performed as the highest water users in WW treatment, 7.6 and 6.6 L/m^2day respectively. In contrast, *C. maritimum* and *S. Sediforme* showed the lowest water consumption, 1.9 and 1.5 L/m^2 day (Table 1). As expected, water consumption was significantly lower in MD and SD in all species. Significant increment of WUE at plant level under SD conditions was only observed in *A. maritimus*. Stomatal conductance and net photosynthesis were significantly reduced in all species, but *B. phoenicoides* (Table 1). In 4 of 5 species, MD RWC were significantly lower than PD RWC under WW plants, with 2 exceptions: *S. pungens* which reduced RWC at MD in all treatments and *S. sediforme* which reported any significant differences between MD and MD RWC. *C. maritimum, L. virgatum* and *S. Sediforme* showed no significant differences in PD and MD RWC between treatments. (Table 1).

Table 1: Total Dry Biomass **(TDB)**, Senescent Biomass **(SB)**, Water Consume **(WC)**, Net photosynthesis **(A)** stomatal conductance **(g)** for control **(C)**, mild drought **(MD)**, severe drought **(SD)**.Values represent means ± s.e.

		TDB (g)	**SB (g)**	**WC (L/ m^{2}día)**	**WUE (g biomass /lH_2O)**	**A (µmol CO_2 / m^{2}s)**	**g (mol H_2O / m^{2}s)**	**RWC MD**	**RWC PD**
A. maritimus	**C**	4.9±0.6^{a}	13.7 ±0.9^{a}	5.5±0.2^{a}	2.2±0.0^{a}	15.4±1.2^{a}	0.2±0.02^{a}	73.9±0.6^{a}	81.2±1.9^{a}**(*)**
	MD	5.3±0.8^{a}	13.5 ±0.6^{a}	4.3±0.0^{b}	2.7±0.1^{b}	10.6±1.3^{a}	0.11 ±0.02^{b}	67.5±3.2^{a}	72.0±3.1^{ab}
	SD	5.0±0.6^{b}	12.0 ±0.2^{a}	3.0±0.1^{c}	3.2±0.1^{c}	1.4±0.1^{b}	0.2 ±0.2^{c}	56.4±1.1^{b}	62.3±4.8^{b}
B. phoenicoides	**C**	24.8±1.0^{a}	7.2 ±0.9^{a}	7.6±0.2^{a}	2.6±0.0^{a}	8.1±2.7^{a}	0.09 ±0.03^{a}	94.7±1.2^{a}	95.4±0.1^{a}**(*)**
	MD	11.8±1.4^{b}	4.9± 0.6^{a}	4.6±0.1^{b}	2.4±0.1^{ab}	3.3±1.9^{a}	0.09 ±0.03^{a}	91.7±1.7^{a}	94.6±0.8^{a}
	SD	7.7±1.9^{b}	6.0 ±0.2^{a}	3.4 ±0.2^{c}	2.8±0.1^{b}	3.7±0.2^{a}	0.09 ±0.03^{a}	8 0.3±4^{b}	89.5±1.2^{b}
C.maritimum	**C**	2.4±0.5^{a}	0.5 ±0.3^{a}	1.9 ±0.1^{a}	1.8±0.2^{a}	12.9±2.7^{a}	0.09 ±0.03^{a}	82.4±1.8^{a}	95.0±2.2^{a}**(*)**
	MD	3.5± 0.9^{a}	1.5± 0.3^{a}	1.6 ±0.0^{ab}	2.9±0.5^{a}	12± 1.8^{a}	0.04 ±0.01^{ab}	83.9±1.8^{a}	91.1±0.9^{a}
	SD	2.4±0.4^{a}	2.3 ±0.4^{a}	1.3±0.1^{b}	2.0±0.2^{a}	2.7±0.7^{b}	0.03 ±0.0^{b}	80.6±3.8^{a}	85.0±3.7^{a}
L. virgatum	**C**	10.5±1.9^{a}	4.7 ±0.7^{a}	6.6±0.2^{a}	3.2±0.1^{a}	19.5±2.5^{a}	0.23 ±0.03^{a}	72.2±3.2^{a}	87.8±2.2^{a}**(*)**
	MD	8.2±0.6^{b}	4.5 ±0.5^{a}	4.9 ±0.1^{b}	3.3±0.2^{a}	14.1 ±1.2a^{a}	0.11 ±0.01^{b}	70.0±2.2^{a}	83.4±3.1^{a}
	SD	6.3±0.8^{c}	5.0 ±0.5^{a}	3.8 ±0.3^{c}	3.4±0.2^{a}	2.5±0.5^{b}	0.01 ±0.00^{c}	70.1±3.5^{a}	83.0±1.8^{a}
S. pungens	**C**	15.1±1.2^{a}	6.6 ±0.9^{a}	5.0 ±0.1^{a}	4.2±0.2^{a}	27±3.2^{a}	0.15 ±0.01^{a}	81.5±2.2^{a}	97.7±0.9^{a}**(*)**
	MD	9.7± 0.7^{b}	3.9± 0.5^{b}	3.6±0.1^{b}	4.1±0.1^{a}	25.7± 2.9^{a}	0.13 ±0.01^{a}	88.7±1.4^{a}	96.6±0.9^{a}**(*)**
	SD	5.4±0.3^{b}	3.1 ±0.2^{b}	1.9 ±0.1^{c}	5.1± 0.5^{a}	7±2.5^{b}	0.04 ±0.01^{b}	74.8±2.0^{b}	97.0±1.0^{a}**(*)**
S. sediforme	**C**	6.1±2^{a}	0 ±0^{a}	1.5±0.1^{a}	2.8±0.5^{a}			87.0±2.0^{a}	85.7±1.8^{a}
	MD	3.6± 0.4^{a}	0 ±0^{a}	0.8±0.1^{b}	3.4±0.4^{a}			80.4±1.2^{a}	76.9±2.3^{ab}
	SD	3.3±0.2^{a}	0 ±0^{a}	0.8 ±0.1^{c}	3.5±0.2^{a}			78.6±3.7^{a}	72.4±3.6^{a}

(n=6)

Along most of the experiment, RA showed satisfactory performance. RA values only declined in SD plants during the last two weeks of the experiment. That decline was specially noticed in *S. pungens*, *B. phoenicoides* and *A. maritimus.*

Our results provide evidence that the studied species are able to withstand stress conditions and maintain near optimal aesthetic appearance under MD conditions by reducing water consumption to a greater extent than biomass production, what would allow to optimize green roof functionality and performance. Senescent biomass production, plant height and

RA results highlight the interest of these species in green roof implementation under Mediterranean conditions. However, the observed biomass reduction and plant damage in grasses and ***A. maritimus*** under SD may limit the interest of these species under sever limitation of water in green roof conditions.

