

30 September - 3 October 2014
University of Évora - Portugal



SOCIEDADE PORTUGUESA
DE FISILOGIA VEGETAL



XII

Portuguese-Spanish
Symposium on:
Plant Water Relations
Water to Feed the World



30 September - 3 October 2014
University of Évora - Portugal



SOCIEDADE PORTUGUESA
DE FISILOGIA VEGETAL



XII

Portuguese-Spanish
Symposium on:
Plant Water Relations
Water to Feed the World



30 September - 3 October 2014
University of Évora - Portugal



SOCIEDADE PORTUGUESA
DE FISILOGIA VEGETAL



XII

Portuguese-Spanish
Symposium on:
Plant Water Relations
Water to Feed the World



30 September - 3 October 2014
University of Évora - Portugal



SOCIEDADE PORTUGUESA
DE FISILOGIA VEGETAL



XII

Portuguese-Spanish
Symposium on:
Plant Water Relations
Water to Feed the World



30 September - 3 October 2014
University of Évora - Portugal



SOCIEDADE PORTUGUESA
DE FISILOGIA VEGETAL



XII

Portuguese-Spanish
Symposium on:
Plant Water Relations
Water to Feed the World



30 September - 3 October 2014
University of Évora - Portugal



SOCIEDADE PORTUGUESA
DE FISILOGIA VEGETAL



XII

Portuguese-Spanish
Symposium on:
Plant Water Relations
Water to Feed the World



30 September - 3 October 2014
University of Évora - Portugal



SOCIEDADE PORTUGUESA
DE FISILOGIA VEGETAL



XII

Portuguese-Spanish
Symposium on:
Plant Water Relations
Water to Feed the World



XII Portuguese-Spanish Symposium on Plant Water Relations Water to Feed the World

BOOK OF PROCEEDINGS

**Évora, 30th of September – 3rd of October
Portugal**

Organising committee

President:	João Santos Pereira (ISA-UL, Lisboa, Portugal)
Secretary:	Margarida M. Vaz (Universidade de Évora, Portugal)
Vocals:	Renato Coelho (Universidade de Évora, Portugal)
	Luís Leopoldo da Silva (Universidade de Évora, Portugal)
	Ana Elisa Rato (Universidade de Évora, Portugal)
	Arturo Torrecillas (CEBAS - CSIC, Murcia, España CEBAS-CSIC)

Scientific committee

João Santos Pereira	ISA-UL, Lisboa, Portugal
M. Manuela Chaves	ISA & ITQB, Lisboa, Portugal
Margarida M. Vaz	Universidade de Évora, Portugal
João Mota Barroso	Universidade de Évora, Portugal
Teresa Soares David	INIAV, Lisboa, Portugal
Jorge Marques da Silva	FCUL-UL, Lisboa, Portugal
M. Manuela David	Universidade do Algarve, Portugal
M. Margarida Oliveira	ITQB, Lisboa, Portugal
Arturo Torrecillas	CEBAS - CSIC, Murcia, España
Aurelio Gómez Cadenas	Universitat Jaume I de Castelló, Barcelona, España
Hipólito Medrano	Universitat de les Illes Balears, España
Robert Savé	IRTA, Barcelona, España
José Enrique Fernández	IRNAS-CSIC, Sevilla, España
Ismael Aranda	INIA-CIFOR Madrid, España
Jaume Flexas	Universitat de les Illes Balears, España
Félix Moreno	IRNAS-CSIC, España

Organising entities



Sponsors



Index

PLENARY CONFERENCES AND THEMATIC CONFERENCES	1
Water Relations in the Irrigation Scheduling of Olive Orchards	2
Water Use in <i>Montado</i> Ecosystems	7
ORAL PRESENTATIONS	13
Genetic Control of Functional Traits Related to Photosynthesis and Water Use Efficiency in <i>Pinus Pinaster</i> Ait. Drought Response: Integration of Genome Annotation, Allele Association and QTL Detection for Candidate Gene Identification	14
Effect of Deficit Irrigation and Elaboration Process of Spanish-Style Green Table Olives on Phytoprostanes Content in Manzanilla de Sevilla Olive Flesh	19
Differences on the Decline of Leaf Hydraulic Conductance with Dehydration in Olive and Almond: Effects on Stomatal Conductance Regulation and Methodological Considerations.	24
More Fruit per Drop: Optimizing Irrigation in “Rocha” Pear Orchards in Central Portugal	29
Short-Term Responses of Water and Carbon Fluxes to Cork Stripping	34
Effects of an Extreme Dry Winter on Cork Oak Woodland: Net Ecosystem Exchange and Phenology Adjustments ..	38
The invasion of <i>Oenothera drummondii</i> on Huelva coastal dunes, previsions of short-time success	43
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	48
POSTERS	53
Phytohormone Profile of Selected Provenances of <i>Pinus Pinaster</i> Aiton.....	54
Drought induced changes in membrane fatty acids and physiological responses of Arabidopsis plants altered in the expression of the phospholipase A gene <i>pPLAIIα</i>	59
Membrane Tolerance in <i>Coffea</i> Species in Response to Environmental Constraints.....	63
Physiological Evaluation of Drought Tolerance in <i>Triticum Durum</i> Genetic Resources	68
Application of Thermal Imaging to Assess the Physiological Status of Papaya Crop (<i>Carica papaya</i> L.) Under Different Deficit Irrigation Regimes	73
Microclimatic Conditions and Gas Exchange in a Vineyard Under Plastic Cover	78
Chloride Nutrition Regulates Water Balance in Plants	82
Physiological Comparison Behaviors between Two Cultivars of <i>Vitis Vinifera</i> (Grenache and Tempranillo Cvars.)...87	
Influence of sustained deficit irrigation on the phenolic composition and quality of grape berries (<i>Vitis vinifera</i> L.) cv. ‘Tempranillo’ and ‘Graciano’	92
Effects of water availability on water use and aesthetic of five Mediterranean species in terms of suitability for green roof implementation	97
Interaction of Light and Water Stress on the Ecophysiological Response of <i>Nothofagus Antarctica</i> (G. Forster) Oerst.....	101
Ecophysiological Studies on <i>Cistus Palhinhae</i> Ingram and <i>Cistus Ladanifer</i> L. in Southern Alentejo	105
Author index	110

PLENARY CONFERENCES AND THEMATIC CONFERENCES

Water Relations in the Irrigation Scheduling of Olive Orchards

Moriana A^{a,b,*}, Corell M^{a,b}, Girón IF^{b,c}, Martín-Palomo MJ^{a,b}, Moreno F^{b,c}

^a Dept. Ciencias Agroforestales, EUITA, University of Seville, Carretera de Utrera Km 1, 41013 Sevilla, Spain;

^b Unidad Asociada al CSIC de Uso sostenible del suelo y el agua en la agricultura (US-IRNAS). Crta de Utrera Km 1, 41013, Sevilla, Spain.

^c Instituto de Recursos Naturales y Agrobiología de Sevilla, IRNAS (CSIC), P.O.Box 1052, 41080 Sevilla, Spain

* e-mail: amoriana@us.es

ABSTRACTS

Olive trees (*Olea europea* L) are traditional Mediterranean specie. The agricultural management of olive orchards has been changed from 90's of the last century. The most important change is the great increase of the irrigation surface. This new water demand has been produced in water scarcity areas. Such conditions and the traditional rainfed management of the orchards have produced very restrictive water used. The traditional irrigation scheduling based on water budget is a useful tool in conditions of full irrigation, but most of the olive orchards are deficit irrigated. In the last decades, plant water status measurements have been suggested in different fruits trees in order to improve the management of deficit irrigation. In this work, results of several experiments in different olive orchards using midday stem water potential and trunk daily diameter are presented. The water stress sensitivity and the real commercial utility are discussed.

INTRODUCTION

Olive tree is traditional rainfed fruit specie in the Mediterranean basin. Olive tree is drought resistant specie that could allow severe level of dehydration and midday water potential around -4.0 MPa are common in scientific papers. Such capacity of drought resistant and the rainfed management have produced that, in the traditional growing zones, deficit irrigation is the most common practise. Water relations have been considered until the end of the XX century as a tool for evaluated the irrigation scheduling strategies in scientific works, but not as real parameter for water management. Plant water status measurements have the advantage of characterised the whole tree and provide a "global point of view" of the irrigation management. However, they have the big disadvantage of the strong relationship with the environment and the plant physiology. The aim of this work is to present the results obtained in several studies of irrigation scheduling in olive trees based on plant water status measurements and discuss and suggest strategies for commercial use.

First steps in the use of plant water status measurements in irrigation scheduling

Water budget is the traditional irrigation scheduling in the field. Deficit irrigation is difficult to control with this information, mainly if the water available is small, such as in olive orchards.

The great capacity of olive trees for dehydrating suggests that water potential is likely one of the most easy and early water stress indicators. Moriana and Fereres (2004) reported that the influence of evaporative demand in the values of midday stem water potential (ψ) is low in no water stress conditions (Figure 1). Then, it would be possible to use the same value of ψ along the summer. Moriana et al (2012) reported several experiments in different conditions (locations and olive cultivars) with the same irrigation scheduling based on stem water potential. This work suggested that -1.2 MPa before pit hardening and -1.4 MPa after pit hardening could be threshold values for full irrigated conditions (Figure 2). These threshold values have been used in other experiments in different conditions and cultivars when a good agreement with the Orgaz et al (2006) model (data not shown). This suggests that though a mild water stress could be assumed, the seasonal physiological response of the orchard would be near to full irrigated conditions.

The determination of the water stress level in deficit irrigation is also affected for the moment and the length of the drought period. From Goldhamer (1999), there is a general agreement about that pit hardening is the most drought resistant phenological stage. The water stress level reported in this period in the literature is very variable with minimum values very low which corresponded with not too high decrease in yield. During the pit hardening period, the fruit is a very important source of carbohydrate and controls the water relations of the whole tree (Martín-Vertedor et al., 2011). Dell'Amico et al (2012) suggested that in low water stress conditions tree water relations delayed the decrease in fruit turgor in table olive. These authors suggested that until values of midday stem water potential around -2.0 MPa fruit growth could be not affected. Unpublished data obtained in a mature hedgerow, showed no significant differences between treatments at -2.0 MPa and -3.5 MPa though a clear trend to reduce yield around 10% (data not shown). Then two levels of water stress could be considered around -2 MPa (a low water stress) and -3.5 MPa (a moderate water stress).

High sensitive irrigation scheduling. Water relations for increasing the accuracy.

Water potential is likely the easiest and cheapest methodology now for commercial purpose but it is not the most accurate. Moriana and Fereres (2002) reported that trunk growth rate (TGR) measured with dendrometers was earlier sensitive to drought conditions than midday stem water potential. Trunk diameter fluctuations are, also, a continuous and automatic measurement which could permit a daily irrigation scheduling. However, this increase in the precision is likely related with an increase in the error. Pérez-López et al (2013) estimated in olive a very high number of sensors (34) to obtain a standard error which were the 10% of the average in the Maximum daily shrinkage (MDS) indicator.

There have been a lot of works with trunk diameter fluctuations parameter from 90's but with different results between species. In olive trees, the relationship between MDS and stem

water potential shows that this indicator is very confusing (Moriana et al 2010). There are no clear differences in MDS in moderate water stress conditions. Full irrigated trees in summer are around maximum values of MDS and water stress trees from -1.5 to probably -2.5 MPa are also in the same range of values. Only with very severe water stress, MDS in stress trees is lower than control.

Maximum daily diameter (MXTD) and trunk growth rate (TGR) are in olive trees more sensitive to drought conditions than MDS. MXTD is the sum of the daily TGR. TGR are more suitable for description of water status, mainly in the period of rehydration (Moriana and Fereres, 2002) than MXTD. MXTD in olive trees, as in other fruits, shows a seasonal pattern link to the fruit development (Moriana et al, 2013; Figure 3). In conditions of enough fruit load, the TGR's seasonal pattern has two different phases, trunk growth, with positives TGR, and constant size period, with TGR values around zero. Such pattern provides very interesting information about the phenological stage of the trees because it is strongly related with the period of massive pit hardening (Pérez-López et al. 2008). Recently, Moriana et al (2013) suggested that a TGR around $-5 \mu\text{m day}^{-1}$ from the beginning of this period until harvest is a useful threshold value in Regulated Deficit Irrigation (RDI).

The most important limitation of this parameter is the lack of relationship between TGR and environmental measurements. Our data during pit hardening period show that TGR is even negative in conditions of full irrigation in some days or periods likely related with very high temperatures (Figure 3), though no significant relationship has been found. Then when TGR is used in irrigation scheduling there is not a clear pattern around this value. In other words, when an irrigation scheduling was based on a threshold value of $-10 \mu\text{m day}^{-1}$ the seasonal pattern of MXTD is not a line with a slope of $-10 \mu\text{m day}^{-1}$, otherwise is a pattern similar to full irrigated control (data not shown). This response and the need of works that provided information with the same irrigation threshold limit the commercial use of these sensors.

All this limitations of the trunk diameter fluctuations indicators show that water relations of this fruit tree are not yet completely solve. Further works than clarify this respond are needed. In addition, it is also very important the experiments in network that provide information of the same threshold value or irrigation scheduling in different conditions.

LIST OF REFERENCES

- Dell'Amico, J., Moriana, A., Corell, M., Girón, I.F., Morales, D., Torrecillas, A., Moreno, F. 2012. Low water stress conditions in table olive trees (*Olea europaea* L) during pit hardening produced a different response of fruit and leaf water relations. *Agric. Water Manage.* 114,11-17
- Goldhamer, D.A. 1999. Regulated deficit irrigation for California canning olives. *Acta Hortic.* 474, 369-372.

- Martin-Vertedor, A.I., Pérez-Rodríguez, J.M., Prieto-Losada, H., Fereres-Castiel, E. 2011. Interactive responses to water deficits and crop load in olive (*Olea europaea* L., cv. Morisca) I. - Growth and water relations. *Agric. Water Manage.*, 98, 941-949.
- Moriana, A. and Fereres, E. 2002. Plant indicators for scheduling irrigation for young olive trees. *Irrigation Science* 21,83-90
- Moriana, A. and Fereres, E. 2004. Establishing references values of trunk diameter fluctuations and stem water potential for irrigation scheduling of olive trees. *Acta Hortic.* 664,407-412.
- Moriana, A., Girón, I., Martín-Palomo, M.J., Conejero, W., Ortuño, M.F., Torrecillas, A., Moreno, F. 2010. New approach for olive trees irrigation schedulings using trunk diameter sensors. *Agric. Water Manage* 97,1822-1828.
- Moriana, A., Pérez-López, D., Prieto, M.H., Ramírez-Santa-Pau, M., Pérez-Rodríguez, J.M. 2012. Midday stem water potential as a useful tool for estimating irrigation requirements in olive trees. *Agric. Water Manage.* 112,43-54.
- Moriana, A., Corell, M., Girón, I.F., Conejero, W., Morales, D., Torrecillas, A., Moreno, F. 2013. Regulated deficit irrigation based on threshold values of trunk diameter fluctuation indicators in table olive trees. *Sci Hort* 164:102-111.
- Orgaz, F., Testi, L., Villalobos, F. J., Fereres, E., 2006. Water requirements of olive orchard-II: determination of crop coefficients for irrigation scheduling. *Irrig.Sci.* 24, 77- 84.
- Pérez-López, D., Moriana, A., Rapoport, H., Olmedilla, N., Ribas, F. 2008 New approach for using trunk growth rate and endocarp development in the irrigation scheduling of young olive orchards. *Scientia Hort*155, 244-251
- Pérez-López, D., Pérez-Rodríguez, J.M., Moreno, M.M., Prieto, M.H., Ramírez-Santa-Pau, M., Gijón, C., Guerrero, J., Moriana, A. 2013. Influence of different cultivars-locations on maximum daily shrinkage indicators: Limits to the reference baseline approach. *Agric. Water Manage.* 127, 31-39

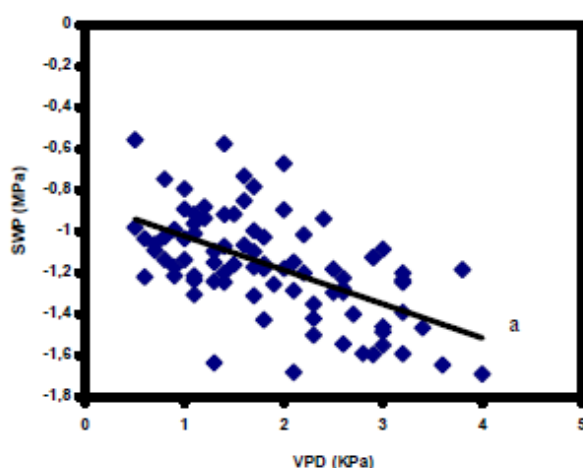


Figure 1. Relationship between midday stem water potential and vapor pressure deficit (VPD) in mature “on” (a), mature “off” (b) and young (c) olive orchards. Source. Moriana and Fereres (2004).

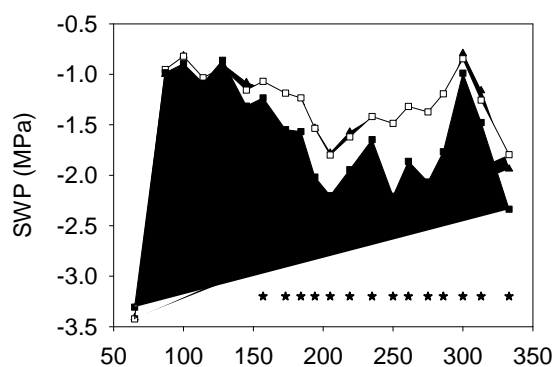


Figure 2. Seasonal pattern of midday stem water potential Badajoz (Spain) cv Morisca. In both experiments the threshold values were the same ▲ Control treatment; □ WI treatment (-1.2 and -1.4 MPa); ■ DI treatment (-2 MPa). Source: Moriana et al (2012)

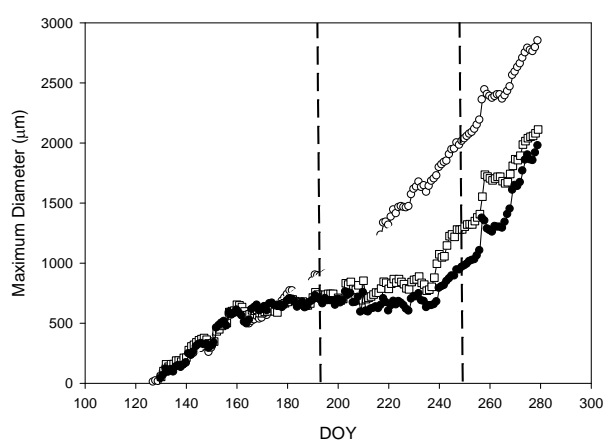


Figure 3. Seasonal pattern of maximum daily diameter during. Solid lines represent Control trees and both dashed lines are regulated deficit treatments. Vertical bars show rain events and vertical lines the periods of pit hardening. Source: Moriana et al 2013

Water Use in *Montado* Ecosystems

Teresa S. David

Instituto Nacional de Investigação Agrária e Veterinária IP (Quinta do Marquês, Oeiras, 2780-159 Oeiras, Portugal); Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa (Tapada da Ajuda, 1349-017 Lisboa, Portugal) Members of the team relevant for the presented results: C.Pinto, J.S. David, J.S.Pereira, N.Nadezhdina, M.O.Henriques, T.Paço, C.Besson, M.C.Caldeira, T.Quilhó, H.Cochard, M.M.Chaves, M.Vaz, F.Valente, L.Gazzarini, I. Ferreira, J.Cérmak

* e-mail:teresa.david@iniav.pt

ABSTRACTS

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 equalled 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 measurements

The work was carried out at three sites: Mitra I (38°32'N, 8°01'W; *Q. ilex*); Mitra II (38°32'N, 8°00'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). 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).

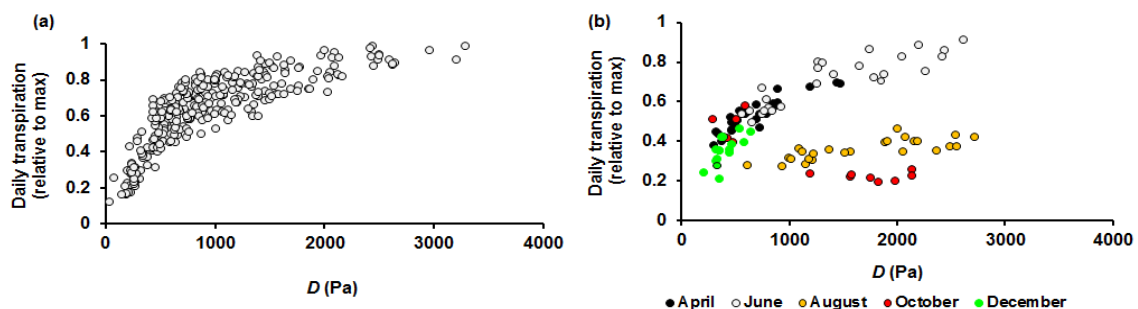


Fig. 1. Relationships between daily tree transpiration (relative to maximum) and D observed at Mitra I and Lezírias (a) and Mitra II (b).

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).

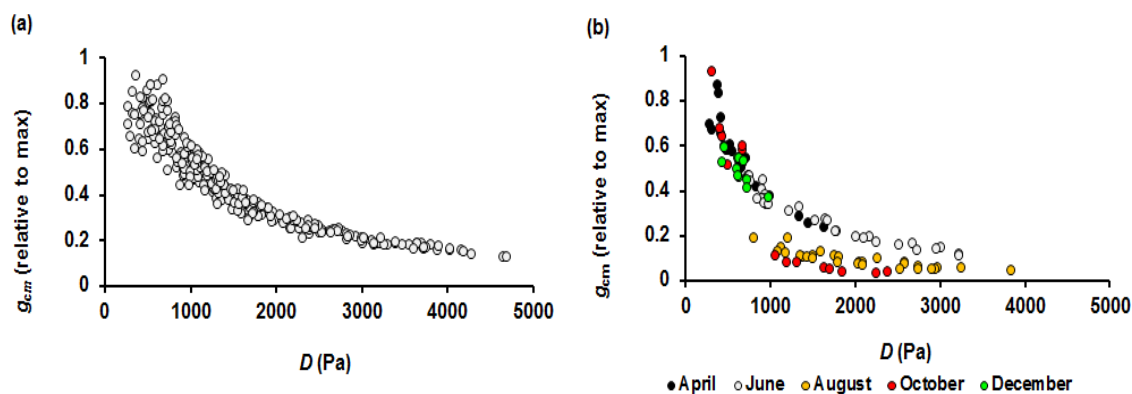


Fig 2 . Relationships between midday canopy conductance (g_{cm} , relative to maximum) and D at Mitra I and Lezírias (a) and Mitra II (b).

