Universitat de Lleida

Use of plant and soil water status sensors to manage deficit irrigation in woody trees under Mediterranean conditions

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Dipòsit Legal: L.964-2013
http://hdl.handle.net/10803/120486

Universitat de Lleida

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RESEARCH & TECHNOLOGY
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Use of plant and soil water status sensors to manage deficit irrigation in woody trees under Mediterranean conditions

PhD thesis

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Lleida (2013)

APPROVAL OF DIRECTOR FOR PHD THESIS

CARMEN BIEL LOSCOS, doctor in Institute of Research Technology Food and agriculture.

AUTHORIZE

The presentation of the next PhD thesis titled "**Use of plant and soil water status sensors to manage deficit irrigation in woody trees under Mediterranean conditions**"
ASHRF ABDELFATAH ALI MOHAMED to get the PhD degree of the Lleida University.

And for the record for the appropriate purposes issuing this authorization

Caldes de Montbui (Barcelona), 05 July, 2013

CARMEN BIEL LOSCOS

AUTHORIZATION OF THE ACADEMIC TUTOR FOR PHDTHESIS

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Luis Serrano

For my dear family

My father and my mother

My wife

And my brothers

Acknowledgements

Acknowledgements

First and foremost I would like to thank my advisor Dra. Carmen Biel. It has been an honor to be under her supervision in my Master thesis. She has taught me all the things related with managing irrigation and its relation with the tree physiological status. Also I want to thank her for her patience as she started with me from zero because I was working in another subject in Egypt. I appreciate all her contributions of time, ideas, and her stimulation to me. I am also thankful for her kind treatment, and the excellent example she has provided as successful professor to me as she treated me like here young brother or friend I never felt that I am her student.

I want to present my sincere thanks to the members of HORTICULTURA AMBIENTAL department in IRTA Torre Marimon as they have contributed immensely to achieve this work and helped me a lot in writing my articles. This group has been a source of friendships as well as good advice and collaboration. I am especially grateful for Dr. Robert Saver because he treated me as his son, also Dr. Xavier Aranda and Dra. Felicidad de Herralde as they treated me as their young brother or friend. Also I want thankful all of my colleges in the office Antonio Vestrella, Merce Guardia, Tony Vilanova and Antonio Molina for their friendship, collaboration and the funny things happened with us in this office. Also I am thank all of my friends the technician persons in our department Beatriz Grau, Immaculada Funes, Marc Ferrer, Eulalia Serra, Cristian Morales, and Julian Cazaña for their help in the field work and their help to learn a lot of new Spanish and Catalan words. Also I would like to thank all of the persons of IRTA Torre Marimon for their help, friendship, and for their kindness treatment specially Francesc Puigdomenech and Pere Planas as they helped me a lot to come to the work by their special cars.

Also I want gratefully thank Dr. Luis Serrano for accepting to be a tutor for this thesis, and I want to thank him for his help and his effort in my master thesis, and I appreciate all his contributions of time.

I gratefully acknowledge the Spanish Ministry of Science and Innovation (MICINN, AGL2007-66279-C03-01/AGR) because it was the funding source to my PhD with the Grant reference (BES-2008-009667).

Also I gratefully thank Prof. Isabel Ferreira for her acceptance to be under her supervision during my stage in Lisbon, Portugal I think it was very useful stage for me and I want to thank her for her hospitality and kindness. And I want to thank all of the members of Biosystems Engineering: Water, Natural Resources and Climate department do ISA for their kind treatment and help during this stage.

Also, I would like to extent all my sincere thanks to my family for all their love, and encouragement, for my parents Abdelfatah and Souad whom raised me with a love of science and supported me economically and morally in all stages of my grades and educational life, I want to thank him for their effort with me as they were the main reason for my success in my life. For my patient wife Khadiga who helped me and encouraged me with her warm love, and kind marital treatment to pass this stage and to my beautiful son Albaraa, for my brothers Alaa, Mohamed, Aml, and Ali for their support and help during all the stages of my grades life, for my father-in-law El shekh Mosleh and my mother-in-law Fatma for their support to me and the facilities that they provided to me to marry their daughter and to be happy. For all my friends whom participated me in all bad and good things of my life and supported me economically and morally specially Mr. Abdelrahman Elsayed, Dr. Elsayed Mansour, Dr. Ali Salamh, Mr. Mohamed Mansour, Dr. Fathy Ali, Eng. Hamed Mansour, Dr. Abdallah abdelazeem and Mr. Salah Ibrahim, and also all my friends in El Hosainya city for their support.

I gratefully acknowledge all of my professors and my colleagues of Horticulture department, Faculty of Agriculture, Zagazig University for their help and support before my coming to here specially Dr. Farid Samy. Dr. Mohamed Mahmoud, Dr. Ahmed Abdallah Mohsen and Dr. Mohamed Momtaz. Also I want to thank Dr. Elsyed Gomaa, Dr. Mohamed el Sayed, Mr.Abdelghany and Mr. Manea abo Abdoh for their help and the facilities that they provided to me in their laboratories during my three years in Egypt. Also I want to thank all of the persons in el Khtara farm, and all of my students whom helped me in my work in the faculty.

Also I want to present all of my deep thank for all of my professors in Abo Sarar School (from1990 to 1995) and Ezwlain School (from 1995 to 1998) for their support and encouragement during all my studying stages as they helped me in building my personality, aims and to love my study.

Also my deep thanks for all the persons and my parents in my village Ezwlain for their support to me as they were always beside me in all good and bad conditions and they were one of the important reason for my success in my life till now.

My deep thanks also to all of my friends here in Barcelona specially in Canovellas as they helped me to pass this stage and they gave me the feel that I live between my parents in my country specially El shekh Mohamed Wadod, Mr Mohamed el essawy, Mohamed Reda, Mohsin, Mr. Abdelhadi Sajid, Mamadou Dialo, and Bo Abdelkader. My time at Barcelona was made enjoyable in large part due to the many friends and groups that became a part of my life. I am grateful for time spent with them.

Thanks for all

Ashraf Abdelfatah Ali

Acknowledgements

Acknowledgements

"If the end of the world approaches, while

one of you has a new seedling in his

hands he must continue planting

it before he leaves his place"

Our Prophet Mohamed –peace be upon him

"Even if I knew that tomorrow the world

Would go to pieces, I would still

plant my apple tree"

Martin Luther

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List of symbols and abbreviations used

CV Coefficient of variation, it is the ratio of the standard deviation (σ) to the mean (μ) (dimensionless number).

DI Deficit irrigation

DOY Day of the year

DMXTD Daily difference between the RDI trees and the control trees for MXTD

ETc Crop evapotranspiration (mm)

ETo Potential evapotranspiration (mm)

Gs stomatal conductance

IN Irrigation need

Kc Crop coefficient

Kr Coefficient related to the percentage of ground covered by the crop

LAI Leaf area index

LVDT Linear Variable Displacement Transducer, it is the sensor that transducers the movement of the trunk/stem in electrical signal.

MDS Maximum Daily trunk Shrinkage, the difference between the maximum daily trunk diameter value (just before sunrise) and the minimum daily trunk diameter value

(During the afternoon) (mm or μm).

MNTD Minimum daily Trunk Diameter. The minimum value of daily trunk diameter, which is reached sometime during the afternoon (mm or μm)

MXTD Maximum daily Trunk Diameter The maximum value of daily trunk diameter, which is reached just before sunrise (mm or μm).

P Precipitation

R^2 coefficient of determination

RDI Regulated deficit irrigation

RMDS relative maximum daily shrinkage

RT relative transpiration

SDI Sustained deficit irrigation

SF Sap flow, estimation of the transpiration flow in a plant ($l\ s^{-1}$ or $l\ d^{-1}$)

SI Signal intensity

TDV Trunk diameter variation, the daily cycle of swelling and shrinking of the trunk/stem

TGR Trunk Growth Rate, the difference between two consecutive maximum daily trunk diameters (mm or μm)

VPD vapour pressure deficit (kPa)

Ψ_s Soil matric potential (kPa)

Ψ_{mid} midday leaf water potential (MPa)

Ψ_{pd} Predawn leaf water potential (MPa)

Ψ_{stem} Midday stem water potential (MPa)

SUMMARY

Nowadays in Spain and many regions in the world face the problem of water scarcity and decreasing of its supplies. Moreover, climatic model projected that water scarcity will become one of the most important problems in many areas of the world. This serious problem supposes an urgent need to improve water use efficiency. Considering this problems, the general objective of this work was to improve irrigation efficiency by using new irrigation management protocol in two different species cherry trees (*Prunus avium*) and plane trees (*Platanus* x *hispanica*) for timber production. By applying different deficit irrigation strategies, based on the dendrometry measurements and other plant based water status indicators combined by soil water status measurements. The essays were conducted in 2009 and 2010; the first parts were conducted in one greenhouse and the second and third parts in the open field at IRTA Torre Marimon facilities. The objectives of the first chapter were to evaluate if MDS is a reliable indicator of cherry plants water status through its relationship with physiological and environmental variables; moreover, to study from these relationships the possibility of derivation a threshold value of MDS that indicated maximum water stress level in order to use it for irrigation scheduling. As expected well irrigated and stressed trees presented significant differences in all physiological variables: stomatal conductance (g_s), midday leaf water potential (Ψ_{md}) and daily sap flow (Q_d); substrate water potential (Ψ_s) decreased till -33kPa and the corresponding MDS value about 0.30 mm. Those values could be considered potential threshold for irrigation scheduling of young cherry trees. Those values corresponded to Ψ_{md} of -2.3 MPa and g_s of 50 mmol m^{-2} s^{-1}, indicative of severe water stress. In part B of this chapter, the objective was to explore if drought affected the daily and hourly pattern of two continuous indicators of water status, trunk diameter variation and sap flow. Stressed trees showed a significant MDS increase and SF decrease respect to irrigated trees. MDS was more sensitive than SF in front of drought stress, mainly due to the higher variability of SF from tree to tree. In the daily cycle values, trunk diameter variation and sap flow were related to each other via a loop that differs according to tree water status. The change in the slope of the relationship between the two varibles between 8 and 16 hours in drought conditions can be an indicator of stress. The aims of the second chapter were to improve managing irrigation in woody trees, cherry and plane tree, by applying a regulate deficit irrigation protocols based on the absolute value of MDS (RDImds) and soil water potential (Ψ_s), combined by reclaimed water in cherry trees. Results indicated no negative effect related with RDI treatments was noticed on cherry or plane trees growth as no any significant differences was noticed in trunk diameter growth rate (TGR), height and relative green cover (RGC)neither in stomatal conductance in both species. Water quality neither affected the grothw of cherry trees. The RDImds treatment combined with Ψ_s saved water without any negative effect on tree growth, with the same behavior when irrigated with reclaimed water or well water. The protocol applied in 2009 would be recommended for young cherry trees, and the protocol applied in 2010 would be recommended for plane trees. In the third chapter, the objective was to evaluate an irrigation scheduling protocol based on the information obtained from soil status Ψ_s and MDS signal ($MDS_{RDI}/MDS_{100\%Etc}$) as precision tools for automated adjustment of deficit irrigation in cherry and plane tree. Results indicated that RDIsignal and Ψ_s protocols had no negative effect on tree growth indicators TGR and RGC; also no clear differences were noticed between well irrigated and RDI_{signal} trees in MDS and $g_{s.}$ Our irrigation scheduling protocol based on MDSsignal and Ψ_s can be considered a valid protocol for schedule irrigation in woody trees as it helped us to save about 20- 30% of water in both species without any negative effect on the trees growth. As well as in the treatment RDImds, the protocol applied in 2009 could recommend for young cherry and protocol used in 2010 could recommend bananas for shade. The latter treatment has disadvantage it takes control, yet avoid the problems of the influence of environmental conditions on MCD treatment RDImds.

RESUM

Avui dia a Espanya i en moltes regions del món s'enfronten al problema d'escassetat d'aigua i la disminució de les seves ofertes. D'altra banda, les projeccions dels models climàtics preveuen que l'escassetat d'aigua es convertirà en un dels problemes més importants en moltes zones del món. Aquest seriòs problema fa que sigui urgent millorar l'eficiència en l'ús d'aigua. Considerant aquest aspecte, l'objectiu general d'aquesta tesis ha estat millorar l'eficiència del reg mitjançant la utilització de nous protocols de reg en dos espècies d'arbres, cirerers (*Prunus avium*) i de platan d'ombra (*Platanus x hispanica*) per a produir fusta. Mitjançant l'aplicació de diferents estratègies de reg deficitari basades en mesures de la màxima contracció diària del diámetre del tronc (MCD) combinat amb la mesura de l'estat de l'aigua al sòl. Els assajos van ser realitzats els anys 2009 i 2010, en un hivernacle (primer capítol) i en camp obert (segon i tercer capítol) a les instal·lacions de l'IRTA a Torre Marimon. Els objectius del primer capítol van ser avaluar si la MCD és un bon indicador de l'estat hídric del cirerer a partir de les relacions amb altres indicadors fisiològics i ambientals; així mateix, es va obtenir un valor llindar d'aquesta variable per a utilitzar-lo en la programació de reg. Com s'esperava els arbres ben regats i els arbres estressats van presentar diferències significatives en les variables fisiològiques: conductància estomàtica foliar (g_s), potencial hídric foliar de migdia (Ψ_{md}) i el flux de saba diari (Qd); el potencial matricial del substrat (Ψ_s) va disminuir a valors de - 33 kPa i el corresponent valor de MCD va ser aproximadament de 0.30 mm. Aquests valors són considerats llindars potencials per a planificar el reg cirerers joves. Aquests valors corresponen a un Ψ_{md} de -2.3 MPa i una g_s de 50 mmol m^{-2} s^{-1}, els quals indiquen estrès sever. En la part B del primer capítol, l'objectiu era explorar si la sequera afecta el patró diàri i horari dels dos indicadors de l'estat hídric mesurats en continu com son la variació del diàmetre del tronc i el flux de saba. Els arbres estresats mostren un increment significatiu de la MCD i un decrement del flux de saba respecte als arbres ben regats. La MCD és més sensible que el SF diàri front l'estrès per sequera, principalment a causa de la variabilitat més gran del SF entre arbres. En els cicles diàris, la variació del diàmetre del tronc i el flux de saba estan relacionats per un bucle que es comporta diferent quan estàn ben regats o estan estressats. La variació del pendent de la relació entre les dues varibles entre les 8 i les 16 hores en situacions de sequera pot ser un indicador d'estrés. Els objectius del segon capítol van ser intentar millorar la gestió de reg en arbres, cirerer i plàtan d'ombra, mitjançant la implantació de nous protocols de reg deficitari regulat (RDI_{mds}) basat en el valor absolut de la MCD i el potencial matricial del sòl (Ψ_s), també es va combinar amb l'us d'aigua regenerada en cirerers . Els resultats indiquen que no va haver cap efecte negatiu associat amb l'aplicació del tractament RDI en el creixement en diàmetre, alçada i cobertura verda relativa, ni en la conductància estomàtica en ambdues especies. La qualitat d'aigua de reg tampoc va afectar al creixement dels cirerers El tractament RDI_{mds} ha estalviat aigua sense cap efecte negatiu sobre el creixement de l'arbre, amb el mateix comportament quan s'ha regat amb aigua regenerada o aigua de pou. El protocol aplicat en 2009 podria ser recomanat per a cirerers joves, i el protocol aplicat en 2010 podria ser recomanat per als plàtans. En el tercer capítol, l'objectiu va ser evaluar un protocol de programació de reg basat en el Ψ_s i la señal de laMCD ($MCD_{RDI}/MCD_{100\%Etc}$) en cirerer i en plàtan d'ombra. Els resultats van indicar que tractaments de RDI basats en la señal de MCD no van tenir efecte negatiu en els indicadors de creixement dels arbres, ni en la MCD ni en la gs. El tractament RDI_{senyal} va estalviar el 20-30% de l'aigua en les dues espècies. Així com en el tractament RDI_{mds}, el protocol aplicat en 2009 es podria recomanar per als cirerers joves i el protocol utilitzat en 2010 es podria recomanar per a plàtans d'ombra. Aquest darrer tractament té la desventatja que es necessita un control, però a la vegada evita els problemes de la influència de les condicions ambientals en la MCD del tractament RDI_{mds}.

