UNIVERSIDAD POLITÉCNICA DE CARTAGENA

DEPARTAMENTO DE PRODUCCIÓN VEGETAL

Tesis Doctoral

Mejora de la productividad del agua y calidad de la cosecha en uva de mesa cv. ‘Crimson Seedless’

Mª del Rosario Conesa Saura

2015

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MEJORA DE LA PRODUCTIVIDAD DEL AGUA Y CALIDAD DE LA COSECHA EN UVA DE MESA cv. ‘CRIMSON SEEDLESS’

Tesis Doctoral

presentada por

Mª del Rosario Conesa Saura

Ingeniero Agrónomo

dirigida por

Alejandro Pérez Pastor y Rafael Domingo Miguel

Profs. Drs. Departamento del Producción Vegetal

para la obtención del Grado de Doctor
con mención internacional por la Universidad Politécnica de Cartagena

-2015-

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SUSTAINABLE IRRIGATION MANAGEMENT IN TABLE GRAPES cv. 'CRIMSON SEEDLESS' USING DEFICIT IRRIGATION STRATEGIES

Report phD Thesis

presented by

Mª del Rosario Conesa Saura

Agricultural Engeenering

supervised by

Alejandro Pérez Pastor y Rafael Domingo Miguel

Profs. Drs. Department of Plant Production

To obtain the phD with the International mention

-2015-

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DT-16

CONFORMIDAD DE SOLICITUD DEAUTORIZACIÓN DE DEPÓSITO DE TESIS DOCTORAL POR EL/LA DIRECTOR/A DE LA TESIS

D. Alejandro Pérez Pastor y D. Rafael Domingo Miguel, Directores de la Tesis doctoral, **'Mejora de la productividad del agua y calidad de la cosecha en uva de mesa cv. *Crimson Seedless*'.**

INFORMAN:

Que la referida Tesis Doctoral, ha sido realizada por Dª María del Rosario Conesa Saura, dentro del programa de doctorado **Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario**, dando mi conformidad para que sea presentada ante la Comisión de Doctorado para ser autorizado su depósito.

La rama de conocimiento en la que esta tesis ha sido desarrollada es:

☐ Ciencias
☐ Ciencias Sociales y Jurídicas
X Ingeniería y Arquitectura

En Cartagena, a 4 de Septiembre de 2015

EL/LA DIRECTOR/A DE LA TESIS

Fdo.: Alejandro Pérez Pastor

EL/LA DIRECTOR/A DE LA TESIS

Fdo.: Rafael Domingo Miguel

COMISIÓN DE DOCTORADO

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CONFORMIDAD DE DEPÓSITO DE TESIS DOCTORAL POR LA COMISIÓN ACADÉMICA DEL PROGRAMA

D. Francisco Artés Hernández, Presidente/a de la Comisión Académica del Programa 'Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario'

INFORMA:

Que la Tesis Doctoral titulada, "**Mejora de la productividad del agua y calidad de la cosecha en uva de mesa cv. *Crimson Seedless***", ha sido realizada, dentro del mencionado programa de doctorado, por Dª. María del Rosario Conesa Saura, bajo la dirección y supervisión de los Drs. Alejandro Pérez Pastor y Rafael Domingo Miguel.

En reunión de la Comisión Académica de fecha 07/09/2015, visto que en la misma se acreditan los indicios de calidad correspondientes y la autorización del Director de la misma, se acordó dar la conformidad, con la finalidad de que sea autorizado su depósito por la Comisión de Doctorado.

La Rama de conocimiento por la que esta tesis ha sido desarrollada es:

☐Ciencias
☐Ciencias Sociales y Jurídicas
X Ingeniería y Arquitectura

En Cartagena, a 8 de Septiembre de 2015

EL PRESIDENTE DE LA COMISIÓN ACADÉMICA DEL PROGRAMA

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Fdo Dr. Francisco Artés Hernández

COMISIÓN DE DOCTORADO

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Nada que se consiga sin esfuerzo y sin trabajo,
es verdaderamente valioso
(J. Addison)

RECONOCIMIENTOS

RECONOCIMIENTOS

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A continuación se detalla la labor realizada durante la presente Tesis Doctoral:

- **Publicaciones listadas en JCR incluidas en la Tesis:**

Conesa, M.R., de la Rosa, J.M., Artés-Hernández, F., Dodd, I.C., Domingo, R., Pérez-Pastor, A. (2015). Long-term impact of deficit irrigation on the physical quality of berries in ‘Crimson Seedless’ table grapes. Journal of the Science of Food and Agriculture. 95 (12): 2510-2520. DOI: 10.1002/jsfa.6983. Impact factor: 1.71 (7/56) q1.

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- **Publicaciones listadas en JCR no incluidas en la Tesis:**

Egea, G., González-Real, M.M., Baille, A., Nortes, P.A., **Conesa, M.R.**, Ruiz-Salléres, I. (2012). Effects of water stress on irradiance acclimation of leaf traits in almond trees. Tree Physiology, 32 (4): 450-463. DOI: 10.1093/treephys/tps016. Impact factor: 3.04 (2/64) q1.

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De la Rosa, J.M., **Conesa, M.R**., Domingo, R., Pérez-Pastor, A. (2014). A new approach to ascertain the sensitivity to water stress of different plant water indicators in extra-early nectarine trees. Scientia Horticulturae, 169: 147-153. **DOI:** 10.1016/j.scienta.2014.02.021. Impact factor: 1.50 (9/23) q2.

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Truque, E., Águila, D., Gómez, P., Aguayo, E., Otón, M., **Conesa, M.R,** Pérez-Pastor, A., Artés, F., Artés-Hernández, F. (2013). Conservación de uva 'Crimson Seedless' bajo atmósfera controlada tras su cultivo en riego deficitario. Horticultura. 306: 66-69. **ISSN**: 1578-8881

Conesa, M.R., Pérez-Pastor, A**.,** de la Rosa, J.M., Robles, J.M., Domingo, R., García-Salinas, M.D. (2013). Sensibilidad estomática de 'Crimson Seedless' al déficit hídrico.VI Introducción a la investigación de la UPCT. pp 61-63. **ISSN:** 1888-8356.

- **Congresos y eventos de difusión científica**

Pagán, E., Pérez-Pastor, A., Domingo, R., **Conesa, M.R.**, Robles, J.M., Botía, P., García-Oller, I., Caro. (2008). Feasibility study of the maximum daily trunk shrinkage for scheduling mandarin trees irrigation X Congreso of the European Society for Agronomy. Bolonia (Italia). Tipo de participación. Comunicación Oral. Publicación: Actas del congreso

De la Rosa, J.M., **Conesa, M.R**., Domingo, R., Pagán, E., Corbalán, M., Pérez-Pastor, A. (2010). Líneas de referencia basadas en la máxima contracción diaria del tronco en nectarinos extratempranos. XXVIII Congreso Nacional de Riegos y Drenajes (AERYD). 15-17 Junio, León (España). Tipo de participación. Comunicación Oral. Publicación: Actas del congreso

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Conesa, M.R., de la Rosa, J.M., Pérez-Pastor, A., Domingo, R., Corbalán, M., Pagan, E. (2012). Effects of different deficit irrigation strategies on the yield and berry quality of table grape, orchad Crimson. II Conference Emuni Research Resouk. 17-18 Abril. Cartagena (España). Tipo de participación. Oral. Publicación: Actas del congreso.

Conesa, M.R. Manejo sostenible del agua de riego en uva de mesa. Primeros resultados. (2012). I Workshop de Investigación Agroalimentaria. Doctorado en Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario. 7-8 Mayo 2012. Cartagena (España). Tipo de participación. Oral. Publicación: Actas del Workshop.

Truque, E., Águila, D.J., Gómez, P.A., Aguayo, E., Otón, M., **Conesa, M.R**, Pérez-Pastor, A., Artés, F., Artés-Hernández, F. (2012). Conservación de uva 'Crimson Seedless' bajo Atmósfera Controlada tras su cultivo en riego deficitario. X Simposio Nacional y VII Ibérico sobre Maduración y Postcosecha de Frutas y Hortalizas. 1-4 octubre. Lleida, (España). Tipo de participación. Oral. Publicación: Actas del congreso

De la Rosa, J.M., **Conesa, M.R**., Domingo, R., García-Salinas, M.D., Gómez-Montiel, J., Pérez-Pastor, A. (2013). Sensibilidad de indicadores del estrés hídrico en nectarino extratemprano. XXXI Congreso Nacional de Riegos y Drenajes (AERYD). 7-8 Junio. Orihuela (España). Tipo de participación. Oral. Publicación: Actas del congreso

Conesa, M.R., Navarro, H., de la Rosa, J., Pérez-Pastor, A., Torres, R. Redes de sensores cableadas e inalámbricas. (2013). Estudio y aplicación en la instrumentación y control de riego en uva de mesa. Seminario Anual de Automática, Electrónica Industrial e Instrumentación (SAAEI). 11-12 Mayo. Tipo de participación. Oral. Publicación: Actas del congreso

Conesa, M.R. Efecto del déficit hídrico en la acumulación de ácido abscísico en uva de mesa cv. ‘Crimson Seedless’. (2013). II Workshop de Investigación Agroalimentaria. Doctorado en Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario. 11-12 Mayo. Cartagena (España). Tipo de participación. Oral. Publicación: Actas del Workshop.

Pérez-Pastor, A., de la Rosa, J.M, **Conesa, M.R**., Domingo, R. (2013). Increases of 40% in water use efficiency attained through a sustained irrigation strategy in a commercial nectarine orchard located in an area of low water availability. The 8th Conference on Sustainable Development of Energy, Water and Environmental System- SDEWES Conference. 25-29 Septiembre. Dubrovnik (Croacia) Tipo de participación. Oral. Publicación: Actas del congreso

Pérez-Pastor, A., de la Rosa, J.M, **Conesa, M.R**., Domingo, R. Feasibility of using reference lines derived from plant water status indicators for irrigation scheduling of early nectarine trees. The 8th Conference on Sustainable Development of Energy, Water and Environmental System- SDEWES Conference. 25-29 Septiembre. Dubrovnik (Croacia) Tipo de participación. Oral. Publicación: Actas del congreso

De la Rosa, J.M, **Conesa, M.R**., Domingo, R., Gómez-Montiel, J., Pérez-Pastor, A. (2013). Sensitivity to water stress of plant water status indicators in early nectarine trees. VIII International Peach Symposium. XXX Junio. Matera (Italia). Tipo de participación. Oral. Publicación: Actas del congreso

Conesa, M.R., García-Salinas, M.D., Pérez-Pastor, A., de la Rosa, J.M., Fernández-Trujillo, J.P. (2013). Deficit irrigation applied during fruit growth of 'Fortune' mandarin improves quality at harvest, during storage and subsequent shelf-life. Symposium of quality of fresh produce herbs, and vegetables- from field to fork. (Warsaw) Polonia, 2013. Tipo de participación. Póster. Publicación: Actas del congreso

Puértolas, J., Alcobendas, R**., Conesa, M.R.,** Dodd I.C. **(2013).** Does long-distance ABA signalling and local root water potential depend on how soil moisture is heterogeneously distributed? XIII Congreso Luso-Espanhol de Fisiología Vegetal. XXX Julio. Lisboa, (Portugal). Tipo de participación. Póster. Publicación: Actas del congreso

Conesa, M.R. Programación del riego en uva de mesa cv. 'Crimson Seedless' a partir de indicadores de planta. III Workshop de Investigación Agroalimentaria. Doctorado en Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario. 9-11 Mayo. Cartagena (España) Tipo de participación. Oral. Publicación: Actas del Workshop.

Conesa, M.R., De la Rosa, J.M., Domingo, R., Torres, R, Soto, F., Navarro, H., García, M., Pérez-Pastor, A. (2014). Programación del riego en uva de mesa cv. 'Crimson Seedless' en base a la máxima contracción diaria del tronco. XXXII Congreso Nacional de Riegos y Drenajes (AERYD). XXX Junio. Madrid (España). Tipo de participación. Oral. Publicación: Actas del congreso

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- **Estancias en el extranjero**

Estancia en el centro de investigación 'Lancaster Environment Center' (LEC) de la Universidad de Lancaster (Reino Unido), supervisada por el Dr. Ian C. Dodd desde el 4 de Enero de 2013 hasta el 11 de Abril de 2013.

- **Participación en otros proyectos de investigación durante la Tesis**

Título del Proyecto: Efecto de los factores edafoclimáticos en el balance de carbono en frutales cultivados en distintas zonas de la Región de Murcia (Ref: CayA22_08). Entidad financiadora: Consejería de Agricultura y Agua de la Región de Murcia. Entidades participantes: UPCT. Duración. 2008/2010. Investigador Principal: Dr. Alejandro Pérez Pastor. Cuantía de la subvención. 11.615.00 €.

Título del Proyecto: Implementation of efficient irrigation management for a sustainable agriculture (Ref: LIFE13 ENV/ES/000539). Entidad financiadora: Unión Europea. Entidades participantes: UPCT, UCO, CEBAS-CSIC, CARM y FENACORE. Duración. 2014/2017. Investigador Principal: Dr. Alejandro Pérez Pastor. Cuantía de la subvención. 840,193.00 €.

- **Dirección de Proyectos Fin de Carrera (PFC) y supervisión de Trabajo Fin Máster (TFM) en máster de investigación TAIDA.**

Título PFC: Estrategias de Riego Deficitario Controlado en uva de mesa cv. Crimson Seedless. Autor: Virginia Palacios Pividal. Director: Alejandro Pérez Pastor. Co/director: **María R. Conesa.** Fecha de defensa: Septiembre de 2013. Calificación. Notable (8.5).

Título TFM: Programación del riego en mandarino basada en el crecimiento del fruto. Efectos sobre la postcosecha. Autor: Oscar Dóncel Méndez Director: Alejandro Pérez Pastor Co/director: Juan P. Fernández-Trujillo. Tutor. **María R. Conesa.** Fecha de defensa: Septiembre de 2012. Calificación. Sobresaliente (9.5).

Título TFM: Estudio comparativo de las relaciones hídricas y producción en uva de mesa bajo Riego Deficitario Controlado (RDC) y Desecación Parcial de Raíces (PRD). Efecto del déficit continuo y controlado. Autor: Cristina Martínez Barba: Alejandro Pérez Pastor. Tutor. **María R. Conesa**. Fecha de defensa: Septiembre de 2015. Calificación. Sobresaliente (9).

- **Premios**

Primer premio Resouk 2012 otorgado por el Campus Mare Nostrum a la conferencia ‘Effects of different deficit irrigation strategies on the yield and berry quality of table grape, orchad Crimson’, celebrada en II Conference Emuni Research Resouk 2012 del 17 al 18 Abril de 2012.

Enlace web: http://www.laverdad.es/murcia/v/20120628/region/mare-nostrum-entrega-premios-20120628.html.

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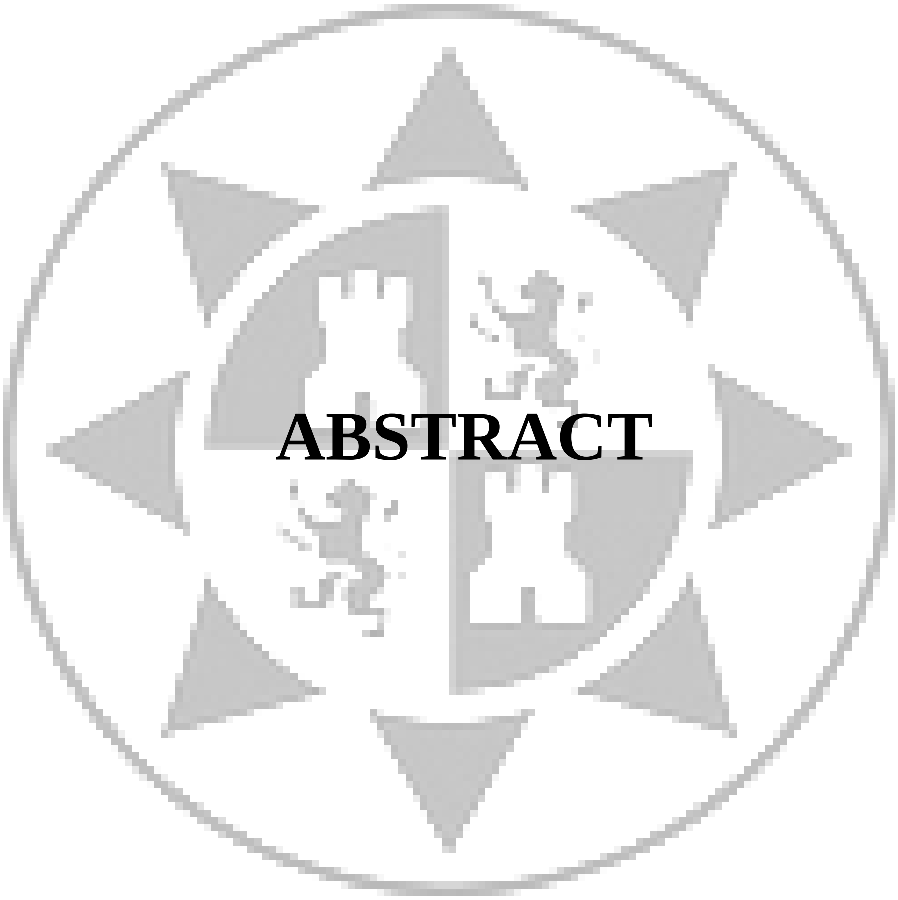


ABSTRACT

ABSTRACT

The research of the current PhD Thesis deals with the evaluation of the agronomic and physiological responses of mature table grapes cv. ‘Crimson Seedless’ to partial root-zone drying (PRD) and regulated deficit irrigation (RDI) with respect to other irrigation treatments that received different amounts of water applied. To this end, four irrigation treatments were established: (i) Control, receiving 110 % of crop standard evapotranspiration, ET_C, throughout the whole growing season following the criteria by the commercial farm; (ii) RDI treatment, irrigated similar to Control levels during pre-veraison and at 50% of the same during post-veraison (considered the non-critical period); (iii) PRD treatment, irrigated in a similar way to RDI but alternating (every 10-14 days) the dry and wet sides of the root-zone, depending on water deficit with respect to field capacity; and (iv) a null irrigation treatment (NI) which only received natural precipitation and occasional supplementary irrigation when the midday stem water potential (ψ_s) exceeded -1.2 MPa. To establish reference equations another full irrigation treatment (110-115% ET_c) was used. Furthermore, the results were extrapolated to a pot experiment in order to determine the physiological behavior of this cultivar, under controlled conditions in a greenhouse.

Chapter I analysed the yield response and chemical quality to long-term deficit irrigation (DI) strategies. No significant differences were found between PRD and RDI with respect to well-watered vines irrigated according to ET_c, thus the application of a greater amount of water was not essential for plant behavior and berry development in ‘Crimson Seedless’ table grapes. Both PRD and RDI treatments supposed a water saving of 35% without compromising total yield and its components. Only NI (which received 72% less water than Control) led to a reduction in yield and the weight of clusters/berries compared with the other irrigated counterparts. Water use efficiency was also increased in all DI treatments as many water restrictions were assessed.

Regarding chemical berry quality, all deficit irrigation treatments increased berry coloration (evaluated subjectively and objectively) which is considered the main issue of this variety for its marketability. Despite the fact that RDI and PRD received the same amount of annual water applied, PRD induced a greater accumulation of skin anthocyanins, resveratrol and antioxidant capacity. Although PRD did not show significant changes in yield response with respect to RDI, the fact that PRD increased the main bioactive compounds analysed that are beneficial to health, underlined the feasibility of the implementation of this strategy by growers.

Chapter II focused on the long-term impact of DI strategies on physical berry quality, with particular attention to the berry firmness, since it is one of the most important characteristics in order to be marketed and for consumer acceptance. Moreover, the storage performance to ascertain the potential shelf-life of this cultivar was reported. RDI and PRD did not noticeably affect physical berry quality after cold storage while the subsequent shelf-life period tended to minimise the difference found at harvest or at the end of cold storage. Furthermore, NI treatment showed the worst sensory scores post-harvest and the most dehydrated clusters and lower berry size. In fact, sensory results were similar in RDI and PRD, which provided grapes that were more acceptable to consumers than well-irrigated vines, mainly due to lower stem browning and higher berry coloration. Remarkably, PRD registered the highest berry shattering, which was correlated with the lower concentration of ABA_{xylem} induced by the grower's strategy.

Thus, the results obtained in Chapters I and II indicate that it is possible to decrease irrigation by applying RDI and PRD to 'Crimson Seedless' table grapes without adversely affecting yield and the physicochemical berry quality.

The physiological response and vegetative growth to DI strategies were described in Chapter III. The analysis of the physiological fluxes (net CO_2 assimilation, A_{CO2} and transpiration rate, E) and their characteristic attributes (stomatal conductance, g_s) determined at leaf scale, under saturating-light conditions, showed a water stress response in accordance to the water stress severity imposed, regardless of irrigation strategy. Comparing post-veraison strategies, PRD induced higher plant and soil water deficit levels than RDI. Nevertheless, PRD neither significantly reduced g_s nor increased ABA_{xylem}

against expectations. These results suggest a greater root development and root density from PRD with respect to RDI for water uptake. As expected, vegetative parameters were adversely affected by the severe deficit reached in NI, while the leaf area index was also modified by PRD. PCA results showed that inter-annual differences detected between irrigation treatments were higher than those observed between phenological periods, especially when RDI and PRD were compared. Furthermore, maximum daily shrinkage (MDS) was the best plant-water status indicator to ascertain irrigation differences before veraison, whereas other conventional plant water status indicators (such as water potential and transpiration rate, E) might be considered for irrigation scheduling during post-veraison.

Different reference lines appeared in Chapter IV from plant water status indicators such as MDS and ψ_s indicators were obtained during pre and post-veraison periods, respectively, for irrigation scheduling in well-irrigated table grapes cv. 'Crimson Seedless'. In this sense, MDS and ψ_s showed better adjustment with mean temperature (T_m) during pre-veraison, while after veraison reference crop evapotranspiration (ET_0) and vapour pressure deficit can also be used. The correlation coefficients in MDS decrease during post-veraison due to changes of stem transpiration, the presence of sugar-demanding sinks and the accumulated $ABA_{xylem.}$ Besides this, under commercial conditions, water savings with respect to conventional scheduling based on ET_c were achieved when the irrigation scheduling was done using SI_{MDS} around unity (in pre-veraison) and maintaining ψ_s as a threshold value in well-watered vines (in post-veraison). Moreover, in this Chapter we also observed that some standard cultural practices such as girdling and the collocation of hail mesh to prevent torrential rainfalls might also modify vine water status.

From a physiological point of view, the results obtained were extrapolated to a pot experiment in Chapter V. Table grapes showed a substantial loss of photosynthetic capacity as the season progressed both growing in the field (as shown in Chapter III) and in a pot experiment (Chapter V). Crimson Seedless displayed different responses to DI strategies, depending on the diurnal course. At predawn (*t1*) and early morning (*t2*), the cultivar

showed near-anisohydric behavior, through a less effective stomatal control of drought, whereas at midday (*t3*), the behavior was near-isohydric. In addition to this, water stress conditions induce avoidance mechanisms to drought, such as stomatal closure, partial defoliation and a reduction in leaf insertion angle. Analysis of the vegetative response does not indicate that PRD vines respond differently, or present a clear distinct adaptive mechanism to water stress with respect to RDI vines. In fact, pruning dry weight was only affected by severe water deficit (NI).

BACKGROUND

BACKGROUND

1. Vines

1.1. Origin and characteristics

The vines belong to the *Rhamnales* order*, Vitaceae* family, *Vitis* genus and *Euvitis* subgenre (Ribéreau-Gayon and Peynaud 1986). The species belonging to the *Vitaceae* family, as vines, are climbing shrubs with a woody stem whose leaves are alternate and usually stipulated and with tendrils opposite them. Herbaceous branches are called shoots and when they are lignificated, they are called branches which can produce fruiting buds. The flowers are small and hermaphrodite. Its inflorescence is bunch composed and the berry fruit with a seed of hard and thick testa. Within the *Euvitis* subgenre, *Vitis Vinifera L.* is the European vine and cultivated species per excellence, and over 90% of the grape varieties produced belong to this species, as it has great qualities such as: succulent large berries, and marketable characteristics. It is also known that they are sensitive to cold, fungal diseases and phylloxera, but resistant to chlorosis (Pérez-Camacho, 1992).

1.2. Crimson Seedless

It is a red late table grape cultivar developed in USDA-ARS in Fresno, California in the early-90s. It is also known as C102-26 selection. As the surname ‘seedless’ suggests, the cultivar corresponds to a variety without seeds. To obtain it, five generations of hybridisation were needed, which initially intervened ‘Sultanina’, and finally crossing Emperor x C33-199.

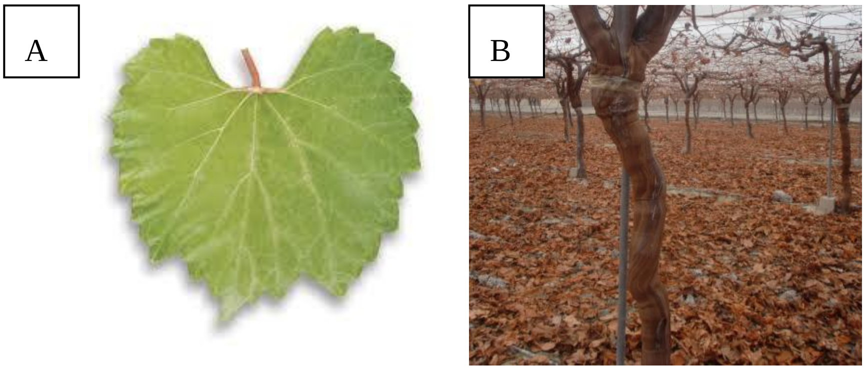
The cultivar has a good productivity, so it must be handled to prevent overloading (Picture 1). Therefore, it needs wide plantation frames. It usually produces berries of medium size (16-19 mm of equatorial diameter), compact, conical, pinkish to purplish red, with two seminal sketches virtually undetectable to eat and lots of small compact clusters (Blanco *et al.,* 2010).

Picture 1. *Detail of the Crimson Seedless orchard (A) and clusters of variety (B)*

The skin is medium thick and its flesh is clear, firm and crisp. The berry's taste is sweet and neutral. It has great market acceptance due to its excellent nutritional properties and its exportable value (Río-Segade *et al.,* 2013). Besides being resistant to disease and having a good aptitude in post-harvest, it also offers good performance in its cold storage and transport resistance. It is therefore considered a very interesting variety for cultivation (Conesa *et al.,* 2012).

1.3. Paulsen 1103 rootstock

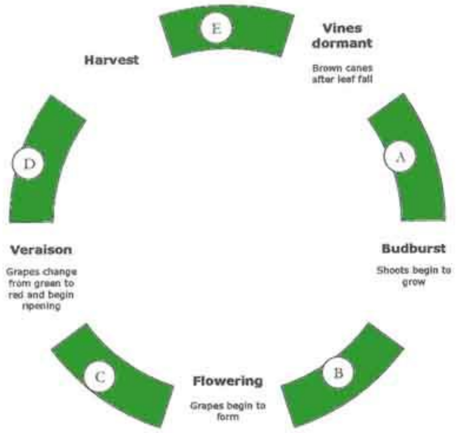
It originated in Sicily and was obtained from the hybridisation between *Vitis Berlandieri R x Vitis Rupestres de Lot* (Picture 2). Among its most important features, its great vigour and good rooting after transplanting should be highlighted (Pérez-Camacho, 1992). Therefore, in most cases it is grafted the same year of planting. Thanks to its resistance to salinity, drought and nematodes this is a pattern that works with excellent results in semiarid areas. It has also been successful in infertile soils and compact clay soils, although it is recommended for soils of medium compactness with cool or wet basement. It tolerates up to 23% of active lime and 30% total lime stone. Its resistance to iron chlorosis is evaluated as average. Magnesium is well absorbed and it is also interesting for its resistance to iron chlorosis and chlorides. However, it is usually developed for its remarkable resistance to salinity (Pérez-Camacho, 1992).



Picture 2. *Details of the leaves (A) and trunk (B) of the rootstock Paulsen 1103*

1.4. Growth cycle of the vine

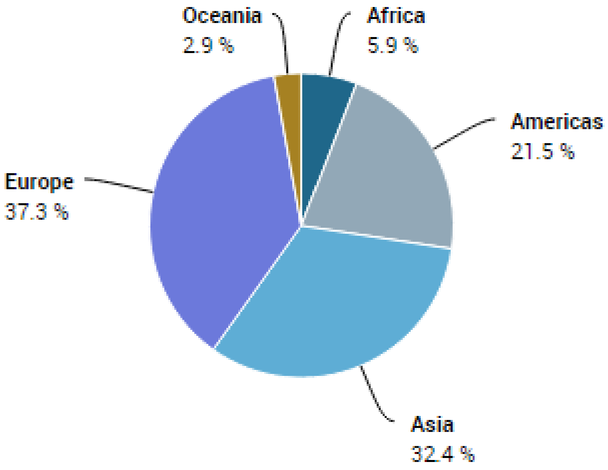
The annual growth cycle of the vines involves many processes and events in the vineyard each year. In the process, each step plays a vital role in the development of grapes with ideal attributes. Annual growth of vines is frequently described using the following stages (reviewed by Karvonen, 2014): 1) budburst; 2) flower cluster initiation; 3) flowering; 4) fruit set; 5) berry development; 6) harvest; and 7) dormancy. Moreover, in red varieties such as 'Crimson Seedless', the veraison, as the changing of berry color is called, had occurred at the onset of maturation (Blanco *et al.,* 2010; Faci *et al.,* 2014), with the means between points 5 and 6 (Picture 3). Moreover, it is also known that prior to veraison is the most sensitive period to water stress in Crimson Seedless (Blanco *et al.,* 2010; Faci *et al.,* 2014). The timing and duration of these events are subject to variations due to the grape variety, local climate and seasonal weather, but the sequence of them does remain constant.



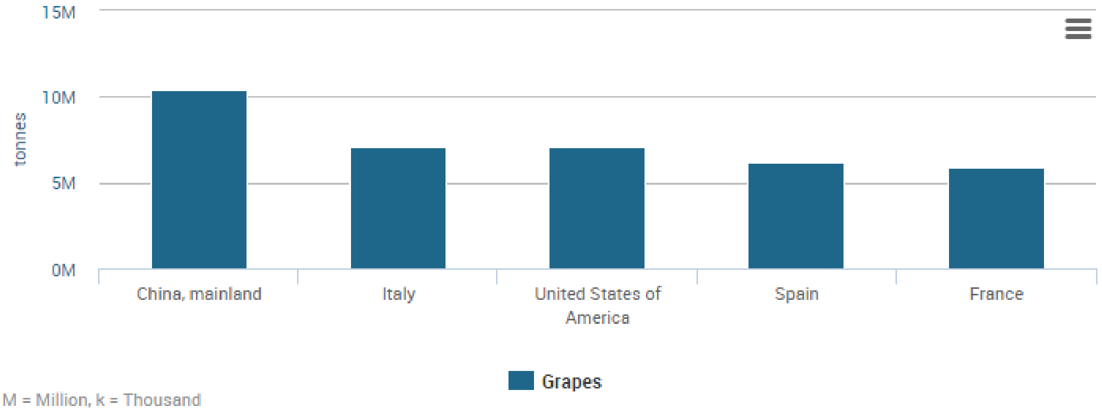
Picture 3. *Annual growth cycle in red varieties. Source: www.thewordwine.com.*

1.5. Current situation and economic importance of table grapes

Europe and Asia (mainly China) are the main producers of grapes with approximately 35% of worldwide production (Picture 4). Spain is the fourth producer country with an average annual production of 6,175,175 t in the period 2011-2013 (FAOSTAT, 2015), as observed in Picture 5. Spain is one of the most important producers of table grapes cultivars in Europe and the province of Murcia is the largest in Spain, with 125,000 t cultivated per year, representing 53% of the national production (MAGRAMA, 2015). In fact, the observed increasing trend with time is due to the establishment of the new seedless varieties, which have increased consumer acceptance (Faci *et al.,* 2014). Besides this, the development of the new seedless varieties allows a unique offer in the Region of Murcia with tasty and attractive grapes for the consumers and profitable and productive techniques for crop growers and the European consumer.



Picture 4. *Average grape production share by region in the period 2011-2013 (FAOSTAT, 2015).*



Picture 5*. Production of top 5 producers in the world of grapes during the period (2011-2013) (FAOSTAT, 2015).*

1.6. Current situation of the water resources in the local area of study

The Segura river basin is located in the south-east of Spain, with a surface area of about 18,870 km^2, and covering four regions: practically the whole of the Region of Murcia, and also parts of Andalucía, Castilla-La Mancha and Valencia (Picture 6).

Picture 6*. Distribution of the Segura Rivera basin (CHS, 2015).*

The Segura basin is characterised by problems of permanent water shortage and over-exploitation of most of its water resources from well or groundwater. Uncertainty is characteristic with regard to the water availability in the basin, with markedly Mediterranean low rainfall, where water for production clearly shows insufficient resources available (structural deficit). The annual rainfall is about 400 mm (CHS, 2015), so precipitation occurs irregularly and much of it occurs with storms.

This rainfall does not meet the average annual potential evapotranspiration estimated at 700 mm, which is a deficit basin in which structural water deficit is estimated at 460 hm^3 (CHS, 2015). Given this permanent water deficit situation, the option for irrigators in the area has been to reduce the acreage, with the risk of increasing favourable conditions for soil erosion and desertification, or below water needs encouraging savings and water productivity. In this critical context, there is an urgent need to encourage the adoption and implementation of alternative management practices that increase the irrigation water productivity (Jones, 2004).

2. Deficit irrigation (DI)

2.1. Concept

DI can be defined as an irrigation strategy in which the amount of water applied is lower than that needed to satisfy the full crop water requirements. DI is aimed at increasing the water use efficiency (WUE) of a crop by reducing or even eliminating irrigations that have little impact on yield. The challenge is to define an optimal irrigation strategy that will minimise the negative impact of the expected stress. Therefore, the correct management of DI requires an understanding of a crop's sensitivity to drought stress and the economic impact any reductions on yield.

In order to quantify the level of DI to be applied it is first necessary to know the full crop evapotranspiration (ET_c) requirements, usually calculated from the equation proposed by Penman-Monteith (Allen *et al.,* 1998) and the crop coefficient. When the reductions in the water applied are lower than ET_c, the crop extracts water from the soil reservoir to compensate this water deficit (Fereres and Soriano, 2007). As a consequence, transpiration (T) and therefore carbon assimilation is limited (Ruiz-Sánchez *et al.,* 2010). Any significant decrease in soil water storage usually has an impact on the water available for the crop and hence on yield and ET_c. This suggests the need to know crop yield responses to water stress before applying DI programs (Kirda *et al.,* 1999).

2.2. Regulated deficit irrigation (RDI)

RDI was developed in the early-80s as a watering strategy to reduce excessive vegetative growth, save water and improve fruit quality (Chalmers *et al.,* 1981). Water reductions need to be imposed at times when tree yield responses are minimally affected by a water deficit (Mitchell *et al.,* 1989). Under RDI, such reductions in irrigations are applied at certain times of the growing season, while fully covering the needs of the crop during the so-called 'critical periods' or phenological stages that are most sensitive to water stress (Lampinen *et al.,* 1995). Therefore, the use of this technique requires knowledge of the periods when a crop is sensitive to DI, which differ from crop to crop, depending on the agronomic and physiological behaviours of each (Buesa *et al.,* 2013). In elaborating RDI strategies, the key is to confine the stress to tolerant periods when yield and fruit quality are not adversely affected (Ruiz-Sánchez *et al.,* 2010). Moreover, RDI requires not only careful selection of time of application but also of the intensity and duration of the application, which all depend on the stage of plant development. Therefore, the calculated yield-loss functions could be provided as a tool for programming a long-term strategy of RDI under water-scarcity conditions (García-Tejero *et al.,* 2013).

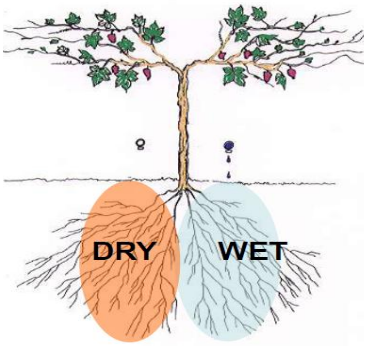
Another very important feature for the implementation of RDI is the ability of trees (vines in our case) to adapt to water stress. In-depth exploration in search of water could be one of the first mechanisms of adaptation to water stress conditions. Furthermore, the osmotic adjustment is an adaptive mechanism that occurs in apple, almond, pistachio, pear and citrus, thereby maintaining cell turgor in low water potentials (Goode and Higgs, 1973; Castel and Fereres 1982).

2.2.1. RDI experiments in vines

When the vine is subjected to water stress, it produces a large decrease in leaf area and shoot growth. Therefore, as the crop cycle advances the sensitivity of growth to water deficit increases (Schultz and Matthews, 1988; Poni *et al.,* 1993). The number of bunches per plant, number of berries per cluster and berry weight are the components of the final performance of the vine which generally decreased as the soil water content is reduced (Matthews and Anderson, 1989). Ferreyra *et al.,* (1999) found that with deficits before veraison, berry diameter is smaller to those produced with deficits after ripening and during continuous irrigation, also producing wilting and delayed ripening. Similar deductions were made by Matthews and Anderson (1988), who conclude that the lack of water before ripening probably inhibits cell division, primarily responsible for the berry growth in the early stages of development. These authors also stated that higher yields are obtained after post-veraison deficit in relation to those deficits applied before veraison. However, studies by Ferreyra *et al.,* (2001) showed that water deficits applied prior to veraison could significantly affect the berry size. In addition, Matthews and Anderson (1989) found that the number of berries per cluster would be determined by the vine water content before veraison, which coincided with the results reported by Puyo (1992). Faci *et al.* (2014) concluded that a moderate adjustment of deficit irrigation induced during post-veraison promoted good crop yield and harvest and also high grape quality in varieties such as ‘Autumn Royal’ and ‘Crimson Seedless’. Moreover, they also observed an improvement in the berry color parameters from ‘Crimson Seedless’ with the use of RDI applied after veraison.

2.3. Partial root-zone drying (PRD)

Partial root-zone drying (PRD), which was first developed in grapevines by Dry *et al.,* (1996) is a variation of deficit irrigation (DI) which involves irrigating only one part of the root zone in each irrigation event, leaving another part to dry to certain soil water content before rewetting by shifting irrigation to the dry side (Picture 7). Thus, PRD is a novel irrigation strategy since half of the roots are placed in drying soil and the other half are growing in irrigated soil (Sepaskhah and Ahmadi, 2010). PRD can reduce leaf transpiration and limit vegetative growth, thereby increasing WUE, which reflects in the dry matter produced per unit of water transpired. Therefore, the soil water content in the wet zone has to be maintained relatively high whilst that in the drying soil zone should not be very low, in order to maintain high soil and plant water status (Wang *et al.,* 2012).



***Picture 7**. Illustration of PRD strategies in grapevines (provided by Dr. Ian Dodd)*

The hypothesis underlying PRD is that root-to-shoot signalling regulates the plant response to drying soil (Stoll *et al.,* 2000), which ultimately reduces plant water use by partly closing the stomata and limiting vegetative growth (Dodd, 2005). Many reports implicate increases in xylem abscisic acid (ABA) concentration in the regulation of stomatal behaviour as the soil dries (Dodd, 2005). Therefore, ABA levels in plants fluctuate widely in response to environmental changes, especially to drought stress (Seki *et al.,* 2007). Moreover, in order to understand ABA signalling in the field, the knowledge on how vertical and lateral soil moisture gradients affect root ABA accumulation is required (Puértolas *et al.,* 2013), because the water redistribution could be modulated by differences in hydraulic resistance within the root zone. Puértolas *et al.* (2015) reported that root ABA accumulation seems to be affected only by the degree of soil drying, regardless of the spatial layout of soil moisture heterogeneity, and not by differential internal water redistribution. Meta-analyses comparing yield at similar irrigation volumes in many cultivars have demonstrated that PRD enhances yield in only 20–40% of experiments (as reviewed by Dodd, 2009). Grapevines irrigated with PRD and RDI, receiving the same amounts of water, revealed some differences in leaf water relations, WUE, crop yield and fruit quality (Romero and Martínez-Cutillas, 2012 and Romero *et al.,* 2015). Similarly, in perennial species, the higher leaf photosynthesis of PRD trees obtained with respect to RDI, could enhance WUE (Pérez-Pérez *et al.,* 2012). Nevertheless, in some deciduous crops (*e.g.* almond), Egea *et al.* (2010) reported that PRD did not produce any physiological advantages compared with conventional deficit irrigation when the volume of water was the same.

2.4. Comparison between RDI and PRD

Earlier reports in grapevines compared both strategies (RDI and PRD). However, the information of that comparison dealing with table grapes is scarce. For example, Romero *et al.* (2015) compared in wine grape cv. Monastrell, the PRD to RDI strategies. PRD improved yield, number of bunches per vine and average weight of berries. PRD addition increased the concentration of amino acids and anthocyanins in berries, and altered their composition by increasing phenols and chromatic characteristics of the wine. Moreover, García-García *et al.* (2012) made a comparison between different RDI and PRD treatments in vineyards and conducted a financial analysis of production. Either PRD or RDI (moderate deficit irrigation), increased the quality of the harvest and could be viable, although productivity diminished compared to well-irrigated vines. They also noted that severe irrigation deficits in both PRD and RDI were unviable. They concluded that for our area and weather, the RDI treatment is more viable than PRD, mainly due to the high cost of irrigation installation.

In this sense, McCarthy *et al.,* (2002) provides a comparison between the RDI and PRD techniques for wine grapes, and it can be seen that PRD has a number of advantages over the technique of RDI (Table 1).

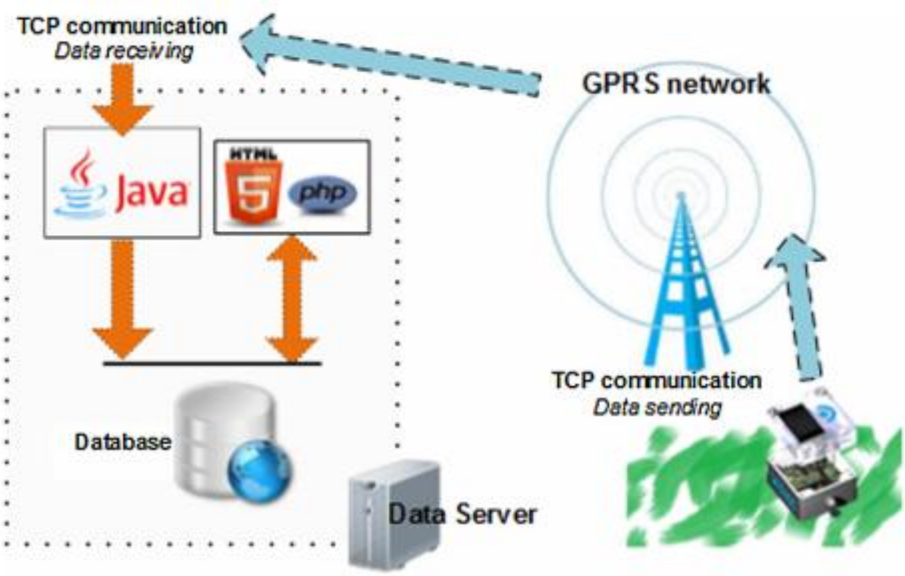
Table 1. *Comparison of regulated deficit irrigation (RDI) and partial root zone drying (PRD) on grapevine, according to McCarthy et al., (2002).*

Regulated Deficit Irrigation (RDI)	**Partial root-zone drying (PRD)**
Berry size control	No effects on berry size
Vegetative growth control	Vegetative growth control
Potential loss of performance	No potential loss of performance
Positive effects on the quality of grapes and wine	Possible improvements in the quality of grapes and wine
Negligible water saving	Significant water saving
The irrigation installation has not been modified	Implies important changes in irrigation installation

3. Use of wireless and wired sensor networks in agriculture

Irrigation scheduling using DI techniques requires a deep control of plant water status every time. To carry this out, instrumentation wired sensor platforms are used between sensors and recording equipment (Conesa *et al.,* 2013). They lack the flexibility to implement sites adequately because the wiring distance limits the location of said sensors, regardless of installation problems or theft which have often been associated. For these reasons, sensors networks, such as wireless, have mainly emerged, whose structure is not centralised on a logger, but provide collaboration between all elements or nodes forming part of the sensor network.

Wireless sensor networks are also characterised by an efficient and independent use of the energy they need to operate, which confers optimal flexibility for use in agriculture. (Picture 8) The nodes of a network of sensors differ according to the role exerted on the network, distinguishing between sensor nodes (or end-device), nodes routers and coordinator nodes (Navarro-Hellin *et al.,* 2015). Sensor nodes are responsible for interacting with the sensor or sensors attached to it, record the information locally and send it to the node coordinator. The coordinator node is responsible for managing the wireless network, dealing with the choice, for each sensor node, of which route is the most suitable for the information from that node to reach the coordinator. To do this, the nodes can support each other so they use routers or nodes collaboratively, whose mission is to support network areas where coverage may be jeopardised.



Picture 8. *Architecture of the system (Adapted from Navarro-Hellín et al., 2015)*

The measurement area that can be covered by a network of sensors depends largely on the network architecture is chosen, the protocol used, and the frequency and power use of the nodes. In all cases it is necessary to strike a balance between power communication and energy independence. Typical sensor networks are usually built on the ZigBee standard, which is a stack of commands supported by several microcontrollers from market firms highly recognised in the field of electronic design (Conesa *et al.,* 2013).

