



UNIVERSIDAD DE CÓRDOBA

## **UNIVERSIDAD DE CÓRDOBA**

DEPARTAMENTO DE AGRONOMÍA

TESIS DOCTORAL

“Relaciones hídricas y manejo del riego en pistachero (*Pistacia vera* L.)”

Doctorando:

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2013

TITULO: *RELACIONES HIDRICAS Y MANEJO DEL RIEGO EN PISTACHERO*  
(*Pistacia vera L.*).

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**TÍTULO DE LA TESIS: Relaciones hídricas y manejo del riego en el pistachero (*Pistacia vera* L.)”**

**DOCTORANDO/A:** M<sup>a</sup> del Carmen Gijón López

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Informan:

D<sup>a</sup> M<sup>a</sup> del Carmen Gijón López ha realizado bajo nuestra dirección el trabajo titulado “**RELACIONES HÍDRICAS Y MANEJO DEL RIEGO EN EL PISTACHERO (*P. vera* L.)**”, y consideramos que reúne los méritos suficientes para optar al grado de Doctor por la Universidad de Córdoba.

Asimismo, del trabajo de investigación desarrollado han derivado las siguientes publicaciones:



**Gijón, M.C., Guerrero, J. , Couceiro, J.F., Moriana, A., 2009.** Deficit irrigation without reducing yield or nut splitting in pistachio (*Pistacia vera* L cv. Kerman on *Pistacia terebinthus* L). *Agricultural water management* 96: 12-22.

Área temática en la base de datos de Referencia: **Water Resources**

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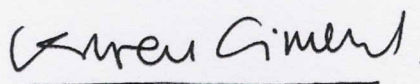
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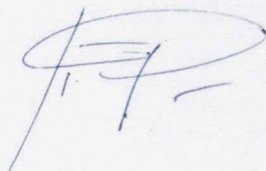
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**Nota:** Al presentar esta Tesis como compendio de publicaciones y con el fin de establecer una coherencia formal a lo largo del presente trabajo, se han editado los trabajos originales, eliminando de cada uno de ellos el apartado de referencias para agruparlas al final del documento.





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# RESUMEN





El cultivo del pistachero se ha convertido en una alternativa a los cultivos tradicionales con un creciente interés en zonas concretas de España, especialmente en Castilla la Mancha. El pistachero es un frutal tolerante a la sequía, cultivado tanto en condiciones de secano como de regadío. Puesto que el agua es un recurso cada vez más escaso, en esta tesis se ha estudiado con diferentes enfoques el comportamiento de este cultivo ante condiciones de riego y de estrés hídrico. En el primer capítulo se presenta la respuesta de la producción a diferentes situaciones de disponibilidad de agua, las cuales se consiguieron aplicando distintos tratamientos hídricos (riego cubriendo el 100% de las necesidades del árbol (Control), riego deficitario controlado (RDC), riego deficitario progresivo con distintas dosis de riego y secano). Los resultados muestran que el riego incrementa la producción y la calidad de los frutos y que no existen diferencias en estos parámetros entre el tratamiento RDC y el control, lo cual apunta a que el riego deficitario controlado (que en este caso ha supuesto un ahorro de agua de aproximadamente un 20%) es una buena práctica para este cultivo. En el segundo capítulo se ha estudiado la respuesta fisiológica de plantas en maceta del cultivar Kerman injertado sobre 3 portainjertos distintos (*Pistacia terebinthus* L., *P. atlantica* Desf. y un híbrido resultante del cruce de *P. atlantica* Desf. x *P. vera* L.) a condiciones de estrés hídrico y posterior recuperación de las mismas. Los resultados de este ensayo permitirán elegir el patrón adecuado dependiendo de las condiciones hídricas que se esperen en la plantación. Los datos obtenidos indican que en condiciones de una alta dotación de riego, el híbrido mostraría el mejor comportamiento en cuanto a los parámetros estudiados, y bajo condiciones de secano la mejor respuesta al estrés hídrico la mostraría el cultivar injertado sobre el patrón *P. terebinthus*. En el tercer capítulo se ha caracterizado la respuesta del potencial hídrico, la fotosíntesis y el intercambio gaseoso al estrés hídrico tanto durante ciclos diarios así como durante las 3 fases fenológicas del desarrollo del fruto. Además se han realizado curvas presión-volumen para analizar la respuesta al estrés hídrico en cada una de las fases fenológicas. Se ha observado que las relaciones hídricas en los árboles varían a lo largo

del ciclo fenológico del cultivo debido al desarrollo de distintos procesos fisiológicos de adaptación a la sequía. A medida que el estrés hídrico aumenta, se va produciendo también un progresivo cierre estomático desde antes del mediodía. Bajo condiciones de estrés hídrico moderado se maximiza la eficiencia en la transpiración debido al mantenimiento de la tasa de fotosíntesis. También se ha observado un mayor grado de ajuste osmótico durante las fases II y III. Estos resultados fundamentan fisiológicamente la mayor resistencia a la sequía de esta especie durante la fase II de desarrollo del fruto.

# ABSTRACT





Pistachio has become an alternative to more traditional tree crops in some areas of Spain, particularly in the Region of Castilla la Mancha. Pistachio is a drought-resistant fruit tree that can be cultivated under rainfed and irrigated conditions. Water can be a scarce resource; this thesis therefore investigates the behavior of this crop under irrigated and water stress conditions. The first chapter deals with how production responds to different water availability: irrigation covering 100% of the needs of the tree (control), regulated deficit irrigation (RDI), sustained deficit irrigation with different irrigation rates, and rainfed conditions. Irrigation was associated with an increased yield and better fruit quality, but no differences were seen in these respects between the RDI and control treatments. This suggests that RDI, which is associated with a water saving of approximately 20%, is a good management option for this crop. The second chapter deals with the physiological responses of potted pistachio plants (cv. Kerman) on three different rootstocks (*Pistacia terebinthus* L, *P. atlantica* Desf., and the hybrid *P. atlantica* Desf. x *P. vera* L.) to water stress and subsequent rehydration. The results suggest which rootstock might provide the best results in an orchard depending on water availability. The hybrid rootstock would appear to be the most adequate under conditions of good water availability, while the challenge of increased water stress under rainfed conditions appears to be best met by the *P. terebinthus* rootstock. The third chapter characterises the response to water stress with respect to water potential, photosynthesis and gaseous exchange, both in terms of daily cycles and during the three phenological stages of fruit development. Pressure-volume curves were produced for each phenological stage. It would appear that water relations in pistachio trees change over the growing season as different physiological processes are brought into play to achieve drought adaptation. Progressive stomatal closure starting before noon was observed as water stress increased. Under moderate water stress conditions, transpiration efficiency was maximised due to the maintenance of the photosynthetic rate. Greater osmotic adjustment was seen during



stages II and III. These results provide physiological evidence of increased resistance to drought in this species during stage II of fruit development.

# INTRODUCCIÓN

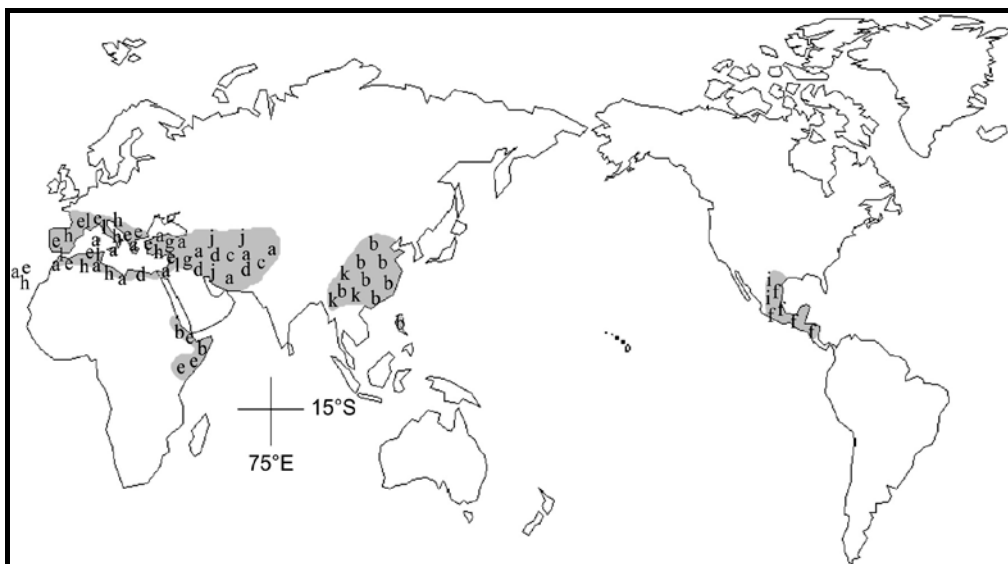




## 1. Importancia económica y distribución

Con la excepción de las especies norteamericanas *P. texana* y *P. mexicana*, las especies del género *Pistacia* se distribuyen principalmente en la región mediterránea, Asia Central y Occidental y Oriente Medio (Fig. 1). Aunque varias especies del género producen semillas comestibles y se usan para producción de aceites y jabones (Hepper, 1992), el pistachero (*P. vera* L.) es la especie con mayor interés comercial, y se cultiva ampliamente en países mediterráneos. El pistacho es el sexto fruto seco en superficie a nivel mundial después del almendro, nogal, anacardo, avellano y castaño (Mehlenbacher, 2003). La producción mundial de pistacho alcanzó las 900.000 toneladas en 2010 (FAOSTAT, 2010), siendo el mayor productor mundial Irán, con 446.000 t, seguido de EEUU (213.000 t), Turquía (128.000 t), Siria (57.500 t) China (48.700 t), Grecia (9000 t), Túnez (2.600 t) e Italia (1.600 t) (FAOSTAT, 2010). Sin embargo todo parece apuntar a que en los próximos años EEUU se situará como primer productor a nivel mundial debido a problemas coyunturales en Irán (Couceiro, com. pers.)

En la Unión Europea, la incertidumbre sobre el respaldo económico para los cultivos tradicionales de la región mediterránea como el olivar y la viña, además de la explotación de terrenos empobrecidos y no aptos para la mayoría de cultivos, han llevado al progresivo incremento en la superficie plantada de pistachero. Desde los últimos 15 años la producción en Grecia e Italia se ha mantenido constante (FAOSTAT, 2010), sin embargo en Turquía (tercer productor mundial) ha aumentado en unas 80.000 t (FAOSTAT). Otro país de la cuenca mediterránea como es Siria (4º productor mundial) ha aumentado en la última década la superficie plantada en 6.000 ha, lo que ha supuesto un incremento en la producción de aproximadamente 7000 t (FAOSTAT, 2010). En España actualmente el área total plantada es de alrededor de 6000 ha, habiendo sido a principios de los 90 casi inapreciable (Couceiro, et al., 2011).



**Fig. 1. Distribución mundial del género *Pistacia*. El sombreado gris indica las localizaciones generales de 11 especies de este género y un híbrido: a = *P. atlantica*, b = *P. chinensis*, c = *P. integerrima*, d = *P. khinjuk*, e = *P. lentiscus*, f = *P. mexicana*, g = *P. palaestina*, h = *P. terebinthus*, i = *P. texana*, j = *P. vera*, k = *P. weinmannifolia*, l = *P. saportae*. Fuente: Tingshuang Yi et al., 2008**

### **Superficie en España.**

Después de introducirse en la Península Ibérica durante la dominación árabe, tras la reconquista, el cultivo del pistachero fue desapareciendo paulatinamente. En la década de los 80, el cultivo se reintroduce en España, primero en Cataluña a través del Centro Agropecuario "Mas Bové" del IRTA de Reus (Tarragona) (Couceiro et al., 2011). El pistachero como cultivo alternativo para Castilla la Mancha se comenzó a estudiar en el año 1986 mediante un proyecto de investigación (Guerrero et al., 2005 y 2008) con el fin de identificar cultivos alternativos al olivar y a la viña y aprovechar terrenos marginales. Fue a partir de 1996 cuando se comenzaron a establecer plantaciones bien organizadas agrónomicamente. De las 6000 ha de pistachero que existen en España actualmente (Couceiro et al, 2011), Castilla-La Mancha es la

región con una mayor superficie dedicada a este cultivo (unas 5000 ha), seguida de Cataluña (400 ha), Andalucía (300 ha), Extremadura (200 ha) y Castilla y León (100 ha) (Couceiro, com. pers.). La mayor parte de las plantaciones son muy jóvenes (8 años después de injerto), con una producción muy baja (unos 400 Kg/ha de media), y el ritmo de crecimiento es de unas 400 ha anuales (Couceiro, com. pers.). España está situada en el décimo lugar del ranking mundial de producción detrás de países como Irán, EEUU, Turquía, Siria, Grecia, Italia, Túnez, Afganistán y Pakistán, aunque con perspectivas de igualar a corto plazo a países como Grecia e Italia.

## **2. Ecología del cultivo**

### **2.1. Requerimientos climáticos**

El pistachero es una especie xerófita que requiere temperaturas moderadamente bajas en invierno. Para que el árbol tenga una óptima y homogénea brotación es preciso la acumulación durante el reposo invernal de un determinado número de horas frío (HF), variable según los cultivares considerados. Estas necesidades oscilan entre las 800 HF en cultivares tempranos como Mateur, Avdat y Aegina (Guerrero et al., 2011) y entre 1000 y 1200 HF en cultivares tardíos como Kerman (Crane y Takeda, 1979; Crane e Iwakiri, 1981; Maranto y Crane, 1982; Guerrero et al., 2011). El cultivo no se debería implantar en regiones donde no se superen las 700 HF (Couceiro, com. pers.).

El pistachero es muy sensible a la humedad ambiental debido al ataque de hongos patógenos que afectan a la parte aérea del árbol tales como *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not, *Alternaria alternata* (Fr.:Fr.) Keissl., *Aspergillus* spp., *Septoria* spp., *Pileolaria terebinthi* Castagne, *Botrytis cinerea* Pers.: Fr. y *Phomopsis* spp. (Michailides, 2008). Una HR media por encima del 40-45% durante los meses de junio, julio y agosto será limitante a la hora de mantener un

estado sanitario satisfactorio de la plantación y obtener una producción rentable (Couceiro et al., 2011). Además, al ser la polinización del pistachero anemófila, un exceso de lluvias durante la floración podría reducirla considerablemente (Crane, 1984).

A pesar de ser un cultivo muy resistente a bajas temperaturas durante el periodo de dormancia (Joley, 1973; Crane y Maranto, 1989; Guerrero et al., 2005), las heladas durante el periodo de floración y cuajado del fruto podrían llegar a ser un factor limitante en este cultivo (Arpaci, et al., 2005, Guerrero et al., 2005), causando pérdidas importantes en la producción. Arpaci et al. (2005) estudiaron la respuesta a las heladas en distintos cultivares de pistachero, observando que el porcentaje de flores afectadas por heladas primaverales fue de un 50% en todas las variedades cuando eran expuestas a -1 °C durante 1 o 2 horas. Cuando se exponían durante 1 h a -3°C el porcentaje de daños ascendía al 70-80%, aumentando hasta casi el 90% cuando la exposición a esta misma temperatura era durante 2-3 horas.

El tiempo térmico necesario (desde el movimiento de las yemas florales hasta cosecha) para una maduración óptima de los frutos se ha determinado en la zona de Castilla-La Mancha en 3400 °-día para las variedades tardías y unas 3000 °-día para las tempranas (Guerrero, 2011). La temperatura base media para el cálculo del tiempo térmico en pistachero es de 2.63 °C

## **2.2. Requerimientos de suelo**

El pistachero puede ser cultivado en un gran número de tipos de suelo (Vargas et al., 1999; Couceiro et al., 2000; Guerrero et al., 2002; Ferguson, 2008) aunque se adapta mejor a suelos poco profundos, pedregosos, moderadamente salinos y calcáreos que cualquier otro tipo de frutal (Ferguson, 2005). En cuanto a la textura, para su óptimo desarrollo, requiere suelos francos ó franco-arenosos bien drenados, y no suele

desarrollarse ni fructificar de forma adecuada en aquellos con más de un 30% de arcilla (Guerrero et al., 2005; Couceiro et al., 2011), debido a la escasa permeabilidad que tienen, provocando encharcamiento cuando las precipitaciones son abundantes y asfixia radicular. Si el encharcamiento se produce durante el invierno los síntomas pueden no percibirse hasta bien entrada la primavera e incluso en el verano (Couceiro, com. pers.).