RESUMEN

Hoy en día en España y en muchas regiones del mundo se enfrentan al problema de la escasez de agua y la disminución de sus suministros. Por otra parte, las proyecciones de los modelos climáticos preveen que la escasez de agua se convertirá en uno de los problemas más importantes en muchas zonas del mundo. La gravedad de problema hace que sea necesario mejorar la eficiencia del uso del agua. Considerando lo anterior, el objetivo general de esta tesis es mejorar la eficiencia del riego mediante la utilización de nuevos protocolos de gestión de riego en dos especies de árboles: cerezos (*Prunus avium*) y plátano de sombra (*Platanus x hispanica*) para producir madera. Mediante la aplicación de diferente estrategias de riego deficitario basadas en las medidas de la máxima contracción diaria del tronco (MCD) combinado con la medida del estado hídrico del suelo. Los ensayos se realizaron los años 2009 y 2010, en un invernadero (primer capítulo) y en campo (segundo y tercer capítulo) en las instalaciones del IRTA en Torre Marimon. Los objetivos del primer capítulo fueron evaluar si la MCD es un buen indicador del estado hídrico del cerezo a partir de las relaciones con otros indicadores fisiológicos y ambientales; asimismo, se obtuvo un valor umbral de esta variable para utilizarlo en la programación del riego Como se esperaba, los árboles bien regados y los estresados presentaron diferencias significativas en todas las variables fisiológicas: conductancia estomática (gs), potencial hídrico de la hoja al mediodía (Ψ_{md}) y flujo de savia diario (Qd); el potencial matricial del substrato (Ψ_s) disminuyó hasta -33, y el correspondiente valor de MCD fue aproximadamente 0.30 mm. Estos valores se consideran umbrales potenciales para la programación del riego de árboles jóvenes de cerezos. Estos valores se corresponden a un Ψ_{md} de -2.3 MPa y una gs de 50mmol m^{-2} s^{-1}, los cuales indican estrés hídrico severo. En la parte B del primer capítulo B, el objetivo fue explorar si la sequía afecta al patrón diario y horario de dos indicadores continuos del estado hídrico como son la variación del diámetro del tronco y el flujo de savia. Los árboles estresados mostraron un incremento significativo de la MCD y un decremento del SF diario respecto a los bien regados. La MCD es más sensible a la sequía que el SF diario, debido principalmente a la mayor variabilidad entre árboles del segundo. En los ciclos diarios, la variación del diámetro del tronco y el flujo de savia horario están relacionados por un bucle que se comporta diferente cuando está regado o estresado. La variación de la pendiente de la relación entre las dos variables entre las 8 y las 16 horas en situaciones de sequía puede ser un indicador de estrés. Los objetivos del segundo capítulo fueron intentar mejorar la gestión del riego en árboles, cerezo y plátano de sombra mediante la implantación de nuevos protocolos de riego deficitario regulado basados en el valor absoluto de MCD (RDI_{mds}) y el potencial matricial de suelo (Ψ_s), también se combinó con el uso de agua regenerada en cerezos. Los resultados indicaron que no hubo ningún efecto negativo relacionado con los tratamientos de RDI en el crecimiento en diámetro y altura del tronco ni en la cobertura verde, ni en la conductancia estomática en ambas especies. El tratamiento RDI_{mds} ha ahorrado agua sin ningún efecto en el crecimiento de los árboles, con el mismo comportamiento cuando se ha regado con agua regenerada o agua de pozo. El protocolo aplicado en 2009 podría ser recomendado para cerezos jóvenes, y el protocolo aplicado en 2010 podría recomendarse para plátanos. En el tercer capítulo, el objetivo fue evaluar un protocolo de riego basado en el Ψ_s y la señal de la MCD ($MCD_{RDI}/MCD_{100\%Etc}$). Los resultados indicaron que tratamientos de $RDI_{señal}$ no tuvieron ningún efecto negativo sobre los indicadores de crecimiento de los árboles ni en la MCD ni en la gs. El tratamiento $RDI_{señal}$ ahorró un 20 - 30% de agua en las dos especies. Així com en el tractament RDI_{mds}, el protocolo aplicado en 2009 se podría recomendar para cerezos jóvenes y el protocolo utilizado en 2010 se podría recomendar para plátanos. Este último tratamiento tiene la desventaja que necesita un control, pero a su vez evita los problemas de la influencia de las condiciones ambientales en la MCD del tratamiento RDI_{mds}.

General introduction

Water plays a central role in the functioning of the biosphere and in supporting all life. According with the report “Water resources in Europe in the context of vulnerability” (EEA report 11, 2012) land use change, water abstraction and climate change are the principal causes of water vulnerability of the ecosystems.

In order to manage the available water in a basin or irrigation sector it is necessary to know the amount of water available (rainfall, surface water and underground water), the land use and the climate models projections. Also, it is essential to know the risk of water scarcity or excess that we can absorb/resist (EEA report 11, 2012).

Agriculture in the Mediterranean basin is complex, due to human transformation and use since a long time ago, high inhabitant density and semi arid climate that developed landscapes consisting in a wide range of habitats (forests, shrub lands, agricultural fields and urbanized areas) of many different sizes and uses (Forman and Godron, 1986; Forman, 1995). As an example of this fragmentation of the land use, in Catalonia in particular, 39.6% of its surface is covered with forests (Ibañez and Burriel 2010) and 30.3% by crops, from which 68% are not irrigated (DAAR, 2007). The rest of the land is occupied by urban areas, water channels, grasslands, shrublands, unproductive coastal areas and high mountains (Anglès, 2009). This pattern is similar in all the Mediterranean area of Iberian Peninsula.

The interrelations between the different habitats create some problems such as water distribution among different social sectors, the excessive fragmentation of agricultural land for infrastructure, development of large impermeable areas, the reduction and use of surface water flows, the overexploitation of groundwater resources, the break of natural biological corridors, the massive introduction of alien species, etc. (Forman 2004).

The role that agricultural land can play in the future depends on the projected climate change scenarios: it can absorb the excess of water drained from urbanized land if the case of an increase of storms and high intensity rainfalls due to the soil acting as a reservoir or plant water extraction; in water scarcity scenarios crop management can help to preserve water (less water spender cultivars, non tillage or changes in the growth cycle) or maintain soil properties (Llebot et al., 2010). Agriculture is the socio-economic sector that manages more water; as an example, in Catalonia about 80% of the water in the inner basin (but only 6% in the coastal basin), is used either by rain fed agronomic practices or by irrigation techniques (Girona et al., 2009; Savé at al., 2009).

In this sense, water scarcity could increase the vulnerability of agriculture (Sebastià et al., 2010). Nowadays, there are notable efforts to improve the water use efficiency at the basin and at the landscape level (Barros et al., 2011; Du et al., 2010; He et al., 2012) because this knowledge is the key to understand the water fluxes, the variability of water quality with respect to its use, or the potential water storage in soil, among others that give shape to the potential water footprint of different crops involved in the same landscape (Hoekstra et al., 2011).

Unfortunately, there is scarce information about water use of woody species used for wood production, landscaping or gardening in the literature.

The European 2020 strategy will try to develop a green economy more environmentally and friendly in the European Union. This strategy wants to ensure that economic sectors such as agricultural (agriculture + livestock), energy and transport, adopt management practices that can keep water ecosystems healthy and resilient and increase the carbon sinks of agro forest systems.

In this way the EU strategy on adaptation to climate change, in the case of Spain, considers that planting trees in dry areas of the Iberian Peninsula can prevent desertification and can help to increase the resilience of the area to projected climate change. However until now reforestation projects have only been partly successful because of the continuous need for water (Trabucco et al. 2008) with extremely low survival rates of trees and high overall costs (EEA 2012)

This serious problem supposes an urgent need to improve water use efficiency (Fereres and Evans 2006; Fernandez and Cuevas 2010; Jones 2004;), by using means of accurate irrigation scheduling based on physiological indicators, which show information on crop water status. Along the last years, there has been a notable increase in the number of papers and reviews related to stress in plants and crops, which can be attributable to the improvement of plant stress detection and the rise in the interest in optimizing plant productivity in a wide sense (Levitt 1980; Larcher 1980, 1995; Bradford and Hsiao 1982; Lichtenthaler 1998; Givnish 1986; Mooney et al. 1991; Alsher and Cumming 1990; Smith and Grifiths 1993).

Plants have a few potential responses in front of stress, but they combine these to reduce the stress consequences. The first are elastic responses that provide high productivity; later, as stress is increasing, restrictions in growth and some adaptations appear; if stress is maintained, survival rate is reduced and death can appear (Schulze et al 2005).

The potential climate change attributable to global change can increase local and general temperature (IPCC, 2007). This increase will not be the same around the world (IPCC 2004); it seems it will be particularly relevant the Mediterranean Basin (Pinyol et al 1998; ACCUA 2011). Predictions arising from different models for generating climate change scenarios showed that the Mediterranean region would be affected by drought periods of medium (4 - 6 months) and long duration (more than 12 months), and 3 to 8 times more frequent than at present (Sheffield and Wood 2008). Also, Mediterranean environments are characterized by a double stress (Terradas and Savé 1992): drought in summer and low temperatures in winter.

Thus, some engineering options (soil preparation, water flow and reservoir arrangements, etc.) are used in conjunction with some agronomic and ecophysiological options (plant hardening, selection of species, plant quality, irrigation, etc.) (Garcia Navarro et al 2004; Espelta et al. 2003; Cortina et al 2006).

Ecophysiology or environmental physiology is a biological discipline which studies the adaptation of organism's physiology to environmental conditions. So ecophysiology also can be used as a tool that permits objective evaluations of plant material, methods and systems to optimize their culture under a wide range of environmental conditions (Prasad 1997; Lange et al 1982, Schulze et al. 2005; Stuart et al 2002).

From a long time the ecophysiological field methods have been used to measure the plant responses to environmental conditions. In the last years, new portable or laboratory methods have been developed which provide easy, fast, repetitive and objective methods for this kind of studies (Slavic1974; Pearcy et al. 1989; Hendry and Grime 1993; Reigosa 2001; Mooney et al 1991).

Information on the plant water status is indeed best provided by physiological indicators, because of their dynamic nature and their direct relation with climatic and soil conditions (Jones 2004; Ortuño et al. 2006; Remorini and Massai 2003). So some parameters must define plant status, plant stress level and the implications that this is producing in plant growth.

For irrigation scheduling procedures involving high-frequency irrigation systems, information about crop evapotranspiration (ETc) is required (Allen et al., 1998), although the application of ETc has some uncertainties due to the effects of tree canopy architecture, the degree of canopy soil cover and soil surface management.

In addition, as stated before, till now many woody crops for timber production of increasing interest in the Mediterranean region had no sufficient information about its water requirements; hence it is currently not possible to schedule irrigation properly (Ortuño et al. 2010).

To solve some of these aspects, many studies tried to apply different deficit irrigation (DI) strategies in fruit crops, for example, in peach (Goldhamer et al. 1999), plum (Intrigliolo and Castel, 2004), apricot (Pérez-Pastor et al. 2009), and kaki (Badal et al. 2010). Depending on the aim and the way DI is applied, they can be divided into two strategies: regulated deficit irrigation (RDI) and sustained deficit irrigation (SDI).

RDI is a strategy aiming at reducing tree growth of vigorous individuals and at saving water, in which irrigation water amounts are reduced during phenological periods not critical for yield or fruit quality, while during the rest of the season full tree water requirements are applied (Chalmers et al. 1981; Mills et al. 1997). RDI has been applied successfully in many fruit species like lemon, almond, apricot, vineyards, peach and olive (Conejero et al. 2011; Domingo et al. 1996; Gispert et al. 2012; Marsal et al. 2004; Pérez-Pastor et al. 2009; Romero et al. 2010 respectively).

Sustained deficit irrigation (SDI) tries to deliver uniform amounts of water, lower than plant requirements during the growing season to promote a gradual development of plant water stress as the season progresses and soil water is depleted (Chalmers et al. 2010; Fereres and Soriano 2007).

Irrigation scheduling strategies based on the use of soil water measurements accompanied by plant-based water status indicators has been investigated in recent years for planning more precise irrigation management programs (Badal et al. 2010; Conejero et al. 2011; Goldhamer and Fereres 2001; Hanson et al. 2000; Intrigliolo and Castel 2004; Ortuño et al. 2009; Vélez et al. 2007).

In the eighties and nineties of the past century, the first approaches to optimize irrigation scheduling were based on evaluating plant water status by determining leaf water potential, either as predawn readings (Domingo et al. 1996), or midday readings (Girona et al. 2006; Naor 2006), as well as stem water potential (McCutchan and Shackel 1992) among others. However, with the development of new sensors based on trunk diameter variation (TDV) or sap flow measurements, promising strategies for irrigation management were developed that used them for detecting water stress and assessing crop water needs (Fernandez et al. 2011; Giorio and Giorio 2003; Ortuño et al. 2004; Ortuño et al. 2010).

It has long been known that plant growth is one of the most sensitive indicators of water stress. Therefore, trunk diameter variations TDV have been used in water relations studies since the 1970's. Subsequently, the appearance of electronic devices permitted accurate measurements of TDV, which related changes in trunk diameter with transpiration (Klepper et al. 1971; Kozlowski 1967; Molz and Klepper 1973). This led to the proposal to use dendrometers for continuous monitoring of plant water status (Huck and Klepper 1976), which would be useful in irrigation decision-making (Goldhamer and Fereres 2001; Huguet et al. 1992).

Several indicators can be derived of TDV daily cycle, such as trunk growth rate (TGR), which is the difference between the maximum diameter of two consecutive daily cycles, and MDS, the difference between maximum and minimum values of a single daily cycle (Goldhamer and Fereres 2001; Huck and Klepper 1977; Kozlowski 1967).

In deciduous fruit trees (Cohen et al. 2001; Marsal et al. 2002), in citrus species (García-Orellana et al. 2007; Ortuño et al. 2006) and in cherry trees (Livellara et al., 2011; Cabibel and Isbérie, 1997), MDS and TGR were found to be responsive to changes in soil moisture availability. In these cases, plant water deficit increased trunk shrinkage and decreased trunk growth, but there is not a unique relation valid for the whole season between plant water status and trunk shrinkage (Fereres and Goldhamer 2003; Intrigliolo and Castel 2005; Marsal et al. 2002). Moreover, crop load itself might directly affect MDS (Intrigliolo and Castel 2007 and references within).

Several authors have demonstrated the possibility of scheduling irrigation based only on daily MDS in many species (Badal et al. 2010; Besset et al. 2001; Bussi et al. 1999; Conejero et al. 2011; Goldhamer and Fereres 2004; Intrigliolo and Castel 2007; Li et al. 1989; Mercier et al. 2009; Ortuño et al. 2009). However, in citrus trees (Vélez et al. 2007) and in mature olive trees (Cuevas et al. 2010), absolute MDS values could not be used as the only variable to schedule irrigation, because it varied largely from day to day, as a result of atmospheric changes.