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. suber* 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 $\delta^{18}O$) 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 (Nadezhkina *et al.* 2008), showed the occurrence of roots connected to soil and groundwater. From root sap flow data, tree stem sap flow was modelled 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).

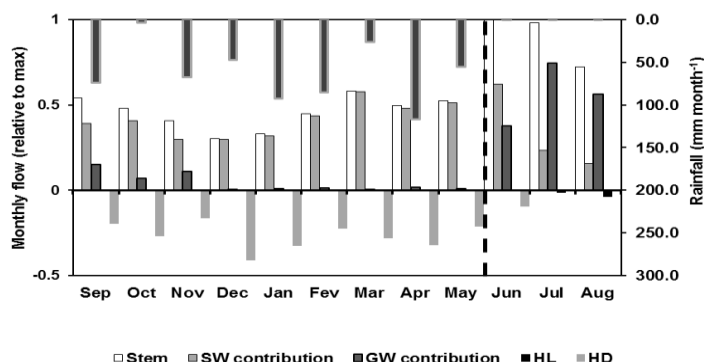


Fig. 3. Monthly flow (relative to maximum) of soil water and groundwater contributions to stem sap flow and relative magnitude of hydraulic lift and hydraulic descent (adapted from David *et al.* (2013)).

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).

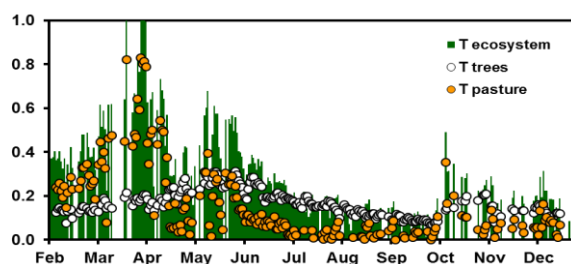


Fig. 4. Partitioning of ecosystem transpiration (relative to maximum) into its components at Mitra II (adapted from Paço *et al.* (2009)).

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; 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 equalled 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 may 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).

ACKNOWLEDGMENTS

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

REFERENCES

- David TS, David JS, Pinto CA, Cermak J, Nadezhdin V & Nadezhdina N 2012. Hydraulic connectivity from roots to branches depicted through sap flow: analysis on a *Quercus suber* tree. *Functional Plant Biology* 39: 103-115.
- David TS, Ferreira MI, Cohen S, Pereira JS & David JS 2004. Constraints on transpiration from an evergreen oak tree in southern Portugal. *Agricultural and Forest Meteorology* 122: 193-205.
- David TS, Henriques MO, Besson CK, Nunes J, Valente F, Vaz M, Pereira JS, Siegwolf R, Chaves MM, Gazarini LC & David JS 2007. Water use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. *Tree Physiology* 27: 793-803.
- David TS, Pinto CA, Nadezhdina N, Kurz-Besson C, Henriques MO, Quilhó T, Cermak J, Chaves MM, Pereira JS & David JS 2013. Root functioning, tree water use and hydraulic redistribution in *Quercus suber* trees: A modeling approach based on root sap flow. *Forest Ecology and Management* 307: 136–146.
- Jarvis PG & McNaughton KG 1986. Stomatal control of transpiration: scaling up from leaf to region. In *Advances in Ecological Research* 15, Macfadyen A, Ford ED (eds). Academic Press: London, 1–49.
- Nadezhdina N, Ferreira MI, Silva R, Pacheco CA 2008. Seasonal variation of water uptake of a *Quercus suber* tree in Central Portugal. *Plant Soil* 305: 105–119.
- Paço TA, David TS, Henriques MO, Pereira JS, Valente F, Banza J, Pereira FL, Pinto C & David JS 2009. Evapotranspiration from a Mediterranean evergreen oak savannah: the role of trees and pasture. *Journal of Hydrology* 369: 98-106.
- Pinto CA, David JS, Cochard H, Caldeira MC, Henriques MO, Quilhó T, Paço TA, Pereira JS & David TS 2012. Drought-induced embolism in current-year shoots of two Mediterranean evergreen oaks. *Forest Ecology and Management* 285: 1-10.
- Pinto CA, Nadezhdina N, David JS, Kurz-Besson C, Caldeira MC, Henriques MO, Monteiro FG, Pereira JS & David TS 2013. Transpiration in *Quercus suber* trees under shallow water table conditions: the role of soil and groundwater. *Hydrological Processes* (doi: 10.1002/hyp.10097).

ORAL PRESENTATIONS

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.

de Miguel M.^{1,2}, Cabezas J-A.^{1,2}, de María N.^{1,2}, Sánchez-Gómez D¹, Guevara M.A.^{1,2}, Vélez M.D.^{1,2}, Sáez-Laguna E.^{1,2}, Díaz L.M.^{1,2}, Mancha J.A.^{1,2}, Barbero M.C.^{1,2}, Collada C.^{2,3}, Díaz-Sala C.⁴, Aranda I¹, Cervera M.T.^{1,2}

1Departamento de Ecología y Genética Forestal. INIA-CIFOR. Ctra. de La Coruña Km 7.5, 28040 Madrid, Spain, demiguel.marina@gmail.com

2Unidad Mixta de Genómica y Ecofisiología Forestal, INIA/UPM, Madrid, Spain.

3ETSIM, Departamento de Biotecnología, Ciudad Universitaria s/n 28040 Madrid, Spain.

4Departamento de Ciencias de la Vida. Universidad de Alcalá. Ctra. de Barcelona Km 33.6, 28871 Alcalá de Henares, Madrid, Spain.

* e-mail: demiguel.marina@gmail.com

ABSTRACTS

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. 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.

ACKNOWLEDGMENTS

This work was supported by MAPINSEQ (AGL2009-10496), PinCoxSeq (AGL2012-35175), SUSTAINPINE (PLE2009-0016) and Procogen (FP7/2007-2013). L Alté and S Ferrándiz are gratefully acknowledged for their assistance.

REFERENCES

- Brendel O., et al., (2002) Genetic parameters and QTL analysis of $\delta^{13}\text{C}$ and ring width in maritime pine, *Plant, Cell & Environment*, 25, 945-953.
- de Miguel M., et al., (2014) 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, *BMC Genomics*, 15, 464.
- de Miguel M., et al., (2012) Functional and genetic characterization of gas exchange and intrinsic water use efficiency in a full-sib family of *Pinus pinaster* Ait. in response to drought, *Tree Physiology*, 32(1), 94-103.
- Guo P., et al., (2008) QTLs for chlorophyll and chlorophyll fluorescence parameters in barley under post-flowering drought, *Euphytica*, 163(2), 203-214.
- Paterson A.H., et al., (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms, *Nature*, 335, 721-726.
- Sarris D., et al., (2007) Recent decline in precipitation and tree growth in the eastern Mediterranean, *Global change biology*, 13(6), 1187-1200.
- Sehgal D., et al., (2012) Integration of gene-based markers in a pearl millet genetic map for identification of candidate genes underlying drought tolerance quantitative trait loci, *BMC Plant Biology*, 12, 9.
- Thavamanikumar S., et al., (2013) Dissection of complex traits in forest trees: opportunities for marker-assisted selection, *Tree Genetics & Genomes*, 9(3), 627-639.
- Thumma B.R., et al., (2001) Identification of causal relationships among traits related to drought resistance in *Stylosanthes scabra* using QTL analysis, *Journal of Experimental Botany*, 52(355), 203.
- Watkinson J.I., et al., (2003) Photosynthetic acclimation is reflected in specific patterns of gene expression in drought-stressed loblolly pine, *Plant Physiology*, 133(4), 1702-1716.
- Whan A., et al., (2010) A quantitative genetics approach to nitrogen use efficiency in sugarcane, *Functional Plant Biology*, 37(5), 448-454.
- Yin Z., et al., (2010) Mapping quantitative trait loci associated with chlorophyll a fluorescence parameters in soybean (*Glycine max* (L.) Merr.), *Planta*, 231, 875-885.

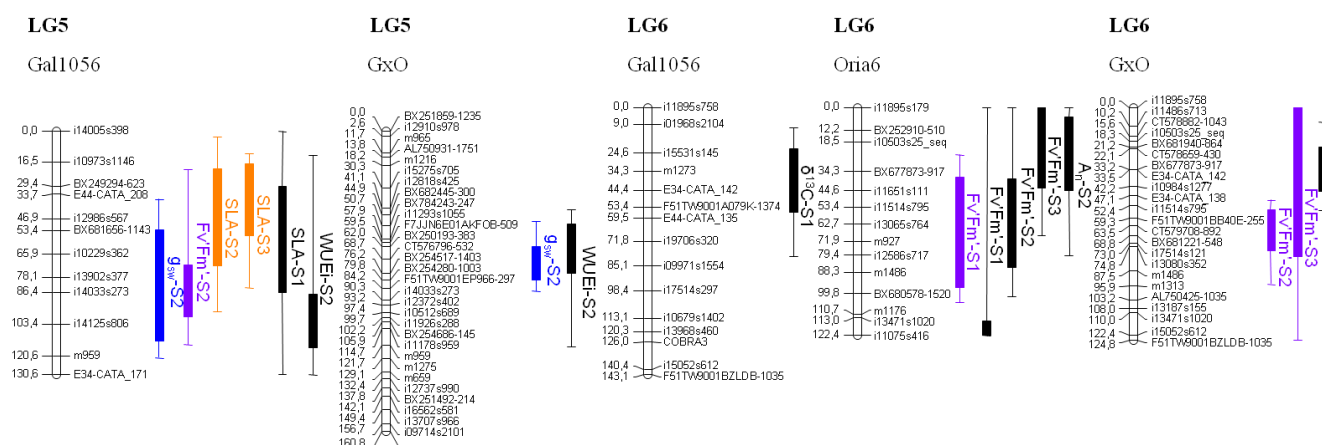


Figure 1. Representation of QTL identified on the parental (Gal1056 and Oria6) and consensus (GxO) linkage maps (LGs 5 and 6). Confidence intervals are indicated by squares (± 1 LOD) and lines (± 2 LOD). Colored QTL are the significant QTL (significant at 99% at chromosome level or 95% at genome wide 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.

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

Collado-González J¹, Moriana A², Girón IF³, Corell M², Medina S¹, Durand T⁴, Guy A⁴, Galano JM⁴, Galindo A⁵, Ferreres F¹, Moreno F³, Torrecillas A^e, Gil-Izquierdo A^{1*}

1 Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology. Centro de Edafología y Biología Aplicada del Segura (CSIC), P.O. Box 164, Espinardo, E-30100 Murcia, Spain

2 Departamento de Ciencias Agroforestales, EUITA. University of Seville, Carretera de Utrera Km 1, 41013 Sevilla, Spain

3 Instituto de Recursos Naturales y Agrobiología de Sevilla (CSIC), P.O.Box 1052, 41080 Sevilla, Spain

4 Institut des Biomolécules Max Mousseron (IBMM), UMR 5247 - CNRS - University Montpellier I and II - ENSCM, Faculty of Pharmacy, Montpellier, France.

5 Departamento de Riego. Centro de Edafología y Biología Aplicada del Segura (CSIC), P.O. Box 164, Espinardo, E-30100 Murcia, Spain

*e-mail: angelgil@cebas.csic.es

ABSTRACTS

Manzanilla de Sevilla olive trees were submitted 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 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 nonenzymatic 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) ($ET_c = ET_o \times K_c \times K_r$). During the 2012 and 2013 growing seasons, plants were irrigated using a drip irrigation system. Control treatment (T0) trees were over-irrigated (125% ET_c) 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 (Ψ_{stem}) 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 $SI = \sum (\bar{\Psi}_{stem} - (-1.4)) \times n$, where $\bar{\Psi}_{stem}$ is the average Ψ_{stem} values for any interval and n is the number of the days in the interval. Olive fleshs were milled with a solution of MeOH and BHA. PhytoPs in olive flesh and olive covering liquid were extracted using a liquid-liquid extraction (LLE) 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-PhytoP + *Ent*-9-L1-PhytoP 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 no enzymatic lipid peroxidation from α -linolenic acid (Thoma et al., 2003), the absence of these PhytoPs induces to think that 16-B1-PhytoP + *Ent*-16-B1-PhytoP and 9-L1-PhytoP + *Ent*-9-L1-PhytoP 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.

PhytoPs	Season	Treatment		
		T0	T1	T2
9-F1t-PhytoP	2012	363.54cA	538.58bA	627.87aA
	2013	383.37cA	431.82bB	468.71aB
9- <i>epi</i> -9-F1t-PhytoP	2012	193.86bA	232.17aA	254.14aA
	2013	154.95bB	184.07abB	201.24aB
16-B1-PhytoP + <i>Ent</i> -16-B1-PhytoP	2012	32.39cA	48.45bA	82.91aA
	2013	29.86cA	41.39bA	58.53aB
9-L1-PhytoP + <i>Ent</i> -9-L1-PhytoP	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.

PhytoPs	Season	Treatment		
		T0	T1	T2
9-F1t-PhytoP	2012	2504.04bA	3587.52aA	3730.31aA
	2013	2627.63bA	3521.58aA	3625.42aA
9- <i>epi</i> -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 (Moriani et al., 2013) but is clearly critical for PhytoPs content in treated and non-treated olive fleshs (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 $\min \Psi_{\text{stem}}$ (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 $\min \Psi_{\text{stem}}$ (MPa) and SI (MPa x day) using all data pooled.

PhytoP	<i>a</i>	<i>b</i>	r^2	<i>n</i>	MSE
<i>PhytoP vs. $\min \Psi_{\text{stem}}$</i>					
9-F1t-PhytoP	8.52	- 209.94	0.7593*	6	49.02
9- <i>epi</i> -9-F1t-PhytoP	58.20	-66.20	0.5556 ^{ns}	6	23.51
16-B1-PhytoP + <i>Ent</i> -16-B1-PhytoP	- 46.44	- 43.58	0.8633**	6	7.29
9-L1-PhytoP + <i>Ent</i> -9-L1-PhytoP	- 21.63	- 18.99	0.8981**	6	2.70
Total content	- 1.34	- 338.60	0.7861*	6	73.56
<i>PhytoP vs. SI</i>					
9-F1t-PhytoP	329.56	5.77	0.8742**	6	35.43
9- <i>epi</i> -9-F1t-PhytoP	158.23	1.87	0.6975*	6	19.40
16-B1-PhytoP + <i>Ent</i> -16-B1-PhytoP	21.14	1.15	0.8977**	6	6.30
9-L1-PhytoP + <i>Ent</i> -9-L1-PhytoP	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.

PhytoPs	Season	Treatment		
		T0	T1	T2
9-F1t-PhytoP	2012	25.54cA	59.34aA	38.68bA
	2013	4.99cB	15.17bB	26.21aB
9- <i>epi</i> -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.

ACKNOWLEDGEMENTS

To R. Pleites and Global Olive and Cooperativa Nuestra Señora de las Virtudes for the management of the industrial processing. This research was supported by MICINN (CICYT/FEDER AGL2013-45922-C2-1-R and AGL2013-45922-C2-2-R) CSIC 201170E041, Fundación Séneca (04486/GERM/06) and CYTED, Action 112RT0460 CORNUCOPIA grants to the authors. JCG and AG were funded by a grant of the FPI and FPU fellowship program, respectively, and SM was appointed under a CSIC research contract.

REFERENCES

- Durand, T. et al. 2009. *Lipids* 44, 875-888.
 Galindo, A. et al. 2014. *Agric. For. Meteorol.* 194, 29-35.
 Loeffler, C. et al. 2005. *Plant Physiol.* 137, 328-340.
 Minghetti, L. et al. 2014. *Free Radical Biol. Med.* (In Press).
 Moriana, A. et al. 2013. *Sci. Hortic.* 164, 102-111.
 Thoma, I. et al. 2003. *Plant J.* 34, 363-37

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

Hernandez-Santana V*, Rodriguez-Dominguez CM, Díaz-Espejo A

Irrigation and Crop Ecophysiology Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC). Avenida Reina Mercedes 10, 41012 Sevilla, Spain.

* e-mail: virginiahsa@irnas.csic.es

ABSTRACTS

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 relationship 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 individuals of olive and almond. Data obtained with two methodologies 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 the potential differences between them. The results showed that the vulnerability curves for K_{leaf} determined by the two different methodologies were indistinguishable, but connecting 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 xylem conduits in the leaves. K_{leaf} was significantly higher in almond than in olive for decreasing Ψ_{leaf} which is 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 Mediterranean climate, which is contrary to previously observed g_s dynamics for both species. Thus, a high K_{leaf} may favour a high g_s in almond and olive but according to our results, K_{leaf} would not determine the stomatal closure due to low Ψ_{leaf} .

INTRODUCTION

A major resistance component of the plant hydraulic system has been demonstrated to be 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 “Dynamic rehydration kinetics method” (DRKM, Blackman and Brodribb, 2011) some uncertainties are still unresolved (Flexas et al. 2013) such as the potential embolism caused by cutting the xylem under tension to make K_{leaf} measurements. Moreover, the extent of the K_{leaf} decline with water stress and its recovery

varies from species to species, even within a particular habitat (Scoffoni et al. 2012) and the knowledge of its relationships to leaf structure and ecological strategy remains incomplete (Blackman et al. 2010). Vein density (VD) is one of the major structural features that contribute 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 two agricultural Mediterranean species with different ecology (olive-*Olea europea* and almond-*Prunus dulcis*) in well irrigated young individuals. Data obtained with two methodologies (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 the potential differences between them.

MATERIALS AND METHODS

Olive (*Olea europea* L., cv 'Arbequina') and almond (*Prunus dulcis* (Mill.) D.A. Webb cv. Guara) shoots were sampled from young potted trees of 4 years irrigated regularly. The trees were located in an orchard nearby Seville (Spain) (37° 15' N, -5° 48' 102). The area has a Mediterranean climate with hot and dry weather from May to September and 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, current year and sun exposed leaves. The shoots were cut under water and then, they were allowed to dehydrate to obtain a wide range of leaf water potential values (Ψ_{leaf}) and allowed to equilibrate for at least 30 mins in dark plastic bags with wet paper towel. The leaves were cut under purified water and rapidly connected to a flowmeter consisting of a silicon tubing containing purified and degassed water. One group of leaves were taken out the water to be connected to the flowmeter for few seconds and another group were connected under water. The tubing connects the leaf to a pressure transducer (Omega Engineering Ltd., UK), which was, in turn, connected to a CR1000 data logger (Campbell, Campbell Scientific Ltd., UK) to register readings every one second to calculate the flow rate through the leaf ($\text{mmol s}^{-1} \text{m}^{-2}$). 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_0) was measured in neighboring leaves. K_{leaf} was also determined in 29 leaves of each species using EFM as described in Scoffoni et al. (2012). For direct comparisons K_{leaf} was determined in 10 leaves of each species using both methods. To construct the vulnerability curve for each species, K_{leaf} was then related to the lowest Ψ_{leaf} water potential which is Ψ_0 in DRKM and Ψ_0 or Ψ_{final} in EFM (Scoffoni et al. 2012). In the above-described methods all Ψ_{leaf} were measured with a Scholander-type pressure chamber (PMS Instrument Company, Albany, Oregon, USA).

Ten leaves of both species similar to the leaves where K_{leaf} was determined were used to quantify the 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 mm² in almond and 11 mm² in olive were taken centrally in the top, middle and bottom thirds of the leaves and ImageJ (Schneider et al. 2012) was used to quantify the vein length.

The fitted functions to our $\Psi_{leaf} - K_{leaf}$ dataset 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 the differences between the models were <0.05. R package was used for all the analyses (R version 2.14.0).

RESULTS AND DISCUSSION

The results showed that the vulnerability curves for K_{leaf} determined by DRKM and EFM were statistically indistinguishable ($P=0.87$ and 0.12 for olive and almond, respectively). The K_{leaf} data obtained by the direct comparison of both methodologies were also similar although DRKM data were slightly lower than EFM data ($r^2=0.79$ 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 for lower Ψ_{leaf} .

The vulnerability curves for K_{leaf} were best described by exponential functions for both species. 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 are also compatible with the fact that almond is able to keep higher g_s than olive for lower Ψ_{leaf} , as shown in other works (Fernández et al. 1997; Rodríguez-Domínguez et al. *in preparation*). High VD would allow the almond leaves to achieve greater K_{leaf} favoring higher g_s . However, K_{leaf} slightly varied for Ψ_{leaf} values that are normally obtained during the day in the field under Mediterranean conditions, contrary to what has been observed for g_s 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 along the day (Fernández et al. 1997; Rodríguez-Domínguez et al. *in preparation*). Thus, a high K_{leaf} may favour greater g_s in almond and olive. However, according to our results, K_{leaf} would not determine the stomatal closure due to low Ψ_{leaf} because both variables would have different trends in the short-term. Further work is needed to assess these hypotheses.

ACKNOWLEDGMENTS

This work was funded by Spanish Ministry of Science and Innovation (AGL2012-34544). The first author benefited from a Juan de la Cierva postdoctoral research fellowship from Spanish Ministry of Science and Innovation.

REFERENCES

- Blackman & Brodribb (2011). Two measures of leaf capacitance... *Funct. Plant Biol.* 38, 118–126
- Fernández et al. (1997). Stomatal control of water use in olive tree leaves. *Plant Soil* 190, 179–192
- Flexas et al. (2013). Leaf mesophyll conductance and leaf hydraulic... *J. Exp. Bot.* 64, 3965–81
- Rodriguez-Dominguez. The contribution of hydraulic and... *In preparation* to New Phytologist
- Sack & Scoffoni (2013). Leaf venation: structure, function, development... *New Phytol.* 198, 983–1000
- Sack & Holbrook (2006). Leaf hydraulics. *Annu. Rev. Plant Biol.* 57, 361–81.
- Schneider et al. (2012). NIH Image to ImageJ... *Nature Methods*, 9, 671–675.
- Scoffoni et al. (2012). Dynamics of leaf hydraulic conductance with water... *J. Exp. Bot.* 63, 643–58.