### **3 - Material Vegetal**

El pistachero es una especie con muy poca capacidad de enraizamiento, por lo que es preciso el uso de portainjertos para su propagación vegetativa. En otras especies, el uso de patrones es opcional y se utiliza como una herramienta para mejorar las características de la variedad en cuanto al tamaño del árbol, su respuesta productiva o su calidad. Diferentes estudios con portainjertos de manzano (Olien y Lakso 1986; Cohen y Naor 2002) y de melocotonero (Weibel et al. 2003) mostraron que éstos influyen en la tasa de crecimiento vegetativo del árbol. Solari *et al.* (2006) confirmaron en melocotonero que el efecto del portainjerto en las relaciones hídricas del árbol y en el crecimiento vegetativo potencial proviene, al menos en parte, de diferencias en la conductividad hidráulica del árbol asociadas con portainjertos específicos. Por otro lado, Rogers y Beakbane (1957), Lockard y Schneider (1981) y Webster (1995) sugirieron en diferentes especies que los portainjertos tienen un efecto en el crecimiento vegetativo a través del estado hormonal del árbol (Kamboj *et al.* 1999), estado de nutrición mineral (Jones, 1971) o estado hídrico (Olien y Lakso, 1986). Los estudios en portainjertos de pistachero son menos numerosos y en general sólo se han centrado en comparar la respuesta productiva y/o la resistencia a enfermedades.

#### **3.1. Portainjertos**

El cultivo del pistachero está basado en variedades clonales injertadas sobre patrones francos de la misma especie o de otras especies del género *Pistacia* e híbridos interespecíficos. La elección del portainjerto



es una de las decisiones más importantes para el desarrollo de la plantación, y es diferente según las zonas de cultivo. Así, *P. atlantica*, *P. integerrima* e híbridos entre *P. atlantica* x *P. integerrima* son los principales portainjertos en California, francos de *P. vera* se utilizan en Turquía y *P. mutica*, *P. khinjuk* y francos de *P. vera* son los más utilizados en Irán (Guerrero, 2011). A la hora de elegir el patrón se debe conocer la profundidad y textura del suelo, disponibilidad de agua, temperaturas mínimas absolutas y compararlas con las resistencias y debilidades de cada pie en cuanto a plagas, enfermedades, frío, etc. Las características más destacadas de las especies que pueden ser utilizadas como portainjertos en función de su adaptabilidad se exponen a continuación.

***Pistacia terebinthus* L.** Especie autóctona de la Península Ibérica llamada vulgarmente *cornicabra* o *terebinto* (Guerrero, 2011). Esta especie crece de forma natural en toda la región mediterránea, extendiéndose por el sur de Europa, norte de África y el suroeste asiático (Guerrero, 2011). Dentro de la distribución general mediterránea, la especie muestra gran variabilidad, por lo que se la puede encontrar desde zonas muy térmicas y casi a nivel del mar hasta en localizaciones frías, en torno a los 1600 m de altitud. No obstante, en el caso de la Península Ibérica, parece mostrar su adaptación óptima entre los 500 y los 1400 m de altitud (Sánchez et al., 2001). Existe gran heterogeneidad en cuanto a su vigor (Ferguson et al., 2008), aumentando éste en zonas más meridionales. Es una especie muy resistente al frío, muy eficiente nutricionalmente y resistente a numerosas plagas y enfermedades, aunque sensible a verticilosis (Ferguson et al., 2005; Guerrero et al., 2005). Por todas estas características este patrón es utilizado en la mayoría de países productores de pistacho (Italia, Grecia, Turquía y Australia). En España la mayor parte de las plantaciones se encuentran injertadas sobre este pie (Guerrero et al., 2007; Couceiro et al., 2011) (Fig. 2).



**Fig. 2. A) Ejemplar de *Pistacia terebinthus* L. Glza. Julio C. Vergara. B) Hoja y frutos de *Pistacia terebinthus* L.**

***P. atlantica* Desf.** Es utilizada en países donde es autóctona (Marruecos, Túnez, Argelia, Irak, Irán, etc) (Zohary, 1952, INRAT, 1981). En EEUU comenzó a utilizarse pero se abandonó debido a su extrema sensibilidad a enfermedades fúngicas (*Phytophthora* spp., *Verticillium dahliae* Kleb., *Armillaria mellea*, etc.) (Ferguson et al., 2008). Es una especie de vigor intermedio. En todos los ensayos llevados a cabo en el Centro Agrario “El Chaparrillo” no se han encontrado diferencias significativas entre este pie y el terebinto en cuanto a producción y calidad de los frutos en árbol (Fig. 3).



**Fig. 3. A) Ejemplar de *Pistacia atlantica* D. localizado en el Atlas-Meseta del Rekkam (Marruecos). Glza. J.C. Vergara. B) Hoja y frutos de *Pistacia atlantica* Desf.**

***P. integerrima* Stewart.** Especie utilizada en EEUU que se conoce comercialmente como PGI (Pioneer Gold I). Es el pie más empleado en este país, aproximadamente en el 95% de las plantaciones (Joley, 1979; Ferguson et al., 1995; Guerrero et al., 2005). Es muy vigoroso (Ferguson et al., 2008), por lo que sería adecuado para zonas con suelos profundos y alta disponibilidad de agua. Es muy sensible al frío y tolerante a enfermedades como la verticilosis (Ferguson, 1993; Avanzato, 1994) (Fig. 4).



**Fig. 4. A) Ejemplar de *Pistacia integerrima* S. B) Hoja y frutos de *Pistacia integerrima* S.**

**UCB-1.** Híbrido americano procedente del cruzamiento ente *P. atlantica* y *P. integerrima*, mediante polinización cerrada. Es el más vigoroso y productivo de todos los portainjetos cuando se cultiva en condiciones óptimas de suelo y estado hídrico (Ferguson, 2008). Es tolerante a la verticilosis y sensible a nematodos (Morgan et al., 1992; Epstein et al., 2004; Ferguson et al., 2005). Es medianamente resistente al frío (Ferguson et al., 2005). Aunque en el mercado actual es el más caro de todos los portainjetos, no se han apreciado diferencias entre sus ventajas y las de *P. integerrima*, con la excepción ya comentada de su vigor, razón por la cual apenas se utiliza en EEUU. En su empleo se debe considerar el pago de derechos al organismo americano que lo ha patentado (Fig. 5).



**Fig. 5. A) Ejemplar de UCB1 (*P. atlantica* x *P. integerrima*). B) Hoja y frutos de UCB 1**

La elección del portainjerto es una fase crucial en el desarrollo futuro del cultivo (Ferguson et al., 2008, Vargas et al., 1995). Existen amplias diferencias en cuanto a producción, vigor y longevidad (Tarango, 1993). Hay pocos trabajos que comparen los diferentes portainjertos, posiblemente el de Ferguson et al. (2008) sea el más completo. Entre las aproximadamente 10 especies del género *Pistacia* que pueden ser utilizadas como portainjertos del pistachero, *P. terebinthus* se define como un patrón poco vigoroso (Krueger y Ferguson, 1995; Vargas y Romero 1998; Ferguson et al, 2008), con baja uniformidad del desarrollo y afinidad por el injerto (Ferguson et al, 2005 a; Guerrero et al., 2005 y 2006 a). No obstante, es el que posee un área de aclimatación más amplia debido a su gran rusticidad caracterizada por su adaptación a diferentes tipos de suelo, a baja pluviometría, temperaturas extremas y a condiciones de salinidad (Tarango, 1993; Couceiro et al., 2000; Ferguson et al., 2008). En el trabajo de Ferguson (2008) se considera *P. terebinthus* como el patrón más resistente a la sequía y al frío, mientras que *P. integerrima* es el más sensible a heladas. UCB1 es más resistente al frío que *P. integerrima* pero menor que *P. atlantica* y *P. terebinthus*. *P. integerrima* y UCB1 son los patrones más tolerantes a enfermedades de suelo de los 4 portainjertos mencionados en este estudio, mientras que *P. terebinthus* es en términos generales resistente a plagas y enfermedades pero es el más sensible a verticilosis (Ferguson et al., 2005; Guerrero et

al., 2005). *Pistacia atlantica* es un patrón desde el punto de vista de cosecha interesante, aunque ha sido abandonado en muchas zonas por su gran susceptibilidad a la verticilosis (Ferguson *et al.*, 2005, 2008). En la actualidad UCB es el patrón considerado más productivo en condiciones de regadío (Ferguson *et al.*, 2005). Germana (1997) comparando distintos portainjertos, observó que *P. atlantica* tiene una transpiración y actividad fotosintética más intensa que *P. terebinthus*, particularmente en plantas estresadas lo que podría hacerlas más susceptibles a estrés hídrico. Guerrero *et al.* (2003) no encontraron un efecto del portainjerto en diferencias productivas al comparar los patrones *Pistacia terebinthus* L., *Pistacia integerrima* L., *Pistacia atlantica* Desf y *Pistacia vera* L. en condiciones de secano. Finalmente los resultados contradictorios al comparar *P. terebinthus* con el resto de portainjertos podría estar relacionada con el origen de las semillas del primero, mucho menos seleccionado que el resto. Guerrero (2011) describe un comportamiento diferente según la localización geográfica de las plantas de terebinto. Las semillas obtenidas en las prospecciones en diferentes lugares de España han presentado diferencias según la población, destacando la población obtenida en Calzada de Calatrava (Ciudad Real) por ser una de las que muestran un mayor vigor, sin diferenciarse estadísticamente de *P. vera*, portainjerto considerado de vigor medio-alto.

### **3.2. Cultivares**

Según estudios realizados por Whitehouse en 1957 se considera a la región de Bagtiz en Turmekistán y las zonas adyacentes de Irán como el centro de diversidad del pistacho, propagándose en la cuenca mediterránea posteriormente por los romanos (Hormaza *et al.*, 1994). Para algunos autores (Ferguson *et al.*, 2008), se podrían distinguir dos grupos de cultivares claramente diferenciados: aquellos cuyo origen se sitúa en el Asia occidental y central, incluyendo los pertenecientes a Irán, y los cultivados en el área mediterránea como Italia (Sicilia), Grecia,

Túnez, etc. Los primeros se caracterizan por sus frutos grandes y redondeados, con el color del grano amarillo verdoso y los segundos por tener frutos más pequeños y alargados, de un sabor y aroma reconocible, con un grano de color verde intenso. En función de la época de floración y maduración del fruto, también se pueden establecer 2 grupos de cultivares: tempranos y tardíos (Guerrero et al., 2005; Guerrero et al., 2010). Comercialmente es habitual el uso de varios cultivares femeninos que permitan diversificar el riesgo. Además, cuando se establece una plantación, a la hora de la elección de variedades también se debe tener en cuenta el mercado actual de este fruto seco, perspectivas de la evolución del consumo a corto, medio y largo plazo y el tipo de productos, tanto tradicionales como novedosos, que se podrían producir con esta materia prima (Guerrero et al., 2010).

El pistachero es un árbol dioico. Existen innumerables cultivares de pistachero, tanto femeninos como masculinos. En la tabla 1 se indican los cultivares femeninos más importantes a nivel mundial, así como sus correspondientes masculinos.

<b>TABLA 1. Relación de cultivares (autóctonos o foráneos) de pistachero (<i>Pistacia vera</i> L.) cultivados en el mundo*. Fuente: J.F. Couceiro.</b>		
	<b>CULTIVARES</b>	
<b>PAÍS</b>	<b>FEMENINOS</b>	<b>MASCULINOS</b>
<b>IRAN</b>	Abdollahi, Aghai, <b>Ahmad Aghaee, Akbari</b> , Amiri, Badami, Badami Ravar, <b>Badami Zarand</b> , Badami Zoodrass, Bagnali, Bilgen, Chaffouri, Dastmaliq, Djavadi, Ebrahimi, Ebrahim Abadi, Eghmis, Fandoghi 48, Fandoghi Riz, Ghafuri, Ghazvini, Ghermeza, Gholam Rezami, Hassan Zadeh, Herati, Hosseini, Imperial de Damgham, Italiaee, Jalab, Jawzi, <b>Kalleghouchi</b> , Kay, Khandani, Khanjari, Noghli, , Shah-passand, , Mirhavy (Moradi), Momtaz, Nish Kalaghi, Ogah, <b>Ohadi</b> , Rafsanjani, Razvine, Rezaee Zoodras, Rezahi, Sadeghi, Safeed, Saleed, Seiffeddini, Sefidet, Shahpessand, Sharifi, Shastie, Soltani, Syrizi, Tbeahimi, Tedjarati, Wahedi, etc.	Innumerables
<b>EEUU (CALIFORNIA)</b>	Aleppo, Bronte, Joley, <b>Kerman</b> , Lassen, Red Aleppo, Sfax, Trabonella, etc.	02-18, 02-16, Peters (Peter), Chico-23,
<b>TURQUÍA</b>	Abiot miwahy, Ashouri, Awhah, Ayimi, Beyaz Ben, Cakmak, Degirmi, El Batoury, El Djalale, El Jalale, Haci Şerifi, Halebi, <b>Kermez (Kimizi, Kirmizi)</b> , Keten Gömleği, Minasian, Muntaz,	Innumerables

	Obiad, Safeed, Sefidi, <b>Siirt</b> , Sultani, Uzun, Vahidi, etc.	
<b>SIRIA</b>	Abou Rieha, Achxiri, Aintab, Al Grahy, Allami (Alemi, Ajamy, Ayimi), Aleppo, Al marawhi, Antaby, <b>Ashoury (Ashoory, Achouri, Red Aleppo)</b> , Baidy, Bataury, bayd Al Tair, Boundoky, Entaby a, Entaby b, Halebi, Jalab, Lathwardy (Lazwardy, Lazaourdi), Mirhavy, Nab Al Dajaml, Obiad, Ouleimy (Oleimy), Red Jalab, Red Oleimy (Red Ouleimy), Red White Ouleimy (White Oleimy), Sen Al Feel, Wardany, White Ashoury, White Turkey, Zaroory, etc.	M-37, M-38, M-47, M-11, M-36, M-57
<b>ITALIA (SICILIA)</b>	Agostana, <b>Bianca (Napoletana)</b> , Bronte, Cappuccia, Cerasola (Girasola), Femminella, Ghiandalora, Gialla, Gloria, Insolia, Notalora, Pignatone, Serradifalco, Silvana, Tardiva, Tard. Serra di Falco, Trabonella, etc.	M-5, Santangilisi, M8, M3, M10, M7, M4, M1, M502, M2, M3, M6, <i>Pistacia terebinthus</i> L.
<b>GRECIA</b>	<b>Agina (Aegenes)</b> , Fountoukati, Nichati, Pontikis, etc	A, B, C y D
<b>TUNEZ</b>	Beta (Vita), El Guettar, Gafsa, Halebi (Jalab), Kasserina, <b>Mateur</b> , Meknassy, Nouri, Safed, Sefid Peste, Sfax, Thyna, etc.	25A, 40A
<b>ISRAEL</b>	Arie, Avdat, Avidon, Bronte, Damghan, Egina, Gazvin, Kastel, Kerman, Larnaka, Rashti, Red Aleppo, Shufra, etc.	Eilon 502 , Ask (semillas de Siria), Enk , Nazaret 1(semillas de Siria) (Nazar, Naz), Alumoth 29
<b>CHIPRE</b>	Ashoury, Kéri (Messaria, Keri), <b>Larnaka (Larnaca)</b> , etc.	M-Especial
<b>AFGANISTAN</b>	Numerosas variedades de fruto pequeño y grano verde procedentes de árboles de semilla (Harati)	Innumerables, procedentes de semilla
<b>TURKMENISTÁN</b>	Kouchka, Kara Taou, Pelengovali, Talass, etc.	
<b>IRAQ</b>	Iraq-2, Iraq-1	
<b>MARRUECOS</b>	Achouri, Mateur, etc.	Gamma gréce, Vera, Beta, Gamma
<b>AUSTRALIA</b>	Bronte, Kerman, Lassen, Sfax, <b>Sirora</b> , Trabonella, etc.	Peters (Peter), Kaz
<b>* En negrita se destacan los cultivares más frecuentes del país</b>		

En España se han importado distintos cultivares, tanto femeninos como masculinos, aunque caben destacar, tanto por su comportamiento agronómico como por sus características organolépticas sobre todo 2:

- **KERMAN:** Es el cultivar prioritario en EEUU y el que ocupa el 90% de las plantaciones en la Península Ibérica (Couceiro, com. pers.). Es uno de los cultivares con frutos de mayor tamaño que se puede ver en los mercados de consumo directo junto a otros cultivares iraníes. Destaca por la blancura de su cáscara, lo que lo hace más



atractivo para su consumo como snack (Guerrero et al., 2005). Su mayor inconveniente es el bajo rendimiento productivo y menor porcentaje de frutos abiertos con respecto a otros cultivares, cuando se cultiva en suelos pobres, sin agua y con una textura del suelo excesivamente arenosa o arcillosa (Couceiro et al., 2011). Su floración es tardía, lo que la hace de gran interés para lugares con potenciales problemas de heladas primaverales, como por ejemplo Castilla-La Mancha (Guerrero et al., 2005; Guerrero et al., 2010) (Fig. 6).