The usefulness of TDV and sap flow measurements for detecting water stress and assessing water needs could be reduced by the atmospheric changes, the age, crop load and the limited information provided at the end of the season because of the loss of elasticity in green tissues of bark, cambium and outer xylem tissues (Cuevas et al. 2010; Moriana et al. 2010; Pérez-López et al. 2008). For this reason a lot of authors suggested alternative variables derived of TDV daily cycle to deal with the influence of atmospheric changes. For example, the use of relative indicators which compare the differential values between deficit and control plant, such as relative diameter trunk shrinkage RDTS (deficit/control) (Ferreira et al. 1997), the difference in maximum daily diameter between deficit and control plants (D_{MXTD}) after considering the values recorded at the beginning of the irrigation season as zero (Fernandez et al. 2011), or the modified MDS introduced by De Swaef et al. (2009) as the daily difference between the maximum diameter of the control tree and the minimum diameter of the stressed tree.

On the other hand, other studies searched for providing alternative sources of fresh water, such as the use of reclaimed water in agriculture, which is considered an important management strategy in areas with limited freshwater resources, because of its potential economic and environmental benefits (Dobrowolski et al. 2008; Martínez et al. 2012; Parsons et al. 2001; Pedrero et al. 2010; Reboll et al. 2000; Zekri and Koo 1993). The most important advantages are that reclaimed water can be used as an economical source for irrigation because of its availability, and that it may reduce fertilizer application because of its nutrient content (Neilsen et al. 1989; Reboll et al. 2000)**.** Moreover, recycling these nutrients may prevent pollution of surface or ground water (Sanderson 1986)**.** However, reclaimed water may have disadvantages depending on its sources and treatments if not treated adequately; its potential disadvantages including serious health risks (Rose and Gerba 1991), salt and heavy metal accumulation (Paranychianakis and Angelakis 2008; Westcot 1988) and new micro pollutants (Calderon et al. 2013),.

Ecophysiology Subprogram (Environmental Horticulture Program) of IRTA has been developed during last years, under the direction of Dra. C. Biel, an important effort to increase the water use efficiency in trees for landscaping and wood production under Mediterranean conditions (Savé et al. 2004; Biel et al. 2005; Biel, et al. 2007; Savé et al. 2007; Abdelfatah et al. 2013). All this contrasted information promoted to focus our work on improving water use efficiency and irrigation scheduling in woody crops.

OBJECTIVES

The overall aim of the present thesis is to improve irrigation water use efficiency by using deficit irrigation strategies supported by plant and soil water status measurements in two woody crops (cherry and plane trees) for timber production under Mediterranean conditions.

In order to achieve this, other additional objectives were:

To develop irrigation algorithms based in the combination between trunk maximum daily shrinkage and soil matric potential.

To test the irrigation schedules experimentally developed in trees under field conditions.

Specific objectives and Thesis structure

Chapter 1A:

1- To evaluate if MDS is a reliable indicator of cherry plants water status through its relationship with physiological and environmental variables.

2- To study from these relationships the possibility of derivating a threshold value of MDS that would indicate maximum water stress level in order to use it for irrigation scheduling.

Chapter 1B:

3- To elaborate information about the hourly pattern of two continuous indicators of water status, MDS and sap flow, and study if these patterns were affected by drought. To evaluate if the relationship between these two indicators can be use in irrigation scheduling.

Chapter 2:

1-To improve cherry and plane tree irrigation management by applying an irrigation scheduling protocol combining maximum daily shrinkage (MDS) and soil water potential (Ψ_s) of the previous day.

2- To assess the effect of the combination of the previous protocol and irrigation with reclaimed water on growth parameters and water relation of cherry trees to test it as an alternative water resource for irrigation.

Chapter 3:

1- To evaluate the regulated deficit irrigation protocol using the MDS signal in comparison with 100%ETc and sustained deficit irrigation.

2- To study the growth and stomatal conductance responses in two tree species to the deficit irrigation treatments.

Thesis structure

The first chapter essay was made in a greenhouse where potted trees were submitted to different drought cycles.

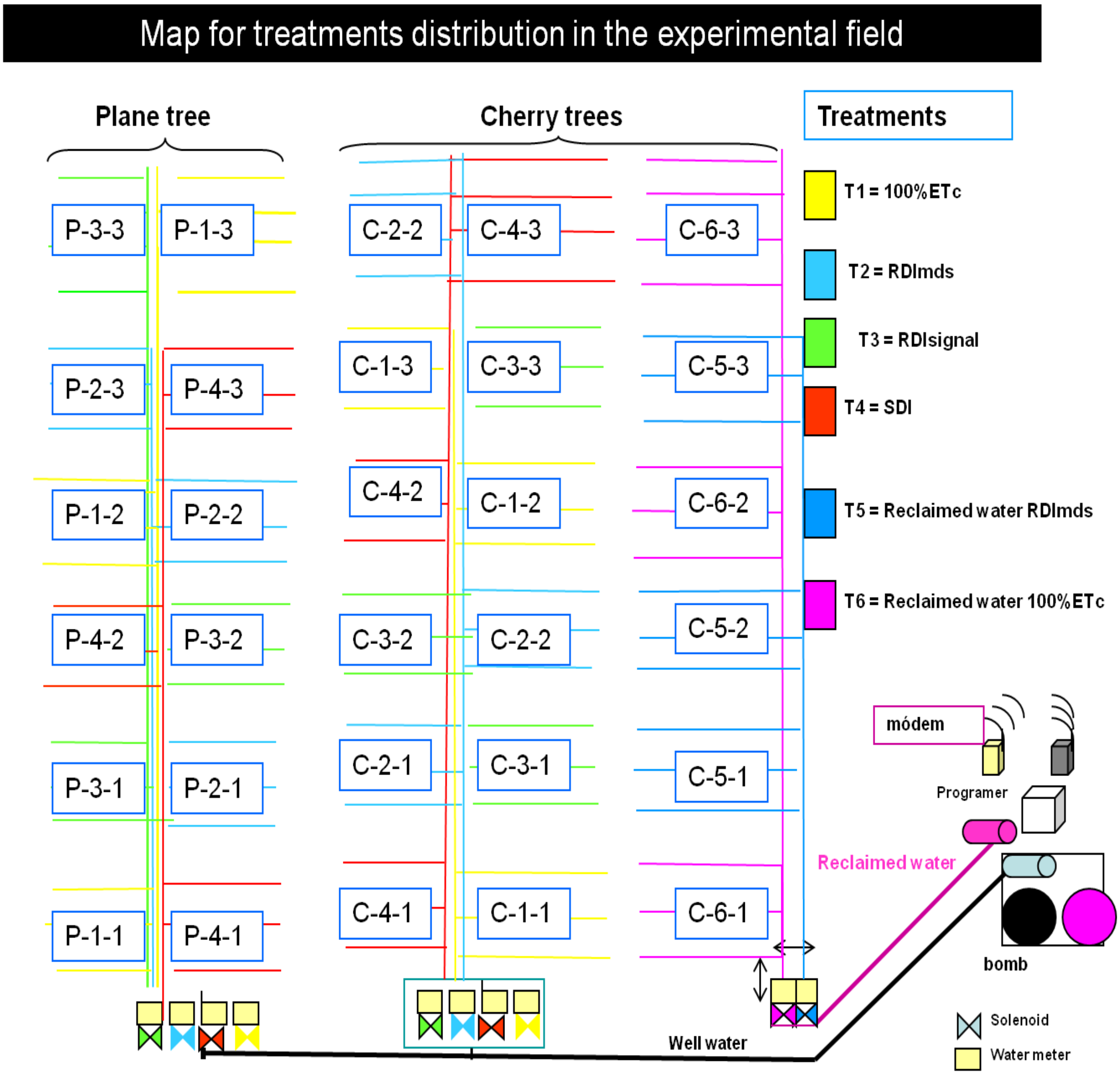
The second and third chapter essays were made in field conditions during 2009 and 2010.

General description of the plot

Experiments described in chapters 2 and 3 were carried out in an orchard located at the facilities of IRTA (Institut Recerca i Tecnologia Agroalimentaries) in Torre Marimon, Caldes de Montbui, Barcelona, Spain; 41.6ºN, 2.1ºW; elevation 176m above mean sea level. One year old cherry trees and two years old plane trees were planted in February 2008. Trees were arranged in north-south oriented rows at 4x4 spacing. The soil was stony, with Sandy-loam, sandy-clay-loam and loam textures, volumetric percentage of stones ranging from 20 to 70 % and organic matter from 1 to 1.68 %.

The orchard was divided into three main plots (see the map below): the two first plots contained cherry or plane trees irrigated with well water (WW) and the third plot cherry trees irrigated with reclaimed water (RW). The plots were subdivided in three blocks within which several irrigation treatments were randomly distributed. In the WW plots, four irrigation treatments were allocated, but only two for the RW plot. Each subplot within a species, irrigation treatment within a block and water quality treatment consisted of 12 trees, thus resulting in 144 trees in each WW plot (12 trees x 4 irrigation treatments x 3 blocks) and 72 in the RW plot (12 trees x 2 irrigation treatments x 3 blocks).

As only two irrigation treatments were present in all three plots (100% of crop evapotranspiration requirements and regulated deficit irrigation based on maximum daily trunk shrinkage), they are analyzed separately in chapter 2. Chapter 3 analyzes the other two treatments, present only in the WW plots.



CHAPTER 1A

Evaluation of the response of maximum daily shrinkage in young cherry trees submitted to water stress cycles in a greenhouse

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Agricultural Water Management 118 (2013) 150– 158

Abstract

In the semiarid zones of the Mediterranean area, such as the eastern coast of the Iberian Peninsula, as in many others in the world, water scarcity and the increase of water use in many socio-economical sectors, suppose an urgent need to improve irrigation management. To assess the feasibility of using maximum daily shrinkage (MDS) of the tree trunk as a plant water stress indicator for irrigation scheduling in cherry trees (*Prunus avium*), an experiment was carried out in 2009 under greenhouse conditions were the relationship of MDS with several environmental and physiological variables was studied. Two years-old cherry trees planted in plastic pots were submitted to several irrigation withholding cycles. Fully irrigated and stressed trees presented differences in all measured physiological variables: leaf stomatal conductance (g_s), midday leaf water potential (Ψ_{md}), and daily sap flow (Q_d), as expected; and also in MDS which, among the variables measured in the plant, correlated best with Ψ_{md} (r^2= 0.49***). Also a strong (r^2=0.65***), two phase relationship was found between MDS and substrate matric potential (Ψ_s) with a linear response of MDS with some scattering till Ψ_s reached about-33 kPa, followed by a stationary phase with a MDS value about 0.30 mm, a potential threshold for irrigation scheduling of young cherry trees. This value corresponded to Ψ_{md} of -2.3 MPa and g_s of 50 mmol m^{-2} s^{-1}, indicative of water stress. Finally, MDS (which can be considered an adequate indicator of cherry tree water status given its strong relation with other indicators of plant water status) was the most sensitive and early indicator of water stress (highest signal to noise ratio) of the measured physiological variables. This high sensitivity makes it a potential candidate indicator for irrigation scheduling in young cherry trees, if the threshold obtained in this work can be validated or any other can be established.

Keywords: Leaf water potential, Sap flow, Stomatal conductance, Substrate matric potential, water deficit indicator.

1. Introduction

Irrigation is, nowadays, the largest freshwater consumer in the world; thus, the scarcity of water supplies and increasing of water demands suppose an urgent need to improve irrigation management (Fereres and Evans, 2006). Improvement of water use efficiency by means of accurate irrigation scheduling based on physiological indicators which show information on crop water status has been long studied: see Jones (2004) and Fernandez and Cuevas (2010) for a review and more recent work by, among others, Naor and Cohen, (2003) in apple trees, Ortuño et al. (2009) in lemon trees, and Moriana et al. (2010) in olive trees.

There are many ways to manage irrigation, but all of them include the amount of water to be applied, when it should be applied and how to do so, and for all of these issues a quantitative approach gives the best results (Fereres et al., 2003). An irrigation scheduling protocol is the quantitative, combined answer to the two first points, conditioned by the methodology used for irrigation, and it includes knowledge, general or specific, of the crop water requirements. Although the tendency of most plants to minimize changes in its water status poses a problem to its use as a measure of water deficit (Jones 2004), the knowledge of the hydric condition of the plant is still proposed for irrigation management with appreciable results because of its dynamic nature, which is directly related with climatic and soil conditions (Remorini and Massai, 2003; Vélez et al., 2007). As a matter of fact, the precise determination of plant water status is considered important to plan irrigation scheduling protocols by some authors (Naor 2006, Intrigliolo and Castel 2007a). The most widely used approach for evaluating plant water status has been to determine leaf water potential, either as predawn readings

(Domingo et al., 1996), or midday readings (Naor, 2000; Girona et al., 2006), as well as stem water potential, (McCutchan and Shackel, 1992) among others.

However, other approaches and technologies to optimize irrigation scheduling are being developed. Among them, those based on monitoring a key variable in the plant have the potential advantage of integrating, in a single measurement, not only the direct effects of the prevailing soil and atmospheric water conditions on water flow through the plant, but also the response of the plant physiological mechanisms controlling water use. Thus, canopy temperature, water content in the trunk, trunk diameter variations and sap flow readings are considered as promising plant-based variables for irrigation control, either alone as such or as relative to values of a control treatment, or in combination with other plant or environmental variables. An additional and crucial advantage of using any of these variables for irrigation scheduling is that they can be automatically monitored (Fereres and Goldhamer, 2003; Jones, 2004; Naor, 2006).

From trunk diameter variations (TDV), which have been used in water relations studies since the 1970's, several indicators can be derived, such as trunk growth rate (TGR), which is the difference between the maximum diameter of two consecutive daily cycles, and MDS, the difference between maximum and minimum values of a single daily cycle (Kozlowski, 1967; Huck and Klepper, 1977; Goldhamer and Fereres, 2001).

The sensitivity of TDV to water stress is well-known since early studies (Molz and Klepper, 1973). In deciduous fruit trees (Cohen et al., 2001; Marsal et al., 2002) and citrus species (García-Orellana et al., 2007; Ortuño et al., 2006), MDS and TGR were found to be responsive to changes in soil moisture availability. In these cases, plant water deficit increased trunk shrinkage and decreased trunk growth, but there is not a unique relation valid for the whole season between plant water status and trunk shrinkage (Fereres and Goldhamer, 2003; Intrigliolo and Castel, 2005; Marsal et al., 2002). Moreover, crop load itself might directly affect MDS (Intrigliolo and Castel, 2007a and references within).

Several authors have demonstrated the possibility of scheduling irrigation based only on MDS measurements (Li et al., 1989; Bussi et al., 1999; Besset et al., 2001, Mercier et al., 2009) but usually base lines or relative to a well irrigated tree are used (Badal et al., 2010; Conejero et al., 2011; Goldhamer and Fereres, 2004; Intrigliolo and Castel, 2007a; Ortuño et al., 2009). However, in citrus trees (Vélez et al., 2007) and in mature olive trees (Cuevas et al., 2010) the absolute MDS values could not be used as the only variable to schedule irrigation. This was because either it varied largely from day to day, as a result of atmospheric changes or because after an increase of MDS as the plant water potential decreased, MDS decreased again, as the plant water potential became more negative (Ortuño et al., 2010; Fernandez and Cuevas, 2010). The relevance of these drawbacks depends on the irrigation protocol context: for instance, the two-phase problem will only appear in the context of low frequency irrigation protocols, and even in those cases it can be taken into account if the algorithm looks for the appearance of high, consecutive MDS values, irrespective of these being followed by low values, which would call for an increased irrigation frequency (Goldhamer and Fereres, 2001). To deal with the influence of atmospheric changes, many previous studies suggested the use of relative indicators which compared the stress plant values with control values, such as relative diameter trunk shrinkage (RDTS, Ferreira et al., 1996 and 1997), the difference in maximum daily diameter between stressed and control plants after considering the values recorded at the beginning of the irrigation season as zero (Fernandez et al., 2011), or the modified MDS introduced by De Swaef et al. (2009) as the difference between the maximum diameter of the control tree and the minimum diameter of the stressed tree, thus potentially combining higher shrinkage in the stress plant with higher diameter growth in the control. Goldhamer and Fereres

(2001) followed a similar approach: they calculated the ratio between stress plant and control plant values, equivalent to RTDS for example, (the signal), but divided it by the coefficient of variation of these values (the noise), obtaining an estimation of the strength of the effect of the stress on the variable in relation to its variability (the sensitivity). This is the approach we followed in the work presented here, because it has the advantage of not relying only on the strength of the signal, that may be low in many cases but: if the signal is high enough, the noise caused by the tree-to-tree variability may not be as critical. However, all these relative methods need the presence of a control treatment that makes them less applicable in practice (De Swaef et al., 2009).