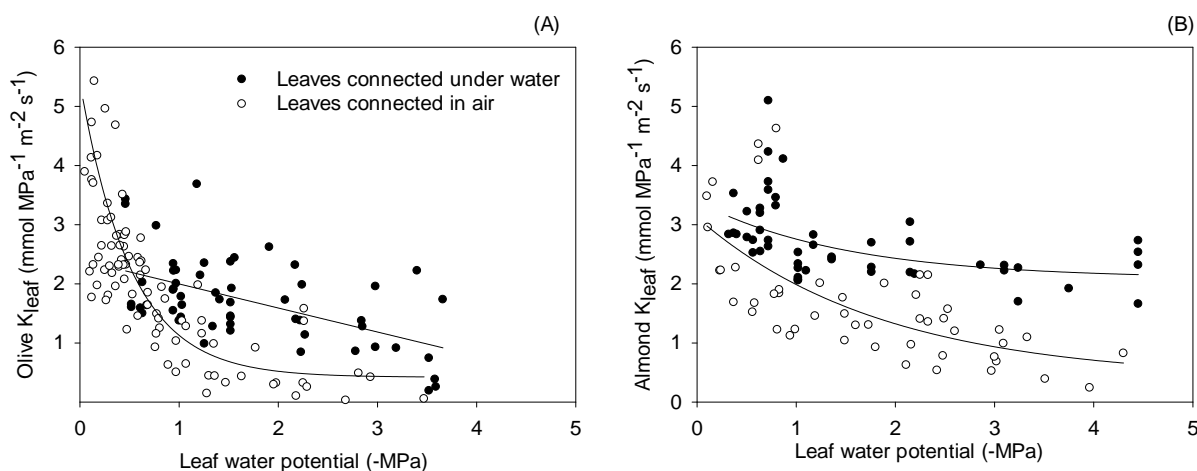


Figure 1. Vulnerability curves for K_{leaf} for (A) olive and (B) almond determined using two approaches (petioles cut under water-black circles or in air-white circles) to test the effect of air entry into xylem conduits upon cutting the petioles.

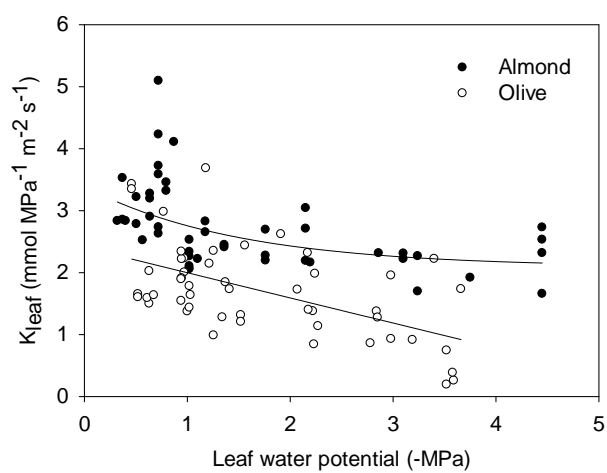


Figure 2. Comparison of vulnerability curves for K_{leaf} for olive (white circles) and almond (black circles) estimated using DRKM.

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

Silvestre S^{1*}, Leão de Sousa M², Ribeiro D³, Pires S³, Rodrigues B³, Arrabaça JD¹, Matos AR¹, Bernardes da Silva A¹, Marques da Silva J¹

1 Universidade de Lisboa, Faculdade de Ciências, Center of Biodiversity, Functional and Integrative Genomics (BioFIG), Campo Grande, 1749-016 Lisboa, Portugal.

2 Campotec, S.A., EN 9, Zona Industrial de Casalinhos de Alfaiata, 2560-393 Silveira, Torres Vedras, Portugal.

3 Hidrosoph, Lda., Rua Alcárcova de Baixo, 54 Sala C, 7000-841 Évora, Portugal.

* e-mail: smsilvestre@fc.ul.pt

ABSTRACTS

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 integrity. 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 optimise water use in irrigated orchards of “Rocha” pear in Central Portugal.

MATERIALS AND METHODS

Four “Rocha” pear orchards (*Brejo*, *Rol*, *Ruiva* and *Junqueira*) 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 CO₂, 25 ± 2 °C), 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 (WUE_i) was calculated as the ratio photosynthesis/stomatal conductance at 200 and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. 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). Conductivity (Cond) was measured with a conductimeter Eutech Ecoscan Con 5 (EUTECH INSTRUMENTS, Singapore). Total conductivity (Cond_T) was measured after heating samples for 90 min at 90 °C and relative conductivity (Cond_{Rel}) calculated as $(\text{Cond}/\text{Cond}_T) \times 100$. Data were analyzed by two-way ANOVA with GraphPad Prism 6.01 (GraphPad Software, USA).

RESULTS AND DISCUSSION

In May, the photosynthetic capacity (A_{max}, 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 CO₂ (LCP) 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 WUE_i in May, but this parameter decreased sharply in Junqueira, whereas it stayed steady in Brejo (Fig. 1E, F). 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 PI_{ABS} 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). 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). 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 higher membrane relative conductivity in July. In all sites except Brejo membrane conductivity increased in July (Fig. 4). 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

We are grateful to PRODER, 4.1 Cooperação para a Inovação, nº 44894 for financial support.

REFERENCES

- Beck, et al. (2007) *J. Biosci.*, 32(3), 501–510.
Lambers, et al. (1998) Plant Physiological Ecology, *The Netherlands: Springer*, pp. 154-209.
Lichtenthaler (1987) *Meth Enzymol* 148, 350-382
Marques da Silva and Arrabaça (2004) *J Plant Physiol*, 161, 551-555.
Nunes et al. (2009) *Ann Appl Biol*, 155, 321-332.
Silvestre, (2014) *J Integr Plant Biol*, 56 (7), 610 – 621.

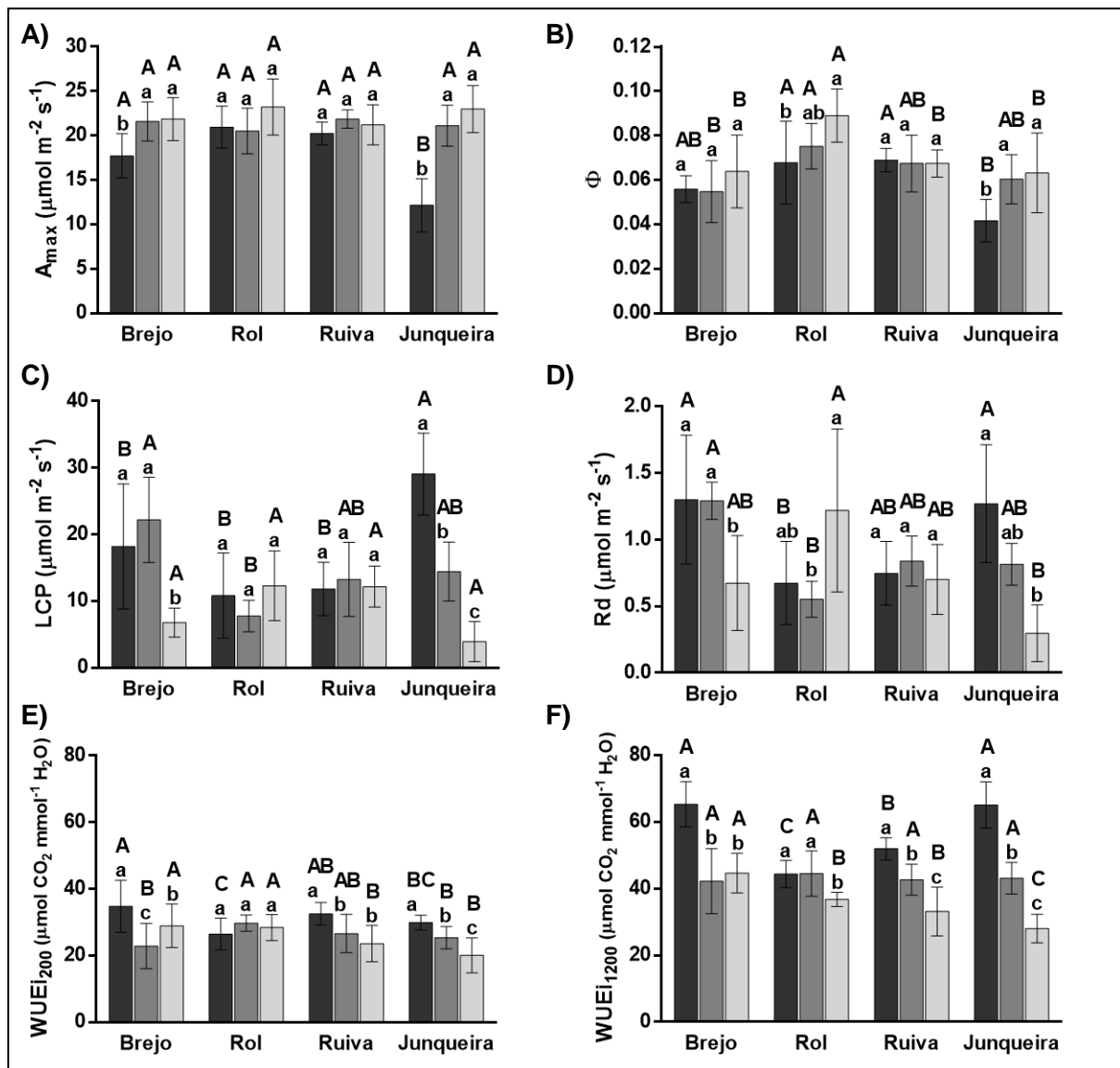


Figure 1. A) Maximum photosynthesis (A_{max}), B) photosynthetic apparent quantum yield (ϕ), C) apparent light compensation point (LCP), D) dark respiration point (R_d), and intrinsic water use efficiency (WUE_i) at E) 200 and F) 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for the four orchards Brejo,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

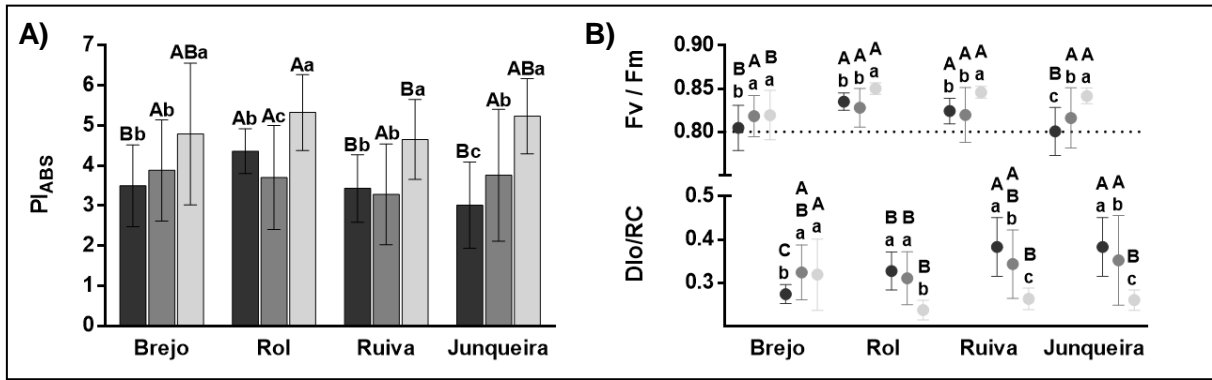


Figure 2. A) Performance index on absorption basis (PI_{ABS}), B) maximum quantum yield of PSII (F_v/F_m), and energy flux of dissipation energy per reaction center (Dio/RC) for the four orchards Brejo, Rol, Ruiva and Junqueira during ■ May, ■ June, and ■ July. Different letters indicate significant differences between orchards (uppercase) and months (lowercase). Values are mean \pm SD.

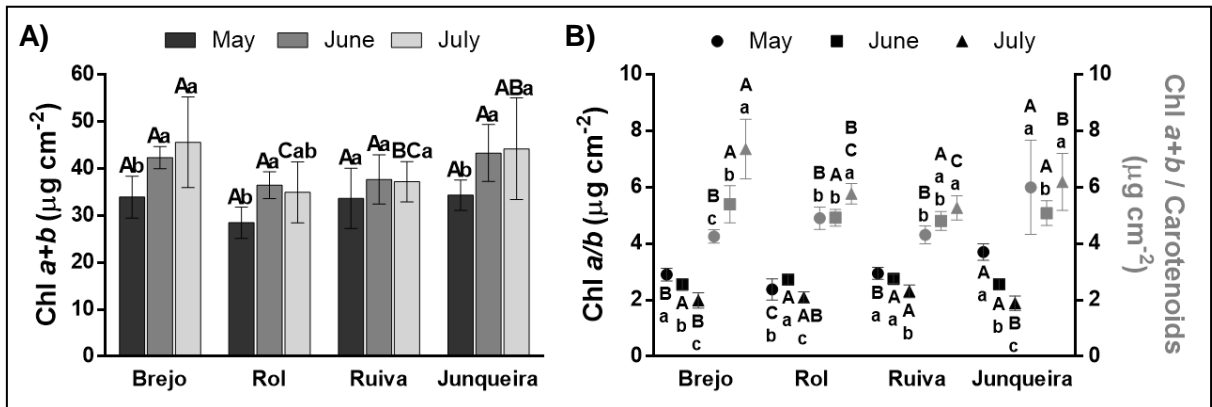


Figure 3. A) Total chl content ($Chl\ a+b$), B) chl a/b ratio ($Chl\ a/b$), and the ratio between total chl content and carotenoids ($Chl\ a+b / Carotenoids$) for the four orchards Brejo, Rol, Ruiva and Junqueira during May, June, and July. Different letters indicate significant differences between orchards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

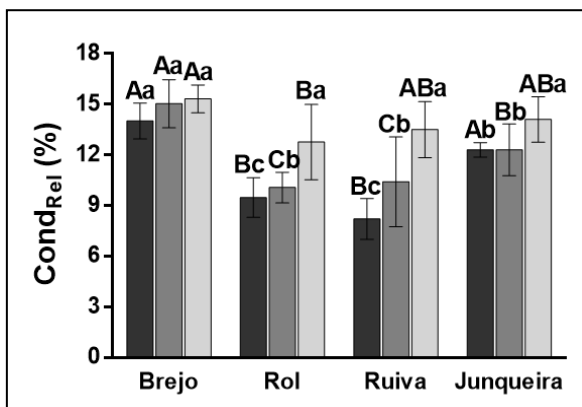


Figure 4. Relative conductivity ($Cond_{Rel}$) for the four orchards Brejo, Rol, Ruiva and Junqueira during ■ May, ■ June, and ■ July. Different letters indicate significant differences between orchards (uppercase) and months (lowercase). Values are mean \pm SD.

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

Correia AC¹, Costa e Silva F.¹, Pinto C², David TS², Hernandez-Santana V³, Pereira JS¹

¹Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal, alexandrac@isa.utl.pt

²Instituto Nacional de Investigação Agrária e Veterinária, Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal

³Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), Av de la Reina Mercedes, 10, 41012 Sevilla, Espanha

* e-mail: alexandrac@isa.utl.pt

ABSTRACTS

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 ecophysiology (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 (7 July 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 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stem respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and stem water loss ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$).

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 1°C 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. 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 CO₂ and H₂O 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 CO₂ and H₂O 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.

REFERENCES

- Camilo-Alves CC, M. I. E.;Ribeiro,N. 2013. Decline of Mediterranean oak trees and its association with *Phytophthora cinnamomi*: a review. *European Journal of Forest Research* 132: 411-432.
- Oliveira GC, A. 2012. How resilient is *Quercus suber* L. to cork harvesting? A review and identification of knowledge gaps. *Forest Ecology and Management* 270: 257-272.

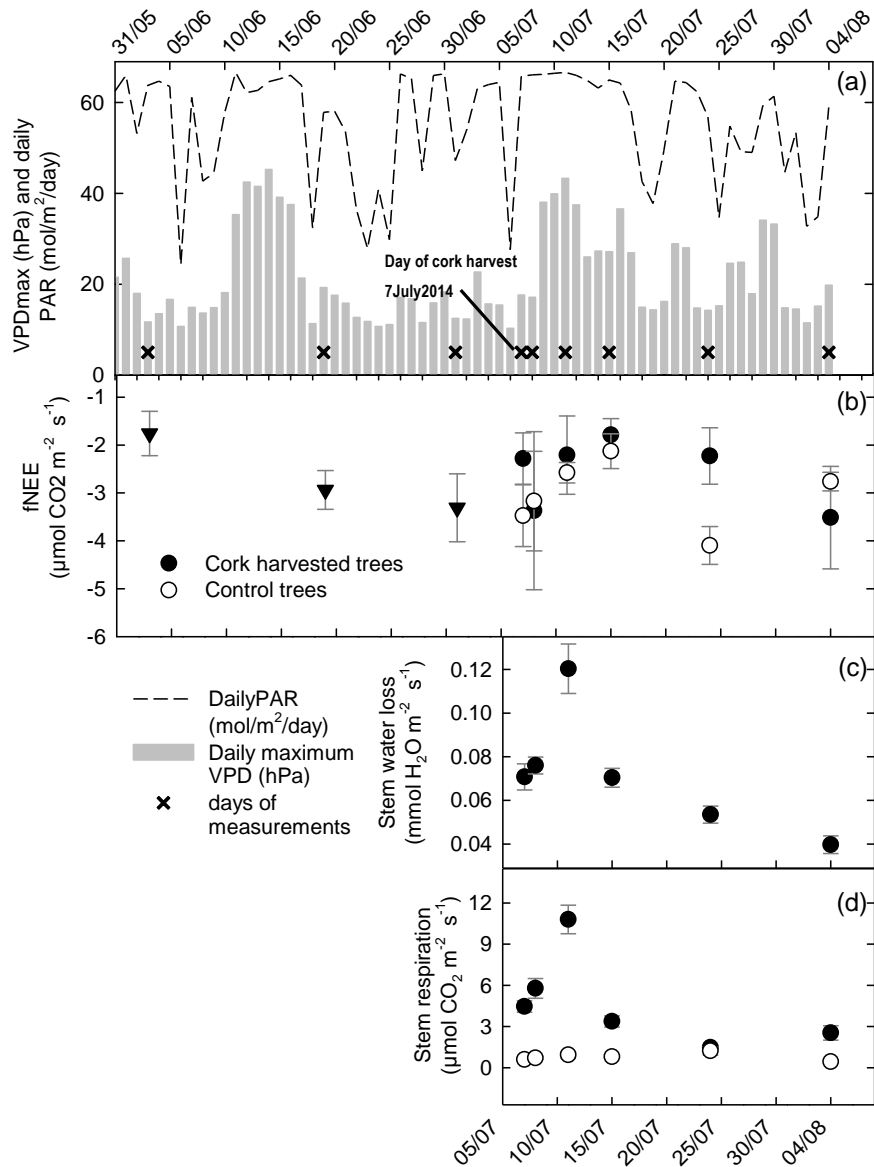


Fig. 1 – (a) Meteorological data during June and July 2014 daily average of total incident photosynthetically active radiation (PAR, mol m⁻² d⁻¹) and maximum daily vapour pressure deficit (VPDmax, hPa), (X) represent the days of measurements (b) Net carbon uptake measured in the light chamber ($\mu\text{mol m}^{-2} \text{ d}^{-1}$) for the days of measurements, (c) Stem water loss ($\text{mmol H}_2\text{O m}^{-2} \text{ d}^{-1}$) for the days of measurements and (d) Stem respiration for the days of measurements ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ d}^{-1}$). Erros bars represent standard errors.

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

Costa e Silva F*, Correia AC, Piayda A, Dubbert M, Werner C, David JS, Pereira JS

CEF, Instituto Superior de Agronomia. Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa (Portugal).

* e-mail: filipecs@isa.utl.pt

ABSTRACTS

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 °C (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 CO₂ 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 m² 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). 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 evergreenness 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). 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 (day 127 to 156, Fig. 2) in opposition to 2011 where stem diameter growth rates were sustained. 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.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of Fundação para a Ciência e Tecnologia, through the fellowships to F. Costa e Silva and A. Correia and through the project “Cork carbon footprint: from trees to products (PTDC/AGR-FOR/4360/2012)”. The authors wish to thank António Gonçalves Ferreira for providing field site facilities at Machoqueira do Grou.

REFERENCES

- Correia, A.C. et al., 2014. Carbon sink strength of a Mediterranean cork oak understorey: how do semi-deciduous and evergreen shrubs face summer drought? *Journal of Vegetation Science*, 25(2): 411-426
- Limousin, J.M. et al., 2009. Long-term transpiration change with rainfall decline in a Mediterranean *Quercus ilex* forest. *Global Change Biology*, 15(9): 2163-2175.
- Misson, L. et al., 2011. Phenological responses to extreme droughts in a Mediterranean forest. *Global Change Biology*, 17(2): 1036-1048.
- Pinto, C.A. et al., 2011. Phenology and growth dynamics in Mediterranean evergreen oaks: Effects of environmental conditions and water relations. *Forest Ecology and Management*, 262(3): 500-508.

Richardson, A.D. et al., 2010. Influence of spring and autumn phenological transitions on forest ecosystem productivity. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 365(1555): 3227-3246.

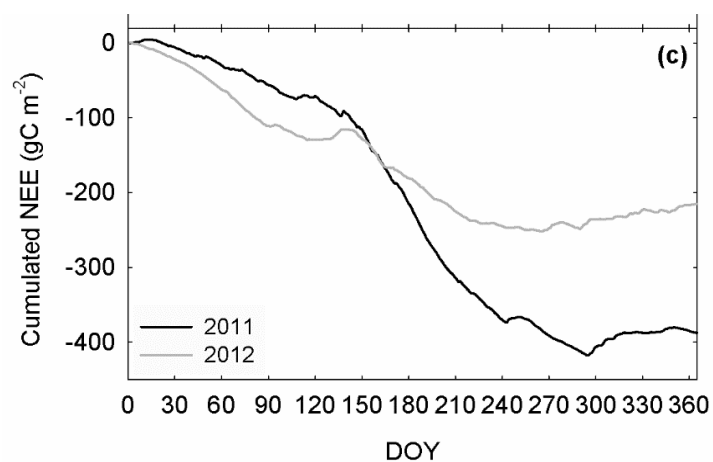


Fig. 1. Cumulated values of net ecosystem exchange (NEE, g C m⁻²) during 2011 and 2012. Negative values represent carbon sequestration in the ecosystem.