- **LARNAKA:** Cultivar prioritario en Chipre. Destaca por su buen comportamiento agronómico en seco (buena producción, bajo índice de vecería, etc.) y la apreciable calidad de sus frutos (elevado porcentaje de frutos abiertos y bajo de frutos vacíos) (Guerrero et al., 2005; Couceiro et al., 2011; Guerrero, 2011). Fruto de forma alargada y de tamaño medio. Su floración es temprana. La tonalidad más oscura de su cáscara en relación a cv. Kerman es un aspecto menos valorado por el mercado para su consumo como snack (Guerrero et al., 2005; Guerrero et al., 2010) (Fig. 6).



**Fig. 6. Frutos de los cultivares Kerman y Larnaka**

Se pueden destacar como polinizadores tempranos a C especial (macho más utilizado con el cultivar femenino Larnaka), y de floración tardía a 02-18, Peter, Guerrero y Chaparrillo (obtenidos estos dos últimos



en el C.A. "El Chaparrillo" y caracterizados como de floración más tardía que la variedad Peter) (Guerrero et al., 2010; Guerrero, 2011).

#### **4. Necesidades hídricas y respuesta al estrés hídrico**

El riego es la práctica agronómica que más incrementa la productividad de un cultivo en medio ambientes semiáridos. Sin embargo el agua es un bien escaso, especialmente para el uso agrícola y la cantidad de agua de riego disponible es cada vez menor. Sólo los sistemas agrícolas más eficientes tendrán probabilidad de recibir las cantidades adecuadas de agua para riego (Fererres *et al.*, 2003).

##### **4. 1. Relaciones hídricas**

Aunque la respuesta al estrés hídrico del pistachero ha sido poco caracterizada, tradicionalmente se ha considerado como un árbol muy resistente a la sequía y salinidad (Behboudian *et al.* 1986; Rieger, 1995). Al igual que en otros cultivos, el riego incrementa la cosecha y además mejora la calidad del fruto (aumenta el número de frutos abiertos en la cosecha y disminuye el de vacíos) y disminuye la vecería (Kanber *et al.*, 1993; Goldhamer *et al.*, 1995). No obstante, la respuesta al riego en este cultivo necesita ser cuantificada adecuadamente (Guerrero et al., 2007).

Las condiciones agronómicas en las que se va a establecer el cultivo del pistachero van a ser generalmente deficitarias en agua. En estas condiciones, el efecto del portainjerto puede ser fundamental en la respuesta de la variedad al estrés hídrico, tanto a nivel fisiológico como en la producción final, por lo que es esencial caracterizar la respuesta de las plantas tanto durante el periodo de sequía como durante el de recuperación del estrés hídrico con diferentes portainjertos.

Los déficits hídricos afectan a una amplia variedad de procesos fisiológicos en las plantas superiores, principalmente a la expansión foliar,

la apertura de los estomas y la asimilación fotosintética de carbono (Robichaux, 1984). En todos estos procesos está implicada la turgencia celular, por lo que mecanismos que favorezcan el mantenimiento de ésta mejorarán el crecimiento en condiciones de baja disponibilidad de agua (Turner y Jones, 1980; Jones et al., 1981; Bradford y Hsiao, 1982).

Las relaciones hídricas pueden variar en función del estado fenológico del árbol. Marsal y Girona (1997) han descrito en peral en ausencia de estrés hídrico variaciones en las relaciones hídricas según estados fenológicos. Tales variaciones pueden explicar la mayor resistencia a la sequía en determinadas fases fenológicas como la de endurecimiento del hueso.

#### **4. 2. Respuesta al riego**

Una herramienta básica para evaluar la eficiencia del riego es la respuesta productiva de los cultivos al agua (Vaux y Pruitt, 1983). La biomasa y el rendimiento de los cultivos están relacionados linealmente con la evapotranspiración del cultivo (ETc). A veces, sin embargo, se observan relaciones no lineales entre el rendimiento y la ETc, por ejemplo en algodón (Orgaz et al., 1992) y olivo (Moriani et al., 2003). Esto puede estar relacionado con el aumento de la resistencia a la sequía durante alguna de sus etapas fenológicas. Durante estos períodos, una reducción de la ET no reduce significativamente el rendimiento (Behboudian y Mills, 1997). Si la respuesta de la producción a la variación en la ET no es lineal, los niveles óptimos de ET deben ser definidos para diferentes especies y precios del agua.

El coeficiente de cultivo (Kc) del pistachero es uno de los más altos dentro de los árboles de hoja caduca, superando el valor de 1 durante la mayoría de la estación de crecimiento (Fereres y Goldhamer, 1990). Por tanto, ya que la mayoría de zonas de cultivo del pistachero son de clima semiárido, es aconsejable el riego deficitario como práctica de ahorro de

agua. A finales de los 80, se diseñaron programaciones de riego deficitario controlado (RDC) para el pistachero injertado sobre *Pistacia atlantica* Desf. en California (Goldhamer *et al.*, 1987; Phene *et al.*, 1987). Estas técnicas de RDC se aplican a especies leñosas y se basan en la distinta sensibilidad de las diferentes fases fenológicas del cultivo al estrés hídrico (Behboudian y Mills, 1997; Ruiz-Sanchez *et al.*, 2010; Behboudian *et al.*, 2011), lo cual permite un ahorro de agua sin disminución significativa de la producción ni de la calidad de ésta. Estos experimentos mostraron diferentes respuestas de la cosecha a las condiciones de estrés hídrico dependiendo del estado fenológico del fruto durante el periodo de estrés. Las distintas fases fenológicas se establecen en función del crecimiento y del peso del fruto (Goldhamer, 1995; Goldhamer y Beede, 2004).

**Fase I:** Al final de la floración y una vez cuajado el fruto, se produce una alta tasa de crecimiento de éste aunque sin haber crecimiento del grano, periodo en el que casi llega a alcanzar su tamaño definitivo, lo cual se puede determinar tanto por el diámetro longitudinal como por el transversal. El peso fresco del fruto tiene una pauta de crecimiento parecida a la de su diámetro (Fig. 7 y 11). En el momento en el que se alcanza el diámetro máximo del fruto, tiene lugar la transición entre la fase I y la fase II.



**Fig. 7. Frutos en fase fenológica I.**

La fase I es sensible al estrés hídrico, determinando el tamaño del fruto. Distintos estudios han mostrado que un estrés hídrico moderado durante la fase I y II (periodo de crecimiento rápido de los frutos y periodo de endurecimiento del endocarpio, respectivamente) aumenta el porcentaje de frutos abiertos, y por tanto la calidad de la cosecha (Goldhamer y Beede, 2004). Sin embargo en experimentos más recientes se ha encontrado un incremento de frutos abiertos pero también de frutos rajados prematuramente en algunos años y en árboles sometidos a tratamientos de riego deficitario controlado durante la fase I (Goldhamer et al. 2004, 2005, 2006. Este rajado es una ruptura del epicarpio y mesocarpio que ocurre antes de la cosecha y que hace al fruto más susceptible de sufrir infecciones de la semilla, reduciendo la calidad de la cosecha y en consecuencia, su valor económico (Figura 8). La apertura normal del fruto se produce en el endocarpio (la cáscara) también antes de la cosecha, y aumenta la calidad y el valor comercial. Doster y Michailides (1997) relacionaron el incremento de frutos rajados con un retraso en el periodo de cosecha, pero no con la influencia del estrés hídrico al comienzo de la estación de crecimiento.



**Fig. 8. Frutos rajados.**

**Fase II:** A este rápido crecimiento del fruto durante la fase I le sigue un período de ralentización del crecimiento del árbol y del incremento

del peso fresco del fruto. En esta fase II tiene lugar el endurecimiento del endocarpio (cáscara dura) (Fig. 9 y 11).



**Fig. 9. Frutos en fase fenológica II (endurecimiento de la cáscara).**

Esta fase es la menos sensible al estrés hídrico, siendo por tanto en la que se puede reducir el aporte de riego (Goldhamer et al. 2004, 2005, 2006; Guerrero et al., 2005). Phene et al. (1987) encontraron que en árboles de pistachero maduros que crecen en suelos poco profundos, una reducción en el riego del 50% de la evapotranspiración del cultivo (ETc) en la fase II no tiene efectos negativos sobre el rendimiento final.

**Fase III:** se inicia con el comienzo del desarrollo del grano, lo que se traduce en un fuerte incremento del peso del fruto, y termina con la cosecha (Fig. 10 y 11).



**Fig. 10. Frutos en fase fenológica III (crecimiento del grano).**

Esta fase es la más sensible al estrés hídrico (Goldhamer et al., 2004, 2005 y 2006), por lo que si se aplica un cierto estrés en la fase II se debe recuperar el árbol en la fase III para evitar un menor crecimiento del grano, que se traduciría en una reducción de la producción (Guerrero et al., 2007).

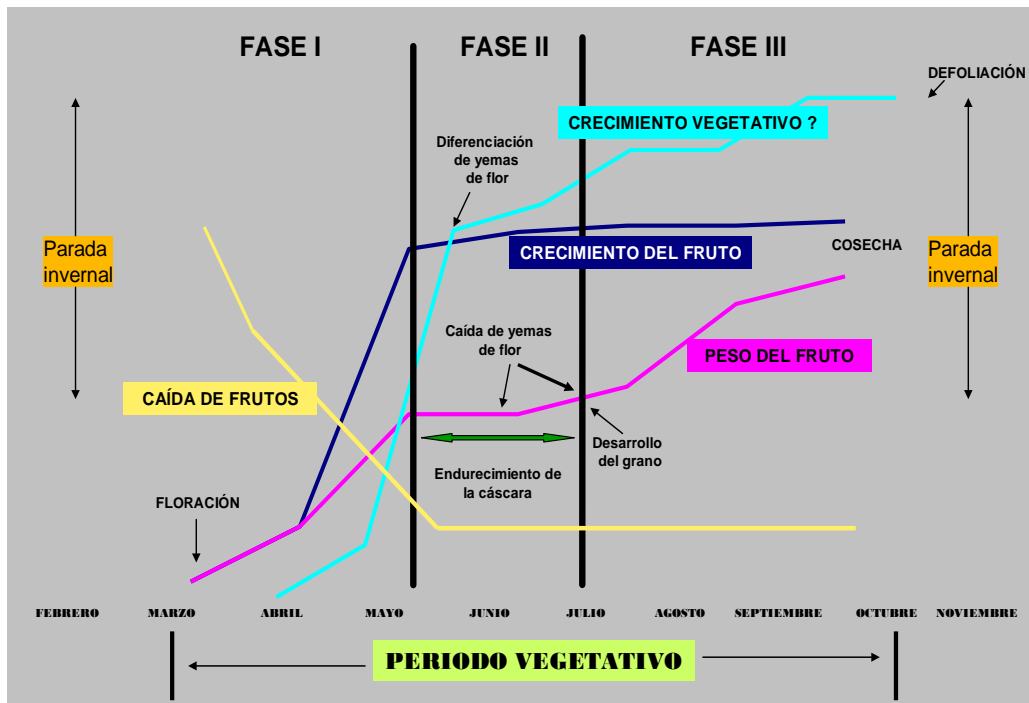


Fig. 11. Ciclo vegetativo y reproductivo del pistachero.



# OBJETIVOS







El objetivo global de esta Tesis ha sido estudiar la respuesta del pistachero (*P. vera* L.) al estrés hídrico. Este objetivo general se ha abordado a través de los siguientes objetivos específicos:

1) Estudiar la respuesta productiva al riego del cultivar Kerman sobre el portainjerto más utilizado en la zona (*Pistacia terebinthus* L.) en una plantación adulta.

2) Analizar la influencia del riego deficitario controlado (RDC), imponiendo estrés hídrico durante las distintas fases de crecimiento del fruto, en la producción y calidad de la cosecha.

3) Investigar la relación entre los frutos rajados prematuramente y las condiciones medioambientales y de estrés hídrico.

4) Estudiar el efecto del portainjerto en la respuesta fisiológica al estrés hídrico del cultivar Kerman, valorando el comportamiento tanto durante la fase de sequía como en la de recuperación del estrés.

5) Caracterizar la respuesta del potencial hídrico y del intercambio gaseoso de árboles de pistachero al estrés hídrico durante los 3 periodos de desarrollo del fruto, identificando los mecanismos fundamentales que ocurren en cada uno de ellos.

6) Establecer, mediante curvas presión-volumen, el grado de resistencia a la sequía del pistachero en cada una de las fases fenológicas.

Los objetivos 1, 2, y 3 se recogen en el artículo de referencia: Deficit irrigation without reducing yield or nut splitting in pistachio (*Pistacia vera* L. cv. Kerman on *Pistacia terebinthus* L. (Capítulo I) (ANEXO)

El objetivo 4 se recoge en el artículo de referencia: Rootstock influences the response of pistachio (*Pistacia vera* cv Kerman) to water stress and rehydration. (Capítulo II) (ANEXO)

Los objetivos 5 y 6 se recogen en el artículo de referencia: Water relations of pistachio (*Pistacia vera* L.) as affected by phenological stages and water regimes. (Capítulo III) (ANEXO)



# CAPÍTULO I





## CAPÍTULO I

**Riego deficitario en el Pistachero (*Pistacia vera* 'cv. Kerman sobre *Pistacia terebinthus* L.) sin reducción en la producción y en la apertura de los frutos.**

**Publicado como:**

**Gijón M.C., Guerrero J., Couceiro J.F., Moriana A. (2009). Deficit irrigation without reducing yield or nut splitting in pistachio (*Pistacia vera* 'cv. Kerman on *Pistacia terebinthus* L). *Agricultural Water Management* 96: 12-22.**



## Resumen

Para la realización de este capítulo se llevó a cabo un ensayo con el cultivo del pistachero (*Pistacia vera* cv. Kerman injertado sobre *Pistacia terebinthus* L.) durante 4 años en el centro de la Península Ibérica, para determinar el efecto del riego deficitario controlado (RDC) en la cantidad y calidad de la cosecha. La estación de crecimiento se dividió en 3 etapas fenológicas: etapa I – desde final de floración hasta final del crecimiento rápido del fruto, etapa II – desde que el fruto alcanza su tamaño máximo hasta el comienzo del desarrollo del grano, y fase III – desde el comienzo de crecimiento del grano hasta cosecha. Los árboles control fueron regados al 100% de sus necesidades totales durante toda la estación de crecimiento, excepto en el periodo de postcosecha. Otros 2 grupos de árboles fueron sometidos a riegos deficitarios del 65% (DI<sub>65</sub>) y 50% (DI<sub>50</sub>), durante toda la campaña de riego, respecto a los árboles control. El tratamiento RDC aplicado a otro grupo fue diseñado para someter a los árboles a un periodo de estrés durante las fases I y II y recuperarlos durante la fase III.; el objetivo fue reducir la dosis de agua e incrementar el porcentaje de frutos abiertos. Un quinto grupo de árboles se mantuvo en condiciones de secano. Parámetros como el potencial hídrico y la conductancia estomática se vieron afectados en DI<sub>50</sub>, DI<sub>65</sub> y secano, principalmente durante las fases II y III, con potenciales hídricos al mediodía por debajo de -2 MPa. Los árboles RDC solo fueron significativamente afectados por el estrés en fase II, mostrando un potencial hídrico al mediodía de -1.4 MPa. Durante la mayoría de los días, la conductancia estomática no fue significativamente afectada en ninguno de los tratamientos de riego. Los frutos del DI<sub>65</sub> y DI<sub>50</sub> fueron significativamente más pequeños en diámetro y su cosecha total fue significativamente más pequeña que en el tratamiento control. Sin embargo no hubo diferencias significativas en el peso seco del grano. Los árboles RDC mostraron una producción y un porcentaje de frutos abiertos similar a los árboles control, aunque recibieron un 20% menos de agua. El porcentaje de frutos abiertos mostró una relación lineal con la



evapotranspiración del cultivo. Sin embargo, aunque el porcentaje de frutos abiertos fue similar en todos los tratamientos, esta variación parece estar relacionada con la cosecha total. Los árboles RDC no mostraron el patrón normal de alternancia (que ocurre claramente en los árboles control). El rajado de los frutos, un proceso que disminuye la calidad de la cosecha, no estuvo relacionado con el estado hídrico, pero sí con temperaturas durante el periodo de muestreo por debajo de 13°C. Los resultados sugieren que el patrón *Pistacia terebinthus* confiere a *Pistacia vera* algún grado de resistencia a la sequía, reduciendo la posibilidad de estrés hídrico, y por tanto, permitiendo un manejo del riego deficitario más severo.

**Palabras clave:** Evapotranspiración del cultivo, frutos abiertos, frutos rajados, conductancia foliar, funciones de producción, potencial hídrico de tronco.