However, this problem has also been addressed in a different way by some authors: if a threshold can be establish for absolute values of MDS, independently of a control treatment, MDS values could be directly used for irrigation management. The potential problems have already been presented, but in practice, and in the short term, where irrigation decisions are taken, the day to day variations in MDS are not a problem: De Swaef et al. (2009) abandons traditional MDS calculation for a modified version because MDS decreases when the stress was still in progress, but MDS values were still higher than the threshold value of about 0.3 mm that could have been derived from the control trees; even if the worst cases, the highest coefficient of variation in the same order of the change in MDS sought to apply irrigation (about 30% and 25%, respectively, in Vélez et al. (2007). However, best results are obtained when the irrigation protocols combine MDS with another variable, like soil water potential, to adjust irrigation frequency and dose: if soil moisture is high, MDS is ignored, any high MDS value being attributable to atmospheric conditions or, anyway, not being sensitive to soil water content, and irrigation is stopped; when soil water potential is below the threshold value, irrigation is scheduled according to MDS threshold values (Bonany et al., 2000). This allowed a 38% water savings in apple trees using -15 kPa and 0.20 mm as soil water potential and MDS threshold values, respectively without affecting yield or fruit quality (J. Bonany, unpublished data). Similarly, Biel et al. (2008) achieved an additional 7% water saving in plane trees (*Platanus* x *hispanica*) without affecting tree growth using -20 kPa and 0.20 mm as thresholds compared with a treatment in which irrigation was scheduled to maintain soil water potential at -20 kPa.

As a prerequisite to implement this approach to irrigation management in cherry trees, MDS should be proven as an adequate indicator of cherry tree water status first, with a high sensitivity to water stress in this species. To achieve that, more traditional water stress indicators such as leaf water potential (Fernandez et al., 1997; Ruiz-Sanchez et al., 1997; Tognetti et al., 2004; Fernandes-Silva et al., 2010) and leaf conductance (Flexas et al., 2002; Ruiz-Sánchez et al., 1997; Tognetti et al., 1998 and 2004) should be measured and related to MDS and their relative sensitivity to water stress should be compared. Moreover, the use of another continuous recording, plant-based variable such as sap flow (Q) in addition to TDV adds more detailed information on the daily evolution of the tree water status.

The general aim of our study was to evaluate the usefulness of MDS to establish irrigation scheduling protocols for young cherry trees. This would include the evaluation of a consistent, quantitative behavior of MDS in front of water stress, and its establishment and test in a specific protocol. This work deals only with the first part of that general purpose, for which two specific objective s were addressed: the assessment of MDS value as a reliable indicator of cherry plants water status through its relationship with different environmental and physiological variables, and the derivation of a threshold value of MDS for water stress from these relationships.

2. Materials and methods

The experiment was developed in 2009 in a greenhouse at IRTA (Institut de Recerca i Tecnologia Agroalimentàries) facilities in Torre Marimon, Caldes de Montbui, Barcelona, (Spain; 41.6ºN, 2.1ºW; altitude 176 m). Sixty two years-old cherry trees (*Prunus avium*) were planted in 25 L plastic pots using a substrate of peat and perlite (2:1, v: v). Trunk diameter and height at the beginning of the essay were 2.2 cm and 1.7 m. Water was supplied by drip irrigation using one emitter of 4 L h^{-1} per tree. In 13th of July 2009 (day of the year, DOY, 194) water withdrawal and recovery cycles started, which lasted till DOY 218 (8th of August).

2.1. Design of stress treatments

Trees were submitted to three watering regimes: Control, well watered trees (CON); stress 1 (S1), submitted to one cycle of water withholding till a substrate water potential of -50 kPa. (Hanson et al., 2000), followed by recovery irrigation to full capacity; and stress 2 (S2), submitted to three consecutive cycles of water withholding and recovery irrigation. The resulting application schedule is shown in Fig.1A. S1 short cycle treatment was applied to identify the effect of water stress on the physiological variables and the response of the trees to a single event of irrigation recovery. S2 treatment was design to observe the effect of repeated event of drought and recovery irrigation cycles, as in a water scarcity irrigation scheduling scenario.

CON trees were irrigated at optimum dose according to the water balance of the previous week and was applied a Kc of 0.6, resulting in approximately 3 L day^{-1} $tree^{-1}$ and throughout the experiment.

In S1 treatment, irrigation was withheld for one cycle up to a substrate matric potential of -50 kPa (from DOY 194 until DOY 197), and recovery irrigation (three days of irrigation with the same dose applied to CON tress, which sufficed to reach container capacity) was carried in just one rewatering cycle. For the rest of the experiment, irrigation was as for CON trees.

Finally, in S2 treatment, irrigation was withheld in three consecutive cycles, up to a substrate matric potential of -50 kPa each. The first cycle spread from DOY 194 to DOY 197, the second cycle from DOY 201 to DOY 204, and the third cycle from DOY 208 to DOY 211. After every water withholding cycle, recovery irrigation was carried out as in S1 treatment.

After the end of the third cycle in S2 trees, a 4 L day^{-1} tree^{-1} dose (which was above water requirements) was applied to all trees to ensure a complete recovery.

2.2. Variables measured

Meteorological variables inside the greenhouse were taken as follows: air temperature and relative humidity above and below the canopy (SS-1, Decagon Devices, USA) and solar radiation above the canopy (PYR pyranometer, Decagon Devices, USA) were recorded every 15 minutes with and Em50 datalogger (Decagon Devices, USA). Daily potential evapotranspiration (ET_0) inside the greenhouse was calculated using the FAO Penman-Monteith equation (Allen et al., 1998) from those data. Substrate matric potential (Ψ_s) was measured in four trees per treatment with ISR electrotensiometers (Irrometer, USA) installed at 20 cm depth, and 15 cm away from the trunk in the substrate of each tree. Data were recorded every 20 min with a CR10X data logger (Campbell, USA).

At noon, leaf water potential (Ψ_{md}) was measured in one mature sun exposed leaf per tree and six trees per treatments using a pressure chamber (Soil Moisture Equipment Corporation, model 3005, USA). Leaves were excised and kept in a plastic container with wet paper and measured in the laboratory. No other water potential measurements, like predawn or stem water potential were considered because of the rapid water stress expected was rapid (see Discussion section).

Leaf conductance (g_s) was measured with a porometer (Decagon SC-1, USA) at midday in one mature sun exposed leaf per tree and six trees per treatment.

TDV was measured on four trees per treatments, using a set of linear variable displacement transducer (LVDT, DF 2.5, Solartron, UK): a single sensor was attached to the trunk of each tree, with a specially manufactured holder made of aluminum and Invar as is usual for these set ups (Li et al., 1989). The LVDTs were located on the north side of the trunk; measurements were recorded every 20 min with data logger (CR10X, Campbell, USA). Indexes derived from the daily TDV cycle were: daily maximum trunk diameter (MXTD); daily minimum trunk diameter (MNTD); daily maximum trunk shrinkage (MDS), calculated as the difference between MXTD and MNTD; and daily trunk diameter growth (TDG), calculated as the difference between two consecutive MXTD.

Sap flow (Q) was measured in four trees per treatment with a stem heat balance T4.2 sap flow meter from Environmental Measuring System (Brno, Czech Republic), in the same trees used for TDV measurements. This system measures total sap flow in a branch or trunk of small dimensions (6 to 20 mm diameter) by keeping a constant temperature difference of 4 °C between two probes place upstream and downstream from a heater. From the energy expended to keep that temperature difference and specific heat of water, water needed to dissipate the energy added to the system can be calculated. One probe was located above the LVDT sensor on each tree. Data were recorded every 20 min with a dedicated UNILOG data logger (Environmental Measuring System, Brno, Czech Republic). At the end of the essay, leaves were removed and leaf area were measured (Winfolia, Regents, CA) to normalize sap flow per unit of leaf area (Q_l) and daily accumulated (Q_d).

2.3. Statistical analysis

The experiment had a completely randomized design with three treatments: each treatment consists of 20 trees in two adjacent rows. Measurements of TDV, sap flow, and substrate matric potential were carried out on four trees of every treatment and six trees were used for leaf water potential, and leaf conductance. Measurements were analyzed by one way ANOVA with one factor (irrigation) and three levels of this factor (CON, S1, S2) and means were separated by LSD range test with a probability of 0.05 (SAS version 9.2). Single and multiple regression analysis between MDS and all physiological and environmental variables were carried out with the Proc Reg procedure (SAS version 9.2). Slope and intercept comparison were made by covariance analysis (JMP 8 statistical package, SAS Inc).

Sensitivity analysis for the different cherry tree water status indicators (MDS, SF, g_s, and Ψ_{md}) was carried out using the method proposed by Goldhamer and Fereres (2001). The sensitivity was calculated as the ratio between the signal intensity (ratio between stressed treatments values and the control values: S1/CON and S2/CON), and the noise (coefficient of variation of each treatment). In the case of SF and g_s, were the values are higher in the controls, the signal was calculated as the ratio of control over stressed values (Badal et al., 2010).

3. Results

Environmental conditions during the experimental period are shown in Fig. 1. Temperatures in the greenhouse presented a typical behavior, with maximal daily average quite higher than outside (40ºC inside the greenhouse vs. 31.2ºC outside), while minimal daily average was similar inside and outside (18.3ºC inside the greenhouse and 17.7ºC outside), as expected form a plastic greenhouse under Mediterranean summer conditions. Daily relative humidity (RH) average reached 60% and solar radiation average experiment (R_s) was 16.34 MJ m^{-2} (Fig. 1B). Finally, daily average of vapor pressure deficit was 1.35 kPa, and daily ET_0 was from 5 to 6 mm day^{-1} during all days of the experiment (Fig. 1C).

Substrate matric potential (Ψ_s) in the control treatment CON was nearly stable between -20 to -22 kPa throughout the irrigation cycles (Fig. 2A). By the end of the first stress cycle, when water was withheld, Ψ_s dropped to -65 kPa and -50 kPa in S1 and S2 respectively (difference not statistically significant). Then, after the first recovery cycle S1 recovered to the original value of Ψ_s. The slow recovery is only apparent: data are the daily average of data recorded every 20 minutes, which results in about one third of data of the first recovery day obtained before the irrigation event (automatic irrigation), thus lowering the average. In the second and third stress cycles in S2, Ψ_s decreased to -50 kPa. Three days after resuming irrigation after the third stress cycle substrate matric potential in S2 trees recovered to the same value of the CON and S1 treatments in DOY 215.

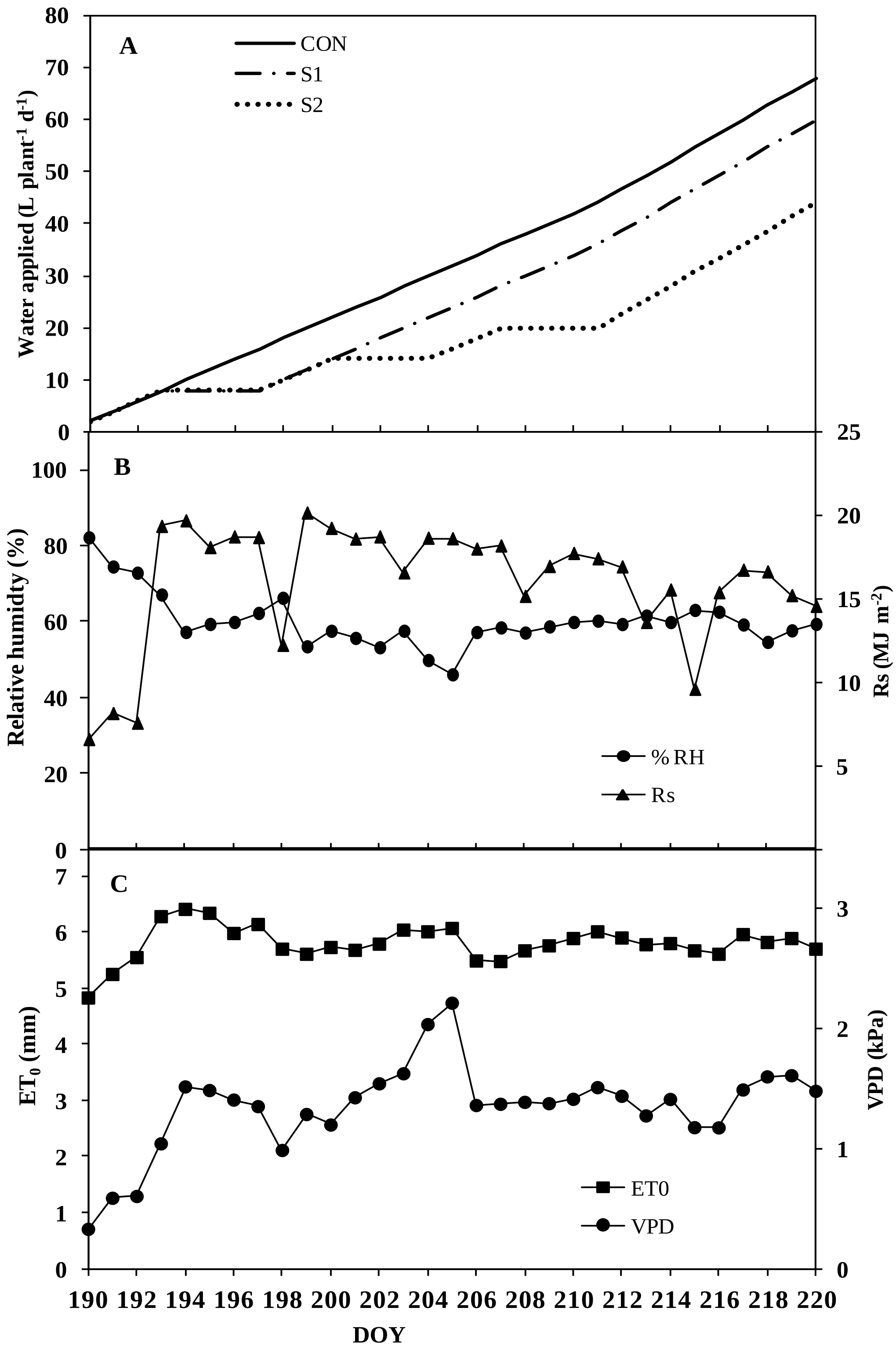

Fig.1. Daily evolution of (A) water applied per treatment and plant, (B) average of air relative humidity and solar radiation inside the greenhouse, and (C) average of air vapor pressure deficit (VPD) and ET_0.

Midday leaf water potential (Ψ_{md}) before irrigation withholding was similar in all three treatments (Fig. 2B). But when irrigation was withheld in the first stress cycle a significant difference between the stressed and control trees was noticed as $\Psi_{md.}$ in the control was nearly stable during all days of experiment but decreased significantly in stressed treatments. Four days after rewatering midday Ψ_{md} in S1 trees recovered, but it continued significantly lower in S2 after the second and third cycles. Four days after resuming irrigation in the third stress cycle, S2 trees recovered to the same value of the CON and S1 treatments (DOY 216).