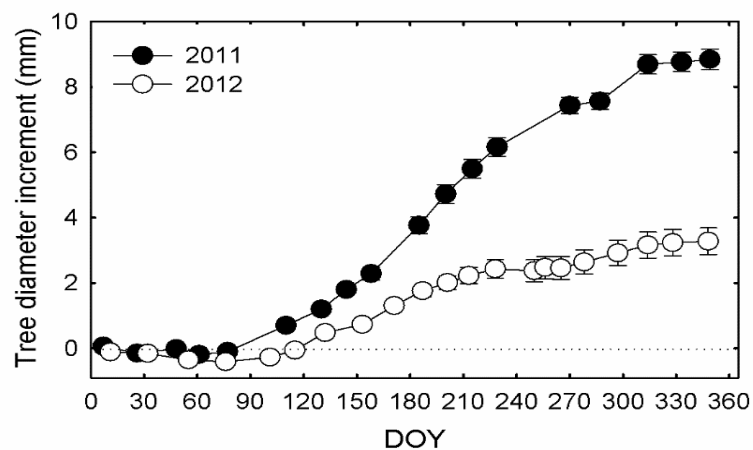


Fig. 2. Tree diameter increment (mm) during 2011 and 2012. Values are means \pm se ($n=9$).

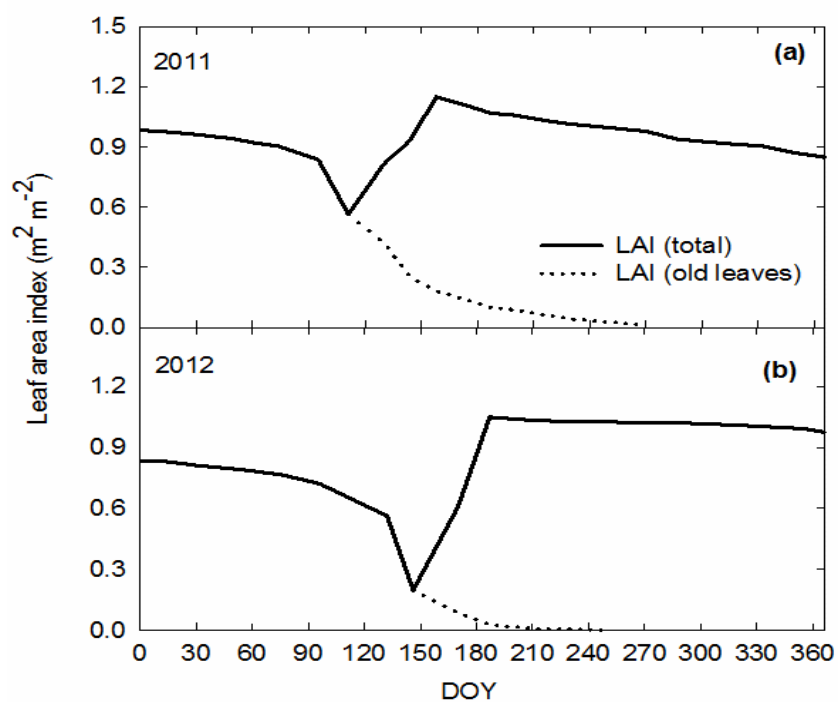


Fig. 3. Tree leaf area index (LAI) during 2011 (a) and 2012 (b). The dash line represents LAI of old leaves matured in the previous spring.

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

Zunzunegui González, M.¹, Ruiz, E.¹, Sert, M.², Diaz-Barradas, M.C.¹ Gallego Fernández, J.B.¹

¹Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Apartado 1095, 41080 Sevilla, SPAIN, zunzu@us.es

²Universidade Estadual de Maringá, Departamento de Biología, Maringá, BRASIL

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. drummondii 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. drummondii* 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. drummondii* 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 (gs) and transpiration rates (E) were made using a portable compact CO₂/H₂O 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/gs). 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/m²). 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. $F_v/F_m = (F_m - F_0)/F_m$, 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. drummondii* always exhibited a less negative water potential than *O. broteri* which evidenced a better water performance in the exotic species

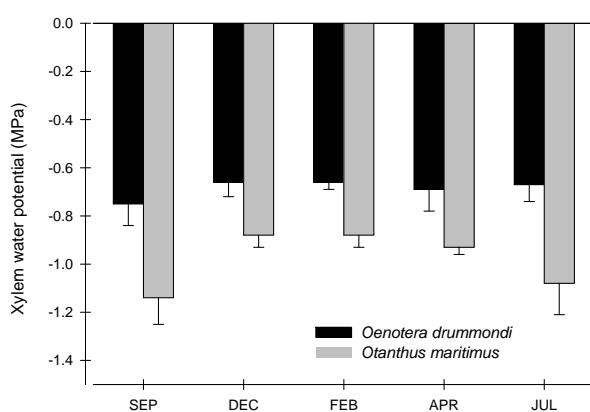


Fig. 1. Average (\pm sd) midday leaf water potential, in the two study species, over the 2013-14 annual cycle.

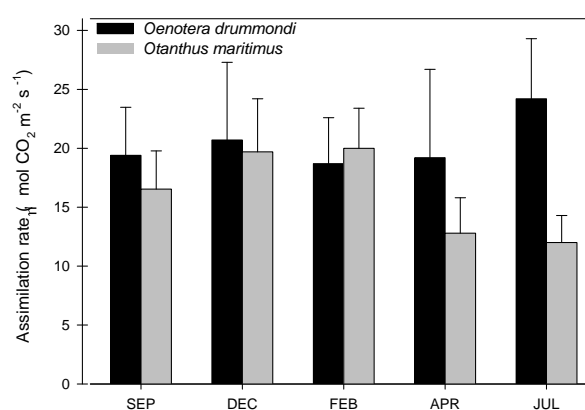


Fig. 2. Average (\pm sd) net assimilation rate in the two study species, over the 2013-14 annual cycle.

O. drummondii 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 $20 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ the whole year, while in the native species figures exhibit a seasonal fluctuation (Fig. 2).

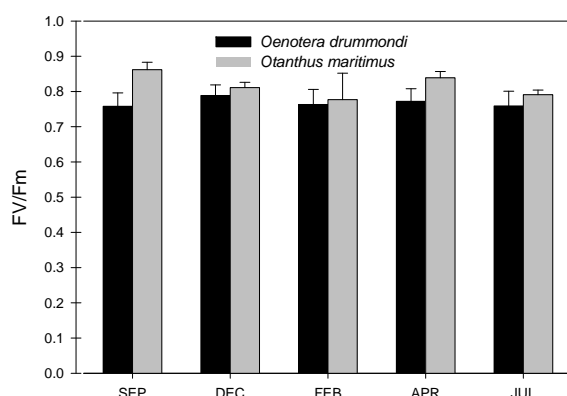


Fig. 3. Average (\pm sd) midday F_v/F_m of dark adapted leaves, in the two study species over the 2013-14 annual cycle.

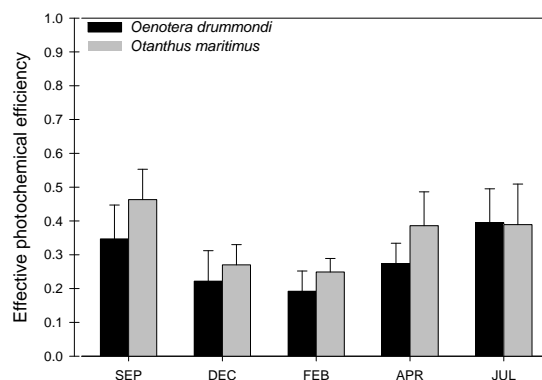


Fig. 4. Average (\pm sd) midday effective quantum yield, in the two study species over the 2013-14 annual cycle.

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

CONCLUSIONS

These first results show that *O. drummondii* 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. drummondii* 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. drummondii*, which transform this species in a dangerous alien invasive; only cold temperatures might be a limiting factor to control the invasion.

REFERENCES

- Bates LS, et al. (1973) Rapid determination of free proline for water stress studies, Plant Soil, 39, 205–207
- Bottollier-Curtet M, et al. (2013) Competition between young exotic invasive and native dominant plant species: implications for invasions within riparian areas, 24, 1033-1042
- Ehrenfeld JG (2010) Ecosystem Consequences of Biological Invasions, Annual Review of Ecology, Evolution, and Systematics. 41, 59-80
- Kourtev PS, et al. (2002) Exotic plant species alter the microbial community structure and function in the soil. Ecology 83, 3152-3166

Levine JM et al., (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proc R Soc Lond B*, 270, 775–781

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148, 350-382

Zunzunegui M, et al. (2005) To live or to survive in Doñana dunes: adaptive responses of woody species under a Mediterranean climate. *Plant and Soil*, 273, 77–89.

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

Rodríguez-Dominguez CM^{1,2}, Buckley TN³, de Cires A², Fernández JE¹, Perez-Martin A¹, Díaz-Espejo A¹

1 Irrigation and Crop Ecophysiology Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC). Avenida Reina Mercedes 10, 41012 Sevilla, Spain. crdominguez@us.es

2 Departamento de Biología Vegetal y Ecología, Universidad de Sevilla, Sevilla, Spain

3 IA Watson Grains Research Centre, Faculty of Agriculture and Environment, The University of Sydney, Narrabri, NSW 2390 Australia

* e-mail: crdominguez@us.es

ABSTRACTS

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:

$$(1) \quad P'_p = \frac{P_p - P_{\min,1}}{P_{\max,1} - P_{\min,1}}, \text{ where } P_{\min,1} \text{ is the minimum value (maximum turgor pressure)}$$

reached at pre-dawn of the decided experimental day (1 = June 25; 2 = August 3) and $P_{\max,1}$ 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:

$$(2) \quad g_{s,\text{model}} = \frac{\chi\beta\tau K(\Psi_s + \pi)}{K + \chi\beta\tau\text{VPD}}, \text{ where } K \text{ is leaf-specific hydraulic conductance, } \chi \text{ is a}$$

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:

$$(3) \quad K_{\text{var}} = \frac{g_s \text{VPD}}{\Psi_s + \pi - \frac{g_s}{\chi\beta\tau}}.$$

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

$$(4) \quad \Psi_{\text{l,model}} = -\left(\frac{g_{s,\text{model}} \text{VPD}}{K} - \Psi_s\right), \text{ and } \pi \text{ is the maximum value estimated as:}$$

$$(5) \quad \pi = \frac{g_s}{\chi\beta\tau} - \Psi_{\text{leaf}}.$$

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). 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. 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). 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.

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

This work was funded by the Spanish Ministry of Science and Innovation (research projects AGL2009-11310/AGR) and co-funded by FEDER programme. The first author benefited from a FPD research fellowship from the Junta de Andalucía. We are grateful to A. Montero and V. Hernandez-Santana for assistance in the field.

REFERENCES

- Buckley & Mott (2013). Modelling stomatal conductance... *Plant Cell Environ.*, 36, 1691 - 1699.
- Buckley et al. (2003). A hydromechanical and biochemical... *Plant Cell Environ.*, 26: 1767 - 1785.
- Dichio et al. (2006). Osmotic regulation in leaves and roots... *Tree Physiol.*, 26, 179 - 185.
- Ehrenberger et al. (2012). Leaf patch clamp pressure probe... *Plant Biol.*, 14, 666 - 674.
- Fernández et al. (2011). Online-monitoring of tree water... *Agr Water Manage.*, 100, 25 - 35.
- Fernández et al. (2013). A regulated deficit irrigation strategy... *Plant Soil*, 372: 279 - 295.
- Jones (2004). Irrigation scheduling... *J Exp Bot.*, 55, 2427 - 2436.
- Rüger et al. (2010). Comparative monitoring of temporal... *Agr Water Manage.*, 98, 283 - 290.
- Torres-Ruiz et al. (2013). Loss of hydraulic functioning at leaf... *Acta Horticulturae*, 991, 333 - 339.

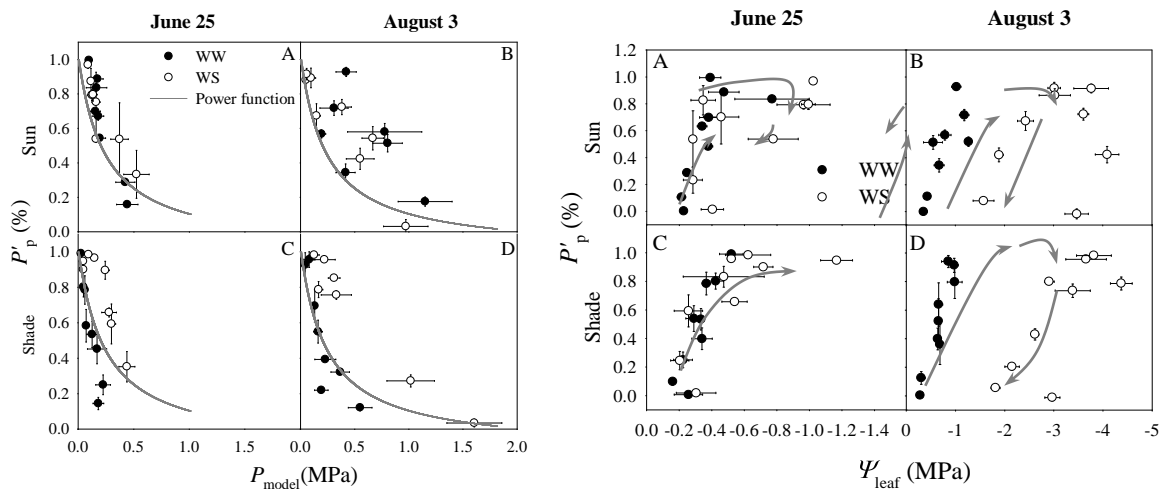


Figure 1 (left). Relationships between the normalized output leaf patch pressure (P'_p , %) and the absolute leaf turgor pressure modeled with the BMF model (P_{model}) on the two experimental dates in sunny and shaded leaves and for the two water treatments (WW and WS). Points are mean and error bars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

Figure 2 (right). Relationships between the normalized output leaf patch pressure (P'_p , %) and the leaf water potential (Ψ_{leaf} , -MPa). Gray arrows indicate the diurnal evolution of the measurements. Rest as in Fig. 1.

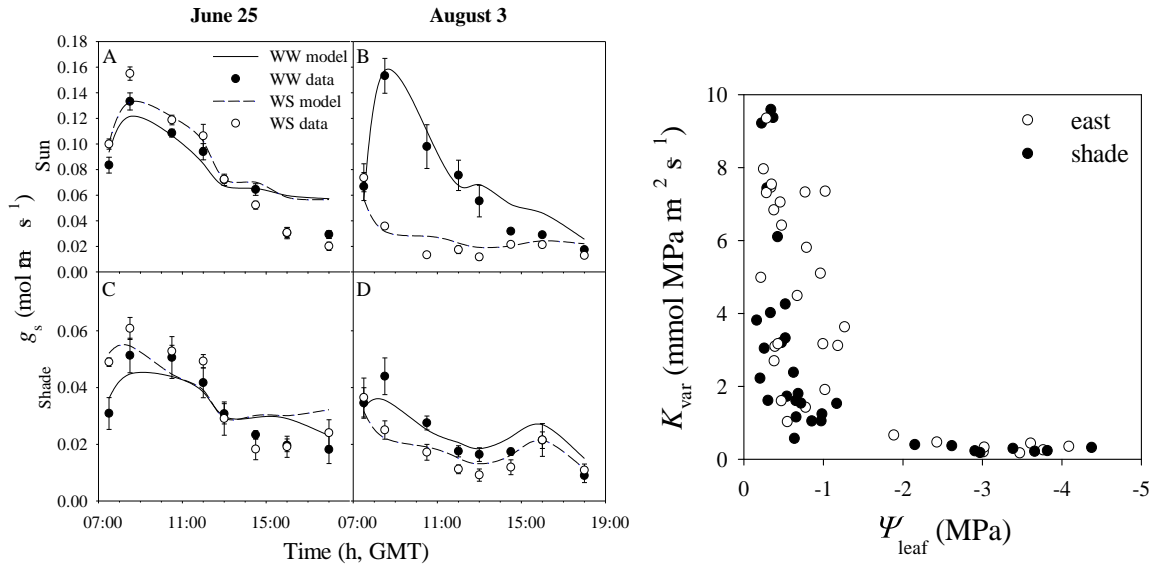


Figure 3 (left). Evolution of g_s data (points) and g_s fitted with the BMF model (lines). Rest as in Fig. 1 $n = 4$ for g_s data. GMT = Greenwich Mean Time.

Figure 4 (right). 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.

POSTERS

Phytohormone Profile of Selected Provenances of *Pinus Pinaster* Aiton.

Delatorre C*, Granda V, Dantas AK, Meijón M, Majada JP, Feito I, Rodríguez A.

Departamento de Biología de Organismos y Sistemas, Facultad de Biología, Universidad de Oviedo, C/
Catedrático Rodrigo Uría s/n, 33071, Oviedo, Asturias, España.

* e-mail: delatorre85@gmail.com

ABSTRACTS

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 °C and dry weight (DW) after one week at 60° 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 CO₂ 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 (epibrassinolide, 24EB; abscisic acid, ABA; indolacetic acid, AIA; benziladenine, BA; castasterone, BK; dihydrozeatin, DHZ; dihydrozeatinriboside, DHRZ; gibberellins GA₁, GA₃, GA₄, GA₇ and GA₉; homobrassinolide, 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/H₂O/concentrated HCl (2:1:0.002, v/v/v) with internal standards (d₇-BA (10 ng); d₆-ABA, d₅-AIA, DHJA, d₃-DHZ, d₆-SA (20 ng); d₅-BK y d₂-GA1 (40 ng)) were added. Next the mixture was agitated for 30 min at 4 °C after which CH₂Cl₂ (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 $\Psi_{\pi 100}$ 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 GA₃- 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 GA₃, the former presenting the lowest levels of GA₃ 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.

REFERENCES

- Granda, V. et al. (2011). "Rapid responses of C14 clone of *Eucalyptus globulus* to root drought stress: Time-course of hormonal and physiological signaling." *Journal of Plant Physiology* 168(7): 661-670.

- Jaime, R. et al. (2014). "Differences in gas exchange contribute to habitat differentiation in Iberian columbines from contrasting light and water environments." *Plant Biology* 16(2): 354-364.
- Nguyen, A. et al. (2002). "Osmotic adjustment in *Pinus pinaster* cuttings in response to a soil drying cycle." *Ann. For. Sci.* 59: 795-799.
- Novak, O. et al. (2008). "Cytokinin profiling in plant tissues using ultra-performance liquid chromatography-electrospray tandem mass spectrometry." *Phytochemistry* 69(11): 2214-2224.
- Ocheltree, T. W. et al. (2013). "Stomatal responses to changes in vapor pressure deficit reflect tissue-specific differences in hydraulic conductance." *Plant, Cell & Environment* 37(1): 132-139.
- Sakamoto, T. et al. (2013). "Auxin signal transcription factor regulates expression of the brassinosteroid receptor gene in rice." *Plant Journal* 73(4): 676-688.
- Scholander, P. F. et al. (1965). "Sap pressure in vascular plants." *Science* 148(3668): 339-346.
- Turner, N. C. (1988). "Measurement of plant water status by the pressure chamber technique." *Irrigation Science* 9(4): 289-308.
- Xi, Z. et al. (2013). "Effects of 24-epibrassinolide on antioxidation defense and osmoregulation systems of young grapevines (*V-vinifera* L.) under chilling stress." *Plant Growth Regulation* 71(1): 57-65.

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

Marques da Silva J, Bernardes da Silva A, Ribeiro AS, Silvestre S, Arrabaça JD, Matos AR.

University of Lisbon, Faculty of Science, Department of Plant Biology and Center for Biodiversity, Functional and Integrative Genomics, Campo Grande, 1749-016 Lisbon, Portugal. armatos@fc.ul.pt

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*, *pPLAII α* , encoding an enzyme that releases fatty acids from membrane lipids. Here we have used plants overexpressing (OE) or silenced (AS) for *pPLAII α* , 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 (ET_o/RC), without changes in absorption (ABS/RC) and trapping (TR_o/RC). Our results indicate that *pPLAII α* 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 submitted 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*, pPLAII α , 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 pPLAII α we analyze 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 cm³ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 23/18°C (day/night) and relative humidity of 70 %. Plants were watered by sub-irrigation 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. \times 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). 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). 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 that and the increased photochemical performance. Drought induced a decrease of F_v/F_m 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). 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 pPLA1 α is required for drought-induced membrane adjustments and the lack of this protein negatively impacts photochemical performance.

ACKNOWLEDGMENTS

This work was funded by the project PTDC/AGR-AAM/103721/2008 from Fundação para a Ciência e Tecnologia, Portugal.

REFERENCES

- Bligh and Dyer (1959) *Can J BiochemPhysiol*, 235, 8–17.
- Gigon, et al. (2004) *Ann Bot (Lond)*, 94, 345-51.
- La Camera (2005) *Plant J*, 44, 810-25.
- Matos and Pham-Thi (2009) *Plant PhysiolBiochem.*, 47(6), 491-503.
- Matos, et al. (2001) *FEBS Letters*, 491(3), 188-92.
- Matos, et al. (2008) *Physiol Plant*, 134(1), 110-120.
- Scherer, et al. (2010) *Trends Plant Sci.*, 15(12), 693-700.
- Silvestre, et al. (2014) *JIntegr Plant Biol.*, 56(7), 610-621.
- Vieira da Silva, et al., (2012) XI Simposio Hispano-Portugués de Relaciones Hídricas en las Plantas, Seville.