### **Abstract**

An irrigation experiment involving the pistachio (*Pistacia vera* cv Kerman on *Pistacia terebinthus* L rootstock) was performed over a four-year period in central Spain to determine the effect of regulated deficit irrigation (RDI) on nut yield and quality. The growth season was divided into three phenological stages: stage I - from sprouting until the end of rapid nut growth; stage II - from maximum nut size until the beginning of kernel growth; and stage III - from the beginning of kernel growth until harvest. Control trees were irrigated to supply their full water needs throughout the growth season, except for the post-harvest period. Sustained deficit irrigation at 65% (DI<sub>65</sub>) and 50% (DI<sub>50</sub>) of control irrigation was provided to two other groups of trees. The RDI provided to a further group was designed to provide a stress period during stages I and II but no water stress during stage III; the aim was to reduce water use and increase the percentage of split nuts. A fifth group of trees was

maintained under rainfed conditions. Water potential and leaf conductance were affected in the DI<sub>65</sub>, DI<sub>50</sub> and rainfed treatments mainly during stages II and III, with midday water potentials below -2.0 MPa. The RDI trees were only significantly water stressed during stage II, showing midday water potentials of around -1.4 MPa. On most days, leaf conductance was not significantly affected in any of the irrigation treatments. The nuts of the DI<sub>65</sub> and DI<sub>50</sub> trees were smaller in diameter and their total yield was reduced compared to the controls. However, no significant differences in kernel dry weight were observed. The RDI trees showed a total yield and percentage of split nuts similar to those of the controls, even though they received around 20% less water. The split nut yield showed a linear relationship with crop evapotranspiration. However, since the percentage of split nuts was similar in all treatments this variation was likely related to the total yield. The RDI trees did not show the normal alternate bearing pattern (which was clearly maintained in the control trees). Early splitting, a process that decreases the yield quality, was not related to water status but to temperatures lower than 13°C. The results suggest that *Pistacia terebinthus* L rootstocks confer *P. vera* scions a degree of drought-resistance, reducing the likelihood of water stress and, therefore, allowing more severe RDI scheduling.

**Keywords:** Crop evapotranspiration, split nuts, early-split nuts, leaf conductance, production functions, stem water potential.

## **Introduction**

Pistachio nuts are an important product of Iran and the USA (the world's first and second producers respectively), but interest in this species as an alternative to traditional fruit crops is growing in other countries. In the European Union, the uncertainty about financial support for traditional Mediterranean crops such as olives and grapes has led to an increase in the area planted with pistachio trees. Over the last 15 years production in Turkey (the world's third producer) has been steady, but in Greece it increased from 5000 t in the early 90s to 9000 t at the beginning of the present century (FAOSTAT, 2006). In Spain the total area now planted with pistachio trees is around 6000 ha, yet at the beginning of the 90s it was almost negligible.

Pistachio is considered a drought and saline-resistant species (Behboudian et al., 1986; Rieger, 1995), and in the Mediterranean basin it is mainly grown in rainfed conditions. However, it is an exceptional candidate for growth under irrigation. As with other trees, irrigation increases the yield, but with pistachio it also improves the quality of the nuts (the percentage of split nuts is higher) and dampens the normal alternate bearing pattern (Kanber et al., 1993; Goldhamer et al., 1995). The irrigation efficiency of this crop may, therefore, be better than that of others (although this needs to be properly quantified). This is of great interest since increasing the amount of irrigated land is difficult; water is scarce and only the most efficient agricultural systems are likely to receive inputs of irrigation water (Fereres et al., 2003).

A basic tool for assessing irrigation efficiency is the crop-water production function (Vaux and Pruitt, 1983). Crop biomass and yield are linearly related to crop evapotranspiration ( $ET_c$ ). Sometimes, however, non-linear relationships between yield and  $ET_c$  are observed, e.g., in cotton (Orgaz et al., 1992) and olive (Moriana et al., 2003). This may be related to the increase in drought resistance shown by most fruit trees during one or more of their phenological stages; during these periods, a reduction in  $ET_c$  does not significantly reduce yield (Behboudian and Mills,

1997). If the response to variation in  $ET_c$  is non-linear, optimal  $ET_c$  levels need to be defined for different fruits and water prices so that deficit irrigation can be programmed.

The crop coefficient ( $K_c$ ) of pistachio is one of the highest of all deciduous fruit trees, exceeding 1.0 for the bulk of the growing season (Fereres and Goldhamer, 1990). Therefore, in most countries where this crop is planted, irrigation has to be in deficit. Regulated deficit irrigation (RDI) is a system of managing the soil water supply (mainly for fruit crops) by imposing periods of water deficit that cause no (or only small) reductions in economic benefits (Behboudian and Mills, 1997). At the end of the 1980s RDI schedules were designed for pistachio in California (Goldhamer et al., 1987; Phene et al., 1987). These experiments showed different responses of nut yield to water stress conditions depending on the phenological stage of the fruit during which they occurred, e.g., water stress had little impact during stage II (nut hardening) (Krane and Iwakiri, 1981; Goldhamer et al., 1987; Phene et al., 1987). A further three year-long experiment with a controlled reduction of irrigation during stage II, and no irrigation during post-harvest (thus providing a water saving of around 20%) confirmed these results (Goldhamer and Beede, 2004). In addition, it was hypothesized that mild water stress during stage I (a period of rapid fruit growth) would increase the percentage of split nuts and therefore the quality of the yield (Goldhamer and Beede, 2004). In more recent experiments, Goldhamer reported an increase in split nut yield but also in early-split nut yield in some years for some - but not all - trees undergoing RDI during stage I (Goldhamer et al., 2004, 2005, 2006). Early splitting is a rupture of the hull that leads to mould infections of the kernel and a reduced marketable yield. Early splitting occurs before harvest and reduces the quality and the value of the yield. Nut splitting is a process that occurs in the endocarp (pit); this also occurs before harvest, but increases the quality and value of the yield. Increases in early-split nuts have been associated with a delay in the harvest period (Doster and Michailides, 1997) but not with the influence of water stress at the beginning of the growth season. All the above investigations were

performed with trees grafted onto commercial Californian rootstocks of *Pistacia atlantica* Desf, UCB and Pioneer Gold I (*Pistacia integerrima* L.). Oddly, *Pistacia terebinthus* L., which is thought to be even more resistant to drought and frost (Ferguson et al., 2005), has been less commonly studied.

The aim of this work was to study, over three experimental growth seasons, the influence of RDI scheduling (imposing water stress conditions during rapid nut growth and nut hardening, i.e., stages I and II), sustained deficit irrigation and rainfed conditions on nut yield and quality. A further objective was to investigate the relationship between early splitting and environmental/water stress conditions. All the trees in the present experiment were *Pistacia vera* cv Kerman on *Pistacia terebinthus* L rootstocks - a rootstock that has been less frequently studied.

## **Materials and Methods**

### ***Experimental site and plant material***

This experiment was conducted between 2003 and 2006 in an eight year-old pistachio (*Pistacia vera* L. cv Kerman on *Pistacia terebinthus* L. rootstock) orchard at the *El Chaparrillo* Research Station, Ciudad Real, Spain (3° 56' W, 39° 0' N; altitude 640 m). The results of the first year of the experimental period (2003) were not taken into account because pistachio has a biannual fruit bearing pattern. The soil at the experimental site is a shallow clay-loam (Alfisol Xeralf Petrocalcic Palexeralfs) with a depth of 1.3 m and a discontinuous petrocalcic horizon between 0.75 and 0.85 m. The volumetric water content of the first 0.3 m is 22.8% at field capacity (soil matric potential -0.03 MPa), 12.1% at wilting point (soil matric potential -1.5 MPa), and for the layer from 0.3 to 1.3 m it is 43.0 and 21.1%, respectively. The trees were spaced 5 x 5 m apart (400 trees ha<sup>-1</sup>). Table 1 shows the annual reference evapotranspiration (ET<sub>0</sub>; the evapotranspiration rate from a hypothetical grass reference crop with

specific characteristics, without water shortage), the  $ET_0$  during the irrigation period, and the rainfall during the year and the irrigated period alone. The  $ET_0$  expresses the evaporating power of the atmosphere for a specific location and time of the year without taking into account crop characteristics or soil factors. The FAO Penman-Monteith method is recommended as the sole method for determining  $ET_0$  (Allen et al., 1998). Rain fell mainly outside of the irrigation period and was below the historic average (397 mm) during 2004 and 2005. The absolute minimum temperatures were  $-7^{\circ}\text{C}$  (2004),  $-8.1^{\circ}\text{C}$  (2005) and  $-9.2^{\circ}\text{C}$  (2006), occurring in March in 2004 and 2005, and in January in 2006. The absolute maximum temperatures were  $40.4^{\circ}\text{C}$  (2004),  $41.4^{\circ}\text{C}$  (2005),  $39.9^{\circ}\text{C}$  (2006), occurring in June, August and July, respectively.

**Table 1 - Environmental conditions, rainfall and reference evapotranspiration ( $ET_0$ ) for the three experimental growth seasons**

	Rainfall irrigated period (mm)	$ET_0$ irrigated period (mm)	Annual $ET_0$ (mm)	Annual Rainfall (mm)
2004	24.8	677.9	1172.9	296.6
2005	17.9	727.5	1326.7	225.0
2006	40.8	714.7	1293.7	417.0

The annual values and the values for the irrigated periods are provided. The irrigated period was from day of the year 155-273 in all years.

The treatments consisted of five different irrigation and rainfed strategies. The phenological stages taken into account in the RDI treatment were those suggested by Goldhamer and Beede (2004):

- stage I - from sprouting until the end of rapid nut growth
- stage II - from maximum nut size until the beginning of kernel growth
- stage III - from the beginning of kernel growth until harvest.

A complete randomised design was used, involving 20 trees per experimental plot with the following treatments:

- Control trees: these received water to cover estimated evapotranspiration ( $ET_c$ ) losses by fully replenishing all soil water extracted. In order to maintain no-water-stress conditions, irrigation was increased by 20% of the evapotranspiration rate if the midday stem water potential ( $\Psi_x$ ) fell below -1.0 MPa.
- $DI_{50}$ : throughout the growing season these trees received water to replace 50% of the  $ET_c$  calculated for the control treatment.
- $DI_{65}$ : throughout the growing season these trees received water to replace 65% of the evapotranspiration calculated for the control treatment.
- Regulated deficit irrigation (RDI): these trees received 50% of the water received by the control trees during stages I (rapid nut growth) and II (nut hardening), and the same amount of water as the control trees during stage III (kernel growth). In each of the three growth seasons of the experiment the water deficit recovery period was started slightly before stage III in order to reduce any delay in the recovery of  $\Psi_x$  (Goldhamer and Beede, 2004; Guerrero et al., 2006b).
- Rainfed conditions.

The orchard was maintained under rainfed conditions until the end of 2002. No irrigation was provided during the post-harvest period in any of the treatments. The irrigation water used was saline and had high concentrations of nitrate ( $EC = 3.4 \text{ dS m}^{-1}$ ;  $SAR = 1.4 \text{ mmol}^{1/2} \text{ l}^{-1/2}$ ;  $\text{NO}_3^- = 66 \text{ mg l}^{-1}$ ;  $\text{pH} = 7.2$ ;  $\text{HCO}_3^- = 362 \text{ mg l}^{-1}$ ). Drip irrigation (five emitters per tree providing  $4 \text{ l h}^{-1}$ ) was provided four days per week.

### ***Irrigation regimes***

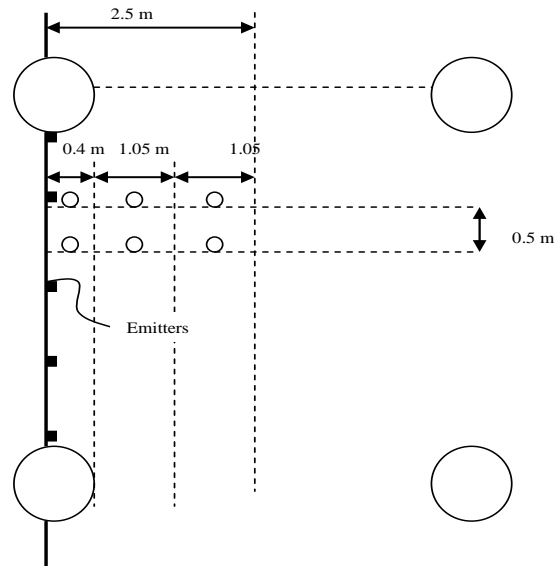
The amount of water provided was calculated on the basis of the crop evapotranspiration ( $ET_c$ ) and the crop coefficient ( $K_c$ ) [according to the FAO method (Doorenbos and Pruitt, 1974)]. The mean  $K_c$  values provided by Goldhamer (1995) for pistachio trees growing under Californian conditions were used – 0.39, 1.06 and 1.14 for stages I, II and

III, respectively – after correction for canopy size (Ferreeres and Goldhamer, 1990). The reference evapotranspiration,  $ET_o$ , was estimated using the Penman-Monteith equation and employing daily data from a nearby automatic weather station (1.5 km from the experimental orchard).

### ***Soil water and evapotranspiration measurements***

The soil water content was measured using a portable capacitance probe (Divinier, 2000, Sentek Pty. Ltd., Australia) employing the default calibration supplied by the manufacturer. The data, therefore, reflect the relative changes in soil water content. It was assumed that, in a 5x5 m square around each tree, the same soil conditions reigned. In addition, it was assumed that the distribution of the water from the emitters was the same. To measure the soil moisture each 5x5 m square was divided into small rectangles of 2.5 m. x 0.5 m (Fig. 1). These were further divided into three moisture zones depending on the distance from the emitters. A 1.5 m-long access tube was then placed at the centre of each moisture zone of two of the rectangles (one close to the emitters, one between the emitters) (Fig. 1). A weighted average of the water content based on the area of each moisture zone (Fig. 1) was then calculated. Weekly measurements were taken every 10 cm between soil depths of 0.1 m and 1 m.





**Fig. 1- Distribution of the capacitance probes in the plot. Large circles represent the trees, the small ones the probes, and the shaded squares the emitters.**

To calculate the crop evapotranspiration ( $ET_c$ ), the growing season [day of the year (DOY) 116 to 293] was divided into the rainy and dry period. During the dry period (DOY 155-273) rain was scarce (Table 2) and deep percolation was assumed to be negligible. The dry period was considered to cover the same period in each year of the experiment. The  $ET_c$  during the dry period was calculated as:

$$ET_c = (SM_1 - SM_2) + I + R \quad (1)$$

where:

- $SM_1$  and  $SM_2$  represent the soil moisture on the first and the last days of the period considered in 1m depth (mm).
- $I$  is the irrigation provided (mm).
- $R$  is the rainfall (mm).

During the rainy season  $ET_c$  could not be determined using equation (1) since deep percolation could not be estimated. The equation to use under such circumstances would be:

$$ET_c = ET_o \times K_c \quad (2)$$

where:

$K_c$  is the crop coefficient (dimensionless).

$ET_o$  is the reference evapotranspiration (mm).

$ET_c$  is the crop evapotranspiration (mm).

However, since pistachio is a deciduous tree the values of  $K_c$  in Eq (2) would be very low during this part of the growth season, as would those of  $ET_o$  and  $ET_c$ . In addition, the values in  $K_c$  should be the same for all the treatments though they may be different for water stress. Therefore, the different irrigation strategies were only compared during the dry season when water needs are significant.

The amounts of water provided in the  $DI_{50}$  and  $DI_{65}$  treatments were actually slightly lower than that suggested since the controls were allowed up to 120%  $ET_c$  if the water potential fell to below  $-1.0$  MPa (Table 2). Each year the maximum  $ET_c$  values corresponded to the control trees and the minimum to the rainfed trees. The  $ET_c$  of the control trees increased over the three years of the experiment due to canopy growth. This increase was also seen in all the other irrigation-treatment trees, but not in the rainfed trees: the  $ET_c$  of the rainfed trees in the last two seasons was half that of the first.

**Table 2 – Water applied and estimated crop evapotranspiration (ET<sub>c</sub>) for the different treatments and years**

		Water applied (mm)	ET <sub>c</sub> (mm)
2004	Control	182.0	210.4
	RDI	141.8	146.3
	DI <sub>65</sub>	111.5	185.3
	DI <sub>50</sub>	90.1	171.3
	Rain fed	0	112.5
2005	Control	277.4	305.6
	RDI	232.5	267.9
	DI <sub>65</sub>	156.9	203.4
	DI <sub>50</sub>	120.3	149.6
	Rain fed	0	49.6
2006	Control	365.4	404.7
	RDI	290.7	295.2
	DI <sub>65</sub>	194.4	248.6
	DI <sub>50</sub>	153.6	213.6
	Rain fed	0	54.8

### ***Plant water relations***

Midday stem water potential ( $\Psi_x$ ) was determined to evaluate the water status of the trees. Fully expanded leaves on branches near the main trunk were covered with aluminium foil for at least 1 h before their removal between 12:00 and 14:00 h. Measurements were made for one leaf per tree from six trees per treatment using a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA). Because pistachio leaves exude turpentine, a piece of blotting paper was used to determine the end point; turpentine cannot moisten blotting paper but the contents of the xylem can.

Abaxial leaf conductance ( $g_s$ ) was measured with a steady-state porometer (Model LICOR-1600, U.K.) between 12:00-14:00 pm using the central foliole of the compound leaf. This measurement was made in sunlight and with fully expanded leaves from the same trees in which  $\Psi_x$  was measured. The number of samples used was 18 (three leaves x 6

trees). Measurements were made in the years 2004 and 2006, but not during 2005 due to technical problems with the porometer.