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Interaction of Light and Water Stress on the Ecophysiological Response of *Nothofagus Antarctica* (G. Forster) Oerst.

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ABSTRACT

Nothofagus antarctica (G. Forster) Oerst. (ñire) occupies a latitudinal gradient ranged from 36° 30´ to 56° 00' S, and extends from sea level to 2,000 m a.s.l. It is the species with widest ecological amplitude in *Nothofagus spp*. from south-America covering in southern Patagonia (Argentina) 431,000 ha and growing mainly between the *N. pumilio* forests and steppe where water conditions are limiting. Productivity and different ecological issues of ñire forests have been previously assessed. However, specific studies related to the functional response to water stress of species are scarce. Therefore, the main subject of present study was to evaluate the functional response and growth of ñire seedlings under the interaction of water stress and two light conditions. Ten seedlings were grown during 6 months in a climatic chamber under a photoperiod 14/10 hours of light/darkness, a temperature range of 25ºC day / 20ºC night, and 65% relative humidity. One shoot on each seedling was shaded from the beginning of the experiment with a shading mesh (transmittance of 5 % of full light). The rest of plant received at the top 800 $\mu molm^{-2}s^{-1}$ PPFD. After five months of well-watering conditions, half of seedlings were submitted to a water stress cycle by one additional month. By the end of experiment, diameter growth at the base of stem seedling was measured. In addition, different leaf functional parameters were recorded: specific leaf área (SLA), net photosynthesis (A_n), stomatal conductance to water vapor (gwv), and different parameters from building P-V curves: osmotic potential at maximum and zero turgor (Π^{100}; Π^0), relative water content at zero turgor (RWC_0), maximum modulus of elasticity (E_{max}), and dry/full-hydrated weight ratio (DW/TW). Plant water status was recorded measuring predawn water potential (Ψ_{pd}). Light and water stress affected most leaf functional parameters with synergic to antagonistic impacts depending on a particular trait.

INTRODUCTION

The deciduous tree species *Nothofagus antarctica* (G. Forster) Oerst. (ñire) has the widest ecological amplitude in *Nothofagus spp*. from south-America (Donoso et al. 2006) occupying a latitudinal range from 36° 30´ to 56° 00' S, and extends from sea level to 2,000 m a.s.l. (Veblen et al. 1996). In southern Argentinean Patagonia the "*ñire*" forests cover an area of 431,000 ha (Collado 2001; Peri and Ormaechea 2013), growing in contrasting environmental conditions ranging from poorly drained sites with high precipitations and enduring flooding to dry sites limiting with the Patagonian steppe. However, most of these forests grow in an ecotone zone between the *N. pumilio* forests and steppe where water conditions severely limits growth of forest trees. Several ecological and productive issues of ñire forests have been studied in southern Patagonia (Lencinas et al. 2002; Peri et al. 2010; Bahamonde et al. 2012; Gargaglione et al. 2013), but specific studies related to the functional response to water stress of species are scarce, especially in southern Patagonia. Some previous reports on the ecophysiological response of the species are those from Peri et al. (2009) in northern Patagonia who studied the photosynthetic response of seedlings of *N. antarctica* to different radiation levels and water availability; Bucci et al. (2013) evaluated hydraulic characteristics of leaves and stems of six species of *Nothofagus*, being one of them *N. antarctica*. In Chile, Dettmann et al. (2013) studied xylem anatomy of four *Nothofagus* including *N. antarctica* and calculated the hydraulic conductivity and Ψ_{50}. Therefore, the main objective of present study was to evaluate the functional response and acclimation of ñire leaves under the interaction of water stress and two levels of light availability.

MATERIAL AND METHODS

Seeds from Tierra del Fuego (54°20´LS – 67°52´LW) were germinated according to existing protocols (Bahamonde et al. 2011) and planted in 100 cc trays. After an initial growth phase of two months in the greenhouse, ten seedlings were transplanted to 2 L pots and grown for 6 months in a climatic chamber under a photoperiod of 14/10 hours of light/darkness, a temperature range of 25ºC day / 20ºC night, and 65% relative humidity. One shoot on each seedling was shaded from the beginning of the experiment with a shading mesh (transmittance of 5 % of full light). Rest of plant received a PPFD at the top of 800 µmolm^{-2}s^{-1}. After five months of well-watering, half of seedlings were submitted to a water stress cycle for one additional month. The diameter at the base of stem seedling was measured at the beginning and the end of the experiment. Thus, relative diameter growth at the base of stem seedling was calculated. In addition, at the end of the experiment different leaf functional and morphological parameters were recorded: specific leaf area (SLA), net photosynthesis (A_n), stomatal conductance to water vapor (g_{wv}). In addition different water parameters from building P-V curves were also recorded: osmotic potential at maximum and zero turgor (Π_{100}; Π_0), relative water content at zero turgor (RWC_0), relative water content of the apoplast (RWC_A), maximum modulus of elasticity (E_{max}), and dry/full-hydrated weight ratio (DW/TW). Plant water status and soil water availability were recorded from measuring predawn water potential (Ψ_{pd}) at the end of the experiment, and soil water content (SWC) at different times during the cycle of watering withdrawal.

RESULTS AND DISCUSSION

Water stress affected negatively plant growth. The diameter relative growth at the stem base was significantly higher in the control compared to the stressed plants (Table 1), which confirms the drought sensitivity of the species previously reported (Gyenge et al. 2011). SLA values were higher in shaded leaves regardless of water status.