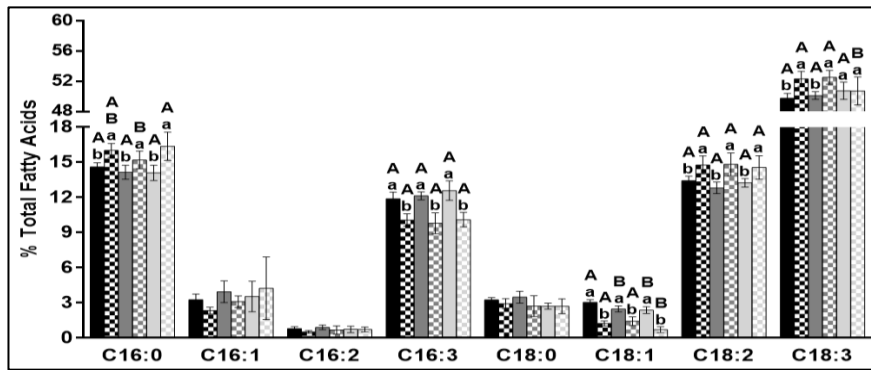


Figure 1. Fatty acid composition of total lipids from fully expanded leaves of *pPLAIIα* overexpressing (■OE), wild type (■WT), and antisense (■AS) lines under control (no pattern) and drought (pattern) conditions. Values are mean \pm SD.

Different letters indicate significant differences between lines (uppercase) and treatment (lowercase); no differences were found in C16:1, C16:2 and C18:0.

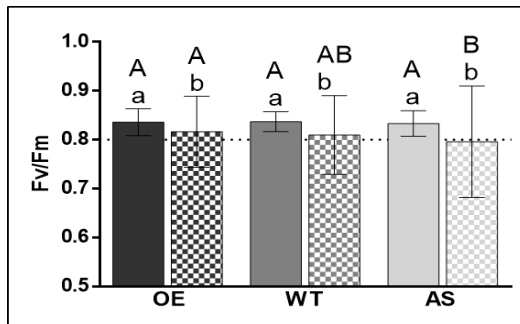


Figure 2. Maximum quantum efficiency of PSII (Fv/Fm) for *pPLAIIα* overexpressing (■OE), wild type (■WT), and antisense (■AS) lines under control (no pattern) and drought (pattern). Values are mean \pm SD. Different letters indicate significant differences between lines (upper) and treatment (lowercase).

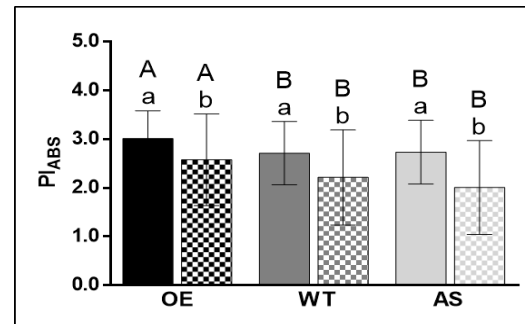


Figure 3. Performance index on absorption basis (PI_{ABS}) for *pPLAIIα* overexpressing (■OE), wild type (■WT), and antisense (■AS) lines under control (no pattern) and drought (pattern). Values are mean \pm SD. Different letters indicate significant differences between lines (upper) and treatment (lowercase).

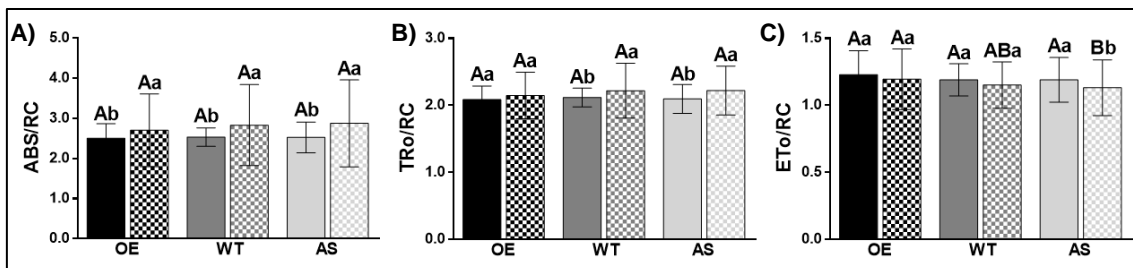


Figure 4. Energy fluxes per reaction center for absorption A) (ABS/RC), B) trapping (TRo/RC), and C) electron transport (ETo/RC) for *pPLAIIα* overexpressing (■OE), wild type (■WT), and antisense (■AS) lines under control (no pattern) and drought (pattern) conditions. Values are mean \pm SD. Different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

Membrane Tolerance in *Coffea* Species in Response to Environmental Constraints

Scotti-Campos P ^{1*}, Pais IP ¹, Fortunato AS ², Lidon FC ³, Ramalho JC ²

1 Unidade de Investigação em Biotecnologia e Recursos Genéticos, Inst. Nac. Inv. Agrária e Veterinária, I.P., Qta do Marquês, Av. República, 2784-505 Oeiras, Portugal.

2 Grupo Interações Planta-Ambiente, Centro Ambiente, Agricultura e Desenvolvimento, Inst. Inv. Científica Tropical, I.P., Qta do Marquês, Av. República, 2784-505 Oeiras, Portugal.

3 Cicege, DCT, Fac. Ciências e Tecnologia, UNL, Qta da Torre, 2829-516 Caparica, Portugal.

* e-mail: paula.scotti@iniav.pt

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 °C, day/night), photoperiod (12 h), RH (65–70%), irradiance (750–850 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the upper part of the canopy) and CO_2 (380 $\mu\text{L L}^{-1}$). 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and CO_2 (ca. 7%) saturating conditions, at 25°C, 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 $\text{DBI} = [(\% \text{monoenes} + 2 \times \% \text{dienes} + 3 \times \% \text{trienes}) / \% \text{saturated FAs}]$.

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). 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.

ACKNOWLEDGMENTS:

This work was supported by Portuguese national funds through Fundação para a Ciência e Tecnologia, under the scope of the project PTDC/AGR-PRO/3386/2012 and the grant SFRH/BPD/47563/2008 (A.S. Fortunato), the latter co-financed through the POPH program subsidized by the European Social Fund.

REFERENCES

- Cakmak I. & Horst J.H. (1991) Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*), *Physiol. Plant.*, 83, 463-468.
- Campos P.S. et al. (1999) Effects of drought on photosynthetic performance and water relations of four *Vigna* genotypes, *Photosynthetica*, 36, 79-87.
- Campos P.S. et al. (2003) Electrolyte leakage and lipid degradation account for cold

sensitivity in leaves of *Coffea* sp. Plants, J. Plant Physiol., 160, 283-292.

Chaves M.M. et al. (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell, Ann. Bot., 103, 551-560.

DaMatta F.M. & Ramalho J.C. (2006) Impacts of drought and temperature stress on coffee physiology and production: a review, Braz. J. Plant Physiol., 18, 55-81.

Ramalho J.C. et al. (1998) Nitrogen dependent changes in antioxidant system and in fatty acid composition of chloroplast membranes from *Coffea arabica* L. plants submitted to high irradiance, Plant Sci., 135, 115-124.

Ramalho J.C. et al. (2013) Sustained photosynthetic performance of *Coffea* spp. under long-term enhanced [CO₂], PLoS ONE, 8, e82712.

Scotti-Campos P. et al. (2013) Physiological responses and membrane integrity in three *Vigna* genotypes with contrasting drought tolerance, Emir. J. Food Agric., 25, 1002-1013.

Scotti-Campos P. et al. (2014) Phospholipids profile in chloroplasts of *Coffea* spp. genotypes differing in cold acclimation ability, J. Plant Physiol., 171, 243-249.

Torres-Franklin M.L. et al. (2009) Omega-3 fatty acid desaturase (FAD3, FAD7, FAD8) gene expression and linolenic acid content in cowpea leaves submitted to drought and after rehydration, Environ. Exp. Bot., 65, 162-169.

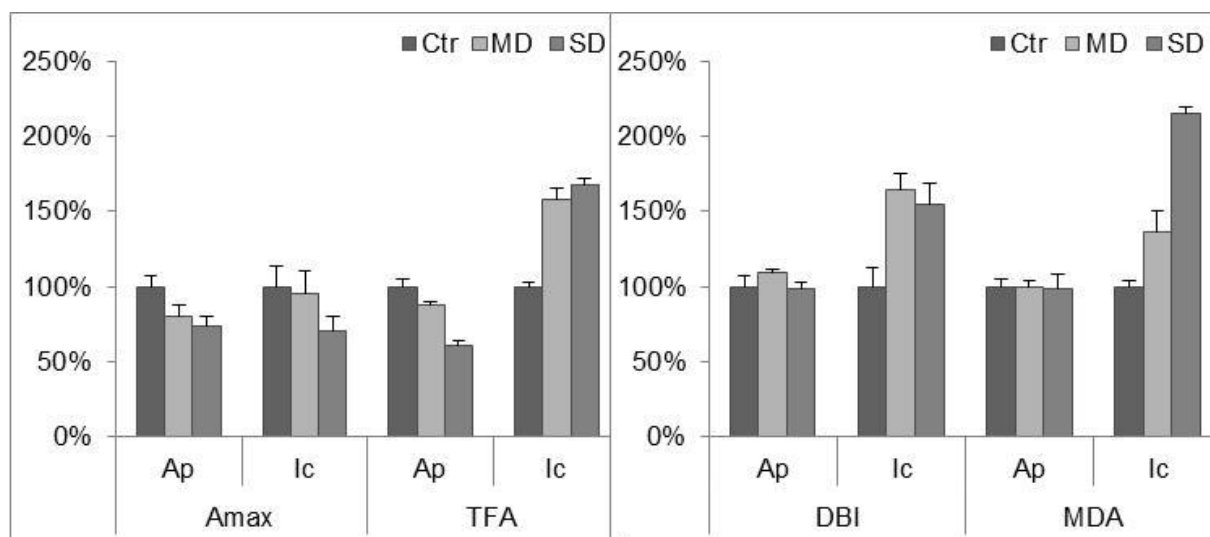


Figure 1 – Effect of moderate (MD) and severe (SD) drought in photosynthetic capacity (A_{max}) and chloroplast total fatty acids (TFA) in two *Coffea* genotypes. Results as % of control, representing the mean \pm SE (n=3-6)

Figure 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 \pm SE (n=3-4).

Table 1 – Changes in main fatty acids of chloroplast lipids in two *Coffea* genotypes subjected to drought. MD: moderate drought; SD: severe drought. Results as % of control, representing the mean \pm SE (n=3).

	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Ap-Ctr	100 \pm 5	100 \pm 9	100 \pm 4	100 \pm 3	100 \pm 0	100 \pm 3
Ap-MD	88 \pm 6	149 \pm 20	110 \pm 6	208 \pm 10	84 \pm 1	103 \pm 3
Ap-SD	104 \pm 18	93 \pm 13	108 \pm 3	173 \pm 6	87 \pm 3	100 \pm 6
Ic-Ctr	100 \pm 7	100 \pm 3	100 \pm 11	100 \pm 5	100 \pm 5	100 \pm 5
Ic-MD	73 \pm 7	128 \pm 9	69 \pm 5	85 \pm 8	109 \pm 13	122 \pm 6
Ic-SD	73 \pm 4	178 \pm 9	76 \pm 10	143 \pm 8	101 \pm 5	118 \pm 6

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

Scotti-Campos P^{1*}, Semedo JN¹, Pais IP¹, Oliveira MM¹, Passarinho JA¹, Santos M¹, Ramalho JC², Almeida AS¹, Costa AR¹, Pinheiro N¹, Coutinho J¹, Maçãs B¹.

1 Unidade de Investigação em Biotecnologia e Recursos Genéticos, Inst. Nac. Inv. Agrária e Veterinária, I.P., Qta Marquês, 2784-505 Oeiras / Apartado 6, 7350-591 Elvas, Portugal.

2 Grupo Interações Planta-Ambiente & Biodiversidade, Centro Ambiente, Agricultura e Desenvolvimento, Inst. Inv. Cient. Tropical, I.P., Qta Marquês, 2784-505 Oeiras, Portugal.

* e-mail: paula.scotti@iniav.pt

ABSTRACTS

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 exchanges, 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. An advanced line, Minimus, 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 CO₂ starvation at the carboxylation sites (Chaves and Oliveira, 2004). Water shortage may induce membrane damage through oxidative stress, which results in cell compartmentation 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.

MATERIAL 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: Gediz/Fgo//..., Minimus_6/Plata_16//Immer/3/Sora/..., Ajaia-12/F3Local(Sel.Ethio.135.85)//...and Arment//2*Sooty_9//..., referred as Gediz, Minimus, Ajaia and Arment, respectively. Seeds were sown in 60 L containers, filled with clay loam soil and maintained in a greenhouse (mean daily values: temperature 23-26°C; 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 exchanges (net photosynthetic rate, P_n ; stomatal conductance, g_s ; transpiration, E) were measured in the morning (10:00-12:00h) using a portable $\text{CO}_2/\text{H}_2\text{O}$ 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 1000 kernel weight (TKW) and test weight (kg/hl) were obtained after oven drying at 35°C for 72 h.

RESULTS AND DISCUSSION

Gas exchanges were compared in well watered ($\square_w \approx -0.65$ MPa) and droughted ($\square_w \approx -1.3$ MPa) plants (Tab. 1). P_n decreased in all varieties, being Arment and Ajaia the less and the most affected (ca. 10% and 28% reductions, respectively). As regards g_s , no differences were found in Minimus. Celta and Arment showed 16% a 23% rises, respectively, while the remaining presented g_s decreases, particularly Cocorit (55%) and Ajaia (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 Ajaia 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 Ajaia 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 Gediz (decreases of 45 and 34%, respectively), which also presented a high electrolyte leakage (45% increase) under stress (Fig. 1), 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 Gediz was observed through decreased kernel weight per spike that resulted from a lower number of kernels per spike (Tab. 2). A significant reduction of TKW occurred in Celta, Gediz, Minimus, Ajaia and Arment (Tab. 2). Concomitantly in these varieties test weight (kg/hl) 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 Minimus 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

This work was funded by Proder project 18998 (Genetic Resources).

REFERENCES

- Chaves M.M. & Oliveira M.M. (2004) Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture, *J. Exp. Bot.*, 55, 2365-2384.
- Coutinho J. et al. (2013) Melhoramento de cereais de outono/inverno em Portugal, *Grandes culturas*, 1, 5-11.
- Dodd I.C. et al. (2011) Genetic and management approaches to boost UK wheat yields by ameliorating water deficits, *J. Exp. Bot.*, 62, 5241-5248.
- Kesavan M. et al. (2013) Seed size: a priority trait in cereal crops, *Physiol. Plant.*, 147, 113-

120.

- Konopka I. (2007) The effect of water stress on wheat kernel size, color and protein composition, *Pol. J. Nat. Sci.*, 22, 157-171.
- Lichtenthaler H.K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, *Methods Enzymol.*, 148, 350-382.
- Matos M.C. et al. (1998) Diurnal and seasonal changes in *Prunus amygdalus* gas exchanges, *Photosynthetica*, 35, 517-524.
- Munns R. et al. (2010) New phenotyping methods for screening wheat and barley for beneficial responses to water deficit, *J. Exp. Bot.*, 61, 3499-3507.
- Pinheiro N. et al. (2013) Durum wheat breeding in Mediterranean environments – influence of climatic variables on quality traits, *Emir. J. Food Agric.*, 25, 962-973.
- Schölander P.F. et al. (1965) Sap pressure in vascular plants. *Science*, 148, 339-346.
- Scotti-Campos P. et al. (2013) Physiological responses and membrane integrity in three *Vigna* genotypes with contrasting drought tolerance, *Emir. J. Food Agric.*, 25, 1002-1013.
- Scotti-Campos P. et al. (2014) Heat tolerance of Portuguese old bread wheat varieties, *Emir. J. Food Agric.*, 26, 170-179.

Table 1 - Leaf water potential (ψ_w), net photosynthesis (P_n), stomatal conductance (g_s), transpiration (E), instantaneous water use efficiency (iWUE) as P_n/E , 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 \pm SE (n=3).

		ψ_w (-bar)	P_n ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	g_s ($\text{mmol m}^{-2}\text{s}^{-1}$)	E ($\text{mmol m}^{-2}\text{s}^{-1}$)	iWUE (μmolCO_2 $\text{mmolH}_2\text{O}^{-1}$)	Chl ($\text{mg g}^{-1}_{\text{dw}}$)	Car. ($\text{mg g}^{-1}_{\text{dw}}$)	C_i ($\mu\text{mol mol}^{-1}$)
Castiço	Ctr.	7.7 \pm 0.3	16.3 \pm 0.4	337.2 \pm 13.3	6.3 \pm 0.3	2.8 \pm 0.2	8.4 \pm 0.2	1.8 \pm 0.1	277.9 \pm 2.6
	WS	12.5 \pm 0.5	12.1 \pm 0.6	282.5 \pm 18.6	5.0 \pm 0.2	2.4 \pm 0.1	7.9 \pm 0.2	1.9 \pm 0.1	305.8 \pm 3.2
Cocorit	Ctr.	7.0 \pm 0.6	16.4 \pm 0.5	424.0 \pm 21.2	6.3 \pm 0.3	2.6 \pm 0.2	6.7 \pm 0.2	1.6 \pm 0.1	291.2 \pm 4.9
	WS	13.3 \pm 0.3	12.6 \pm 0.6	190.6 \pm 11.5	2.7 \pm 0.1	4.9 \pm 0.1	6.8 \pm 0.4	1.7 \pm 0.1	256.3 \pm 4.1
Celta	Ctr.	6.7 \pm 0.3	14.8 \pm 0.2	371.6 \pm 33.2	5.7 \pm 0.3	2.8 \pm 0.2	8.5 \pm 0.6	1.9 \pm 0.1	297.2 \pm 8.4
	WS	12.5 \pm 0.3	11.0 \pm 0.8	431.8 \pm 40.7	5.0 \pm 0.3	2.2 \pm 0.1	8.5 \pm 0.4	2.1 \pm 0.1	334.0 \pm 2.8
Gediz	Ctr.	5.7 \pm 0.4	14.2 \pm 0.1	336.0 \pm 8.3	6.0 \pm 0.2	2.4 \pm 0.1	11.3 \pm 0.8	2.3 \pm 0.2	293.7 \pm 2.6
	WS	14.5 \pm 0.5	10.7 \pm 0.2	265.1 \pm 12.8	5.3 \pm 0.2	2.0 \pm 0.1	6.2 \pm 0.3	1.5 \pm 0.0	313.2 \pm 2.5
Minimus	Ctr.	6.3 \pm 0.3	14.0 \pm 0.2	278.2 \pm 23.6	5.6 \pm 0.4	2.8 \pm 0.2	5.0 \pm 0.3	1.2 \pm 0.1	269.0 \pm 6.2
	WS	12.0 \pm 0.0	11.8 \pm 0.2	268.7 \pm 9.5	5.6 \pm 0.1	2.1 \pm 0.0	5.5 \pm 0.5	1.3 \pm 0.1	305.9 \pm 1.8
Ajaia	Ctr.	5.7 \pm 0.3	14.8 \pm 0.4	292.9 \pm 24.5	6.6 \pm 0.3	2.3 \pm 0.1	8.1 \pm 0.8	1.9 \pm 0.2	266.9 \pm 4.9
	WS	14.2 \pm 0.4	10.7 \pm 0.5	192.7 \pm 7.5	3.3 \pm 0.1	3.2 \pm 0.1	7.7 \pm 0.8	1.9 \pm 0.2	291.5 \pm 1.6
Arment	Ctr.	6.7 \pm 0.3	15.2 \pm 0.3	394.9 \pm 15.6	6.6 \pm 0.3	2.4 \pm 0.1	7.8 \pm 0.6	1.7 \pm 0.1	300.3 \pm 2.3
	WS	11.2 \pm 0.4	13.7 \pm 0.1	487.2 \pm 10.5	7.0 \pm 0.0	2.0 \pm 0.0	8.2 \pm 0.4	2.0 \pm 0.1	329.2 \pm 1.4

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 decrease (%) in relation to control.

	Castiço	Cocorit	Celta	Gediz	Minimus	Ajaia	Arment
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

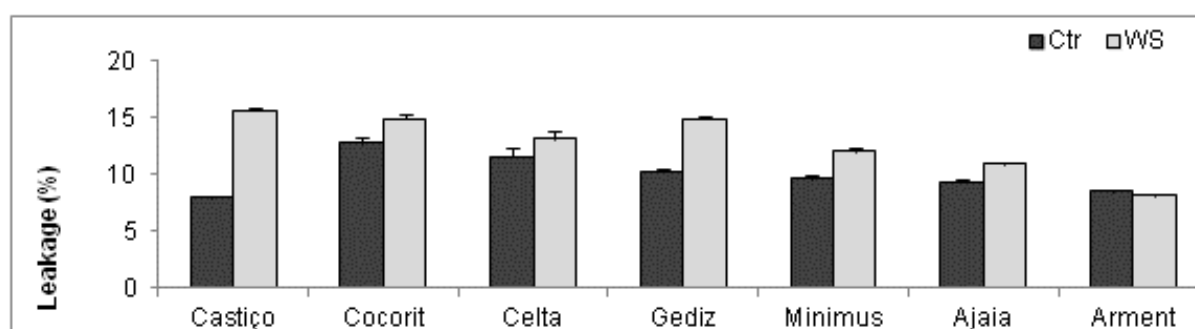


Figure 1 - Changes in electrolyte leakage values in leaf sections of seven *T. durum* varieties, under control (Ctr) or water stress (WS) conditions imposed after anthesis. Results are means \pm SE (n=3).

Application of Thermal Imaging to Assess the Physiological Status of Papaya Crop (*Carica papaya* L.) Under Different Deficit Irrigation Regimes

Campostrini E^{*1}, Lima RSN¹, García-Tejero I², Costa JM³, Lopes TS¹, Vaz M⁴, Chaves MM³

1 Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). Av. Alberto Lamego, 2000, Campos dos Goytacazes, RJ, Brasil.

2 IFAPA-Centro 'Las Torres-Tomejil'. Ctra. Sevilla-Cazalla Km. 12,2. 41200. Alcalá del Río, Sevilla, Espanha.

3 Plant Molecular Ecophysiology Laboratory (LEM), Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República – EAN, 2780-157, Oeiras, Portugal.