Leaf conductance (g_s) (Fig. 2C) at midday solar time showed a similar behavior but appeared more dependent on particular environmental conditions of the measurement day. In stressed treatments S1 and S2, it was significantly lower than the control treatment when water was withheld in the first stress cycle. Three days after the first recovery cycle S1 and S2 recovered to the same value of the control. The second stress cycle coincided with high VPD values (Fig. 1C), resulting in a remarkable decrease of g_s in all treatments, leaving not much room to a greater decrease in S2 trees, which anyway recovered after rewatering. In the third stress cycle g_s decreased significantly in S2. Five days after re-irrigation, S2 recovered to the same value of S1 and control treatments and g_s values in all treatments were nearly similar at DOY 217.

Sap flow (Q) presented some variations with respect the behaviors described up to this point: Q daily values (Q_d, Fig. 3A) in stressed trees decreased significantly in the first stress cycle and recovered to the same value as in the CON treatment when they were rewatered. However, in S2, after the first decrease in the first stress cycle, Q_d stayed significantly lower than CON treatment during all days of the experiment. However, three days after the third recovery cycle (DOY 220) all treatments recovered to the values of the control.

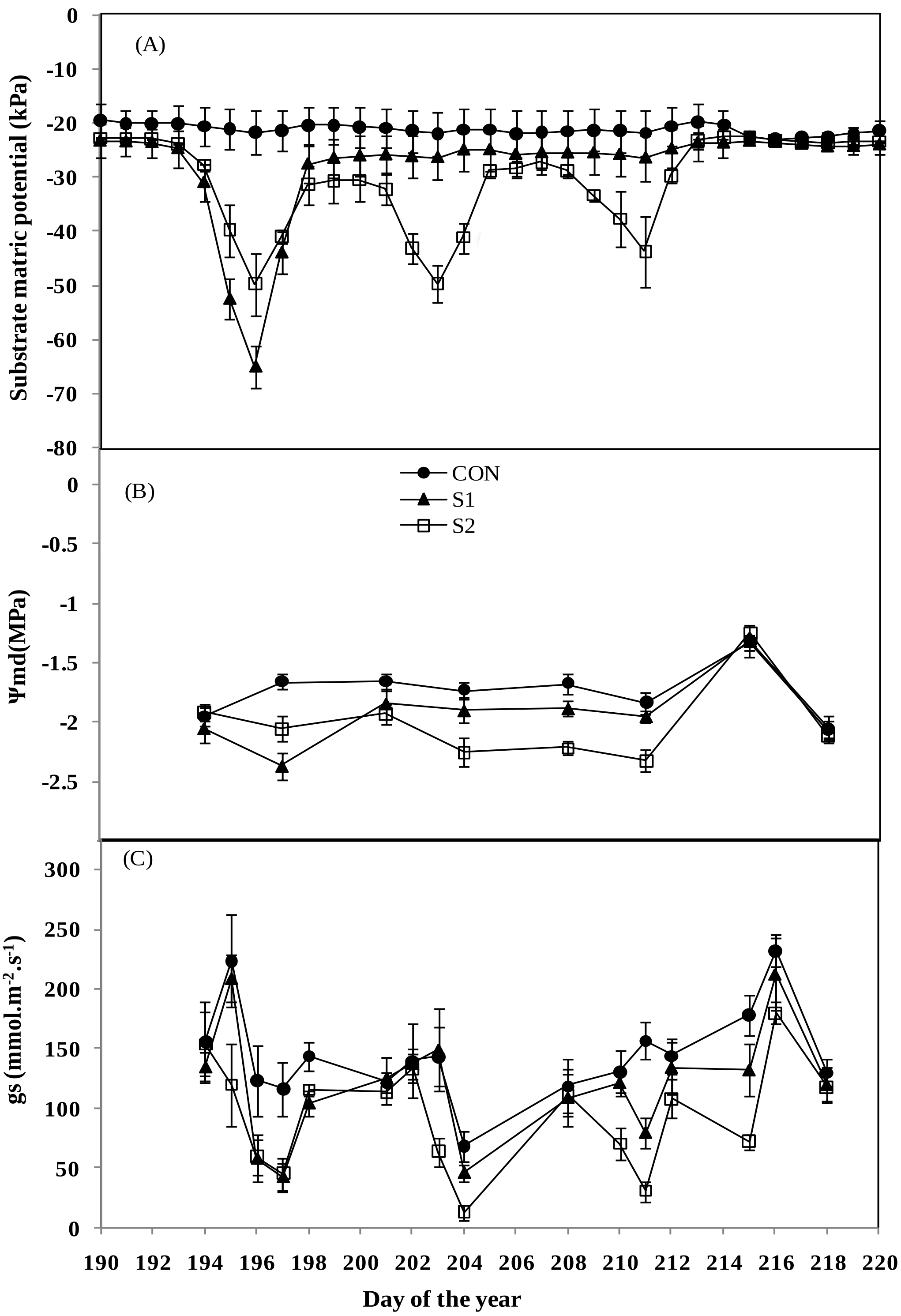

Fig.2. Patterns of (A) substrate matric potential (Ψ_s), (B) midday leaf water potential (Ψ_{md}), (C) and leaf stomatal conductance (g_s). Values of substrate matric potential Ψ_s are the daily average of 4 sensors per treatment and Ψ_{md} and g_s values are the average of 6 leaves per treatment, the vertical bars represent the standard error.

MDS behaved somewhat similarly to Q_d, although the direction of the changes was opposite, as expected (Fig. 3B): although MDS values of stressed trees were not significantly different from CON trees during the recovery periods, S2 was generally above CON (while Q_d was below) from the beginning of the first stress cycle. Finally, after six days of reirrigation in the third recovery cycle, S2 recovered to the same values of CON, and S1 treatments (DOY 220).

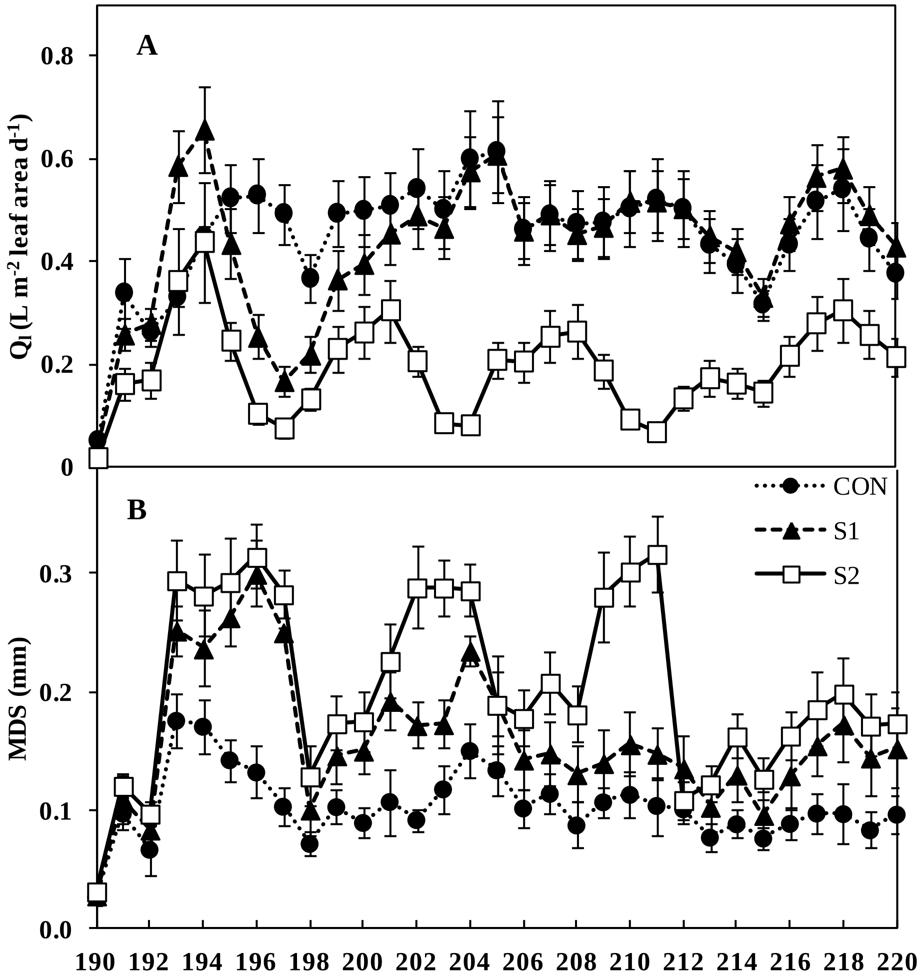

Fig.3. Time course evolution of (A) daily sap flow (Q_d) and (B) maximum daily shrinkage (MDS). Values are the daily average of 4 measurements in both variables and the vertical bars represent the standard error.

Regression analysis was carried out to study the relationship between MDS and environmental and physiological variables. There were negative relationships between

MDS and Ψ_{md} and g_s and presented similar intercepts and slopes in all treatments according to COVARIANCE analysis (Fig. 4). Midday leaf water potential showed the highest correlation with MDS (Fig. 4A). A strong relationship was also found between MDS and g_s (Fig. 4B).

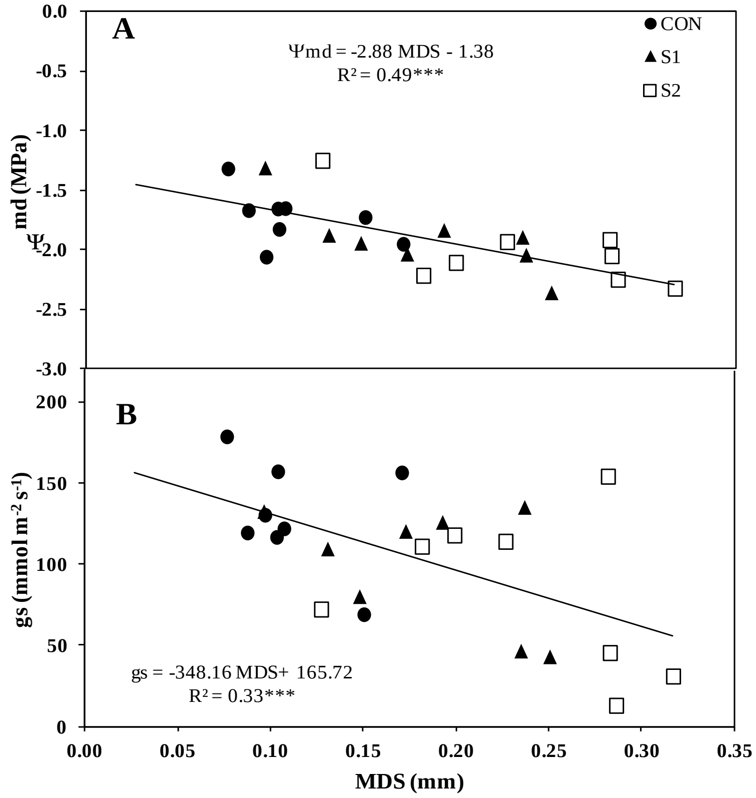

Fig.4. Relationship between maximum daily shrinkage (MDS) with: (A) midday leaf water potential (Ψ_{md}) and (B) leaf stomatal conductance (g_s). Values of Ψ_{md} and g_s are the average of n=6 and MDS and SF are the average of n=4 for every day of measure. And (***) Significant at $P < 0.0001$.

In addition, MDS and substrate matric potential Ψ_s presented a negative relationship with high correlation (Fig. 5A). This relationship was strongly linear with some scattering till Ψ_s reached about -33 kPa, but after that MDS values stayed stable around 0.30 mm with the continuation of stress till -60kPa. The relationship between MDS and the environmental variables ET_0, R_s and VPD were also high, but these presented different intercepts and slopes in all treatments (Fig. 5B, C, and D). Positive and strong relationships were found between MDS and ET_0, R_s, and VPD in S2 trees, which presented the strongest correlations, except for ET_0 which was higher in S1 trees, while CON trees presented the weakest correlations.

Stepwise multiple regressions of MDS against Ψ_{md}, g_s, SF, Ψ_s, ET_0, and VPD, indicated that Ψ_s and ET_0 explained 73% of MDS variability, as Ψ_s explained 40% and ET_0 explained 33% table (1).

Therefore MDS could then be estimated as in the next equation:

$$MDS= -0.006\ \Psi_s + 0.086\ ET_0 - 0.494 \qquad (1)$$

Table.1. Results of the stepwise multiple regression analysis performed with MDS as dependent variable and environmental and physiological parameters as independent variables. The variables left in the model are significant at 0.1500 levels.

Summary of Stepwise						
Variable Entered	Coefficient error	Standard Error	Partial R-Square	Model R-Square	F Value	Pr > F
Ψs	-0.00638	0.0013	0.4751	0.4751	19.91	0.0002
ET0	0.19541	0.0448	0.2514	0.7265	19.30	0.0003

The sensitivity analysis of the plant water stress variables to deficit irrigation (Figure 6) indicated that the signal/noise ratio of MDS was the first to increase in treatments S1 and S2, although Ψ_{md} presented the highest values in the last day of the second and third cycles. The highest signal, however, was presented by Q_d, but its higher variability (noise) resulted in a lower sensitivity to water stress. Similarly, stomatal conductance presented a signal comparable to MDS, but the resulting sensitivity was much lower.

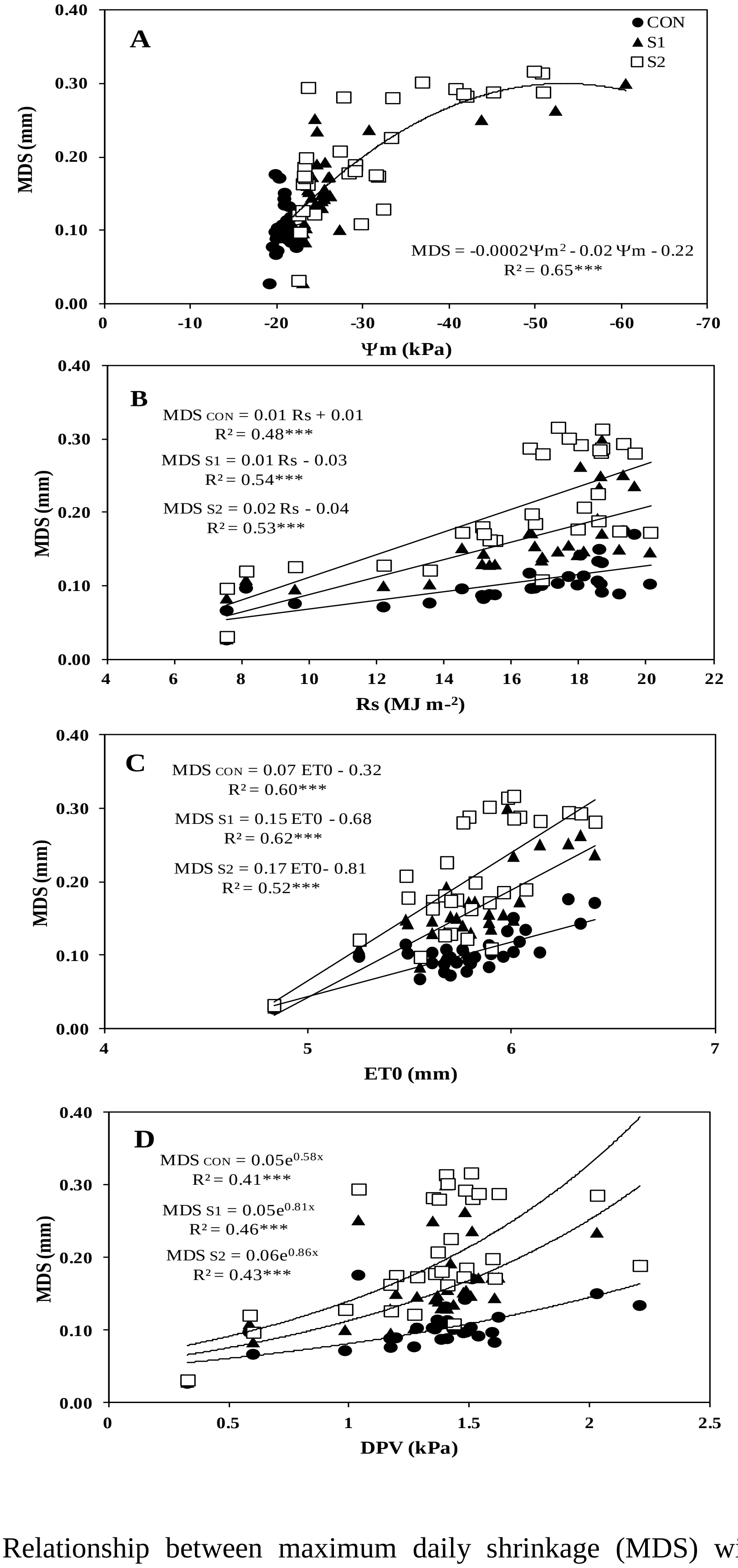

Fig.5. Relationship between maximum daily shrinkage (MDS) with: (A) substrate matric potential (Ψ_s); (B) solar radiation (R_s); (C) daily reference evapotranspiration (ET_0); (D) average air vapor pressure deficit (VPD). Values are the average of n=4 for MDS. And (***) Significant at P < 0.0001.