Table 1. Functional parameters of *N. antarctica* seedlings growing under light and water stress.

	Full light/WW	Shaded/WW	Full light/WS	Shaded/WS
SLA	22.4 b	38.0 a	26.0 b	38.3 a
A_n	8.27± 1.12a	2.43 ± 0.48 ab	5.64 ± 2.39 a	0.91± 0.42 b
g_{wv}	0.186 ± 0.028 a	0.061 ± 0.008 b	0.050 ± 0.027 b	0.016 ± 0.006 b
C_i	315 ± 4 a	315 ± 23 ab	183 ± 27 b	302 ± 6 ab
Π^{100}	-1.43 ± 0.18 a	-1.15 ± 0.23 a	-1.13 ± 0.20 a	-0.92 ± 0.18 a
Π^0	-1.95 ± 0.23 a	-1.55 ± 0.29 a	-1.60 ± 0.25 a	-1.34 ± 0.23 a
RWC_0	0.81 ± 0.03 a	0.80 ± 0.04 a	0.84 ± 0.04 a	0.80 ± 0.03 a
DW/TW	0.31 ± 0.01 a	0.26 ± 0.01 b	0.28 ± 0.01 a	0.26 ± 0.01b
Emax	5.89 ± 1.18 a	5.35 ± 1.52 a	4.34 ± 1.32 a	3.36 ± 1.18 a

Similarly, Varela (2010) reported a trend of higher values of SLA in seedlings of *Nothofagus nervosa* and *N. obliqua* growing under shade compared to plants at full sun, but there was not an effect of different water levels on SLA either the interaction light x watering treatments was significant. Light was the main driver in prompting changes in SLA. A_n was affected by shade, but no statistical significant differences were detected under water stress, even though there was a trend to be lower in stressed plants (Table 1). However, for stomatal conductance there were statistically significant differences, with lower values due to both water stress and light limitation compared to control plants ($P < 0.05$ both main effects, and $P < 0.05$ interaction term). Peri et al. (2009) reported a decrease of A_n in seedlings of *N. antarctica* either with shade and water stress. Our results suggest that *N. antarctica* seedlings closed the stomata to avoid water loss, but probably the level of water stress was not high enough to imply a decrease in photosynthesis. The differential impact of water stress according to light environment on A_n and g_{wv} translated in significant differences for Ci, being significant the interaction term ($P < 0.05$). In the last, this result would point out to a differential impact of water stress in gas exchange regulation according to light environment. In relation to water parameters, only light affected to DW/TW, while not differences were observed for rest of parameters (Table 1). There are not antecedents about the effect of water or light stress on these parameters for *N. antarctica*. However, Varela (2010) informed that water stress did not generate an osmotic adjustment in *N. obliqua* and *N. nervosa*, close related species to *N. antarctica*.

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Response to salinity in young olive trees of three Iberian varieties

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ABSTRACT

Soil salinization is a problem in the Mediterranean region. This paper reports a research on the response to salt in two year-old olive trees (Olea europaea L.) of three Iberian varieties: Arbequina, Cobrançosa and Galega Vulgar.

Plants were grown in plastic pots containing approximately 9 Kg of a sandy granitic soil, on a greenhouse at the University of Évora since February 2010. The experiment went from February to April 2012. As a rule, plants were watered every other day alternating salt solution (0 mM, 80 mM or 200 mM NaCl) or tap water.

After three months irrigation with the different NaCl solutions, soil electric conductivity and soil water content were significantly higher on salt-irrigated pots. Salt also decreased significantly stomatal conductance (g_s) and mid-day leaf water potential (Ψ), Cobrançosa having in general higher g_s and Ψ but lower SLA than the two other varieties. Chlorophyll content of leaves was not affected by salt after this three months exposure to NaCl but was significantly higher on Arbequina and lower on Cobrançosa.

In general, hyperspectral reflectance indexes did not show significant correlations with salt irrigation, except for the Photochemical Reflectance Index (PRI) which was clearly lower on plants of all three varieties irrigated with salt. Interestingly, Cobrançosa showed frequently vegetation indexes different from the other two varieties.

INTRODUCTION

The problem of soil salinity has been an increasingly important issue due to the decrease in good quality irrigation water. The Mediterranean region is no exception.

Salinity affects plant growth by osmotic stress, ion toxicity or both (Munns and Tester, 2008). The area occupied by intensive and hedgerow olive orchards in the Mediterranean region has greatly increased over the last years (Santos et al., 2013). These orchards need frequently high levels of irrigation during summer which may lead to an increase in soil salinity due to the high evaporative demand. Therefore, investigation on the tolerance to salinity of the different olive-tree varieties is of particular importance.

This paper reports a research on the response to salt in two year-old olive trees (*Olea europaea* L.) of three Iberian varieties: Arbequina, Cobrançosa and Galega Vulgar.

MATERIALS AND METHODS

Plants were grown in 10 L plastic pots containing approximately 9 Kg of a sandy granitic soil, on a greenhouse at the University of Évora since February 2010. The experiment went from February to April 2012. As a rule, plants were watered every other day alternating salt solution or tap water. There were three levels of salinity in the irrigation water, 0 mM, 80 mM or 200 mM NaCl, corresponding to about 0,05 dS m^{-1}, 7,3 dS m^{-1} or 19 dS m^{-1}, respectively (6 plants per salinity level in a total of 18 plants of each variety). After 3 months, several parameters were assessed. Soil water content and salinity were measured with a portable conductivity meter (Hanna HI9835, USA). In addition, mid-day leaf stomatal conductance (g_s) was measured with a AP4 porometer (Delta-T Devices, Uk), mid-day leaf water potential (Ψ) with a pressure chamber (PMS Instrument Company, USA), percentage of water, relative water content, specific leaf area (SLA), leaf chlorophyll content measured with a CL-01 chlorophyll meter (Hansatech Instruments, UK) and different indices derived from hyperspectral leaf signature (Table 1), measured with a FieldSpec Spectroradiometer (ASD Inc., USA).