### ***Nut development***

Nut development was monitored via the measurement of kernel dry weight, nut diameter and the percentage of early splitting in all three years of the experiment. However, since the results for the three growth seasons were similar only the data for 2006 are presented. Sixty inflorescences per treatment were randomly selected at the beginning of each growth season. The diameters of their eventual nuts were recorded periodically from the beginning of nut formation, and the percentage of early-split nuts was recorded periodically from the beginning of August until harvest. Finally, from the beginning of July, a further 60 nuts per treatment were sampled every two weeks and the kernels dried at 70°C until a constant weight was achieved.

### ***Nut yield***

The yield of each treatment was evaluated in terms of total nut, split nut, and total blank nut (including aborted and blank nut) yields [kg per hectare (always peeled and dried)].

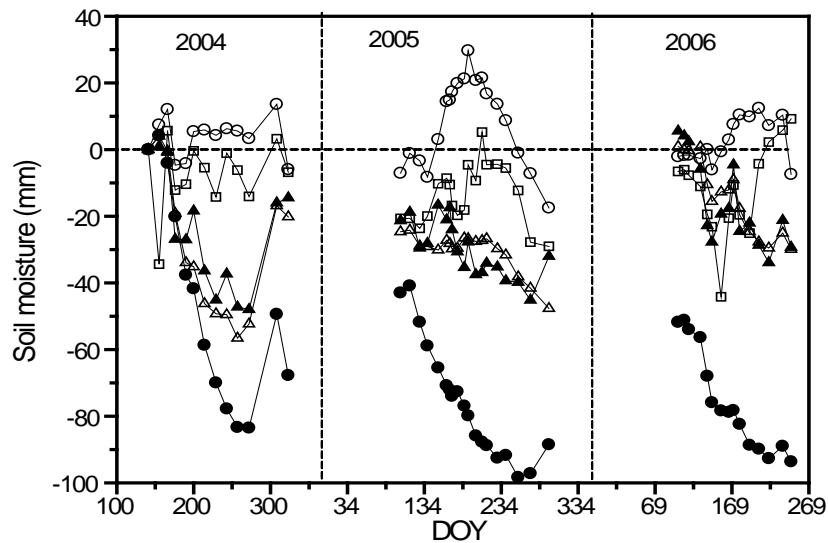
### ***Regressions and statistical analyses***

The main treatment effects were examined by ANOVA. Differences between means were analysed using the Tukey test, with a significance  $P < 0.05$ . Polynomial and linear regressions were performed to investigate the relationship between yield and  $ET_c$ .

## Results

### *Plant water relations*

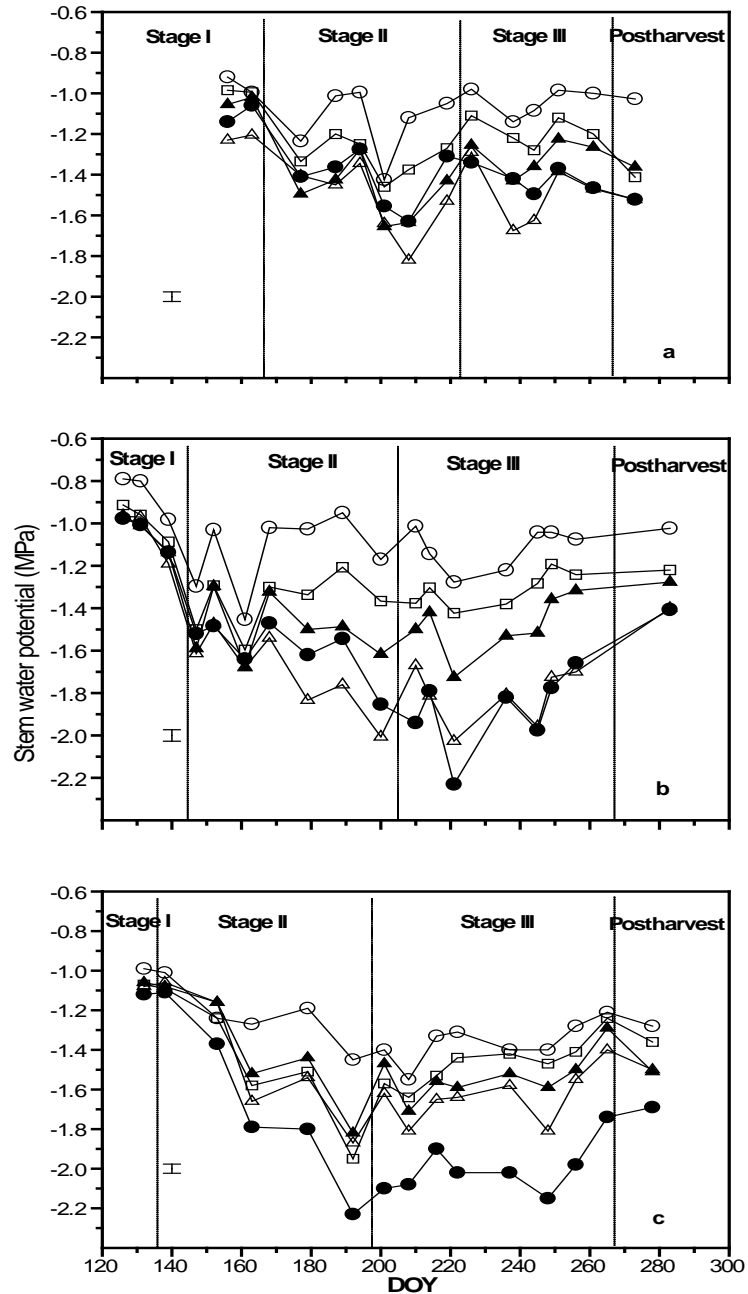
Fig. 2 shows the patterns of soil moisture for the three seasons of the experiment. Since the default calibration of the capacitance probe was used, all data are relative to the starting measurements recorded at the beginning of the first season (year 2004), which were tared. For all three seasons the soil moisture was similar in the control treatment, with a slight reduction at the end of each season and a recovery with the beginning of the autumn–winter rains. The increase in soil moisture during the second season (year 2005) indicated that the soil was probably not at field capacity in most seasons (see Fig. 2). The soil moisture content in the remaining treatments was clearly lower than in the control treatment. In the RDI treatment, soil moisture showed a seasonal pattern, with a reduction at the beginning of the season and a recovery at the end. Nevertheless, this increase did not always allow the soil moisture content to recover to that seen in the control treatment or even that recorded for the same RDI treatment at the beginning of the experiment. The soil moisture patterns in the  $DI_{65}$ ,  $DI_{50}$  and rainfed treatments followed the same trend but differed from that seen in the RDI treatment. A continuous reduction in soil moisture was seen in all three study seasons, and full recovery was not always achieved with the autumn-winter rains. The slope of the reduction in soil moisture was steepest in the rainfed treatment. The difference in the soil moisture content between the beginning of the experiment and the end in the rainfed treatment was around 80 mm, while in the  $DI_{65}$  and  $DI_{50}$  treatments it was around 30 mm, and in the control and RDI treatments it was 0 mm.



**Fig. 2. Variation in soil water content from the beginning of the experiment. Dashed lines separate the different seasons (DOY: day of the year). ○ = Control; □ = RDI; ● = Rainfed; ▲ = DI<sub>65</sub> △ = DI<sub>50</sub>**

The patterns of midday stem water potential ( $\Psi_x$ ) in each treatment were similar in all three seasons (Fig. 3). The value of  $\Psi_x$  at the beginning of the season in the control trees was around -1 MPa, with a slight reduction during stage II of nut development, although recoveries were seen in 2004 and 2005 (Fig. 3a, b). In 2006 the reduction in  $\Psi_x$  was stronger and remained at -1.3 MPa over stages II and III (Fig. 3c). Compared to the other treatments, significant differences were seen during stage II and early stage III (Fig. 3a, c). Only during 2005 there were significant differences in  $\Psi_x$  between the controls and the different treatments during stage I (Fig. 3b). Minimum values of  $\Psi_x$  below -2.0 MPa were recorded during stage III in 2005 and 2006 in the rainfed trees (Fig. 3b, c), and of -1.8 MPa in 2004 in the DI<sub>50</sub> trees (Fig. 3a). At the end of stage III in all seasons, the rainfed and DI<sub>50</sub> trees showed a recovery of their  $\Psi_x$  values, even though no rain fell. This recovery was, however, incomplete, with values never reaching those obtained in the control treatment. The  $\Psi_x$  values for the RDI trees were significantly lower than

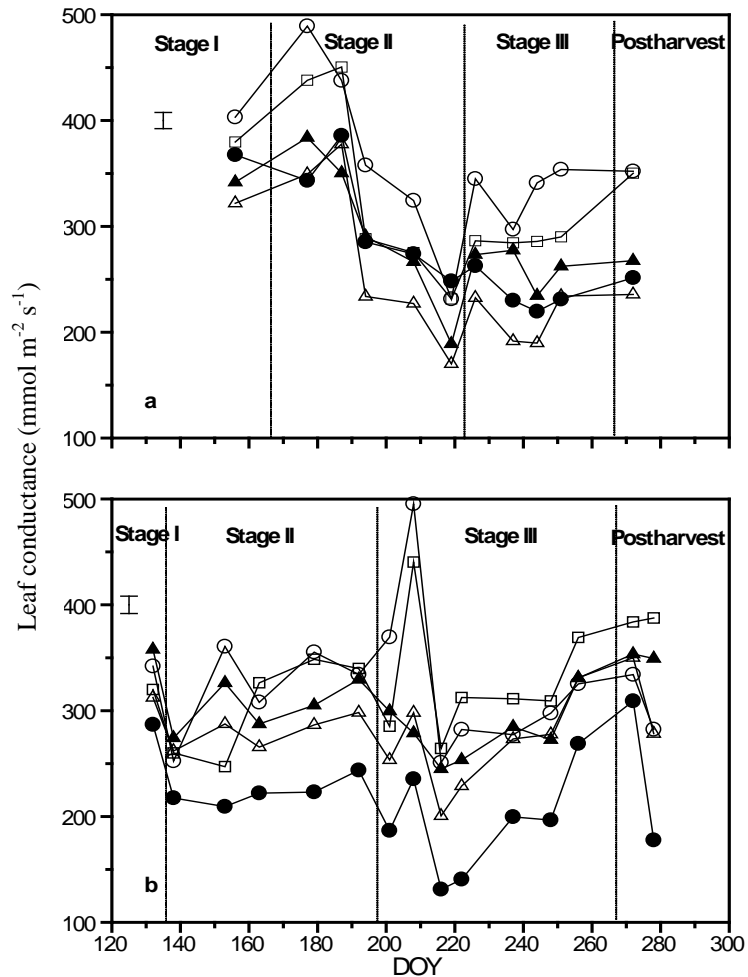
those for the control trees during stage II, but recovery during stage III was fast and no significant differences were seen between these trees and the controls in this phenological stage in any year.



**Fig. 3-** Seasonal pattern of midday stem water potential during the three years of the experiment (a) 2004; (b) 2005; (c) 2006. The periods corresponding to each phenological stage are shown. Each point represents the average of six measurements. The mean standard error is shown in each figure. Statistical differences are reported in the text ( $P < 0.05$ . Tukey test) ○ = Control; □ = RDI; ● = Rainfed; ▲ = DI<sub>65</sub> △ = DI<sub>50</sub>



Midday leaf conductance was significantly affected by the irrigation treatment (Fig. 4). During 2004, its seasonal pattern showed maximum values in all treatments at the beginning of the year, a slight reduction during stage II, and steady values during stage III (Fig. 4a). In the same year, the values of the  $DI_{65}$ ,  $DI_{50}$  and rainfed trees were always significantly lower than those of the control trees, but no differences were seen between the RDI and control trees (Fig. 4a). The seasonal pattern in 2006 was different to that of 2004. Values were steady in all treatments throughout the season but lower than those recorded in 2004. During 2006, the values for the rainfed trees were significantly lower than those of the control trees for the entire season (Fig. 4b). The  $DI_{65}$  and  $DI_{50}$  trees had lower values than the control trees but these differences were often not significant. The values for the RDI trees were similar to those of the control trees throughout 2006.



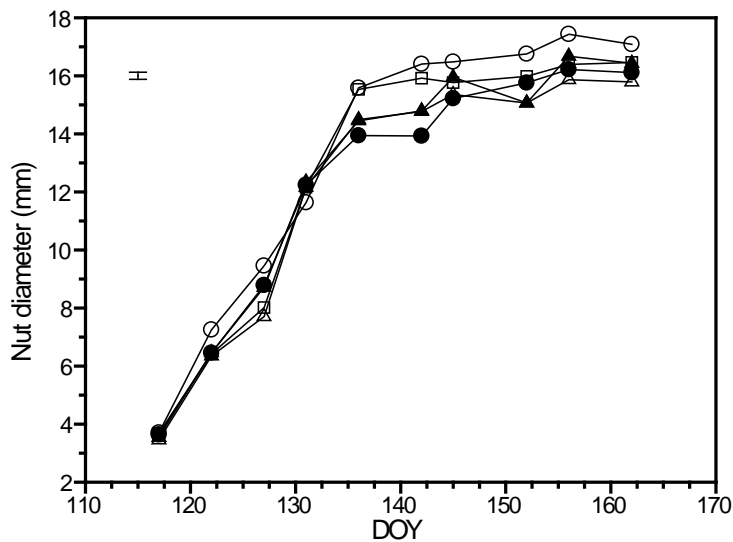
**Fig. 4- Seasonal pattern of midday leaf conductance during the 2004 (a) and 2006 (b) seasons. The periods corresponding to each phenological stage are shown. Each point represents the average of 12 measurements. The mean standard error is shown in each figure. Statistical differences are reported in the text ( $P < 0.05$ , Tukey test)**

○ = Control; □ = RDI; ● = Rainfed; ▲ = DI<sub>65</sub> △ = DI<sub>50</sub>

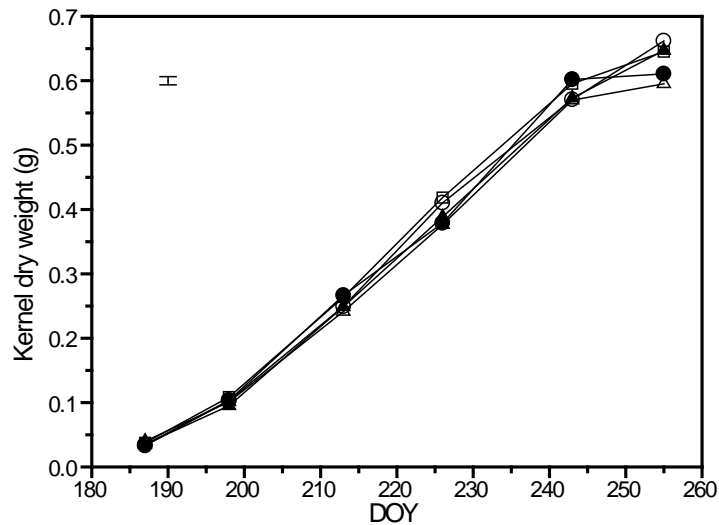
### ***Nut development***

The nut diameter results were similar for all three years of the experiment; therefore, only the data for 2006 are presented (Fig. 5). The period of rapid nut growth (stage I) varied from 22 days, from full bloom,

in 2006 to 49 days in 2004. The maximum diameter was around 18 mm and the difference between the control and rainfed nuts was significant at the end of this stage (although differences greater than 2 mm were never seen). The control trees had the largest diameter nuts and the rainfed the smallest; the remaining treatments all produced nuts of a similar, intermediate size. The increase in kernel dry weight was also similar for all three years; thus again, only the data for 2006 are shown (Fig. 6). The accumulation of dry matter was linear from the end of July or the beginning of August until harvest; no significant differences were observed between treatments.