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INTEREST AND OBJECTIVES

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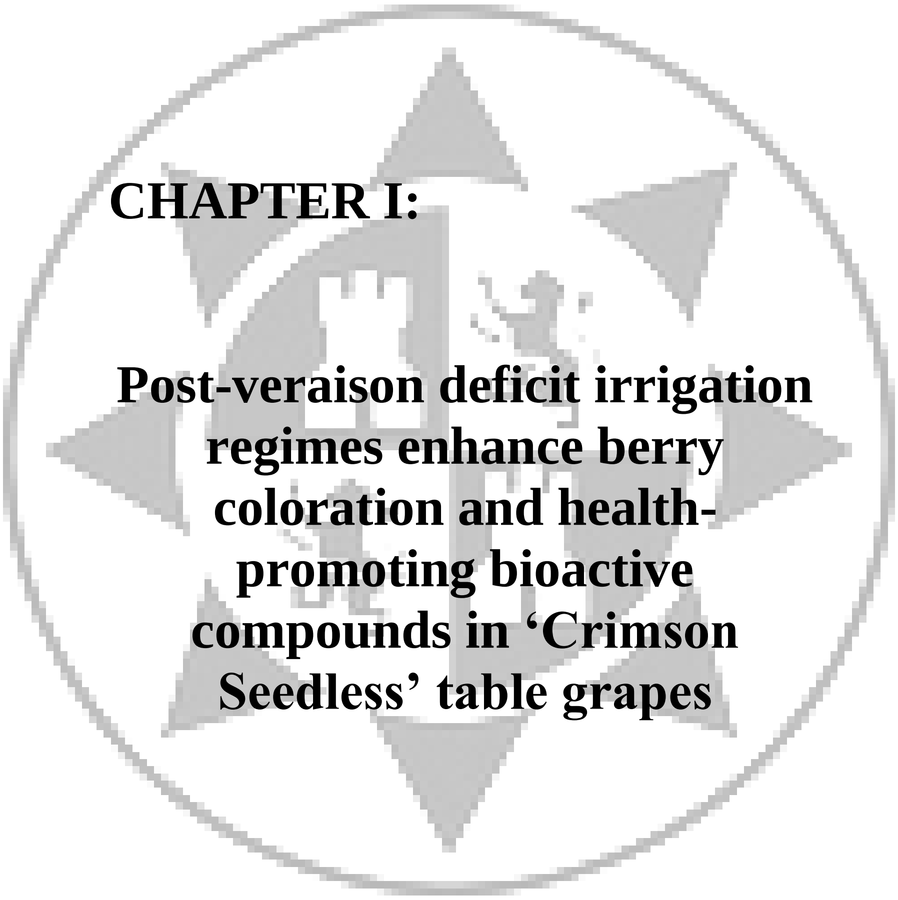
Irrigated agriculture is known as the primary user of diverted water globally, reaching a proportion that exceeds 70–80% of the total in arid and semiarid zones. Since forecasts of water withdrawals predict sharp increases in future demand, it is obvious that irrigated agriculture will become a primary consumer of water especially in emergency drought situations. Moreover, other factors such as: (i) the booming of population across the world, and (ii) the progress of climate change, induce an increasing food production and more water deficit situations. Therefore, the challenge for the coming years will be to increase or at least maintain fruit production and quality with less irrigation water. This could be achieved through the implementation of different irrigation strategies capable of increasing irrigation water efficiency. Thus, the determination of crop water requirements is essential to apply deficit irrigation (DI). In fact, the demand for seedless varieties (e.g. 'Crimson Seedless') has increased considerably in recent years as a result of an increase in international demand and new plantings.

The most common methods for applying irrigation at rates lower than crop requirements are regulated deficit irrigation (RDI) and partial root drying (PRD). RDI is based on the fact that imposing water stress during those phenological stages has minimal effects on yield while it could also lead to an improvement in the quality of the products. On the other hand, PRD involves the deliberate wetting and drying of alternate sites of the root zone so that the production of specific root-sourced chemical signals will be optimised inducing partial stomatal closure and thereby increasing water use efficiency. Besides this, among chemical signals, the production of abscisic acid (ABA) in the drying roots is widely believed to play a dominant role in regulating plants' stomatal conductance.

A great deal is already known about the comparison between RDI and PRD effects in grapevines when both techniques received the same amount of irrigation. For example, earlier reports revealed differences in leaf water relations, water use efficiency, crop yield, and fruit quality. However, studies about RDI and PRD in the literature on table grapes are scarce. Indeed, little information exists about ‘Crimson Seedless’ and the main issue of this cultivar; the lack of berry coloration, which can limit its marketability.

For all of the above reasons, the specific objectives for this Thesis are:

- Determine the long-term (2011-2013) effects of different post-veraison deficit irrigation (DI) strategies (mainly regulated deficit irrigation (RDI), partial root-zone drying (PRD), and the comparison of both) on the agronomical response and physicochemical berry quality, highlighting their influence on berry coloration **(Chapters I and II).**
- Evaluate the effects of DI strategies on the overall berry quality and the content of bioactive compounds that are beneficial to health **(Chapter I).**
- Determine the DI strategies’ performance after cold storage and quantify the potential shelf-life **(Chapter II).**
- Evaluate the physiological response and water relations to long-term DI strategies (2011-2013). Investigate the influence of post-veraison effects of RDI and PRD strategies based on the information provided by several plant-based water status indicators **(Chapter III).**
- Establish reference equations from meteorological variables in well-watered vines. Evaluate the suitability of maximum daily shrinkage (MDS) and midday stem water potential (Ψ_s) as criteria of irrigation scheduling during pre and post-veraison, respectively **(Chapter IV).**
- Quantify the possible involvement of ABA as a component of the berry-ripening process and its influence on the water relations assessed **(Chapters I, II, III and IV).**
- Clarify the results obtained in the field to ‘Crimson Seedless’ plants grown in pots from a physiological point of view **(Chapter V).**



CHAPTER I:

Post-veraison deficit irrigation regimes enhance berry coloration and health-promoting bioactive compounds in ‘Crimson Seedless’ table grapes

Chapter I: Post-veraison deficit irrigation regimes enhance berry coloration and health-promoting bioactive compounds in 'Crimson Seedless' table grapes

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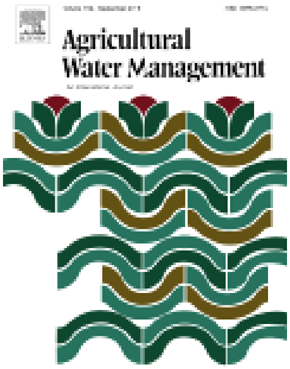
Running title: Post-veraison deficit irrigation applied to table grapes



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Abstract

The impact of different post-veraison deficit irrigation regimes on yield, berry coloration and bioactive compounds in a commercial vineyard of 'Crimson Seedless' cv. was evaluated during three consecutive years (2011-2013). Four irrigation treatments were assayed: (i) a Control, irrigated at 110% of seasonal crop evapotranspiration (ETc), (ii) regulated deficit irrigation (RDI) irrigated similar to Control levels during pre-veraison and at 50% of the same during post-veraison (a non-critical period); (iii) partial root drying-zone (PRD), irrigated in a similar way to RDI but alternating (every 10-14 days) the dry and wet sides of the root-zone, and (iv) a null irrigation treatment (NI) which only received natural precipitation and occasional supplementary irrigation when the midday stem water potential (Ψ_s) exceeded −1.2MPa. Total yield and fruit quality at harvest were not significantly affected by RDI or PRD. Only NI led to a reduction in yield and the weight of clusters and berries to compare with the other irrigated counterparts. All deficit irrigation treatments enhanced berry coloration and provided a higher crop yield in the first pick harvest compared with the Control treatment. Although RDI and PRD received similar annual volumes of water, PRD induced a greater accumulation of skin anthocyanins and resveratrol, while increasing the soluble phenolic content and antioxidant capacity evaluated at harvest. However, the higher values of anthocyanins observed in PRD could not be explained by higher values of xylem abscisic acid (ABA_{xylem}) because is the phloem which feeds berries during veraison. Overall, our results demonstrate a strong relationship between the total amount of water supplied during the growing season and the main parameters related to yield, water use efficiency and bioactive compounds that are beneficial to health.

Keywords

Yield; anthocyanins; Total antioxidant capacity; resveratrol; flavonoids; water use efficiency

Abbreviations

DI, deficit irrigation; Control, full irrigation; RDI, regulated deficit irrigation; PRD, partial rootzone drying; NI, null irrigation; T, temperature; VPD, vapour pressure deficit; ET_0, reference crop evapotranspiration; k_c, crop coefficient; ET_c, crop evapotranspiration; TSS, total soluble solids; TA, titratable acidity, MI, maturity index; EC, electrical conductivity; L*, lightness; C*, chrome; °h, hue angle; SPC, soluble phenolic content; GAE, gallic acid equivalent; TAC, total antioxidant capacity; AsAE, ascorbic acid equivalent; ABA_{xylem}, xylem abscisic acid; S-ABA, exogenous abscisic acid; Ψ_s, midday stem water potential; θ_v, soil volumetric water content; WUE, water use efficiency; WA, amount of water applied; Y_r, total relative yield; WA_r, relative amount of water applied.

1. INTRODUCTION

New table grape cultivars (*Vitis vinifera L.*) with a commercial high value are constantly appearing because “seedlessness” has stimulated consumer acceptance worldwide. Approximately 80% of Spanish seedless table grapes are produced in warm-climate of the southeast of the country, where the red-table cv. ‘Crimson Seedless’ is one of the most important from an economic point of view. Its characteristic red peel is a consequence of the accumulation of anthocyanins in cells. However, reaching a commercially acceptable red color is problematic, probably because of the high summer temperatures which prevent proper color development (Peppi *et al.,* 2006; Ferrara *et al.,* 2014). Some early studies reported that flavonols stabilize the anthocyanin molecule through co-pigmentation (Singh Brar *et al.,* 2008). Thus, both anthocyanins and flavonols belong to phenolic compounds. They are known to have antioxidant capacity and, in this sense, they have beneficial effects on human health. Antioxidant compounds are able to protect cells from oxidative stress, reducing the effects of neurodegenerative disease such as Alzheimer’s (Dixon and Pasinetti, 2010) and helping to prevent cardiovascular diseases. Some studies also mention their anti-inflammatory activities and anticarcinogenic effects (Doshi *et al.,* 2015). In particular, resveratrol, one of the most important phenolic compound present in grapes, has shown anti-atherosclerosis, anticoronary diseases and anticancer properties, which make it a particularly attractive food ingredient for human health (Flamini *et al.,* 2013). Moreover, flavonols may also show antidiabetic activity (Doshi *et al.,* 2015). Therefore, knowledge of the total antioxidant capacity (TAC) and phenolic profile is essential to health-promoting compounds.

Besides, it is known that the hormone abscisic acid found in the xylem (ABA_{xylem}) is accumulated in grape skins at the same time as anthocyanins and other phenolic compounds also increase (Coombe and Hale, 1973), although they have little effect on total soluble solids (TSS) or titratable acidity (TA) (Peppi *et al.,* 2006).

Environmental constraints and cultural practices have a greater influence on the phenolic compostion and anthocyanins (Flamini *et al.,* 2013). For example, soil water availability has been described as one of the most important constrains limiting grape production and fruit quality (Williams and Matthews, 1990). One way to counter water shortages is to apply deficit irrigation (DI) strategies, among which regulated deficit irrigation (RDI) and partial root-zone drying (PRD) have been the most commonly assessed. RDI, as defined by Chalmers *et al.* (1981), is based on reducing irrigation during certain periods of the growth cycle when the crops have low sensitivity to water stress. In the case of table grapes, a water deficit is generally applied after veraison, the onset of maturation, since reductions in irrigation before veraison can promote a smaller berry size and lower yield (Conesa *et al.,* 2015). The application of RDI to table grapes decreases water usage with little or no impact on crop yield (Blanco *et al.,* 2010; Faci *et al.* 2014), although, to date, the management of RDI has been driven by the need to control vine vigour and maximise fruit quality rather than the need to improve vineyard water use efficiency (Edwards and Clingeleffer, 2013). PRD is a variation of DI that requires approximately half of the root system to be maintained in a dry state, while the remainder of the root system is irrigated (Dry *et al.,* 1996). The key point behind PRD is to expose part of the root system to the drying soil, leading the roots in this dry part to produce a signal so that the remaining roots in the wetted soil can maintain the water supply of the crop (Kang and Zhang, 2004). PRD also depends on the fact that root-to-shoot signalling (especially ABA_{xylem}) regulates the plant response to drying soil (Stoll *et al.,* 2000). A comparison between PRD and RDI in grapevines reported little or no improvement in crop yield and fruit quality when PRD was used rather than RDI (Romero *et al.,* 2012, 2014). The mechanism involved in the differential yield responses of PRD and RDI were reviewed by Dodd (2009), but no studies have looked at the impact of PRD and RDI on the yield and bioactive compounds of table grapes. The hypothesis is that a controlled water stress applied during post-veraison can improve berry coloration in red-varieties by increasing the bioactive compounds accumulation involved in the berry-ripening process (Peppi *et al.,* 2007). The exogenous application of abscisic acid (S-ABA) is being investigated as a novel strategy to improve the quality of grapes (Ferrara *et al.,* 2013, 2014). Although S-ABA is commonly sprayed on developing clusters to stimulate berry coloration, changes in root-to-shoot ABA_{xylem} signalling induced by variations in soil moisture dynamics may affect berry quality and bioactive compounds.

For these reasons, a 3-year long experiment was carried out on table grapes to (i) determine the effects of different post-veraison DI strategies on yield components, fruit quality and the bioactive compounds involved in the berry-ripening process; and (ii) compare the agronomical response of table grapes to PRD with that observed under a conventional RDI strategy.

2. MATERIALS AND METHODS

2.1. Site description and experimental design

The experiment was conducted over three consecutive years (2011-2013) at a commercial vineyard (*Vitis vinifera L.*) of 10-year-old ‘Crimson Seedless’ vines grafted onto Paulsen 1103 (4x4 m spacing) located in Cieza (Murcia, SE Spain). The experimental field conditions are described in detail in

Conesa *et al.* (2015). Daily meteorological variables (T, temperature; RH, relative humidity; and Prec, precipitation) were recorded by an automatic weather station (CI42-www.siam.es) near the experimental site. The air vapor pressure deficit (VPD) was calculated each day using T and RH data. Daily reference crop evapotranspiration (ET_0) was computed according to the FAO-56 Penman-Montheith equation (Allen *et al.,* 1998). Crop evapotranspiration (ET_c) was determined weekly from the product of ET_0 and the crop coefficient or kc (between 0.2 and 0.8), as proposed by Williams *et al.* (2003).

Four irrigation treatments were assessed: (i) a control treatment (Control) irrigated to satisfy maximum crop water requirements (ETc-110%) throughout the whole growing season; (ii) a RDI treatment, irrigated as the Control except post-veraison, when the vines were irrigated at 50% of the level used for the Control; (iii) a PRD treatment, irrigated as RDI (the same amount of water) but alternating the dry and wet sides of the root-zone every 10-14 days, when 75% of the soil field capacity (≈ 34% determined as gravimetric sample) was reached in the dry root-zone; and (iv) a null irrigation (NI) treatment, which received only rain water and additional irrigations when daily measured midday stem water potential (Ψ_s) was more negative than the established threshold value of -1.2 MPa (Conesa *et al.,* 2012).

The experimental layout was a randomized complete block design with four block-replicates per irrigation treatment. Each replicate consisted of three adjacent rows of vines with six vines per row. The four central vines of the central row were monitored, while the others served as guard vines. A total of 288 vines were involved in this experiment. The vines were fertilised with 105-98-207 kg ha^{-1} $year^{-1}$ of N, P_2O_5 and K_2O, respectively. Canopy management and standard cultural practices included girdling, pruning (based on leaving 8-10 spurs per vine), weed control, and the exogenous applications of S-ABA were the same for all the vines of the experiment, and were carried out by the technical department of the commercial orchard following usual criteria for the area. During the three post-veraison seasons assayed, two applications of S-ABA of 2 L ha^{-1} were sprayed on clusters of the whole experiment to enhance coloration of the berries as well to increase the amount of harvestable clusters at the first pick. This effect was evident and significant in all treatments after 48 hours.

2.2. Vines and soil water status

Midday stem water potential (Ψ_s) was determined every 7-10 days from June to November on six sunny leaves per irrigation treatment (two leaves per replicate of three replications) with a Scholander-type chamber (Soil Moisture Equipment Corp. Model 3000, CA, USA) following the recommendations of Hsiao (1990). For Ψ_s determination (from 12.30 h to 13.30 h GMT), selected mature leaves near the trunk were wrapped in small black polyethylene bags and covered with silver foil at least 2 h prior to measurement. Soil volumetric water content (θ_v) was measured from 10 cm down to a maximum depth of 1 m every 0.1 m with a frequency domain reflectometry (FDR) probe (Diviner 2000®, Sentek Pty. Ltd., South Australia). The effective root depth was 0-50 cm because the soil layer below 60 cm was mainly hard clay (Conesa *et al.,* 2015). Four access tubes (1 per replicate) were installed within the emitter wetting area on randomly selected trees. Frequent measurements were taken between 10.00 and 12.00 h GMT during the three seasons assayed.

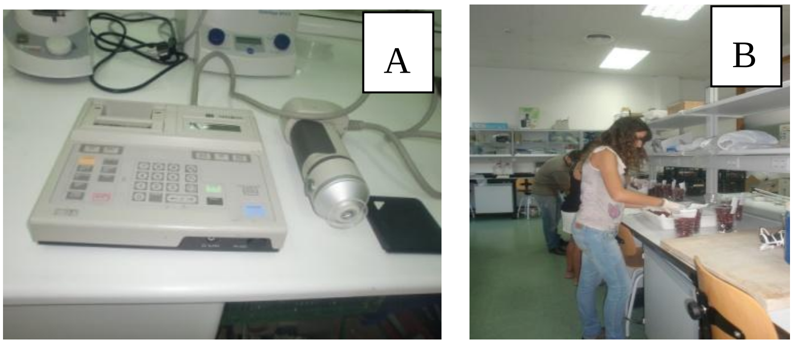
2.3. Yield components and water use efficiency

Berry equatorial diameter was determined weekly from fruit set to harvest by digital calliper (Mitutoyo, CD-15D) on 60 tagged berries (15 berries per replicate). Total yield (expressed as kg per vine) and number of clusters per vine was determined at the time of commercial harvest (from early-September to mid-November) in the all vines treated (72 per treatment) (Picture 1). The exact commercial picking data depended on the year and usually ranged among 3-4 harvestable picks. Weighting was carried out with 60 kg scales (Scaltec, Model SSH 92) with an accuracy of ± 2 g. Average cluster weight (expressed in g) was determined as the yield/number of clusters ratio. Average berry weight was determined using a precision balance (Gram Precision Serie SV-612 CM-R) with an accuracy of ± 0.001 g on the same 100 berries which were used to determine the chemical traits. The number of berries per cluster was calculated as the ratio between the average weight of clusters and the average berry weight. Crop yield (expressed as percentage) was determined as the relation between the total yield obtained in each irrigation treatment and the production registered in each harvestable pick. Water use efficiency (WUE) was calculated as the ratio between yield and total irrigation applied.

Picture 1. *Assessment of total yield at the field*

2.4. Quality traits

Immediately after harvesting, all samples were transported by ventilated car to the laboratory in about 1 h, where they were analyzed. The objective color parameters were recorded in samples of 15 berries per replicate (60 berries per treatment) on three equidistant points of the equatorial zone using a Minolta CR-300 colorimeter (Minolta, Osaka, Japan) (Picture 2A). The CIE L*a*b* system, and the mean values of lightness (L*), red/greenness (a*) and blue/yellowness (b*) coordinates were obtained. Results were expressed in trichromatic coordinates, lightness (L*), chroma [$C^* = (a^*2 + b^*2)^{1/2}$] and hue angle [$^{o}h = \tan^{-1} (b^*/ a^*)$]. Eight panelist (5 men and 3 women; aged 27-65) conducted the classification of subjective color of berries (expressed in percentage) following 5-point categories from different levels of red-color and intensity. For its better identification, this classification was grouped in three categories (Table 1) (Picture 2B).



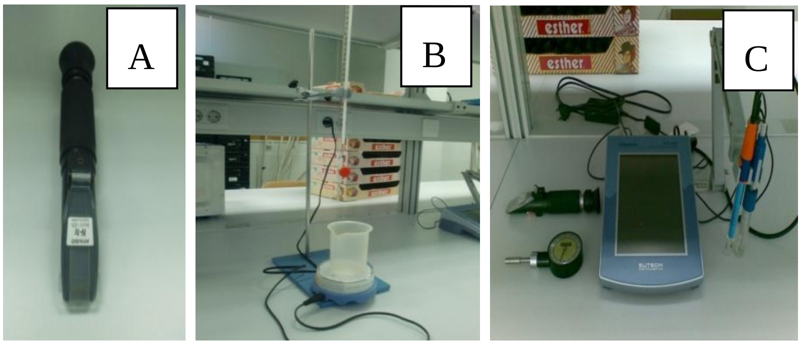
Picture 2. *Minolta CR-300 colorimeter (A) and (B) different assessors to evaluate the subjective color berries*

Table 1. *Classification scale for determining subjective color percentage*

Category	Color	Uniformity
I-II	Pale Pink	Low[z]
III-IV	Moderate-Red	Medium/High
V	Red-Purple	High

[z] *Green zone in the superior basal of the berries*

A total of 400 berries per treatment (100 berries per replicate) were used in the above classification. Then, the same samples were pressed with a juicer (Braun, Model MR-6500, Krongber, Germany) and filtered. TSS values were determined in the berry juice using a hand refractometer (Atago N1, Japan), and expressed as ºBrix (Picture 3A). The TA of the berry juice was determined by titrating 5 mL of juice with 0.1 N NaOH and expressed as g L^{-1} of tartaric acid (Picture 3B). The maturity index (MI) was expressed as the TSS/TA ratio. The pH and electrical conductivity (EC) at room temperature (25ºC) were determined with a Cyberscan instrument (Model PCD-6500, Nijkerk, Netherlands) (Picture 3C).



Picture 3. *Hand refractometer (A) acid titrator instrument (B) and pH meter and conductivity meter (C) used in the experiment*

2.5. Bioactive compounds

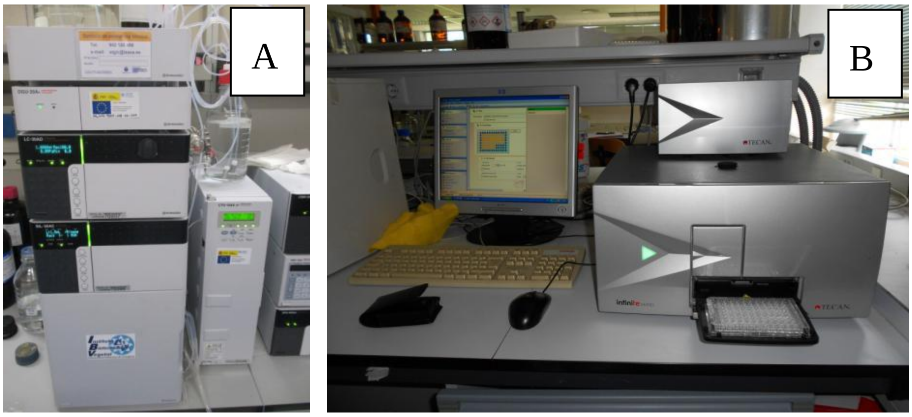
In addition to berry quality traits at harvest, which was assessed in 2011 and 2012, the bioactive compounds were examined during the year 2013. For all the analyses, peel and pulp from the grapes samples were separated, frozen in liquid N_2, ground to a fine powder with a mincer (IKA, A 11 basic, Berlin, Germany) and stored at -80 ºC until analysis. All measurements were evaluated in the peel using three replicates per irrigation treatment.

2.5.1. Soluble phenolic content (SPC)

Aliquots (0.10 g) of frozen, ground peel was placed in glass bottles and 3 mL of methanol (MeOH) /water (7:3, v/v) were added. The extraction was carried out for1 h in an orbital shaker (Stuart, Staffordshire, UK) at 200 x *g* in darkness inside a polystyrene box filled with ice. Then, 1.5 mL of the extracts was transferred to three 1.5 mL microcentrifuge tubes and centrifuged at 15,000 x *g* for 10 min at 4ºC. The amount of SPC in the supernatant obtained was determined according to Swain and Hillis (1959) with slight modifications, as described in Falagán *et al.* (2014). SPC was expressed as gallic acid equivalents (GAE) per 100 g fresh weight (f.w.).

2.5.2. Extraction and quantification of individual phenolic and anthocyanin compounds

Five grams of frozen peel and pulp samples were homogenized with 10 mL of a water:MeOH solution (2:8, v/v) containing 2 mM NaF, according to Tomás-Barberán *et al.* (2001). Homogenates were centrifuged (11,500 rpm, 15 min, 4 °C) and the supernatants were filtered through a 0.45 µm filter. Then, 20 µL of the extracts were analyzed using a UPLC LC-30AD system (Shimadzu Corporation, USA Manufacturing INC, Canby OP, USA) equipped with a degasser (DGU-20A), an autosampler (SIL-30AC), a column oven (CTO-10AS), a communications module (CMB-20A) and a diode array detector (SPDM-20A). The column used was a Gemini NX (250 mm × 4.6 mm, 5 µm) C18 column (Phenomenex, Torrance CA, USA) (Picture 4). The mobile phases consisted of 95% water + 5% MeOH (A); 88% water + 12% MeOH (B); 20% water + 80% MeOH (C); and 100% MeOH (D), following the gradient detailed in Tomás-Barberán *et al.* (2001).



Picture 4. *HPLC (A) and spectofotometer used (B)*

For the quantification of individual phenolics and anthocyanins, external standards were used, according to Artés-Hernández *et al.* (2006). Flavonols were quantified as mg of quercetin 3-glucoside per 100 g f.w. (mg qu 3-glc/100 g f.w.), flavan-3-ols as mg of catechin per 100 g f.w. (mg catechin/100 g f.w.), stilbenoids as mg of resveratrol per 100 g f.w. (mg resveratrol/100 g f.w.) and anthocyanins as mg cyanidin 3-glucoside per 100 g f.w. (mg cy 3-glc/100 g f.w.) (Picture 4).

2.5.3. Total Antioxidant capacity (TAC)

The extraction procedures used were described as for SPC. The TAC was determined according to Benzie and Strain (1996), with the modifications reported in Falagán *et al.* (2014). Results were expressed as ascorbic acid equivalent (AsAE) per100 g f.w.

2.6. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using SPSS (v.9.1) to discriminate between irrigation treatments. When there was a significant difference ($P < 0.05$), means were separated using Duncan’s multiple range test. The two pair-wise comparisons and the interaction treatment x year were also analyzed. Correlation analyses were performed to determine the relationship between the treatments, and the coefficient of determination evaluated the goodness-of-fit of associations among the parameters studied.

3. RESULTS

3.1. Environmental conditions and irrigation volume applied

Each growing season extended from bud-break (early April) to the end of harvest (mid-November). T, VPD, and ET_0 showed upward trends in the three years assayed, until the onset of veraison, reaching maximum average values of 39.4 ± 1.46, 2.91 ± 0.16, 7.57 ± 0.32, respectively (Figs. 1A-C).

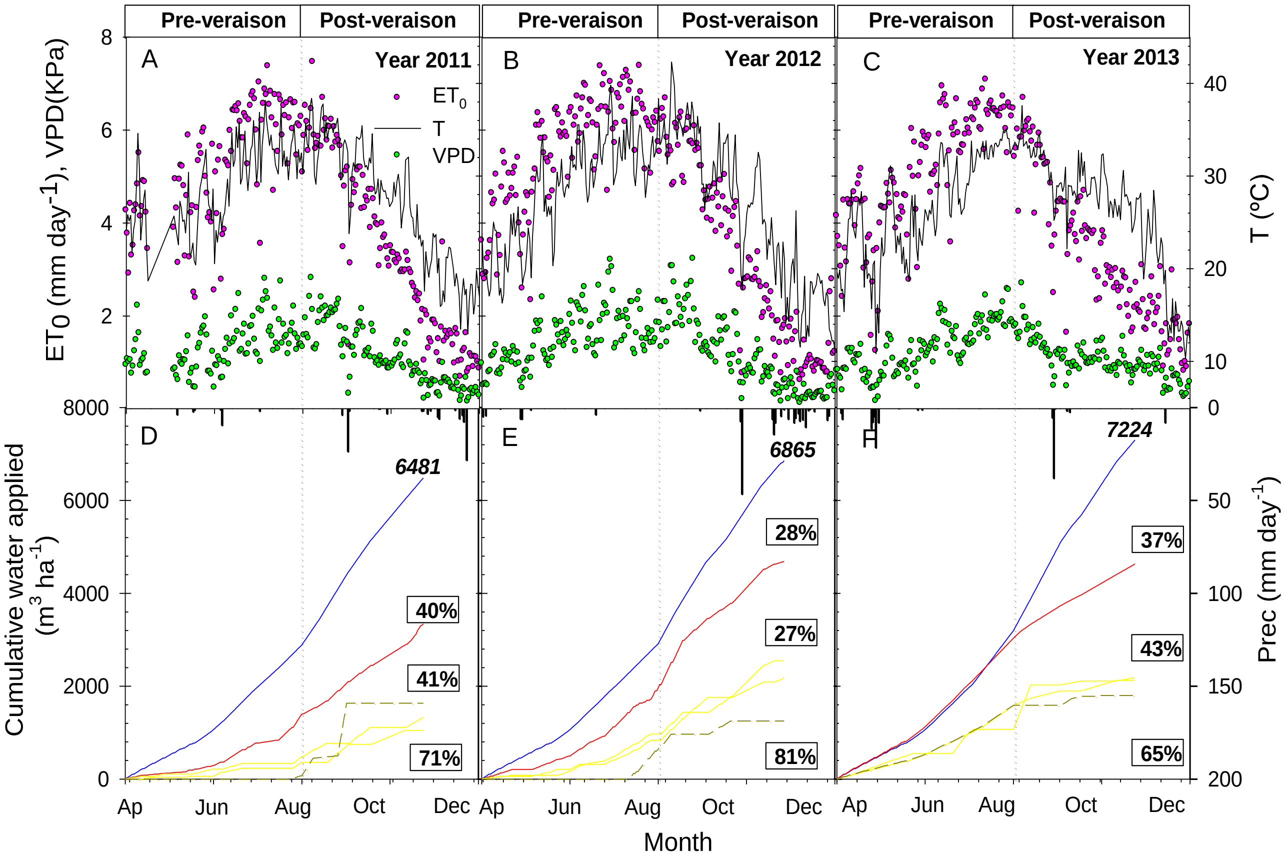


Figure 1. Seasonal evolution of daily reference crop evapotranspiration (ET_0), vapour pressure deficit (VPD) and temperature during the three years assayed (2011-2013) (A-C). Seasonal variation of cumulative water applied and daily precipitation during the same years (D-F). Squares represent the percentage of the reductions of water applied in the moderate deficit irrigation treatments (RDI, PRD) and severe (NI) to respect Control. Precipitation events are shown as vertical bars originating from the x-axis.

Total annual ET_0 values ranged from 1195 to 1274 mm; 2012 was the wettest year, with 375 mm of seasonal precipitation. Dynamics of these environmental variables started to fall in mid- September, coinciding with the harvest period and the lowest climatic demand (Figs. 1A-C).

During the three years studied, irrigation was applied from April to October. The cumulative amount of water applied in the control treatment was 6481, 6865 and 7224 m^3 ha^{-1} in 2011, 2012 and 2013, respectively. For the RDI treatment, water restriction with respect to Control represented 40, 28 and 37%

in 2011, 2012 and 2013, respectively (Figs. 1D-F). These amounts were practically similar for the PRD treatment, whereas NI showed the highest water reductions (71, 81 and 65%) with respect to Control during the same years (Figs. 1D-F).

3.2. Soil water and vine water status

For the 3 years assayed, the θ_v, in Control vines was maintained above field capacity at 0-50 cm depth, averaging 35.6 ± 0.5% (Fig. 2). As expected, the RDI treatment only showed significant differences from the Control during the post-veraison period, with θ_v, values that were 9, 20 and 5% lower than the Control in 2011, 2012 and 2013, respectively. In each year studied, these reductions were slightly greater (expressed as the average of wet and dry side θ_v, values) in the PRD treatment - about 13, 21 and 20%, respectively (Figs. 2G-I). Thus, although both RDI and PRD treatments received the same total volume of water (Fig. 1), PRD suffered a more severe water stress during the three post-veraison seasons. Meanwhile, the NI treatment showed significant differences in θ_v, with respect to the Control during pre and post-veraison periods, averaging values for the 3 years of 27.1 ± 3.1 and 30.3 ± 1.3, respectively.

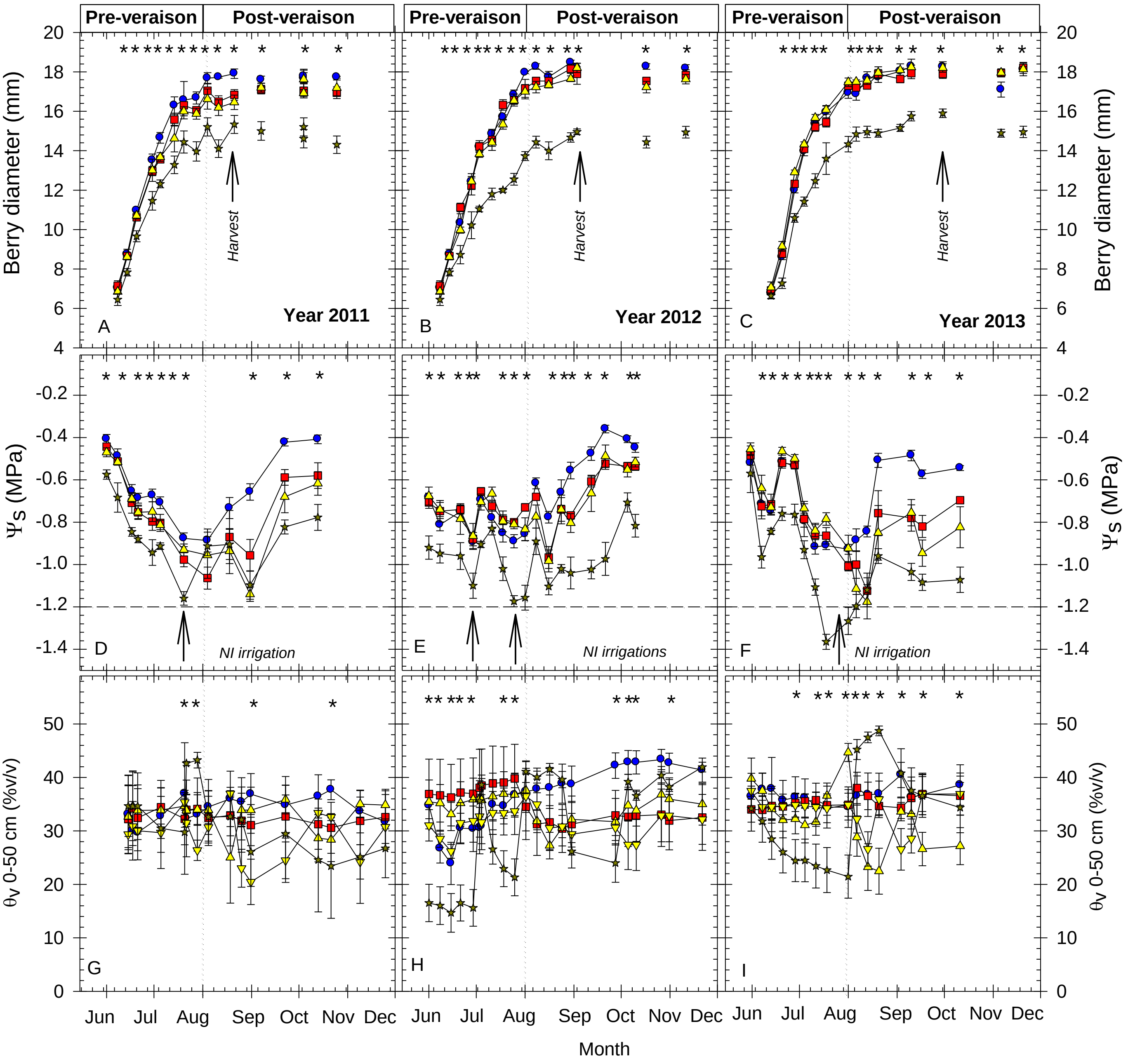


Figure 2. *Seasonal evolution of berry equatorial diameter (A-C), midday stem water potential (Ψ_s, MPa) (D-F) and soil water volumetric content in the profile 0-50 cm (G-I) for all irrigation treatments (●, Control, 🟥, RDI, ▲, PRD and ★, NI) during the three years assayed (2011-2013). Each point is the mean ± SE of n= 60 fruits, n= 6 leaves and n= 4 FDR probes per irrigation treatment, respectively. Asterisks indicate statistically significant differences between treatments by Duncan's multiple range test ($P < 0.05$).*

Control vines registered quite similar Ψ_s values during the study period, with an average value for the three years of -0.65 and -0.68 MPa in pre and post-veraison, respectively (Figs. 2D-F). After veraison, the moderate deficit treatments (RDI and PRD) decreased the values of Ψ_s by around 0.2 MPa compared with Control vines. The severe treatment (NI) showed the lowest Ψ_s values, with an average reduction of 0.2 and 0.3 MPa compared with Control in pre and post-veraison, respectively. When Ψ_s values of NI approached the threshold value of -1.2 MPa, supplementary irrigation was applied. In such cases, the NI treatment exhibited a rapid recovery in both Ψ_s and θ_v, and showed values close to those of the Control treatment. In this way, the previous reductions obtained in the NI respect to Control during post-veraison, were slightly lower than the real conditions submitted in this treatment, because they coincided with the latter supplementary irrigations. Berry equatorial diameter was clearly affected by the severe deficit of the NI treatment, promoting a mean reduction up to 12% compared to Control berries. However, no significant effect on berry equatorial diameter was found in RDI and PRD compared with the Control, reaching 18 mm, approximately (Figs. 2A-C).

3.3. Yield components and water use efficiency

Mean values of total yield, individual mean weight and the number of clusters were significantly higher in 2011 and did not show significant differences among treatments (Table 2). Total yield was not affected by RDI or PRD in any of the 3 years studied. However, the most severely stressed treatment (NI) presented significant yield reductions in 2012 and 2013, lower values that could be explained by the low mean weight of clusters and berries observed in this treatment.

Table 2. *Mean values of total yield , number of clusters, mean weight of clusters, number of berries and mean weight of berries evaluated at harvest during the study period (2011-2013) for all the irrigation treatments: Control (full irrigation treatment), NI (null irrigation treatment, severe deficit); RDI (regulated deficit irrigation treatment, moderate deficit) and PRD (partial rootzone drying, moderate deficit) treatments.*

	Yield components				
Year and Treatment	**Yield (kg vine^{-1})**	**Number of clusters**	**Mean weight of clusters (g)**	**Number of berries**	**Mean weight of berries (g)**
2011					
Control	73	148	493.24	86	5.73 a
RDI	84	158	529.54	92	5.72 a
PRD	78	137	571.78	105	5.42 a
NI	67	135	498.77	124	4.02 b
2012					
Control	79 b	127	622.04 c	68	5.99
RDI	72 b	135	533.33 bc	53	6.00
PRD	62 ab	146	425.78 ab	50	5.23
NI	45 a	130	345.26 a	34	5.40
2013					
Control	68 b	152	446.87 a	67	5.61 b
RDI	66 b	144	457.09 a	64	5.82 b
PRD	65 b	137	474.07 a	68	6.17 b
NI	45 a	142	316.15 b	58	4.57a
Treatment	***	n.s	***	n.s	***
Year	**	***	***	*	n.s
Treatment x Year	n.s	n.s	n.s	n.s	n.s

*Means within columns followed by a different letter were significantly different according to Duncan multiple range test (P<0.05). *. **. *** significant effect at P = 0.05. 0.01 or 0.001, respectively; n.s = not significant.*

Interestingly, the number of clusters and berries was not significantly affected by any of the irrigation regimes in any of the years. Comparing RDI and PRD, no significant difference in total yield or the components of the same were found (Table 2). Moreover, the interaction treatment x year was not affected by any of the treatments.

The highest accumulated yield for the whole period (2011-2013) was obtained in RDI, followed by the Control, PRD and NI. A good linear relationship was obtained between the accumulated yield, normalised with respect to the yield obtained in the Control (Y_r, in %) and the corresponding value of the amount of water applied (WA), normalised with respect to the water registered in the Control (WA_r, in %). The regression line [$Y_r = -52.06 + 1.4WA_r$, $r^2 = 0.90$, $P < 0.05$] shows that in the severe water deficit (NI), the loss of yield was 28% in response to a 72% reduction in water applied. Furthermore, deficit irrigation treatments increased crop yield compared with Control vines at the first harvesting date (Fig. 3), an effect that was particularly pronounced in 2013.

All deficit irrigation treatments presented higher WUE values than the Control. Among them, only the NI treatment was significantly different in the 3 years assayed (Fig. 4). The correlation between the average value of WUE and the WA showed a linear trend [$WUE = 18.89 – 0.00018WA$, $r^2 = 0.97$, $P<0.01$]. Within the range of WA (from 190 mm year^{-1} in NI, to 686 mm year^{-1} in Control), the relationship predicts an increment in WUE with respect to Control of 165%, 169% and 235% for RDI, PRD and NI, respectively.

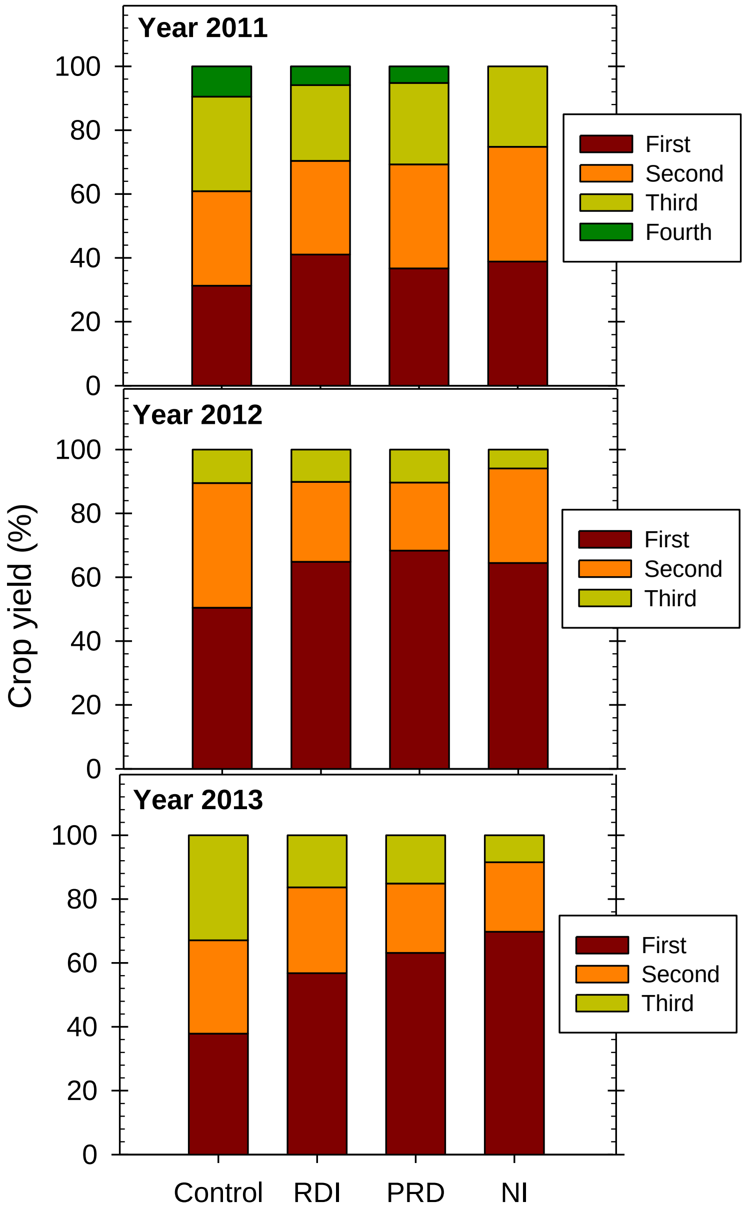


Figure 3. *Crop yield (%) evaluate at each pick (first, second, third and fourth) of the harvest period in all water treatment (Control, RDI, PRD and NI) during the years 2011 (A), 2012 (B) and 2013 (C), respectively.*

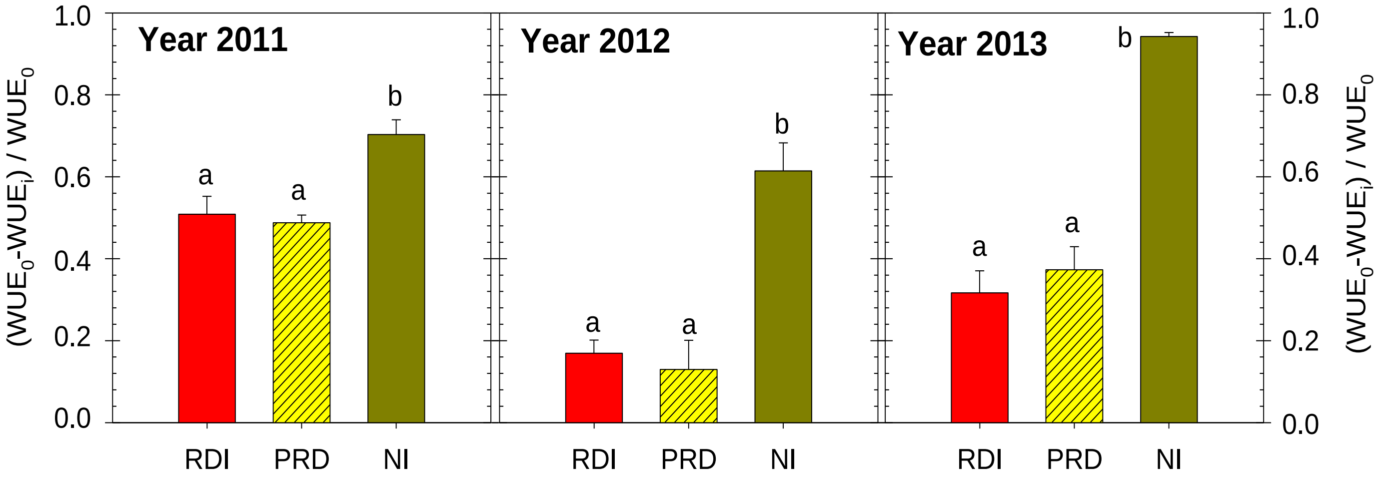


Figure 4. *Water use efficiency (WUE) determined for the deficit irrigation treatments with respect to Control in the three years assayed (2011-2013). Data from Fig. 1 and Table 2 were used for the analysis. Subscripts 'i' and 'o' refer to deficit and fully irrigated, respectively. In each panel, columns with different letters denote significant differences according to Duncan's multiple range test (P < 0.05).*

3.4. Fruit quality

Overall, fruit quality at harvest was affected differently by each deficit irrigation treatment, depending on the growing season. TSS significantly increased in 2011. However, no significant inter-annual differences were found between treatments as regards TA, MI and CE (Table 3). The pH was clearly affected by deficit irrigation and also by the year considered. Both TSS and TA were strongly correlated with the berry diameter, and increases in TSS imply decreased TA in large berries (data not shown). With regard to skin color parameters, C* and L* changed significantly with the irrigation treatment and year studied (Table 3). However, ºh was only significantly higher in the Control during 2012, and so was unaffected by the irrigation treatment, year or their interaction. Of note is the fact that RDI and PRD treatments provided the most intense redness (reflected by lower C* values) in 2011 and 2013, respectively. In contrast, L* values were highest in the Control during the same years. The interaction treatment x year was more significant for C* than the remainder of the parameters studied, reflecting its greater sensitivity to water deficit (Table 3). Furthermore, the classification of subjective color led to approximately 80% of RDI, PRD and NI berries being included in the category III-IV, considered as the optimum in terms of marketability (Table 4).