Table 1. List of vegetation indexes analysed in this work.

Vegetation Index		***Equation***
SIPI	Structure Insensitive Pigment Index	R800-R445/R800-R680
MSI	Moisture Stress Index	R1599/R819
NDII	Normalized Difference Infrared Index	(R819-R1649)/(R819+R1649)
NPQI	Normalized phaeophytinization index	(R415-R435)/(R415+R435)
WBI	Water Band Index	R900/R970
WI	Water Index	R900/R1600
NDVI	Normalized Difference Vegetation Index	(R750-R705)/(R750+R705)
$NDVI_{680}$	Normalized Difference Vegetation Index	(R810-R680)/(R810+R680)
PRI	Photochemical Reflectance Index	(R531-R570)/(R531+R570)
REIP	Red Edge Inflexion Point Index	R700+40((Re-R700)/(R740-R700))
CRI_2	Carotenoid Reflectance Index 2	(1/R510) - (1/R700)
ARI_2	Anthocyanin Reflectance Index 2	R800 [(1/R550) - (1/R700)]
NDLI	Normalized Difference Lignin Index	(logR1754)-(logR1680)/(logR1754)+(logR1680)
PSRI	Plant Senescence Reflectance Index	(R680 - R500)/R750

RESULTS

After three months irrigation with the different NaCl solutions, soil electric conductivity and soil water content (Table 2) were significantly higher on salt-irrigated pots.

Table 2. Salinity and water content in the soil after 90 days irrigation with different NaCl solutions. Values are mean ± SE (*n* = 6). For each parameter, different letters indicate statistical difference ($p < 0,05$).

	NaCl	**Arbequina**	**Cobrançosa**	**Galega**
Soil CE (dS m^{-1})	**0 mM**	0,18 ± 0,02 $^{\mathbf{e}}$	0, 18 ± 0,01 $^{\mathbf{e}}$	0,17 ± 0,02 $^{\mathbf{e}}$
	80 mM	1,02 ± 0,10 $^{\mathbf{cd}}$	0,62 ± 0,13 $^{\mathbf{de}}$	1,02 ± 0,11 $^{\mathbf{cd}}$
	200 mM	1,88 ± 0,27 $^{\mathbf{ab}}$	1,47 ± 0,09 $^{\mathbf{bc}}$	2,06 ± 0,18 $^{\mathbf{a}}$
Soil water content (%)	**0 mM**	0,12 ± 0,01$^{\mathbf{bc}}$	0,13 ± 0,03 $^{\mathbf{bc}}$	0,11 ± 0,02 $^{\mathbf{c}}$
	80 mM	0,12 ± 0,01 $^{\mathbf{bc}}$	0,13 ± 0,01 $^{\mathbf{bc}}$	0,14 ± 0,02 $^{\mathbf{bc}}$
	200 mM	0,22 ± 0,07 $^{\mathbf{a}}$	0,17 ± 0,02 $^{\mathbf{b}}$	0,17 ± 0,04 $^{\mathbf{b}}$

Table 3 shows the effects of NaCl on plants. Salt irrigation decreased significantly stomatal conductance (g_s), Cobrançosa having in general higher g_s than the two other varieties. Mid-day leaf water potential (Ψ) was also significantly lower on salt-irrigated plants and again Cobrançosa exhibited slightly higher values compared with the other two varieties.

Relative water content was not significantly affected by salt irrigation. Salt had also no clear effect on the specific leaf area (SLA), but Cobrançosa had on average higher SLA than the two other varieties. Chlorophyll content of leaves was not affected after this three months exposure to NaCl but was significantly higher on Arbequina and lower on Cobrançosa.

Table 3. Mid-day stomatal conductance (g_s, mmol m^{-2} s^{-1}, *n* = 48), water potential (*Ψ*, MPa, *n* = 6), relative water content (RWC, *n* = 6), specific leaf area (SLA, *n* = 6) and relative chlorophyll content (arbitrary units, *n* = 30) of plants after 90 days irrigation with different NaCl solutions. Values are mean ± SE. Different letters indicate statistical difference ($p <$ 0,05).

	NaCl	**Arbequina**	**Cobrançosa**	**Galega**
Stomatal conductance (g_s)	**0 mM**	63 ± 7 ^{b}	109 ± 9 ^{a}	63 ± 6 ^{b}
	80 mM	27 ± 3 ^{cd}	36 ± 5 ^{c}	18 ± 3 ^{cd}
	200 mM	13 ± 2 ^{d}	17 ± 3 ^{cd}	9 ± 1 ^{d}
Mid-day leaf water potential (*Ψ*)	**0 mM**	-1,33 ± 0,05 ^{a}	-1,12 ± 0,05 ^{a}	-1,29 ± 0,07 ^{a}
	80 mM	-1,94 ± 0,13 ^{bc}	-1,34 ± 0,07 ^{a}	-1,57 ± 0,05 ^{ab}
	200 mM	-2,18 ± 0,25 ^{c}	-1,72 ± 0,07^{abc}	-2,28 ± 0,26 ^{c}
Relative water content (RWC)	**0 mM**	0,84 ± 0,02	0,81 ± 0,03	0,84 ± 0,02
	80 mM	0,79 ± 0,03	0,82 ± 0,03	0,78 ± 0,02
	200 mM	0,82 ± 0,02	0,82 ± 0,01	0,79 ± 0,01
Specific leaf area (SLA)	**0 mM**	3,02 ± 0,16 ^{b}	3,96 ± 0,37 ^{ab}	3,26 ± 0,17 ^{ab}
	80 mM	3,12 ± 0,24 ^{b}	4,28 ± 0,53 ^{a}	3,45 ± 0,17 ^{ab}
	200 mM	3,69 ± 0,39 ^{ab}	3,19 ± 0,18 ^{b}	3,47 ± 0,17 ^{ab}
Relative chlorophyll content	**0 mM**	139 ± 5 ^{abc}	129 ± 5 ^{bc}	146 ± 5 ^{ab}
	80 mM	153 ± 7 ^{a}	145 ± 4 ^{ab}	128 ± 6 ^{bc}
	200 mM	136 ± 6 ^{abc}	121 ± 5 ^{c}	144 ± 7 ^{ab}

In general, hyperspectral reflectance indexes (Table 4) did not show significant correlations with salt irrigation. From all the fifteen vegetation indexes analysed (Table 1.), only the Photochemical Reflectance Index (PRI) was clearly lower on plants of all three varieties irrigated with salt (Table 4).