4 Departamento de Biologia Instituto de Ciências Agrárias e Ambientais Mediterrânicas ICAAM, Universidade de Évora, Portugal

* e-mail: campostenator@gmail.com

ABSTRACTS

The aim of this work was to study the physiological responses of young plants of Papaya (*Carica papaya* L.) when subjected to different irrigation regimes, in particular by establishing relationships between leaf temperature and leaf gas exchange parameters (stomatal conductance to water vapour, g_s , net photosynthesis, A_n). The trial was conducted in a greenhouse, from beginning of February to April of 2012. Four irrigation treatments were applied: i) a full irrigated treatment (FI), keeping the soil at field capacity; ii) a partial root-zone drying, which received 50% of total water applied in FI to only one side of the root system, alternating sides each 7 days (PRD); iii) a regulated deficit irrigation (RDI), in which was applied the 50% of FI, in both sides of plant; iii) and a non-irrigated treatment (NI). Leaf gas exchange measurements were done every 3 days. Additionally, leaf temperature (T_{leaf}) was monitored at the middle of the experiment (9 days after treatments (DAT), and at the end of maximum stress (14 DAT), by using a thermal imaging camera. Significant negative relationships were found between T_{leaf} and leaf gas exchange parameters, which permitted to define threshold values of T_{leaf} causing significant limitations to A_n and g_s . Our results show that thermal imaging can be used as fast and non-invasive technique to monitor the physiological status of papaya plants.

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most important tropical crops, and is cultivated in many tropical and subtropical areas of the world (Campostrini et al, 2010). Up to day, Brazil with 34,213 ha and 52,3 t ha⁻¹ on average, is the third producer of papaya in the world, representing 17.5% of total world production (FAOSTAT, 2012). Water availability is one of the most limiting factors, this being determined by natural climatic conditions of the growing areas, and the processes linked to climate change. The understanding of the physiological processes related with plant responses to drought is fundamental to minimize the negative impacts under these conditions. Thermography emerges as a remote and non-destructive

option tool to assess the crop water status in many crops and even support the irrigation scheduling under limiting irrigation conditions, based on the inverse relationship between leaf temperature and transpiration rate (Kummerlen et al. 1999; Jones and Vauhan 2010). This technique allows the graphical representation of spatial distribution of plant's surface temperature by sensing the infrared radiation emitted (and reflected) by the crop. However, to optimize the use of leaf temperature (T_{leaf}) or some of the most used thermal indexes such as the crop water stress index (CWSI) or ΔT (this being the difference between T_{leaf} and air temperature T_{air}), it is necessary to define the main relationship with other studied physiological variables such as leaf water potential Ψ_{Leaf} , stomatal conductance to water vapour (g_s) or net photosynthesis (A_n). The aim of this work was to establish the main effects of different irrigation strategies on papaya's physiology, in particular on the changes operated at leaf temperature level and to establish the most significant relationships between T_{leaf} and the g_s and A_n , and identify threshold values of T_{leaf} affecting leaf gas exchange.

MATERIAL AND METHODS

The trial was conducted in a greenhouse at the Universidade Estadual do Norte Fluminense-UENF, Goytacazes Campus, Rio de Janeiro, Brazil (21°44'47"S 41°18'24"W). In this study we used 96 days-old plants of papaya (*Carica papaya* cv. Gran Golden), grown in two 15-L polyvinyl chloride pots, connected to each other, with the root split equally between the two pots, such that water exchange between the two compartments was prevented. The substrate used in the pots was soil, sand, and cattle manure (2:1:2). After this, plants were kept at field capacity (FC) until the following irrigation treatments were applied: **T1**- Fully irrigated (FI): both sides of the split root were kept at 100% FC; **T2**- Non-irrigated (NI): irrigation was interrupted on both sides of the split root during 14 days, following which the plants were again irrigated for six days; **T3**- partial root-zone drying (PRD): water was initially applied daily to one side of the root (100% FC), and no irrigation was applied on the other side of the root (100% FC applied to each root half), being the irrigation alternated between the two root sides every 7 days; **T4**- regulated deficit irrigation (RDI): both sides of the split root were simultaneously irrigated at 50% FC. From the beginning of the experience, and every 3 days, gas-exchange measurements (g_s and A_n) were done, using a IRGA (Li-6400, Licor, USA), on ten plants per treatment, and two fully expanded leaves per plant. Leaf temperature (T_{leaf}) was measured by using a ThermoCAM™ Flir i50 (Flir Systems Inc., USA), 7.5µm–13µm, having a resolution of 140 × 140 pixels), with an emissivity set at 0.96. The camera was placed at 0.3 m distance from the same leaves used in gas-exchange measurements. Measurements were done at three moments of the experiment: i) at the beginning ($T_{\text{air max}}$: 22°C, RH_{min} : 80%) , when A_n values of NI plants were close to 50% of the A_n measured in FI [9 days after treatment (DAT)]($T_{\text{air max}}$: 40°C, RH_{min} : 30%); ii) when A_n

values were close to zero (maximum stress, previous to recovery)(14 DAT)($T_{\text{air max}}$: 40°C, RH_{min} : 30%). Thermal images were analyzed with the software FLIR QuickReport 1.2 (FLIR Systems, Inc., USA).

RESULTS AND DISCUSSION

Leaf gas exchange measurements were in accordance to the irrigation treatments applied (Fig. 1). The highest values of g_s and A_n were found in FI plants, and the lowest in NI plants, which evidenced the most stressed behaviour, and only partially recovered after irrigation with the same amount of water of FI (14 days after the beginning of the stress period) (Fig. 1). Except for the first 3 days ($\psi_{\text{soil}} = -10$ KPa) and 12 DAT (overcast day), RDI and PRD treatments showed significant differences in A_n , g_s and E when compared with FI treatment. PRD treatment in severe stress (14 DAT) had a similar trend to NI. We observed that during clear days ($1000 \mu\text{mol m}^{-2} \text{s}^{-1}$) with high VPD (5kPa) A_n , g_s in PRD and RDI treatment were more affected than in overcast days. Regarding the values of T_{leaf} , these had a opposite trend to the gas exchange measurements. Reduced values of A_n and g_s promoted an increase of T_{leaf} (Figure 2 and 3). Before the start of the treatment no significant differences in T_{leaf} between plants were found (Figure 2). At at 9 DAT however, NI and PRD plants showed the highest T_{leaf} , whereas RDI had similar values to FI plants (Figure 2).

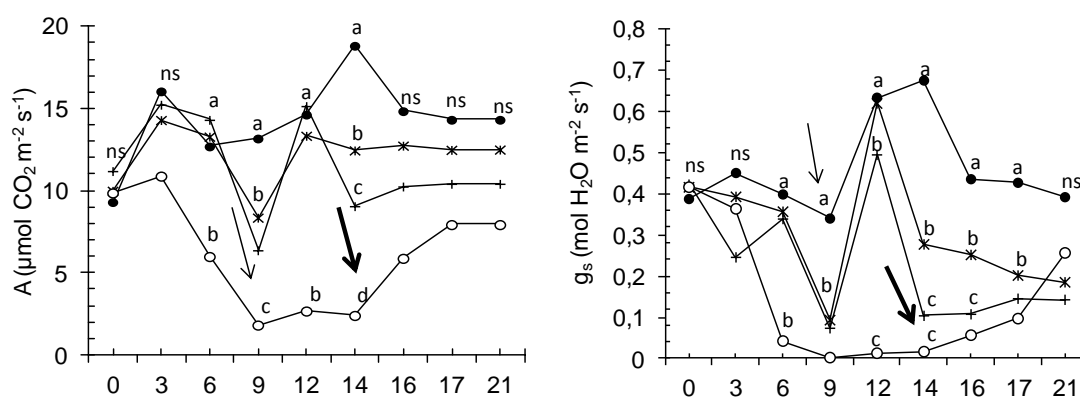


Figure 1. Net photosynthesis (A_n) and stomatal conductance to water vapour (g_s) of papaya plants, grown in greenhouse and cultivated in pots under four irrigation regimes: FI, NI, PRD and RDI. Lower case letters refer to mean values followed by the same letter are not significantly different ($P=0.05$) based on Tukey's multiple range test. Arrows indicate the first alternating between the two root sides of the PRD (9 DAT) pots and rehydration of NI treatment (14 DAT), respectively.

Our results showed as well a significant negative correlation between T_{leaf} and the leaf gas exchange parameters (Figure 3). Similar results were reported by several authors for

different species, such as almond trees (García-Tejero et al. 2012), citrus (García-Tejero et al. 2011) or grapevine (Jones et al., 2002; Costa et al., 2012).

From this experiment, conducted under semi-controlled conditions, we may conclude that thermal imaging is an alternative method to assess papaya's physiological status in response to water-saving irrigation strategies. However, the results of the present study must be complemented and validated by field experiments, with the goal of defining more robust relationships and establish protocols to optimize the use of thermal imaging and get the most adequate results in papaya.

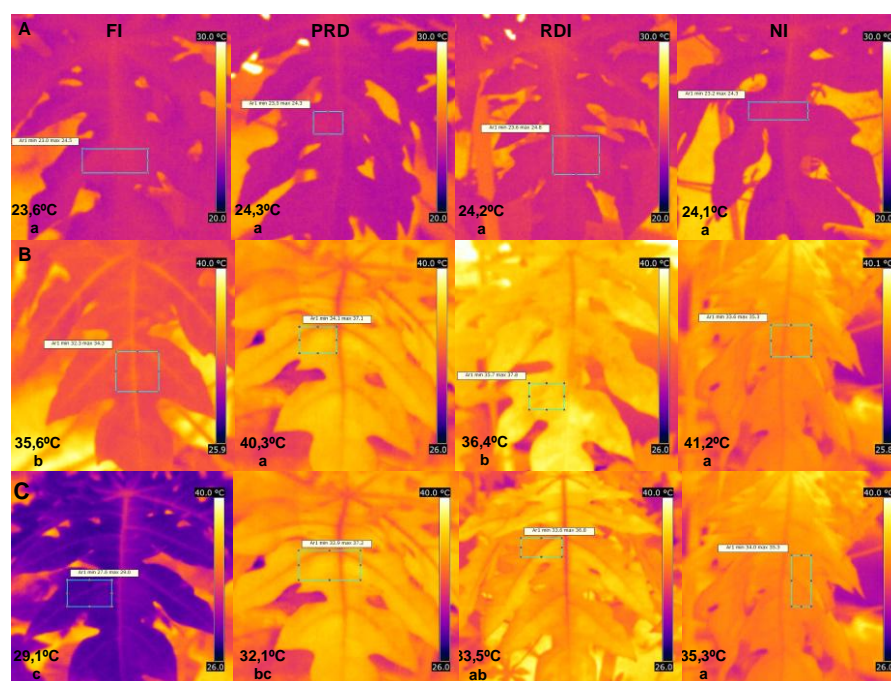


Figure 2. Thermal images of individual leaves of papaya, grown in the greenhouse and cultivated in pots under four different irrigation regimes: FI, NI, PRD and RDI. Plants were observed (A) just before applying treatments, (B) 9 days after the treatment (DAT) and (C) at 14 DAT. Lower case letters refer to mean values followed by the same letter are not significantly different ($P=0.05$) based on Tukey's multiple range test.

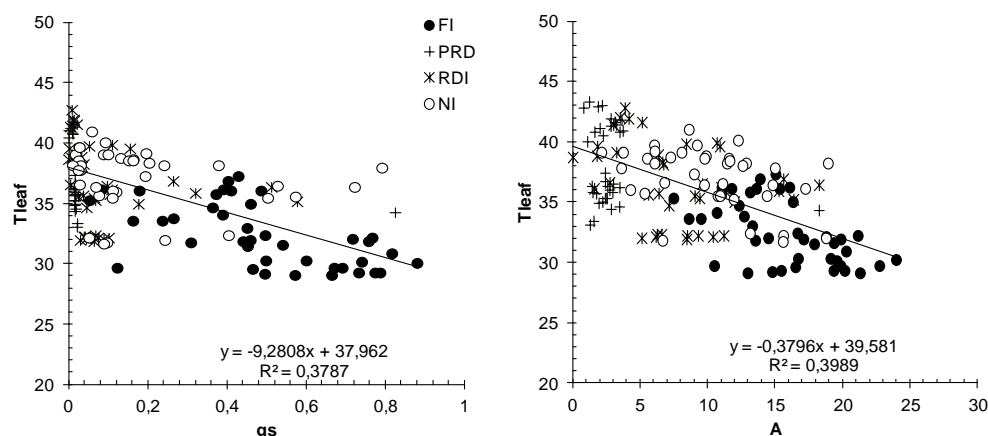


Figure 3. Relationship between leaf temperature (T_{leaf}) and net photosynthesis (A_n), stomatal conductance to water vapour (g_s) of papaya plants in greenhouse and cultivated in pots under four different irrigation regimes: FI, NI, PRD and RDI. The data are collected 9 days after treatments (DAT) and at 14 DAT.

References

- Campostrini, E., Glenn, D.M., Yamanishi, O.K., 2010. Papaya: environment and crop physiology. In: (Ed.: Fabio da Matta). *Ecophysiology of Tropical Tree Crops* (Agriculture Issues and Policies). 1st ed. New York: Nova Science Publishers, v. 1, p. 287-308.
- Costa J.M., Ortuno M.F., Lopes C.M., Chaves M.M. (2012). Grapevine varieties exhibiting differences in stomatal response to water deficit, *Functional Plant Biology* 39, 179–189.
- FAOSTAT (2012) FAOSTAT. Available at: <http://faostat.fao.org/site/567/default.aspx#ancor>.
- García-Tejero, I., Durán-Zuazo, V.H., Muriel-Fernández, J.L., Jiménez-Bocanegra, J.A. (2011) Linking canopy temperature and trunk diameter fluctuations with other physiological water status tools for water stress management in citrus orchards. *Functional Plant Biology* 38, 106–117.
- García-Tejero I., Durán-Zuazo V.H., Arriaga, J., Hernández A., Vélez L.M., Muriel-Fernández, J.L. (2012). Approach to assess infrared thermal imaging of almond trees under water-stress conditions. *Fruits* 67, 463-474
- Jones, H.G; Vaughan, R.A. (2010) Remote sensing of vegetation: principles, techniques and applications. Oxford, UK: Oxford University Press.
- Kümmerlen B., Dauwe S., Schmundt D., Schurr U. (1999). Thermography to measure water relations of plant leaves. In: Jähne B, Haußecker H, Geißler P, eds. *Handbook of computer vision and applications*, Vol. 3. Systems and applications. London, UK: Academic Press, 763–781.

Microclimatic Conditions and Gas Exchange in a Vineyard Under Plastic Cover

Esteves BS^{1*}; Sousa EF¹; Lousada LL¹; Pessanha DP¹; Bressan-Smith RE¹; Campostrini E¹; Fernández JE^{2*}

1 Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). Av. Alberto Lamego, 2000, Campos dos Goytacazes, RJ, Brasil.

2 Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC), Avenida Reina Mercedes n.º 10. 41012-Sevilla

* e-mail: barbbarase@gmail.com; jefer@irnase.csic.es

ABSTRACTS

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 decreasesolar 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 21° 30' 58" S and longitude 41° 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 (Figure 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 (°C), relative humidity of the air (%) and photosynthetic active radiation ($\mu\text{mol m}^{-2} \text{s}^{-1}$). 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.

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 $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$, 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). 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. 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 increase 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. $0.3 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ 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.

REFERENCES

- Allen, R.G. et al. (1998) Crop evapotranspiration-Guidelines for Computing Crop Water Requirements. Irrigation and Drainage. FAO, pp. 56.
- Barradas, V. L., et al. (2005) Transpiration and canopy conductance in Young apricot (*Prunus armenica* L.) trees subjected to different PAR levels and water stress. Agricultural Water Management, v.77, p.323-333.
- Beckmann, M. Z., et al. (2006) Radiação solar em ambiente protegido cultivado com tomateiros nas estações verão-outono do Rio Grande do Sul. Ciência Rural, v.36, p.86-92.
- Cardoso, L.S., et al. (2008) Alterações microclimáticas em vinhedos pelo uso de cobertura de plástico. Pesquisa Agropecuária Brasileira, v.43, n.4, p.441-447.
- Chavarria, G. et al. (2009) Microclima de vinhedos sob cultivo protegido. Ciência Rural, v.39 (n.7), p. 2029-2034.
- Chaves, M.M., et al. (2002). How plants cope with water stress in the field? Photosynthesis and Growth. Annals of Botany, v.89, p.907-916.
- Flexas, J. et al. (2002) Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. Funct. Plant Biol., v.29, p.461-471.
- Tanny, J. (2013) Microclimate and evapotranspiration of crops covered by agricultural screens: A review. Biosystems Engineering, n.114, p.26-43.



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

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 ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	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.

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

Days after pruning	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)				A_N ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)			
	Without cover	plastic	With cover	plastic	Without cover	plastic	With cover	plastic
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, $p \leq 0.05$)

Chloride Nutrition Regulates Water Balance in Plants

Franco-Navarro JD¹, Brumós J², Rosales MA¹, Vázquez-Rodríguez A¹, Sañudo BJ¹, Díaz-Rueda P¹, Rivero C¹, Talón M², Colmenero-Flores JM¹

1 Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNASE). Consejo Superior de Investigaciones Científicas (CSIC). Avda. Reina Mercedes 10, 41012-Sevilla, Spain.

2 Instituto Valenciano de Investigaciones Agrarias. Centro de Genómica. Carretera Moncada - Náquera, Km. 4,5. 46113-Moncada (Valencia), Spain.

* e-mail: chemacf@irnase.csic.es

ABSTRACTS

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); NO₃⁻ salts (N); and SO₄²⁻ + PO₄³⁻ 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.* (2013a,b). Mean values \pm 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**). 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**).

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**). 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 (Ψ_p) 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**). 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.

ACKNOWLEDGEMENTS

This work was supported by the Spanish Ministry of Science and Innovation-FEDER grant AGL2009-08339/AGR.

REFERENCES

- Brumós, J., et al. (2010). Cl^- homeostasis in includer and excluder citrus rootstocks: transport mechanisms and identification of candidate genes. *Plant, Cell & Environment* 33, 2012-2027.
- Franco-Navarro, J.D., et al. (2013a). Chloride nutrition: novel functions in water relations. *XI Simposio hispano-portugués de relaciones hídricas en las plantas*. IRNASE-CSIC, Sevilla.
- Franco-Navarro, J.D., et al. (2013b). Chloride nutrition at macronutrient levels regulates development, water balance and drought resistance in tobacco plants. *Environment Workshop 2013*. UNIA-UMA-CSIC, Baeza.
- Johnson C.M., et al. (1957) *Plant Soil*, **8**, 337-353.
- Flowers, T.J. (1988). Chloride as a nutrient and as an osmoticum. In: Tinker B LA, ed. *Advances in plant nutrition*, Vol. 3. New York: Praeger, 55-78.
- Johnson, C.M., et al. (1957). Comparative chlorine requirements of different plant species. *Plant Soil* 8, 337-353.
- Marschner, H. (1995). *Mineral Nutrition of Higher Plants*, 2nd ed. London: Academic Press.
- Terry, N. (1977). Photosynthesis, growth, and role of chloride. *Plant Physiology* 60, 69-75.
- White, P.J. & Broadley, M.R. (2001). Chloride in Soils and its Uptake and Movement within the Plant: A Review. *Annals of Botany* 88, 967-988.
- Zimmermann, D., et al. (2008) A novel, non-invasive, online monitoring, versatile and easy plant-based probe for measuring leaf water status. *Journal of Experimental Botany* 59, 3157-3167.

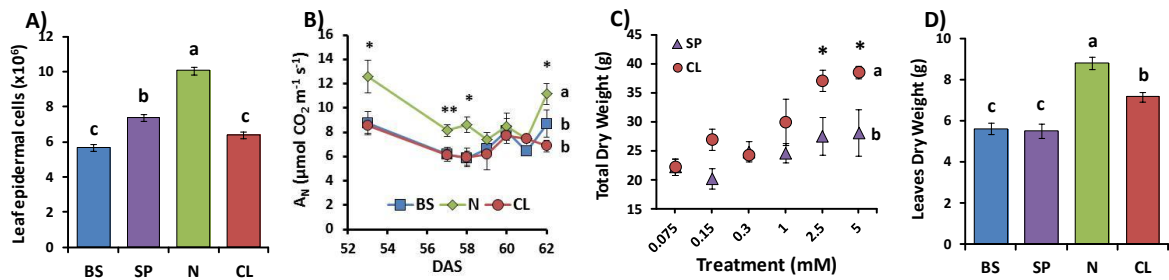


Fig. 1. Effect of Cl⁻ nutrition on photosynthesis, cell division and growth. (A) Cell division rate, quantified as the number of epidermal cells per leaf. **(B)** Effect on net Photosynthetic rate (A_N) measured in fully expanded and photosynthetic leaves. **(C)** Total plant dry weight. **(D)** Leaves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

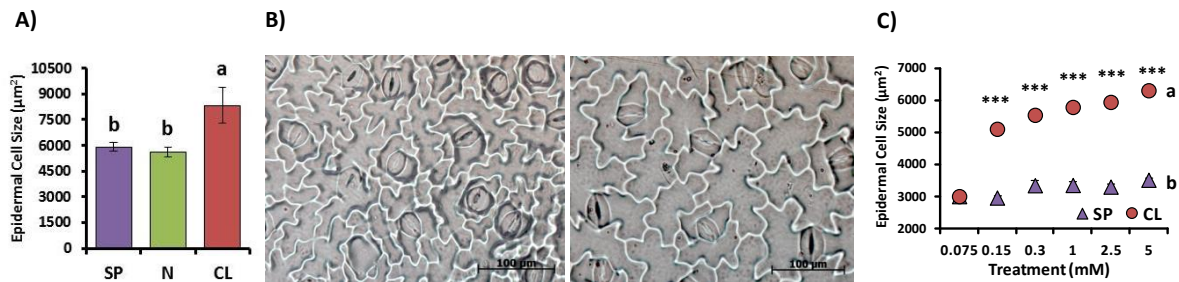


Fig. 2. Effect of Cl⁻ nutrition on cell elongation. (A,C) Epidermal Cell Size. **(B)** Epidermal cell size observed in microscopy images of leaf epidermal impressions of SP-treated plants (left) and Cl⁻-treated plants (right).