Table 3. *Mean values for the chemical parameters (TSS, total soluble solids; TA, titratable acidity, MI, maturity index; ph and EC, electrical conductivity) and skin color parameters (hº, hue angle; C*, chrome; L*, lightness) evaluated at harvest during the study period (2011-2013) for all the irrigation treatments: Control (full irrigation treatment), NI (null irrigation treatment, severe deficit); RDI (regulated deficit irrigation treatment, moderate deficit) and PRD (partial rootzone drying, moderate deficit) treatments*.

	Quality traits							
	Chemical parameters					Skin color parameters		
Year and Treatment	**TSS (ºBrix)**	**TA (g L^{-1})**	**MI**	**pH**	**EC**	**h°**	**C***	**L***
2011								
Control	19.16 a	3.91	49.30	3.31 c	3.49	57.70	13.54 c	30.91 c
RDI	19.20 a	3.99	48.64	3.00 b	3.48	57.46	9.15 a	26.26 a
PRD	19.03 a	3.95	48.42	3.12 bc	3.46	55.58	10.21 a	26.21 a
NI	19.70 b	3.92	51.06	2.90 a	3.43	57.52	11.60 b	27.53 b
2012								
Control	18.03	3.97	46.93	3.70	3.47	57.81 b	12.45	29.36 b
RDI	19.22	4.27	46.53	3.60	3.57	57.69 a	13.55	29.62 ab
PRD	19.14	3.93	49.90	3.63	3.40	57.64 a	13.02	27.91ab
NI	19.35	4.00	49.83	3.60	3.10	57.62 a	13.38	27.73 a
2013								
Control	18.60	4.85	38.57	3.48 b	3.17	57.84	17.59 d	29.20 b
RDI	18.93	4.40	43.79	3.35a	3.02	57.58	11.99 b	24.43 a
PRD	18.33	4.20	43.77	3.46 b	2.47	55.74	9.03 a	26.24 a
NI	19.07	3.75	51.86	3.43 ab	3.03	57.50	14.31 c	24.94 a
Treatment	n.s	n.s	n.s	***	n.s	n.s	***	***
Year	n.s	n.s	*	***	***	n.s	***	***
Treatment x Year	n.s	n.s	n.s	*	n.s	n.s	***	**

*Means within columns followed by a different letter were significantly different according to Duncan multiple range test (P<0.05). *. **. *** significant effect at P = 0.05. 0.01 or 0.001, respectively; n.s = not significant.*

Table 4. *Classification of subjective color from berries expressed as a percentage of all the irrigation treatments: Control (full irrigation treatment), NI (null irrigation treatment, severe deficit); RDI (regulated deficit irrigation treatment, moderate deficit) and PRD (partial rootzone drying, moderate deficit) treatments.*

		Subjective color (% Berries)			
Year	**Category**^{Z}	**Control**	**RDI**	**PRD**	**NI**
2011	**I-II**	17	17	17	14
	III-IV	82	82	81	84
	V	1	1	2	2
2012	**I-II**	30	25	21	7
	III-IV	63	75	77	75
	V	7	0	2	18
2013	**I-II**	49	27	18	19
	III-IV	51	73	82	73
	V	0	0	0	7
2011-2013	**I-II**	32	23	19	13
	III-IV	65	77	80	77
	V	3	0	1	9

^{z}Category: I-II: pale-pink (low color); III-IV: moderate-red (optimal color); V: red-purple (excessive color)

3.5. Bioactive compounds

The main bioactive compounds analyzed at harvest in grape peel are shown in Figure 5. As regards the flavonols (Fig. 5A), NI berries presented the highest concentration (0.43 ± 0.05 mg qu 3-glc/100 g f.w.) followed by PRD and Control berries (0.31 ± 0.01, 0.27 ± 0.01) and lastly those of the RDI treatment (0.11 ± 0.01 mg qu 3-glc/100 g f.w.). For flavan 3-ol, PRD followed by NI induced a significantly higher content than the other irrigation strategies (Fig. 5B).

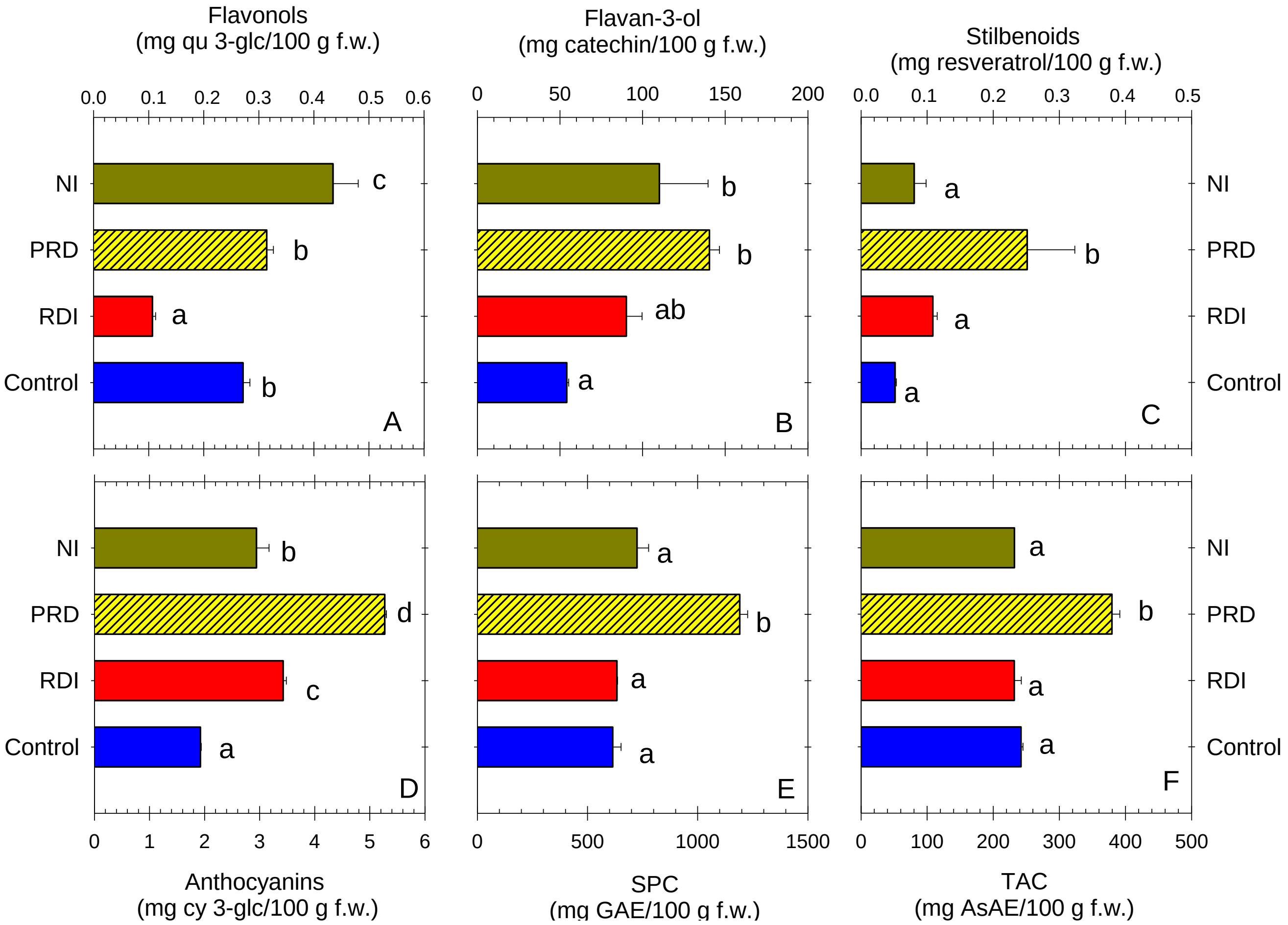


Figure 5. *Mean values of flavonol (A), flavan-3-ol (B), resveratrol (C), anthocyanins (D), SPC, soluble phenolic content (E), and TAC, total antioxidant capacity (F) determined at harvest in berries of (Control, RDI, PRD and NI). Data correspond with the year 2013. Bars are the means ± SE (n = 3). Vertical bars indicate the standard error. Columns with different letters denote significant differences according to Duncan's multiple range test ($P < 0.05$).*

Indeed, the flavan-3-ol content in PRD was nearly 3-fold higher than in the Control samples (140.41 ± 6.15 *versus* 54.05 ± 1.23 mg catechin/100 g f.w.). In the case of the stilbenoid content, PRD berries also showed the highest content compared with the other treatments (Fig. 5C), where no differences were found among Control, RDI and NI (0.25 ± 0.07 *versus* 0.08 ± 0.01 mg resveratrol/100 g f.w.). The PRD treatment also presented the highest anthocyanins values (5.27 ± 0.03 mg cy 3-glc/100 g f.w.), followed by the RDI (3.42 ± 0.06 mg cy 3-glc/100 g f.w.), NI (0.23 mg cy 3-glc/100 g f.w.) and

Control (1.92 ± 0.01 mg cy 3-glc/100 g f.w.) treatments (Fig. 5D). As can be seen, flavan-3-ol showed the most abundant bioactive compounds in grape peel. As expected from previous results, PRD showed a higher SPC content than the other irrigation treatments (Fig. 5E). Finally, the TAC in grape peel was 35% higher in PRD berries (390 mg AsAE mg/100 g f.w.) than in those subjected to RDI, NI or Control (Fig. 5F).

4. DISCUSSION

Recently, post-veraison RDI strategies have been successfully used in vineyards demanding high quality table grapes in order to solve a variety of problems, such as minimizing berry cracking in ‘Autumn Royal’ (Blanco *et al.,* 2010; Faci *et al.,* 2014) and improving the coloration of ‘Crimson Seedless’ (Faci *et al.,* 2014). However, despite increasing interest in PRD to improve grape and wine quality (Chaves *et al.,* 2007; Intrigliolo and Castel, 2009), few studies have examined table grapes. To the best of our knowledge, no report on the effects of RDI and PRD on bioactive compounds can be found in the literature.

Control vines exhibited Θ_v values above field capacity and the vine water status values assessed through Ψ_s were within the range of non-stress conditions for vines (Sellés *et al.,* 2004; Conesa *et al.,* 2012). Before veraison, Control, RDI and PRD showed similar θ_v, and Ψ_s, whereas during post-veraison, both water stress treatments (RDI and PRD) were 0.2 MPa lower than in the Control (Fig. 2). Despite receiving the same amount of water applied, the post-veraison reductions in θ_v, with to respect Control were slightly greater in PRD than in RDI. However, this was not observed in the trend of $\Psi_{s.}$ presumably due to a higher stomatal closure. Indeed, alternating cycles in PRD can maintain favourable plant water relations in the wet side, whereas dehydration in the other side will induce chemical signalling (mainly ABA_{xylem}) that leads the leaves to reduce stomatal conductance and/or growth (Chaves *et al.,* 2010). The NI treatment promoted a reduction of 0.2 and 0.3 MPa in the values of Ψ_s with respect to the Control during pre and post-veraison, respectively (Fig. 2). Therefore, the severe water stress (0.2 MPa) reached in NI during pre-veraison promoted a reduction in the berry diameter (Fig. 2). Before veraison, carbohydrates are imported through the xylem for use in seed development, cell division, and berry growth (Thomas *et al.,* 2006; Chaves *et al.,* 2010). Thus, the lower supply of water and carbohydrates in NI during this period could possibly have induced a decrease in mesocarp cell turgor, reducing berry expansion (Chaves *et al.,* 2010), and affecting berry growth at the end of the season.

RDI and PRD treatments received an average reduction of ~35% of the total water applied to the Control (Fig. 1), without compromising the main yield components assayed (Table 2). However, the yield response in NI (~72% lower water applied than Control) was clearly affected by the severe deficit reached and the cumulative effect of the deficit was evident as the experiment progressed (Table 2). This negative impact could be due to the lower photosynthetic capacity (data not shown), which may lead to a reduction in photoassimilates available for grape growth (Chaves *et al.,* 2010), as well as increased cluster transpiration rates and subsequent berry dehydration (Santos *et al.,* 2005).

Crop yield was also higher in the deficit irrigation treatments (NI, PRD and RDI), the harvestable clusters at the first pick increasing compared with Control during the 3-years assayed (Fig. 3). The skin color parameters (mainly C*), but also the subjective color parameters (assessed by the panellists), pointed to an increase in the intensity of the red color in NI, PRD and RDI compared with the Control. Furthermore, all deficit irrigation treatments increased WUE compared with the Control – the greater the intensity of the deficit, the greater the increase of WUE (Fig. 4). Nevertheless, an excessive reduction in the water applied (as observed in NI), results in severe losses of yield whatever suppressed this advantage (Medrano *et al.* 2015).

DI, compared to full irrigation, may also improve berry quality due to an increase in bioactive compounds (Sofo *et al.,* 2012). As previously observed, the moderate deficit of the PRD treatment promoted the highest value of stilbenoid, anthocyanin, SPC and TAC. It also induced higher values of flavonoids such as flavan-3-ols. Phenolic compounds of the grape are divided into non-flavonoid (*i.e.* stilbenoids) and flavonoid compounds (*i.e.* flavan-3-ols and anthocyanins) (Teixeira *et al.,* 2013). Resveratrol has been attracting attention recently for its benefits to human health (Smoliga *et al.,* 2011; Artero *et al.,* 2015). Its concentration was lower than that of the flavonoids analyzed in this study (Fig. 5). Stilbenoids are mainly synthesized in the skin at the mature stage (Teixeira *et al.,* 2013) and are highly influenced by water stress. In our case, PRD provoke the greatest response in stilbenoid synthesis compared with Control, while RDI and NI made no difference (Fig. 5C).

A similar response was observed in the flavonoids group. The accumulation of anthocyanin was a result of their biosynthesis in skin tissues during veraison reaching a maximum around the harvesting period (Kyraleou *et al.,* 2015). Anthocyanins are the biggest group of water-soluble natural pigments in plants, and are responsible for intense red colors (Hernández-Herrero and Frutos, 2014). In this sense, we observed a close relation between anthocyanin content and skin C* (Fig. 6) and PRD inducing the highest values in both. Water stress clearly affected the total anthocyanin level (Castellarín *et al.,* 2007; Bucchetti *et al.,* 2011) (Fig. 5D). Indeed, vines are able to detect different levels of stress, activating metabolic biosynthetic pathways with more or less intensity, as occurred in the PRD treatment (Kyraleou *et al.,* 2015). Theoretically, in response to the dry soil, PRD produced hormonal signals (mainly ABA_{xylem}) that are responsible for the biosynthesis of anthocyanins in skin through the stimulation of anthocyanin hydroxylation, probably as a result of the up regulation of the gene encoding the enzyme F3′5′H (Castellarin *et al.,* 2007). However, the poor linear correlation [Anthocyanins = 7.49 – 0.0012 ABA_{xylem}, $r^2 = 0.32$, $P = 0.05$] observed between anthocyanins (analyzed in skin) and ABA_{xylem} (from the sap of the leaves) indicates that after veraison, there is no direct link from the xylem to the berries since, at this stage, the connectivity of berry to the vine is via the phloem (Thomas *et al.,* 2006). In this sense, Zarrouk *et al.* (2012) reported that, unlike in leaves, where ABA levels are normally well correlated with the stress degree, ABA levels in the berries fluctuate during maturation, reflecting its role in berry development and ripening.

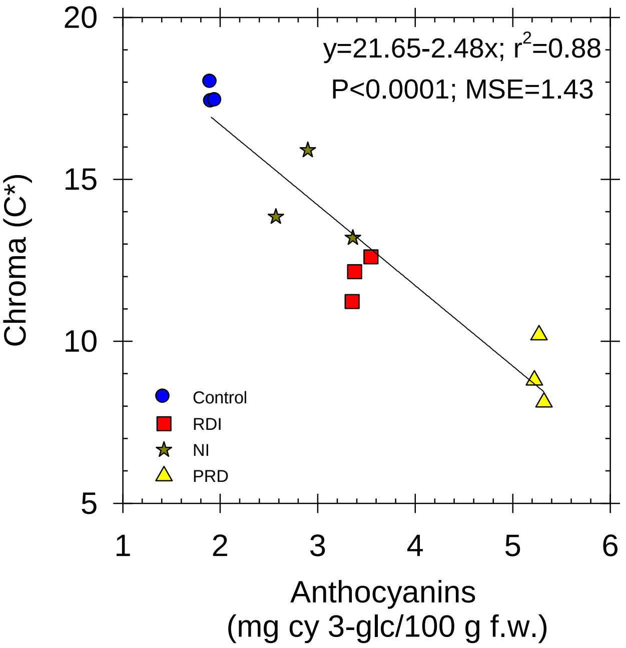


Figure 6. *Relationship between anthocyanins content and the skin Chroma (C*) evaluated at harvest in for all water treatments (●, Control, 🟥, RDI, △, PRD and ★, NI). Values of anthocyanins were the average of the three replicates per treatment. Results of C* were the mean values of 60 berries per treatment.*

The content of SPC was also higher in the PRD treatment (Fig. 5E). In this sense, the alternation of the wet side in PRD provoked a stronger perception of water stress than RDI, so that phenolic biosynthesis pathways were activated as a defense mechanism. This effect was particularly related to root development in PRD. The alternation involved in PRD induced roots of the dry side to extend in depth. This fact could promote a higher area of roots in the surface without water and, as a consequence, the vines would present higher deficit levels. All these compounds are potentially antioxidant due to their ability to scavenge reactive oxygen species. PRD contained the highest amount of bioactive compounds (Fig. 5F) which have antioxidant potential. Subsequently, PRD also showed a higher TAC than the rest of the treatments. We conclude that the application of greater amounts of water (as in Control) is not essential for plant performance and berry development. Indeed, moderate water deficit irrigation (RDI and PRD in this case) can maintain or even improve fruit quality. Both PRD and RDI treatments supposed a saving water of 35% without compromising total yield and its components, while increasing WUE (by about 30%) compared with full irrigation. Moreover, berry coloration increased (evaluated objectively and subjectively) in all deficit irrigation treatments. Compared with a conventional RDI strategy, the main bioactive compounds evaluated (anthocyanins, resveratrol and antioxidant capacity) were highest in PRD, underlining the interest of this technique in field conditions.

5. ACKNOWLEDGEMENTS

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CHAPTER II:

Long-term impact of deficit irrigation on the physical quality of berries in ‘Crimson Seedless’ table grapes

Chapter II: Long-term impact of deficit irrigation on the physical quality of berries in 'Crimson Seedless' table grapes

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Abstract

In table grapes, berry firmness influences consumer acceptance so it is important to avoid berry shattering and dehydration during their postharvest life. Since studies of irrigation effects on table grape quality are comparatively rare, sensory evaluation aimed to identify high-quality berries obtained under different deficit irrigation (DI) treatments. A three-year study examined the effects of DI strategies on some physical quality attributes at harvest, after 28 days of cold storage at 0ºC and after an additional shelf-life period of 3 d at 15ºC. Control vines were irrigated to ensure non-limiting water conditions (110% of crop evapotranspiration, ET_c), while both regulated deficit irrigation treatment (RDI) and partial rootzone drying (PRD) treatments applied 35% less water post-veraison. The null irrigation treatment (NI) only received natural precipitation (72% less water than Control vines). Total yield and physical quality at harvest were not significantly affected by RDI or PRD. Only severe deficit (NI) decreased berry size, and this treatment had the most dehydrated berries and the worst sensory scores postharvest. After cold storage, increased berry shattering of the PRD treatment was correlated with lower leaf xylem ABA concentration at the time of harvest. Overall quality, especially stem browning, determined the shelf-life, and longer storage duration tended to diminish treatment differences. Only NI clusters showed lower quality than their irrigated counterparts. Neither RDI nor PRD had any noticeable effect on berry quality at the end of cold storage and shelf-life, with the slight differences detected between these treatments related to stem browning and dehydration. Sensory results were similar in RDI and PRD, which provided grapes more acceptable to consumers than the Control. Thus, it is possible to decrease irrigation of table grapes without adversely affecting berry physical quality.

Keywords: *Vitis vinifera L.,* water stress, abscisic acid, firmness, shattering, storage performance

1. INTRODUCTION

The cultivation of seedless table grapes (*Vitis vinífera L*) has considerably increased in recent years as a result of increased international demand and new plantings. Spain is the largest producer in Europe of table cultivars and the province of Murcia is the largest in Spain, with 125,000 t cultivated per year, representing 53% of national production (MARM, 2012). 'Crimson Seedless' is a late red-purple seedless table grape widely cultivated for its enormous export value (Conesa *et al.,* 2012b). The fruit is characterized by its excellent eating properties, which include a crisp berry texture and sweet flavor (Faci *et al.,* 2014). The most important characteristic in table cultivars for them to be marketed is firmness, since this parameter provides objective information about their physical properties (Lee *et al.,* 1980; Río-Segade *et al.,* 2013). Moreover, pulp compactness and berry skin consistency are important for customer acceptance of the product (Crisosto and Mitchell, 2002) while knowledge of any firmness indices like skin thickness might also provide fundamental information about when the grapes can be harvested (Sato *et al.,* 1997).

From a storage point of view, berry shattering, decay and stem browning are some of the most important factors limiting their marketability (Cantin *et al.,* 2007; Mahajan *et al.,* 2010). The most robust characteristic of deterioration is loss of firmness, or softening, which may influence not only berry quality but also storage life, transportability and resistance to rotting (Wue *et al.,* 1992). Fruit softening is associated with changes in the cell wall composition and the activity of degradation enzymes, as reported in detail in other grape varieties (Deng *et al.,* 2005). Moreover, softening has also been associated with the flow of carbohydrates and osmotically active nutrients to the fruit due to competition for the accumulated reserves between vegetative and reproductive growth and the differences in the movement of solutes as a result of phytohormonal action (Ruiz *et al.,* 1994).

The need to optimize available water resources in Mediterranean areas has led to development of new water saving techniques, which have increased crop water use efficiency. Among such strategies is regulated deficit irrigation (RDI), as defined by Chalmers *et al.* (1981), which is based on reducing irrigation during certain periods of the growth cycle when the crops have a low sensitivity to water stress, from veraison in the case of table grapes (Conesa *et al.,* 2012). Partial rootzone drying (PRD, Dry *et al.,*1998) is a technique that requires approximately half of the root system be allowed to dry, while the remainder of the root system is irrigated (Chaves *et al.,* 2007). Much is known about the effects of both techniques on shoot physiology and berry composition for many cultivars of *Vitis*, although little information exists for *'Crimson Seedless'*. Moreover, most scientific contributions related with firmness indices deal with ripening stages (Abbal *et al.,* 1992; Faci *et al.,* 2014; Río-Segade *et al.,* 2013) but no information exists concerning the effects of deficit irrigation on the physical properties of the berries of this table grape cultivar at harvest and during storage at different temperatures.

Minimum values of stem water potentials (Ψ_s) of −0.85 MPa reached during pre-veraison promote reductions in berry growth, whereas after veraison berry growth is insensitive to higher vine water deficits (minimum Ψ_s of −1.6 MPa) (Thomas *et al.,* 2006). These changes in growth may be related to berry water relations, since mesocarp cell turgor pressure decreases approximately at veraison and becomes insensitive to water deficits post-veraison (Matthews *et al.,* 2009).

An alternative view is that berry growth and quality may be regulated by changes in chemical compounds induced by water stress. ABA is an essential hormone that regulates crop responses to various environmental challenges including drought, salt and cold stresses (Leung *et al.,* 1998). In grape berries, ABA has been considered to promote ripening and also regulate several processes concerning anthocyanin biosynthesis in colored cultivars (Jeong *et al.,* 2004; Antolín *et al.,* 2008). Most of the work on PRD irrigation in grapevine has focused on the effects of root-to-shoot ABA signalling on stomatal aperture and leaf expansion (Stoll *et al.,* 2000), which may limit vegetative growth thereby favouring reproductive growth but without signal penetration into the fruit (Jeong *et al.,* 2004). This hypothesis is supported by the observation that xylem transport to berries decreases after veraison (Bondada *et al.,* 2005; Antolín *et al.,* 2008; Niculcea *et al.,* 2013). Nevertheless, different irrigation techniques can alter ABA concentrations measured in berries (Bondada *et al.,* 2005), which may alter berry quality.

This work aimed to evaluate the long-term effects of both water saving strategies (RDI and PRD) applied during post-veraison on the physical quality, especially berry firmness at harvest, during cold storage and after a subsequent simulated retail sale period. The possible involvement of ABA as a component of the berry-ripening process and fruit quality was also assessed.

2. MATERIALS AND METHODS

2.1. Experimental conditions and irrigation treatments

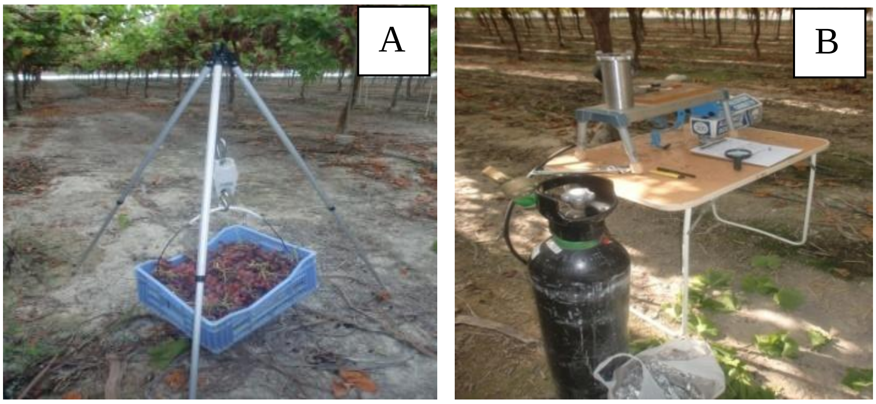
The study was carried out in a commercial vineyard located in Cieza (Murcia, Spain) during three consecutive growing seasons (2011-2013). The plant material consisted of 72 vines (*Vitis vinifera, L.)* per treatment of cv *'Crimson Seedless'* grafted onto Paulsen 1103 (*V.Berlandieri R.*× *V. Rupestres du Lot*), spaced at 4 x 4 m. The vineyard was trained on an overhead trellis system and was covered with a net made of a thread warp of high-density poly-ethylene to protect the vines (from hail, birds, and insects) at a height of ≈ 3.0 m above ground level, just above the canopy level. The vines (ten-years-old at the beginning of the trial) were drip irrigated using one drip irrigation line for per row, with four emitters of 4 L h^{-1} each per vine. The upper soil layer comprised ~ 60 cm of clay-silt-loam (37% clay, 46% silt, 17% sand) with a bulk density of 1.25 g cm^{-3}, organic matter content of 2.1 % and soil pH of 8.6. Below this layer, the substrate was mainly a clay hard soil layer. The irrigation water, sourced from the Tagus-Segura Transfer system, had an average electrical conductivity ($EC_{25ºC}$) close to 1.3 dS m^{-1}.

Four irrigation treatments were imposed: (i) a Control treatment (Control) irrigated at 110% of crop evapotranspiration (ET_c) to ensure non-limiting soil water conditions; (ii) a regulated deficit irrigation treatment (RDI), irrigated as the Control except during post-veraison (a non-critical period), when the vines were irrigated at 50% of the level used for the Control; (iii) a partial rootzone drying treatment (PRD) irrigated as RDI but alternating (every 10-14 days) the dry and wet sides of the rootzone, when 75% of the soil field capacity (≈ 34%, determined as gravimetric sampling) was reached in the dry rootzone, and (iv) a null irrigation (NI) treatment, which received only rain water and occasional supplementary irrigation when the stem water potential (Ψ_s) exceeded -1.2 MPa (Table 1).

Crop evapotranspiration (ET_c) was determined weekly from the product of reference crop evapotranspiration ($ET_{0,}$ Allen *et al.,* 1998) and the crop coefficient (K_c) reported by Williams *et al.* (2003). Treatments were distributed according to a completely randomized block design with four replications. Each repetition consisted of three rows of 6 vines each. Standard cultural practices such as pruning, girdling, weed Control, fertilization and the exogenous applications of S-ABA during post-veraison to improve berry coloration were the same for the trees of all the treatments, and were carried out by the technical department of the commercial orchard following usual criteria for the area. The amount of S-ABA used was 4 L ha^{-1} divided in two applications on the whole of the plot.

2.2. Total yield, plant water status and leaf xylem ABA concentration

Total yield was determined as the average cluster weight of 72 vines for each treatment (18 vines per replicate) (Picture 1A). Midday (12:00 h solar time) stem water potential (Ψ_s) was monitored weekly under field conditions with a pressure chamber (Soil Moisture Equipment Corp. Model 3000) on 6 sunny leaves per irrigation treatment (two leaves per replicate of 3 replications) from close to the main trunk according to the procedure described by Hsiao (1990) (Picture 1B). Xylem sap was collected every fortnight (prior to alternating the wet- and dry-zones of PRD plants) at predawn (between 05:00 and 6.30 hours GMT) by detaching a leaf, measuring predawn water potential and then applying an overpressure of between 0.3 and 0.5MPa for 1-3 min with a glass pipette. Sap was immediately transferred to an Eppendorf tube, frozen in liquid nitrogen and stored at -20 ºC prior to ABA measurement with radioimmunoassay (Quarrie *et al.,* 1988), using the monoclonal antibody AFRC MAC 52.



***Picture 1.** Measurement of total yield (A) and plant water status with pressure chamber at the field (B)*

2.3. Postharvest storage and shelf-life

Having assessed berry quality at harvest in 2011 and 2012, during 2013 the commercial postharvest life was also examined. Harvested clusters (2 October, 2013) from each irrigation treatment were immediately transported about 120 km by air conditioned van to the Universidad Politécnica de Cartagena and were immediately air precooled at 0ºC.

Clusters from all treatments were harvested with total soluble solids content of 19.5 ºBrix and titratable acidity of 3.5 expressed as g of tartaric acid per L^{-1}, respectively. The following morning, clusters were selected on the basis of uniform size, color, firmness and freedom from evident diseases (Picture 2). They were randomly distributed into two boxes per irrigation treatment with three replicates per box and three clusters per replicate (about 500 g each).

The cold storage and shelf life experiment lasted up to 28 days at 0ºC and 90±2% relative humidity (RH) in air, plus an additional retail shelf-life period of 3 days at 15ºC and 60±5 %.

Picture 2. *Distribution of clusters in box for the postharvest experiment*

2.4. Quality analyses and determinations

Skin thickness

Skin thickness was measured at harvest in 60 berries per irrigation treatment (20 berries per replicate) by removing the skin with a sharp knife. It was washed in order to assure that no pulp was present in the skin before it was measured. After that the skin thickness was measured with a manual Spherometer (Model 74-115064, Interapid, Bern, Switzerland).

Geometrical characteristics

To geometrically characterize the berries, the equatorial and polar diameters were determined with a digital caliper (Picture 3) at harvest and during storage in 20 berries per replicate (60 berries per treatment).

Picture 3. *Measurement of berries geometrical characteristics with digital calliper*

By simulating the berry shape as an ellipsoid (Rio-Segade *et al.,* 2013), the area, volume and the rate area/volume were calculated following the models described by Cheung and Yen (1996) with some modifications.

Suppl. Figure 1 shows an ellipse in x-y coordinates. The letters ‘a’ and ‘b’ represent the major and minor axes of the ellipsoid, respectively. Area can be obtained by rotating the ellipse on the x axis.

$$dA = 2\pi y d(x) \qquad \text{[Equation 1]}$$

By integrating the various elements (-a) to (a), we can obtain the expression of the surface area:

$$A = 2\Pi\int_{-a}^{a} y(x)dx = 2\Pi\int_{-a}^{a}\frac{b}{a}(a^2 - x^2)^{1/2}dx = 2\Pi\frac{b}{a}\int_{-a}^{a}(a^2 - x^2)^{1/2}dx = ab\pi^2 \qquad \text{[Equation 2]}$$

The berry volume was also calculated as an ellipsoid and was obtained by integrating the differential volume (-a) to (a):

$$V = \Pi\int_{-a}^{a} y(x)^2 dx = \Pi\int_{-a}^{a}\left[\frac{b}{a}(a^2 - x^2)^{1/2}\right]^2 dx = \Pi\frac{b^2}{a^2}\int_{-a}^{a}(a^2 - x^2)dx = \frac{4}{3}\pi ab^2 \qquad \text{[Equation 3]}$$

As summarized, the equations obtained were:

$$A(cm^2) = ab\Pi^2 \qquad \text{[Equation 4]}$$

$$V(cm^3) = \frac{4}{3}\Pi ab^2 \qquad \text{[Equation 5]}$$

$$Rate\,\frac{A}{V} = \frac{3}{4}\frac{\Pi}{b} \qquad \text{[Equation 6]}$$

Firmness

Berry, pulp and skin firmness were determined at harvest, after cold storage and at the end of the shelf life. Berry firmness (B_F) was recorded as the maximum force to break the berry skin in the equatorial zone using a texture analyzer LFRA 1500 (Brookfield, USA) (Picture 4) equipped with a cylindrical probe of 4 mm diameter and a test speed of 10 mm s^{-1}, which travelled 5 mm until skin breakage. Meanwhile, a piece of skin was removed with a sharp knife to measure pulp firmness (P_F) under the same conditions, with a probe displacement until breakage of 3 mm. Skin firmness (S_F) was calculated as the difference between berry and pulp firmness and expressed the contribution of the skin to the berry turgor. In all cases 20 berries per replicate (60 berries per treatment) were used. The results were expressed in Newtons (N).

Weight loss, decay and berry shattering

Weight losses and decay were recorded after cold storage and shelf-life using a scale with an accuracy of 0.01 g (Great Accuracy ST, Barcelona, Spain) and expressed as a percentage of initial fresh weight. Decay was mainly identified as *Botrytis cinerea* according to the literature and our previous reports (Artés-Hernández *et al.,* 2004; 2006) and the frequency of berries afflicted was recorded. To quantify berry shattering, clusters were manually moved for 3 s. Detached berries were weighed and expressed as % of initial fresh weight (Artés-Hernández *et al.,* 2006). All measurements determined in three clusters per treatment and replicate (n=9).

Picture 4. *Texture analyzer LFRA 1500*

Sensory analyses

Sensory evaluation was performed at harvest, after cold storage and at the end of shelf life period by a panel consisting of eight trained assessors (aged 27–65) screened for their sensory ability according to international standards (Eggertj and Zook, 1986). Visual appearance, flavour, eating texture and overall quality were determined on a 5-point hedonic scale representing acceptance: 1(very poor), 2 (poor), 3 (acceptable, limit of marketability), 4 (good) and 5 (very good) while stem browning, off-flavours and berry softness were determined by the following 5-point hedonic scale in intensity of disorder: 1 (extreme), 2 (severe), 3 (moderate, limit of marketability), 4 (slight) and 5 (none) based on Artés-Hernández *et al.* (2006) (Picture 5).

PANEL DE CATA PARA LA EVALUACIÓN SENSORIAL

Evaluador: Producto: UVA

Fecha: Estado de Conservación:

MUESTRA		A	B	C	D	E	F	G	H	I	J	K
Apariencia Visual	Aceptación											
Sabor												
Textura al masticar												
Calificación Global												
Pardeamiento Raspón	Alteraciones											
Aromas extraños												
Ablandamiento Grano												
Sabores extraños												

Escalas de puntuación:

Escala de aceptación de 1 a 5	Escala de alteraciones de 1 a 5
1: muy malo	1: Extremo
2: malo	2: severo
3: aceptable como límite de consumo	3: moderado (aceptable límite consumo)
4: bueno	4: ligero
5: muy bueno	5: ninguno

Observaciones del catador:

Picture 5. *Sample of panel used by determining the sensory analyses*

2.5. Statistical analyses

Data were subjected to analysis of variance (ANOVA) using SPSS (v.9.1) to discriminate between irrigation treatments. Values were subjected to the least significant difference test (Duncan) at P<0.05. The two pair-wise comparisons between treatment x year and treatment x storage period, as well as the influence of irrigation treatment, storage + shelf-life period were analyzed. Correlation analyses were performed to determine the relationship between the treatments, and the coefficient of determination evaluated the goodness-of-fit of associations among physical parameters.

3. RESULTS

3.1. Water applied, plant water status and total yield

Weather conditions were characteristic of semiarid areas and averaged (2011-2013) 253 mm precipitation (P) and 1251 mm crop reference evapotranspiration (ET_0) (Table 1).

Table 1. *Seasonal values (April to October) of reference crop evapotranspiration (ET_0), precipitation, and irrigation water applied to the different irrigation treatments: Control; NI, null irrigation (severe deficit); RDI, regulated deficit irrigation (moderate deficit) and PRD, partial rootzone drying (moderate deficit).*

	2011		**2012**		**2013**		**Average**		
	(mm)	%Red[1]	(mm)	%Red[1]	(mm)	%Red[1]	(mm)	SE[2]	%Red[1]
ET_0	1195		1274		1253		1241		
P	188		375		195		253		
Control	648		686		722		685	21	
NI	186	71	133	81	251	65	190	34	72
RDI	387	40	495	28	455	37	446	32	35
PRD	385	41	502	27	409	43	432	36	37

[1] *percentage reduction in water applied (%Red.) relative to Control treatment*
[2] *SE indicates the standard error*

In the three years, the average irrigation water applied to the Control treatment was 685 mm, while the RDI, PRD and NI treatments received 35%, 37% and 72% less than the Control, respectively (Table 1). During pre-veraison, the mean values of Ψ_s in RDI and PRD treatments were close to the Control (≈−0.65 MPa) and NI was 0.2 MPa lower (Table 2). In the post-veraison period, deficit irrigation decreased Ψ_s by around 0.15 MPa in RDI and PRD, and 0.3 MPa in NI respectively, compared to Control plants (Table 2). Similarly, NI had the lowest values of Ψ_{pd} thought the experiment. Values of Ψ_s and Ψ_{pd} of RDI and PRD treatments significantly differed from NI before veraison and to Control and NI after this ripening event, respectively. Before veraison, the mean values of leaf xylem ABA concentration ($[ABA]_{xylem}$) in all treatments were below 2 µM (data not shown), increasing after post-veraison as soil water availability decreased (Table 2). Although PRD treatment had the lowest absolute values of $[ABA]_{xylem}$ (<3 µM) during the experiment, no significant effects of treatment or season time (pre and post-veraison) were detected.

Table 2. *Stem water potential at midday (Ψ_s) and at predawn (Ψ_{pd}) during both phenological stages (pre- and post-veraison) under the different irrigation treatments: Control; NI, null irrigation (severe deficit); RDI, regulated deficit irrigation (moderate deficit) and PRD, partial rootzone drying (moderate deficit).*

	Pre-veraison				**Post-veraison**			
	Control	**RDI**	**PRD**	**NI**	**Control**	**RDI**	**PRD**	**NI**
Ψ_s (MPa)								
2011	-0.61a^z	-0.64a	-0.65a	-0.73b	-0.62a	-0.77b	-0.87b	-0.86b
2012	-0.68a	-0.64a	-0.65a	-0.92b	-0.71a	-0.76b	-0.76b	-0.98c
2013	-0.67a	-0.69a	-0.64a	-0.91b	-0.72a	-0.84b	-0.89b	-1.07c
Average	-0.65	-0.65	-0.64	-0.85	-0.68	-0.79	-0.84	-0.97
Ψ_{pd} (MPa)								
2011	-0.08a	-0.08a	-0.08a	-0.10a	-0.08a	-0.11b	-0.12b	-0.14b
2012	-0.08a	-0.10a	-0.11a	-0.21b	-0.06a	-0.10b	-0.12b	-0.22c
2013	-0.10a	-0.11a	-0.12a	-0.28b	-0.08a	-0.12b	-0.12b	-0.32c
Average	-0.09	-0.10	-0.10	-0.20	-0.07	-0.11	-0.12	-0.23

^{z} Means within rows followed by a different letter within each season (pre- and post-veraison, respectively) indicate significant differences according to Duncan multiple range test ($P<0.05$).

During the first year of the study, there were no treatment differences in yield, due to excessive soil water content in the vineyard at the beginning of the experiment (data not shown), which decreased as the season progressed. Yield of RDI and PRD plants did not differ from the Control during the three seasons. However, the yields obtained in the NI treatment were significantly lower (around 43% and 34% lower than the Control in the years 2012 and 2013, respectively) (Fig. 1).

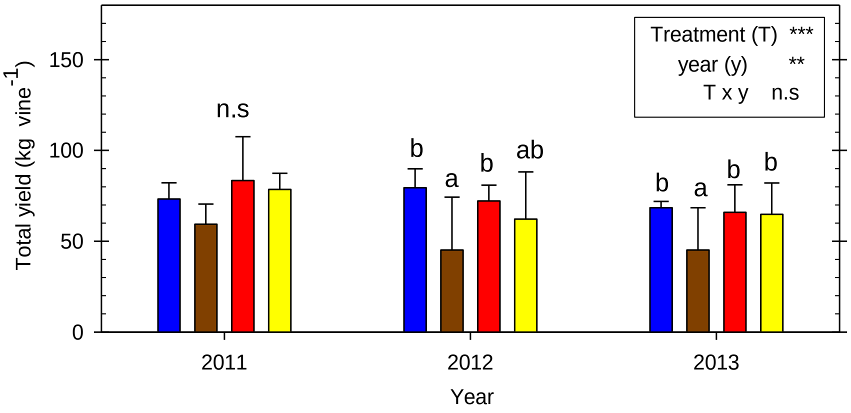


Figure 1. *Mean values of total berry yield (kg vine^{-1}) at harvest in 2011-2013 for the vines in Control (🟦), NI (🟫, null irrigation) RDI (🟥, regulated deficit irrigation) and PRD (🟨, partial rootzone drying) treatments. Bars are means ± SE of 4 replicates (n=18 vines). Different letters within a year indicate significant differences according to a Duncan multiple range test ($P<0.05$). Inset indicates P-values from a two-way analysis of variance using Treatment (T) or year (y) as factors, and the interaction T x y. n.s. = not significant; **, $P<0.01$; ***, $P<0.001$.*

3.2. Influence of water deficit on berry properties at harvest

The mean value of skin thickness was 0.33 mm in Control berries, and remained quite constant in all growing years and irrigation treatments (Table 3). As expected, the most severe irrigation treatment (NI) decreased berry equatorial diameter, whereas PRD and RDI values were similar to the Control in all three years. Smaller berry sizes were also associated with the NI treatment even though the difference was only significant compared to Control values in 2011 and 2012. No differences in berry volume and area were found among the four irrigation treatments during 2013. Interestingly, the area to volume (A/V) ratio was highest in NI in all three years, reflecting the smaller width than obtained in the other irrigation treatments (Table 3). Across all treatments, irrigation volume applied and berry volume (cm^3) were correlated (Fig. 2B), although above 580 mm irrigation the berry volume remains fairly constant.

Table 3. *Mean values for the geometrical and firmness parameters of berries at harvest observed during the study period (2011-2013).*

	Geometrical characteristics						**Firmness parameters**		
	Equatorial diameter (cm)	**Polar Diameter (cm)**	**Area (A) (cm^2)**	**Volume (V) (cm^3)**	**Ratio A/V**	**Skin Thickness (mm)**	**B_F (N)**	**P_F (N)**	**S_F (N)**
2011									
Control	1.76 b	2.72 b	11.86 b	4.45 b	2.67 a	0.35 a	9.69 a	2.73 b	6.96 a
NI	1.50 a	2.17 a	8.08 a	2.58 a	3.14 b	0.32 a	8.47 a	2.19 a	6.09 a
RDI	1.72 b	2.67 b	11.38 b	4.18 b	2.74 a	0.31 a	8.98 a	2.37 ab	6.50 a
PRD	1.71 b	2.59 b	10.38 b	3.89 b	2.79 a	0.33 a	8.04 a	2.31 a	5.73 a
2012									
Control	1.82 b	2.63 b	11.84 b	4.57 b	2.59 a	0.34 a	12.67 ab	2.91 a	9.75 a
NI	1.53 a	2.14 a	8.14 a	2.66 a	3.08 b	0.33 a	10.19 a	2.20 a	7.99 a
RDI	1.74 b	2.45 b	10.57 b	3.91 b	2.71 a	0.35 a	11.53 ab	2.61 a	8.91 a
PRD	1.82 b	2.52 b	11.37 b	4.41 b	2.58 a	0.36 a	13.30 b	2.94 a	10.36 a
2013									
Control	1.71 ab	2.36 a	9.97 a	3.62 a	2.76 b	0.31 a	10.96 b	3.63 a	7.33 b
NI	1.63 a	2.10 a	8.45 a	2.92 a	2.90 c	0.26 a	7.70 a	4.23 a	3.48 a
RDI	1.79 bc	2.39 a	10.64 a	4.06 a	2.62 ab	0.30 a	10.63 b	3.25 a	7.38 b
PRD	1.82 c	2.34 a	10.53 a	4.07 a	2.58 a	0.26 a	9.48 ab	2.69 a	6.78 b
Treatment (T)	***	*	***	***	***	n.s	**	n.s	**
Year (y)	**	n.s	n.s	n.s	*	*	***	*	***
T x Y	*	n.s	n.s	n.s	*	n.s	n.s	n.s	n.s

*^{z} Means within columns followed by a different letter were significantly different according based on Duncan multiple range test (P<0.05). *. **. *** significant effect at P=0.05. 0.01 or 0.001. respectively; ns = not significant.*

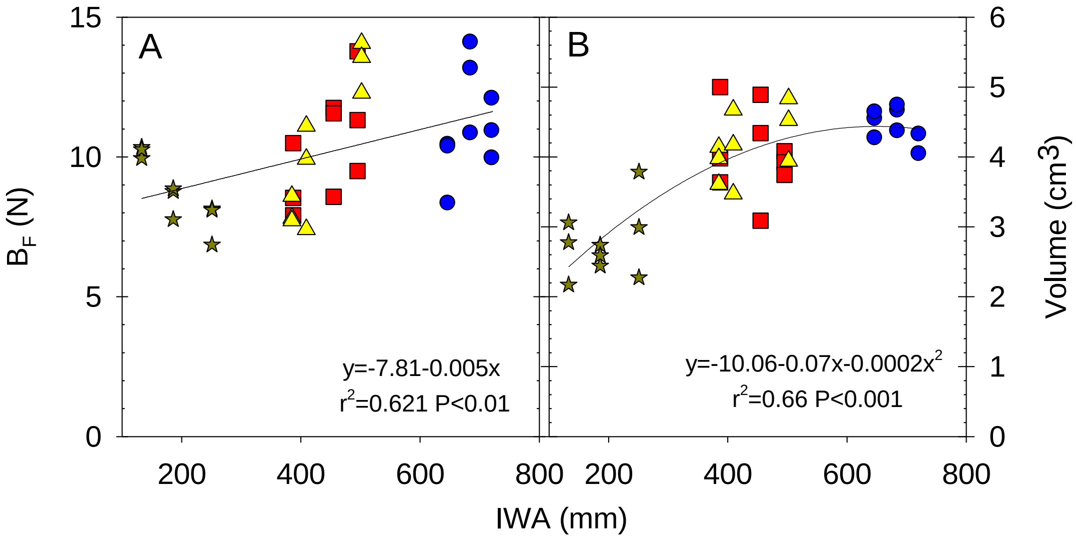


Figure 2. *Relationship between irrigation water applied (IWA) and (A) berry firmness (B_F) and (B) volume. Each point are means ± SE (n=20 berries) of 3 replicates per treatment. Symbols refer to individual Control (●), NI (★) null irrigation (severe deficit), RDI (🟥) regulated deficit irrigation (moderate deficit) or PRD (△), partial rootzone drying (moderate deficit) berries from different seasons years (2011-2013). Regressions were fitted where significant, with r^2 and P-values given.*

At harvest, berry firmness parameters were usually higher in the Controls than in NI, although differences were only statistically significant in the third year of the experiment, while similar to RDI and PRD values (Table 3). The greatest differences among treatments were found for berry firmness (B_F) and for skin firmness (S_F) in the third year, whereas pulp firmness (P_F) was practically unaffected by moderate (RDI and PRD) and severe (NI) water deficit. Increased irrigation water applied (IWA) correlated significantly with increased B_F ($P<0.01$; $r^2=0.62$) (Fig. 2A) although the strength of the correlation was much lower ($r^2= 0.32$ and 0.15) for P_F and S_F, respectively (data not shown). Of particular note was the close dependence between S_F and B_F (Fig. 3). Across all treatments, berry firmness (B_F) and berry volume (V) were weakly correlated.