Interestingly, Cobrançosa showed frequently vegetation indexes different from the other two varieties, higher Moisture Stress Index (MSI), Normalized Phaeophytinization Index (NPQI) and Normalized Difference Vegetation Index (NDVI) but lower Normalized Difference Infrared Index (NDII), Water Index (WI), Structure Insensitive Pigment Index (SIPI), Carotenoid Reflectance Index (CRI$_2$), Anthocyanin Reflectance Index (ARI$_2$) and Plant Senescence Reflectance Index (PSRI).

Table 4. Vegetation indexes measured on individual leaves after 90 days irrigation with different NaCl solutions. Values are mean ± SE (n = 6). Different letters indicate statistical difference (p < 0,05).

	NaCl	**Arbequina**	**Cobrançosa**	**Galega**
Photochemical Reflectance Index (**PRI**)	**0 mM**	0,026 ± 0,003 ^{a}	0,024 ± 0,003 ^{ab}	0,027 ± 0,002 ^{a}
	80 mM	0,019 ± 0,005^{abc}	0,018 ± 0,003^{abc}	0,009 ± 0,006 ^{c}
	200 mM	0,005 ± 0,012 ^{bc}	0,009 ± 0,003 ^{c}	0,011 ± 0,009 ^{c}
Moisture Stress Index (**MSI**)	**0 mM**	0,49 ± 0,04 ^{b}	0,61 ± 0,04 ^{a}	0,49 ± 0,03 ^{b}
	80 mM	0,49 ± 0,05 ^{b}	0,58 ± 0,04 ^{ab}	0,52 ± 0,02 ^{ab}
	200 mM	0,57 ± 0,05 ^{ab}	0,49 ± 0,03 ^{b}	0,52 ± 0,02 ^{ab}
Normalized Phaeophyt. Index (**NPQI**)	**0 mM**	0,02 ± 0,01 ^{b}	0,05 ± 0,01 ^{a}	0,02 ± 0,01 ^{b}
	80 mM	0,02 ± 0,01 ^{b}	0,06 ± 0,01 ^{a}	0,02 ± 0,01 ^{b}
	200 mM	0,03 ± 0,01 ^{b}	0,06 ± 0,01 ^{a}	0,02 ± 0,01 ^{b}
Normalized Difference Vegetation Index (**NDVI**)	**0 mM**	0,64 ± 0,02 ^{ab}	0,66 ± 0,02 ^{ab}	0,70 ± 0,02 ^{a}
	80 mM	0,62 ± 0,04 ^{b}	0,66 ± 0,02 ^{ab}	0,61 ± 0,04 ^{b}
	200 mM	0,61 ± 0,02 ^{b}	0,67 ± 0,01 ^{ab}	0,66 ± 0,04 ^{ab}
Normalized Difference Infrared Index (**NDII**)	**0 mM**	0,27 ± 0,03 ^{a}	0,18 ± 0,03 ^{b}	0,27 ± 0,03 ^{a}
	80 mM	0,27 ± 0,04 ^{a}	0,20 ± 0,03 ^{ab}	0,24 ± 0,02 ^{ab}
	200 mM	0,21 ± 0,04 ^{ab}	0,27 ± 0,02 ^{a}	0,24 ± 0,02 ^{ab}
Water Index (**WI**)	**0 mM**	2,14 ± 0,15 ^{ab}	1,70 ± 0,10 ^{c}	2,14 ± 0,14 ^{ab}
	80 mM	2,19 ± 0,23 ^{a}	1,80 ± 0,14 ^{bc}	1,96 ± 0,10 ^{abc}
	200 mM	1,87 ± 0,15 ^{abc}	2,09 ± 0,11 ^{ab}	1,98 ± 0,09 ^{abc}
Structure Insensitive Pigment Index (**SIPI**)	**0 mM**	1,00 ± 0,00 ^{ab}	0,99 ± 0,00 ^{c}	1,00 ± 0,00 ^{ab}
	80 mM	1,00 ± 0,00 ^{a}	0,99 ± 0,00 ^{c}	1,00 ± 0,00 ^{a}
	200 mM	1,00 ± 0,00 ^{ab}	0,99 ± 0,00 ^{c}	1,00 ± 0,00 ^{b}
Carotenoid Reflectance Index (**CRI$_2$**)	**0 mM**	16,1 ± 1,4 ^{a}	12,8 ± 1,2 ^{ab}	15,1 ± 2,3 ^{ab}
	80 mM	16,8 ± 1,3 ^{a}	11,1 ± 1,8 ^{b}	16,7 ± 2,8 ^{a}
	200 mM	17,5 ± 1,6 ^{a}	13,5 ± 2,3 ^{ab}	15,1 ± 1,9 ^{ab}
Anthocyanin Reflectance Index (**ARI$_2$**)	**0 mM**	0,26 ± 0,08 ^{a}	-0,05 ± 0,09^{b}	0,23 ± 0,12 ^{a}
	80 mM	0,46 ± 0,13 ^{a}	-0,09 ± 0,04^{b}	0,39 ± 0,10 ^{a}
	200 mM	0,29 ± 0,17 ^{a}	-0,02 ± 0,11^{b}	0,47 ± 0,05 ^{a}
Plant Senescence Reflect. Index (**PSRI**) × 10^{3}	**0 mM**	2,5 ± 0,6 ^{a}	-0,6 ± 0,8 ^{bc}	2,0 ± 1,1 ^{a}
	80 mM	3.5 ± 0.2 ^{a}	-1.5 ± 0.1 ^{c}	2.0 ± 0.1 ^{a}
	200 mM	2.0 ± 0.2 ^{a}	-1.5 ± 0.1 ^{c}	1.6 ± 0.1 ^{ab}

DISCUSSION

As expected, soil salinity reduced significantly water potential (Ψ) and stomatal conductance (g_s) of all plants. The fact that Cobrançosa showed higher Ψ and g_s than the other two varieties can not be attributed to differences in irrigation, as soil water content was similar in pots of all three varieties. Also, total leaf area of plants of Cobrançosa was just marginally lower than that of the other varieties. One possible explanation is that Cobrançosa shortened the time span of their stomatal opening therefore reducing water lost, despite having higher g_s, and thus increasing leaf water potential.