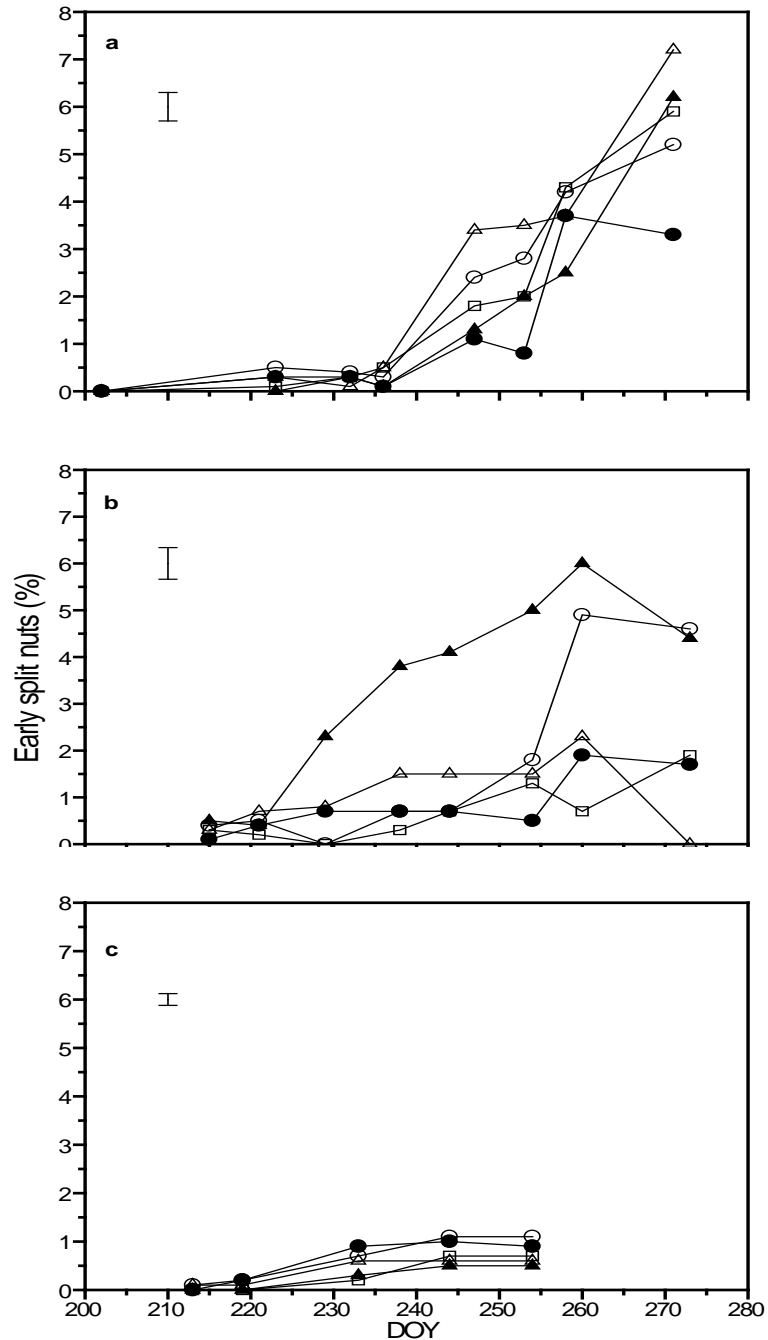
**Fig. 5- Nut diameter growth during stage I in the 2006 season. Each point represents the average of 60 measurements. The mean standard error is shown in each figure. Statistical differences are reported in the text ( $P < 0.05$ , Tukey test) ○ = Control; □ = RDI; ● = Rainfed; ▲ = DI<sub>65</sub> △ = DI<sub>50</sub>**



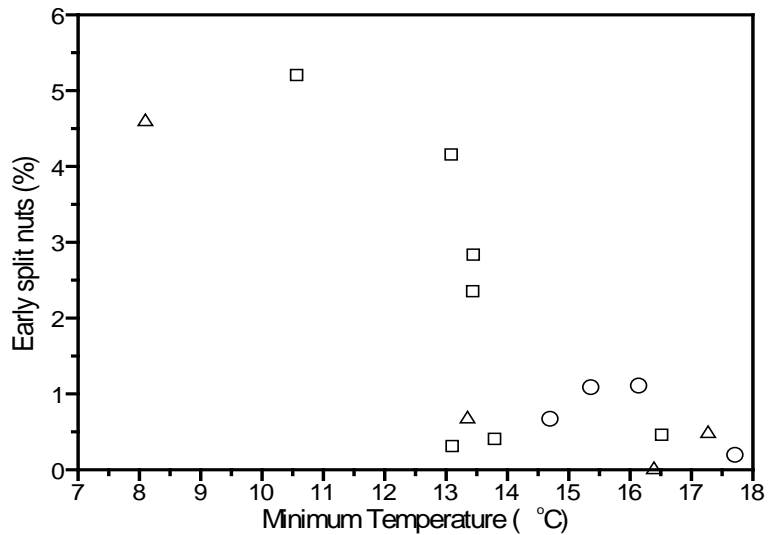
**Fig. 6- Kernel dry weight pattern during stage III i in the 2006 season. Each point represents the average of 60 measurements. The mean standard error is shown in each figure. No significant differences exist (Tukey test) ○ = Control; □ = RDI; ● = Rainfed; ▲ = DI<sub>65</sub> △ = DI<sub>50</sub>**

The pattern of early splitting was different between years but the effect of irrigation was not always clear (Fig. 7). In all three growth seasons an increase in the percentage of early spitting occurred at the end of August, but to very different extents. In 2004, the percentage of early-split nuts in all treatments increased from mid August until the end of September, although this problem was significantly less serious in the rainfed trees (Fig. 7a). Similar results were obtained in 2005, although the increase in early-split nuts peaked in mid-September (Fig. 7b). In 2005, the DI<sub>65</sub> and control treatments produced the highest percentages of early-split nuts (significantly greater than in the other treatments). In both years (2004 and 2005) the maximum yield of early-split nuts reached around 6% of the total (in the RDI, DI<sub>65</sub> and DI<sub>50</sub> treatments in 2004, and in the control and DI<sub>65</sub> treatments 2005). In 2006 the increase in early-split nuts in all treatments took place mainly in mid August, with no increase during September (Fig. 7c). In addition, no significant differences were seen among the irrigation treatments and controls. The

maximum percentage of early-split nuts in the 2006 season was around 1% - six times lower than the maximum of the previous years. When the percentages early split nuts of the three seasons for the control trees were compared to the average minimum temperature in the period between sampling, a sharp increase was seen when this fell below 13°C (Fig. 8). No relationship was seen with air humidity.



**Fig. 7- Percentage of early-split nuts for 2004, 2005 and 2006. Each symbol represents the average of 60 measurements. The mean standard error is shown in each figure. Statistical differences are reported in the text ( $P < 0.05$ . Tukey test)  $\circ$  = Control;  $\square$  = RDI;  $\bullet$  = Rainfed;  $\blacktriangle$  = DI<sub>65</sub>  $\triangle$  = DI<sub>50</sub>**



**Fig. 8- Increase in the percentage of early-split nuts in the control treatment with respect to the average minimum temperature recorded between nut sampling points. Each point is the average for 60 inflorescences. (○) 2006; (△) 2005 (□) 2004**

### ***Nut yield***

All trees showed the typical alternate bearing pattern except the RDI trees (Table 3). In the “off” year (2005) a reduction was seen in the yield of more than 50% with respect to the “on” years in the rainfed, control and DI<sub>65</sub> trees, while in the DI<sub>50</sub> treatment this reduction was around 30%. No significant differences were seen between the treatments during the “on” years though the total split nut yield, total yield and nut load of the DI<sub>50</sub> trees were around 40% of the control. This may be related to the high coefficient of variation of the results (> 40%). In the “off” season (2005), the total yield, blank yield and nut load of the RDI trees were significantly greater than in the control and rainfed treatments. The water productivity (the ratio between yield and ET<sub>c</sub>) was significantly greater in the rainfed trees (especially in 2006), while no clear differences

were found between the irrigated treatments in any year (although the DI<sub>50</sub> trees showed a trend towards having the lowest).

**Table 3 – Yield and yield components for the three growth seasons**

	Total yield (Kg ha <sup>-1</sup> )	Split nut (Kg ha <sup>-1</sup> )	Blank nut (Kg ha <sup>-1</sup> )	Dry nut weight (g)	Nut load	WPTY (Kg ha <sup>-1</sup> mm <sup>-1</sup> )	WPSN (Kg ha <sup>-1</sup> mm <sup>-1</sup> )
2004							
Control	1766 ± 189.9	1242 ± 141.4	119 ± 25.2	0.7a	6112 ± 651	8.4ab ± 0.9	5.9ab ± 0.7
65%	1707 ± 268.3	1108 ± 190.3	119 ± 28.9	0.7ab	6296 ± 914	9.2ab ± 1.4	6.0ab ± 1.0
50%	887 ± 274.4	618 ± 159.7	58 ± 15.6	0.7bc	3341 ± 982	5.2b ± 1.6	3.6b ± 0.9
Rain fed	1380 ± 172.3	1043 ± 109.7	106 ± 25.3	0.6c	5751 ± 677	12.3a ± 1.5	9.3a ± 1.0
RDI	1435 ± 258.8	1161 ± 234.7	63 ± 16.8	0.7ab	5083 ± 824	9.8ab ± 1.8	7.9ab ± 1.6
2005							
Control	498 b ± 112.1	366 ± 107.4	26 b ± 9.4	0.8	1483b ± 326	8.0ab ± 0.8	5.2ab ± 0.8
65%	754 ab ± 230.0	295 ± 122.4	99 ab ± 29.2	0.9	2139ab ± 607	13.5a ± 1.4	8.2a ± 1.1
50%	677 ab ± 235.9	319 ± 64.1	85 ab ± 40.3	0.8	2148ab ± 777	4.5b ± 1.6	2.1b ± 0.4
Rain fed	538 b ± 189.0	293 ± 106.8	36 b ± 17.8	0.8	1614ab ± 543	10.8ab ± 3.8	5.9ab ± 2.2
RDI	1442 a ± 265.5	802 ± 240.3	170 a ± 37.0	0.9	4191a ± 766	5.4ab ± 1.0	3.0b ± 0.9
2006							
Control	2447 ± 253.1	1589 ± 230.1	78 ± 7.5	0.8	8082 ± 760	6.0b ± 0.6	3.9b ± 0.6
65%	2756 ± 282.9	1675 ± 218.5	103 ± 19.6	0.7	9167 ± 857	11.1b ± 1.1	6.7b ± 0.9
50%	1683 ± 370.2	1053 ± 178.2	84 ± 14.8	0.7	5798 ± 1249	7.9b ± 1.7	4.9b ± 0.8
Rain fed	2004 ± 345.8	1370 ± 293.8	72 ± 13.1	0.7	6803 ± 1175	36.6a ± 6.3	25.0a ± 5.4
RDI	1914 ± 393.3	1237 ± 238.6	124 ± 27.3	0.7	6273 ± 1146	6.5b ± 1.3	4.2b ± 0.8

Different letters, within column and in the same season, indicate significant differences ( $P < 0.05$ ; Tukey test). The results are presented as means (left) ± standard errors (right). The water productivity of the total yield (WPTY) and the water productivity of the split nut yield (WPSN) are the ratios between the total yield and split nut yield, respectively, and crop evapotranspiration ( $ET_c$ ).

The absence of significant yield losses in all the irrigation and rainfed treatments compared to the controls may be explained by the different crown volumes of the trees in the different treatments. Over the three years of the experiment these differences became smaller, even though the crown volumes of the DI<sub>65</sub>, RDI and rainfed trees remained somewhat larger. The difference between the mean crown volume of the rainfed and control trees for 2004/2005 was 2.5 m<sup>3</sup> per tree, while for 2005/2006 it was 1.9 m<sup>3</sup>.

Another factor that might have explained the absence of yield losses described above is the alternate bearing pattern. To better analyse the relationship between yield and  $ET_c$ , two different transformations of the yield data were performed. First, the mean yields obtained in each

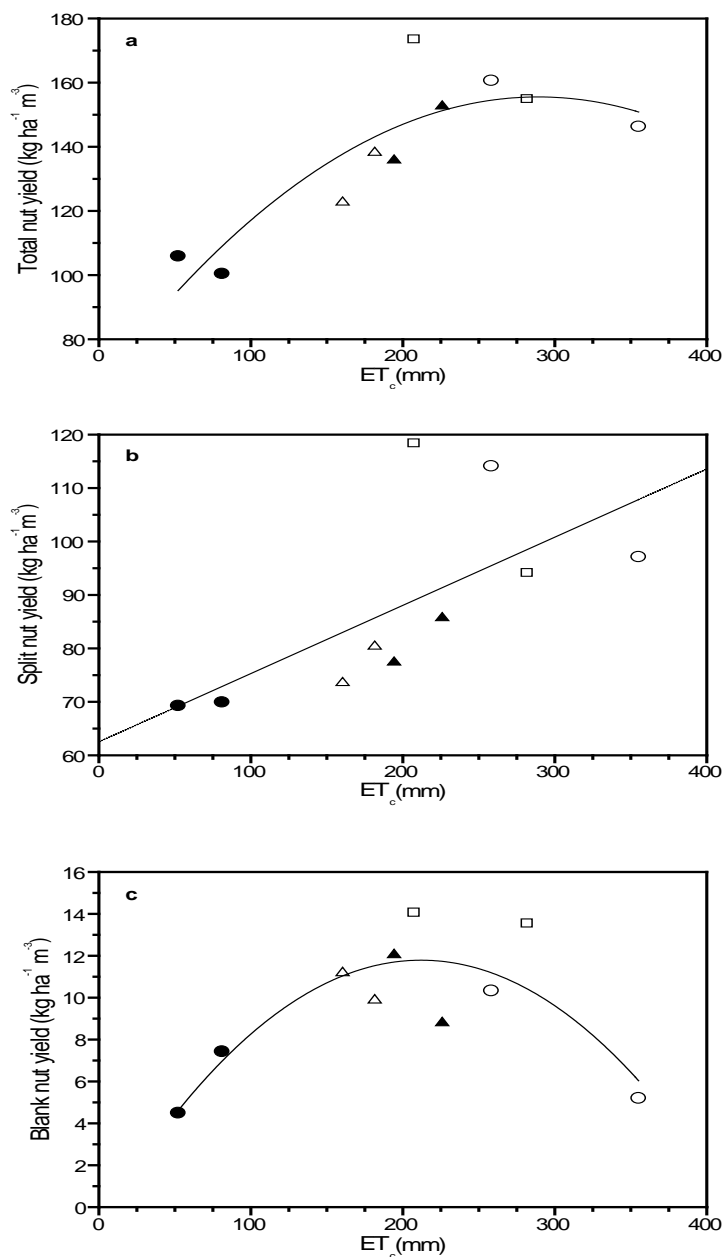


treatment for 2004/2005 and 2005/2006 were calculated and used instead of annual yields (thus reducing the influence of the alternate bearing pattern) to determine the relationship with  $ET_c$ . Secondly the yield was normalised with respect to the crown volume in each treatment.

Biannual total nut yield increased from 100 to 160 kg ha<sup>-1</sup> m<sup>-3</sup> as  $ET_c$  increased between 100 mm and 250 mm (Fig. 9a). When  $ET_c$  values were higher than 250 mm the total yield obtained remained almost constant at around 160 kg ha<sup>-1</sup> m<sup>-3</sup>. In all seasons the  $ET_c$  values obtained in the RDI treatment were around this 250 mm maximum, while those of the control treatment were slightly higher; the yield of these trees, however, was the same.

The relationship between the split nut yield and  $ET_c$  showed a linear pattern (Fig. 9b). The slope was smaller than that between  $ET_c$  and the total nut yield (Fig. 9a). The split nut yield varied from around 70 kg ha<sup>-1</sup> m<sup>-3</sup> in the rainfed trees to 100 kg ha<sup>-1</sup> m<sup>-3</sup> in the control trees. However, the percentage of split nuts in the total yield varied (mainly between 60% and 70%) with no significant differences between treatments within years.

The blank nut yield was very low and its relationship with  $ET_c$  unclear (Fig. 9c). Values varied from 4 kg ha<sup>-1</sup> m<sup>-3</sup> to 14 kg ha<sup>-1</sup> m<sup>-3</sup>, although most clustered around 10 - 14 kg ha<sup>-1</sup> m<sup>-3</sup>. The percentage of blank nuts varied from 4 to 11% of the total nut yield with no significant differences between treatments.



**Fig. 9- The influence of  $ET_c$  on yield differs according to the yield component considered. The yield data provided are biannual and relative to average crown volume - a) total nut yield ( $\text{kg ha}^{-1} \text{m}^{-3}$ ), b) split nut yield ( $\text{kg ha}^{-1} \text{m}^{-3}$ ) and c) blank nut yield ( $\text{kg ha}^{-1} \text{m}^{-3}$ ) - and are plotted against crop evapotranspiration ( $ET_c$ , mm).  $\circ$  = Control;  $\square$  = RDI;  $\bullet$  = Rainfed;  $\blacktriangle$  =  $DI_{65}$   $\triangle$  =  $DI_{50}$ . The best fit relationships are: a) total nut yield;  $Y = 65.6 + 0.62ET_c - 1.1 \cdot 10^{-3}ET_c^2$ ;  $R^2 = 0.75^{**}$ ; Resid. Mean Square = 177;  $n = 10$ , b) split nut yield;  $Y = 62.5 + 0.13ET_c$ ;  $R^2 = 0.42^*$ ; Resid. Mean Square = 201;  $n = 10$ , c) blank nut yield;  $Y = -0.85 + 0.12 ET_c - 3 \cdot 10^{-4}ET_c^2$ ;  $R^2 = 0.70^*$ ; Resid. Mean Square = 4;  $n = 10$**

## Discussion

The total yield of the experimental trees was positively related to  $ET_c$  until values of around 250 mm (Fig. 9). Research into yield responses to decrease in  $ET_c$  have shown that most water production functions for row crops are linear (Vaux and Pruitt, 1983). In contrast, similar investigations with fruit trees strongly suggest that there are developmental periods during which water stress is not detrimental to yield - thus RDI can be used (Behboudian and Mills, 1997). Moriana et al. (2003), working with mature olive trees, reported a relationship between fruit yield and  $ET_c$  with an almost constant yield after 750 mm. In the present work, a mean crown volume of 10 m<sup>3</sup> per tree is related to a rate of variation in the water-productivity function (the slope of Fig. 9a) in the range of 4.0 - 0.7 kg ha<sup>-1</sup> mm<sup>-1</sup> - one sixth of that reported for mature olive trees (Moriana et al., 2003). Olive trees are considered more drought-resistant than pistachio (Rieger, 1995); the latter therefore shows a lower water productivity. However the increase in the total and split nut pistachio yields seen in the present work (Fig. 9) may compensate for this.