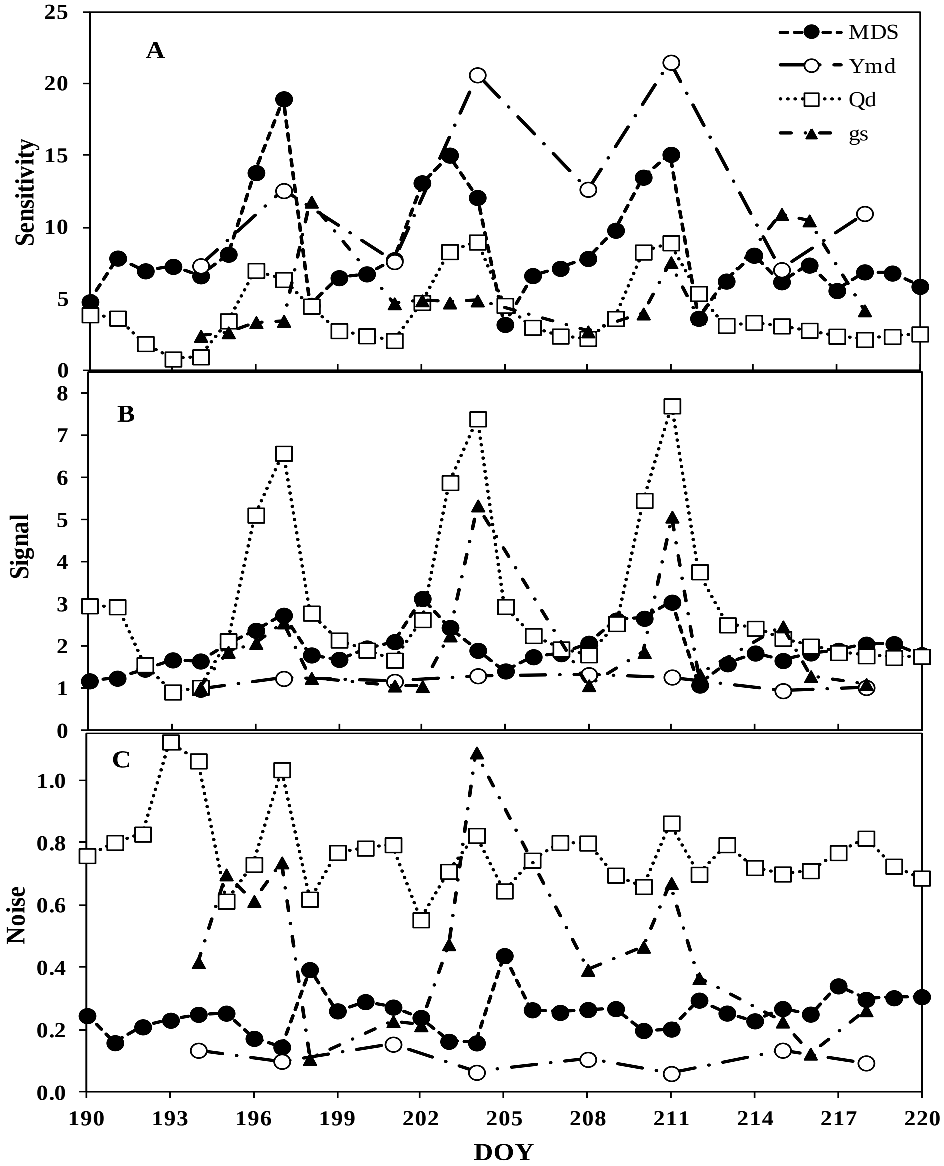

Fig. 6. Time course of: (A) sensitivity (Signal to Noise ratio), (B) signal and (C) noise for the different variables in S2 treatment: Maximum daily trunk shrinkage (MDS), midday leaf water potential (Ψ_{md}), daily sap flow (Q_d), leaf stomatal conductance (g_s)

4. Discussion

The values of Ψ_s for CON, S1 and S2 were consistent with the hard summer environmental conditions, high air temperature and low humidity of our Mediterranean greenhouse essay (Fig. 1B and C), and with the treatments imposed. They agree with lack and presence of water stress, respectively, for CON trees and S1, S2 trees (Hanson et al., 2000; Gonzalez et al., 2009; Thompson et al., 2007). In fact, all measured water status variables presented low values that correspond with a water stress situation along the experiment in both S1 and S2. No water potential measurements other than Ψ_{md} (predawn or stem water potential) were considered because expected water stress was rapid, as indeed occurred, so we sought variables that would respond rapidly. We chose Ψ_{md} as we expected it would pick up the maximal effect during the day, which would be related to MDS, as maximum shrinkage usually happens at o nearly after midday. In other words, in a rapid water stress, temporal effects of water flow through the plant that would be (partially) recovered during the night should be taken into account as it is not clear, even in the field, if the signal for drought adaptation is a cumulative measure of water status or maximum daily deficit (Jones, 2007). Our assumption was supported by the correlation between MDS and leaf water potential at midday being the strongest among the physiological variables.

Ψ_{md} and g_s decreased once in S1 and three times in S2 during their respective drought stress cycles (Fig. 2B), with no apparent cumulative effect in S2, as the three cycles were similar and began with similar values after a complete recovery at the end of each recovery cycle, with the slight differences being attributable to the particular environmental conditions of each cycle: for instance, the second cycle presented a value of g_s in DOY 204 much lower than the equivalent days of cycle one and three, but this day presented also the highest VPD and ET_0 values of the experiment, and anyway all drought cycles of S2 and S1 reach g_s values that indicate a severe water stress (Flexas and Medrano, 2002). Moreover, if a cumulative effect had been present, we would have expected an even lower value in cycle three, which was not observed. However, in DOY 215, three days after the last drought cycle in S2 and recovery irrigation above substrate water depletion, S2 plants showed reduced gs values that were not consistent with the recovered values presented by Ψ_s and Ψ_{md}. This is a known behavior even in potted plants, where no delay generally exists in soil moisture recovery after irrigation, and has received a number of explanations with the common factor of a control of stomatal conductance by the root (Pérez-López et al., 2008). This control has been attributed to abscisic acid produced in the root and suddenly reaching the leaves when xylem flow is completely reestablished (Hsiao, 1973, Lovisolo et al. 2008), which has been explained as a safety mechanism that allows the plant to regain full turgor more efficiently (Ruiz-Sanchez et al., 1997). Another set of explanations, not necessarily contradictory between them nor with the hormonal hypothesis, would be the slow recovery of xylem cavitation in the roots (Maherali et al., 2006), or the trunk and petioles (Clearwater and Goldstein, 2005; Cochard et al., 2008; Lo Gullo et al., 2003; Tognetti et al., 1998) that would restrict complete reestablishment of xylematic flow.

Water stress reduced Q_d in S1 and S2 with respect to CON tress (Fig. 3A) which corresponds to the physical constraint of plant transpiration. It was clearly noticed that Q_d in S2 did not recover to the same value of the CON trees and slight differences were found between Q_d values in drought cycles and recovery cycles. Moreover, Q_d recovery after the last drought cycle took longer than for Ψ_{md} or even g_s. This is in agreement with the occurrence of cavitation in xylem vessels explained above that would hinder sap flow, combined with the hard summer environmental conditions of our

Mediterranean greenhouse experiment that makes difficult a complete recovery during a single, short, summer night.

The narrow relationship for cherry trees between MDS and plant water status is indicated by the sharp increase of MDS values after withdrawing irrigation in S1 and S2 treatments in all drought irrigation cycles. The strong relationship found between MDS and Ψ_s (figure 5A) reflected the sensitivity of MDS to decreased substrate water content, resulting in increased MDS values after irrigation withdrawal in stressed treatments. The value of 0.30 mm at which MDS stabilized in the second phase of this relationship can thus be considered as a threshold for irrigation scheduling. Many previous studies suggested different MDS thresholds in different species: 0.53 mm for olive trees (Moriana et al., 2000), 0.24 mm for lemon trees (Ortuño et al., 2006), or 0.16 mm for grapevines (Intrigliolo and Castel, 2007b). In our case, the suggested threshold corresponded to Ψ_{md} of -2.3MPa indicative of water stress (McCutchan and Shackel, 1992; Shackel et al., 1997; Naor and Cohen, 2003; Intrigliolo and Castel, 2004, 2005; Ortuño et al., 2006; Doltra et al., 2007) and g_s 50 mmol m^{-2} s^{-1}, indicative of the onset of severe stress (Flexas et al., 2002; Flexas and Medrano, 2002).

The strong regression between MDS and Ψ_s, is reinforced by the stepwise regression analysis, which results in 73% of the MDS variability being explained by Ψ_s and ET_0. This would suggest a strong dependency of MDS on the environment. However, it is not surprising that the multiple regression procedure picks the two main environmental variables driving water movement through the plant, ET_0 and Ψ_s, as all other variables depend on them. In contrast with these regressions, the relationship of MDS with R_s, ET_0 and VPD resulted in different slopes in the three treatments. The correlations being similar between the three treatments in every particular regression of MDS with a specific variable, the analysis of the slopes suggests a stronger influence of the stress with respect to the environmental conditions, as changes in the slopes are much higher that the slopes themselves: in most cases, the slope at least doubles between controls and the stress treatments, while the slopes are one to several orders of magnitude below that.

Contrary to MDS, daily trunk diameter growth (difference between two consecutive maxima) presented an undesirable behavior under our approach: the sensitivity was much higher in the absence of water stress, as the noise increased much more than the signal as stress progressed (data not shown). Fernandez et al. (2011) were able to obtain good results in olive trees based on the evolution of maximum trunk diameter, but they used the difference between stressed and control trees to calculate the difference, and the course of their experiment was much slower than ours, as corresponds to field-grown olive trees.

Probably more relevant that the good relationship between MDS and edaphoclimatic conditions is the strong relationship between MDS and classical physiological indicators of plant water status, like g_s and, especially, Ψ_{md} (Fig. 4A). The strongest of these correlations was found for Ψ_{md}, confirming the view of Molz and Klepper (1973) that the main factor controlling MDS is the leaf water potential, which determines the driving force for water transport from the phloem and related tissues (cambium and green tissues of the bark, made up predominantly of parenchyma cells), as well as from the living tissues of the outer xylem, to the xylem, which results in trunk radial shrinkage (Brough et al., 1986; Čermák and Nadezhdina, 1998; Zweifel et al., 2000),

In the frame of the sensitivity approach proposed by Goldhamer and Fereres (2001) and Naor and Cohen (2003) to compare the sensitivity of different plant-based indicators to detect water stress, we can indicate that MDS and Ψ_{md} were the most sensitive indicators (Fig. 6). The literature is not clear about this relationship, as Goldhamer and Fereres (2001) found MDS to be more sensitive than plant water potential in mature almond trees, although they used stem Ψ; Badal et al. (2010) showed that MDS was a very sensitive indicator of plant water status in kaki trees, and MDS was found to be the most in peach and lemon trees (Conejero et al., 2007; Ortuño et al., 2005; Ortuño et al., 2006). However, Shackel et al. (1997) found the contrary for almond and prune trees, along with Goldhamer et al. (1999) for peach, and Intrigliolo and Castel (2004) for plum tree. Anyway, both variables appear in the first ranks in all these works, and finally the election of one or the other in an irrigation protocol will have to choose between the simple but labor-intense measure of water potential (either midday, pre-dawn or stem) and the automation prone but technically demanding measure of TDV. What our work can add to the contradictory literature is that, in potted young cherry trees, MDS has resulted a good candidate indicator of plant water status and, if a threshold (like the one obtained in this work) can be established, it could be tested in irrigation scheduling protocols, and has already assayed in almond trees (Goldhamer and Fereres, 2004), peach trees (Conejero et al., 2011), and lemon trees (García-Orellana et al., 2007; Ortuño et al., 2009).

5. Conclusions

MDS appears good candidate indicator for young cherry trees irrigation scheduling with a suggested threshold of 0.30 mm as it performed well as an indicator of water status, responded sharply to irrigation withdrawal and the subsequent decrease of substrate matric potential and was the most sensitive indicator of water stress.

Acknowledgements

This research was supported by the Spanish Ministerio de Ciencia e Innovación (MICINN, AGL2007-66279-C03-01/AGR) and a Grant to Ashraf Abdelfatah the author (BES-2008-009667). Also we thank J. Cazaña, B. Grau, M. Ferrer, C. Morales y E. Serra for their help in our field work. We acknowledge the constructive and helpful contribution of the anonymous referees.

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CHAPTER 1B

Relationship between Sap Flow, Maximum Daily Shrinkage and Other Ecophysiological Parameters in *Prunus avium* Subjected to Water Stress

This chapter is a reduced version of the article published in:
C. Biel, A. Abdelfatah, F. de Herralde, R. Savé and X. Aranda
Proceeding of 8th Syposium of Sap flow.

Acta Hort. 951, ISHS 2012

Abstract

Daily sap flow and maximum daily shrinkage (MDS) are two parameters that can be used for irrigation decision-making. In this subchapter we try to understand the different behavior of the two parameters at different timescales. The data come from the anterior assay (Chapter 1A) An assay was raised under controlled conditions where drought was imposed by irrigation withdrawal in cherry plants to compare their response of MDS with that of other ecophysiological parameters commonly used in water relations studies. Results showed contrasting relationships between MDS and daily sap flow depending on the degree of water stress that could be due to stomatal regulation. At a daily timescale, trunk diameter variation and sap flow were related to each other via a loop that differs according to tree water status. MDS resulted more sensitive to drought than sap flow, but the simultaneous use of both indicators rendered complementary information sensitive to the water status of the plants.

Keywords: cherry trees, stomatal conductance, leaf water potential, drought stress indicators, irrigation

1. Introduction

Several studies in fruit trees have shown a relationship of either maximum daily shrinkage (MDS) or sap flow with other water status indicators like leaf or stem water potential, or stomatal conductance (Ortuño et al., 2005, 2006; Conejero et al., 2007; Remorini and Massai, 2003; Intrigliolo and Castel, 2004 among others). Only few studies have tried to extract more information from the combination of these two sensors (Cermark et al., 2007; Fernandez and Cuevas, 2010; Fernández et al., 2011; Sevanto et al., 2008; Steppe et al., 2006, 2008). Under well watered conditions, during the day, the slope of the relationship between sap flow and trunk diameter variation is negative and differs between species (Sevanto et al., 2008). Under drought stress conditions, this relationship could be different depending on the evolution and severity of water stress. Moreover this behavior could be affected by the stomatal response and the vulnerability to embolism of species.