Hyperspectral reflectance index PRI showed a positive correlation with chlorophyll content (0,55, *p*<0,001) but a negative correlation with salinity (-0,44, *p*<0,001). However, the relationship between salinity and chlorophyll was not significant (-0,1) which suggests that PRI may have been affected directly by salt in the leaves as pointed out in previous studies (Zinnert et al. 2012). There were also clear differences in vegetation indexes between Cobrançosa and the other two varieties, Arbequina and Galega. Focusing on water-related indexes, Cobrançosa showed higher Moisture Stress Index (MSI) and lower Water Index and Normalized Difference Infrared Index (NDII), all these suggesting lower water content. Nevertheless the relative water content of leaves was not significantly different from one variety to the other, and the water percentage was even slightly (but not significantly) higher in Cobrançosa. There seems to be some characteristics of Cobrançosa leaves that mask the reading of these indexes.

Pigment-related indexes were also determined. NPQI, the Normalized Phaeophytinization Index, which is an indicator of chlorophyll degradation, was much higher in Cobrançosa than on the other two varieties. Actually, Cobrançosa showed significantly lower chlorophyll content than the other two varieties, somehow contradicting the higher NDVI found in this variety. SIPI was also lower on Cobrançosa, suggesting lower carotenoid content, also supported by the lower CRI_2 observed on this variety but in someway contradicting the lower Plant Senescence Reflectance Index (PSRI). Strangely, Anthocyanin Reflectance Index (ARI_2) showed negative values in Cobrançosa.

Determination of actual pigment content in the leaves would be necessary to verify these data. The response of Cobrançosa to salt, clearly different from that of Arbequina or Galega, needs further investigation.

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Effects of different irrigation levels on the quality of Trincadeira grapes – Mediterranean conditions

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ABSTRACT

This work reports the research on Trincadeira grape vine subject to three increasing levels of irrigation: i) non irrigation, ii) farmer irrigation or iii) full irrigation. During grape's maturation, berry size and quality parameters were analyzed. Only in September all analysed parameters were significantly different: non-irrigated plants had smaller berries and lower brix, anthocyanins and polyphenols than irrigated ones.

Keywords: irrigation, grapes, brix, anthocyanins, polyphenols.

INTRODUCTION

Irrigation is a useful tool for vineyards of the Mediterranean region, but the availability of water, which can be positive to ensure the plant metabolism and production, can also have negative effects on the quality of grapes by dilution of soluble solids in the berries (Girona *et al*., 2006; Santesteban and Royo, 2006). Controlled irrigation has positive effects on the synthesis and concentration of phenolic compounds, soluble solids and anthocyanins promoting improvements in the quality of wines in color, aroma and flavor, due to the increased ratio skin/pulp of the berries (Acevedo-Opazo *et al*., 2010).

The development of appropriate irrigation strategies proves to be very important, both to ensure productivity and a good management of water resources. This work aims to contribute to the study of the effects of irrigation on the main quality characteristics of grapes.

METHODOLOGY

This research was performed on a vineyard located in Alto Alentejo region (lat: +38,551017º; long: -7,882821º), Portugal, in plants of Trincadeira variety. A trial was established with three irrigation treatments: i) rain-fed (21 mm), ii) farmer irrigation (83 mm) or iii) full irrigation (108 mm), mm of watering being total allocation from July to September, including precipitation.

In July, August and September, fifty berries were randomly harvested from the 13 plants of each treatment. They were weighed (fresh weight) and dried (dry weight) at 48ºC for 15 days. Samples were collected in August, at stage II and in September, at stage III for

analysis of the most used parameters to directly assess the quality of the fruit: brix, anthocyanins and polyphenols. Brix was determined by a manual refractometer (ALLA, France). The determination of total anthocyanins followed Ribéreau-Gayon and Stonestreet (1965) method, and phenolic compounds were dosed according to Curvelo-Garcia (1988), using a spectrophotometer Perkin Elmer, model Lambda 25 UV/Vis (Shelton, USA).
IBM SPSS Statistics 22 was used for statistical analysis.

RESULTS

Average berry size and water content was determined from July to beginning of September. Berry water percentage was not affected by irrigation throughout the experimental period. Mean dry weight in July was 0,15g/berry and there were no differences between irrigations. In August there were also no differences but in early September non-irrigated plants had 12% significantly smaller berries than irrigated ones (Table 1).

Table 1. Grape dry weight (n = 50) and quality parameters (n = 6 or 8) of berries from the plants subject to the three levels of irrigation. For each parameter, different letters indicate significant statistical differences (p < 0,05).

	August			September		
	Non irrigation	Farmer irrigation	Full irrigation	Non irrigation	Farmer irrigation	Full irrigation
Dry weight to 50 berries (g)	0,59 ± 0,1	0,59 ± 0,1	0,70 ± 0,1	0,62 ± 0,1 ^{b}	0,70 ± 0,1 ^{ab}	0,71 ± 0,1 ^{a}
Brix (ºBx)	12,3 ± 1,5	12,0 ± 1,9	12,0 ± 1,5	14,7 ± 0,2 ^{c}	15,0 ± 0,1 ^{b}	15,3 ± 0,2 ^{a}
Anthocyanins (mg/L)	100 ± 48	109 ± 58	115 ± 54	178 ± 8 ^{b}	176 ± 2 ^{b}	209 ± 6 ^{a}
Polyphenols (abs)	8,07 ± 1,0	7,88 ± 0,6	7,84 ± 0,8	9,36 ± 0,9 ^{b}	9,09 ± 0,2 ^{b}	10,43 ± 0,1 ^{a}

Brix, anthocyanin and polyphenols were also assessed (Table 1). In August, different applied irrigations had no significant effects on these parameters, but in September there were marked differences between irrigations. All these quality parameters were significantly higher in fully-irrigated plants and lower on non-irrigated ones.