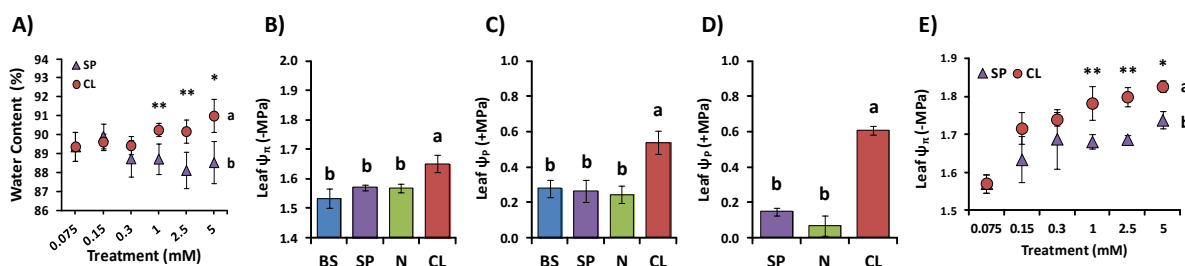


Fig. 3. Effect of Cl⁻ nutrition on leaf osmotic potential and turgor, and water content. (A) Leaf Water Content. **(B,E)** Mature Leaf Osmotic potential; **(C,D)** Mature Leaf Turgor potential; **(B,C,E)** Pre-dawn values; **(D)** Midday values.

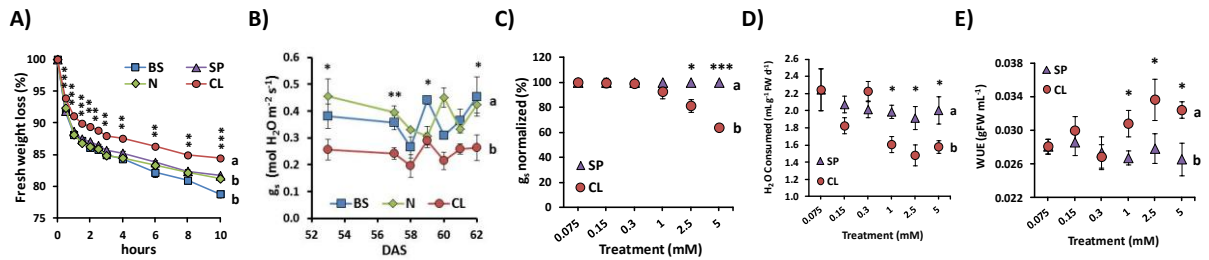


Fig. 4. Effect of Cl^- nutrition on leaf transpiration. (A) Fresh weight loss in detached leaves. **(B,C)** Stomatal conductance; **(D)** water consumption related to fresh biomass; **(E)** Integrated Water Use Efficiency;

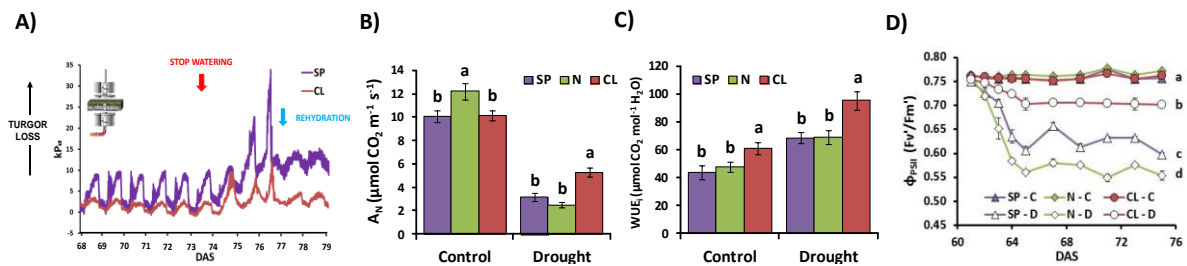


Fig. 5. Effect of Cl^- nutrition on drought resistance. (A) Leaf turgor measurement using the magnetic leaf patch clamp pressure probe (Zimmermann et al., 2008). Red arrow indicates stop watering and blue arrow indicates rehydration. **(B,C)** 3-days averaged data measured from plants subjected to 2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

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

Martorell S^{1*}, Díaz-Espejo A², Tomás M¹, Pou A¹, El Aou-uad H¹, Escalona JM¹, Vadell J¹, Flexas J¹, Ribas-Carbó M¹, Medrano H¹.

¹ Research Group on Plant Biology under Mediterranean Conditions, Departament de Biologia, Universitat de les Illes Balears, Carretera de Valldemossa Km 7.5, 07122 Palma de Mallorca, Illes Balears, Spain

² Irrigation and Crop Ecophysiology Group, Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS, CSIC). Avenida Reina Mercedes 10, 41012 Sevilla, Spain

* e-mail: sebastia.martorell@uib.es

ABSTRACTS

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 regulation of leaf water relations parameters under water stress were observed between both cultivars. Results indicated that there is a clear relationship between hydraulic conductance (K_h) and stomatal regulation. ABA can exert a changing role on the stomatal control during different stages of grapevine growth period. The combination of both signals, ABA and K_h , control the stomatal aperture in a different way between the two varieties leading to have differential 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, level of water stress and its duration and intensity, 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 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. In 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 summer 2011 and 2012. Plants were two years old (planted in 2011) 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 on 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 ($\Psi_{\square, FT}$), 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 measured at the initial phase of water stress treatments (later June dates for each season). Five replicates per treatment were measured.

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 of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ of photosynthetic photon flux density, ambient air temperature and a CO_2 concentration of $400 \mu\text{mol mol}^{-1}$. 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):

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

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 Ψ_{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 occurred 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). Also, a lower ABA concentration was found constitutively in Tempranillo (Figure 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 of this behavior between both cultivars (Figures 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.

REFERENCES

- Bota et al. (2001). Genetic variability of photosynthesis and water use in Balearic grapevine cultivars. *Ann. Appl. Biol.* 138, 353-361.
- Flexas et al. (2010). Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. *Aust. J. Grape Wine R.* 16: 106-121
- Gómez-Cadenas, A., et al., (2002). Direct analysis of abscisic acid in crude plant extracts by liquid chromatography-electrospray/tandem mass spectrometry. *Phytochem. Anal.* 13, 228-234.
- Jones (2006). Climate and terroir: Impacts of climate variability and change on wine. *Fine Wine and Terroir—The Geoscience Perspective*. Geoscience Canada, eds Macqueen RW, Meinert LD (Geological Association of Canada, St John's, Newfoundland), pp 1–14.
- Lovisolo et al. (2002). Whole-plant hydraulic conductance and root-to-shoot flow of abscisic acid are independently affected by water stress in grapevines. *Funct. Plant Biol.* 29, 1349-1356.
- Medrano et al., (2003). A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Funct. Plant Biol.* 30, 607-619.
- Mullins et al. (1992). *Biology of the grapevine*. Cambridge University Press.
- Pou et al. (2012). Anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric behaviour. *Plant Soil*, 359, 335-349.
- Schultz (2003). Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown *Vitis vinifera* L. cultivars during drought. *Plant Cell Environ*, 26, 1393-1405.
- Tomás et al. (2012). Water-use efficiency in grapevine cultivars grown under controlled conditions: effects of water stress at the leaf and whole-plant level. *Aust. J. Grape Wine Res.* 18, 164-172.
- Tyree & Hammel (1972). Measurement of turgor pressure and water relations of plants by pressure bomb technique. *J. Exp. Bot.* 23, 267-282
- Van Leeuwen et al. (2013). Why climate change will not dramatically decrease viticultural suitability in main wine-producing areas by 2050. *P. Natl. Acad. Sci.* 110, E3051-E3052.

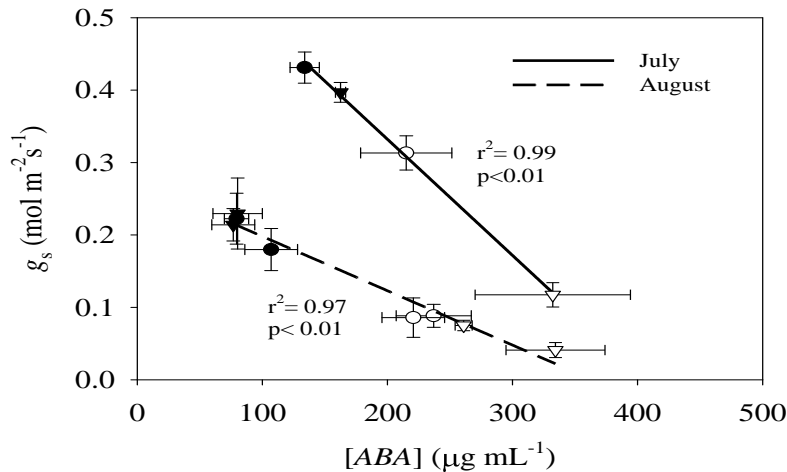


Figure 1 Stomatal conductance (g_s) versus abscisic acid xylem sap concentration ($[ABA]$) under well watered conditions (full symbols) and water stress (blank symbols) between both cultivars. Grenache is represented by triangles and Tempranillo by circles. Two sampling dates were differentiated, July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

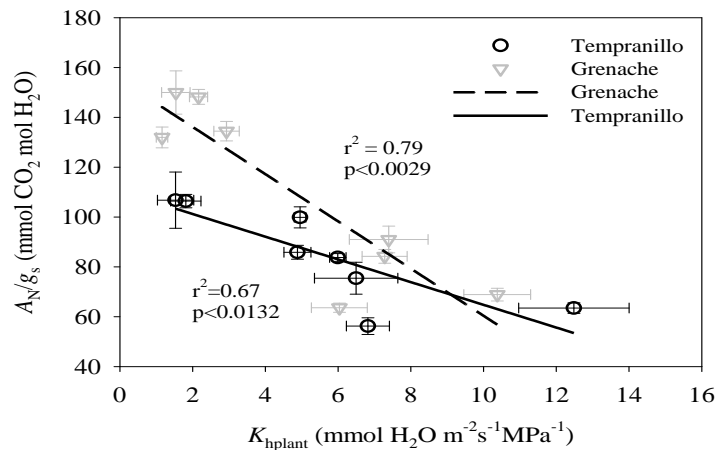


Figure 2: Relationship between plant hydraulic conductance (K_{hplant}) and water use efficiency (A_N/g_s) only for water stress treatments. Circles represent Tempranillo variety and triangles Grenache. Each regression line represents a different variety including data from 2011 and 2012. Regression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

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

Niculcea M¹, Martínez-Lapiente L², Guadalupe Z², Sánchez-Díaz M¹, Ayestarán B², Antolín MC¹

¹ Grupo de Fisiología del Estrés en Plantas (Dpto. de Biología Ambiental), Unidad Asociada al CSIC (EEAD, Zaragoza, ICVV, Logroño). Facultades de Ciencias y Farmacia, Universidad de Navarra, c/ Irunlarrea 1, 31008, Pamplona, Spain.

² Instituto de Ciencias de la Vid y del Vino (Universidad de La Rioja, Gobierno de La Rioja y CSIC) c/ Madre de Dios 51, 26006 Logroño, Spain

E-mail: cantolin@unav.es

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 22°Brix) [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 ($P < 0.05$).

Measurement	'Tempranillo'		'Graciano'	
	Control	SDI	Control	SDI
Leaf area ($\text{m}^2 \text{ plant}^{-1}$)	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 ($\text{cm}^2 \text{ g}^{-1}$)	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 ($^{\circ}\text{Brix}$)	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 (Figure 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). 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).

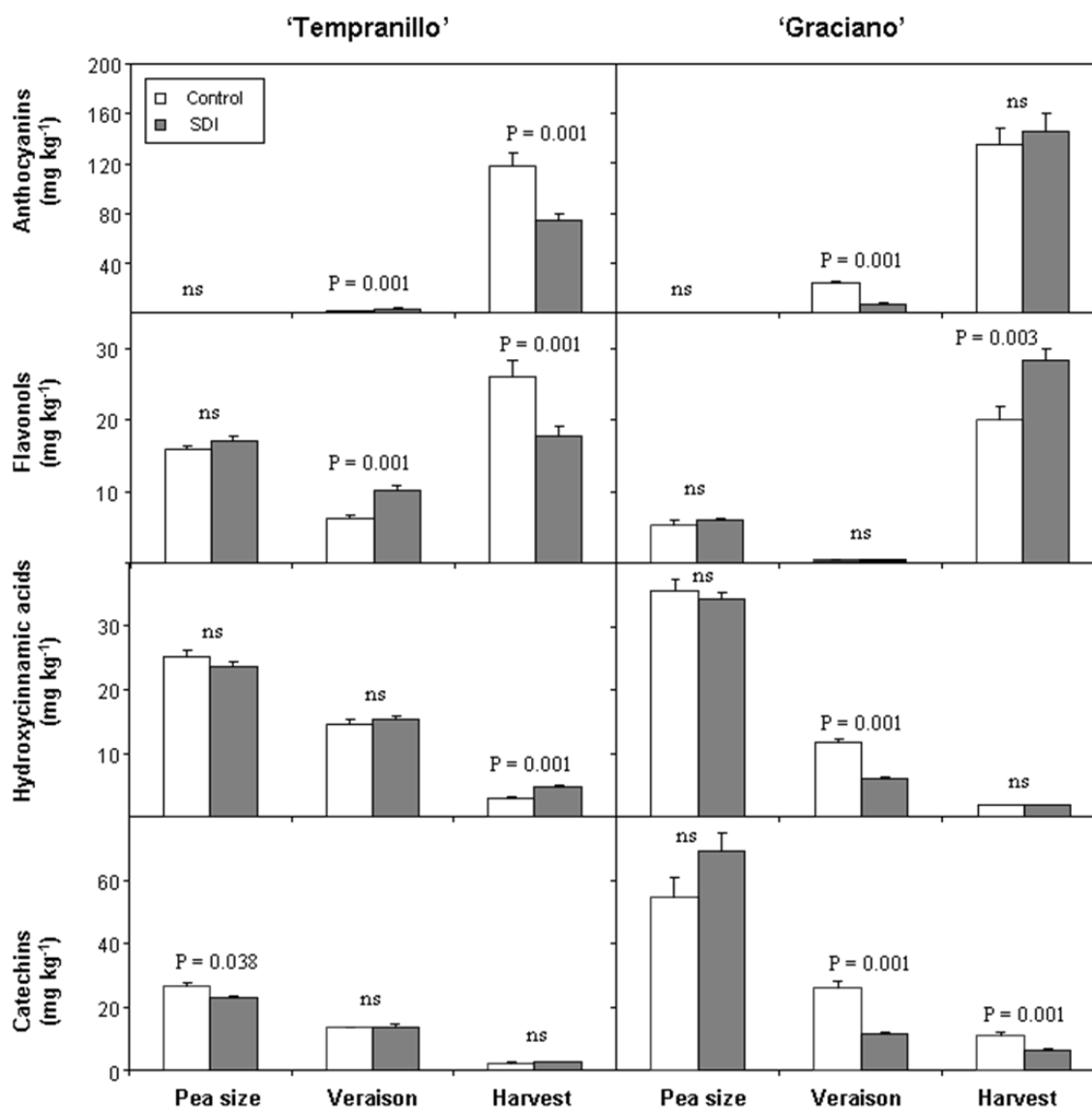


Figure 1. Phenolic composition at different stages of berry growth and ripening of 'Tempranillo' and 'Graciano' grapevines subjected to different irrigation treatments: full irrigation (Control), or sustained deficit irrigation (SDI). Values represent means \pm SE (n=25). Within each phenological stage 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.

ACKNOWLEDGEMENTS

Project funded by Ministerio de Ciencia e Innovación (MCINN BFU2011-26989) of Spain. M. Niculcea was the recipient of a grant from Asociación de Amigos de la Universidad de Navarra.

REFERENCES

- Chatonnet et al. (1993). Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wine. J. Sci. Food Agr. 62: 191-202.
- Chaves et al. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. Ann. Bot. 105: 661-676.
- Coombe (1995). Adoption of a system for identifying grapevine growth stages. Aust. J. Grape Wine Res. 1: 104-110.
- Guadalupe and Ayestarán (2008). Changes in the color components and phenolic content of red wines from *Vitis vinifera* L. cv. Tempranillo during vinification and aging. Eur. Food Res. Technol. 228: 29-38.
- Niculcea et al. (2014a). Involvement of berry hormonal content in the response to pre-and post-veraison water deficit in different grapevine (*Vitis vinifera* L.) cultivars. Aust. J. Grape Wine R. 20: 281-291.
- Niculcea et al. (2014b). Characterization of phenolic composition of *Vitis vinifera* L cv. 'Tempranillo' and 'Graciano' subjected to deficit irrigation during berry development Vitis (in press).
- OIV (2014). Compendium of international methods of analysis of wines and musts (Paris).

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
<i>Anthocyanins</i> (% of total)	<i>3-Monoglucosides</i>	86.7 a	72.2 b	83.4 a	77.9 b
	<i>3-Acetyl-glucosides</i>	2.5 b	3.4 a	7.0 a	6.9 a
	<i>3 p-Coumaroyl-glucosides</i>	10.8 b	24.4 a	9.6 b	15.2 a
<i>Flavonols</i> (mg kg^{-1})	Myricetin 3-O-glucoside	20.5 a	12.4 b	ND	ND
	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
<i>Hydroxycinnamic acids</i> (mg kg^{-1})	<i>c</i> -caftaric acid	6.3 a	2.6 b	ND	ND
	<i>t</i> -caftaric acid	12.8 b	2.7 a	9.7 a	7.1 b
	<i>c</i> -coutaric acid	ND	ND	2.8 a	1.5 b
	<i>t</i> -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

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

Azeñas V., Medrano H., Gulías J.

Research group in plant biology under Mediterranean conditions. Departament of Biology. Universidad de las Islas Baleares, Ctra. Valldemossa Km. 7.5. 07122 Palma de Mallorca, Spain.

ABSTRACT

Green roof implementation has particular features benefits and difficulties. Mediterranean zones still represent an important challenge since rainfall distribution is very heterogeneous along the year in these areas, what leads to frequent drought periods not only in summer but also during spring and autumn. Therefore it is necessary to provide species knowledge 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 maritimus***, ***Brachipodium phoenicioides***, ***Crithmum maritimum***, ***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. maritimum*** 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. maritimum*** and ***S. sediforme*** showed no significant variation of RA along the experiment in any treatment.

INTRODUCTION

Green roofs are considered new green infrastructure, 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 *et al.* 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. sedifforme***) 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, 80m asl. It was performed during spring and summer 2013, meteorological data were recorded by a meteorological station at the experimental site.

Plant selection: Five species from the Balearic Islands: ***A. maritimus*, *B. phoenicoides*, *C. maritimum*, *L. virgatum*, *S. sedifforme*** 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), 18 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. RA was performed as a mean of a weekly estimation aesthetical parameters. 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 SB, even though SB varied significantly among species, ***S. sediforme*** showed 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/m²day respectively, in contrast, ***C. maritimum*** and ***S. Sediforme*** showed the lowest water consumption, 1.9 and 1.5 l/m² 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).

C. maritimum, ***L. virgatum*** and ***S. Sediforme*** showed no significant differences in PD and MD RWC between treatments, also MD RWC were significantly lower than PD RWC in 4 of 5 species 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 PD RWC (Table 1). 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. maritimum***.

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 severe limitation of water in green roof conditions.

ACKNOWLEDGEMENTS This work is part of the project INNPACTO (IPT 2011-1017-310000) funded by the Spanish Government. V. Azeñas has been granted by Agencia Española de Cooperación Internacional para el desarrollo (AECID).

REFERENCES

- Farrell C., Szota C., Williams N.S.G. & Arndt S.K. (2013) High water users can be drought tolerant: using physiological traits for green roof plant selection. *Plant and Soil* 372, 177–193.
- MacIvor J.S. & Lundholm J. (2011) Performance evaluation of native plants suited to extensive green roof conditions in a maritime climate. *Ecological Engineering* 37, 407–417.
- Nagase A. & Dunnett N. (2010) Drought tolerance in different vegetation types for extensive green roofs: Effects of watering and diversity. *Landscape and Urban Planning* 97, 318–327.
- Speak a. F., Rothwell J.J., Lindley S.J. & Smith C.L. (2013) Reduction of the urban cooling effects of an intensive green roof due to vegetation damage. *Urban Climate* 3, 40–55.