The split nut yield was also positively related to  $ET_c$  (Fig. 9b). The best fit was linear, rather than polynomial as for the total nut yield, but the wide scatter of the data limits the conclusions that can be drawn. This linear relationship suggests high water efficiency in terms of split nut yield; this agrees with the results of Phene et al. (1987) who reported pistachio trees to show high sensitivity to water stress. However, this response has been reported to be strongly related to the period when water stress occurs. In the present work the increase in split nuts was mainly related to the increase in total yield since the percentage of splitting was similar in all treatments; certainly, no clear effect of water stress during stages I and III was seen. The Kerman cultivar is very sensitive to water stress in stage III, to which its response is a reduction in the percentage of split nuts (Goldhamer and Beede, 2004). Mild water stress during stage I, however, increases the percentage of split nuts (Doster and Michailides, 1995, Goldhamer and Beede, 2004; Goldhamer et

al., 2004; 2005). The lack of agreement between the results of the former researchers and those of the present work suggests that the present rootstocks increase the drought-resistance of the scion compared with *Pistacia atlantica* or UCB rootstocks used in the former authors' work. *Pistacia terebinthus* has been reported as the most drought-resistant pistachio rootstocks (Ferguson et al., 2005; Guerrero et al., 2006a). Therefore, to increase the yield of split nuts when using *P. terebinthus* rootstocks, greater water stress might be advisable during stage I. This agrees with the findings of Kanber et al. (1993), who worked with cultivars and rootstocks different to those used in the present and in Goldhamer's work; these authors also reported no significant differences in the percentage of split nuts between rainfed and irrigated trees.

Regulated deficit irrigation reduced the amount of water applied by some 20% (Table 2) without difference in nut yield (Table 3 and Fig. 9), although the water status of the trees was affected during stage II (Figs 3 and 4). In addition, this treatment provided an almost steady total yield and no alternate bearing pattern was seen, unlike in the remaining treatments (Table 3). Though the mechanism of alternate bearing has not been completely elucidated, it may be the result of carbohydrate competition between the nuts of one season and the forming buds that will produce nuts in the following season (Stevenson et al., 2000; Stevenson and Shackel, 1998; Ferguson et al., 2005). The greater the nut yield, the greater the carbohydrate competition and, therefore the greater the bud fall for the same water status conditions. However, this pattern can be dampened if a balance between the carbohydrate source (photosynthesis) and sink (number of buds) is achieved. The present results suggest that moderate water stress during stages I and II may reduce the number of flower buds produced in RDI treatment, but the recovery during stage III of this treatment (Fig. 3) reduces bud fall due to improved assimilation.

These advantages of RDI in pistachio production could be limited, however, by the increase of an early splitting (Goldhamer et al., 2004). In the present work, early splitting was not clearly related to irrigation treatment, although the rainfed trees commonly showed the least early

splitting (Fig. 7). For rainy seasons, Goldhamer et al. (2006) reported no significant differences between treatments in terms of early splitting. The results of these authors, plus the absence of an early splitting response to irrigation in our work, suggest that factors in addition to water status must influence this process. For Pistachio trees, harvesting begins when the hull can easily be removed. Doster and Michailides (1997, 1998) reported an increase in early-split nuts in cv Kerman at a delayed harvest in September in two commercial orchards in California. This was probably related to environmental conditions more than water status. The present data strongly suggest that such environmental influence(s) may exist since an increase in the percentage of early-split nuts was always seen by the beginning of September (Fig. 7), even though the phenological stages reached in the different seasons were different (Fig. 3). According to the present data, temperature may be involved in this (Fig. 8). Minimum temperatures below 13°C are commonly recorded from the beginning of September in the area of the experimental orchard, the very time when the percentage of early-split nuts increased sharply (Fig. 7).

## Conclusions

The present study shows that irrigation increases the total nut and split nut yields of pistachio. However, the increase in split nuts is mainly related to the increase in the total yield more than to the splitting process, since the percentage of split nuts in all irrigation treatments was similar. These results suggest that *P. terebinthus* L rootstocks may increase the drought resistance of cv Kerman scions, and therefore reduce the effect of water stress during stage I.

The increase in total yield with the increase in  $ET_c$  was not linear and the RDI treatment allowed for a 20% water saving with no variations in yield and a very appreciable dampening of the alternate bearing pattern. The RDI strategy followed was designed to reduce the water supply during stages I and II by 50% of  $ET_c$  and to increase nut splitting. According to the conclusions above, when the rootstock *P. terebinthus* L. is

used the water stress imposed (at least during stage I) should be more severe in order to increase the split nut yield. The reduction in the alternate bearing in the RDI treatment was probably a product of a balance between the flower buds induced during stages I and II (period of water stress) and the assimilation capacity during bud fall in stage III (period of water recovery).

Early-split nut production showed a similar pattern in all the seasons with a sharp increase at the beginning of September that was not clearly related to water stress. When minimum temperatures were below 13°C, the percentage of early splitting sharply increased. Thus, under the present conditions, temperature was the main factor affecting its onset.

### **Acknowledgments**

We thank Professor Elias Fereres for his assistance in the design of the irrigation treatments, and Ana Rivero for the skilful technical assistance. This work was supported by INIA project RTA-03-008 (Spanish Education and Science Ministry) (2003 to 2005 seasons), and by project PAI-06-100 of the Castilla-La Mancha Regional Government (2006 season).



## CAPÍTULO II







## CAPÍTULO II

**Influencia del portainjerto en la respuesta del pistachero (*Pistacia vera* L. cv Kerman) al estrés hídrico y posterior recuperación.**

**Publicado como:**

**Gijón M.C., Giménez C., Pérez-López D., Guerrero J., Couceiro J.F., Moriana A. (2010). Rootstock influences the response of pistachio (*Pistacia vera* L. cv. Kerman) to water stress and rehydration. *Scientia Horticulturae* 125: 666-671.**



## Resumen

El cultivo del pistachero requiere el uso de portainjertos, ya que el injerto es la única forma de propagación vegetativa para este cultivo. Los patrones comerciales principales son *Pistacia integerrima* L, *Pistacia atlantica* Desf, *Pistacia terebinthus* L. y *Pistacia vera* L. El pistachero es considerado como un cultivo resistente a la sequía y salinidad; sin embargo, existe poca información que describa la respuesta varietal sobre los distintos portainjertos al estrés hídrico. Algunos estudios han sugerido que *Pistacia terebinthus* L. es el patrón más resistente a la sequía y el frío. El efecto de los patrones en las relaciones hídricas de la planta injertada es crucial para mejorar el rendimiento de los cultivos bajo condiciones de estrés hídrico y para desarrollar una mejor estrategia de riego. En este trabajo se ha estudiado la respuesta fisiológica al estrés hídrico de plantas de pistachero (*Pistacia vera* L. cv. Kerman) injertado sobre tres patrones diferentes *Pistacia terebinthus* L, *P. atlantica* Desf. y un híbrido del cruzamiento *P. atlantica* Desf. x *P. vera* L.. Se evaluaron las respuestas fisiológicas durante un ciclo de sequía y la posterior recuperación de estas plantas en maceta. Los parámetros medidos fueron humedad del suelo, diámetro del tronco, área foliar, número de hojas, peso seco de hojas y tallos, potencial hídrico xilemático, conductancia estomática de las hojas. Los resultados mostraron diferentes respuestas del cv. Kerman según el patrón sobre el que había sido injertado. El patrón híbrido estuvo asociado con un alto grado de control estomático y una menor senescencia foliar (en términos de síntomas visuales y pérdida de hojas) comparado con *P. atlantica* y *P. terebinthus*, a pesar de mostrar un crecimiento de los brotes más vigoroso. *P. terebinthus* también mostró un control estomático muy efectivo, aunque estuvo asociado con una senescencia foliar más rápida en términos de mayor pérdida de hojas. *P. atlantica* se asoció con el crecimiento de brotes menos vigorosos y niveles similares de estrés hídrico, tal como ocurrió con los otros portainjertos en condiciones de elevada demanda evaporativa, que se asoció con un menor control

estomático. La selección del patrón más eficaz para las diferentes condiciones ambientales es discutida.

**Palabras clave:** Conductancia foliar, Crecimiento de la hoja, *Pistacia atlantica*, *Pistacia terebinthus*, crecimiento del tronco, potencial hídrico y relaciones hídricas.

### Abstract

Pistachio cultivation requires the use of rootstock because grafting is the only form of vegetative propagation. The main commercial rootstocks are *Pistacia integerrima* L, *Pistacia atlantica* Desf, *Pistacia terebinthus* L. and *Pistacia vera* L. Pistachio is considered to be a drought and saline-resistant crop; however, there is little information describing varietal responses of rootstocks to water stress. Some studies have suggested that *Pistacia terebinthus* L. is the most drought and cold resistant rootstock. The effect of the rootstock on the water relations of the grafted plant is crucial for improving crop performance under water stress conditions and for developing the best irrigation strategy. This work studied the physiological response to water stress of pistachio plants (*Pistacia vera* L. cv. Kerman) grafted onto three different rootstocks *Pistacia terebinthus* L, *P. atlantica* Desf. and a hybrid from crossbreeding *P. atlantica* Desf. x *P. vera* L.). Plant physiological responses were evaluated during a cycle of drought and subsequent recovery in potted plants. Parameters measured were soil moisture, trunk diameter, leaf area, leaf number, leaf and stem dry weight, stem water potential, leaf stomatal conductance. The results showed different responses of cv. Kerman depending on the rootstock onto which it had been grafted. The hybrid rootstock was associated with a high degree of stomatal control and less leaf senescence (in terms of visual symptoms and leaf number loss) compared to *P. atlantica* and *P. terebinthus*, despite being associated with the most vigorous shoot growth. *P. terebinthus* also enabled very effective

stomatal control, but was associated with the most rapid leaf senescence in terms of a greater loss of leaves. *P. atlantica* was associated with less vigorous shoot growth and similar levels of water stress as occurred with the others rootstocks under conditions of high evaporative demand, which was associated with lower stomatal control. The selection of the most effective rootstock choice for different environmental conditions is discussed.

**Keywords:** Leaf conductance, leaf growth, *Pistacia atlantica*, *Pistacia terebinthus*, trunk growth, water potential, water relations.

## Introduction

Pistachio is an important crop in Iran and USA, which are the major world producers, while in other countries it is becoming an interesting alternative to traditional crops. In the Mediterranean region, uncertainty about the future of economic support from the European Union to traditional crops, such as olive and vineyards, and the use of low fertility soils for agriculture has resulted in a large increase in the area planted with pistachio. In Spain, the total planted area was almost negligible in 1990, and currently is about 4,000 ha. While the production in Turkey (third world producer) has been steady during the last 15 years, in Greece it has increased from 5,000 t in the early 90's to 9,000 t at the beginning of the twenty-first (FAOSTAT, 2007).

Pistachio is considered a drought and saline-resistant crop (Behboudian et al., 1986; Rieger, 1995), and in the Mediterranean basin it is mainly grown under rainfed conditions. However, as with other tree species, irrigation increases yield. In pistachio, irrigation also improves nut quality (higher percentage of splitted nuts) and dampens the alternate bearing pattern (Kanber et al., 1993; Goldhamer et al., 1995). The benefits of irrigation in this crop may be higher than in others crops;

however, there is a requirement properly quantify these responses. The good performance under dryland conditions and the favourable response to irrigation are very important considering that water is a scarce resource and in the future only the most efficient agricultural systems are likely to receive inputs of irrigation water (Feres et al., 2003).

Pistachio cultivation requires the use of rootstocks, and grafting is the only form of vegetative propagation. The main pistachio rootstocks are *Pistacia integerrima* L., *Pistacia atlantica* Desf, *Pistacia terebinthus* L. and *Pistacia vera* L. The most used rootstock in Iran is *Pistacia vera* L., in the USA is *Pistacia integerrima* L. and a hybrid between *Pistacia integerrima* and *Pistacia atlantica* Desf (UCB), and in the Mediterranean basin is *Pistacia terebinthus* L. Most scientific studies with pistachio trees have been done with cultivars grafted onto Californian commercial rootstocks, such as *Pistacia atlantica* Desf, UCB and *Pistacia integerrima* L. There are few reported studies comparing different rootstocks. Ferguson et al. (2005) reported that *Pistacia terebinthus* L. was the most drought and cold resistant rootstock, while *Pistacia integerrima* L. was susceptible to frost but tolerant to verticillium. *Pistacia atlantica*, which was once one of the most popular rootstocks in California, has been discarded in many places because of its high susceptibility to verticillium (Ferguson et al., 2005). Nowadays, UCB is considered the best commercial rootstock under irrigation (Ferguson et al., 2005). Germana (1997) comparing different rootstocks, observed that *P. atlantica* has higher transpiration and photosynthetic activity than *P. terebinthus*, particularly in stressed plants, which could make it more susceptible to drought stress. Guerrero et al. (2003) found no rootstock effect on production when comparing *Pistacia terebinthus* L., *Pistacia integerrima* L., *Pistacia atlantica* Desf and *Pistacia vera* L. under rainfed conditions.

In other woody crops, the use of rootstocks is optional, they may be grown without rootstock, and they are mainly used because the improving some of the cultivar characteristics as tree size, crop yield or yield quality. Different studies on apple (Olien and Lakso 1986, Cohen and

Naor 2002) and peach (Weibel et al. 2003) showed that specific rootstocks had an important influence on vegetative growth rate. Solari et al. (2006) confirmed in peach that rootstock effects on the tree water relations and vegetative growth are derived, at least partially, from differences in the tree hydraulic conductance associated with specific rootstocks. In addition, several hypothesis reviewed by Rogers and Beakbane (1957), Lockard and Schneider (1981) and Webster (1995) suggest that rootstock can affect vegetative tree growth through hormonal effects (Kamboj et al. 1999 ), mineral nutrition (Jones, 1971) or water status (Olien and Lakso, 1986).

The pistachio response to water stress has not been adequately characterized. There are few works studying the water relations of this species. Behboudian et al. (1986) subjected potted Pistachio plants (*P. vera* L. cv. Kerman grafted on *P. atlantica* Desf.) to water and saline stress and reported some photosynthetic activity even at midday stem water potential ( $\psi_x$ ) between -5 and -6 MPa. In this work, they concluded that the stress response of pistachio plants was better than some other fruit trees and other typical xerophytes species (Behboudian et al., 1986).

In recent years, a breeding program has been developed to improve pistachio rootstocks at El Chaparrillo Research Station, Ciudad Real, Spain. This program is being conducted using two techniques: first, crossings have been made between traditional rootstocks, and second, germplasm from different areas of Castilla-La Mancha (central Spain) and northern Andalucía (southern Spain) has been collected to be used in the breeding program (Guerrero et al. 2003). At present, the most promising individuals are being tested. One of the best rootstocks obtained in this program is the second generation, open-pollinated seed crossing *Pistacia atlantica* Desf. x *Pistacia vera* L., which shows a high vigour (Guerrero et al. 2007). This is the rootstock referred to as 'hybrid' in current study. Initial assessments of collected germplasm have shown differences depending on the area they come from, the one from Calzada de Calatrava (Ciudad Real, Spain) showing the highest vigour. This germplasm has



been used in our previous testing to establish the characteristics of *P. terebinthus* as a rootstock (Guerrero et al. 2007).

As pistachio is planted in Spain mainly in dryland areas, the effect of rootstock in the cultivar response to water stress under these conditions will be of major importance. Rootstock effects are important for both physiological responses of the shoot variety and its productivity. The objective of this work was to study the effect of three different rootstocks (*P. terebinthus* L., *P. atlantica* and a hybrid from the crossbreeding of *P. atlantica* x *P. vera* ) on the physiological responses to water stress of pistachio plants (*P. vera* L. cv Kerman). Plant responses were evaluated during cycle of drought and recovery.