DISCUSSION

Three levels of irrigation, the farmer irrigation, below it (rain-fed) and above (full irrigation), were applied to a Trincadeira vineyard.
Except for water percentage, plants subject to full irrigation (above farmer irrigation) showed higher values in all parameters considered (berry weight, brix, anthocyanins and

polyphenols), as compared to the farmer irrigated or rain-fed plants in the month of September.

The smaller berry size on rain-fed vines, possibly due to decrease in turgor (Matthews *et al*. 1987), indicates that, on this particular year, water was a limiting factor for berry growth.

Brix of rainfed plants was significantly lower than that of farmer-irrigated or fully-irrigated plants. This may be due to the fact that lower water endowments influenced stomatal opening and therefore the process of photosynthesis.

The content of anthocyanins and polyphenols was significantly higher in fully-watered fruits as compared to other irrigations.

Contradicting these results, several authors (e.g. Acevodo-Opazo *et al*., 2010; Van Leeuwen *et al*., 2004; Dry *et al*. 2001) report that the imposition of a period of water stress favor the accumulation of anthocyanins. Other authors also say that grapes which receive less water have higher quality because they have a higher skin/pulp ratio, favoring the accumulation of phenolic compounds (e.g. Koundouras *et al*., 2006; Santesteban and Royo, 2006).

Given the results, it appears that water was a limiting factor at least at the farmer irrigation level; plants would therefore be under some degree of water stress which resulted in lower values for these quality parameters.

CONCLUSIONS

This research shows that the quality of the grapes from plants subject to farmer-defined irrigation proved worse than the ones from fully-irrigated plants. Farmer is therefore recommended to increase water allocation.

The apparently contradictory results may suggest that even fully-irrigated plants were still in water stress, i.e., the photosynthetic activity and thus sugar content was limited due to water shortage. These results need farther investigation.

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Ecophysiological Studies on *Cistus Palhinhae Ingram* and *Cistus Ladanifer L.* in Southern Alentejo

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ABSTRACT

Cistus palhinhae Ingram is an endemic plant from Portugal, with a non-consensual taxonomical position. Some authors consider it as a variety or sub-species of *C. ladanifer* L.. Until now, the knowledge about *C. palhinhae* is limited, so we pretend to compare the ecophysiology and anatomy of both species in the same area. This study was performed at 3 different times, in 2007 and 2008, in Cape Sardão region, where the two species occur. Crown structure , leaf water potential and leaf area index were assessed. According to our results, the ecophysiological processes are different in the studied species, especially the ones associated with the xeric features: *C. palhinhae* shows marked xeromorphic adaptations whereas *C. ladanifer* is a semi-deciduos sclerophyll shrub.

INTRODUCTION

Cistus palhinhae Ingram is an endemic plant from Portugal. Its distribution is limited to Abano beach, near Lisbon, (Capelo, personal communication) and to the area between Vila Nova de Mil Fontes and Sagres (SW Portugal), within the Natural Reserve of Costa Vicentina (ICNF, 2006). It is described as very similar to *Cistus ladanifer* L., "but of lesser stature (50 cm), denser canopy leaves 20-60 mm, pristine white flowers and 6-locular ovary" (Franco, 1971). *C. ladanifer* is widely distributed in the western Mediterranean region, from Portugal and Morocco to Algeria and Riviera (Castroviejo et al., 1995). In 1991 Demoly categorized *C. palhinhae* as *C. ladanifer* subsp. *sulcatus*, based on the distribution and habitat of the two species. Proksch and Gulz (1984) studied the flavonoids patterns, of the two *taxa*, found to be similar. Wuerpel (1973) compared the number of valves per fruit and the anatomical features of the leaves, concluding that the leaves allow a better distinction between the two species.

The Mediterranean climate, with rainy winters and dry summers, underlie the presence of woody sclerophyll evergreen plants and sclerophyll semi-deciduous plants with seasonal dimorphism (Aronne & Micco, 2001; Larcher, 1995*;* Werner *et al.*, 1999). Plant water balance depends namely on the processes of root absorption, water transport and transpiration. It varies daily and seasonally. One way to minimize water losses is by reducing the leaf area (Pereira, 2003). Another strategy observed in "cushion" plants that occur in open areas highly exposed to wind is the high branching and the closed nature of the crown structure which creates a microclimate under the canopy enabling moisture concentration, due to its resistance to aerodynamic diffusion (Larcher, 1995).

MATERIAL AND METHODS

The study area was located in in Cape Sardão, Beja, Portugal. , The two study sites were approximately 4 km apart, *C. palhinhae* close to shoreline and *C. ladanifer more inland*. The bioclimate, according to the climatological station of Santiago do Cacém (Queiroz et al., 1999) is Pluviestacional Mediterranean Ocean, and bioclimatic level is superior mesomediterranean with a Sub-humid ombrotip (Rivas-Martinez, 1995). The geology of the two sites is included in Formação da Brejeira of Baixo Alentejo Flysch Group lithostratigraphic unit (succession of thin deposits of deep continental shelf). It consists of a sequence of impure quartzite, quartz-greywacke and slates interspersed, occupying a range of 5 to 10 km (Basile, 2008).

Leaf water potential was measured at predawn and at solar noon (maximum and minimum daily values) at three different times of the year: 24th and 25th of August 2007; 1st of March 2008; 7th and 8th of June 2008. A portable pressure chamber (PMS Instrument Co., Corvallis, Oregon, USA) was used. At each sampling time, 3 leaves per plant from 3 different plants of each species were used. In the first and last sampling sessions, one individual of each species was subjected to a stratified cut following Norman & Campbell (1989). Leaves were stored in bags in different layers and stored at 4 ºC until the next day. Their fresh weight was evaluated on a Sartorius, BP310S scale. Leaf area was assessed on an electronic leaf area meter (LI-3000-3050A, Li-COR Inc., Nebraska, USA). Leaf dried weight was calculated after several days on a dry oven with forced ventilation, at 80 ºC.