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 \pm s.e. (n=6)

	TDB (g)	SB (g)	WC (l / m ² día)	WUE (g biomass / l H ₂ O)	A (μ mol CO ₂ / m ² s)	g (mol H ₂ O / m ² s)	RWC MD	RWC PD
<i>A, maritimus</i>	4,9 \pm 0,6 ^a	13,7 \pm 0,9 ^a	5,5 \pm 0,2 ^a	2,2 \pm 0,0 ^a	15,4 \pm 1,2 ^a	0,2 \pm 0,02 ^a	73,9 \pm 0,6 ^a	81,2 \pm 1,9 ^a (*)
	5,3 \pm 0,8 ^a	13,5 \pm 0,6 ^a	4,3 \pm 0,0 ^b	2,7 \pm 0,1 ^b	10,6 \pm 1,3 ^a	0,11 \pm 0,02 ^b	67,5 \pm 3,2 ^a	72,0 \pm 3,1 ^{ab}
	5,0 \pm 0,6 ^b	12,0 \pm 0,2 ^a	3,0 \pm 0,1 ^c	3,2 \pm 0,1 ^c	1,4 \pm 0,1 ^b	0,2 \pm 0,2 ^c	56,4 \pm 1,1 ^b	62,3 \pm 4,8 ^b
<i>B, phoenicoides</i>	24,8 \pm 1,0 ^a	7,2 \pm 0,9 ^a	7,6 \pm 0,2 ^a	2,6 \pm 0,0 ^a	8,1 \pm 2,7 ^a	0,09 \pm 0,03 ^a	94,7 \pm 1,2 ^a	95,4 \pm 0,1 ^a (*)
	11,8 \pm 1,4 ^b	4,9 \pm 0,6 ^a	4,6 \pm 0,1 ^b	2,4 \pm 0,1 ^{ab}	3,3 \pm 1,9 ^a	0,09 \pm 0,03 ^a	91,7 \pm 1,7 ^a	94,6 \pm 0,8 ^a
	7,7 \pm 1,9 ^b	6,0 \pm 0,2 ^a	3,4 \pm 0,2 ^c	2,8 \pm 0,1 ^b	3,7 \pm 0,2 ^a	0,09 \pm 0,03 ^a	80,3 \pm 4 ^b	89,5 \pm 1,2 ^b
<i>C, maritimum</i>	2,4 \pm 0,5 ^a	0,5 \pm 0,3 ^a	1,9 \pm 0,1 ^a	1,8 \pm 0,2 ^a	12,9 \pm 2,7 ^a	0,09 \pm 0,03 ^a	82,4 \pm 1,8 ^a	95,0 \pm 2,2 ^a (*)
	3,5 \pm 0,9 ^a	1,5 \pm 0,3 ^a	1,6 \pm 0,0 ^{ab}	2,9 \pm 0,5 ^a	12 \pm 1,8 ^a	0,04 \pm 0,01 ^{ab}	83,9 \pm 1,8 ^a	91,1 \pm 0,9 ^a
	2,4 \pm 0,4 ^a	2,3 \pm 0,4 ^a	1,3 \pm 0,1 ^b	2,0 \pm 0,2 ^a	2,7 \pm 0,7 ^b	0,03 \pm 0,0 ^b	80,6 \pm 3,8 ^a	85,0 \pm 3,7 ^a
<i>L, virgatum</i>	10,5 \pm 1,9 ^a	4,7 \pm 0,7 ^a	6,6 \pm 0,2 ^a	3,2 \pm 0,1 ^a	19,5 \pm 2,5 ^a	0,23 \pm 0,03 ^a	72,2 \pm 3,2 ^a	87,8 \pm 2,2 ^a (*)
	8,2 \pm 0,6 ^b	4,5 \pm 0,5 ^a	4,9 \pm 0,1 ^b	3,3 \pm 0,2 ^a	14,1 \pm 1,2 ^a	0,11 \pm 0,01 ^b	70,0 \pm 2,2 ^a	83,4 \pm 3,1 ^a
	6,3 \pm 0,8 ^c	5,0 \pm 0,5 ^a	3,8 \pm 0,3 ^c	3,4 \pm 0,2 ^a	2,5 \pm 0,5 ^b	0,01 \pm 0,00 ^c	70,1 \pm 3,5 ^a	83,0 \pm 1,8 ^a
<i>S, pungens</i>	15,1 \pm 1,2 ^a	6,6 \pm 0,9 ^a	5,0 \pm 0,1 ^a	4,2 \pm 0,2 ^a	27 \pm 3,2 ^a	0,15 \pm 0,01 ^a	81,5 \pm 2,2 ^a	97,7 \pm 0,9 ^a (*)
	9,7 \pm 0,7 ^b	3,9 \pm 0,5 ^b	3,6 \pm 0,1 ^b	4,1 \pm 0,1 ^a	25,7 \pm 2,9 ^a	0,13 \pm 0,01 ^a	88,7 \pm 1,4 ^a	96,6 \pm 0,9 ^a (*)
	5,4 \pm 0,3 ^b	3,1 \pm 0,2 ^b	1,9 \pm 0,1 ^c	5,1 \pm 0,5 ^a	7 \pm 2,5 ^b	0,04 \pm 0,01 ^b	74,8 \pm 2,0 ^b	97,0 \pm 1,0 ^a (*)
<i>S, sediforme</i>	6,1 \pm 2 ^a	0 \pm 0 ^a	1,5 \pm 0,1 ^a	2,8 \pm 0,5 ^a			87,0 \pm 2,0 ^a	85,7 \pm 1,8 ^a
	3,6 \pm 0,4 ^a	0 \pm 0 ^a	0,8 \pm 0,1 ^b	3,4 \pm 0,4 ^a			80,4 \pm 1,2 ^a	76,9 \pm 2,3 ^{ab}
	3,3 \pm 0,2 ^a	0 \pm 0 ^a	0,8 \pm 0,1 ^c	3,5 \pm 0,2 ^a			78,6 \pm 3,7 ^a	72,4 \pm 3,6 ^a

Lettering denotes significant differences between treatments within each species , (*) represent differences between MD and PD values, ($p < 0,005$).

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

Bahamonde HA^{1,2*}, Sánchez-Gómez D⁴, Gyenge J^{1,3}, Peri PL^{1,2,3}, Aranda I⁴

1 Instituto Nacional de Tecnología Agropecuaria, INTA, Buenos Aires, Argentina.

2 Universidad Nacional de la Patagonia Austral, Unidad académica Río Gallegos, UNPA UARG, Argentina

3 Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Buenos Aires, Argentina

4 Instituto Nacional de Investigaciones y Tecnologías Agrarias y Alimentarias, Centro de Investigación Forestal (INIA-CIFOR), Carretera de La Coruña km 7.5 Madrid, España

* e-mail: bahamonde.hector@inta.gob.ar

ABSTRACTS

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 25°C day / 20°C 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 $\mu\text{mol m}^{-2}\text{s}^{-1}$ 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 area (SLA), net photosynthesis (A_n), stomatal conductance to water vapor (g_{wv}), 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 (RWC₀), 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 25°C day / 20°C 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 $\mu\text{mol m}^{-2}\text{s}^{-1}$. 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. 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 C_i , 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*.

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	$8.27 \pm 1.12a$	$2.43 \pm 0.48 ab$	$5.64 \pm 2.39 a$	$0.91 \pm 0.42 b$
g_{wv}	$0.186 \pm 0.028 a$	$0.061 \pm 0.008 b$	$0.050 \pm 0.027 b$	$0.016 \pm 0.006 b$
C_i	$315 \pm 4 a$	$315 \pm 23 ab$	$183 \pm 27 b$	$302 \pm 6 ab$
Π^{100}	$-1.43 \pm 0.18 a$	$-1.15 \pm 0.23 a$	$-1.13 \pm 0.20 a$	$-0.92 \pm 0.18 a$
Π^0	$-1.95 \pm 0.23 a$	$-1.55 \pm 0.29 a$	$-1.60 \pm 0.25 a$	$-1.34 \pm 0.23 a$
RWC_0	$0.81 \pm 0.03 a$	$0.80 \pm 0.04 a$	$0.84 \pm 0.04 a$	$0.80 \pm 0.03 a$
DW/TW	$0.31 \pm 0.01 a$	$0.26 \pm 0.01 b$	$0.28 \pm 0.01 a$	$0.26 \pm 0.01b$
E_{max}	$5.89 \pm 1.18 a$	$5.35 \pm 1.52 a$	$4.34 \pm 1.32 a$	$3.36 \pm 1.18 a$

REFERENCES

- Bahamonde, H.A., Peri, P.L., Monelos, L.H., Martínez Pastur, G. 2011. Aspectos ecológicos de la regeneración por semillas en bosques nativos de *Nothofagus antarctica* en Patagonia Sur, Argentina. *Bosque* 32: 20-29.
- Bahamonde, H.A., Peri, P.L., Alvarez, R., Barneix, A., Moretto, A., Martínez Pastur, G. 2012. Litter decomposition and nutrients dynamics in *Nothofagus antarctica* forests under silvopastoral use in Southern Patagonia. *Agrofor Syst* 84: 345-360.
- Bucci, S.J., Scholz, F.G., Campanello, P.I., Montti, L., Jimenez-Castillo, M., Rockwell, F.A., La Manna, L., Guerra, P., Lopez Bernal, P., Troncoso, O., Enricci, J., Holbrook, M.N., Goldstein, G. 2013. Hydraulic differences along the water transport system of South American *Nothofagus* species: do leaves protect the stem functionality? *Tree physiology* 32: 880-893.
- Collado, L. 2001. Los Bosques de Tierra del Fuego. Análisis de su estratificación mediante imágenes satelitales para el inventario forestal de la Provincia. *Multequina* 10: 1-16.
- Dettmann, S. Pérez, C.A., Thomas, F.M. 2013. Xylem anatomy and calculated hydraulic conductance of four species with contrasting distribution in South-Central Chile. *Trees* 27: 685-696.
- Donoso, C., Steinke, L., Premoli, A. 2006. *Nothofagus antarctica*. Pp. 401-410 In: Donoso, C. (Ed.). Las especies arbóreas de los bosques templados de Chile y Argentina. Autoecología. Marisa Cuneo Ediciones, Valdivia, Chile.
- Gargaglione, V., Peri, P.L., Rubio, G. 2013. Partición diferencial de nutrientes en árboles de *Nothofagus antarctica* creciendo en un gradiente de calidades de sitio en Patagonia Sur. *Bosque* 34: 291-302
- Gyenge, J., Fernández, M.E., Sarasola, M., Schlichter, T. 2011 Stand density and drought interaction on water relations of *Nothofagus antarctica*: contribution of forest management to climate change adaptability. *Trees* 25:1111–1120.
- Lencinas, V.M., Martinez Pastur, G., Cellini, J.M., Vukasovic, R., Peri, P.L., Fernandez, M.C. 2002. Incorporación de la altura dominante y la clase de sitio a ecuaciones estándar de volumen para *Nothofagus antarctica*. *Bosque*, 23: 5-17.
- Peri, P.L., Martínez Pastur, G., Lencinas, M.V. 2009. Photosynthetic response to different light intensities and water status of two main *Nothofagus* species of southern Patagonian Forest, Argentina. *Journal of forest science* 55: 101-111.
- Peri, P.L., Gargaglione, V., Martínez Pastur, G., Lencinas, M.V. 2010. Carbon accumulation along a stand development sequence of *Nothofagus antarctica* forests across a gradient in site quality in Southern Patagonia. *Forest Ecol Manag* 260: 229-237.
- Peri, P.L., Ormaechea, S.G. 2013. Relevamiento de los bosques nativos de ñire (*Nothofagus antarctica*) en Santa Cruz: base para su conservación y manejo. Ediciones INTA web. <http://inta.gob.ar/documentos/libro-2013relevamiento-de-los-bosques-nativos-de-ñire-nothofagus-antarctica-en-santa-cruz-base-para-su-conservacion-y-manejo2013d>. Accessed 10 December 2013
- Varela, S.A. 2010. Respuestas morfológico-fisiológicas ante distintos tipos e intensidades de estrés en dos especies patagónicas del género *Nothofagus* con aptitud comercial. Tesis de Maestría, Universidad de Buenos Aires. 157 pp.
- Veblen, T.T., Donoso, C., Kitzberger, T., Rebertus, A.J. 1996. Ecology of southern Chilean and Argentinean *Nothofagus* forests. Pp. 293–353 In: Veblen, T., Hill, R., Read, J. (Eds.) The Ecology and Biogeography of Nothofagus Forests, Yale University Press, New Haven.

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

Mattioli M, Pereira IP*, Santos-Silva C

ICAAM, Universidade de Évora, Apartado 94, 7002-554 Évora, Portugal

* e-mail: ipp@uevora.pt

ABSTRACTS

Cistus palhinhae Ingram is an endemic plant from Portugal, with a non-consensual taxonomical position. Some authors consider it as a variety or sub-specie of *C. ladanifer* L.. Until now, the knowledge about *C. palhinhae* is limited, so we pretend to compare ecophysiological and anatomically both species in the same area. The study was performed in 3 sampling sessions in Cape Sardão region, where the two species occur. Plant cover structure, water potential and leaf area index were assessed. According to our results, ecophysiological processes are different between the studied species, especially the ones associated with the xeric features: *C. palhinhae* possesses marked xeromorphic adaptations, differing from *C. ladanifer* 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 on the southwest 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*, that revealed to be similar. Wuerpel (1973) compared the number of valves per fruit and 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). Plants water balance depends on the processes of absorption, conduction and transpiration, having daily and seasonal variations. One way to minimize water losses is by reducing the leaf area (Pereira, 2003). There are other strategies such as "cushion" plants that occur in open areas highly exposed to wind. This greatly branched and closed structure 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 Portugal, Beja, in Cape Sardão, with two sites separated by approximately 4 km, the nearest to shoreline include *C. palhinhae* distribution and the farthest *C. ladanifer*. 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, in different times of 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. For each sampling session 3 leaves from different plants of each species were used. In the first and last sampling session, one individual of each species was selected to perform a stratified cut following Norman & Campbell (1989) method. Leaves were store in bags, by layer, and stored at 4 °C until the next day. Leaves fresh weight was measured on a Sartorius, BP310S scale. Leaf area was assessed on an electronic leaf area meter (LI-3000-3050A, Li-COR Inc., Nebraska, E.U.A.). Leaf dried weight was then calculated after several days on a dry oven with forced ventilation, at 80 °C.

Four indexes were calculated: LAI (green leaves uniface area (m²/m²)); LAI_{ac} (added LAI from top to the bottom of the plant); SLA (Porter & Jong 1999; Vendramini *et al.*, 2002; Wilson *et al.*, 1999) and LWC (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).

Water potential comparison means was performed through Student 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 scores where registered in the summer whereas the highest scores were measured 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). 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**, since had a significant seasonal variations (Larcher, 1995), like *C. albidus* and *C. monspeliensis* (Werner *et al.*, 1999). Comparing SLA and LWC indexes (Figure 1), was perceptible that *C. ladanifer* had higher seasonal variation values. Generally, *C. palhinhae* presented higher LWC values while SLA values were similar between 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 of *C. ladanifer* leaf area and the lower water potential values observed in summer suggest an intense 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. For instance, leaf area indexes 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 lack of *C. palhinhae* accurate data and detail occurrence maps it is not possible to state which is the most consistent taxonomic category. It is only possible to disprove Demoly (1991) theory since the two species are not geographically isolated, despite having different habitats.

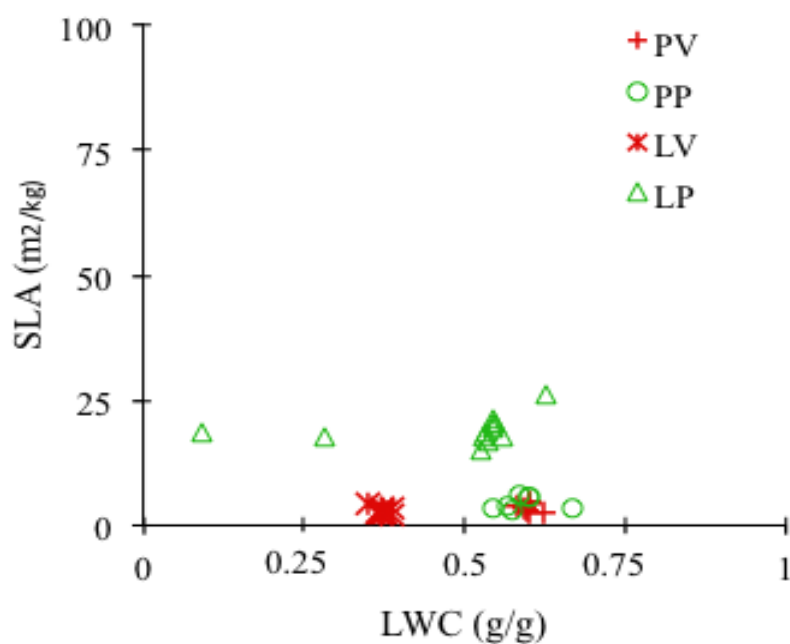
REFERENCES

- Aronne, G. & Micco, V. 2001. Seasonal dimorphism in the Mediterranean *Cistus incanus* L. subsp. *incanus*. *Annals of Botany* **87**: 789-794.
- Basile, Caterina 2008. *Interacção fracturação/dobramento na deformação de sequências turbidíticas; o exemplo do carbónico na Zona Sul Portuguesa*. Tese não publicada. Universidade de Évora. Évora. Portugal.
- Castroviejo *et al.* 1995. *Flora Ibérica* (Vol. III). Fareso, S. A. Madrid. pp. 318-337.
- Demoly, J.P. 1991. Notes et nouveautés nomenclaturales sur le genre *Cistus* L.. *Anales Jardín Botánico de Madrid* **49**(1) :150-152.

- Franco, J. A. 1971. *Nova Flora de Portugal – Continente e Açores* (Vol. III). Sociedade Astória. Lisboa. pp. 458-461.
- Garnier, E. & Laurent, G. 1994. Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. *New Phytologist* **128**: 725-736.
- ICNF, 2006. Plano Sectorial da Rede Natura 2000
- Larcher, Walter 1995. *Physiological Plant Ecology* (3rd edition). Springer. Berlin.
- Lo Gullo & Salleo (1988). Different strategies of drought resistance in three Mediterranean sclerophyllous trees growing in the same environmental conditions. *New Phytologist* **108** (3): 267-277.
- Norman, J. N. & Campbell G. S. 1989. Canopy structure. In: *Plant Physiological Ecology: field methods and instrumentation* (ed. R. W. Pearcy, J. R. Ehleringer, H. A. Mooney and P. W. Rundel), Capman & Hall. London. pp. 301-325.
- Pereira, I. 2003. *Influência da copa de Quercus rotundifolia Lam. na estrutura do coberto, interceptação da radiação, relações hídricas e microclima em Cistus salviifolius L.* Dissertação de Doutoramento. Universidade de Évora. Évora. Portugal.
- Porter & Jong 1999. A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. *New Phytologist*, **143**: 163-176.
- Proksch, P & Gulz, P. 1984. Methylated Flavonoids from *Cistus ladanifer* and *Cistus palhinhae* and their taxonomic implications. *Phytochemistry*, **23**(2): 470-471.
- Queiroz et al. 1999. *O Clima de Portugal* (Fascículo XL, Vol. 4 – 4^a região). Instituto Nacional de Meteorologia e Geofísica. Lisboa.
- Rivas-Martinez 1995. Classificación bioclimática de la terra. *Folia Botánica Matriensis* **16**:1-32
- Roderick et al. 1999. The relationship between leaf composition and morphology at elevated CO₂ concentrations. *New Phytologist* **143**: 63-72.
- Simões, M. P. 2002. Dinâmica de biomassa (carbono) e nutrientes em *Cistus salviifolius* L. e *Cistus ladanifer* L. . Influência nas características do solo. Tese de Doutoramento. Universidade de Évora, Évora. Portugal.
- SPSS Inc. 2006. *Statistical Package for Social Sciences*. Version 15. Chicago, IL.
- Vendramini et al. 2002. Leaf traits as indicators of resource-use strategy in Floras with succulent species. *New Phytologist*, **154**: 147-157.
- Werner et al. 1999. Two different strategies of Mediterranean macchia plants to avoid photoinhibitory damage by excessive radiation levels during summer drought. *Acta Oecologica* **20** (1): 15-23.
- Wilson et al. 1999. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytologist*, **143**: 155-162.
- Wuerpel, Charles. 1973. Further notes on *Cistus palhinhae* (Cistaceae). *Kew Bulletin*, **29**(1): 15-17.

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

		Summer	Winter	Spring	Seasonal amplitude
<i>C. palhinhae</i>	Ψ _{máx.}	-1.8	-0.5	-0.9	1.3
	Ψ _{mín.}	-2.7	-1.2	-2.0	1.5
	Daily amplitude	0.9	0.7	1.1	
	LAIac	1.53	-	2.63	1.1
<i>C. ladanifer</i>	Ψ _{máx.}	-4	-0.2	-1.8	3.8
	Ψ _{mín.}	-5.1	-1.7	-2.9	3.4
	Daily amplitude	1.1	1.5	1.1	
	LAIac	0.94	-	2.09	1.15

**Figure 1** – Specific 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).

Author index

- Almeida AS 68
 Aranda I 14, 101
 Arrabaça JD 29, 59
 Bahamonde HA 101
 Barbero M.C. 14
 Bernardes da Silva A
 29, 59
 Bressan-Smith RE 78
 Brumós J 82
 Buckley, TN 48
 Cabezas J-A 14
 Campostrini E 73, 78
 Cervera M.T. 14
 Chaves MM 73
 Collada C. 14
 Collado-González J 19
 Colmenero-Flores JM .. 82
 Corell M 2, 19
 Correia AC 34, 38
 Costa AR 68
 Costa e Silva F 34, 38
 Costa JM 73
 Coutinho J 68
 Dantas AK 54
 David JS 38
 David TS 7, 34
 de Cires A 48
 de María N 14
 de Miguel M 14
 Delatorre C 54
 Díaz L.M. 14
 Díaz-Espejo A ... 24, 48, 87
 Díaz-Rueda P 82
 Díaz-Sala C. 14
 Dubbert M 38
 Durand T 19
 El Aou-uad H 87
 Escalona JM 87
 Esteves BS 78
 Feito I 54
 Fernández JE 48, 78
 Ferreres F 19
 Flexas J 87
 Fortunato AS 63
 Franco-Navarro JD 82
 Galano JM 19
 Galindo A 19
 García-Tejero I 73
 Gil-Izquierdo A 19
 Girón IF 2, 19
 Granda V 54
 Guevara M.A. 14
 Guy A 19
 Gyenge J 101
 Hernandez-Santana V
 24, 34
 Leão de Sousa M 29
 Lidon FC 63
 Lima RSN 73
 Lopes TS 73
 Lousada LL 78
 Maças B 68
 Majada JP 54
 Mancha J.A. 14
 Marques da Silva J
 29, 59
 Martín-Palomo MJ 2
 Martorell S 87
 Matos AR 29, 59
 Mattioli M 105
 Medina S 19
 Medrano H 87
 Meijón M 54
 Moreno F 2, 19
 Moriana A 2, 19
 Oliveira MM 68
 Pais IP 63, 68
 Passarinho JA 68
 Pereira IP 105
 Pereira JS 34, 38
 Perez-Martin A 48
 Peri PL 101
 Pessanha DP 78
 Piayda A 38
 Pinheiro N 68
 Pinto C 34
 Pires S 29
 Pou A 87
 Ramalho JC 63, 68
 Ribas-Carbó M 87
 Ribeiro AS 59
 Ribeiro D 29
 Rivero C 82
 Rodrigues B 29
 Rodríguez A 54
 Rodriguez-Dominguez
 CM 24, 48
 Rosales MA 82
 Sáez-Laguna E 14
 Sánchez-Gómez D14, 101
 Santos M 68
 Santos-Silva C 105
 Sañudo BJ 82
 Scotti-Campos P 63, 68
 Semedo JN 68
 Silvestre S 29, 59
 Sousa EF 78
 Talón M 82
 Tomás M 87
 Torrecillas A 19
 Vadell J 87
 Vaz M 73
 Vázquez-Rodríguez A . 82
 Vélez M.D. 14
 Werner C 38