The aim of this chapter was to extract more information about the hourly pattern of two continuous indicators of water status MDS and sap flow and if this pattern was affected by drought. And to study the relationship between these two continuous indicators in order to use in irrigation scheduling

1. Material and methods

Forty, three years old, cherry trees (Prunus avium CYL-01) were installed in a greenhouse at IRTA facilities (Caldes de Montbui, Spain) in 25 L pots with peat:perlite (2:1 v:v) as a substrate.

Irrigation treatments

Irrigation was supplied daily with emitters of 4 L h-1 providing a dose proportional to evaporative demand (60% ET0). From 13/07/2009 (day of the year = DOY 194) plants were distributed in two treatments: control (daily irrigated) and one stress group where water was withdrawn 3 times (3 cycles stress) until substrate water potential reached -50 kPa. They were then irrigated again for three days until the matric potential reached the values of daily-irrigated plants (recovery point). The days of the year of maximum stress (end of drought cycle) were the 197, 204 and 211. The days of recovery were 201, 208 and 215 and full recovery 220.

Measurements

The irrigation treatment and the values of trunk diameter variation, sap flow, substrate matric potential, stomatal leaf conductance, and Midday leaf water potential used in this chapter are explained in the chapter 1A.

Statistical analysis

Regressions between sap flow and the other parameters were calculated with Proc Reg (SAS, version 9.2, USA). Three models were sequentially compared using an analysis of the variances explained by each model: a common slope and intercept, a common slope and different intercepts, and different slopes and intercepts. The models were developed using all data and separating between daily irrigated, drought and recovery after drought. Hourly relationships between sap flow and trunk daily variations were calculated for each tree and treatment.

1. Results and discussion

The relationship of sap flow with other plant parameters was also analyzed (Fig. 1 and 2). In all cases, the model best adjusting the relationships considered a common slope for all treatment conditions, with different intercepts (i.e. common slope between parameters but with different ranges for each treatment condition), with leaf water potential presenting the highest correlation followed by stomatal conductance (R^2=0.67, p<0.001 and R^2=0.65 p<0.001 respectively) for complete models including correlation and different intercepts.

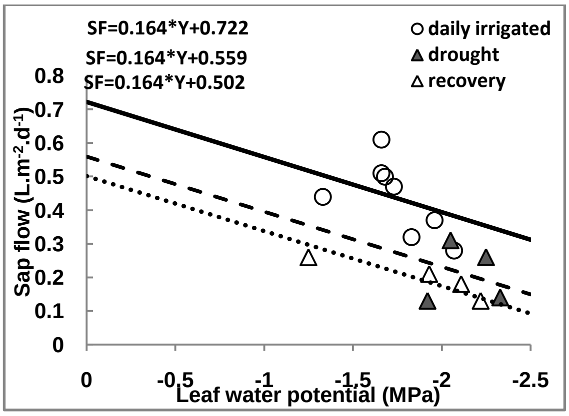

Fig. 1. Relationship between leaf water potential and daily sap flow. Each value is the average of 4 values for sap flow and 6 values for leaf water potential. For the complete model R^2 =0.67, p<0.001.

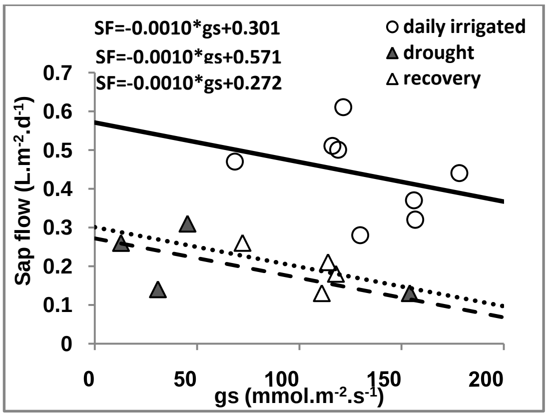

Fig. 2. Relationship between stomatal conductance and daily sap flow. Each value is the average of 4 values for sap flow and 6 values for stomatal conductance. For the complete model R^2 =0.65, p<0.001.

The relationship between daily sap flow and MDS is complex (Fig. 3) due to the stomatal regulation mentioned above: in general, with a well watered soil, both sap flow and MDS will reflect de evaporative demand and will be positively correlated (Sevanto et al., 2008); however, as soil water content decreases and a lower water potential develops in the leaves and the stem, stomata will gradually close, decreasing sap flow while MDS is still high according to the low water potential, which results in a negative correlation between sap flow and MDS. The different positive slopes shown by daily irrigated and recovery points in Fig. 3 indicate that stomatal control lasts for some time during the latter.

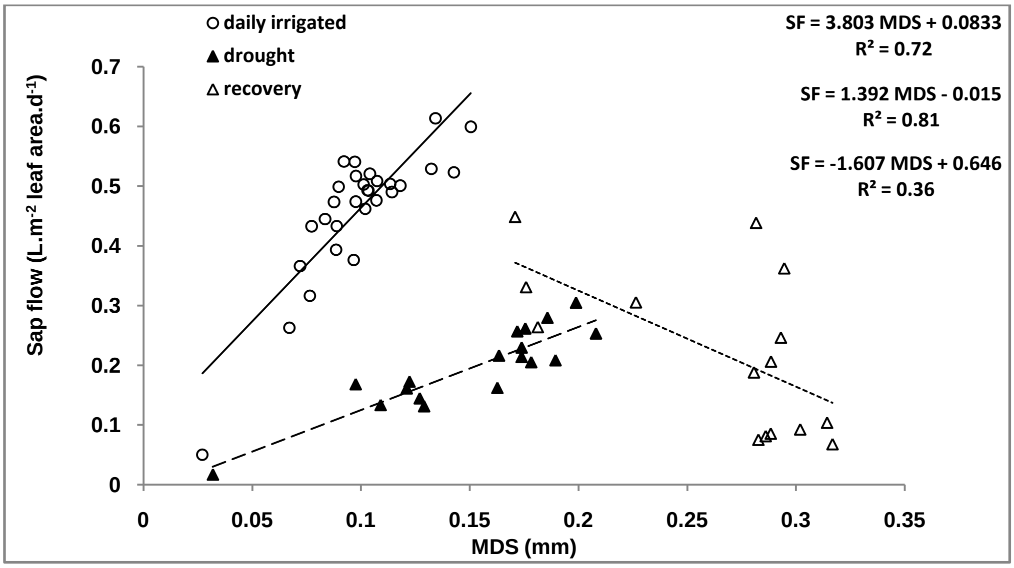

Fig. 3. Relationship between MSD and daily sap flow. Each symbol is the mean of four trees per treatment.).

When examined at the diurnal level, an uncoupling between MDS and sap flow appears, reflecting the axial and radial movement of water, from soil to the atmosphere through the xylem and between xylem and surrounding living tissues, respectively (Fernández and Cuevas 2010; Herzog et al., 1995; Sevanto et al., 2008; Steppe et al. 2006). As the axial movement is affected by stomatal control, different patterns can be observed between daily irrigated, stressed and recovering plants. Before sunrise, trunk diameter swelling can be observed with no sap flow in daily irrigated plants (Fig. 4), reflecting water redistribution inside the plant. From sunrise to afternoon, sap flow increases as stomata open and DPV increases, followed by trunk diameter shrinkage, which reflects the movement of water from storage tissues (cambium, phloem, living tissues of the bark) to the xylem following a water potential gradient; and during the afternoon, trunk diameter swelling (refilling of storage tissues) is accompanied by a decrease of sap flow till next sunrise (Fig. 4).

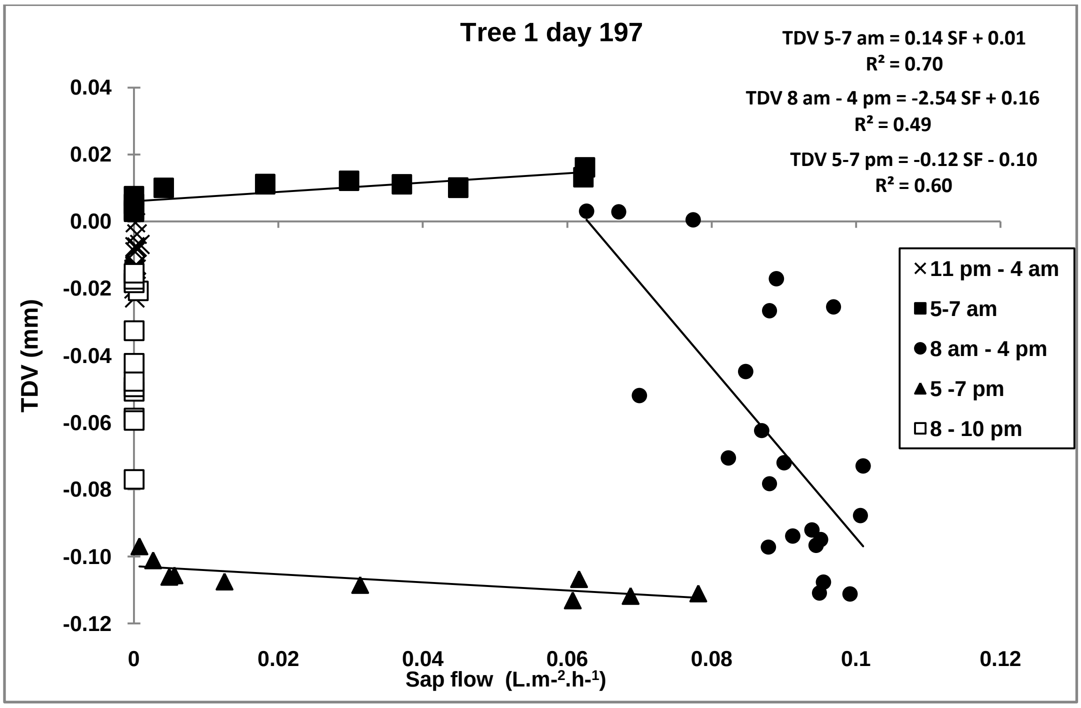

Fig. 4. Relation between diurnal courses of the trunk diameter variation (TDV) and sap flow in a daily irrigated tree (control plant).

Stressed plants behave differently (Fig. 5): the second day of water withdrawal shrinkage of trunk diameter and an increase of sap flow took place from sunrise until 8:00 a.m. when a maximum is achieved; then, sap flow decreases quite quickly arriving to 0 at 10:30 a.m. while the trunk diameter is still shrinking (Figure 5). Those different behaviors have been previously observed (Fernandez et al., 2011) and could be explained by stressed plants opening their stomata in the early morning, when air temperature and DPV conditions are not limiting; but as the air temperature and DPV increase together with lower substrate water potential, they cause stomatal closure and sap flow decreases dramatically. After recovery irrigation, the time course returned to that of daily irrigate plants, although with much lower values of sap flow: maximum was achieved at 9:30 a.m. and maintained until 7:00 p.m. (Fig. 6).

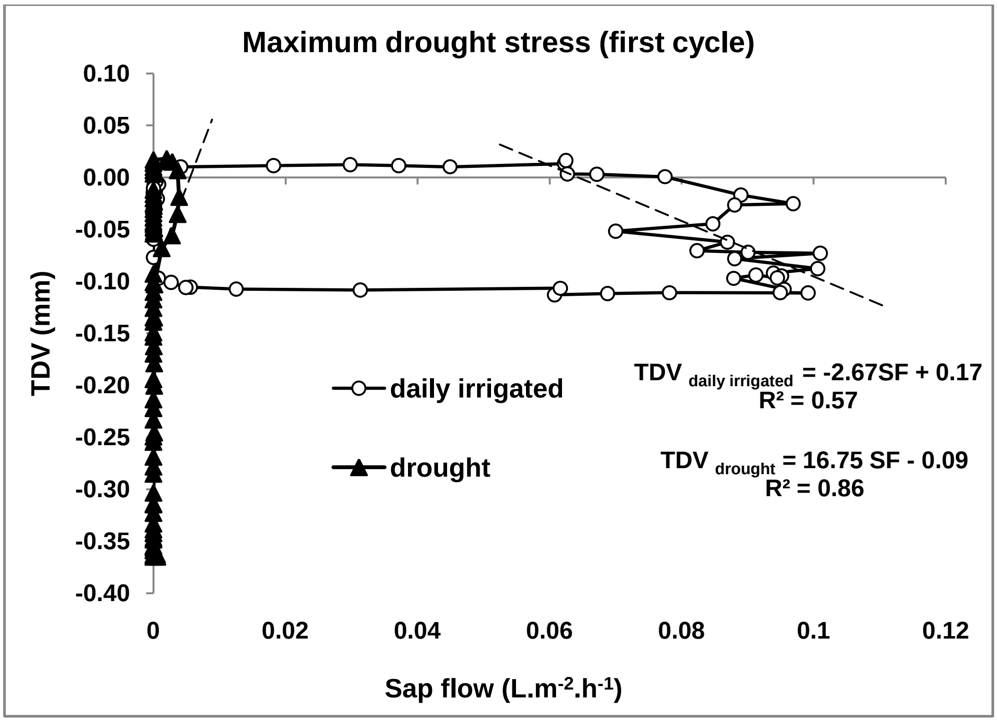

Fig. 5. Relation between diurnal courses of the trunk diameter variation (TDV) and sap flow in daily irrigated and water stress plants the day of maximum stress. Equations shown the relationship of TDV and sap flow between 8 am and 4 pm in both treatments.

Figures 4 to 6 make also evident another difference that could be useful to characterize the water status of the tree: the slope of the right part of the figure (morning to afternoon) is always negative in daily irrigated trees (Figs. 4 to 6), but slightly positive or vertical in water stressed trees (Fig. 5), moreover Table.1 indicated that the slope average in stressed trees were significantly higher than daily irrigated trees;

Table 1. Relation between diurnal courses of the trunk diameter variation (TDV) and sap flow in daily irrigated and water stress plants the day of maximum stress. The relationship between TDV and sap flow between 8 am and 4 pm in 4 trees in both treatments.

Treatments	Slope average	Stander Error
CON	-5.4802	0.933382
S2	20.139	1.464338

Figure 6 shows still a positive slope for the recovery days, which agrees with the persistence of stomatal control discussed in Fig. 3. In summary, the combination of both methodologies reveals contrasting patterns between drought stressed plants and non-stressed (either daily irrigated or recovered plants) that can be used to characterize plant water status.

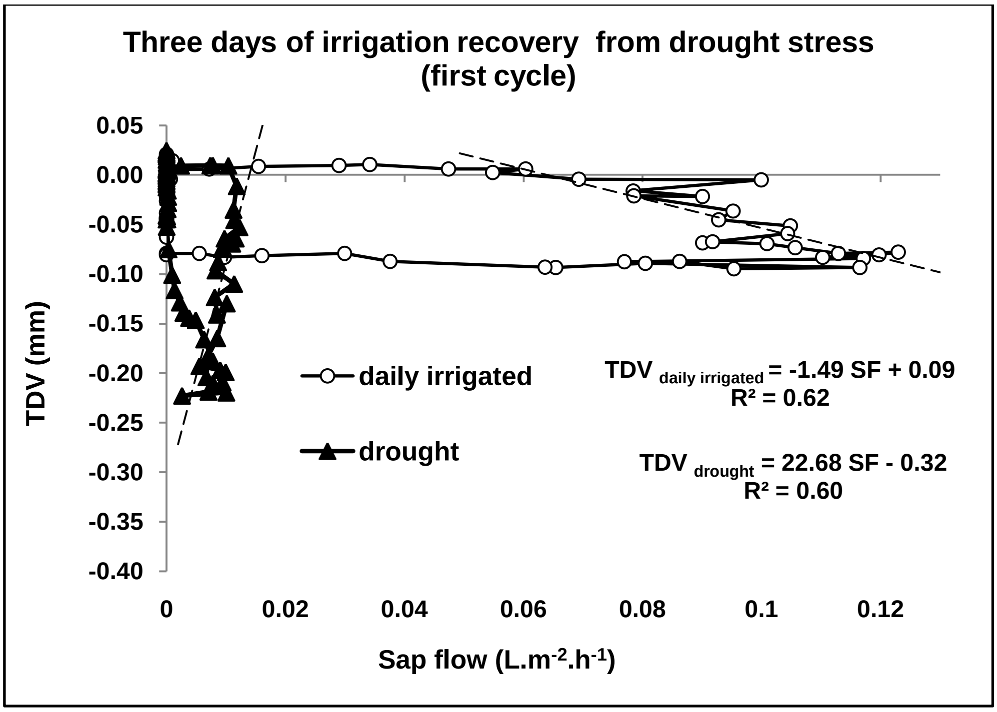

Fig.6. Relation between diurnal courses of the trunk diameter variation (TDV) and sap flow in daily irrigated and water stress plants after three days of irrigation recovery. Equations showed the relationship of TDV and sap flow between 8 am and 4 pm in both treatments.