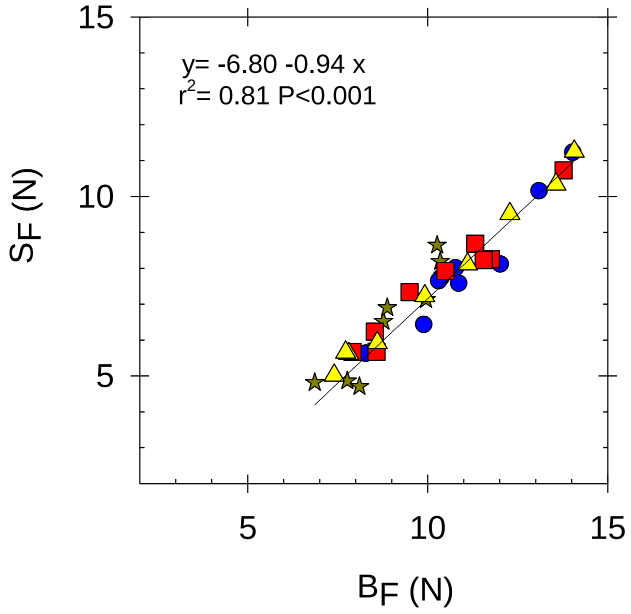


Figure 3. *Relationship between B_F and S_F. Symbols refer to individual Control (●), NI (★) null irrigation (severe deficit), RDI (🟥) regulated deficit irrigation (moderate deficit) and PRD (△), partial rootzone drying (moderate deficit) berries. A linear regression was fitted, with r^2 and P-values given.*

3.3. Influence of water deficit on cold storage and shelf-life

Berry firmness parameters seemed to remain stable during postharvest, except in RDI grapes in which the values decreased following harvest (Fig. 4A). After cold storage, the highest mean values of B_F were observed in Control berries, whereas RDI, PRD and NI values had decreased by 28%, 9% and 40%, respectively, compared to Control values. P_F increased during storage in the Control berries, while S_F increased in Control and also in PRD berries (Fig 4B and C). Moreover, the differences observed in the berry volume, B_F and S_F of the NI treatment at harvest compared with the Control treatment were maintained after 28 d at 0ºC (Fig 4A, C and D).

Increasing temperature to 15ºC, simulating the retail sale period, reduced the differences found at harvest and at the end of cold storage. As expected, all firmness parameters decreased at the end of the shelf-life period (Fig. 4). However, the marketing conditions did not influence berry volume, with NI grapes presenting the lowest values throughout the experiment (Fig. 4D). The irrigation treatment effect (T) was significantly different for all the studied parameters with the exception of P_F (Table 4). The storage condition (Sc) was just significant as regards the polar diameter, while the interaction T x Sc affected the equatorial diameter and the A/V ratio (Table 4).

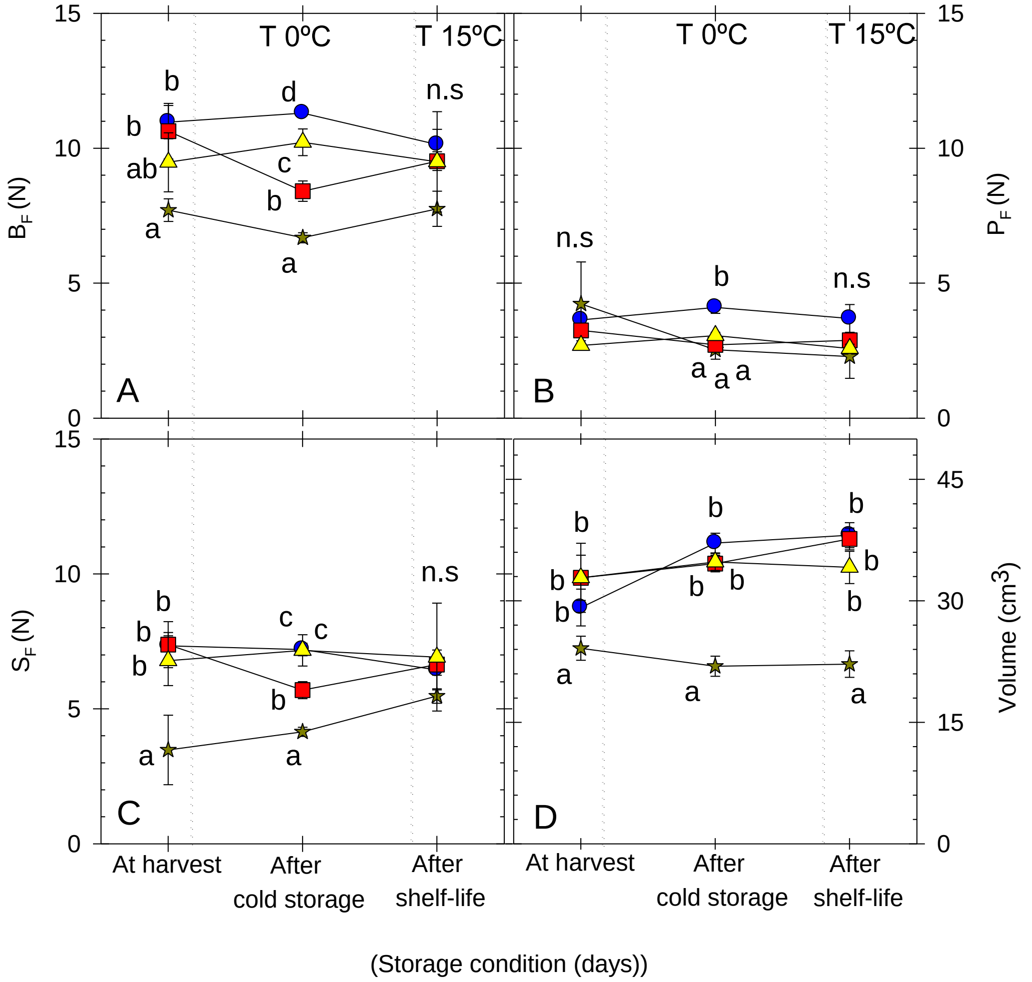


Figure 4. *Seasonal pattern of (A) berry firmness (B_F), (B) pulp firmness (P_F), (C) skin firmness (S_F) and (D) volume at harvest, after cold storage for 28 days at 0ºC or after an additional shelf-life period of 3 days at 15ºC in Control (●), NI (★) null irrigation (severe deficit), RDI (🟥) regulated deficit irrigation (moderate deficit) or PRD (△), partial rootzone drying (moderate deficit) berries. Vertical dashed lines delimit the storage periods. Data are means ± SE (n=20 berries) of 3 replicates per treatment, with different letters at each time indicating significant differences according to a Duncan multiple range test (P<0.05).*

The absolute values of weight loss agreed with the lower values of B_F in RDI treated clusters after cold storage and in Control ones at the end of shelf-life, respectively (Table 5). Accordingly, weight loss (WL) and berry firmness (B_F) were significantly linearly related ($WL = 20.106 - 2.003\ B_F$; $r^2=0.90$ $P<0.001$).

Berry shattering increased with storage time (Table 5). At the end of cold storage, the PRD treatment registered the highest level of berry shattering (5.7%), while NI and Control values were similar at ≈ 2% and RDI showed the lowest values (Table 5). This trend changed after the shelf-life treatment, when NI grapes showed the highest degree of shattering (6.4%), followed by PRD, RDI and Control, respectively. Decay was higher in the Control clusters during cold storage (Table 5). At the end of the shelf-life, an increased incidence of decay was observed in RDI and NI, while PRD clusters maintained the same low percentages in both storage conditions (≈ 0.20%).

Table 4. *Mean values for the quality parameters using treatment and storage condition (28 d at 0ºC and 3 d at 15ºC) as factors.*

Source	**d.f.**[z]	**Sum of squares**							
		Equatorial diameter (cm)	**Polar diameter (cm)**	**Area (cm^2)**	**Volume (cm^3)**	**Ratio A/V**	**B_F (N)**	**P_F (N)**	**S_F (N)**
Treatment (T)	2	0.339 ***	0.978 *	56.370 ***	14.808 ***	0.012 ***	55.415 ***	5.725 n.s	42.176 **
Storage condition (Sc)	3	0.005 n.s	0.686 *	10.142 n.s	1.075 n.s	1.070 n.s	2.064 n.s	2.111 n.s	0.618 n.s
T x Sc	6	0.057 ***	0.067 n.s	5.697 n.s	1.971 n.s	0.140 **	10.768 n.s	5.849 n.s	11.481 n.s
Residual	24	0.039	1.984	39.069	6.044	0.103	45.329	20.911	52.499
Total variance explained (%)	35	91.875	65.199	74.012	79.814	92.797	71.475	62.328	67.039

[z] *Degrees of freedom*

[z] *Means within columns followed by a different letter were significantly different according based on Duncan multiple range test (P<0.05).*

. **. * significant effect at P=0.05. 0.01 or 0.001. respectively; ns = not significant.*

Table 5. *Berry shattering (%); decay (%) and weight loss (%) after a cold storage period of 28 d at 0ºC (CS) and after a subsequent shelf-life period of 3 d at 15ºC (SL) in Control; NI, null irrigation (severe deficit); RDI, regulated deficit irrigation (moderate deficit) and PRD, partial rootzone drying (moderate deficit). Data of the 2013 experiment. P-values comparing irrigation treatments within a storage condition are shown. Data are mean values ±SE of 9 clusters.*

		Control		**NI**		**RDI**		**PRD**		**p-value[z]**	
	Time	**After CS**	**After SL**	**After CS**	**After SL**	**After CS**	**After SL**	**After CS**	**After SL**	**After CS**	**After SL**
Berry Shattering (%)	Mean	2.30	0.95	2.06	6.36	1.13	3.14	5.71	4.84	*	*
	SE	0.96	0.55	0.77	0.65	0.45	0.45	0.71	1.43		
Decay (%)	Mean	0.46	1.08	0	1.13	0	0.65	0.21	0.24	*	n.s
	SE	0.28	1.01	0	1.12	0	0.39	0.12	0.12		
Weight loss (%)	Mean	4.44	6.34	4.47	6.18	5.77	5.10	5.29	5.14	n.s	n.s
	SE	0.28	0.58	0.37	0.62	0.53	0.31	0.61	0.36		

[z] *indicates significant effect according to a Duncan multiple range test (P<0.05). SE means standard error.*

Figure 5. shows the main changes in sensory attributes recorded postharvest. Visually, the grapes of PRD and RDI treatments were more attractive and those of the Control and NI less so, probably as a result of the less intense red color and smaller berry size, respectively (Fig. 5A). No relevant differences between the treatments were observed for flavor during the assayed storage conditions (Fig. 5C). Moreover, no skin and/or pulp browning developed in any treatment at any sampling time, while eating texture remained quite constant and well above the marketability threshold in all treatments (data not shown). No noticeable off-flavours or softness disorders were detected in any treatment at any time during the experiment (data not shown). Stem browning increased during cold storage (Fig. 5B), and was higher in the most severe irrigation treatment (NI). The initial overall quality agreed with the visual appearance (Figs. 5A and D).

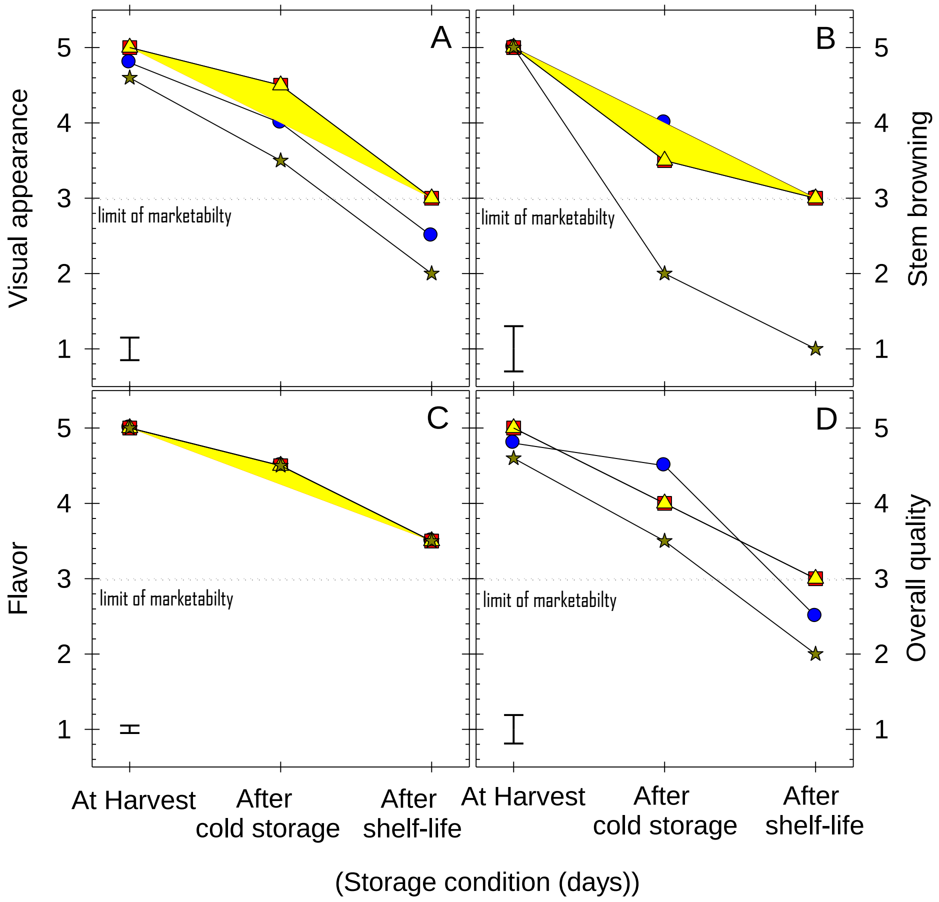


Figure 5. *Sensory score for (A) berry visual appearance (B) stem browning, (C) berry flavor and (D) overall quality of clusters stored up to 28 days at 0ºC (cold storage) plus an additional shelf-life period of 3 days at 15ºC in Control (●), NI*

(★) null irrigation (severe deficit), RDI (🟥) regulated deficit irrigation (moderate deficit) and PRD (△), partial rootzone drying (moderate deficit) clusters. Symbols are the mean of three clusters per replicate (n = 9) with the standard error of the mean in the lower left corner.

After cold storage, the grapes from all the irrigation treatments were above the limit of marketability, the higher values obtained for the Control probably being due to the fresher appearance of the stem. RDI and PRD berries had an attractive intense red color but their stems were slightly more browned than Control ones. Meanwhile, NI recorded the lowest berry size and the most dehydrated stems.

After shelf life, only RDI and PRD grapes could be regarded as marketable, although very close to the limit of marketability (Fig. 5D), an observation that was crucial for establishing the maximum shelf-life period for this experiment. The Control treatment produced a good berry size and moderate stem browning even though the color intensity of the berries was not sufficiently attractive according to the panelists. The NI stems were extremely dehydrated and breakable, while NI berries presented the smallest size and the most intense red-color. No differences were observed between RDI and PRD treatments as regards the sensory parameters studied, both treatments producing what were considered the best grapes in this respect.

4. DISCUSSION

Vines of '*Crimson Seedless'* received a sustained water deficit post-veraison (a non-critical period for crop yield, Conesa *et al.* 2012b) via different DI strategies. Many studies have shown that moderate water deficit applied to grapevines can save water and improve berry quality (Matthews and Anderson, 1989; Chaves *et al.,* 2007; Romero *et al.,* 2012), but many studies evaluated chemical composition of the berries rather than physical attributes.

RDI and PRD treatments received ≈35% less water (Table 1), without compromising total yield (Fig. 1) or the physical quality of the berries at harvest

(Table 3) and throughout a long commercial postharvest period (Table 4). While PRD has out yielded RDI treatments in some studies including grapevine (reviewed in Dodd, 2009), PRD increased berry skin anthocyanin concentration independently of whether vines had greater (Antolin *et al.,* 2006) or lesser (Dos Santos *et al.,*2003) vegetative vigour. Differential responses of PRD/RDI crops may result from three possible mechanisms (Dodd, 2009): (i) different soil water availability due to differences in soil evaporative losses; (ii) differences in root-to-shoot phytohormonal (ABA) signalling; and (ii) different resource allocation caused by the alternating wet/dry cycles in PRD. Nevertheless, in '*Crimson Seedless'* vines, there were no yield or quality differences between PRD and RDI vines consistent with the similar soil water availability (Table 2).

The water status of the Control, RDI and PRD treatments before veraison, as assessed by midday stem water potential (Ψ_s), was similar in the three years of study (Table 2) and characteristic of well-irrigated vines of this cultivar (Conesa *et al.,* 2012a; Sellés *et al.,* 2004). During post-veraison, Ψ_s declined (~ 0.2MPa) in RDI and PRD vines compared to Control, while the NI treatment exhibited a more intense water deficit (around 0.3MPa) (Table 2). Similar Ψ_s values for DI strategies were reported by Williams *et al.* (2010) in '*Thomson Seedless' cv*, with deficit irrigation advancing the date of bud-break compared to well-watered vines. Mean values of Ψ_{pd} and Ψ_s were similar between PRD and RDI at the same irrigation volumes applied (Table 2), although higher Ψ_{pd} was detected in PRD vines after veraison in a pot experiment (Antolín *et al.,* 2008), presumably due to greater stomatal closure.

In previous field experiments with grapevine, alternating irrigation between the wet and dry rootzone sides increased ABA transport to the shoot (Romero *et al.,* 2012). When the same volumes of water are applied (PRD=RDI), higher Ψ_{pd} values in PRD treatment (Romero *et al.,* 2012a and b) would explain lower [ABA] or alternately limited water flow from dry side of PRD plants/greater water flow from the irrigated side of PRD plants (Antolin *et al.,* 2008) but this is inconsistent with the similar Ψ_{pd} of PRD and RDI vines here (Table 2). Alternately, greater penetration of the soil wetting front to deeper in the soil profile, as indicated by greater soil water content in the depth 60-100 cm (data not shown) may indicate decreased evaporative losses as a result of applying the same irrigation volume to a smaller soil surface area. Therefore, pre-harvest treatment (RDI and PRD) effects on [ABA] can be attributed to the changes resulting from the irrigation technique.

Berries from the Control, PRD and RDI treatments surpassed the minimum equatorial diameter required for marketing (≈ 1.6 cm, CARM www.carm.es). As expected, berry volume was clearly affected by the severe continuous deficit of the NI treatment. However, no significant differences were found between the RDI and PRD values and those of the Control (Table 3). '*Crimson Seedless'* cv. berries reached 85% of their final size between fruit set and veraison (Sellés *et al.,* 2004), which might explain the similar final size of Control, PRD and RDI berries since the deficit irrigation was applied post-veraison when there is relatively little (15% of final size) berry growth. Berry volume did not increase if more than 580 mm irrigation water was applied (Fig 3B), suggesting that the Control treatment was over-watered. In addition, Antolin *et al.,* (2008) reported that a decrease in Ψ_{pd} from −0.4 MPa to −0.9 MPa applied throughout veraison decreased berry volume by 30%, consistent with the current study.

Fruit firmness is one of the most important factors which determine postharvest quality and consumer acceptance (Mahajan *et al.,* 2010). Of the firmness parameters measured, berry firmness (considered a measure of freshness, Rio-Segade *et al.,* 2013) was the most affected by the DI strategies applied (Table 3). At harvest, the lowest B_F values were recorded in the most water-stressed treatment (NI) during the three-years of study (Table 3), as in El-Ansary *et al.,* 2005 under similar conditions. Berry softness in '*Dattier*' cv. reflected decreased turgor pressure (Bernestein and Lustig, 1981) and variations in berry firmness were independent of changes in equatorial diameter, as in our results. Furthermore, the low correlation between B_F and P_F underlined the limited contribution of the skin to overall berry firmness (Fig. 4).

After cold storage, the B_F tended to be higher in the Control treatment (Fig. 2A). The declining trend of B_F during storage was more pronounced in RDI, coinciding with weight loss after cold storage (Fig. 2 and Table 5). The hardness of '*Búlida'* apricot submitted to RDI decreased during chilled storage due to structural changes in the middle lamella as well as degradation of cell wall components (Pérez-Pastor *et al.,* 2007). The additional shelf-life period tended to minimize treatment differences in all firmness parameters found at harvest and after cold storage (Fig. 2 and Table 4). Similar results were obtained in '*Fortune'* mandarins exposed to deficit irrigation treatments (Conesa *et al.,* 2014), likely due to epidermal deterioration and an increase in cell wall elasticity (Matthews *et al.,* 2009).

In general, cumulative water loss during postharvest handling caused berry shattering and shriveling. Also, there is a high correlation between cluster water loss and stem browning. When water loss reached more than 2% in '*Flame Seedless'*, *'Perlette'*, *'Thompson Seedless'*, *'Ruby Seedless'* and *'Fantasy Seedless'*, the stems showed symptoms of browning after approximately 7 days in storage (Crisosto and Mitchell, 2002).

Stem browning increased during storage (Fig. 5B), and was higher in the most severe irrigation treatment (NI), probably due to the enzymatic degradation of the stem cell structure caused by the low amount of water received (Martínez-Hernández *et al.,* 2013). Moreover, enzymatic browning in fruits, mostly from the action of polyphenol oxidase (PPO) and peroxidase (POD), can cause undesirable quality changes during handling, processing and storage (Cano *et al.,* 1997). However, the browning potential of some plant products can be directly related to the phenol levels or a combination of both enzymatic activities (PPO and POD) and phenols (Cano *et al.,* 1997). Storage under modified atmosphere can prevent extreme browning. SO_2-treated grapes showed very good visual appearance and lower stem browning after both storage periods without significant differences with Control harvested clusters (Artés-Hernández *et al.,* 2006).

Berry shattering increased during cold storage (Table 5) and at the end of this period, the PRD clusters showed the highest rate of berry shattering (≈5 %). Cantin *et al.* (2007) found no relationship between berry shattering and firmness when lower values of B_F (around 3–4 N) were detected. High values of berry shattering reached in PRD after cold storage coincided with the lower absolute values of $[ABA]_{xylem}$ at the end of post-veraison (Fig. 6), with both parameters strongly correlated when all treatments were compared [$47.58-0.025+4E-0.6x^2$; $r^2=0.98$; $P<0.001$]. However, at the end of shelf-life, the NI treatment had the highest level of berry shattering (Table 5), thus other factors such as stem browning and weight loss should be considered (Fig. 5 and Table 5).

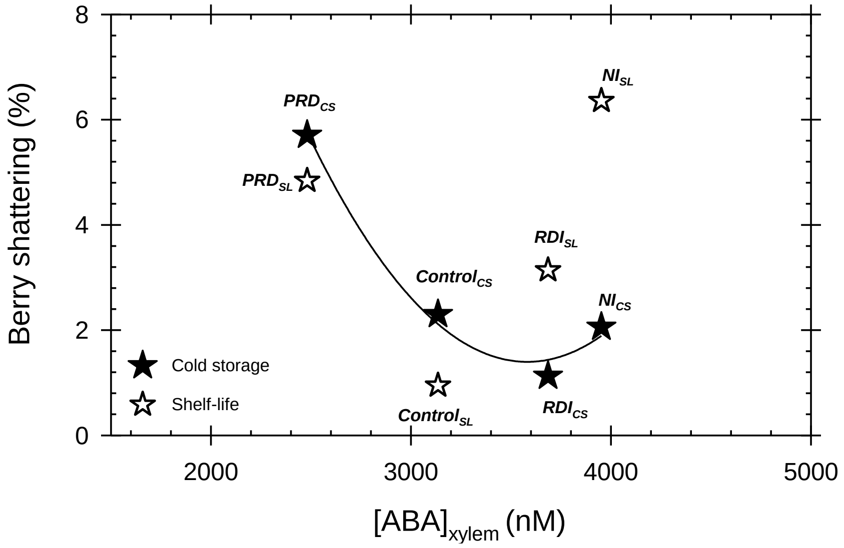


Figure 6. *Relationship between leaf xylem ABA concentration and the percentage of berry shattering post-veraison at the end of cold storage (★) and the additional shelf-life period (☆) in all treatments (Control, RDI, PRD and NI). Values of [ABA] are the average of the seasonal evolution of [ABA] in the field (n=5) from 6 samples for each measurement. Results of berry shattering (%) were the mean values of 9 clusters at the end of cold storage (after 28 d at 0ºC) and shelf-life (3 d at 15ºC). Error bars omitted for clarity.*

After cold storage, the overall quality, visual appearance, and flavor were above the threshold of marketability in all irrigation treatments (Fig 5.A, C and D). The overall quality of the DI grapes determined the shelf-life period. When only the RDI and PRD treatments were above the limit of marketability, the storage experiment ceased. Therefore, a shelf-life period of 3 d at 15°C with 60% RH, representing a sharp increase in temperature and similar sharp decrease in RH, was enough to reduce the differences between treatments compared with those registered at the end of cold storage. Therefore, temperature and RH should be kept at the recommended levels during storage to ensure the longest postharvest life. Martínez-Hernández *et al.* (2013) reported that a high temperature might induce greater water loss, which would be encouraged by air storage. In terms of the sensory analysis, RDI and PRD treatments performed best.

5. CONCLUSIONS

Post-veraison DI strategies applied 35% less water without compromising total yield or physical quality of PRD and RDI vines of '*Crimson Seedless*' table grapes, consistent with the similar soil water availability. Berry volume was only affected by the preharvest severe water deficit ($\Psi_s = -0.97$ MPa) reached in the NI treatment. RDI and PRD did not noticeably affect quality after cold storage while the subsequent shelf-life period tended to minimize the differences found at harvest or at the end of cold storage between NI and the remaining irrigation treatments. RDI berry firmness rapidly declined associated with a higher weight loss after cold storage. PRD resulted in the highest percentage of berry shattering, which was correlated with the lower absolute values of [ABA] induced by the grower's irrigation strategy. At the end of the shelf-life, NI clusters showed the highest rate of berry shattering and obtained the poorest sensory evaluation, mainly due to higher stem browning and dehydration. Generally, most of the physical parameters tested were more affected by pre-harvest irrigation treatment differences than by postharvest storage conditions.

6. ACKNOWLEDGEMENTS

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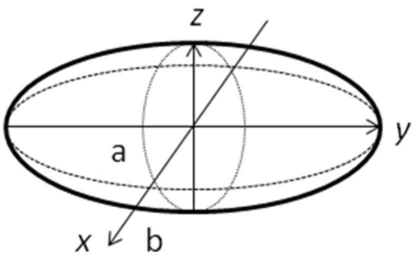
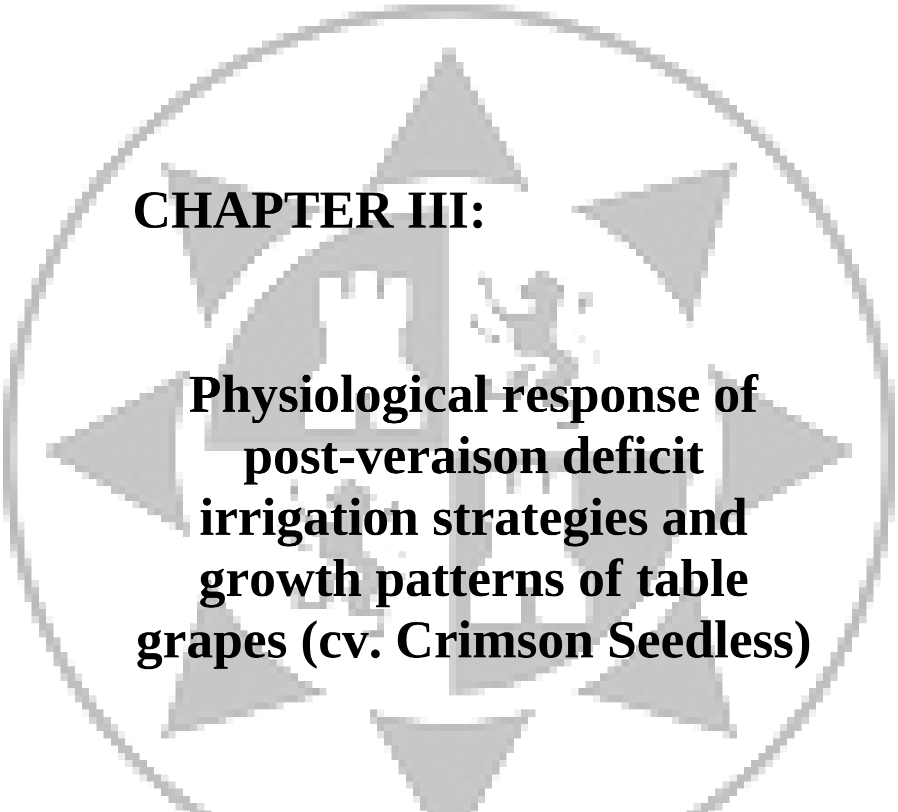


Supplementary Figure 1*. Details of major ‘a’ and minor ‘b’ axis of ellipsoid*



CHAPTER III:

Physiological response of post-veraison deficit irrigation strategies and growth patterns of table grapes (cv. Crimson Seedless)

Chapter III: Physiological response of post-veraison deficit irrigation strategies and growth patterns of table grapes (cv. Crimson Seedless)

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Abstract

To determine whether partial root-zone drying (PRD) optimized leaf gas exchange and soil–plant water relations compared with regulated deficit irrigation (RDI), a 3 year long-experiment was conducted on a commercial vineyard of 'Crimson Seedless' table grapes (*Vitis vinifera L.)*. Four different drip irrigation regimes were imposed: (i) a Control treatment (full irrigated), irrigated at 110% of seasonal crop evapotranspiration (ET_c), (ii), a regulated deficit irrigation (RDI) treatment irrigated similar to Control before veraison and at 50% of the Control treatment during post-veraison, (iii) a partial root-zone drying (PRD) irrigated similar to RDI but alternating (every 10-14 days) the dry and wet side of the root-zone, and (iv) a null irrigation treatment (NI) which only received the natural precipitation and occasional supplementary irrigations when the midday stem water potential (Ψ_s) exceeded -1.2 MPa. PRD induced higher plant and soil water deficit levels than RDI. However, PRD did not significantly reduce stomatal conductance (g_s) and neither xylem ABA concentration or water use efficiency were increased, probably due to a higher root development in depth and root density from PRD and also may be greater water uptake from roots in wet part of the soil profile. Vegetative growth was only decreased by severe deficit irrigation (NI) to except total leaf area index (LAI) that also affected in PRD. Moreover, the use of trunk diameter fluctuations indices (TDF) to ascertain vine water status might also worth before veraison. PRD can be considered a useful strategy in semiarid areas with limited water resources because sustained water use maintained assimilation rates despite greater stress than conventional RDI strategy due to root morphological adjustment

Keywords: Partial root-zone drying; leaf gas exchange; water relations; leaf area index; $[ABA]_{xylem}$; *Vitis vinifera*

Abbreviations: DI, deficit irrigation; RDI, regulated deficit irrigation; PRD, partial root-zone drying, $A_{CO2,}$ net CO_2 assimilation rate; g_s, stomatal conductance; E, transpiration rate; $A_{CO2}/g_{s,}$ intrinsic water use efficiency; $A_{CO2}/E_{,}$

instantaneous water use efficiency; $[ABA]_{xylem}$, xylem abscisic acid; S-ABA, exogenous abscisic acid; θ_v, soil volumetric water content; Ψ_{pd}, predawn leaf water potential; Ψ_s, stem water potential at midday; Ψ_o, leaf osmotic potential; Ψ_{os}, leaf osmotic potential at full turgor; Ψ_t, leaf turgor potential; LAI, leaf area index; TCSA, trunk cross-section area; ΔTCSA, annual increment trunk-section area; PE, productivity efficiency.

1. INTRODUCTION

Crimson Seedless (*Vitis vinifera L.*) is one of the most commercially important and cultivated table grapes cv. in south-eastern Spain, where the predominant climate conditions are those typical of a semi-arid zone: rain scarcity and high evaporative demand (Faci *et al.,* 2014). Under these conditions, irrigation aims to regulate soil water availability to the vines. Table grapes need more water than wine grapes because they require a greater leaf cover, fruit production and larger berry size for fresh consumer (Williams and Ayars, 2005; Silva-Contreras *et al.,* 2012). However, previous work (Conesa *et al.,* 2016) showed that the application of a greater amount of water above maximum crop evapotranspiration (ET_c-110%) is not essential for fruit production and berry development.

Applying deficit irrigation (DI) practices can manipulate the season-to-season water variation to maintain the yield and quality standards required by the fruit market (Ruiz-Sánchez *et al.,* 2010). Within DI, the main techniques are regulated deficit irrigation (RDI) and partial root-zone drying (PRD). Both consist of reducing partially the irrigation during periods of low water stress sensitivity during the growing season (Chalmers *et al.,* 1981; Dry *et al.,* 1996). Table grapes are generally considered tolerant of water stress, where the period from fruit setting to veraison is the most critical stage, since this determines yield and fruit quality. Thus, RDI and PRD have to be applied during post-veraison, when adverse effects on productivity are minimised (Conesa *et al.,* 2016).

The soil water deficit imposed by both techniques (RDI and PRD) might alter vine physiology and also plant hydraulic and chemical signalling systems, thereby affording commercial benefits such as saving irrigation water, increasing water use efficiency (WUE) and decreasing excessive vegetative vigour (Romero *et al.,* 2014). Stomatal conductance (g_s) is decreased by the synthesis of chemical signals (predominantly abscisic acid - ABA) in the roots in response to drying soil, and their subsequent transport to the leaves via the transpiration stream to effect stomatal closure (Puértolas *et al.,* 2015). Globally, in alternate PRD, one part of the root-zone is irrigated at a time, with the wet and dry parts of the root zone periodically alternated to enhance ABA signalling transiently (Dodd *et al.,* 2006) and/or prevent excessive soil drying diminishing the transport of chemical signals to the shoot (Romero *et al.,* 2012). By this way, stomatal closure tend to be used as dominant factor in limiting transpiration and preventing subsequent damage to a hydraulic system (Beis and Patakas, 2010), even though prolonged stomatal closure also limits photosynthetic activity and thereby carbohydrates partionining to berries (Chaves *et al.,* 2010). Indeed, the increase of ABA-induced by greater stomatal closure- might reduce canopy transpiration, through a limitation of photosynthetic carbon gain even while improving WUE (Dodd *et al.,* 2015). Therefore, during prolonged drying cycles in PRD, the limitation of ABA transport from roots and the consequent decline in xylem ABA concentration following alternation of wet and dry parts of the root system, maybe is responsible for the enhance yield of PRD plants compared with conventional RDI plants (Dodd *et al.,* 2010; 2015).

The initial response of plants to water stress is growth reduction, even before any decrease in photosynthetic assimilation (Beis and Patakas, 2015). Canopy development and vegetative growth are highly sensitive to water deficit even more than fruit growth. Indeed, reduced canopy structure can result in lower leaf area, which may be insufficient to develop berries when a low vigour is observed (Ruiz-Sánchez *et al.,* 2010). Moreover, the decrease of vegetative growth under RDI or PRD might be also due to a loss of cell expansion through a decrease in cell turgor (Chaves *et al.,* 2010)

Earlier studies that compared PRD and RDI under the same irrigation volumes revealed differential physiological and biochemical responses in grapevines (Romero *et al.,* 2012, 2014; Beis and Patakas, 2015), but there is little information on table grapes. While there is no reason to suppose table grapes and wine grapes should differ in their physiological responses to PRD and RDI, irrigation management between grapevines *vs.* table grapes is different. As an example in grapevines, Romero *et al.,* (2012), observed that PRD maintained higher leaf area post-veraison, increase root water uptake and gas exchange but decreased WUE causing differences in xylem ABA concentration compared with RDI. Furthermore, the implementation of PRD by growers in favor of conventional RDI strategy requires positive effects on the agronomic performance of PRD, especially due to its high cost of installation and complex irrigation management. Recently, Conesa *et al.,* (2016) reported that PRD enhanced berry coloration and health-promoting bioactive compounds (*e.g.* anthocyanins, resveratrol and antioxidant capacity) compared to RDI. However, whether these differences were due to physiological (leaf gas exchange and water relations) differences in the responses of PRD *vs*. RDI vines have not been investigated in table grapes. For these reasons, a three-year long experiment was conducted on 'Crimson Seedless' table grapes growing in a semiarid climate of south-eastern Spain, to compare physiological responses and vegetative growth of RDI and PRD vines that received the same irrigation volume.

2. MATERIAL AND METHODS

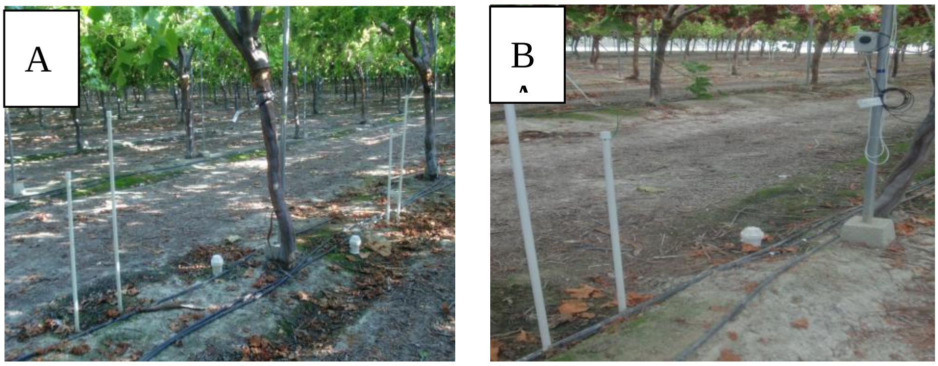
2.1. Experimental conditions, plant material and irrigation treatments

The experimental design has been described in detail in Conesa *et al.* (2015 and 2016). Briefly, this research was carried out in a 1-ha vineyard at the Cieza, Murcia (SE Spain, 38°15'N; 1º33'W). The table grapes were 11-year-old Crimson Seedless (*Vitis Vinifera L.*), grafted onto 1103 Paulsen rootstock. The training system was a bilateral cordon trellised to a three-wire vertical system. The vine rows ran N–NW to S–SE and the planting density was 4 m between rows and 4 m between vines (625 vines ha^{-1}). The experiment involved four different irrigation treatments that were applied during three consecutive years

(2011–2013). A control treatment (Control) irrigated to satisfy maximum crop water requirements (ET_c-110%) through the whole growing season; (ii) a RDI treatment, irrigated as the Control except during post-veraison, when the vines were irrigated at 50% of the level used for the Control; (iii) a PRD treatment, irrigated as RDI (received the same irrigation amount), but alternating the dry and wet sides of the root-zone every 10-14 days, when the 75% of the soil field capacity (~34 % determined as gravimetric sample) was obtained in the dry root-zone; and (iv) a null irrigation (NI) treatment, which only received the rainfalls and additionally irrigations when the daily stem water potential (Ψ_s) overcome the established threshold value of -1.2 MPa (Conesa *et al.,* 2012; 2016). The soil characteristics, climate parameters, fertilization and standard cultural practices at the experimental site have been also reported in Conesa *et al.* (2015 and 2016).

2.2. Soil water status

Soil volumetric water content (θ_v) was measured from 10 cm down to a maximum depth of 1 m every 0.1 m with a frequency domain reflectometry (FDR) probe (Diviner 2000$^{\circledR}$, Sentek Pty. Ltd., South Australia). Three access tubes (1 per each replicate, n=3) were installed within the emitter wetting area on randomly selected trees (Picture 1). In PRD treatment, FDR probes at both sides of the root system were also installed (2 per each replicate, n=6). Measurements were taken every 7-10 days between 10:00-12:00 hours during the experimental period.



Picture 1*. Distribution of the FDR probes in PRD treatment (A) and other irrigation treatments: Control, RDI and NI (B).*

2.3. Water relations and ABA$_{xylem}$

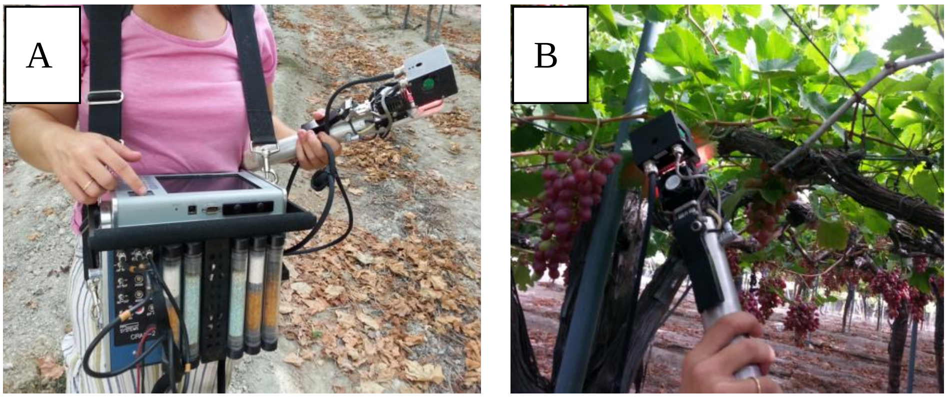
Pre-dawn and midday stem water potentials (Ψ_{pd} and Ψ_s) was monitored every 7-10 days with a pressure chamber (Model 3000, Soil Moisture Equipment, Santa Barbara, CA) on at least two leaves per replicate and three replicates per irrigation treatment (n=6), located on the middle third of the branches, with a pressure chamber (Soil Moisture Equipment Co., Model 3000) following the recommendations of Hsiao (1990). For $\Psi_{s,}$ leaves were enclosed in a plastic bag and placed in the chamber within 20 s of collection. Xylem sap was collected after measuring Ψ_{pd} by applying an over-pressure of between 0.3 and 0.5 MPa for 1-3 min with a glass pipette. Sap was immediately transferred to an Eppendorf tube, frozen in liquid nitrogen and stored at -20ºC prior to ABA measurement with radioimmunoassay (Quarrie *et al.,* 1988) using the monoclonal antibody AFRC MAC52. After measuring $\Psi_{pd,}$ the leaves were frozen in liquid nitrogen and osmotic potential (Ψ_o) was measured after thawing the samples and extracting the sap, using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci *et al.* (1991). Leaf turgor potential (Ψ_t) was estimated as the difference between leaf osmotic (Ψ_o) and predawn water potentials (Ψ_{pd}). Leaf osmotic potential at full turgor (Ψ_{os}) was measured on leaves adjacent to those used to measure Ψ_{pd}. The leaves were excised with their petioles and placed in distilled water overnight to reach full saturation before being frozen in liquid nitrogen (-196 °C) and stored at -30 °C, following the same methodology as for Ψ_o. Osmotic adjustment was estimated as the difference between the Ψ_{os} of stressed and Control vines. In order to estimate the intensity of stress endured by deficit irrigation treatments, the water stress integral was calculated from the values of Ψ_s, according to the equation defined by Myers (1988).

$$S_\Psi = \left| \sum_{i=0}^{i=t} (\overline{\Psi}_{i,i+1} - \Psi_c) n \right| \qquad \text{[Equation 1]}$$

where t is the number of measurements of Ψ_s $\Psi_{i,i+1}$ is the mean Ψ_s for any measurement I and i+1; Ψ_c is the maximum Ψ_s measured during each phenological period (pre and post-veraison); n is the number of days in the interval. All values were referred to Control treatment. S_Ψ obtained in the whole season is the sum of those observed in pre- and post-veraison.