Four vegetation indexes were calculated: LAI (green leaves uniface area (m^2/m^2)); LAI_{ac} (added LAI from top to the bottom of the plant); SLA (specific leaf area, i.e., leaf area per unit leaf mass, Porter & Jong 1999; Vendramini *et al*., 2002; Wilson *et al*., 1999) and LWC (leaf water content; Garnier & Laurent, 1994). Using SLA and LWC layers values a dispersion graphic was shaped, combining the two indexes, as Roderick *et al.* (1999) proposed in *RSBS – model leaf* (Roderick *et al.,* 1999; Vendramini *et. al.,* 2002).

Mean leaf water potential was compared between species through Student's t-test for paired values, using SPSS (2006) program.

RESULTS AND DISCUSSION

C. palhinhae leaf water potential ranged between -0.4 and -3.0 MPa while the amplitude in *C. ladanifer* was among -0.2 and -5.3 MPa (Table 1). The most negative values were observed in the summer and the less negative in winter. The two species behaviour followed the same tendency ($r^2 = 0.923$). However the two species presented highly significant differences in leaf water potential values ($p < 0.001$, N = 54, t = -8.456).

Table 1– Leaf water potential (MPa) and LAI$_{Ac}$ for each species (three individuals mean).

		Summer	Winter	Spring	Seasonal amplitude
C. palhinhae	$\psi_{\text{máx.}}$	-1.8	-0.5	-0.9	1.3
	$\psi_{\text{mín.}}$	-2.7	-1.2	-2.0	1.5
	Daily amplitude	0.9	0.7	1.1	
	LAIac	1.53	-	2.63	1.1
C. ladanifer	$\psi_{\text{máx.}}$	-4	-0.2	-1.8	3.8
	$\psi_{\text{mín.}}$	-5.1	-1.7	-2.9	3.4
	Daily amplitude	1.1	1.5	1.1	
	LAIac	0.94	-	2.09	1.15

Seasonal leaf water potential amplitude values were different for each species. *C. palhinhae* presented small variations on leaf water potential and can be classified as **hydrostable** (Larcher, 1995), like other mediterranean species: *Laurus* spp. (Lo Gullo & Salleo, 1988), *Arbutus unedo* and *Quercus coccifera* (Werner *et al.,* 1999). *C. ladanifer* is **hydrolabil**, showing significant seasonal variations (Larcher, 1995), like *C. albidus* and *C. monspeliensis* (Werner *et al.,* 1999). The comparison between SLA and LWC (Fig. 1) showed that *C. ladanifer* had higher seasonal variation. Generally, *C. palhinhae* had higher LWC values but SLA values where similar in both species. These results suggest that *C. palhinhae* can be roughly regarded as a **succulent** plant, whereas *C. ladanifer* is a typically **sclerophyll** plant (Roderick *et al.* 1999).

C. ladanifer presented LAI$_{ac}$ values below 1 (Pereira, 2003; Simões, 2002; Werner *et al.*, 1999). The decrease in leaf area and the lower water potential values observed in summer in *C. ladanifer* suggests that this species underwent an intense water stress situation, possibly related to its ecological limits. On other hand, *C. palhinhae* presented, even in adverse conditions, higher LAI$_{ac}$ values compared to semi-deciduous species.

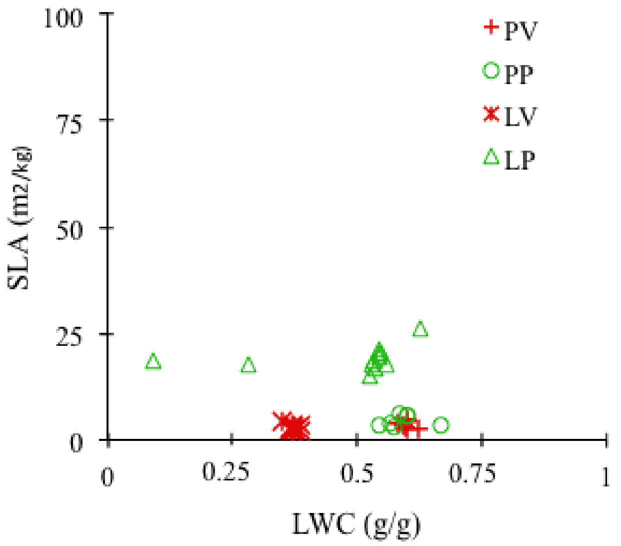

Fig. 1. *Specif Leaf Area* (SLA) vs *Leaf Water Content* (LWC) in Summer and Spring (PV – *C. palhinhae* Summer; PP – *C. palhinhae* Spring; LV – *C. ladanifer* Summer; LP – *C. ladanifer* Spring; each point represents a different layer).

Leaf area index and average leaf thickness resemble those of *Atriplex argentina*, *Maytenus vitis-idaea* and *Lycium elongatum* typified as leaf succulents (Vendramini et al 2002; Roderick et al, 1999). They also share salt resistance, since the presence of salt is extremely relevant in their habitats. However, in the case of *C. palhinhae* the salt is only present in the air and soil by salt spray deposition, while the other mentioned species are more exposed to salt due to its proximity to inner salt lakes (Vendramini et al., 2002). It was observed that the largest differences between the two species were obtained in the summer. This reflects once again the difference in strategies between the two species in response to environmental factors that limit their growth.

CONCLUSIONS

C. palhinhae and *C. ladanifer* have different ecophysiological strategies. Due to the lack of accurate data and detailed occurrence maps for *C. palhinhae* it is not possible to conclude which is the most consistent taxonomic category. It is only possible to disprove Demoly's theory (1991) since the two species are not geographically isolated, despite having different habitats.

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