2. Conclusions

Sap flow relationships with leaf water potential and stomatal conductance showed higher coefficient of determination (r^2) than in MDS. MDS is more sensitive than SF in front of drought stress, mainly due to the higher variability of SF from tree to tree, resulting in a higher noise. On the other hand, the strength of the signal is also higher for MDS than for SF due to stomatal regulation, which also results in significant but contrasting relationships between total daily sap flow and MDS depending on the degree of stress.

The simultaneous use of both techniques renders complementary information sensitive to the water status of the plant, due to the uncoupling of both parameters along the day. This behavior can be used as an early indicator of water stress but it is necessary to asses in field conditions where water withdraws effect in soil water content and plant water is slower than in a pot with organic substrate.

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CHAPTER 2

Assessment of irrigation scheduling combining MDS thresholds and soil water tension in cherry and plane tree

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Submitted to the journal of Environmental and Experimental Botany

Abdelfatah A, Aranda X, Savé R, Herralde F, Biel C. Assessment of irrigation scheduling combining MDS thresholds and soil water tension in cherry and plane tree. Environ Exp Bot. In Press 2013

CHAPTER 3

MDS signal combined with soil water potential are reliable indicators for irrigation scheduling modulation in *Prunus avium* and *Platanus x hispanica* trees.

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Submitted to Agricultural Water Management.


Abdelfatah A, Aranda X, Savé R, Herralde F, Biel C. MDS signal combined with soil water potential are reliable indicators for irrigation scheduling modulation in Prunus avium and Platanus x hispanica trees. Agr Water Manage. In Press 2013

GENERAL DISCUSSION

GENERAL DISCUSSION

The main objective of this work was to improve the irrigation scheduling of trees using plant and soil sensors, in order to increase water productivity and to reduce the water footprint of the tree growing process.

In the first chapter, the work is focused on investigating if MDS can be used as a reliable indicator for young cherry trees water status? For this reason, we look for the threshold values of MDS and soil matric potential that could indicate a moderate water stress. In the second and third chapter we consider different indicators and protocols in field conditions.

We chose MDS because the diurnal course of stem radius fluctuation represents the sum of all external and internal conditions affecting tree water relations as indicated by Zweifel and Hasler (2001) in Norway spruce and Cermak et al. (2007) in Douglas-fir trees Turcotte et al. (2011) in black spruce. In addition, they indicated that automatic measurements of radius variations in the stem can, therefore, provide an effective and sensitive proxy for the water status of trees. Moreover, Molz and Klepper (1973), Siau (1984), and Brough et al. (1986) showed that stem radius fluctuations are mainly determined by water content changes within the bark; also the fluctuations of xylem size in their study accounted for only about 10% of the entire stem radius changes.

In order to find out if MDS could be a water status indicator, a water stress essay was applied to cherry trees in a pot and greenhouses conditions. Sensitivity of MDS and others ecophysiological parameters indicators was studied.

All measured water status variables presented low values in water stressed plants in both S1 and S2. However, MDS was more sensitive to water restriction than all other plant based variables, this may be related with the decrease of substrate water potential which affected on bark and xylem water content. This in agreement with Zweifel et al.

(2000) as they indicated that the stem radius changes are coupled to the bark water content, and the bark water content is determined by water potential gradients within the xylem. Finally, water potential gradient is coupled to the course of transpiration as well as to the soil water potential.

Also, we compare MDS with Ψ_{md} as a water status indicator because we expected it would pick up the maximal effect during the day, which would be related to MDS as maximum shrinkage usually, happens at or nearly after midday (Jones 2007). In other words, in a rapid water stress, temporal effects of water flow through the plant that would be partially recovered during the night should be taken into account as it is not clear, even in the field if the signal for drought adaptation is a cumulative measure of water status or maximum daily deficit (Jones 2007).

To know which physiological variable controlling MDS, the relationships between MDS and physiological variables of plant water status, like g_s and, especially, Ψ_{md} were calculated in irrigated plants and water stress plants. The strongest correlations was found for Ψ_{md}, confirming the view of Molz and Klepper (1973) that the main factor controlling MDS is the leaf water potential, which determines the driving force for water transport from the xylem to phloem and related tissues.

Moreover, to compare the sensitivity of different plant-based indicators to detect water stress, we studied the sensitivity approach proposed by Goldhamer and Fereres (2001) and Naor and Cohen (2003). We can indicate that MDS and Ψ_{md} were the most sensitive indicators. However, Goldhamer and Fereres (2001) found MDS to be more sensitive than plant water potential in mature almond trees, although they used stem Ψ. Badal et al. (2010) showed that MDS was a sensitive indicator of plant water status in kaki trees.

The model indicated by the multiple regressions (MDS= -0.006 Ψ_s + 0.086 ET_0 - 0.494) reflected the correlation between MDS and Ψ_s and we considered this a good result as it would help us to use both to irrigation scheduling modulation.

The second part of the first chapter was a comparison between Sap flow sensitivity to water stress, as another indicator used to monitor irrigation scheduling in other species (Fernandez et al. 2008; Fernandez et al. 2011; Fernandez et al. 2001; Ferreira et al. 2012), and MDS. Our results showed that MDS was more sensitive than SF in front of drought stress, mainly due to the higher variability of SF from tree to tree, resulting in a higher noise. This may be related with the behavior of MDS and sap flow within the daily cycle as we noticed that the movement of water whether it was axial or radial, from soil to the atmosphere through the xylem and between xylem and living elastic tissues of the bark, respectively affected by stomatal control and VPD during the day (Fernandez and Cuevas 2010; Herzog et al. 1995; Sevanto et al. 2008; Steppe et al. 2006; Zweifel et al. 2000). Consequently, we observed trunk diameter swelling with no sap flow in daily-irrigated plants before sunrise, reflecting water redistribution inside the plant. After sunrise to afternoon, sap flow increased as a result of increasing DPV and stomata opening, followed by trunk diameter shrinkage, this was related with movement of water from living tissues of the bark to the xylem potential gradient.

In some woody species, MDS has some limitations for monitoring water stress and scheduling irrigation, related with atmospheric changes tree age and crop load (Cuevas et al. 2010; Moriana et al. 2003; Pérez-López et al. 2008) and largely varied day today (Vélez et al. 2007). Therefore, to avoid these problems in our work we used soil water potential (Ψ_s) beside MDS to schedule irrigation in cherry and plane tree trees as indicated by Hanson et al. (2000), Intrigliolo and Castel (2004) and Badal et al. (2010) and also we evaluated previous days values to help the decision-making process.

Therefore in the second chapter, we investigated using a protocol for improving irrigation scheduling in woody trees modulated by absolute values of MDS combined with soil water potential in cherry and plane tree. These values of MDS were based on MDS thresholds indicated in the first chapter. Also, managing irrigation with reclaimed water as an alternative of fresh water for irrigation was studied in cherry trees.

Two different protocols were applied in 2009 and 2010 with the objective of adjust water supplied in the former year. Within our results, it was clearly noticed 2009 had lower rainfall than 2010, and control trees received only 3% higher irrigation than 2010. In addition, higher evaporative demands in 2010 related with LAI, leaded to RDI treatments received more water in 2010 than in 2009, not only relative to 100% ET_c treatments, but also in absolute terms.

Also by following the modulation factor in every protocol, indicated in Table 1, we found that the numbers of days applied in each treatment in 2010 protocol were more than 2009. As we noticed that 2009 protocol was applied 41, 40, and 41 days in Cherry WW, Plane tree WW, and Cherry RW respectively, compared with 49, 51, 49 days in every treatment in 2010. The majority of irrigation decision in 2009 protocol was based on Ψ_s values in cherry trees WW and RW, while in 2010 protocol the majority of irrigation decisions were based on MDS values.In plane in both protocols the majority of irrigation decision based on both MDS and Ψ_s , because the majority of these days the valus of both of them waere between the threshold intervals of the decision table. Therefore we can indicate that the method applied in 2009 is better than 2010 protocol as it helped us to save water. If we had any doubt at the moment of take our irrigation decisions we had to return to the values of Ψs and MDS in the previous days to take correct decision.

Generally, tree growth TGR and leaves coverage were higher in 2010 than 2009, this is logic result because the trees are two years old in 2009, and they were still in an exponential growth phase.

Reclaimed water had no adverse impact on cherry trees growth indicators as it was similar in the two water qualities, despite high range of nitrogen levels in ground water. These results could be affected by some problems in the irrigation system distribution of the well water in August of both years. The presence of nitrogen in the underground water is not an exceptional situation in rural areas with a high agricultural pressure. Reboll et al. (2000) also found higher levels of nitrates in groundwater, that they attributed to aquifer contamination. In fact, RW has also a fertilizing condition, due to its levels of P, but this alteration was smaller than that for N in WW.This level of nitrogen was not determinal to growth of citrus trees (Zekri and Koo 1993; Reboll et al. 2000) and in olive trees (Segal et al. 2011).

RDImds treatment had no negative effect on the trees growth in both years of treatment. This in agreement with Intrigliolo et al. (2011) as they indicated RDI did not affect on pomegranate trees trunk growth. In addition, no clear effect was noticed in MDS, g_s or relative green cover in the two years of experiment. While consistently lower trunk growth and higher MDS in RDI treatment were observed in peach trees (Marsal et al. 2002), and in plume trees (Intrigliolo and Castel 2004) as they related it with the progressive soil desiccation. These previous studies were confirmed by Badal et al. (2010) for kaki, when water restriction is not severe. Also, using a protocol for modulation irrigation contains Ψ_s beside MDS as indicated in the decision table saved 24%, 33%, and 15% of water applied in cherry irrigated with well water and reclaimed water and Plane tree respectively in 2009. In addition by applying the modified form of this protocol in 2010 we saved 20%, 8%, and 25% this may be because the trees were bigger and extracted more water despite the atmospheric evaporative demand was lower.

Therefore if we want to choose one of these protocols we must to take in our consideration the species and the climatic conditions in the year when we want to apply the protocol, for example if we have dry year and young trees we can recommend 2009 protocol, while if we have rainy year and big trees and it can extract a lot of water we can recommend 2010 protocol.

In the present case we can conclude that 2009 protocol could be recommende for irrigation scheduling modulation in young cherry trees, and 2010 protocol could be recommended for adult plane tree trees.

In the third chapter, we applied a protocol based on the daily calculation of RDIsignal. The irrigation dose modulation applied was a percent of ET_c corrected by the intervals of MDSsignal with control treatment and Ψ_s indicated in the decision table (Table 1) in chapter 3.

Within, our suggested intervals of RDIsignal in the decision table in the two protocols for irrigation modulation in 2009 and 2010 we noticed that the daily signal of MDS have good capability for adjusting the schedule in very short time, and it had the potential for complete automation. As this decision table was effectively applied in 60% and 70% of the total days of irrigation period in cherry and plane trees, respectively in 2009 protocol. Compared with 64% of the total days of the irrigation period in both cherry and plane trees in 2010 protocol, moreover, during the weekends and rain events irrigation were not applied. Moreover RDIsignal treatment saved about 30% and 25% of water applied in 100%ETc treatment in cherry and plane trees respectively in 2009 protocol. While 2010 protocol saved about 19% and 28% in cherry and plane trees respectively.

No negative effect was noticed on cherry trees growth represented in TGR, trunk cross sectional area and relative green cover. These responses in all of these indicators were similar in RDIsignal and SDI with less water applied. The only differences were noticed in plane tree in relative green cover in 2009 as SDI was significantly lower than RDIsignal. Moreover, in SDI treatment management not used sensors, then, we can save money and time. But long term application could affect tree growth.

The results of the chapter 1 showed that MDS had the fastest and more sensitive response to water stress and, thus, a potential indicator to be used in irrigation modulation protocols. This indicator was tested in the assays presented in chapter 2 and 3 for cherry and plane trees. The results of this implementation open the door for a complete automation of the protocol, as proposed by Goldhamer and Fereres (2001) and Conejero et al. (2011) for other species such as almond and peach, respectively. However, we must admit that it has some limitations related with the cost of the sensors and its installation and maintenance. Some of these limitations may be overcome by adjusting the number of sensors, but in the end, the decision will rely on the balance between cost and advantages. The increasing pressure to reduce water use for agricultural purposes and to decrease the water footprint of its products may change the current balance.

By comparing the two protocols applied in chapter 2 (RDImds) and 3 (RDIsignal), we can demonstrate that both of them saved more water than control treatments, to the same extent later protocol saved more water than the former. RDIsignal protocol avoid the problem of using only absolute values of MDS resulting of the changes in environmental conditions. The only limitation to apply RDIsignal protocol that it is necessary a control treatment.

Therefore, we can indicate that if deficit irrigation management is carried out with the protocols that has been tested in this work it is possible to increased water productivity and to reduce the water footprint of the tree growing process.

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

The work developed in this thesis intended to improve irrigation scheduling by applying different deficit irrigation strategies based on plant based indicators combined by soil water status. Also, these strategies were combined by reclaimed water as an alternative source for fresh water for irrigating one species in one part of this work. The conclusions arisen thus far are:

Chapter 1A

1- MDS is a good plant based indicators of water status of young cherry trees. We can suggest a MDS threshold of 0.30 mm. Moreover, MDS has a strong relationship with others plant base indicator like midday leaf water potential and stomatal conductance.

2- Between all plant based water stress indicators, MDS was the most sensitive indicator of water stress for cherry tree as it responded sharply to irrigation withdrawal and the subsequent decrease of substrate matric potential.

Chapter 1B

1- Our results showed that sap flow relationships with leaf water potential and stomatal conductance showed tighter relationship than with MDS. MDS is more sensitive than SF in front of drought stress. On the other hand, the strength of the signal is also higher for MDS than for SF due to stomatal regulation.

2- The information obtained of both indicators can be used as an early indicator of water stress, but it is necessary to assess in field conditions because the effect of water stress on plant water status is slower than in plants in pots with organic substrate.

Chapter 2

1. The protocols based on MDS and Ψ_s is considered a reliable protocol for irrigation scheduling in woody trees as it helped us to save water without any negative effect on trees growth either in well or reclaimed water treatments. Moreover, in cherry trees the 2009 protocol saved more water than 2010 protocol while in plane trees, 2010 protocol saved more water than 2009 protocol.

2. Therefore, depend on the site environmental conditions we can choose different protocol for schedule irrigation, with our results, 2009 protocol can be recommended for cherry trees, and 2010 protocol for plane trees. Moreover, these protocols helped us to increase water productivity and reduce the water footprint of the tree growing process.

Chapter 3

1- By applying such protocols based on MDSsignal helped us to save water without any negative effect on the trees growth and physiological status. Also, these protocols helped us to increase water productivity and reduce the water footprint of the trees growing process.

2- Therefore, we can indicate using an irrigation modulation protocol based on RDIsignal could be promising protocol for woody trees irrigation scheduling, moreover it can be reduce the problems of the variations of MDS absolute values with climatic conditions. The only limitation for applying RDIsignal protocols is the need for control treatment.

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