2.4. Leaf gas exchange

Gas exchange measurements were taken every 7-10 days between 09:00 and 11:30 in daylight hours on at least two leaves per replicate and three replicates per irrigation treatment (n=6) exposed to the sun. Maximum net CO_2 assimilation rate ($A_{CO2,}$ µmol m^{-2} s^{-1}), maximum stomatal conductance (g_s mmol m^{-2} s^{-1}), and transpiration rate ($E_{m,}$ mmol m^{-2} s^{-1}) were measured at a photosynthetic photon flux density (PPFD) ≈ 1500 µmol m^{-2} s^{-1}, near constant ambient CO_2 concentration (Ca ≈ 380 µmol mol^{-1}) and leaf temperature (Tleaf ≈ 30 °C) with a portable gas exchange system CIRAS-2 (PP Systems, Hitchin, Hertfordshire, UK) (Picture 2). Instantaneous and intrinsic water use efficiency was calculated as the ratio between A_{CO2} and E_m (µmol $mmol^{-1}$), and A_{CO2} and g_s (µmol mol^{-1}), respectively.



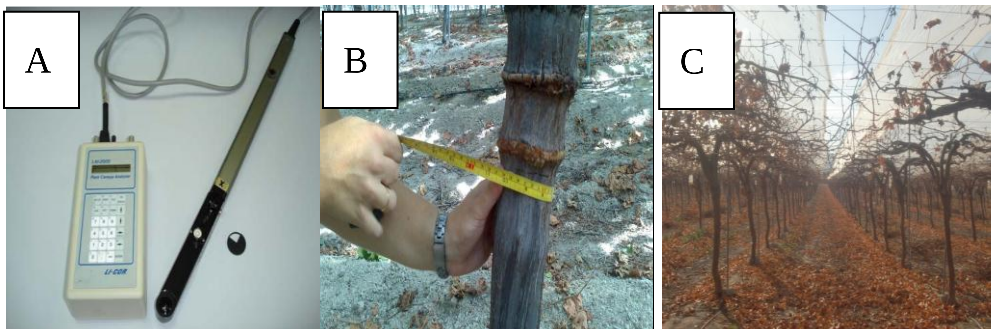
***Picture 2**. Detail of the portable gas exchange system CIRAS-2 (A) and the leave measurement at the experimental field (B)*

2.5. Vines growth patterns

Vines growth measurements were carried out during the experimental period to evaluate the influence of the irrigation treatments on plant vegetative behavior. Micrometric trunk diameter fluctuations (TDF) first described Kozlowski and Winget (1964), were monitored throughout the experimental period in six selected trees, using a set of linear variable displacement transducers (LVDT; Solartron Metrology, Bognor Regis, UK, model DF ± 2.5 mm, precision ± 10 μm) installed on the trunk northern side at 120 cm above-ground and mounted on holders built of aluminium and invar – an alloy comprising 64 % Fe and 35 % Ni that has minimal thermal expansion. Several indices were derived from trunk diameter fluctuations according to Goldhamer and Fereres, (2001): maximum daily trunk diameter (MXTD), minimum daily trunk diameter (MNTD), maximum daily shrinkage (MDS = MXTD – MNTD) and trunk daily growth rate (TGR, calculated as the difference between MXTD of two consecutive days). The vines used for Ψ_s monitoring were also used for TDF determinations

Leaf area index (LAI $m^2_{leaf}\,m^{-2}_{soil}$) was measured in one vine per replicate before veraison using a canopy analyzer instrument LAI 2000® (Li-Cor, Lincoln, Nebraska, USA) (Picture 3A). Previously, a grid of 16 points (each spaced 0.5m) was established on the ground around the vine selected. The final measurement was the average of these 16 points.

Trunk perimeter was measured by tape-measure (Picture 3B) before harvesting on 6 vines per replicate at a marked location around 1.2 m from the soil surface to determine trunk cross-section area (TCSA, cm^2). The annual increment in TCSA (ΔTCSA) was calculated as the difference between two consecutive TCSA measurements. The productivity efficiency (PE) was also calculated as the ratio between yield and $TCSA^{-1}$ Pruning weight was determined annually during winter dormancy in all the vines of the experiment (Picture 3C).



Picture 3. *Detail of LAI 200 instrument (A) trunk perimeter measurement (B) and pruning at winter time (C)*

2.6. Data collection system

Data from LVDT sensors were collected using wireless technology. The sensor nodes were provided by the company WIDHOC (www.widhoc.com) and send data every 20 minutes, approximately, to a coordinator node which was connected to a PC. Each node was provided by one SD card to store the data and it was feeding by lithium polymer batteries (5000 mAh) and small solar panels (5V/80 mA) which allowed a virtually unlimited autonomy. Three nodes per irrigation treatment (one per replicate) were used.

2.7. Statistical analysis

The experimental layout was a randomized complete block design with three block-replicates per irrigation treatment. Each replicate consisted of three adjacent rows of vines with six vines per row. The four central vines of the central row were used for monitoring the vine water relations assessed, while the others served as guard vines. The data were analyzed by one-way ANOVA using Statgraphics Plus for Windows version 5.1 (Manugistics, Inc., Rockville MD, USA). *Post hoc* pairwise comparison between all means was performed by Duncan´s multiple range test at $p<0.05$.

Principal component analysis (PCA) was performed to identify the influence of deficit irrigation: moderate (RDI and PRD) and; severe (NI) on the plant water status indicators studied, using the ‘CANOCO for Windows’ program v4.02 (ter Braak and Smilauer, 1999). PCA results were presented in ordination diagrams, which included arrows representing ‘species’ (in our case, plant water status indicators) and ‘samples’ or points representing irrigation treatments. A rule to interpret the diagram is that the fitted value is positive whenever the projection point of a site lies between the origin of coordinates and the arrow point, and negative whenever the origin lies between the projection point and the arrow point. Therefore, those irrigation treatments whose projection is nearer the arrow point are more closely related to the plant water status indicators which represent this arrow.

3. RESULTS AND DISCUSSION

3.1. Water applied, climate conditions and soil water applied

The average amount of water applied in the Control treatment during the three years of study was 685 mm. The water saved in RDI and PRD to respect well-watered vines was practically similar averaging 35% whereas in NI treatment the reduction to respect Control treatment was higher (72%), corresponded to a severe water deficit (Table 1). There were some differences in the meteorological conditions (ET_0 and rainfall) among the different growing seasons, being the year 2012 the rainiest. Higher atmospheric evaporative demands of the atmosphere (ET_0) were registered in pre-veraison (from early-June to early-August).

Table 2 shows the mean soil volumetric water content (θ_v) values for each one of the irrigation treatments assessed during the study period. In the Control treatment, θ_v values were maintained above field capacity at 0-50cm depth, averaging 35.61 % during the study period. As expected, θ_v values in RDI and PRD treatments were significantly lower than Control during the post-veraison period (from early-August to the end of October). PRD exhibited higher soil water deficits than RDI due to the alternating of the dry side during post-veraison. In this sense, the average values of θ_v ranging between 27 and 35% at both sides of the root system. Furthermore, the NI treatment registered significant differences in θ_v compared to the Control, as expected, supposing a reduction of 17% during the three years assessed (Table 2).

Table 1. *Reference evapotranspiration (ET_0), precipitation (P), and irrigation water applied (from April to the end of October) in Control (full irrigation treatment); RDI (regulated deficit irrigation, moderate deficit); PRD (partial rootzone drying, moderate deficit), and NI (null irrigation treatment, severe deficit) during the three years assessed (2011-2013).*

Year	Phenologial period	ET_0 (mm)	P (mm)	Irrigation (mm)			
				Control	RDI	PRD	NI
2011	**Pre-veraison**	390	63	285	135	157	0
	Post-veraison	886	125	363	252	228	186
	Whole-season	1195	188	648	387 (40)^z	385 (41)	186 (71)
2012	**Pre-veraison**	388	209	288	190	210	60
	Post-veraison	886	166	398	305	292	73
	Whole-season	1274	375	686	495 (28)	502 (27)	133 (81)
2013	**Pre-veraison**	393	130	315	300	263	156
	Post-veraison	860	65	407	155	146	95
	Whole-season	1253	195	722	455 (37)	409 (43)	251 (65)
2011-2013	**Average**	1241	253	685	446 (35)	432 (37)	190 (72)

^{z}*In parentheses percentages of irrigation water saving with respect to the Control treatment*

***Table 2**. Mean values of soil volumetric water content (θ_v, %) in the profile 0-50 cm for Control (full irrigation); RDI (regulated deficit irrigation); PRD_{right} (partial rootzone drying in the side, moderate deficit), PRD_{left} (partial rootzone drying in the left side, moderate deficit) and NI (null irrigation treatment, severe deficit) during the three years assessed (2011-2013). RDI vs. PRD_{total} was also compared individually.*

Year	**Phenologial period**	**θ_v (%)**						
		Control	**RDI**	**PRD_{right}**	**PRD_{left}**	**NI**	**RDI**	**PRD_{total}**
2011	**Pre-veraison**	32.55 a	33.38 a	33.92 a	30.44 a	32.82 a	33.38 a	32.18 a
	Post-veraison	35.23 c	31.92 b	32.15 b	28.49 a	28.09 a	31.92 a	30.32 a
	Whole-season	34.06c	32.56 b	32.93 b	29.35 ab	29.78 a	32.56 a	31.14 a
2012	**Pre-veraison**	31.94 b	37.52 c	35.85 c	31.84 b	28.09 a	37.52 b	33.84 a
	Post-veraison	40.86 b	32.04 a	32.99 a	30.90 a	30.45 a	32.04 a	31.94 a
	Whole-season	36.19 b	34.91 b	34.49 b	31.39 ab	28.52 a	34.91 b	32.94 a
2013	**Pre-veraison**	35.99 b	34.83 b	35.51 b	34.89 b	30.45 a	34.83 a	35,20 a
	Post-veraison	37.47 d	35.86 c	27.86 ab	31.95 b	28.93 a	35.86 b	29.90 a
	Whole-season	36.64 d	35.24 c	32.16 b	33.60 b	29.84 a	35.24 b	32.88 a
2011-2013	**Average**	35.63	34.24	33,2	31.45	29.38	34.24	32.32

Means within rows followed by a different letter were significantly different according to Duncan multiple range test ($P<0.05$).

3.2. Leaf water relations

Data on the seasonal changes (pre-post-veraison and the whole season) of water stress integral (S_Ψ) obtained by stem water potential at midday measurements are shown in Figure 1. During the three pre-veraison years assessed, the S_Ψ in RDI and PRD remained constant to 0 as they received the same irrigation amount than Control. However, differences in the accumulated deficit between RDI and PRD were detected in post-veraison where PRD treatment showed significant highest S_Ψ values than RDI. Considering the whole season, PRD only differed to RDI during the year 2011 and 2012. As expected, NI registered the highest accumulation and duration of stress to compare with RDI and PRD, regardless of the phenological period considered. In this sense, NI treatment presented the highest levels of S_Ψ in the year 2012 (≈ 50 MPa*day) and the lowest in 2011 (≈ 30 MPa*day).

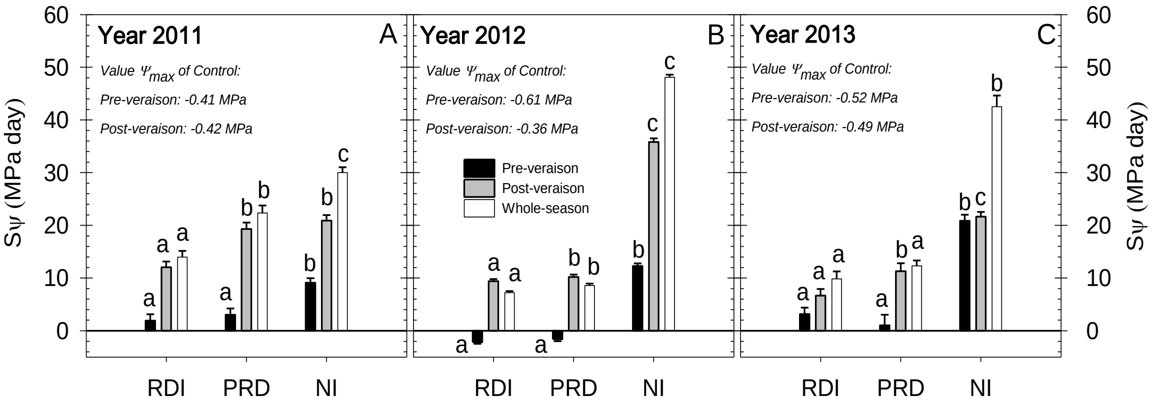


***Figure 1**. Water stress integral (S_Ψ) in deficit irrigation treatments: RDI (regulated deficit irrigation, moderate deficit); PRD (partial rootzone drying, moderate deficit); and NI (null irrigation treatment, severe deficit) during the years 2011 (A); 2012 (B) and 2013 (C), respectively. Black, grey and white bars correspond to pre-veraison, post-veraison and the whole season, respectively. Different letters indicate statistically significant differences between treatments by Duncan's multiple range test ($P<0.05$).*

The predawn leaf water potential (Ψ_{pd}) values for the Control treatment were around -0.08 MPa in the three studied years (Figs. 2 A-C). Ψ_{pd} only showed significantly differences in the pre-veraison of NI treatment during the years 2012 and 2013. However, during post-veraison, all deficit irrigation treatments exhibited moderate (in RDI, PRD) and severe (NI) deficit throughout the study period, averaging values of -0.14 and -0.28 MPa, respectively. Regarding leaf osmotic and osmotic saturated potentials (Ψ_o and Ψ_{os}), only significant differences in RDI, PRD and NI to respect Control were detected during the year 2011 (Fig. 2D). The amount of solutes accumulated in RDI and PRD was not enough to compensate the deficit reached after veraison, whereas NI treatment maintained this trend during both phenological periods (Fig. 2G). Concerning the leaf turgor, RDI and NI differed in the values of Ψ_t compared with Control treatment during the year 2013. In this sense, the irrigation management induced in PRD suggested an improvement in the recovery of the leaf turgor despite of decreasing irrigation (Fig. 2L).

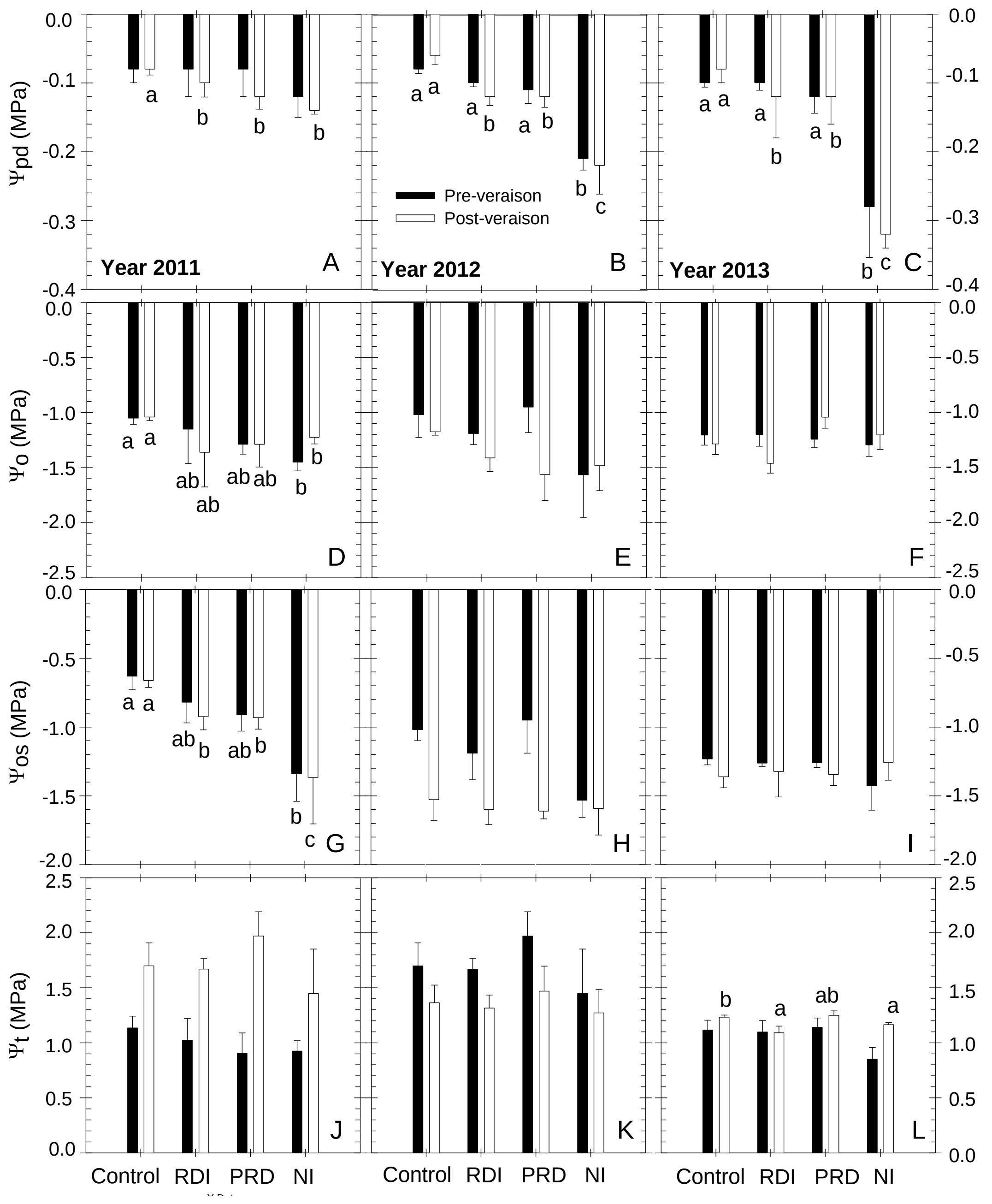


__Figure 2__. Seasonal evolution of (A-C) predawn leaf water potential (Ψ_{pd}), (D-F) leaf osmotic potential (Ψ_o), (G-I) leaf osmotic potential at full turgor (Ψ_{os}), and (J-L) leaf turgor potential (Ψ_t) during the three years assayed (2011-2013) for all the irrigation treatments: Control (full irrigated treatment); RDI (regulated deficit irrigation, moderate deficit); PRD (partial rootzone drying, moderate deficit); and NI (null irrigation treatment, severe deficit). Values are means ± SE during each phenological period of pre-veraison (black bars) and post-veraison (white bars). Different letters indicate statistically significant differences between treatments by Duncan's multiple range test ($P<0.05$).

3.3. Gas exchange parameters and ABA_{xylem}

Table 3 showed the mean gas exchange values obtained during pre, post-veraison and the whole season during the three years assessed (2011-2013). Results on net CO_2 assimilation (A_{CO2}) only showed some differences in the pre-veraison and the whole season of 2012, being the NI treatment which registered the lowest values. Stomatal conductance (g_s) was the gas exchange parameter more affected by deficit irrigation showed significantly differences among treatments during the whole season of the three studied years. Specifically, the reductions in sensitive stomata were highest in NI and lowest in PRD. Therefore, RDI showed highest stomatal closure than PRD in all the three years assessed (2011-2013). The transpiration rate (E) was only significantly lower in NI during the pre-veraison of 2011. Intrinsic (A_{CO2}/g_s) and instantaneous (A_{CO2}/E) water use efficiency performed similar due to the strong relationship obtained when both were compared [$A_{CO2}/E= 0.007+0.0042\ A_{CO2}/gs$, $r^2=0.88$; $P<0.001$]. It is also observed that gas exchange parameters were more affected by differences occasioned by seasonality, regardless of the water availability. Meanwhile, the interaction treatment x year (T x y) was not significant in all of the gas exchange parameters studied (Table 3).

According to the stomatal closure, PRD showed values closer to Control, when RDI and PRD were compared. This fact, are in agreement with the absolute values of the xylem abscisic acid (ABA_{xylem}) obtained in this treatment, although were not significant (Fig. 3). Interestingly, the two exogenous applications of S-ABA to increase berry coloration by the commercial farm, promoted a sharply increment in the seasonal evolution of $ABA_{xylem,}$ reaching a mean value close to 9000 nM. However, the latter increase was quickly reduced after harvest (at the end of September) and it was maintained by about the same levels to those observed in pre-veraison (Fig.3).

***Tabla 3**. Means values for the gas exchange parameters for all the irrigation treatment: Control (full irrigation treatment); RDI (regulated deficit irrigation, moderate deficit); PRD (partial rootzone drying, moderate deficit), and NI (null irrigation treatment, severe deficit) evaluated at pre-veraison, post-veraison and the whole season during the study period (2011-2013).*

	Pre-veraison					**Post-veraison**					**Whole-season**				
Year and Treatment	**A_{CO2}**	**g_s**	**E**	**A_{CO2}/g_s**	**A_{CO2}/E**	**A_{CO2}**	**g_s**	**E**	**A_{CO2}/g_s**	**A_{CO2}/E**	**A_{CO2}**	**g_s**	**E**	**A_{CO2}/g_s**	**A_{CO2}/E**
2011															
Control	6.3	103.1 bc	2.0 bc	60.9	3.3	7.7	104.5	2.7	74.5	2.8	7.0	103.8 ab	2.3	67.7	3.1
RDI	5.9	78.3 b	1.7 b	75.6	3.5	4.7	72.3	2.2	84.1	2.5	5.3	75.3 a	1.9	79.9	3.0
PRD	6.9	164.6 c	3.1 c	50.0	2.3	5.3	155.8	3.9	36.5	1.4	6.1	160.2 b	3.5	43.3	1.8
NI	4.6	41.9 a	1.1 a	116.1	5.1	5.5	127.3	2.8	70.8	2.3	5.1	84.6 a	1.9	93.4	3.7
2012															
Control	9.9 a	309.4	3.6	31.9	2.7	8.0	365.1 c	4.4	23.5	1.8	8.9 b	355.5 b	4.2	26.3	2.2
RDI	9.7 a	345.8	3.9	29.1	2.5	8.5	292.2 b	3.8	28.3	1.9	9.1 b	300.7 b	3.7	30.1	2.4
PRD	9.1 a	328.1	3.8	28.9	2.4	8.1	288.0 b	3.9	28.4	2.0	8.6 b	308.0 b	3.9	28.7	2.2
NI	5.7 b	233.5	2.9	28.3	2.2	6.3	182.0 a	3.3	38.4	1.9	6.0 a	207.8 a	3.1	33.4	2.0
2013															
Control	7.1	192.5	2.9	43.9	2.7	6.6	243.9	4.5	27.1	1.4	7.2	242.1 c	4.1	28.4	1.7
RDI	7.3	240.3	3.8	30.0	2.0	6.5	150.8	3.4	43.1	1.8	6.7	171.7 ab	3.2	47.6	2.5
PRD	6.9	187.1	3.2	40.0	2.3	6.1	258.6	4.6	23.9	1.3	6.5	222.8 bc	3.9	31.9	1.8
NI	6.4	142.3	2.6	45.3	2.6	4.6	157.7	3.8	29.7	1.2	5.4	150.0 a	3.2	37.5	1.9
Analysis of variance:															
Treatment (T)	*	*	n.s	n.s	n.s	n.s	*	n.s	n.s	n.s	**	***	n.s	n.s	n.s
Year (y)	**	***	***	***	n.s	**	***	*	**	n.s	***	***	***	***	*
T x y	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

*Means within columns followed by a different letter were significantly different according to Duncan multiple range test ($P<0.05$). *, **, ** significant effect at $P=0.05$; $P=0.01$ or 0.001, respectively. n.s= not significant. Net CO_2 assimilation rate ($A_{CO2,}$ $\mu mol\ m^{-2}\ s^{-1}$); Stomatal conductance (gs, $mmol\ m^{-2}\ s^{-1}$), Transpiration rate (E, $mmol\ m^{-2}\ s^{-1}$; Intrinsic water use efficiency ($A_{CO2}/g_{s,,}$ $\mu mol\ mol^{-1}$); Instantaneous water use efficiency (A_{CO2}/E, $\mu mol\ mmol^{-1}$).*

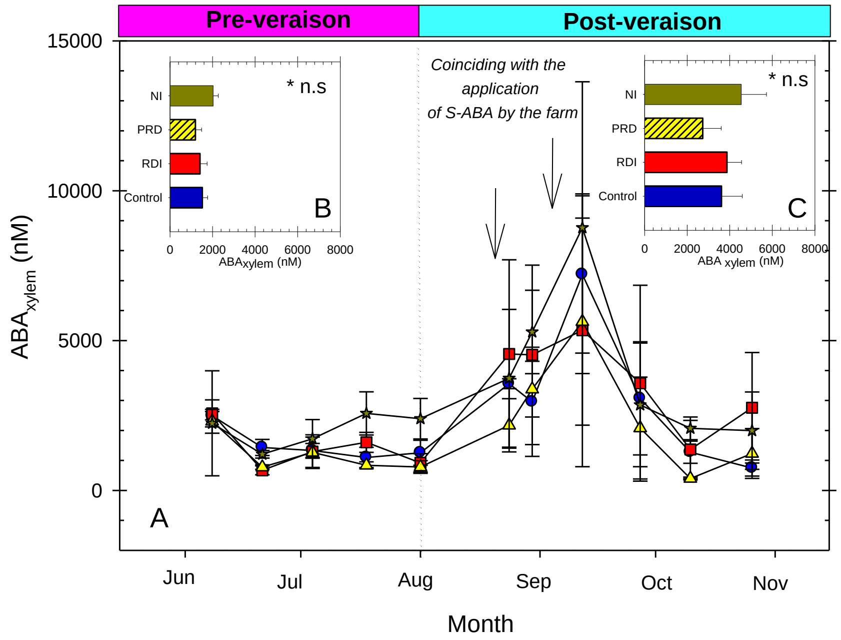


Figure 3. *Seasonal evolution of (A) xylem abscisic acid (ABA_{xylem}) for all the irrigation treatments (●, Control, 🟥, RDI, △, PRD and ★, NI). (B) Means values of ABA_{xylem} for (B) pre-veraison and (C) post-veraison, respectively. Arrows indicate the time of the application of exogenous xylem ABA (S-ABA) by the commercial farm.*

Diurnal time courses of gas exchange and vine water status in two typical post-veraison days (24th August and 3th September) from the years 2012 and 2013, respectively, are shown in Fig. 4. The daytime in the year 2013 presented more stable Ψ_s values than in 2012 and the highest significantly differences among treatments were found at mid-afternoon in both years (Fig 4G-H). Indeed, RDI, PRD and NI treatments dropped sharply during the morning (~09:00–13:00 hours) and did not start to recovery until late afternoon in both years (Fig. 4G-H). Particularly, in Control vines, Ψ_s was more stable during the daytime in the year 2013, whereas the severe deficit imposed in NI was more pronounced in the year 2012. The A_{CO2} was slightly higher in Control than in the deficit treatments even though these differences were less than those corresponding with g_s. Compared PRD and RDI vines, differences in A_{CO2} and g_s were minimized at 14:00 h in respect to midmorning and afternoon times. Although, no clear differences were found in daily mean limitation of g_s between RDI and PRD, the latter vines showing faster evening recovery of gas exchange than in RDI treatment, especially in the year 2012. As expected, NI vines showed the mean lowest values of A_{CO2} and g_s assimilation rates during the whole day in respect to Control, RDI and PRD (Table 3 and Fig. 4).

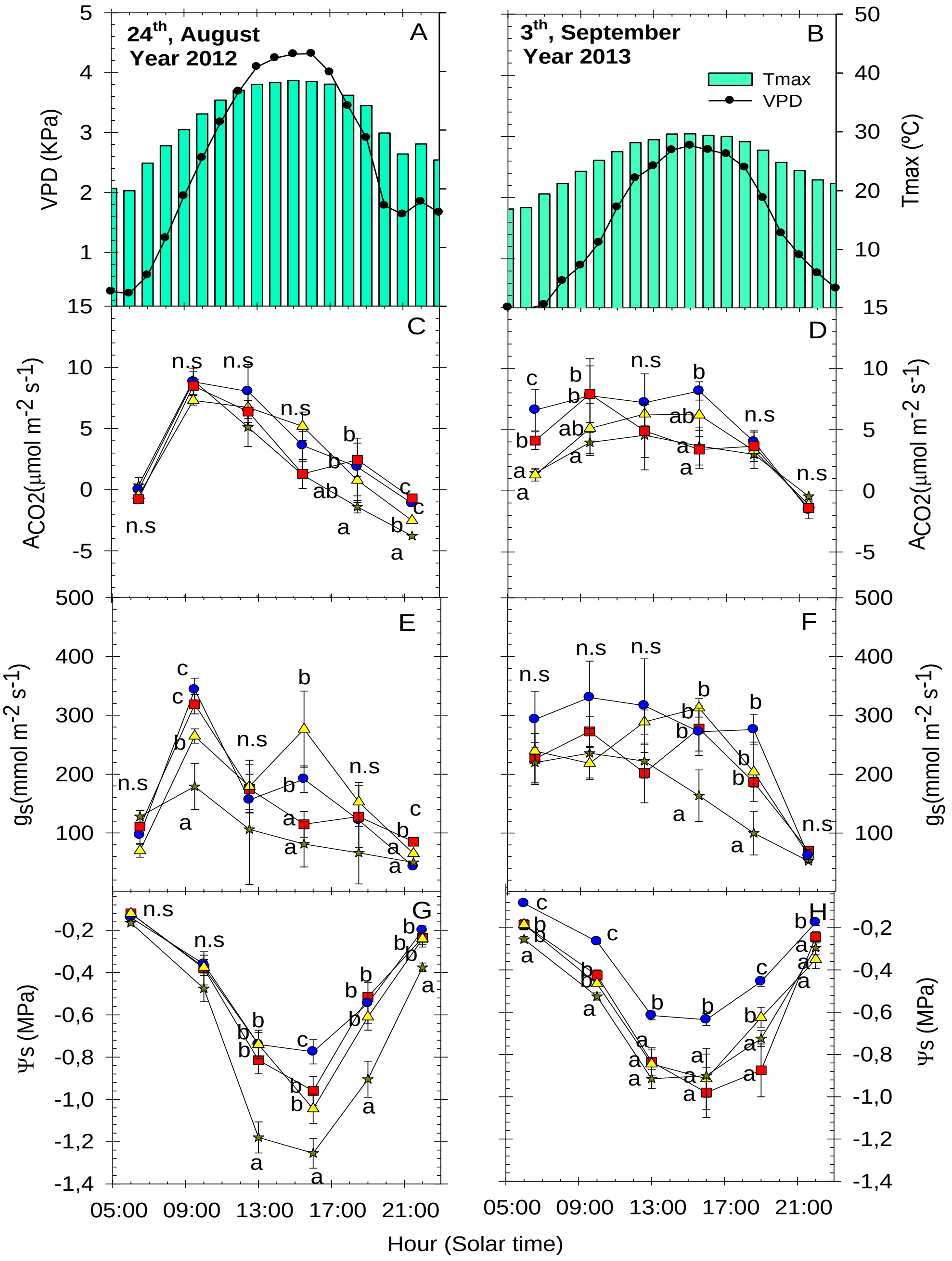


Figure 4. *Daily evolution conducted during two post-veraison days from the years 2012 and 2013 of (A-B) vapour deficit pressure (VPD) and maximum temperature (T_{max}), (C-D) net CO_2 assimilation rate (A_{CO2}), (E-F) stomatal conductance (g_S), and (G-H) stem water potential (Ψ_S). Values are means ± SE of 6 leaves per irrigation treatment (● , Control, ■ , RDI, △ , PRD and ★ , NI). Different letters indicate statistically significant differences between treatments by Duncan's multiple range test (P<0.05).*

3.4. Vegetative growth patterns

Differences in trunk growth rate (TGR) between irrigation treatments were more pronounced in the pre-veraison of 2012, with Control vines presented the highest values (≈ 120 µm day^{-1}) (Figs. 5A-B). Regarding of the treatment considered, TGR appears to stop 7-10 days before veraison and during the period around veraison it even reached negative values in both years (Fig. 5A-B). After veraison, independently of soil water availability, TGR started to decrease in all treatments, reaching more stable values by around 0 and 10 µm day^{-1}. MDS values showed a clear increasing tendency until veraison. However, MDS evolution exhibited a drop after veraison, remained nearly constant their values by around 50 and 100 µm (Figs. 5C-D). According to the deficit applied, significantly differences in the MDS of NI to respect the other irrigation treatments were observed before veraison, especially in the year 2013. Nonetheless, no clear differences were found in the MDS of RDI and PRD compared with Control after veraison (when deficit is applied). Moreover, the relationship between g_s and MDS was only appreciated in some punctual measurements due to the variability on the g_s measurements (Figs. 5E-F). Noteworthy, the severe stomatal closure observed in NI presented a rapid recovery as a result of the supplementary irrigations.

Pruning dry weight was more affected by the season considered than by deficit irrigation even though the absolute values were lower in NI (Table 4). Leaf area index were significantly more affected by deficit irrigation than by seasonality. Indeed, both PRD and NI were the irrigation treatments which more reduce the canopy level. In agreement with LAI results, the increment in the trunk cross-section area was lower in NI, confirmed that the initial response to deficit irrigation affected previously to vegetative growth than berry development. Furthermore, the productivity efficiency (PE), which reflected the level of crop load to respect the vegetative growth, showed lower values in the NI treatment followed by PRD and RDI. Generally, vegetative growth patterns were affected by both deficit irrigation and by the year considered (Table 4).

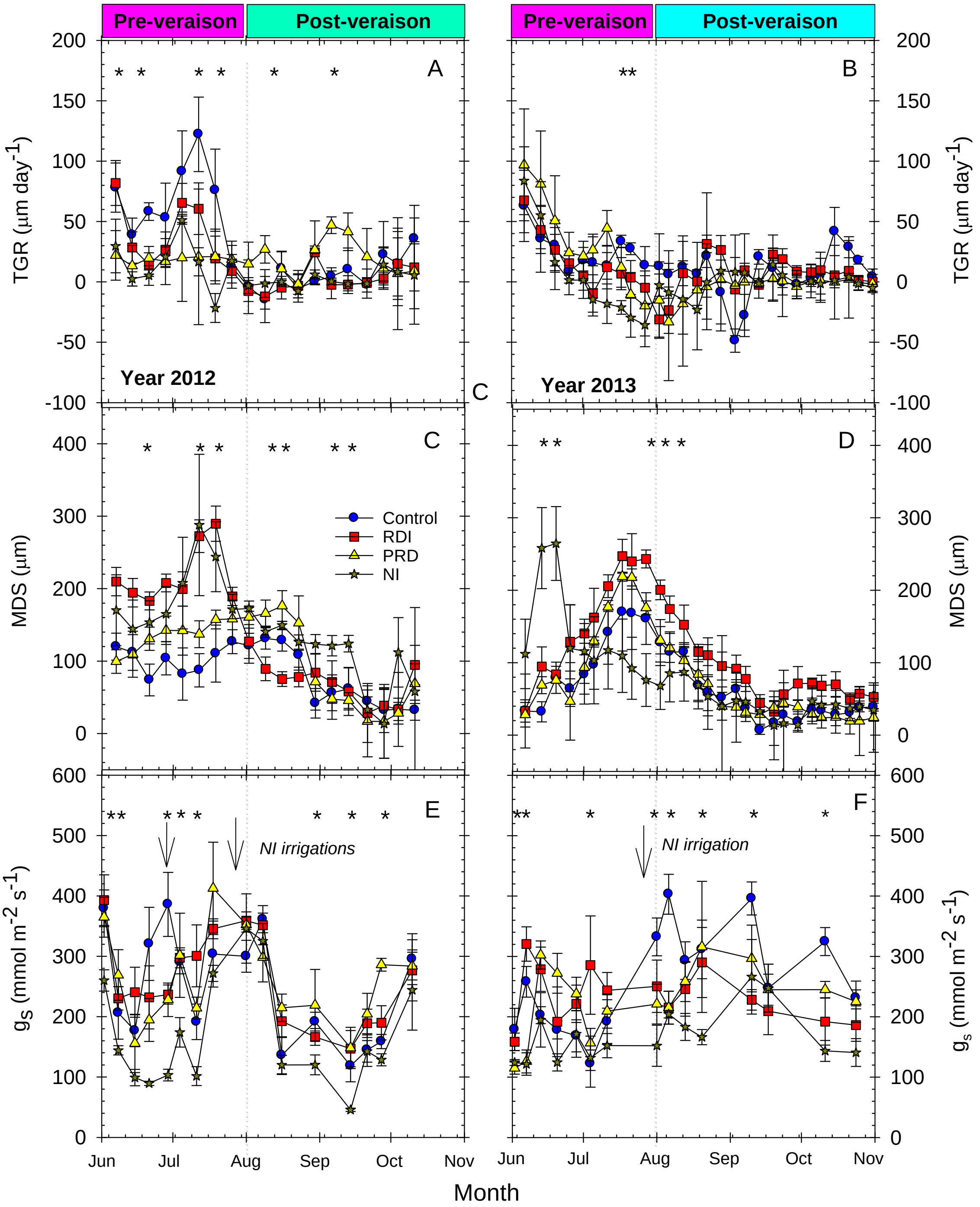


***Figure 5**. Seasonal evolution of (A-B) trunk growth rate (TGR), (C-D) maximum daily shrinkage (MDS) and (E-F) stomatal conductance for all the irrigation treatments (● , Control, ■ , RDI, ▲ , PRD and ★ , NI) during the years 2012 and 2013, respectively. Each point of MDS and TGR represents weekly means ± SE from 6 LVDT sensors per treatment. Each point of g_s is mean ± SE from 6 leaves per treatment. Arrows indicate the time when the supplementary irrigations in NI treatment were applied. Asterisks indicate statistically significant differences between treatments by Duncan's multiple range test (P<0.05).*

Table 4. *Seasonal evolution of pruning weight, leaf area index (LAI), annual trunk cross-section area (ΔTCSA) and productivity efficiency (PE) calculated as the ratio between yield and ΔTCSA in Control (full irrigation treatment); RDI (regulated deficit irrigation, moderate deficit); PRD (partial rootzone drying, moderate deficit), and NI (null irrigation treatment, severe deficit).*

Year and Treatment	**Pruning ($kg\ vine^{-1}$)**	**LAI (%)**	**ΔTCSA ($cm^2\ y^{-1}$)**	**PE ($kg\ cm^{-2}\ y^{-1}$)**
2011				
Control	7.18	83.57 b	4.92 b	14.83 a
RDI	6.82	84.55 b	2.70 ab	31.11 b
PRD	6.57	75.26 ab	2.90 ab	26.89 b
NI	5.64	61.98 a	0.90 a	74.44 c
2012				
Control	6.90	71.64 b	4.31 b	18.32 a
RDI	7.12	66.26 b	3.10 ab	23.22 a
PRD	6.27	67.73 b	2.98 ab	20.80 a
NI	5.22	41.15 a	1.18 a	38.13 b
2013				
Control	4.43	82.15 b	3,41 b	19.94 a
RDI	4.55	79.79 b	3,30 b	20.01 a
PRD	4.46	72.99 ab	3.40 b	19.11 a
NI	3.32	69.71 a	0.88 a	51.13 b
Analysis of variance:				
Treatment (T)	n.s	***	***	***
Year (y)	***	*	n.s	*
Txy	*	***	n.s	n.s

*Means within columns followed by a different letter were significantly different according to Duncan multiple range test (P<0.05). *. **. *** significant effect at P = 0.05. 0.01 or 0.001, respectively; n.s = not significant.*

3.5. Principal component analysis (PCA)

PCA results was used to find patterns in the data in order to classify any combination of variables that could explain the effects of the irrigation treatments on plant water status indicators. The results illustrated clear differences between pre- (Fig. 6) and post-veraison periods (Fig. 7), respectively. In pre-veraison, the gradient from Factor 1 (X-axes) was mainly related to gas exchange parameters: g_s, E and A_{CO2} (on the positive side) and

A_{CO2}/g_s and A_{CO2}/E (on the negative side). The gradient from Factor 2 was defined by MDS on the positive side of the Y-axes and $\Psi_{pd,}$ TGR and Ψ_s on the negative side (Fig. 6). The Factor 2 grouped the values of NI treatment on the positive side and practically separated their samples from the other irrigation counterparts (Control, RDI and PRD).

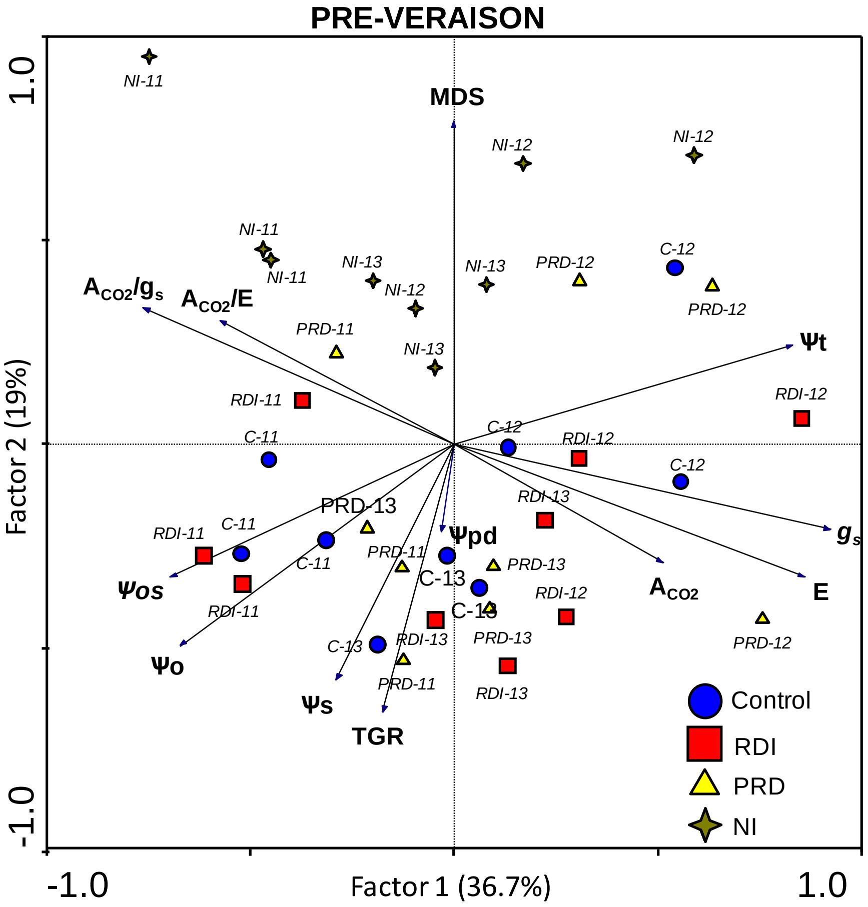


__Figure 6.__ PCA results for irrigation treatments samples during the three pre-veraison years assessed (2011-2013).

The post-veraison deficit irrigation imposed in RDI and PRD altered the PCA results (Fig. 7). Factor 1, which explained the 35.1 % of the total variance, scattered the values of the RDI and PRD, motivated by inter-annual variations. Due to this variability any parameter can be defined as a good indicator of post-veraison deficit. Nevertheless, Factor 2 (25%) clearly separated the samples from Control to NI treatment, suggesting that the severe deficit was more conditioned by E and $\Psi_{o.}$ The gradient from Factor 2 was defined by MDS, $\Psi_{pd,}$

and Ψ_s on the positive side of the Y-axes and TGR and E on the negative side. Remarkably, TGR did not have significant relevance on the analysis (Fig. 7).

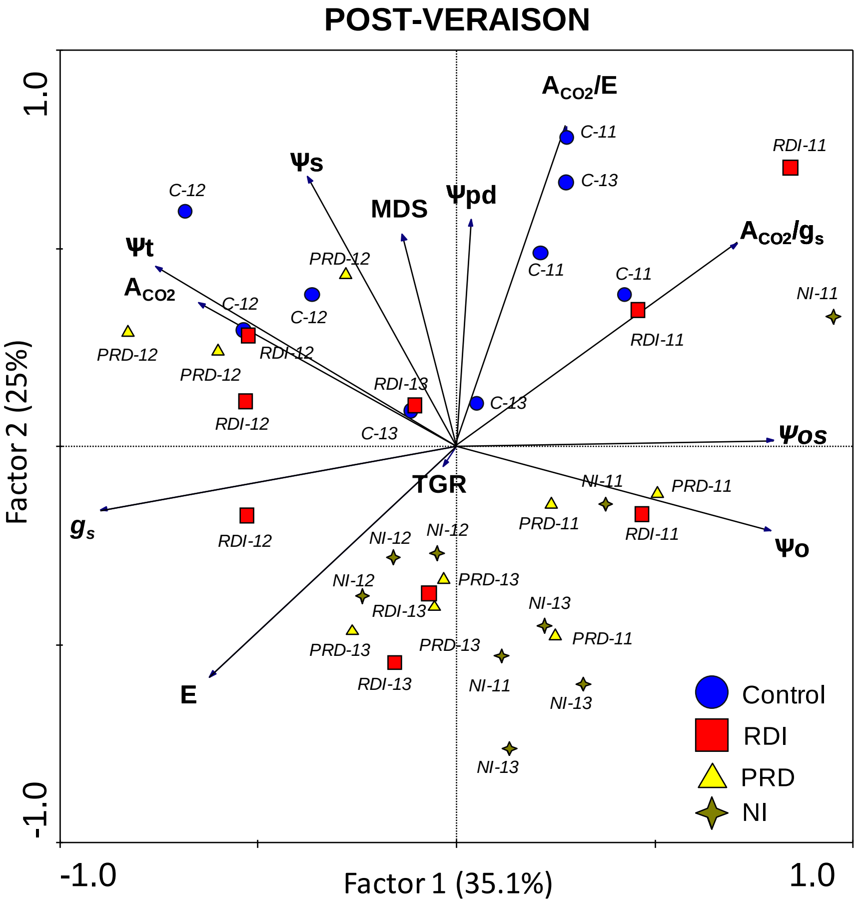


Figure 7. *PCA results for irrigation treatments samples during the three post-veraison years assessed (2011-2013).*

4. DISCUSSION

Although PRD and RDI received the same amount of irrigation volumes during the post-veraison period (50% of Control) and throughout the experiment (≈ 35% less water than Control, Table 1), both treatments had different water uptake patterns (see Conesa *et al.,* 2016) and hence, accumulated water stress (Fig. 1), but no differences between RDI and PRD on predawn water potential measurements (Conesa *et al.,* 2015). In this sense, PRD showed highest severe levels of deficit at both scales (soil and leaf evaluated at midday) than RDI and also differed to well-watered vines (Table 2 and Fig. 1). Usually, under PRD the roots extended deeper through the soil away from the dried soil surface (Dry *et al.,* 2000). Therefore, irrigation events in PRD might penetrate to a deeper level, since twice the amount of water was applied to a single side of the vines compared with the RDI treatment which was watered on both sides. These findings suggested that the accumulation of ABA in water-stressed roots from PRD was sufficient to maintain root development at low water potentials by restricting ethylene production (Sharp, 2002).

It is commonly know that plants respond to water stress by increasing root development but reducing shoot growth due to the accumulation of xylem abscisic acid (ABA_{xylem}) by the root system when it is water stress. Therefore, when water stress occurs, the ABA_{xylem} is transported root-to-shoot to regulate stomatal conductance (Speirs *et al.* 2013), thereby increasing water use efficiency (Flexas *et al.,* 2010). Generally, when plants are exposed to drying soil, xylem ABA concentrations increase causing stomatal closure and reduced transpiration rates (Stoll *et al.,* 2000). Nonetheless, in this study, we could not identify any direct correlation in PRD and RDI between the soil water availability (Table 2) and their physiological behavior (Table 3), which is in contrast to previous recent studies conducted on grapevines (Chaves *et al.,* 2010; Romero *et al.,* 2012) and other species (Wakrim *et al.,* 2005; Ahmadi *et al.,* 2010). Although PRD plants had greater post-veraison soil and plant water deficits than RDI plants, there was less influence on gas exchange parameters (including WUE) and ABA_{xylem} (Table 3). In plants exposed to PRD, xylem ABA concentration was best explained not just by soil moisture levels, but the proportion of sap flow from roots in drying soil (Pérez-Pérez and Dodd, 2015). Moreover, an increment on the water use efficiency in PRD compared to RDI and Control was not observed against expectations (Medrano *et al.,* 2015). These controversial results might explain because the distribution of soil moisture in PRD growing under field settings depends on multiple factors such as the soil type and the environmental conditions of the year (Romero *et al.,* 2014). Further studies of PRD in grapevines demonstrated that halving the water application of Control plants was sufficient to partially close the stomata and prevent severe deficit because half of the roots still receive water (Dry *et al.,* 1996). This fact, together with a promoting root growth to a greater depth (as we comment above), might explain this lack of correlation. Besides this, the more rapid afternoon recovery of g_s in PRD treatment compared with RDI suggested an increase in the water uptake due to a higher root growth to a greater depth (Fig. 4), which can also be related with the higher potential to recover the Ψ_t at the end of the experiment (Fig. 2L). Similarly, plant water status (as diagnosed by changes in trunk diameter) differed between PRD and RDI almond trees during the afternoon (Egea *et al.,* 2011). Therefore, in PRD roots sense the soil drying can induce a reduction in the soil and plant water status but simultaneously the roots in wet soil absorb sufficient water to maintain a high water status in shoots (Sepaskhah and Ahmadi, 2010).

Concerning trunk growth throughout the use of trunk diameter variations, differences among irrigation treatments were found before veraison whereas after veraison changes in stem diameter were diminished (Fig. 5). Particularly, trunk growth ceased 7-10 days approximately before veraison and it reached values close to zero or even negative. Similar results were reported by Intrigliolo and Castel (2007) who found in non-irrigated grapevines the latter stopped at ≈ 20 days before veraison in both non-irrigated and well-irrigated grapevines (but less pronounced in the last ones). The same authors did not found any relationship between grapevine water status and TDF-indices, due to high competition for photoassimilates between fruit and vegetative growth (to the detriment of the latter) and due to the decrease in elasticity of the trunk tissues, which reduced the values of MDS and TGR. Moreover, a work in the same experimental plot (reported in the Chapter IV) reported the interest of ABA_{xylem} accumulation on this effect.

Although PRD has less effect on gas exchange than RDI, vegetative growth (determined by LAI) was more affected. In 2011 and 2013, PRD vines had 10% and 7% lower LAI respectively than RDI vines (Table 4). While this may simply be accounted for by greater water stress experienced by PRD vines (Fig 1), limited ABA export by roots of PRD vines may decrease leaf expansion (Dodd *et al.,* 2009) by allowing greater production of ethylene (a growth inhibitor) in the shoot (Belimov *et al.,* 2009). Lower LAI increased light interception by berries, which in turn might have a positive effect on the berry color and dry matter, suggested that LAI was a powerful factor in controlling berry fruit quality. Gómez-del-Campo *et al.,* (2002) suggested that grapevines productivity could be modeled as a linear function of average leaf are during the growth cycle. In our case (see Conesa *et al.,* 2016), PRD was able to maintain its crop yield and even improve quality when it was compared with RDI and Control treatment.

PCA results from pre-veraison (Fig. 6) and post-veraison periods (Fig.7) showed different patterns in agreement with the intensity of deficit applied. In pre-veraison, MDS might be considered as the best indicator in order to ascertain the plant-water status as reflected the values of NI treatment on the positive side from Y-axes. In contrast, during post-veraison, PCA results only showed a clear tendency of the combinations evaluated when a severe deficit was compared (NI-Control), being the transpiration (E) and $\Psi_{o,}$ which well-defined the changes on the plant water status. The inter-annual post-veraison variations in RDI and PRD can be motivated by differences in the environmental conditions. As an example view, the cloud of points of RDI and PRD treatments from the year 2012 were practically grouped on the negative side of the X-axes. This year (2012) was characterized to represent lower water restrictions to respect Control treatment in RDI (28%) and PRD (27%) regarding to the other years of the experiment. However, the Fig. 7 showed interesting items such as less importance on the analysis for TGR (smaller length arrow) what can confirm the unsuitability of trunk diameter fluctuations to ascertain water stress after veraison.

5. CONCLUSIONS

When PRD and RDI received the same amount of irrigation in table grapes different physiological behavior were found. PRD showed higher water deficits levels in soil and plant scales, even though less reduced the stomatal closure and ABA_{xylem} triggering than RDI in response of the drying soil. This fact reflected a more efficiency on the water uptake in PRD compared with RDI, probably due to a morphological root adjustment. Total leaf area was the vegetative parameter more affected by PRD as there were no differences in TCSA or trunk growth rate. Moreover, the trunk diameter fluctuations indices (MDS and TGR) only can be considered as a good water stress indicators before veraison to ascertain plant water status. Therefore PRD seems a suitable irrigation technique for table grapes to sustain water, and modifying growth (Table 4) and improve berry physical quality (Conesa *et al.,* 2015) and bioactive compounds (Conesa *et al.,* 2016) compared with conventional RDI.

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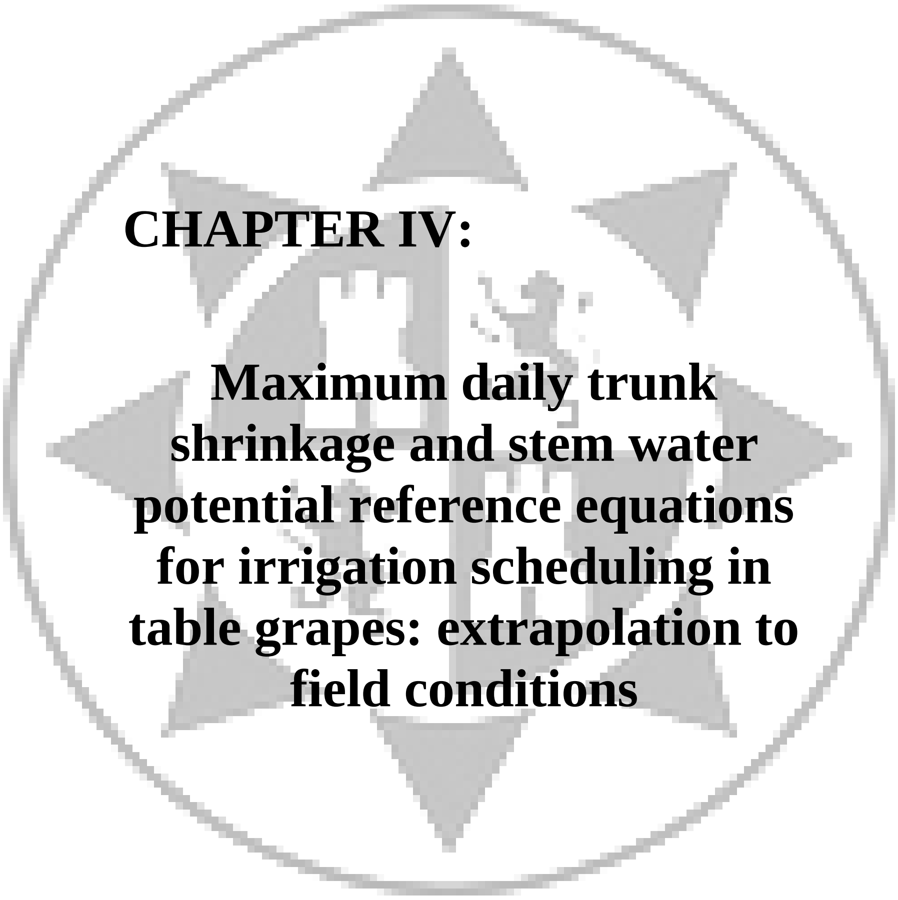

CHAPTER IV:

Maximum daily trunk shrinkage and stem water potential reference equations for irrigation scheduling in table grapes: extrapolation to field conditions

Chapter IV: Maximum daily trunk shrinkage and stem water potential reference equations for irrigation scheduling in table grapes: extrapolation to field conditions

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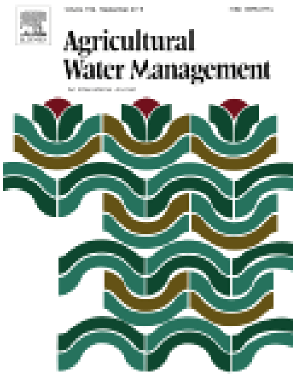

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Abstract

A two-year experiment was conducted to investigate the suitability of reference lines for irrigation scheduling based on maximum daily trunk shrinkage (MDS) and midday stem water potential (Ψ_s) in a commercial orchard of late table grape cv *'Crimson Seedless'* grafted on Paulsen 1103 (*V. berlandieri R.*× *V. rupestres du Lot*). Vines were irrigated (from April to October) above their full crop water requirements (110% of crop evapotranspiration, ET_c) in order to obtain non-limiting soil water conditions. The reference equations obtained for MDS and Ψ_s with meteorological factors differed between the pre and post-veraison periods. Before veraison, MDS was the most reliable indicator for assessing the water status of vines, whereas Ψ_s correlated better with meteorological variables after veraison. The sensitivity of MDS to ascertain the plant water status decreased during post-veraison due to its dependence on growth and to daily fluctuation of stem diameter, which can also be induced by transpiration and changes in the accumulation of xylem abscisic acid (ABA_{xylem}). Mean temperature (T_m) was the environmental variable that best correlated with MDS and Ψ_s during pre-veraison. However, post-veraison reference lines can be obtained for MDS and Ψ_s using reference evapotranspiration (ET_0) and mean daily vapour pressure deficit (VPD_m). The use of the use of MDS signal intensity (SI_{MDS}) around the unity and Ψ_s around -0.65MPa were the best criteria for irrigation scheduling in well-irrigated 'Crimson Seedless' table grapes growing in a semiarid climate of south-eastern Spain, during pre and post-veraison periods, respectively.

Keywords

Wireless sensors; crop load; grape veraison; plant-based water status indicators; water relations

1. INTRODUCTION

Vines are widely cultivated in Mediterranean areas where water is scarce. Facing an increasing world population and competition with other water-using sectors for the limited water resources available, the use of precise irrigation techniques has led the scientific community to develop new technologies for scheduling irrigation. In the case of grape production, how to best manage the amount of water applied continues to receive attention in many regions of the world. For example the use of regulated deficit irrigation, as termed by Chalmers *et al.,* (1981), has been assessed to control vegetative growth and improve the consistency of fruit production and quality in grapes (Goodwin and Jerie, 1992)

One way to know the intensity of any water stress imposed is to use plant-based water stress indicators related with climatic and soil conditions, as well as crop productivity (Ortuño *et al.,* 2010). Stem water potential (Ψ_s) has traditionally been the most widely used indicator for irrigation scheduling in fruit trees because it is affected in addition to transpiration by water availability (Shackel *et al.,* 1997). However, the equipment used to measure this parameter cannot be integrated into an independent irrigation scheduling process (Puerto *et al.,* 2013), and a significant input of labour is necessary to properly monitor the water status of the plant (Pagán *et al.,* 2012).

Trunk diameter fluctuation (TDF, Kozlowski and Winget, 1964) can be continuously and automatically recorded, which represents a clear advantage over the conventional indicator of Ψ_s. TDF included two main components - (i) size increments due to growth; and (ii) size fluctuations due to water movement in tissues inducing a daily cycle of shrinkage (from the beginning of the day) and swelling (from the mid-afternoon) which occurs in all plants (Corell *et al.,* 2013; Zweifel *et al.,* 2014). Increments due to growth can be attributed to the activity in the cambium which exists between the bark and the differentiated wood. The cambium builds new cells towards the centre of the stem, which are mainly directed to xylem and it builds cells towards the periphery of the stem which mainly differentiate to phloem. Size fluctuations due to changes in transpiration induced negative pressure in the xylem leads to a dehydration of living tissues. Moreover, when the transpiration is high the stem loses water from elastic tissues, mainly the bark and the cambium including dividing and enlarging cells as well as phloem (Zweifel *et al.,* 2014). This fact might promote the early detection of water stress (even in a mild stress).

Among the TDF-derived indices that are useful for detecting the plant water status, trunk growth rate (TGR) and maximum daily shrinkage (MDS) are the most widely assessed because they are the most sensitive and show the greater variability, as mentioned by many studies available in the literature (Goldhamer and Fereres, 2001). MDS has been successfully used as a management tool for irrigation scheduling in several crops (reviewed in Ortuño *et al.,* 2010, and in Fernández and Cuevas, 2010). However, the fact that the plant water status indicates the effects of weather conditions and the availability of water in the soil has led to the use of MDS signal intensity ($SI_{MDS}= MDS_{observed}/ MDS_{estimated}$), as proposed by Goldhamer and Fereres (2001), rather than absolute MDS values. SI values above unity indicate increasing water stress levels whereas SI values of unity indicate the absence of stress (de la Rosa *et al.,* 2015). Reference values from plant-based water stress indicators are usually obtained by maintaining reference plants under non-limiting soil water supply. To interpret the actual/observed values of the indicators studied, the concept of a reference line has been developed, which can be defined as an equation that predicts MDS values from one meteorological variable in non-limiting soil water conditions. Many contributions have reported reference lines for MDS in respect to different meteorological data, with vapor pressure deficit (VPD) and air temperature (T) being the most commonly used. MDS *versus* VPD achieved the best fit for almond (Egea *et al.,* 2009), early nectarine (de la Rosa *et al.,* 2013), plum (Intrigliolo and Castel, 2007a) and apple (Liu *et al.,* 2011), whereas the best correlation for citrus crops was the relation MDS *versus* T (Ortuño *et al.,* 2009; Pagán *et al.,* 2012). A reference line can also be affected by many factors such as phenological stage (Fereres and Goldhamer, 2003), tree size (Intrigliolo and Castel, 2006), crop load (Intrigliolo and Castel, 2007a), salinity (Pagán *et al.,* 2012) and the time of the day when MDS occurs (de la Rosa *et al.,* 2013). However, to our knowledge, no reference lines for table-grapes have been reported so far, so it is essential to check the above factors in this cultivar.

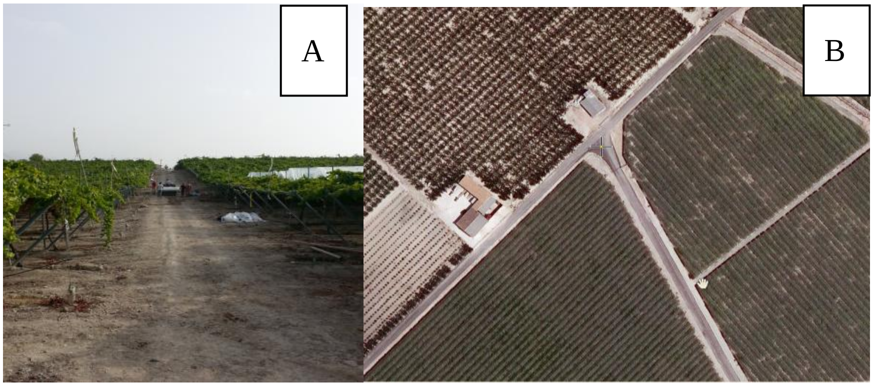
Prior studies conducted in grapevines showed no relationship between plant water status and TDF parameters (MDS and TGR) after veraison, probably due to strong competition between fruit development and vegetative growth during this period (Intrigliolo and Castel, 2007b). Berries might act as a dominant sink for carbon partitioning compared with other vine organs. Indeed, this and a decrease in elasticity of the trunk tissues lead to decrease in MDS and TGR (Sellés *et al.,* 2004) during post-veraison (or as the season progresses). For young trees, and in periods of rapid stem growth, TGR could be a better indicator than MDS to assess plant water status (Fernández and Cuevas, 2010). For example, in well watered 2-year-old olive trees, Moriana and Fereres, (2002) reported that TGR seems to be more sensitive to water stress than MDS. MDS may become a better indicator for detecting plant water status when trunk growth slows as the tree matures. However, when trunk growth is negligible, TGR cannot be used as an indicator of plant water stress (Fernández and Cuevas, 2010). Pérez-Lopez *et al.,* (2008) observed that the TGR values reached in mid-summer in olive were not related with plant water status or air temperature, but coincided with maximum fruit endocarp expansion. Although MDS and TGR showed high plant-to-plant variability, in most cases the signal intensity was high enough to ensure an acceptable degree of sensitivity, and even higher than the sensitivity provided by Ψ_s (Ortuño *et al.,* 2010). Nevertheless, in grapevines, the above suggests that after veraison Ψ_s might be more sensitive than MDS and TGR to water stress.

Consequently, this work describes a two-year experiment in late table grapes to assess: (1) the suitability of MDS and Ψ_s reference equations based on different meteorological variables for irrigation scheduling in both years, (2) the short-term (intra-year) and long-term (inter-year) stability of the relationships considered, and (3) the feasibility of using SI_{MDS} and threshold values of Ψ_s as a control parameter for irrigation scheduling, during pre- and post-veraison, respectively. The effects of girdling and the use of a hail mesh are also discussed.

2. MATERIALS AND METHODS

2.1. Site description

The study was carried out over two consecutive growing seasons (2012 and 2013) in a commercial orchard of 11-year old drip-irrigated table grapes cv. *Crimson Seedless* grafted onto Paulsen 1103 rootstock at a spacing of 4 m x 4 m (Picture 1A). The vineyard with an area of 1 ha was located in Cieza (Murcia, SE Spain; 38º15’N;1º33’W) (Picture 1B). The vines were trained to an overhead trellis system at a height of ≈ 3.0 m above ground level. More details about the experimental field conditions are described in Conesa *et al.,* (2015).



***Picture 1**. Overview of the experimental plot 'in situ' (A) and SigPac (B).*

The soil down to 60 cm depth was clay-silt-loam (37% clay, 46% silt, 17% sand) with a bulk density of 1.25 g cm^{-3}, organic matter content of 2.1 % and soil pH of 8.6. Below 60 cm, the substrate was mainly hard clay. The irrigation water, from the Tagus-Segura water transfer system, had an average electrical conductivity ($EC_{25ºC}$) close to 1.3 dS m^{-1}. The irrigation system consisted of a single drip line per vine row and four pressure-compensated emitters (4 l h^{-1}) per vine.

Daily reference crop evapotranspiration (ET_0, mm day^{-1}) was calculated using the FAO Penman–Monteith equation as follows (Allen et al. 1998):

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma(900/(T+273))u_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)}$$

[Equation 1]

where *Rn* is the net radiation at the surface (MJ m^{-2} day^{-1}), *G* is the soil heat flux (MJ m^{-2} day^{-1}), *T* is the mean air temperature at 2 m height (ºC), *u2* is the wind speed at 2m height (m s^{-1}), e_s is the saturation vapour pressure (kPa), e_a is the actual vapour pressure (kPa), $e_s - e_a$ is the saturation vapour pressure deficit (kPa), *Δ* is the slope vapour pressure curve (kPa ◦C^{-1}), and *γ* is the psychrometric constant (kPa ◦C^{-1}).

Crop coefficients (K_c) were reported by Williams *et al.,* (2003). Maximum crop evapotranspiration (ET_c) was obtained as the product of ET_0 and K_c. Vines were irrigated above the ET_c in order to ensure non-limiting soil water conditions over the study period. The total irrigation amount applied was 732 mm and 635 mm in 2012 and 2013, respectively. The vines were fertilised with 105-98-207 kg ha^{-1} $year^{-1}$ of N, P_2O_5 and K_2O, respectively. Standard cultural practices such as pruning, girdling, covering with a hail mesh, fertilization, phytosanitary treatments, and the exogenous application of abscisic acid (S-ABA) during post-veraison in mid-August (two applications of 4 L ha^{-1} each), were the same for all the vines of the experiment, and were carried out by the technical department of the commercial vineyard following the usual criteria for the area. Particularly, trunks were girdled with a double bladed 4.8 mm knife at the beginning of June to stimulate the carbohydrates partionining to berries. The collocation of the hail mesh to prevent the torrential precipitations had occurred at the end of August.

The experimental vineyard received seven treatments (each with three replicates) in a randomized complete block design. Each replicate consisted of three adjacent vine rows and 6 vines per row. Measurements were taken only in the vines of the central row, the other vines serving as borders. However, in this experiment, only one irrigation treatment (corresponding to well-water irrigated vines, 110% ETc) was used (a total amount of 72 vines).

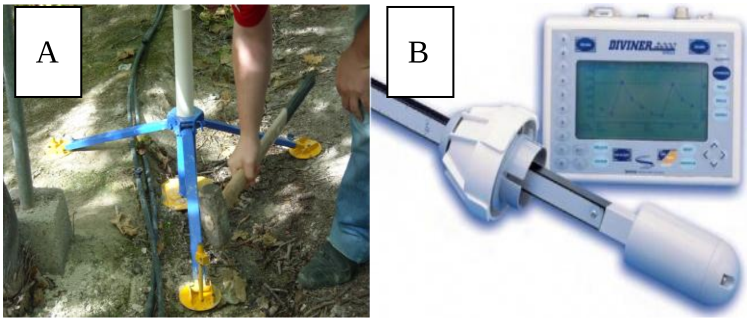
2.2. Measurements

Hourly climatic data (temperature, *T*, relative humidity, *RH*, daily reference evapotranspiration, ET_0 and precipitation, *P*) were recorded by an automatic weather station of the Servicio de Información Agraria de Murcia, located 8.5 km from the experimental field (CIA-42, www.siam.es). Hourly vapour pressure deficit (VPD) was calculated from air temperature and relative humidity values.

The soil volumetric water content (θ_v) was measured from 10 cm to a maximum depth of 1 m every 0.1 m with a frequency domain reflectometry (FDR) probe (Diviner 2000®, Sentek Pty. Ltd., South Australia) (Picture 2). Three access tubes (one per replicate) were installed within the emitter wetting area (~ 25 cm from the main trunk) on randomly selected vines. Measurements were taken from 10:00 h to 12:00 h during the experimental period. Summed θ_v values through the monitored soil profile were used to calculate the level of relative extractable water (REW), defined by the equation (Granier, 1987):

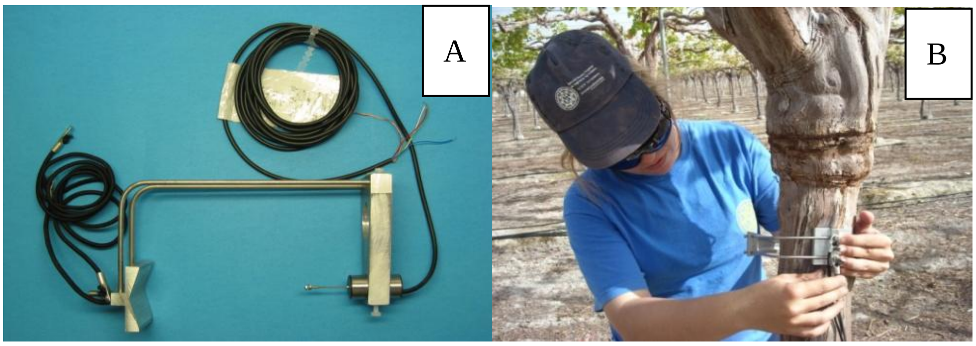
$$\mathrm{REW} = \frac{\mathrm{R} - \mathrm{R}_{\mathrm{min}}}{\mathrm{R}_{\mathrm{max}} - \mathrm{R}_{\mathrm{min}}} \qquad \text{[Equation 2]}$$

where R (%) is the actual soil water content, R_{MIN} (%) the minimum soil water content measured in dry conditions, and R_{MAX} (%) the maximum soil water content obtained in each probe. The values of R_{MIN} and R_{MAX} were 15% and 42%, respectively.



Picture 2. *Installation of access tubes of FDR probes (A) and the model Diviner 2000 used to determine REW (B).*

Micrometric trunk diameter fluctuations (TDF) were monitored throughout the experimental period in six selected vines (2 vines per replicate), using a set of linear variable displacement transducers (*LVDT*; Solartron Metrology, Bognor Regis, UK, model DF ± 2.5 mm, precision ± 10 µm) installed on the northern side of the trunk, 120 cm above-ground and mounted on holders built of aluminium and invar – an alloy comprising 64% Fe and 35% Ni that has minimal thermal expansion (Picture 3). Several indices were derived from trunk diameter fluctuations according to Goldhamer and Fereres, (2001): maximum daily trunk diameter (MXTD), minimum daily trunk diameter (MNTD), maximum daily shrinkage (MDS = MXTD – MNTD) and trunk daily growth rate (TGR, calculated as the difference between MXTD of two consecutive days).



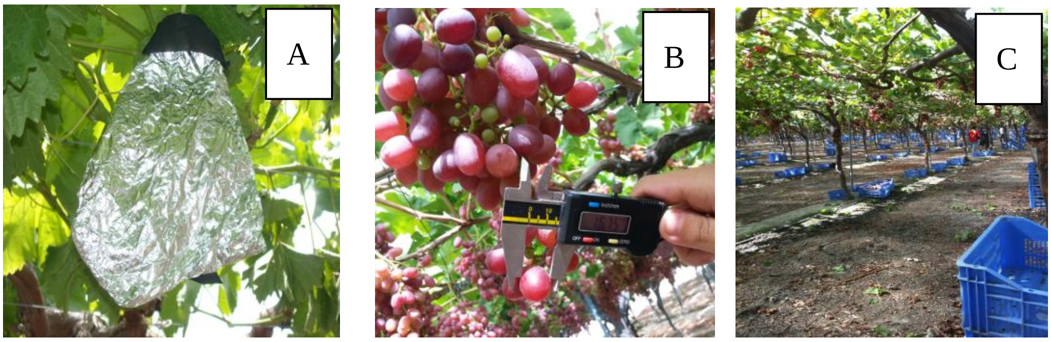
Picture 3. *Detail of LVDT sensor (A) and its collocation at the field (B).*

Midday (12:00 h solar time) stem water potential (Ψ_S) was measured every 7-10 days on six sunny leaves (2 leaves per replicate), in the same vines which were monitored with *LVDT* sensors. Leaves were covered with aluminum foil for at least 2h before being used (Picture 4A). Measurements were performed with a pressure chamber (Soil Moisture Equipment Corp. Model 3000) according to the experimental protocol recommended by Hsiao (1990).

Berry equatorial diameter was determined every 7-10 days from fruit set (early June) to first harvest time (mid-September) with a digital calliper (Mitutoyo, CD-15D) using 45 randomly chosen berries (15 berries per replicate) (Picture 4B).

Trunk perimeter was measured in 18 vines (6 vines per replicate) from the central row at the end of each growing season. Trunk cross-sectional area (TCSA) was estimated considering that the section of the trunk is a perfect circle. Measurements were taken 1 m below the graft union by tape-measure. Mature clusters were harvested from the beginning of September to the end of last harvest (early-November). The exact commercial picking date depended on the year. Total yield (expressed as kg per vine) and crop level (expressed as number of clusters per vine) was obtained for all the vines of the experiment. (Picture 4C). Crop load was calculated as the ratio of kg per vine to TCSA.

Fruit quality was measured in 100 berries per replicate (3 replicates per treatment) at the time of harvest. Total soluble solids (TSS) were determined in berry juice using a hand refractometer (Atago N1, Japan). Values were expressed as ºBrix. The titratable acidity (TA) of the berry juice was determined by titrating 1 mL of juice with 0.1 N NaOH and expressed as g L^{-1} of tartaric acid. For ABA analysis, three replicates, each consisting of 10 µl of sap, were used, following the procedure (radioimmunoassay) described by Quarrie et al. (1988).

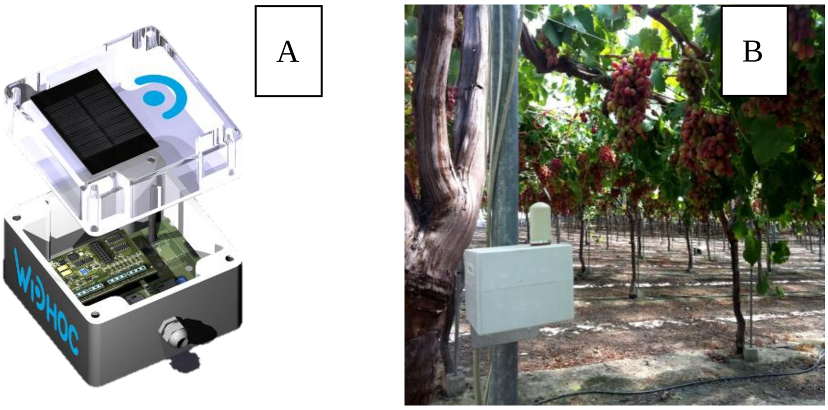


Picture 4. *Detail of leave covered for determining* Ψ_s *(A), berry diameter measurement with calliper (B), and total yield (C).*

2.3. Data collection system

Data from the *LVDT* sensors were collected continuously by means of a wireless sensor network (WSN). WSN is a technology that consists of sensor nodes that can be distributed in a flexible way. These nodes are battery powered so they are energy-independent and the information received from the sensors is sent wirelessly. The nodes send data at regular intervals (approximately every 20 minutes) to a coordinator node, which is connected to a PC where the data is processed and stored (Picture 5). The power system of the nodes is based on lithium polymer batteries of 5000 mAh and small solar panels of 0.4 W (5V/80 mA), allowing virtually unlimited autonomy. In addition, the sensor nodes can store a large amount of data as they use SD cards. The information stored on the node can be sent in real time or in bursts of several samples to optimize the battery usage

The WSN deployed in this experiment uses Zigbee technology and is configured as a collaborative network, whereby some nodes work in sensor mode, others act as routers (covering greater distances between sensor nodes) and one is the coordinator node, collecting all the data from the sensor nodes. This configuration allows a highly flexible installation, since the sensors can be installed in the optimal vines with no data loss or wiring problems (Picture 5).



***Picture 5.** Detail of the aspect extern of nodes (A) and their installation in the experiment (B).*

In this experiment one node per repetition (n=3) was installed to monitor the *LVDT* sensors, two nodes were configured as routers and one coordinator node collected all the data (Picture 6).

***Picture 6.** Nodes deployment within the experimental plot.*

2.4. Reference equations

Over the 2-year study period, relationships by means of regression equations between TDF-derived indices and stem water potential (Ψ_s) were assessed with selected variables related to the evaporative demand of the atmosphere (for the period from early June to end of November, after the last harvest, each year). The variables used were daily reference evapotranspiration (ET_0), global solar radiation (R_s), daily mean, maximum and midday air temperature between 10:00 and 15:00 h solar time, (T_m, T_{mx}, and T_{md}) and daily mean, maximum and midday air vapour pressure deficit between 10:00 and 15:00 h solar time (VPD_m, VPD_{mx} and VPD_{md}), respectively. Moreover, the relationships were assessed for individual phenological stages (pre and post-veraison), the whole season in the years 2012 and 2013 and also for the whole study period (the sum of the years 2012 and 2013). The effects of girdling and the hail mesh over the plant water status indicators were also assessed.

2. 5. Irrigation scheduling based on SI_{MDS} and Ψ_s

The growing season was divided into two periods according to veraison or berry color: (i) pre-veraison (from April to August) and (ii) post-veraison (from August to November).

The irrigation scheduling in the year 2012 was based on ET_c, regardless of pre- and post-veraison periods considered.

During pre-veraison 2013, the irrigation scheduling was based on the best fitting reference equation obtained in 2012. The amount of irrigation water applied was checked weekly to maintain SI_{MDS} at around unity (SI_{MDS} = 1), applying two criteria:

i) SI>1 (4 days or more) → increase irrigation by 10%

ii) SI≤1 (4 days or more)→ reduce the irrigation by 10%

During post-veraison 2013 the irrigation was scheduled for every 7-10 days to provide values of Ψ_s representing non-limiting soil-water conditions. In order to know the amount of water to be applied, the following equation was used:

$$Irrigation \cdot Applied\,(\%) = \frac{\psi_{observed} - \psi_{threshold}}{\psi_{threshold}} \times 100 \qquad \text{[Equation 3]}$$

where $\Psi_{observed}$ was the mean Ψ_s values each day of measuring and $\Psi_{threshold}$ was -0.65 MPa (Conesa *et al.,* 2012).

2.6. Statistical analysis

Analysis of variance (one way ANOVA) was used to discriminate the year effect on the vine size, yield and crop load. Relationships between plant water status indicators and meteorological variables were explored through linear and non-linear regression analyses. Analysis of covariance was used to determine differences between linear regressions. The agreement of the regressions among variables as well as of model validations was evaluated through the coefficient of determination (r^2) and the mean square error (MSE). Data were analyzed using the statistical software package Statgraphics Plus (v.5.1).

3. RESULTS

3.1. Seasonal evolution of soil and plant-water-status indicators

The seasonal patterns of daily reference evapotranspiration (ET_0), mean daily temperature (T_m) and mean vapour pressure deficit (VPD_m) were similar both years (Figs. 1A-B), reaching maximum seasonal values by early August and the minimum at the end of November. Interestingly, the greatest day-to-day variability was observed in VPD_m (Figs. 1A-B). ET_0 and precipitation had average annual values of 1274 and 375 mm, respectively, during 2012. Evaporative demand was similar in 2013 (1253 mm) but precipitation was lower (195 mm) (Figs. 1A-B).

Vines irrigated to ensure non-limiting soil water conditions maintained $REW_{0\text{-}100}$ values close to unity throughout the irrigation season in both of the studied years, with mean seasonal values of 0.98 and 0.95 in 2012 and 2013, respectively (Figs 1C-D). The mean volumetric soil water content (θ_v) was almost constant in the top 0-40 cm of soil, with values close to that corresponding to field capacity content (~340 mm m^{-1}) in both years (data not shown).

The annual trunk growth determined by the values of MXDT was around ~2.5 mm in the two studied years, and the equatorial diameter of berries was about ~18 mm at the time of harvest in this experiment (Figs. 1E-F). In the year 2012, both growth variables reached their maximum value approximately simultaneously, but in the year 2013, there seemed to be a delay in the berry growth dynamics compared to stem growth, although, the growth rate reduction was normal at the beginning of post-veraison (early-August), when berries reached 95% of their final size (Figs. 1E-F).

Seasonal TDF-indices trends were similar to the atmospheric demand, except after girdling and after veraison. After girdling, the usefulness of the TDF-indices to detect variations diminished through a decrease in MDS and TGR values (Fig. 1). Approximately, 20 days after of girdling (mid-July), the usefulness of the trunk was recovered, as reflected by an increase in MDS and TGR values (consistent with the increase in evaporative demand). After that, TGR decreased again until late July, whereas the berry equatorial diameter continued to increase until veraison. During post-veraison, there was also a pronounced decrease in the dynamics of MDS and TGR (Fig. 1).

TGR did not remain constant through the active growth season (Figs. 1G-H), a clear decrease to values close to zero was observed at the onset of veraison. MDS values decreased sharply, registering approximately 100 µm during post-veraison (Figs. I-J). Reflecting the evaporative demand, the average seasonal values for Ψ_s were –0.65MPa and –0.68MPa in 2012 and 2013, respectively. Ψ_s tended to be higher in low demand periods, whereas minimum values (–0.87 and –0.93 MPa in 2012 and 2013, respectively) were observed in high-demand stages, coinciding with veraison (early- August). A gradual fall in the value of Ψ_s during 2013 was also evident (Figs. 1K-L).

Interestingly, the hail mesh (late-August) influenced the above parameters. Indeed, Ψ_s values increased and MDS decreased, both tending to stabilize at the end of the season (Fig. 1). Furthermore, during pre-veraison the mean values of leaf xylem ABA concentration (ABA_{xylem}) were 1522.86 ± 248.91 nM, increasing after post-veraison (3612.77 ± 977.38 nM) in agreement with the increase of the evaporative demand (Figs. 1A-B) and also due to the S-ABA applied by the technical department of the commercial farm during this period to increase berry color (data not shown).

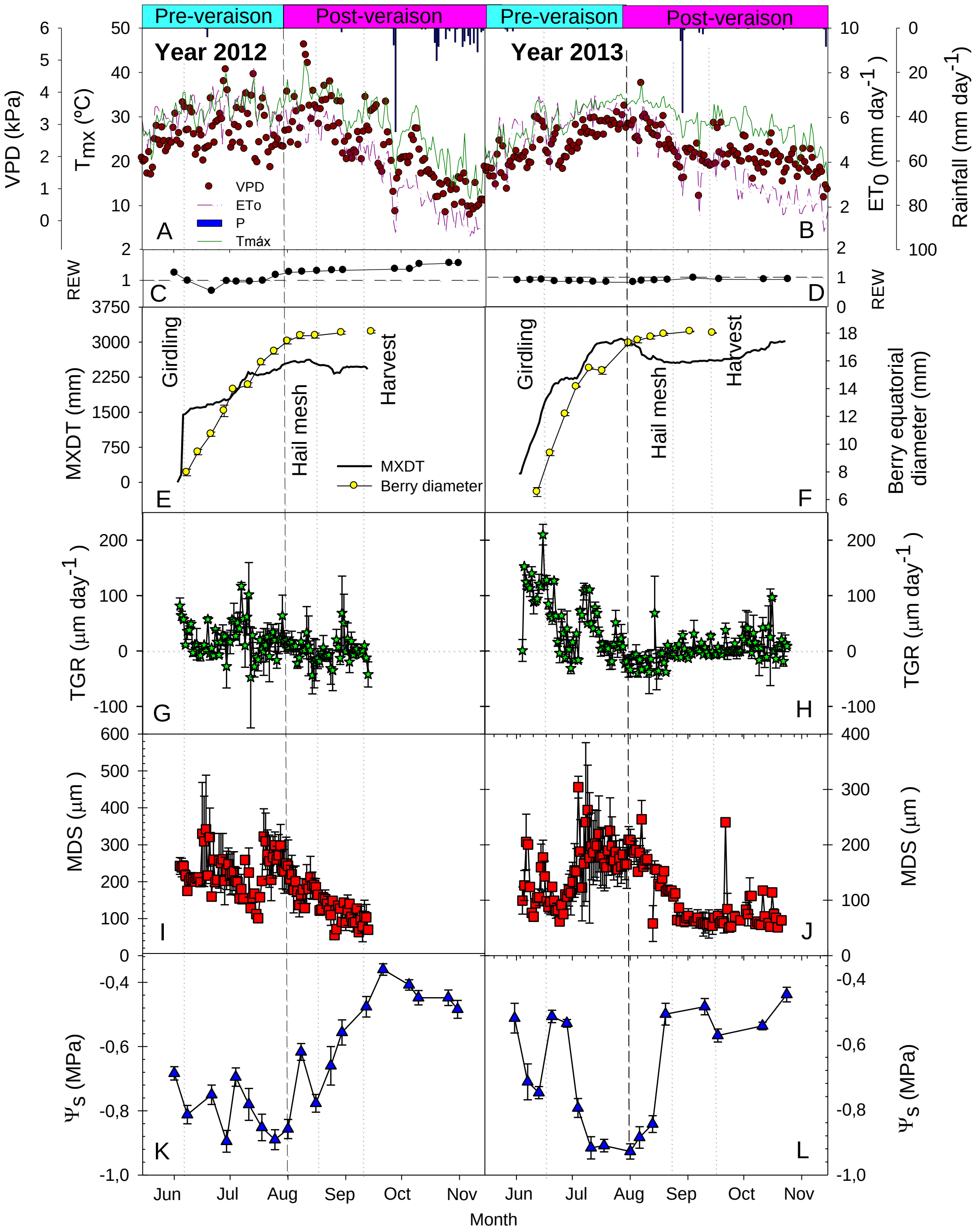


__Figure 1.__ Seasonal evolution of (A, B) reference evapotranspiration (ET_0), air vapour pressure deficit (VPD), daily maximum air temperature and rainfall; (C,D) relative extractable water (REW); (E,F) maximum daily trunk diameter (MXDT) and berry equatorial diameter; (G,H) trunk daily growth rate (TGR); (I,J) maximum daily trunk shrinkage (MDS); and (K,L) midday stem water potential (Ψ_S) during the years 2012 and 2013, respectively. Vertical lines delimit the phenological periods of pre and post-veraison and also indicate when girdling (early-June), the collocation of the hail mesh (late-August) and harvest (mid-September) had occurred. Data are means ± SE of 45 berries, 6 LVDT sensors, and 6 leaves, respectively.

3.2. Vegetative growth and yield components and quality

No inter-annual differences in trunk cross sectional area (TCSA), crop level (number of clusters per vine) or productivity efficiency (PE, calculated as kg per cm^2 TCSA), were found (Table 1). However, total yield in 2012 was 15% lower than in the second year as a result of the lower mean weight of clusters. Similarly, the total soluble solids (TSS) content was higher in 2013, but with no significant difference between years. Titratable acidity (TA) decreased significantly with the greater total yield (Table 1).

Table 1. *Mean trunk sectional area (TCSA), fruit yield, crop level, productivity efficiency (PE), mean weight of clusters, titratable acidity (TA) and soluble solids content (TSS) during the years 2012 and 2013.*

	TCSA	Yield	Crop level	PE	Mean Weight	TA	TSS
Year	**(cm^2)**	**(kg vine^{-1})**	**(number of clusters vine^{-1})**	**(kg cm^{-2} TCSA)**	**(g)**	**(g L^{-1})**	**(ºBrix)**
2012	67.5	61.4	143	0.90	429	5.21	18.67
2013	70.7	72.8	156	1.02	466	4.26	19.22
Significant	n.s	*	n.s	n.s	*	*	n.s

*The last row shows significant differences between years according to the analysis of variance. **P<0.01. *P<0.05. n.s: not significant.*

3.3. Reference equations for MDS and Ψ_s

Associations were analyzed between MDS and Ψ_s and the climatic variables mentioned in section 2.4 from Material and Methods (Tables 2 and 3). The results showed that MDS and Ψ_s were clearly affected by environmental conditions: increases in MDS coincided with more negative Ψ_s values (Fig. 1). However, TGR was not significantly correlated with any climatic variable in either of the two years studied. When TGR (mean of the years 2012 and 2013) was analyzed with mean temperature (T_m) and mean vapour pressure deficit (VPD_m) the r^2 values obtained were 0.0021 (P-value = 0.3914) and 0.0001 (P-value = 0.8179), respectively.

Overall, T was the climatic variable that best correlated with MDS (r^2 ranging from 0.21 to 0.66) in both years (Table 2). When daily mean values (T_m) were considered, the coefficients of determination (r^2) and MSE values indicated a strong correlation ($r^2 = 0.67$) but quite similar MSE and r^2 to the others forms of T (T_{mx} and T_{md}). Best-fit linear annual regressions of Ψ_s *versus* the meteorological variables showed that this parameter was best correlated with ET_0 and T. In contrast, the lowest correlation found with MDS and Ψ_s were with R_s and $VPD_{m.}$

Table 2. *Intercept (a), slope (b), coefficient of determination (r^2), number of data points (n) and mean square error (MSE) of best fit first-order linear equations (y= a+ bx) between maximum daily trunk shrinkage (MDS) and selected meteorological variables.*

Stage	**Season**	**a**	**b**	**r^2**	**n**	**MSE**
MDS *vs.* ET_0						
PRE-V	**2012**	-164.84	56.11	0.46***	108	5155
	2013	-45.84	31.28	0.45***	106	2100
POST-V	**2012**	-44.15	34.21	0.30***	43	1537
	2013	-14.55	26.52	0.39***	64	1511
SEASON	**2012**	-146.84	52.93	0.44***	151	4125
	2013	-34.11	29.21	0.43***	170	2186
	2012+2013	-74.76	38.87	0.44***	321	3311
MDS *vs.*VPD_m						
PRE-V	**2012**	-12.86	106.53	0.39***	108	5764
	2013	-8.79	88.1	0.49***	106	2347
POST-V	**2012**	76.16	33.41	0.16**	43	1832
	2013	-24.82	98.18	0.56***	64	1086
SEASON	**2012**	20.84	77.1	0.28***	151	5309
	2013	-13.4	90.64	0.51***	170	1859
	2012+2013	52.04	37.72	0.21***	321	4657

*Continued below

Stage	Season	a	b	r^2	n	MSE
MDS *vs.* VPD$_{mx}$						
PRE-V	**2012**	-20.7	138.12	0.37***	108	5993
	2013	3.35	57.05	0.60***	106	1837
POST-V	**2012**	73.45	44.36	0.16**	43	1844
	2013	-32.99	57.15	0.38***	64	1545
SEASON	**2012**	13.55	101.84	0.27***	151	5393
	2013	3.73	50.78	0.46***	170	2045
	2012+2013	63.75	36.31	0.12***	321	5176
MDS *vs.* VPD$_{md}$						
PRE-V	**2012**	-29.32	85.62	0.43***	108	5384
	2013	-18.24	68.02	0.50***	106	2338
POST-V	**2012**	71.55	24.28	0.17**	43	1803
	2013	-44.83	73.33	0.49***	64	1260
SEASON	**2012**	22.3	54.29	0.27***	151	5407
	2013	-13.9	63.61	0.45***	170	2116
	2012+2013	-2.08	61.27	0.37***	321	3716
MDS *vs.* T$_{m}$						
PRE-V	**2012**	-202.75	16.38	0.66***	108	3249
	2013	-152.13	12.13	0.64***	106	1650
POST-V	**2012**	-78.15	8.4	0.21***	43	1676
	2013	-201.08	13.06	0.42***	64	1438
SEASON	**2012**	-135.21	12.38	0.46***	151	3961
	2013	-135.06	11.21	0.49***	170	1930
	2012+2013	-147.49	12.34	0.48***	321	3031
MDS *vs.* T$_{mx}$						
PRE-V	**2012**	-227.53	13.26	0.60***	108	3746
	2013	-167.88	10.09	0.58***	106	1940
POST-V	**2012**	-63.1	5.97	0.22***	43	1702
	2013	-285.66	12.71	0.47***	64	1305
SEASON	**2012**	-142.48	9.63	0.41***	151	4341
	2013	-155.39	9.15	0.46***	170	2069
	2012+2013	-162.25	9.83	0.44***	321	3292
MDS *vs.* T$_{md}$						
PRE-V	**2012**	-225.59	13.47	0.63***	108	3510
	2013	-106.83	8.5	0.53***	106	2175
POST-V	**2012**	-55.07	5.86	0.23***	43	1677
	2013	-282.9	13.3	0.49***	64	1260
SEASON	**2012**	-142.5	9.84	0.43***	151	4184
	2013	-100.88	7.77	0.42***	170	2224
	2012+2013	-132.29	9.22	0.45***	321	3215

Continued below

Stage	Season	a	b	r^2	n	MSE
MDS *vs.* R_s						
PRE-V	**2012**	-96.41	0.83	0.15***	108	8110
	2013	-40.76	0.51	0.19***	106	3760
POST-V	**2012**	-2.48	0.55	0.12***	43	1918
	2013	-18.61	0.5	0.23***	64	1922
SEASON	**2012**	-57.74	0.72	0.14***	151	6354
	2013	-12.59	0.44	0.19***	170	3109
	2012+2013	-34.29	0.57	0.18***	321	4914

*ET_0= daily reference evapotranspiration. VPD = daily air vapor pressure deficit. T= daily air temperature. R_s = global solar radiation. Subscripts m, mx and md indicate daily mean, maximum and midday (mean for the period 11:00-15:00 h solar time), respectively. **, Significance at P<0.01; and *** P<0.001, respectively.*

The above relationships differed according to the phenological period (pre and post-veraison) (Tables 2 and 3). Before veraison, MDS showed a better correlation with climatic data, whereas Ψ_s did so after veraison. Interestingly, the coefficients of determination of MDS with all the climatic variables assessed strongly decreased in the post-veraison period (Table 2).

Table 3. *Intercept (a), slope (b), coefficient of determination (r^2), number of data points (n) and mean square error (MSE) of best fit first-order linear equations (y= a+ bx) between stem water potential (Ψ_s) and selected meteorological variables.*

Stage	Season	a	b	r^2	n	MSE
Ψ_s *vs.* ET_0						
PRE-V	**2012**	-0.960	0.024	0.02n.s	8	0.0071
	2013	0.109	-0.139	0.29n.s	7	0.0223
POST-V	**2012**	-0.248	-0.075	0.66**	10	0.0093
	2013	-0.148	-0.114	0.84**	7	0.0065
SEASON	**2012**	-0.226	-0.089	0.73***	18	0.0087
	2013	-0.247	-0.087	0.46**	14	0.0177
	2012+2013	-0.235	-0.085	0.61***	32	0.0123

**Continued below*

Stage	Season	a	b	r^2	n	MSE
Ψ_s *vs.* VPD$_m$						
PRE-V	**2012**	-0.667	-0.059	0.11n.s	8	0.0640
	2013	-0.420	-0.205	0.25n.s	7	0.0234
POST-V	**2012**	-0.319	-0.157	0.61**	10	0.0106
	2013	-0.017	-0.529	0.87**	7	0.0052
SEASON	**2012**	-0.325	-0.182	0.57***	18	0.0127
	2013	-0.284	-0.296	0.50**	14	0.0164
	2012+2013	-0.591	-0.039	0.07n.s	32	0.0295
Ψs *vs.*VPD$_{mx}$						
PRE-V	**2012**	-0.668	-0.075	0.09n.s	8	0.0065
	2013	-0.324	-0.172	0.36n.s	7	0.0200
POST-V	**2012**	-0.309	-0.208	0.61**	10	0.0107
	2013	-0.110	-0.241	0.38n.s	7	0.0249
SEASON	**2012**	-0.310	-0.242	0.62***	18	0.0125
	2013	-0.307	-0.166	0.47**	14	0.0174
	2012+2013	-0.410	-0.144	0.44***	32	0.0177
Ψs *vs.* VPD$_{md}$						
PRE-V	**2012**	-0.643	-0.051	0.16n.s	8	0.0061
	2013	-0.332	-0.184	0.36n.s	7	0.0200
POST-V	**2012**	-0.292	-0.109	0.57**	10	0.0118
	2013	-0.025	-0.326	0.52n.s	7	0.0194
SEASON	**2012**	-0.314	-0.128	0.48***	18	0.0171
	2013	-0.298	-0.190	0.53**	14	0.0154
	2012+2013	-0.373	-0.125	0.38***	32	0.0197
Ψs *vs.* T$_m$						
PRE-V	**2012**	-0.344	-0.017	0.23n.s	8	0.0055
	2013	0.633	-0.059	0.65*	7	0.0109
POST-V	**2012**	-0.062	-0.021	0.49*	10	0.0138
	2013	0.749	-0.060	0.74*	7	0.0103
SEASON	**2012**	0.026	-0.028	0.48**	18	0.0172
	2013	0.539	-0.053	0.58***	14	0.0127
	2012+2013	0.102	-0.033	0.46***	32	0.0172
Ψs *vs.* T$_{mx}$						
PRE-V	**2012**	-0.402	-0.011	0.21n.s	8	0.0057
	2013	0.342	-0.036	0.52*	7	0.0149
POST-V	**2012**	-0.077	-0.015	0.41*	10	0.0161
	2013	0.711	-0.045	0.65*	7	0.0138
SEASON	**2012**	-0.032	-0.019	0.35**	18	0.0215
	2013	0.454	-0.038	0.57***	14	0.0136
	2012+2013	0.009	-0.021	0.34***	32	0.0209

**Continued below*

Stage	Season	a	b	r^2	n	MSE
Ψs *vs.* T_{md}						
PRE-V	**2012**	-0.425	-0.011	0.20n.s	8	0.0058
	2013	0.373	-0.038	0.53*	7	0.0147
POST-V	**2012**	-0.003	-0.002	0.60**	10	0.0146
	2013	0.640	-0.044	0.69*	7	0.0124
SEASON	**2012**	-0.091	-0.002	0.65***	18	0.0202
	2013	0.532	-0.042	0.57***	14	0.012
	2012+2013	-0.010	-0.066	0.35***	32	0.0207
Ψs *vs.* R_s						
PRE-V	**2012**	-1.202	0.001	0.14n.s	8	0.0067
	2013	-25.821	0.005	0.26n.s	7	0.0232
POST-V	**2012**	-0.003	-0.002	0.60**	10	0.0109
	2013	-0.069	-0.002	0.67*	7	0.0131
SEASON	**2012**	-0.091	-0.002	0.65***	18	0.0114
	2013	-0.278	-0.001	0.28*	14	0.0237
	2012+2013	-0.157	-0.001	0.49***	32	0.0162

*ET_0= daily reference evapotranspiration. VPD = daily air vapor pressure deficit. T= daily air temperature. R_s = global solar radiation. Subscripts m, mx and md indicate daily mean, maximum and midday (mean for the period 11:00-15:00 h solar time), respectively. n.s. not significant; *, Significance at P<0.05; **, P<0.01; and *** P<0.001, respectively.*

The stability of the relationship MDS and Ψ_s individually *versus* T_m can be seen in Fig. 2.

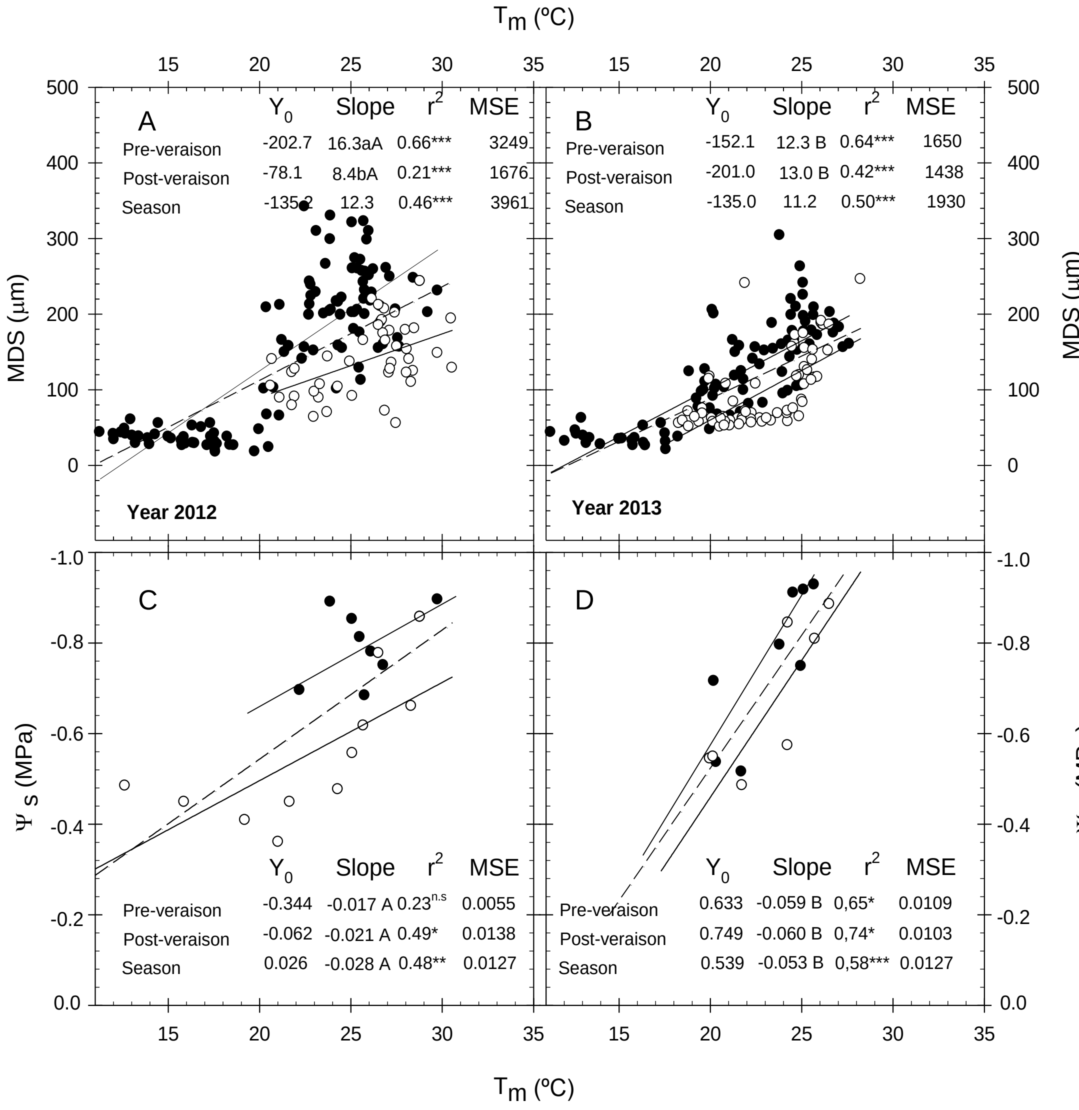


Figure 2. *Relationship between maximum daily trunk shrinkage (MDS) and mean temperature (T_m) for pre-veraison (•), post-veraison (○) and all the season in the years 2012 (A) and 2013 (B). Relationship between midday stem water potential (Ψ_s) and mean temperature (T_m) for pre-veraison (•), post-veraison (○) and the whole season in 2012 (C) and 2013 (D). Each point is the average of 6 LVDT sensors and 6 leaves, respectively. MSE mean square error. n.s = not significant, *** Significance at P<0.001, ** P<0.01, * P<0.05. Parameters for best fit first-order linear equations (y = slope x +y_0) are indicated. Capital and lower case letters indicate significant inter-and intra-year differences, respectively, for pre- and post-veraison and the whole season.*

For MDS in the year 2012, the slope derived for post-veraison was significantly lower (mean reduction of 48%) than that derived for pre-veraison, whereas during 2013, the difference was minimal and the slope was similar in pre- and post-veraison. Interestingly, inter-year differences between both phenological periods were found (Figs. 2A-B). For Ψ_s *versus* T_m, no intra-year differences were observed (Figs. 2C-D), although significant inter-year differences were detected when pre-, post-veraison and the whole-season were compared (Figs. 2C-D). In this sense, the slope for the entire season significantly increased during the observation period (around 47% higher in 2013 respect to 2012).

3.4. Relationship between MDS and Ψ_s

A strong correlation was found between the daily values of MDS and Ψ_s [MDS= -66.9 – 321.9 Ψ_s; r^2= 0.67; $P<0.001$] when pooling data for the two years studied (Fig. 3). The relationship showed different slopes and intercepts, depending on the phenological stage: the slope corresponding to veraison (from July to August) was non-significant (P-value=0.5401) and lower (~96%) than the slopes obtained in pre- (P-value= 0.042) and post-veraison (P-value= 0.008), respectively (Fig. 3).

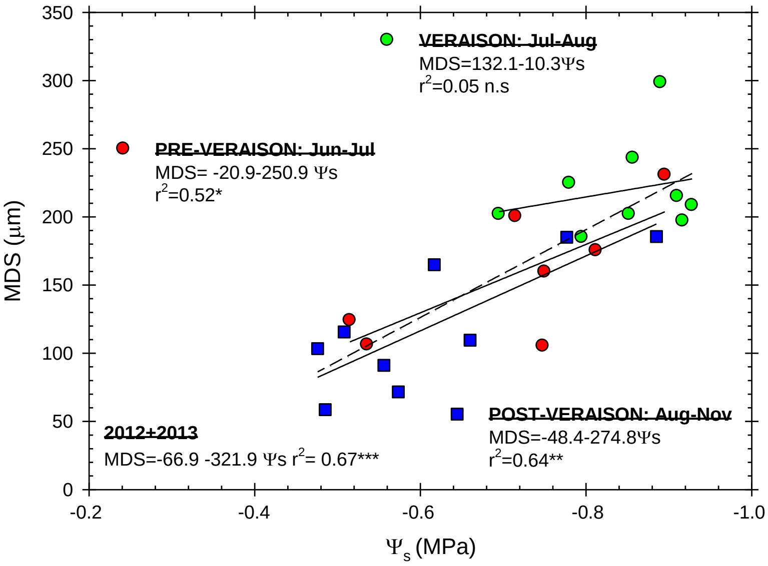


Figure 3. *Relationship between maximum daily trunk shrinkage (MDS) and midday stem water potential (Ψ_s) during 2012 and 2013 for the months (●) June-July (●) July-August and (■) August-November. The discontinuous line corresponds to the mean relationship in both years. n.s = not significant, *** $P<0.001$, ** $P<0.01$, * $P<0.05$.*

3.5. Irrigation scheduling based on IS_{MCD} and Ψ_s

The reference equation MDS *versus* T_m obtained in the pre-veraison of 2012 was used for irrigation scheduling during the pre-veraison of 2013 in order to maintain SI_{MDS} ~ 1 (Fig. 4). SI averaged 0.84 during this period and MDS_{obs} reached maximum values of ~ 210 µm (Fig. 4B). Moreover, the amount of irrigation water applied was reduced by about 17 % with respect to the water applied in the previous year (Fig 4A), while similar ET_0 and precipitation values were registered (368 mm and 0.8 mm, respectively). During post-veraison 2013, the irrigation was scheduled by maintaining a threshold Ψ_s of about -0.65MPa, using Equation 3. For example, for a given $\Psi_{observed}$ value of -0.85MPa, the corresponding increase ($\Psi_{observed} - \Psi_{threshold}$) was 0.20 MPa. To compensate for this difference, the amount of irrigation water applied was increased by 30%. The SI_{MDS} averaged values lower than unity (0.54) as did well watered vines. Moreover, the irrigation water applied during post-veraison was around 8% less in 2013 compared with 2012 (Fig. 4).

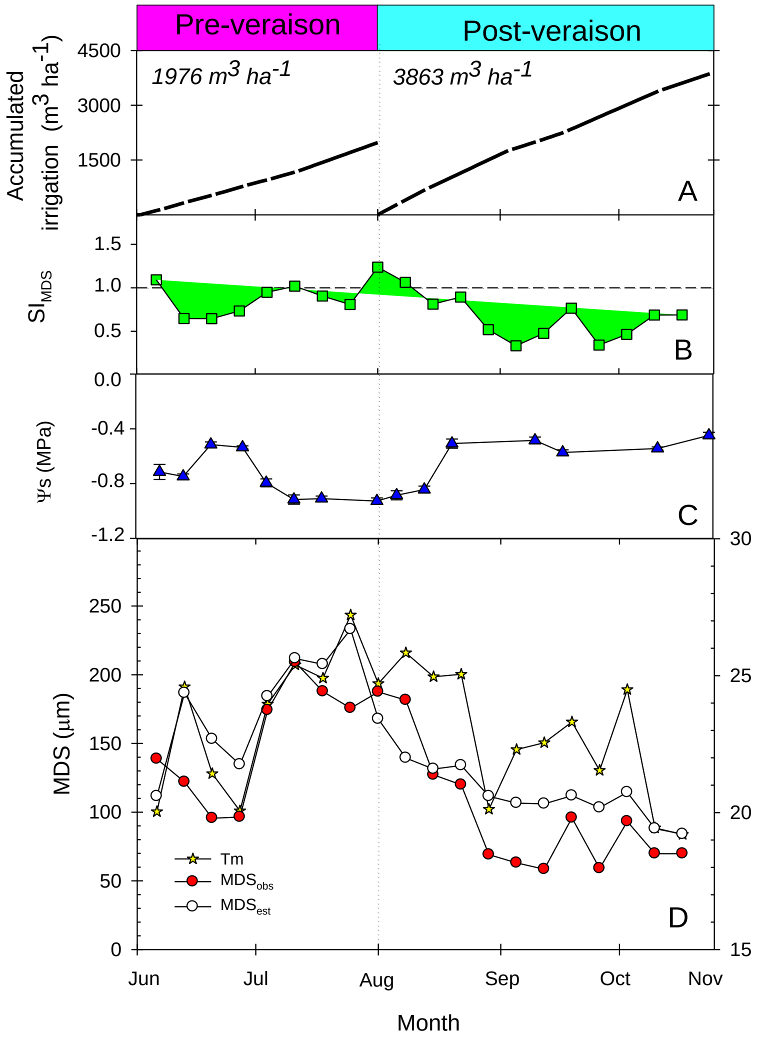


Figure 4. *Seasonal evolution of the (A) accumulated irrigation applied; (B) signal intensity (SI); (C) midday stem water potential (Ψ_s) and (D) weekly MDS_{est}, weekly MDS_{obs} and T_m during 2013. Each point is the mean of 6 sensors LVDT. The discontinuous line delimits the phenological periods of pre and post-veraison, respectively.*

MDS_{obs} presented a similar dynamic to the MDS_{est}, demonstrating the goodness of the relationship between MDS and T_m. MDS_{obs} was closely related with MDS_{est} (r^2= 0.77) (Fig. 5). Moreover, when the phenological stages (pre- and post-veraison) were considered, the lowest coefficient of determination was obtained for post-veraison (r^2= 0.36).

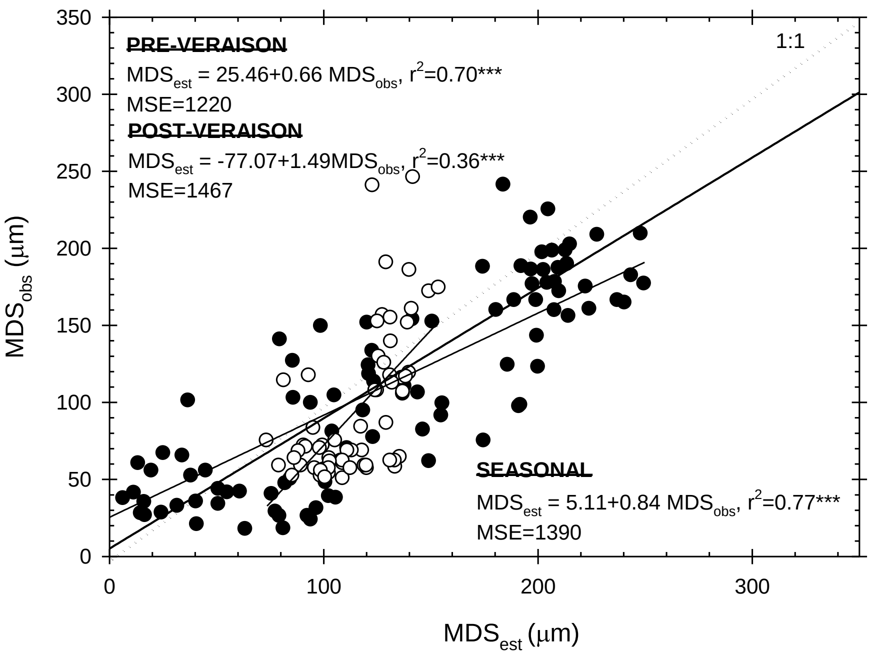


***Figure 5.** Observed (MDS_{obs}) versus estimated (MDS_{est}) maximum daily trunk shrinkage during 2013. MDS_{est} was derived from the equations obtained during 2012 (MDS vs. T_m) for pre and post-veraison, respectively. Dotted line gives the 1:1 relationship. Each point indicates both phenological periods: pre-veraison (•) and post-veraison (◦), respectively. *** $P<0.001$.*

4. DISCUSSION

The full vine water requirements applied throughout the study period allowed REW values to be maintained close to unity (Figs. 1C-D), and Ψ_s values (Figs. 1K-L) within the range of non-stress conditions for table grapes (Conesa *et al.,* 2012; Sellés *et al.,* 2004). Stomatal conductance values (g_s) averaging 250 µmol m^{-2} s^{-1} during the study period indicated that the vines were properly irrigated (Conesa *et al.,* 2012). To our knowledge, no reference lines nor the use of SI_{MDS} have been reported to date for irrigation scheduling in table grapes. Taking into account that MDS and Ψ_s were obtained under unlimited water conditions, during pre-veraison, the mean temperature (T_m) best explained the changes in MDS and Ψ_s (Tables 2 and 3). However, during post-veraison VPD and ET_0 can be used to obtain linear reference lines with both MDS and Ψ_s, mainly due to efficient water movements from stores within the vine when evaporative demand increases (Ortuño *et al.,* 2009). The irrigation scheduling based on SI_{MDS} was successful before veraison, reducing the amount of water applied (~17%) compared with conventional scheduling based on ET_c for 2012 (Fig. 4). After veraison, the use of Ψ_s was the most reliable plant water status indicator for irrigation scheduling (reducing the amount of water by ~8% with respect to ET_c from 2012). Moreover, the results showed an increase in the total yield and berry quality (since higher TSS and lower TA promote higher levels of maturity index, Conesa *et al.,* 2012) during the second year (Table 1), underlining the advantages of irrigation scheduling based on SI_{MDS} and Ψ_s compared with ET_c .

Changes in trunk diameter may be related with changes in the water content of the whole plant or with climatic conditions (Huguet *et al.,* 1992). The fact that TGR and climatic variables were uncorrelated in well-watered vines might be due to the high day-to-day variability of the values of meteorological variables (*e.g.* VPD) (Figs. 1G-H) and the influence of other factors such as crop phenology (Berman and DeJong, 2003) or carbon availability (Daudet *et al.,* 2005). Therefore, the seasonal pattern of TGR was more dependent on the phenological period (pre- and post-veraison) than MDS. Our results revealed the unsuitability of using TGR for establishing reference lines for irrigation scheduling of table grapes as reported by Egea *et al.,* (2009) in mature almond. The high sensitivity of MDS to weather conditions (Figs. 1I-J) agrees with previous reports in other crops (reviewed by Ortuño *et al.,* 2010 and Fernández and Cuevas, 2010). MDS can be used as a good indicator of transpiration intensity when the soil water content is not strongly depleted. Indeed, increases in MDS have been associated with decreases in water potential in pomegranate trees (Galindo *et al.,* 2013).

Standard cultural practices such as girdling and the use of a hail mesh affected the plant water status indicators (Fig. 1). Stem girdling is known to improve fruit yield and quality (Cohen, 1981), thereby preventing the translocation of photosynthates from the source to sinks located below the girdle until the wound heals. The decrease observed in MDS after girdling was probably due to the increased amount of photosynthates available to fruits enabling them to increase their size (Cohen, 1981). Our results showed that the recovery time needed by stem tissues to transpire properly again after girdling is about 20 days. In Thomson Seedless, Williams and Ayars (2005) found that after vines were girdled, water use decreased for a period of approximately 4 weeks.

Meanwhile, the hail mesh reduced the water requirements of the vines as shown by the increased values of Ψ_s (Figs. 1K-L). The conditions below this mesh are very different to those outside. Therefore, the ET_0 estimated by the Penman-Montheith equation is higher than the real conditions. An important point of this equation is the advective component (wind), which is much lower under the hail mess than it would be without it (or outside). Moreover the hail mesh slightly reduced solar radiation, so that the radioactive component would also have been smaller.

The regressions of MDS and Ψ_s with climate data differed when the season considered was divided into phenological stages (Tables 2 and 3). Before veraison, the best fit with environmental variables was found with MDS, whereas during post-veraison the best fit was with Ψ_s values. After veraison, Intrigliolo and Castel (2007b) did not report any relationship between grapevine water status and MDS, due to high competition for photoassimilates between fruit and vegetative growth (to the detriment of the latter) and due to the decrease in elasticity of the trunk tissues, which lowered the values of MDS (Egea *et al.,* 2009). The effect of sugars on xylem diameter fluctuation might be smaller than their effect on phloem diameter fluctuation. Stem growth diameter was measured over bark, which means that the diameter changes of the elastic living bark would have played a major role in the measurements rather than xylem diameter changes. In this sense, deformation of tissues is mainly induced by the dynamics in water potential within the plant (Zweifel *et al.,* 2014). In well-watered vines changes in transpiration should maintain higher levels of water potential. Indeed, the same authors reported that the resulting gradients in water potential not only affect the movement of water up the tree (vines in our case), but also determine the water movement in the radial direction between xylem and bark, so that these changes usually affect the values of MDS more than those of Ψ_s. Moreover, increments due to growth between the bark and the differentiated wood, might promote a separation of the external bark as the season progressed (wood aging).

On the other hand, it is known that chemical compounds, such as xylem abscisic acid (ABA_{xylem}) among others, play a role in drought stress responses, stomatal closure, and performance as activators of gene expression of the enzymes involved in anthocyanin biosynthesis (Ferrara *et al.,* 2013). In this study, we detected that the ABA_{xylem} accumulated during post-veraison could also be related with changes in the trunk growth rate. Figure 6 shows the relationship between MDS and ABA_{xylem}. In this sense, changes in MDS were explained by 45% of the amount of ABA_{xylem} accumulated during the whole season. During pre-veraison, higher values of MDS were associated to lower values of ABA_{xylem}, while after veraison the increase in ABA_{xylem} together with the application of exogenous S-ABA (late-August) reduced MDS (mean values below to 70 µm). Assuming that increases in ABA_{xylem} reduced stomatal function (Stoll *et al.,* 2000), if the stomatal closure is higher, vines will be better hydrically and MDS values will be lower, as a result. Furthermore, another primary action of ABA_{xylem} resulted in changes in cytoplasmatic ionic concentrations, which can induce the accumulation of proline (Ober and Sharp, 1994). Thus, an increase in proline can reduce plant growth (reviewed by Hardikar *et al.,* 2011) through: (i) an osmotic effect (causing water stress), (ii) the toxic effect of ions and (iii) imbalance in the uptake of essential nutrients. Therefore, higher levels of ABA_{xylem} might influence the variations in TDF-derived indices (MDS and TGR) as a result of increasing proline levels and a decreasing in stomatal conductance.

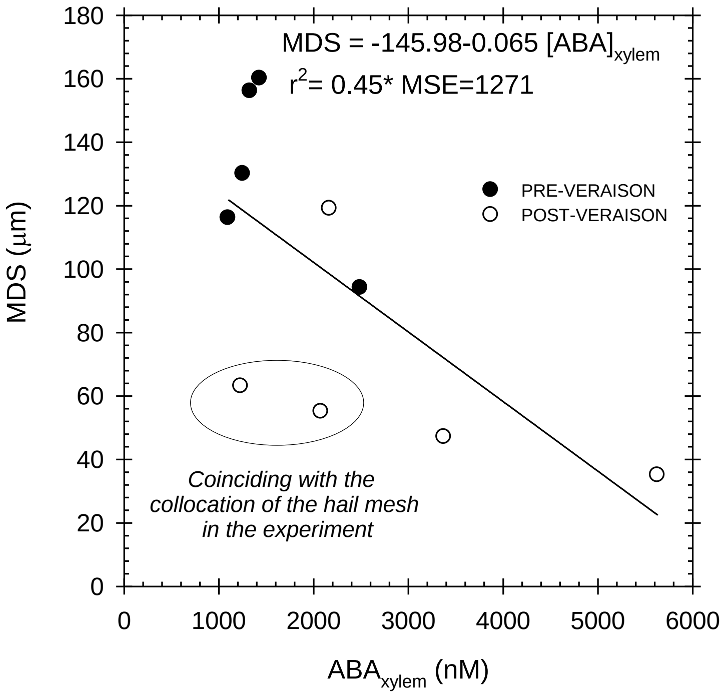


Figure 6. *Relationship between daily values of maximum daily trunk shrinkage (MDS) and the accumulated ABA_{xylem} in pre (•) and post-veraison (○), respectively. Data correspond to measurements from 2013. Data are means ± SE of 6 leaves and 6 LVDT sensors. The collocation of the hail mesh had occurred at the beginning of September.*

As water availability for the table grapes was unlimited in this study, it is logical that mean temperature explains that part of MDS that includes diameter growth. However, in other fruit crops such as almond (Egea *et al.,* 2009), plum (Intrigliolo and Castel, 2006), peach (Conejero *et al.,* 2011) and early nectarine (de la Rosa *et al.,* 2013), good agreement was found with VPD. The different performance observed in the relation between MDS and VPD may be attributed to behavioural discrepancies in the stomata response to VPD and the changes in water potential due to transpiration (Egea *et al.,* 2009). Furthermore, the presence of the hail mesh to protect the vines can be related to this (Fig. 1) since a microclimate is generated below this layer (similar to the effect of the canopy in citrus trees), increasing the relative humidity (RH) and consequently, altering VPD values. During post-veraison, the scatter of data around the regression lines was significantly reduced for Ψ_s as indicated by the lower MSE values (Table 3). This reflects the greater sensitivity of Ψ_s to the vine water status resulting from changes in the climatic conditions at this phenological stage (Fig 1 and Table 3).

The total yield was significantly higher (15%) in 2013 (Table 1). Thus, the reference line obtained in our study was crop load-dependent (promoted by a high production), since the higher yield observed in the second year significantly affected the relationship between plant-based water status indicators and T_m (Fig. 2). Similarly, Intrigliolo and Castel (2007a) observed noticeable differences in both MDS and TGR as a function of crop load (high crop load increased MDS by 34% and decreased TGR by 48%). De Swaef *et al.,* (2014) reviewed the influence of crop load on MDS in peach trees by reference to the effects on plant water status, the stem phloem carbon concentration, stem phloem turgor and the tissue elasticity. The same authors reported that crop load affects these factors via many different processes that interfere with the plant water and carbon status, such as leaf area, stomatal conductance, photosynthesis, root growth, fruit water and carbon transport, among others.

The irrigation scheduled during the pre-veraison of 2013 based on $SI_{MDS} \approx 1$ (Fig. 4) using the wireless technology had a positive effect on the amount of irrigation water that was applied in this period, promoting a good impact on the plant water relations observed during post-veraison. Maintaining values of Ψ_s (around -0.65 MPa) for well-watered vines in 2013 led to a slight reduction in the irrigation water compared with 2012 (Fig. 4). Moreover, the annual increase observed in the slope of the relation Ψ_s *versus* T_m may be due to previous irrigation management and the higher crop load registered (Figs. 2C-D and Table 1). In this study, we observed that the most suitable period for automated scheduling based on SI_{MDS} is from June to veraison (August) corresponding with the 50% of the crop water requirements. Finally, the use of two different equations (pre and post-veraison) to predict MDS did not significantly enhance the whole-season regression (Fig. 5).

5. CONCLUSIONS

In conclusion, our study highlights the suitability of MDS and Ψ_s, for establishing reference lines for scheduling irrigation in table grapes during pre- and post-veraison, respectively. MDS was a reliable plant water status indicator before veraison due to their dependence on growth in addition to daily fluctuation of stem diameter which can also motivated by either transpiration or changes in the accumulation of ABA_{xylem}. Cultural practices such as girdling (early-June) and the collocation of a hail mesh to prevent torrential rainfalls (late-August) also affected MDS and Ψ_s. Finally, a SI_{MDS} equal to unity and the maintenance of Ψ_s around -0.65 MPa can be used a feasibility scheduling irrigation in well-watered vines during pre- and post-veraison periods, respectively, throughout water savings and higher total yield and berry quality, with respect to conventional scheduling based on ET_c

6. ACKNOWLEDGEMENTS

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CHAPTER V:

Changes induced by water stress in the water relations, stomatal behaviour and morphology of table grapes (cv. Crimson Seedless) grown in pots

Chapter V: Changes induced by water stress on water relations, stomatal behavior and morphology of table grapes (cv. Crimson Seedless) grown in pots

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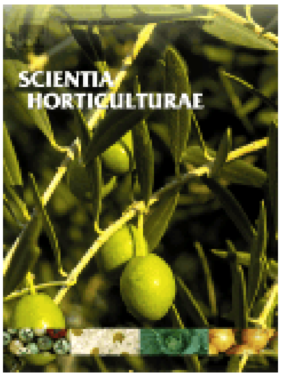
Running title: Water stress applied to table grapes grown in pots



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Abstract

The response of different deficit irrigation strategies on physiological and morphological parameters on table grapes (cv. Crimson Seedless) grown in pots was studied to evaluate how such strategies could be safely used for hardening and to ascertain their tolerance to drought. Five preconditioning treatments were applied: (i) CTL-1 and CTL-2; both irrigated daily to field capacity; (ii) DI, watered to 50% of CTL-1; (iii) PRD_{FIX}, permanently watered to 50% of CTL-1 in a pot, and (iv) PRD_{ALT}, the root was split into two pots, and the pots alternatively watered to 50% of CTL-1 when the volumetric substrate water content (Θ_V) reached 12%. 30 days after the application of the preconditioning treatments, plants were subjected to drought for 7 d, except for CTL-1. After that, plants were re-irrigated and their recovery was studied for 7 d. Crimson Seedless displayed different responses to water stress, depending on the diurnal course. At predawn (*t1*) and early morning (*t2*), the cultivar showed near-anisohydric behavior, through a less effective stomatal control of drought, whereas at midday (*t3*) the behavior was near-isohydric. Although the total amount of irrigation water was the same for DI, PRD_{ALT} and $PRD_{FIX,}$ the plants from PRD_{FIX} had a reduced photosynthetic activity, probably due to a limited sap flow. Water stress conditions induced avoidance mechanisms to drought such as stomatal closure, partial defoliation and a reduction in leaf insertion angle. Osmotic adjustment was only observed in un-preconditioned plants (CTL-2) but it was not enough to reach full turgor, probably due to a loss of wall elasticity. In addition, PRD_{FIX} and CTL-2 plants suffered serious dehydration damages to biomass accumulation and plant quality, regardless of the recovery of gas exchange parameters. PRD_{ALT} and DI can be used as safe techniques for irrigation scheduling.

Keywords: drought stress, recovery, leaf gas exchange; CSWI; turgor potential; *Vitis Vinifera*;

Abbreviations: DI, deficit irrigation; PRD, Partial root-drying zone; $A_{CO2,}$ net CO_2 assimilation rate; g_s, stomatal conductance; $A_{CO2}/g_{s,}$ intrinsic water use efficiency; Tc, canopy temperature; Ta, air temperature; Tc-Ta, canopy to air temperature difference; Θ_V, volumetric substrate water content; ψ_{pd}, pre-dawn leaf water potential; ψ_s, stem water potential at mid-day; ψ_o, leaf osmotic potential; ψ_{os}, leaf osmotic potential at full turgor; ψ_p, leaf turgor potential; LIA, leaf insertion angle; CSWI, crop stress water index; DM, dry matter; DW, Leaf dry weight; LA, leaf area; SLW, specific leaf weight; SLA, specific leaf area

1. INTRODUCTION

The increasing demand for seedless varieties of table grapes for fresh consumption in arid and semiarid environments where water is a limiting factor have already created the need for the development of deficit irrigation strategies (DI). In fact, the application of DI strategies is a common practice in vineyards for the control of canopy growth and the improvement of fruit quality, with water shortage considered a major source of stress in these areas. The plant's response to water scarcity is dependent on the mechanism of their adaptation, such as avoidance, resistance or tolerance against drought stress (Ruiz-Sánchez *et al.,* 2000). Therefore, knowing the mechanisms involved in the physiological behaviors and degrees of canopy development are essential for the successful implementation of these irrigation strategies (Chaves *et al.,* 2010). It is widely known that the deliberate withholding of irrigation water by deficit irrigation techniques such as regulated deficit irrigation (RDI, Chalmers *et al.,* 1981) and partial rootzone drying (PRD, Dry *et al.,* 1996) can be effective management strategies for manipulating crop water use. More specifically, in PRD, approximately half of the root system is maintained sufficiently watered whilst the other part is allowed to dry. The physiological behavior of PRD is related to the triggering of a root-shoot signaling mechanism in response to the drying soil, producing signaling molecules which are transported via the xylem to the leaves, thus provoking a partial stomatal closure (Ruiz-Sánchez *et al.,* 2010).

One way to determine the degree of tolerance to drought could be to measure the ability of a plant to sustain leaf gas exchange and CO_2 assimilation rates during a recovery period after water stress. Stress cycles and recovery from stress are prevalent processes that occur under natural conditions in different seasons and due to different agricultural practices, including irrigation (Gómez-Bellot *et al.,* 2013a). Tolerance to drought stress is generally characterized by a reduction of stomatal conductance (g_s) together with the development of low osmotic potential (ψ_o) through osmotic adjustment, which helps to maintain turgor potential (ψ_t) (Lawlor and Tezara, 2009). Recovery usually promotes an increase in the leaf water potential followed by a recovery of g_s, which may be associated with the re-establishment of hormonal balances (Chaves *et al.,* 2011). Also, the degree of water stress imposed and the timing and duration of these stresses have specific effects on both the speed and the extent of recovery, which can alter the entire functioning and growth of the plant (Lawor and Tezara, 2009; Gómez-Bellot *et al.,* 2013a).

In general terms, vines are considered drought-tolerant plants, characterized by diverse hydraulic and stomatal behaviors, depending on the cultivar (Schultz, 2003, Chaves *et al.,* 2010). Based on their water potentials in response to water limitations, Tardieu and Simoneau (1998) classified grapevine cultivars as both isohydric and anisohydric. Isohydric plants maintain constant midday leaf water potential when water is non-limiting as well as under drought conditions. This is done by reducing stomatal conductance as necessary to limit transpiration, with leaf water potential rarely decreasing more than -1.5MPa (Lovisolo *et al.,* 2010). In contrast, anisohydric plants have a more variable leaf water potential and maintain their stomata open and photosynthetic rates high for longer periods, even in the presence of decreasing leaf water potential or increasing atmospheric water demand (Lovisolo *et al.,* 2010; Sade *et al.,* 2012). The differences in stomatal control of isohydric and anisohydric plants are likely due to differences in the perception of chemical signals, of which the xylem's abscisic acid (ABA) is considered the most important. The changes in the diurnal pattern of water potentials under drought conditions can be related to the water-conducting capacity and stomatal behavior, which may also involve hydraulic signals and cavitation of xylem vessels (Pou *et al.,* 2008). However, Lovisolo et al. (2010) reported similar decreases in g_s and A_{CO2} in response to water stress, which promoted similar values of intrinsic water use efficiency (A_{CO2}/g_s). Attia *et al.* (2015) also found that isohydric plants had reduced leaf conductivity (K_{leaf}), g_s and E whereas anisohydric plants only maintained high K_{leaf} and E under drought.

Several studies have highlighted the importance of studying the degree of water stress on grapevine leaves' structural attributes and gas exchange parameters (Chaves *et al.,* 2010; Lovisolo *et al.,* 2010; Romero *et al.,* 2014). However, the physiological mechanisms involved in the cycles of stress and recovery of table grape plants have been poorly studied. This work aimed to assess the ability of young table grape plants to harden by the application of different water stress preconditioning treatments. The physiological responses, water relations and plant growth were studied to ascertain any differences in behavior of table grapes during tolerance to drought as well as during recovery.

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

Table grape plants (cv 'Crimson Seedless') grafted onto Paulsen 1103 rootstock (n = 45) with an initial height of 60 cm, were transplanted on July 12th, 2014 into 5 L polyethylene pots (18 x 18 x 25 cm) containing a substrate of coconut fibers. In order to maintain similar experimental conditions, the root system of the all plants in the experiment was previously divided into two similar halves, and each half was buried in different pots as described in the Suppl. Fig. 1. The pots were placed in a plastic greenhouse in the Higher Technical School of Agricultural Engineering (Technical University of Cartagena) experimental farm located in La Palma (Murcia, Spain) (Picture 1).

Picture 1. *Distribution of pots inside of the greenhouse*

The micro-climatic conditions (T, maximum air temperature, PAR, photosynthetically active radiation at mid-day; and RH, relative humidity), were recorded with an automatic weather station (model AWS310, Vaisala, Finland), installed inside the greenhouse. Temperatures in the greenhouse were usually typical of the season, with the maximum daily average quite higher than the outside (40 ºC inside the greenhouse *vs*. 33.5 ºC outside), as expected from a plastic greenhouse under Mediterranean summer conditions. During the hardening period, T was 33.1 ºC, whereas the PAR and RH averaged values of 770 W m^{-2} and 62.1%, respectively (Fig. 1A). During the stress/recovery period, the environmental conditions values were slightly lower than the hardening period. T was 29.2ºC, and PAR and RH had averaged values of 368.5 W m^{-2} and 68.9%, respectively. The drip irrigation system per plant consisted of two 2 L h^{-1} emitters each (one emitter was installed in each pot, 4 L h^{-1} per plant) (Suppl. Fig 1). Plant fertilization (30:10:10 N, P, K plus microelements) was applied through the drip irrigation system at the same time as the irrigation. Both irrigation and application of fertilizers were usually done at night (before predawn).

2.2. Preconditioning treatments and irrigation periods

For conditioning the plants during the establishment of the new root system into two pots, they were drip irrigated daily for one month with identical doses of water and vegetative nutrient solution. After, five irrigation treatments were imposed: (i) control treatments, CTL-1 and CTL-2, irrigated daily to satisfy the maximum plant requirements of gravimetric substrate field capacity ($ϴ_{FC}$ ≈ 26%) during the experiment, and only during the preconditioning period, respectively; (ii) deficit irrigation treatment (DI), irrigated at 50% of CTL-1 on both sides of the root system (*i.e.* irrigation applied to both pots); (iii) fixed partial rootzone drying (PRD_{FIX}), irrigated at 50% of CTL-1 but always applied on one side of the root system (*i.e.* irrigation applied to the same pot); and (iv) alternated partial rootzone drying (PRD_{ALT}), irrigated at 50% of CTL-1 but alternatively applied to both sides of the root system. The irrigation was alternated when the volumetric substrate water content ($ϴ_V$) of the dry pot was reduced up to 12% (corresponding to the wilting point of the substrate). The latter preconditioning treatments were applied for 30 days (*hardening period*), and then the irrigation was suppressed for 7 days in all of them (*stress period*), except for the CTL-1 treatment, which was always irrigated around field capacity (initial conditions). After that, the irrigation in all plants was increased for 7 days, as the CTL-1 treatment conditions (*recovery period*).

2.3. Water relations

Volumetric substrate water content ($ϴ_V$) was determined in 3 pots per treatment using GS3 probes (Decagon Devices, Inc). For inserting the probes inside the pots, three 6mm diameter holes (2 cm apart) were punched through the wall of the pot, at the midpoint of each pot (Suppl. Fig. 1). Three GS3 probes per treatment were installed in one pot of three different plants from the CTL-1, CTL-2, DI and PRD_{ALT} treatments and in both pots of the PRD_{FIX} treatment (n=6 probes) to also monitor the dry side. Measurements were recorded daily every 30 s and 15 min with a CR1000X datalogger (Campbell Scientific, Inc., Logan, USA).

Water potentials and gas exchange measurements were obtained at three different times (t) of the day (solar time): Predawn (*t1*) evaluated at 5.30-6.30 h; early morning (*t2*) evaluated at 10:00-11:00 h; and midday (*t3)* evaluated at 13:00-14:00 h.

Pre-dawn leaf water potential (ψ_{pd}) was measured at *t1* and stem water potential (ψ_s) evaluated at *t2* and *t3* on two mature leaves per plant and three plants per treatment (n= 6 leaves), using a pressure chamber (Soil Moisture Equipment Co., Model 160 3000). Leaves were fully expanded and were selected at random from the middle third of the shoots. Leaves for ψ_s measurements at *t2* and *t3* were covered with plastic and aluminium foil for at least 2 h before being used. After measuring $\psi_{pd,}$ the leaves were frozen in liquid nitrogen and osmotic potential (ψ_o) was measured after thawing the samples and extracting the sap, using a WESCOR 5520 vapour pressure osmometer (Wescor Inc., Logan, UT, USA), according to Gucci *et al.* (1991). Leaf turgor potential (ψ_t) was estimated as the difference between leaf osmotic (ψ_o) and predawn water potential at *t1* (ψ_{pd}).

Leaf osmotic potential at full turgor (ψ_{os}) was measured on leaves adjacent to those used to measure predawn water potential (ψ_{pd}). The leaves were excised with their petioles and placed in distilled water overnight to reach full saturation before being frozen in liquid nitrogen (-196 °C) and stored at -30 °C, following the same methodology as for ψ_o. Osmotic adjustment was estimated as the difference between the ψ_{os} of stressed and control plants.

Gas exchange parameters were measured from a similar number and type of leave as for leaf water potential. Leaf net CO_2 assimilation rate ($A_{CO2,}$ μmol m^{-2} s^{-1}) and stomatal conductance (g_s mmol m^{-2} s^{-1}), were measured at a photosynthetic photon flux density (PPFD) ≈ 1500 µmol m^{-2} s^{-1}, near constant ambient CO_2 concentration (*C*a ≈ 350 µmol mol^{-1}) and leaf temperature (*T*leaf ≈ 30 °C) with the portable gas exchange system CIRAS-2 (PP Systems, Hitchin, Hertfordshire, UK). Intrinsic water use efficiency was calculated as the ratio between A_{CO2} and g_s (µmol mol^{-1}).

Canopy temperature was determined with a digital infrared thermometer (Model GM320) on the same leaves where gas exchange parameters were obtained. The crop water stress index (CWSI) was calculated following the equation proposed by Idso *et al.* (1981):

$$\mathrm{CWSI} = \frac{(\mathrm{dT} - \mathrm{dT}l)}{\mathrm{dTu} - \mathrm{dT}l} \qquad \text{[Equation 1]}$$

where dT is the difference between canopy temperature (Tc) and air temperature (Ta): Tc-Ta. dT*u* is the upper limit of the air temperature and canopy temperature difference, and dT*l* is the lower limit of the air temperature and canopy temperature difference. The values for the CWSI range from zero to one, where zero indicates no stress and one indicates maximum stress.

2.4. Vegetative growth and plant quality measurements

To determine the change in petiole angle, or epinasty, the angle between the leaf petiole and the stem (leaf insertion angle, LIA) was measured with a transparent protractor on 10 random leaves per plant and three plants per treatment.

At the end of the hardening, stress and recovery periods, three plants per irrigation treatment were used to obtain the dry weight of the shoots (leaves and stem) and roots. Plant height was previously determined with a measuring tape (Picture 2A). All these tissues were oven-dried at 80 ºC until they reached a constant weight in order to measure their respective dry matter (DM) (Picture 2B). From the leaf dry weight (DW) and leaf area (LA), measurements of the specific weight area (SLW) and specific leaf area (SLA) were also determined for each plant. LA was measured using a cylindrical hole punch instrument (Picture 3C) in 10 cylinder-samples per replicate and two replicates per plant (n=3).

$$SLW(g\,m^{-2}) = \frac{DW\ (g)}{LA(m^2)} \qquad \text{[Equation 2]}$$

$$SLA(m^2 g) = \frac{1}{SLW} \qquad \text{[Equation 3]}$$

Finally, all the plants were visually evaluated at the end of the experiment following the quality scale described in Gómez-Bellot *et al.* (2013b): (1) PIC, percentage of plants in ideal condition; (2) PAC, percentage of plants in acceptable condition; (3) PDB, percentage of plants with dry branches; and (4) DP, percentage of dry plants.

Picture 2. *Detail of the high measurement (A) different weight tissues, e.g. roots (B) and cylindrical hole punch instrument used in the experiment (C).*

2.5. Statistic analysis

The experimental design consisted of three replicates per treatment, randomly distributed within the greenhouse. Each replicate consisted of three different plants. The data were analyzed by one-way ANOVA using Statgraphics Plus for Windows version 5.1 (Manugistics, Inc., Rockville MD, USA). *Post hoc* pairwise comparison between all means was performed by Duncan´s multiple range test at $p<0.05$.

3. RESULTS

3.1. Hardening period

Substrate water content values (Θ_v) in the reference treatment (CTL-1) were close to field capacity throughout the irrigation cycles (Fig 1B). CTL-2 remained nearly stable (between 34 and 26%) during the hardening period. However, the preconditioning deficit treatments of DI, PRD_{FIX} and PRD_{ALT} averaged Θ_v values of 27, 55 and 50% lower than CTL-1, respectively (Fig. 1B). PRD_{FIX} exhibited a substantial depletion in Θ_v, averaging values of 14.3 and 10.2%, in the wet and dry pot, respectively. This could also be observed in the alternated irrigation in PRD_{ALT} when Θ_v in the dry pot reached 12%. Despite of the Θ_v differences observed, all the stress treatments (DI, PRD_{FIX} and PRD_{ALT}) resulted in moderate plant water deficits, as indicated by the stem water potential (ψ_s) values measured at midday (*t3),* which were around -0.7 MPa (Fig. 1C).

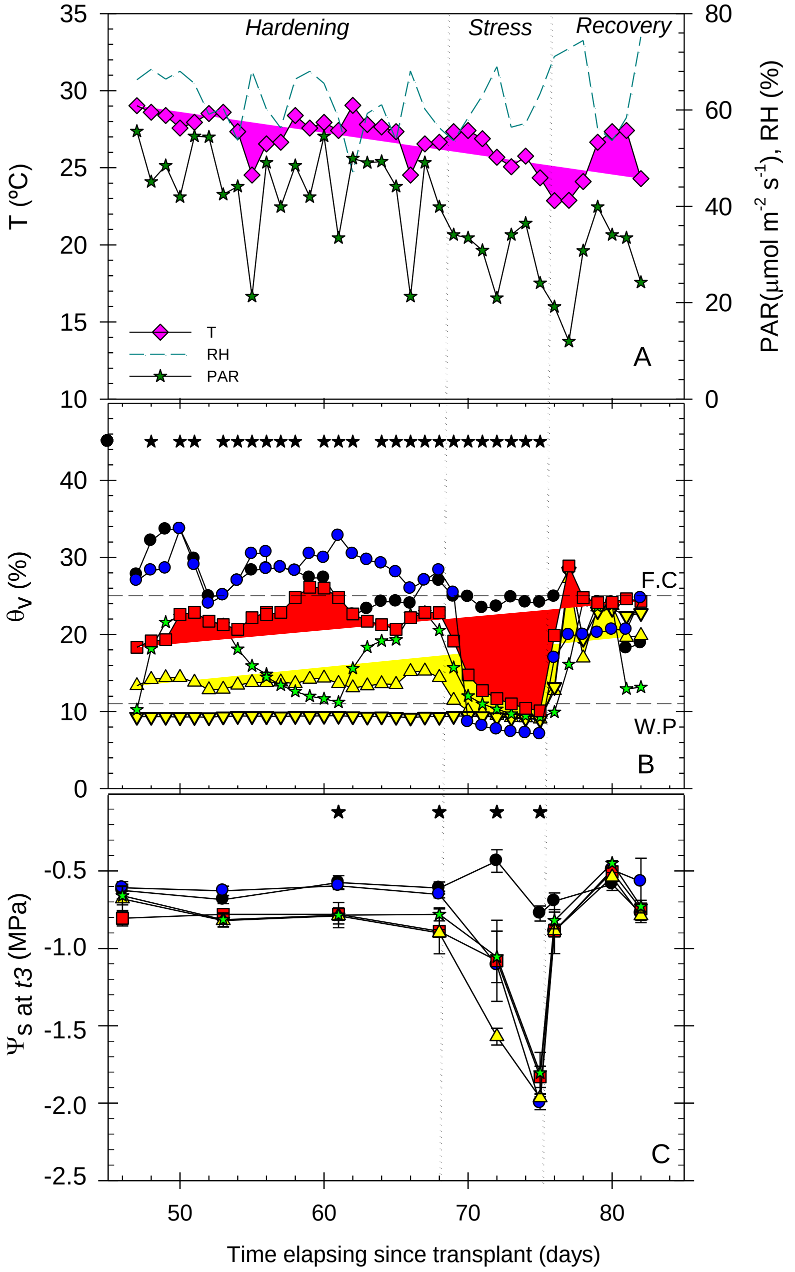

Figure 1. *Evolution of (A) temperature (T), relative humidity (RH), and photosynthetically active radiation (PAR), registered inside of the greenhouse; (B) volumetric substrate water content (Θ_V) and (C) stem water potential (ψ_S), obtained at midday (t3) in all the irrigation treatments: CTL-1 (●), CTL-2 (●), DI (■), PRD_{FIX} of wet pot (▲), PRD_{FIX} of dry pot (▼), and in PRD_{ALT} (★). Asterisks indicate significant differences between irrigation treatments according to Duncan's multiple range test (P<0.05). Error bars in figure B were omitted for clarity. FC and WP indicate the field capacity and the wilting point of the substrate, respectively.*

Similar to those observed in ψ_s, a sharp reduction in leaf angle insertion (LAI) of DI, PRD_{FIX} and PRD_{ALT} treatments were obtained, being more pronounced in PRD_{FIX} (Table 2).

Water deficit also lowered the predawn leaf water potential (ψ_{pd}) measured at *t1* (Fig. 2A and 3A). However, no significant differences in leaf osmotic potential (ψ_o) and leaf turgor potential (ψ_t) were found between treatments during the hardening period (Fig. 2B and D). Only the PRD_{ALT} significantly induced decreases in leaf osmotic potential at full turgor (ψ_{os}) with respect to CTL-1, with a value of around -1.2 MPa (Fig. 2C).

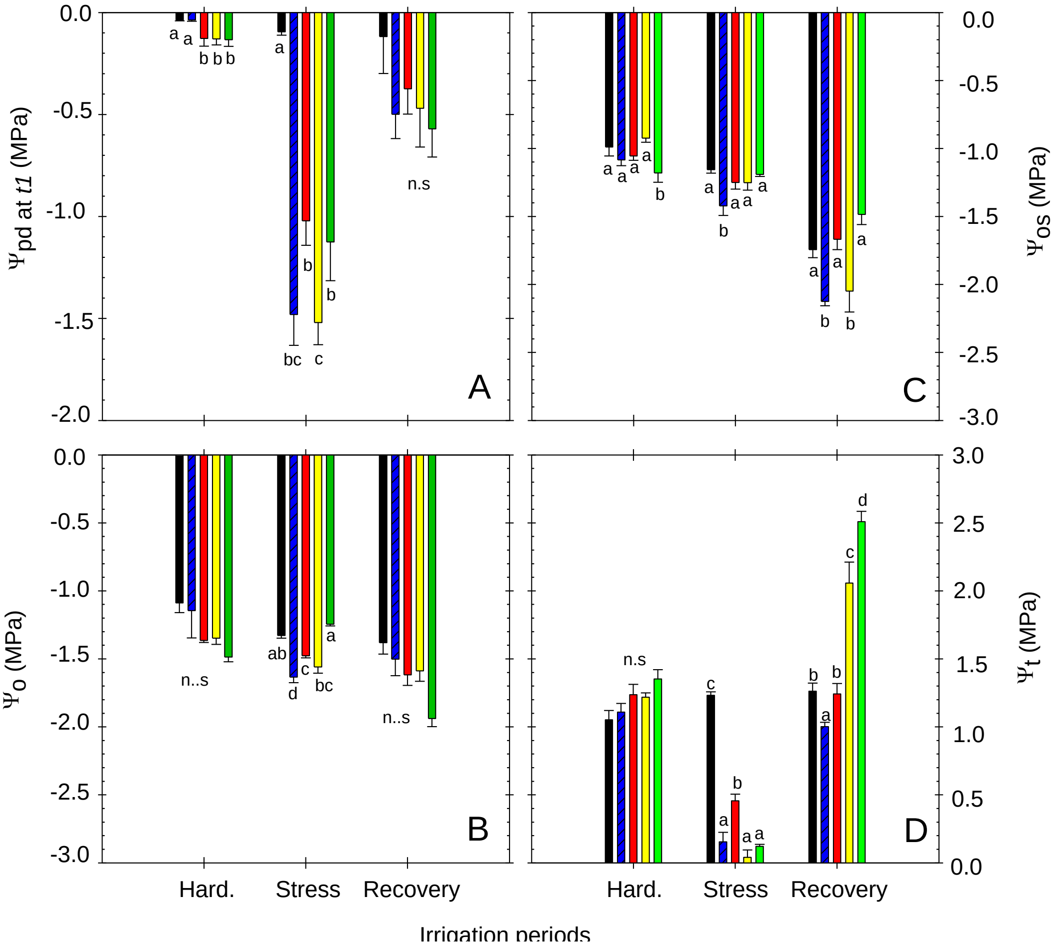

Figure 2. *Mean values of (A) predawn water potential assessed at predawn (t1) evaluated at 5.30-6.30 h (solar time) (ψ_{pd}), (B), leaf osmotic potential (ψ_o), (C) leaf osmotic potential at full turgor (ψ_{os}), and (D) leaf turgor potential (ψ_t), in all the irrigation treatments: CTL-1 (▬), CTL-2 (🟦), DI (🟥), PRD_{FIX} (🟨) and PRD_{ALT} (🟩), at the end of the hardening, stress and recovery periods. Each point is the average of three replicates (n=6 leaves). Vertical bars indicates means ± ES.*

The diurnal patterns of the gas exchange parameters showed the maximum differences between irrigation treatments at early morning or *t2* (Fig. 3A, D and G). Stomatal conductance (g_S) was significantly reduced as compared to CTL-1 (Table 1) due to the effects of water deficit (DI, PRD_{FIX} and PRD_{ALT}). However, the net CO_2 assimilation rate (A_{CO2}) only significantly decreased in PRD_{ALT}. Moreover, the difference between canopy and air temperature (Tc-Ta) increased as a result of water deficit (Table 1).

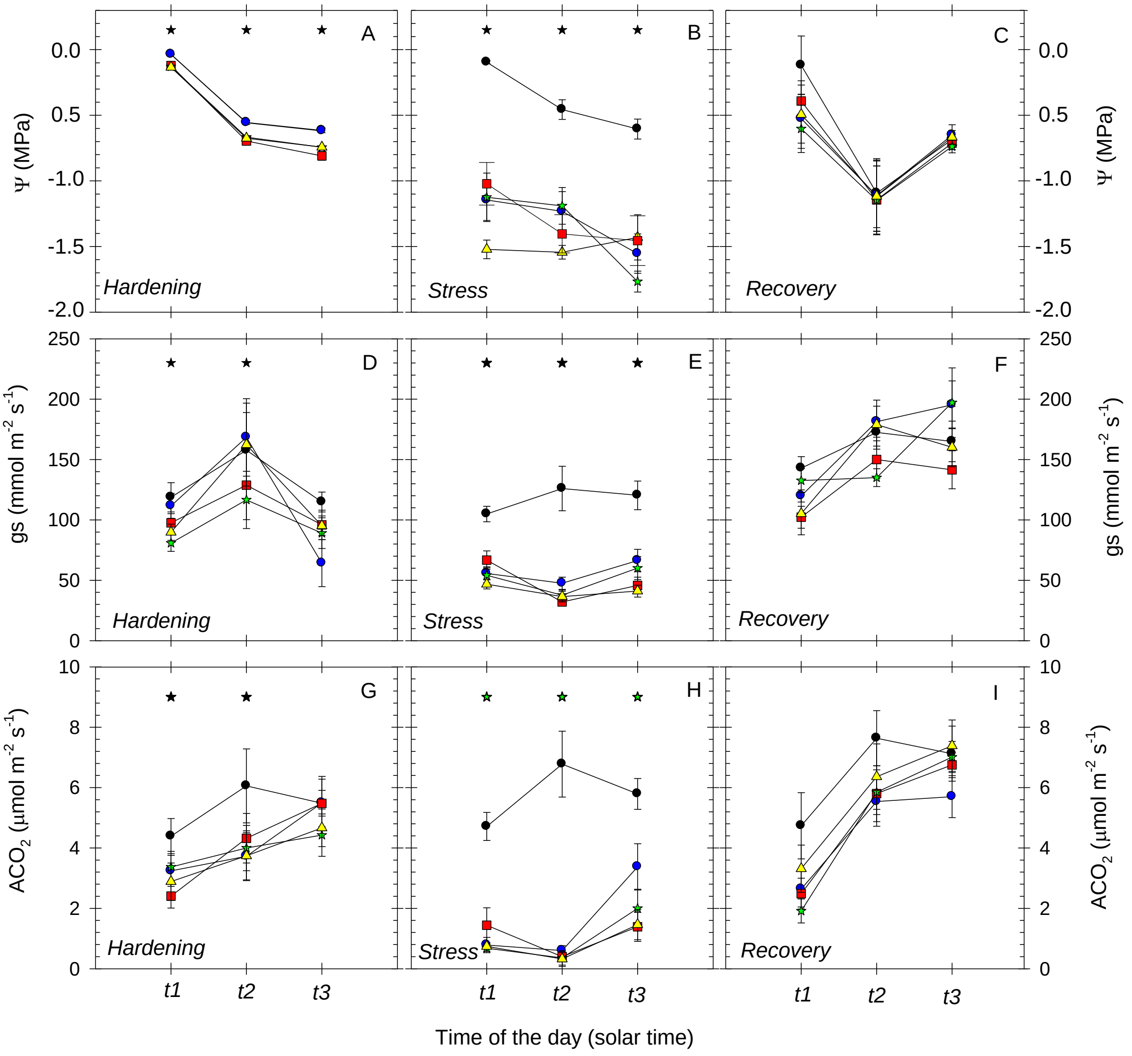

Figure 3. *Mean values of water potential (ψ), Net CO_2 assimilation (A_{CO2}), and stomatal conductance (g_S) at three times of the day: predawn (t1) evaluated at 5.30-6.30 h (solar time); (B) early morning (t2) evaluated at 10:00-11:00 h (solar time); and (C) midday (t3) evaluated at 13:00-14:00 h (solar time) during hardening (A, D and G), stress (B, E and H) and recovery (C, F and I) periods in all the irrigation treatments: CTL-1 (●), CTL-2 (●), DI (■), PRD_{FIX} (▲) and in PRD_{ALT} (★). Asterisks indicate significant differences between irrigation treatments according to Duncan's multiple range test (P<0.05).*

Table 1. *Gas exchange parameters (g_s, ACO_2 and ACO_2/g_s), canopy temperature minus air temperature (Tc-Ta) and the crop water stress index (CWSI) in 'Crimson Seedless' plants under different irrigation treatments (CTL-1, CTL-2, DI, PRD_{FIX} and PRD_{ALT}) at the end of the hardening (H) stress (S) and recovery (R) periods assessed at early-morning (t2). Values are means ± SE (n=6 leaves).*

		Treatments					**ANOVA**	
Measure	**Period**	**CTL-1**	**CTL-2**	**DI**	**PRD_{FIX}**	**PRD_{ALT}**	**P-v Treatment**	**P-v Period**
g_s (mmol m^{-2} s^{-1})	***H***	101.5 b	94.4 b	77.6 a	74.4 a	75.1 a	*	n.s
	S	124.4 a	60.8 b	45.8 b	41.0 b	60.0 b	***	
	R	151.2	148.3	119.0	125.9	158.4	n.s	
ACO_2 (µmol m^{-2} s^{-1})	***H***	5.5 a	5.0 aB	4.9 abB	4.2 abB	3.4 bB	n.s	*
	S	6.3 a	2.8 bA	1.5 bA	1.4 bA	2.1 bA	***	
	R	7.5	5.1 B	5.3 B	5.6 B	5.5 B	n.s	
ACO_2/g_s (µmol mol^{-1})	***H***	59.8	62.6	58.5	65.2	69.1	n.s	n.s
	S	59.1	50.6	44.8	29.2	37.0	n.s	
	R	44.8	54.6	52.7	40.8	38.6	n.s	
Tc-Ta (ºC)	***H***	-0.1 a	-0.1 a	1.5 b	2.0 b	2.2 b	***	n.s
	S	-0.6 a	1.4 ab	1.9 b	2.3 b	2.2 b	**	
	R	-0.8	-0.1	0.8 b	0.9 b	0.9	n.s	
CWSI	***H***	--	0 aA	0.24 b	0.32 bA	0.38 b	***	**
	S	--	0.45 abC	0.29 a	0.66 bB	0.29 a	***	
	R	--	0.13 B	0.29	0.29 A	0.30	n.s	

Means within a row without a common lowercase letter are significantly different as calculated by the Duncan's test among irrigation treatments (P≤ 0.05). Means within a column without a common capital letter are significantly different as calculated by the Duncan's test among periods (P≤ 0.05).

*[z] ANOVA indicates the P-value (P-v, probability level) for irrigation treatments (CTL-1, CTL-2, DI, PRD_{FIX} and PRD_{ALT}) and periods (hardening, stress and recovery), respectively; ns, not significant; * P≤ 0.05; ** P≤ 0.01;***P≤ 0.001.*

3.2. Stress/recovery period

As expected, Θ_v decreased during the stress period and it was near permanent to the wilting point (around 10 %) in all stress treatments (Fig 1B).

CTL-2 plants, which had not been preconditioned and those from PRD_{FIX}, had the lowest values in both the ψ_{pd} and ψ_s assessed at midday or *t3* (Fig. 1C and 2A). Indeed, in PRD_{FIX} values of ψ_{pd} were 0.4 and 0.5 MPa lower than the other preconditioning treatments (DI and PRD_{ALT}, respectively) (Fig. 2A). No significant differences were found in ψ_{os} values between CTL-1 and the preconditioned treatments (Fig 2C). However, the CTL-2 treatment showed the lowest values of ψ_{os} (-1.42 MPa) and ψ_o (-1.63 MPa) (Fig. 2B and C), hence, the highest amount of osmotic adjustment was observed in this treatment (Fig 2C). The ψ_t was close to zero in the stress treatments (Fig. 2D). DI plants had the lowest decrease in the values of ψ_t as compared to the other stress treatments. Plants from the DI and PRD_{ALT} treatments exhibited a smaller decrease in the LIA as compared to CTL-2 and PRD_{FIX} (Table 2). Also, the root:shoot ratio increased in all stress treatments, due to an increase in the dry matter of roots and higher defoliation during the drought period (Table 2).

The daily changes in gas exchange parameters showed similar patterns in all the stress treatments (Fig. 3). All of them had a plateauing effect on g_s as a result of limited stomatal opening (Fig 3E). Moreover, the A_{CO2} also decreased in drought-exposed plants in relation to the CTL-1 treatment (Fig. 3H), without having significant effects on the intrinsic efficiency (A_{CO2}/g_s) (Table 1). Remarkably, all the preconditioning treatments had an increased Tc-Ta value. As a consequence, the CSWI had the highest values in PRD_{FIX} (≈ 0.60), followed by CTL-2 (Table 1).

At the end of the recovery period, ψ_{pd} and ψ_s measured at midday (*t3)* reached similar values in all the stress treatments to those of the CTL-1 treatment (Fig. 1C and 2A). No differences between irrigation treatments were found in the values of $\psi_{o,}$ even though those observed were lower in CTL-2 and $PRD_{FIX,}$ as compared to CTL-1. In addition to this, the ψ_t in CTL-2 was maintained significantly lower than CTL-1 after the irrigation was restored. The negative influence of the water stress can be reversed as observed in the diurnal course of gas exchange parameters (Fig. 3F and I).

Table 2. *Leaf insertion angle (LIA) and growth parameters in 'Crimson Seedless' plants under different irrigation treatments (CTL-1, CTL-2, DI, PRD_{FIX} and PRD_{ALT}) at the end of the hardening (H) stress (S) and recovery (R) periods. Values are means ± SE (n=3 plants).*

		Treatments					ANOVA^{z}	
							P-v	**P-v**
Measure	**Period**	**CTL-1**	**CTL-2**	**DI**	**PRD_{FIX}**	**PRD_{ALT}**	**Treatment**	**Period**
LIA (%)	***H***	81.1 b	77.2 b	50.8 a	47.1a	55.6 a	***	
	S	82.6 d	37.0 a	45.4 c	40.5b	52.9 c	***	
	R	84.6 c	66.6 ab	77.3 bc	63.4 a	80 c	**	n.s
SLW (g m^{-2})	***H***	40.9 B	44.8 C	43.5 B	52.5 C	39.3	n.s	
	S	31.2 A	32.3 B	31.4 A	30.0 A	33.3	n.s	
	R	36.9 A	27.2 A	31.2 A	42.3 B	31.3	n.s	*
SLA (m^{2} g^{-1})	***H***	0.02	0.03	0.02	0.02	0.02	n.s	
	S	0.03	0.03	0.03	0.03	0.03	n.s	
	R	0.02	0.04	0.03	0.02	0.03	n.s	n.s
Height (m)	***H***	4.2	3.3	3.4	3.2	3.1	n.s	
	S	4.1	4.2	3.2	3.9	3.3	n.s	
	R	4.9	3.6	3.5	3.6	3.4	n.s	n.s
Leaves DM (%)	***H***	22.6 A	29.1 A	27.9 A	25.6	25.0 A	n.s	
	S	24.0 A	24.1 A	24.8 A	23.3	25.2 A	n.s	
	R	50.1 B	50.9 B	51.7 B	27.2	50.2 B	n.s	***
Stem DM (%)	***H***	37.8 A	41.8 A	43.1 A	44.8 A	43.5 A	n.s	
	S	38.8 A	41.0 A	42.2 A	43.3 A	43.5 A	n.s	
	R	63.9 B	70.2 B	60.9 B	60.4 B	60.4 B	n.s	***
Roots DM (%)	***H***	45.8 AB	44.1 A	42.5 A	61.7	49.0 A	n.s	
	S	30.1 A	55.6 B	58.8 B	60.6	50.4 A	n.s	
	R	54.9 B	65.2 B	55.6 B	64.3	60.8 B	n.s	**

Means within a row without a common lowercase letter are significantly different as calculated by the Duncan's test among irrigation treatments (P≤ 0.05). Means within a column without a common capital letter are significantly different as calculated by the Duncan's test among periods (P≤ 0.05).

*^{z}ANOVA indicates the P-value (P-v, probability level) for irrigation treatments (CTL-1, CTL-2, DI, PRD_{FIX} and PRD_{ALT}) and periods (hardening, stress and recovery), respectively; ns, not significant; * P≤ 0.05; ** P≤ 0.01;***P≤ 0.001.*
SLW, specific leaf weight; SLA, specific leaf area; DM, Dry matter

It is interesting to note that pooling data from the experiment resulted in different curvilinear relationships when g_s and water potential (ψ) at the three times of the day were compared (Fig. 4). Two points of clouds can be clearly differentiated, in which values of ψ close to -1.5 MPa observed in CTL-2, DI, PRD_{FIX} and PRD_{ALT} correspond to the drought/stress period. Tending to the results, the three correlations varied on the diurnal course, suggesting near-anisohydric behavior in *t1* and *t2* and near-isohydric behavior in *t3*, respectively.

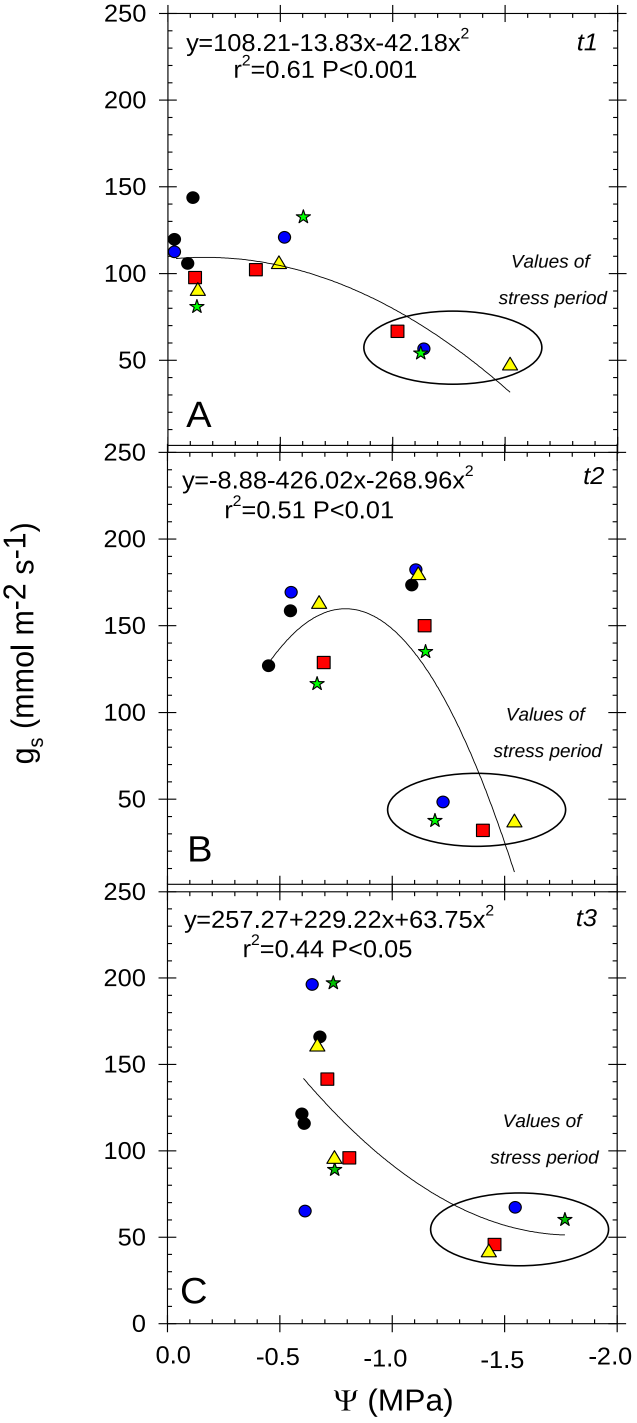

Figure 4. *Relationships between stomatal conductance (g_s) and water potential (ψ) assessed at three times of the day: (A) predawn (t1) evaluated at 5.30-6.30 h (solar time); (B) early morning (t2) evaluated at 10:00-11:00 h (solar time); and (C) midday (t3) evaluated at 13:00-14:00 h (solar time) in all the irrigation treatments: CTL-1 (●), CTL-2 (●), DI (■), PRD_{FIX} (▲) and in PRD_{ALT} (★). Each point is the mean of the hardening, stress and recovery periods, respectively. Values of each irrigation treatment inside of the circle correspond to the stress period.*

At the end of the experiment, water deficit had a significant effect on biomass accumulation (Table 2) as shown by an increase of the percentage of the dry matter in the all tissues analyzed (roots and shoots) compared to CTL-1. In this sense, the specific leaf weight (SLW) experienced a decrease throughout the experiment (Table 2). Lastly, the plant growth quality represented in Figure 5, showed a higher percentage of dry plants (DP) in PRD_{FIX} and CTL-2 treatments whereas DI was the only stress treatment that maintained some percentage of plants (around ≈ 20%) in ideal conditions (PIC) .

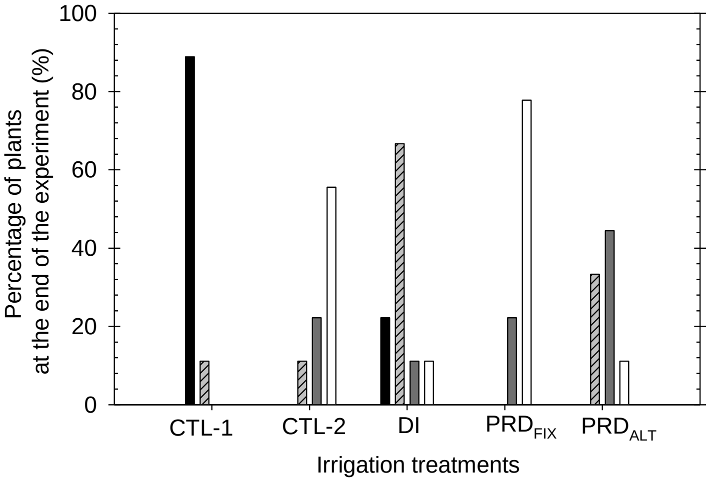

Figure 5. *Percentage of plants sorted according to the visual characteristics of 'Crimson Seedless' plants submitted to different irrigation treatments at the end of the experiment to total evaluated plants (n=15). PIC (▬), percentage of plants in ideal conditions PAC (▨), percentage of plants in acceptable conditions; PDB (▬), percentage of plants with dry branches; and DP (▭), percentage of dry plants.*

CTL-1 **CTL-2** **DI** **PRD_{FIX}** **PRD_{ALT}**

Picture 3. *Visual appearance of the plants in each irrigation treatment (CLT-1 CTL-2, DI, PRD_{FIX} and PRD_{ALT}) at the end of the period of stress / recovery.*

4. DISCUSSION

As seen in other potted species, substrate water limitation had an impact on their stomatal behavior, morphology and dry matter partitioning between roots and shoots. Although the exact effect can be different due to the degree and the intensity of the water stress imposed (Álvarez *et al.,* 2012). In this study we also observed that the manner of applying the stress (*i.e.* fixed or alternated) might also alter these effects.

During the hardening period, the PRD_{FIX} treatment showed a higher depletion of substrate water content (Θ_v) than PRD_{ALT} and DI (Fig. 1). Previous reports on contrasting stomatal behavior between PRD and DI strategies when both received the same irrigation volume hypothesized that re-watering the dry part of the root system transiently increased the root-to-shoot ABA signaling and also sap flow (Dodd *et al.,* 2008; Romero *et al.,* 2014). Thus, maintaining the pot dry during the experiment in PRD_{FIX} ($\Theta_v \approx 10\%$) might have reduced the amount of sap flow, limiting the concentration of xylem ABA, which is transported from the root to the shoot, thus reducing g_s. Moreover, Puértolas *et al.,* (2015) reported that the spatial distribution of substrate moisture heterogeneity can not only influence root ABA accumulation, but also the root growth and root hydraulic conductivity. The preconditioning treatments ($PRD_{FIX,}$ PRD_{ALT} and DI) showed a reduction in the water relations studied and also in the leaf insertion angle (Table 1 and 2). However, A_{CO2} only significantly decreased in PRD_{ALT}, probably due to the lower values of the dry matter of roots observed, which although not significant (Table 2), confirmed that under moderate deficit levels, the transpiration losses are limited, maintaining its leaf productivity.

At the end of the stress period, plants that had not been preconditioned (CTL-2) and those from PRD_{FIX} reached the highest water stress levels as indicated by the ψ_{pd} at *t1* and turgor values (Fig 2A and D). Despite of the fact that the degree of water stress imposed only resulted in an osmotic adjustment in CTL-2 (Fig. 2C), all stress treatments had a decrease in g_s, which suggests that ‘Crimson Seedless’ plants have sensitive stomata.

Progressively, the results of Tc -Ta and also CWSI increased during the drought period as substrate moisture was a limiting factor. Therefore, both of them could be used as good indicators of plant water status in vineyards (Bellvert *et al.,* 2015).

All stress treatments increases the percentage of shoot and root dry matter coinciding with a reduction in SLW, and also confirmed by the root/shoot ratio (Table 2). The redistribution of dry matter in favor of the roots at the expense of the shoots is likely due to the plant's need to maintain enough surface leaf area under drought conditions in order to increase the water uptake from the substrate and to reduce the evaporative surface area (Sánchez-Blanco *et al.,* 2009).

When severe water stress is imposed, the recovery is possible via different growth mechanisms to tolerance or avoidance to drought such as defoliation (Ruiz-Sánchez *et al.,* 2000), and also with the recovery of the photosynthetic processes (Egea *et al.,* 2012; Gómez-Bellot *et al.,* 2013a) (Fig. 3). In contrast, un-preconditioned plants from CTL-2, do not develop enough osmotic adjustment or other tolerance/avoidance safety mechanisms to maintain the leaf's full turgor (Fig. 2D). The plant quality of both CTL-2 and PRD_{FIX} resulted in severely affected and practically dried plants at the end of the experiment (Fig. 5), even though PRD_{FIX} positively regained its turgor (Fig. 2D). The response observed in CTL-2 suggests changes or losses in tissue extension capacity (or elasticity adjustment), as a result of not having been previously preconditioned. Neumann (1995) reported that wall hardening not only increases the ability of expanding cells to maintain turgor pressure, but also acts to inhibit rates of cell expansion growth, which can reduce leaf area for limiting the losses due to transpiration. However, the authors underlined that wall hardening responses to intermittent cycles of irrigation (stress and recovery, in our case) could be also undesirable (Neumann 1995). In this sense, we should also add the possibly negative effect of the fixed irrigation in PRD (permanent dry side) through limiting the active roots area which also limited the amount of sap flow from roots to shoots, especially when it is compared to PRD_{ALT} (dry side alternated). Thus, the recovery of turgor of the preconditioning treatments (DI, PRD_{ALT} and PRD_{FIX}) can be explained by (i) transfer of reserve water from the apoplast to the symplast either from the cell walls or from the vessel lumens by cavitation or (ii) metabolic loss of dry matter and gain of water (Levitt, 1986).

The analysis of seasonal and diurnal leaf gas exchange revealed that 'Crimson Seedless' exhibited near-anisohydric and near-isohydric stomatal behavior (Figs. 3 and 4). Intraspecific differences in stomatal sensitivity have been related to faster leaf area development with a faster substrate water depletion and consequently early stomatal closure (Chaves *et al.,* 2010). Schutz (2003) demonstrated the link between g_s and the hydraulic conductance during the diurnal period. The authors hypothesized that the differences in water-conducting capacity of stems (especially petioles), may be at the origin of this behavior, with the highest hydraulic conductance being more sensitive to cavitation and thus inducing stomatal closure at higher leaf water potentials. In addition, Lovisolo *et al.,* (2010) reviewed that the same variety could have different stomatal behaviors depending on the experimental conditions, for example plants grown in pots or under the field conditions (as a result of the substrate water heterogeneity). In other potted plants experiment, Tramontini *et al.,* (2014) found different responses to water stress in two grapevine cultivars. Syrah, displaying a near-anisohydric response to water stress, and the opposite in Cabernet Sauvignon, with the stomatal behavior being near-isohydric, both dependent on the influence of vineyard growing conditions or seasonality. However, it is the first time that near- anisohydric and near-isohydric behaviors have been described occurring within the same cultivar. Thus, other factors such as the influence of the genotype on the response to water deficits should be considered in future experiments.

5. CONCLUSIONS

In conclusion, table grape plants under preconditioning treatments responded by reducing photosynthetic activity, biomass accumulation and plant growth. After the stress period, the negative effects on water relations and gas exchange parameters (mainly g_s) were compensated for under the recovery period. Plants exposed to moderate water stress conditions developed avoidance/tolerance mechanisms to drought that were mainly based on stomatal closure, reduction of leaf insertion angle and defoliation. Un-preconditioned plants (CTL-2) were not able to compensate for turgor loss, had growth inhibition and the most severe dehydration damages together with those observed in PRD_{FIX}. Moreover, the stomatal behavior was conditioned to the diurnal basis or the time of the day when the stress was applied. These findings should be borne in mind for irrigation scheduling of this cultivar under commercial field conditions.

6. ACKNOWLEDGEMENTS

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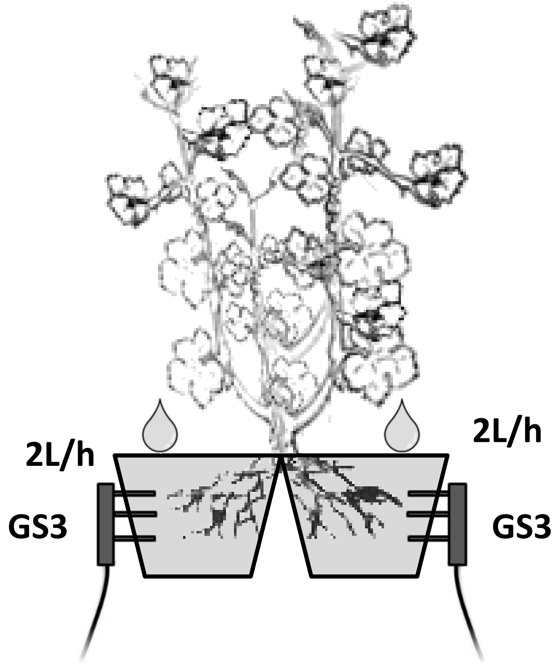

Supplementary Figure 1. Schematic representation of the experimental growth conditions. The locations of GS3 soil moisture probes are also depicted.

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

The general conclusions of the current PhD Thesis were as follows:

- Leaf area index (LAI) was the vegetative growth parameter most affected by post-veraison deficit irrigation (DI). This can be considered a positive aspect by decreasing the competition for assimilates between vegetative apexes and reserve tissues.

- The stomatal behavior of ‘Crimson Seedless’ was subjected to growing conditions, seasonality or phenological stage and the diurnal basis as we could observe in a pot experiment.

- The results showed that it is possible to develop seasonal reference equations of the maximum daily trunk shrinkage (MDS) and midday stem water potential (ψ_s) as a function of climatic variables. Specifically, the first parameter was the most reliable indicator for ascertaining the vine water status before veraison, whereas ψ_s did it after veraison. In both cases, the mean temperature (T_m) is the independent variable that leads to more accurate estimates of both parameters during pre-veraison, whereas after veraison reference equations can be obtained with values of reference crop evapotranspiration or daily mean vapour pressure deficit.

- To our knowledge, until now, publications have not developed irrigation scheduling using trunk diameter sensors in table grapes as well as the use of the signal intensity of MDS (SI_{MDS}), in real farm conditions. We observed that irrigation scheduling during pre-veraison is possible through SI_{MDS} equal to unity in this cultivar. MDS loses sensitivity after veraison due to changes in carbohydrates balance, transpiration and the accumulation of $ABA_{xylem.}$ ψ_s can be used as a plant-based water status indicator to assess the vine water status after veraison instead of MDS.

- Water use efficiency (WUE) was improved by about 30% in RDI and PRD with a water saving of 35% without adversely affecting total yield and berry quality. The increased WUE and the reduction in the water applied in NI (72%) with respect to Control were higher than those observed in RDI and PRD.

However, the excessive losses of yield observed in this treatment suppressed these advantages.

- All deficit irrigation treatments (RDI, PRD and NI) increased berry coloration and provided higher crop load at the first pick harvest compared with Control (full irrigated). This was also reflected by an increase in the accumulation of anthocyanins. This fact can promote several advantages to the commercial farm

- DI strategies provoked a signal in the vine, activating biosynthesis pathways. Indeed, berries subjected to DI showed increased SPC, TAC and flavonoids contents improving their functional berry quality.

- Concerning the postharvest experiment, stem browning determined the potential shelf-life, and longer storage duration tended to diminish treatment differences. Moreover, the highest percentage of berry shattering observed in PRD, was highly correlated with the lower absolute values of ABA_{xylem} induced by the grower’s irrigation strategy.

- From a commercial point of view, our findings showed important advantages that suggest the possible implementation of RDI and PRD strategies in commercial vineyards of ‘Crimson Seedless’ table grapes mainly related to saving water and berry color, which limit the marketability of this cultivar. Specifically, these benefits can be summarised in three aspects: (i) remarkable water savings (35%) without adversely affecting total yield and berry quality, together with a reduction in the energy cost (not quantified in this Thesis); (ii) the greatest increase in the berry coloration can limit the application of artificial color treatments (e.g. S-ABA) and thus, cost-saving product application, and dealing with this (iii) the possibility to obtain better market prices due to the increase in crop yield.

- Comparing PRD with RDI strategies, no clear positive effects on the physiological behavior of PRD were detected, even though showed better root morphological adjustment. Moreover, berries from PRD had a worse cold storage performance motivated by higher berry shattering. Nevertheless, the main bioactive compounds evaluated (resveratrol, antioxidant capacity) and above all the phenolic composition, through anthocyanins content, were higher in PRD. Assuming that the main issue of ‘Crimson Seedless’ is to reach a commercially acceptable red color, the integration of PRD by growers in favour of the conventional RDI strategy can be considered.

ABBREVIATIONS AND SYMBOLS

LIST OF ABBREVIATIONS

ABA$_{xylem}$: Xylem abscsic acid
ANOVA: Analysis of variance
A: Area
A/V: Ratio area/volume
A$_{CO2}$: net CO_2 assimilation rate
A$_{CO2}$/g$_s$: Intrinsic water use efficiency
A$_{CO2}$/E: Instantaneous water use efficiency
AsAE: Ascorbic acid equivalents
B$_F$: Berry firmness
cv.: cultivar
Control: Full irrigation (well-irrigated)
Cº: Chrome
cm: Centimetre
CS: Cold Storage
CWSI: Crop water stress index
df: degree of fredom
dS: Decisiemens
DI: Deficit irrigation
DP: Percentage of dry plants
DM: Dry matter
DW: Dry weight
e.g.: Exempli gratia
E: Transpiration rate
EC: Electrical conductivity
ET$_{0P-M}$: Crop reference evapotranspiration by Penman-Monteith
ETc: Crop evapotranspiration
f.w.: Fresh weight
FAO: Food and Agriculture Organization of the United Nations
FAOSTAT: Food and Agriculture Organization of the United Nations. Statistics division
FDR: Frequency domain reflectrometry
g$_s$: Stomatal conductance
GAE: Gallic acid equivalents
h: Hour
HUE (ºh): Angle Hue
HPLC: High-Performance Liquid Chromatography
Kc: crop coefficient
Kg: Kilogram
KPa: Kilopascal
L: Liter
L*: Lightness
LA: Leaf area
LAI: Leaf area index
LIA: Leaf insertion angle
LDVT: Linear variable differential transformer

m: Metre
mg: Miligram
min: Minute
mL: Milliliter
mmol: Milimolar
MDS: Maximum daily shrinkage
MI: Maturity index
MPa: Megapascal
MeOH: Methanol
MXTD: Maximum daily trunk shrinkage
MNTD: Minimum daily trunk shrinkage
ns: No significance
NI: Null irrigation
P: Significant level
PAC: Percentage of plants in acceptable conditions
PAR: Photosynthetically active radiation
PDB: Percentage of plants in with dry branches
PIC: Percentage of plants in ideal conditions
ppm: Parts per million
PRD: Partial root-zone drying
PRD_{FIX}: Partial root-zone drying fixed
PRD_{ALT}: Partial root-zone drying alternated
P-value: Significant level
P_F: Pulp firmness
REW: Relative extractable water
RDI: Regulated deficit irrigation
RH: Relative humidity
S-ABA: Exogenous abscisic acid
SL: Shelf-life
SLW: Specific weight area
SLA: Specific leaf area
SPC: Soluble phenolic content
S_F: Skin firmness
T: Temperature/Treatment
TSS: Total soluble solids
TA: Titratable acidity
TAC: Total antioxidant capacity
Ta: Air temperature
Tc: Canopy temperature
Tc-Ta: Canopy to air temperature difference
TCSA: Trunk cross-sectional area
TDF: Trunk diameter fluctuation
TGR: Trunk growth rate
T_m: Mean temperature
T_{md}: Midday temperature
T_{mx}: Maximum temperature
V : Volume
VPD : Vapour pressure deficit
VPD_m: Mean vapour pressure deficit
VPD_{md}: Midday vapour pressure deficit
VPD_{mx}: Maximum vapour pressure deficit
v/v: Volume/volume
vs.: Versus

WA: Amount of water applied

WA_r: Relative amount of water applied.

WUE: Water use efficiency

Y: Total yield/year

Y_r: Total relative yield

LIST OF SYMBOLS

Ψ_o: Leaf osmotic potential

Ψ_{pd}: Pre-dawn leaf water potential

Ψ_s: Stem water potential at midday

Ψ_t: Turgor potential

Ψ_{os}: Osmotic potential at full turgor

Θ_v: Volumetric water content (soil and substrate)

µmol: Micromoles

%: Percentage

ºC: Degree Celsius