## **Materials and Methods**

### ***Site description and experimental design***

The experiment was conducted during the summer of 2007 at *La Entresierra* Research Station, Ciudad Real, Spain (3 ° 56 'W - 39 0' N; altitude 640 m). One year old pistachio plants (*Pistacia vera* L. cv Kerman) grafted onto three different rootstocks, *Pistacia terebinthus* L., *P. atlantica* Desf. and a hybrid from the crossbreeding of *P. atlantica* x *P. vera* (hereafter referred to as 'hybrid') were used. The hybrid was obtained in the breeding program at the El Chaparrillo Research Station. Thirty plants were planted in the spring of 2007 in 50 L pots filled with a mixture of gravel, sand and peat (5, 80 and 15% respectively), and placed outdoors. The experiment took place from "day of the year" (DOY) 190 until DOY 225. Reference evapotranspiration (ET<sub>o</sub>) was calculated according to Allen et al. (1998), and rainfall data were obtained from a nearby (aprox. 500 m) meteorological station.

The experimental design was a completely randomized split plot design with 5 replicates. The main factor was the rootstock and the secondary factor was irrigation. The irrigation treatments were full

irrigation (Control) and no irrigation (Stress). The different combination of the two factors will be named as follow:

- *P. terebinthus*-Control (PTC)
- *P. terebinthus* -Stress (PTS )
- *P. atlantica*-Control (PAC)
- *P. atlantica*-Stress (PAS)
- Hybrid-Control (HC)
- Hybrid-Stress (HS)

From DOY 190, the control and stressed plants were drip irrigated until slight drainage occurred. Each pot had 4 drippers (4 L h<sup>-1</sup>) and was irrigated every afternoon. To determine pot weight at field capacity, all pots were weighted early in the morning, always at the same time, at least three times per week (Monday, Wednesday and Friday). In plants subjected to water stress, irrigation was withdrawn from DOY 204 until 218 when they were re-watered to study re-hydration. Once the stress period was completed, pots were re-watered up to field capacity. The weight at field capacity at the end of the experiment was different to that at the beginning of the experiment, probably due to the decreasing water retention capacity of the peat as it dried out. Soil moisture measurements were taken at 10, 20 and 30 cm depth with a portable capacitance probe (Diviner, 2000, Sentek Pty. Ltd., Australia) placed approximately 15 cm away from the stem. As the results were similar to those obtained by the weighing method, only the latter will be shown on the Results section.

### **Measurements**

Trunk diameter was measured in all plants once a week with a digital gauge, 1 cm above the grafting point. Measurements were taken early in the morning, from 183 to 225 DOY. At the end of the experiment,

leaf area (LA) data were obtained using a leaf area meter (LI-3100C, Lincoln, Nebraska, USA), and leaf and stem biomass was determined drying the plant organs in an oven at 70°C until constant weight. Prior to each biomass determination, visual leaf damage caused by water stress was visually evaluated on a scale of 1 to 5. Value 1 corresponded to leaves without wilting symptoms, value 3 to when 50% of leaf surface presented wilting symptoms and value 5 indicated leaves completely wilted.

Stem water potential ( $\Psi_x$ ) was periodically determined, six measurements were made throughout the experiment to evaluate plant water status. Fully expanded leaves were covered with aluminium foil at least one hour before measurement, and  $\Psi_x$  determined at midday using a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA). Each time, one leaf per plant and per replicate was used. Abaxial leaf conductance ( $g_s$ ) was measured with a steady-state porometer (LICOR-1600, UK) between 12:00 and 14:00 local time, on the central leaflets of the composite leaf. Measurements were taken in all plants (two measurements per plant) on fully expanded leaves receiving direct sunlight, three times a week throughout the experiment.

Vapour pressure deficit (VPD) was calculated from data of a meteorological station around 500 m from the place where the experiment was performed.

### ***Statistical analysis***

The main effects of the two factors were examined by ANOVA and means were compared using the test of Tukey, with a significance  $P < 0.05$ . Significant differences are identified with different letters. The Statistix 8.0 (Analytical software, USA) was used for the statistical analysis.

## Results

### *Evapotranspiration and plant water requirements*

During the experimental period (DOY 190 - 225), ETo ranged from 5.3 to 8.5 mm d<sup>-1</sup>. During the water stress period (DOY 204 - 218), except on day 212, ETo was more steady, with values around 7 mm d<sup>-1</sup> (Fig 1). Precipitation was low, just two events were recorded on day 216 (0.7 mm) and day 217 (5 mm) at the end of the stress period (Fig 1). The pots had an initial mean weight of 58 kg (100% in Fig. 2) when the soil was at field capacity, and those of the stressed plants lost on average 17.8, 17.0 and 10.5 kg (PTS, HS and PAS, respectively) from the beginning of the experiment to the time of maximum stress (DOY 218; Fig. 2). The recovery of the soil water content was very fast. Two days after the beginning of the rehydration, PAS pots recovered 94.7% of their initial weight, followed by the HS (91.6%) and PTS (90.4%) (Fig. 2). After a week, the 3 rootstocks had recovered almost their initial weight: 97.7, 96.3 and 95.7% (PAS, HS and PTS, respectively) (Fig. 2).

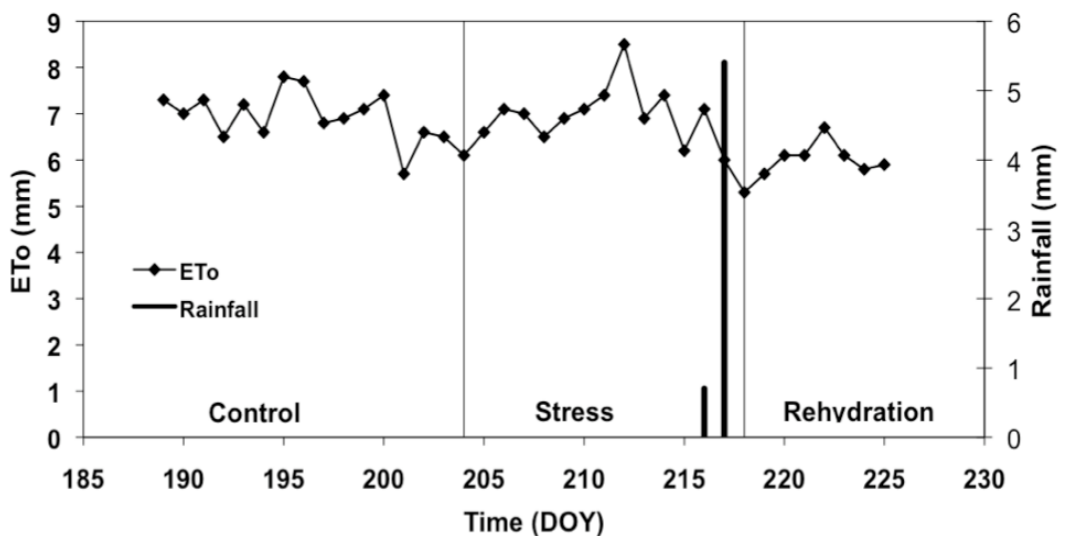


Fig. 1. Rainfall and reference evapotranspiration (ETo) during the experiment. The two vertical lines represent the water stress period.

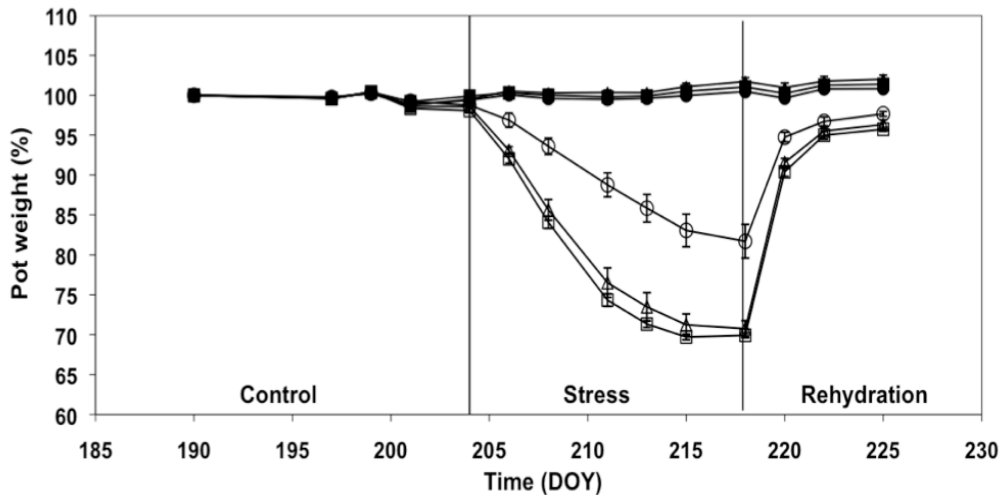


Fig. 2. Pot weight throughout the experiment as a percentage of the weight at field capacity, for all groups. Each point represents the mean of 5 measurements. The two vertical lines represent the water stress period. Bars represent the standard error. ● = PAC; ○ = PAS; ■ = PTC; □ = PTS; ▲ = HC △ = HS.

### *Plant biomass and trunk and leaf growth*

Trunk diameter increased with time in all water treatments and rootstocks. Fig. 3 shows the values of the trunk diameter as a fraction of the diameter at the beginning (TD/TDi) of the experiment for each treatment and rootstock. When plants were well irrigated, there was no effect of the rootstock on the diameter trunk growth. Although *P. atlantica* and the hybrid showed higher final values (1.40) than *P. terebinthus* (1.25), differences were not significant.

Water stress affected trunk diameter earlier in PTS and HS than in PAS, showing a decrease in the slope of TD/TDi with time in relation to well-watered plants with the same rootstock. However, it was only at the end of the stress period (218 DOY) when significant differences were found among rootstocks, with smaller TD/TDi values in PTS than in PAS and HS.

The response of plants to re-watering on DOY 218 was faster in PAS and PTS than in HS, although all the rootstocks showed no significant

differences between control and stressed plants at the end of the rehydration period.

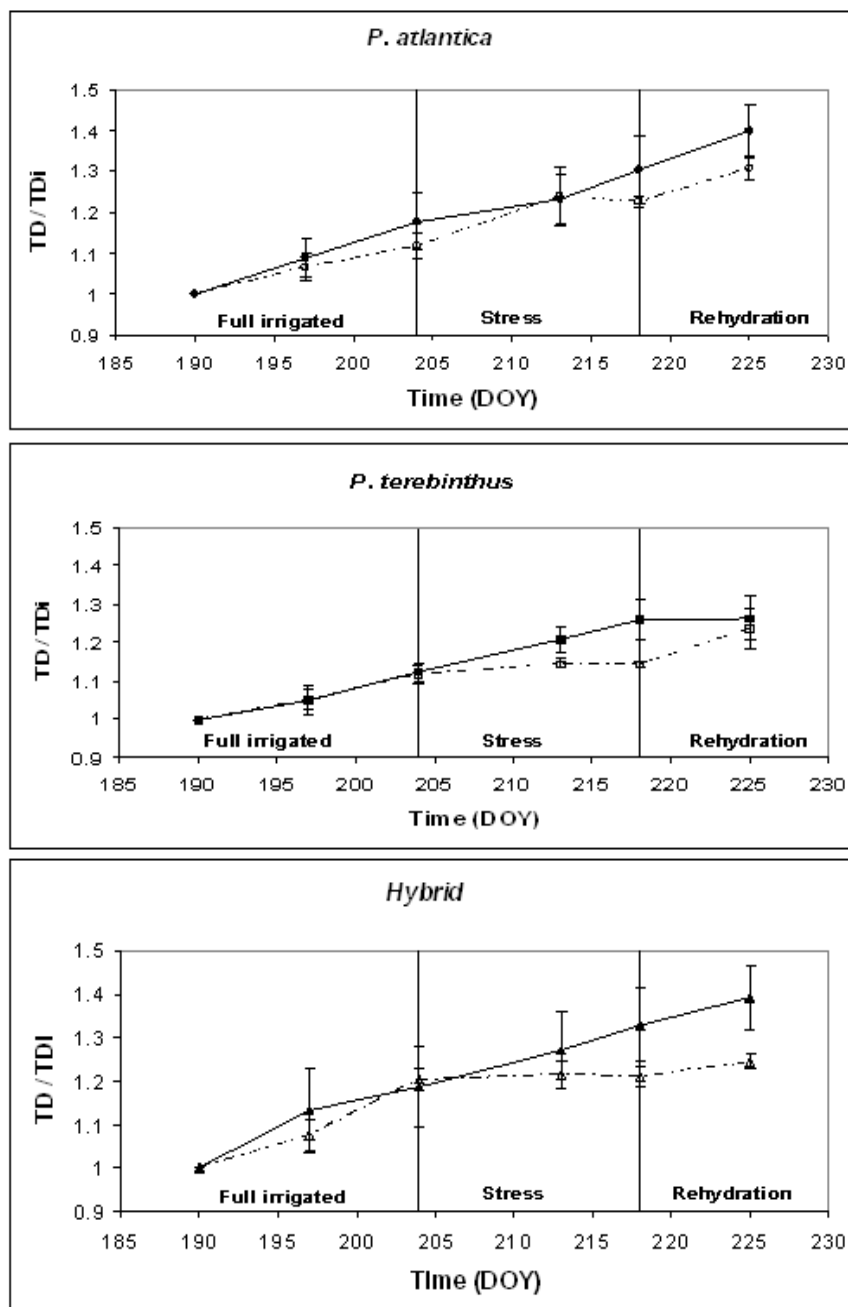
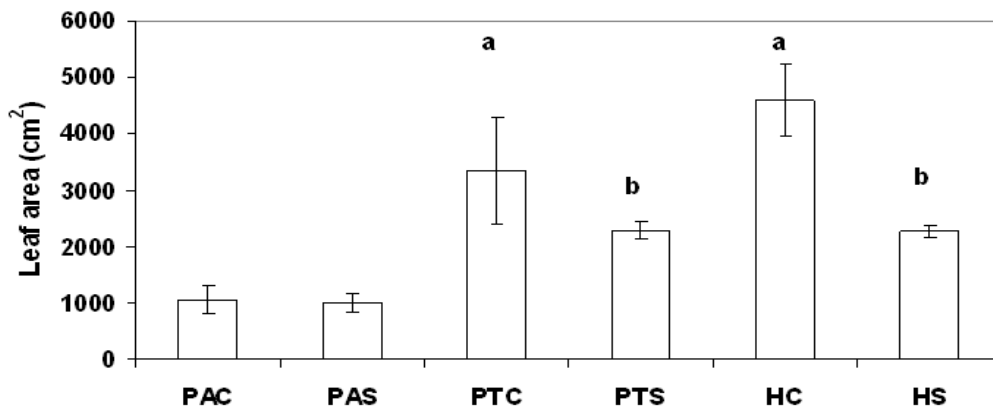


Fig. 3. Seasonal pattern of the trunk diameter as a fraction of the initial value (TD/TDi), for the three rootstocks and two water treatments. Each point is the average of 5 measurements. Bars represent the standard error. ● = PAC; ○ = PAS; ■ = PTC; □ = PTS; ▲ = HC △ = HS.

Another parameter that characterises growth is leaf area. In control conditions, PTC and HC showed a significantly higher leaf area than PAC. Differences between PTC and HC, even though the hybrid was approximately 30 % higher, were not statistically significant (Fig. 4). Water stress mainly affected leaf area development of PTS and HS, producing significant reductions in leaf area of both these treatments. The largest leaf area decrease occurred in the HS, being about 50%, while in PTS it was 32%. On the contrary, there was no significant effect on leaf area development in PAS, with differences between the control and stress treatments being only 6%.



**Fig. 4. Leaf area for all the rootstocks and water treatments at the end of the experiment (DOY 225). Data are the averages of 5 replicates. Bars represent the standard error. Different letters indicated significant differences between control and stress treatment in each rootstock ( $P < 0.05$ , Tukey Test).**

Fig. 5 shows the final leaf number as a fraction of that at the beginning of the experiment (LN/LNi, Fig. 5a) and the leaf dry weight and stem dry weight (Fig. 5b) at the end of the experiment for all the rootstocks and water treatments. LN/LNi was calculated in order to avoid the effect of the variability in leaf number among plants at the beginning of the experiment on the possible differences between water treatments at the end of the stress period. It was not possible to use the same approach for leaf and stem dry weight as it involved destructive measurements. As

it was observed in leaf area, in the control treatment, HC and PTC showed significantly higher leaf and stem dry weight than PAC (Fig. 5b), while the ratio LN/LNi was similar for all the rootstocks. Water stress resulted in a significant reduction in LN/LNi in PTS and HS, but not in PAS (Fig. 5a). PAS also showed similar values of final leaf and stem dry weight than PAC. However, although leaf and stem DW of stressed plants of the hybrid were lower than those of control plants, the differences were not statistically significant, which was probably due to the initial variability of this parameter among plants. The response of PTS plants to water stress was intermediate, with leaf DW being affected but not stem DW, although once again the differences were not statistically significant (Fig. 5b).



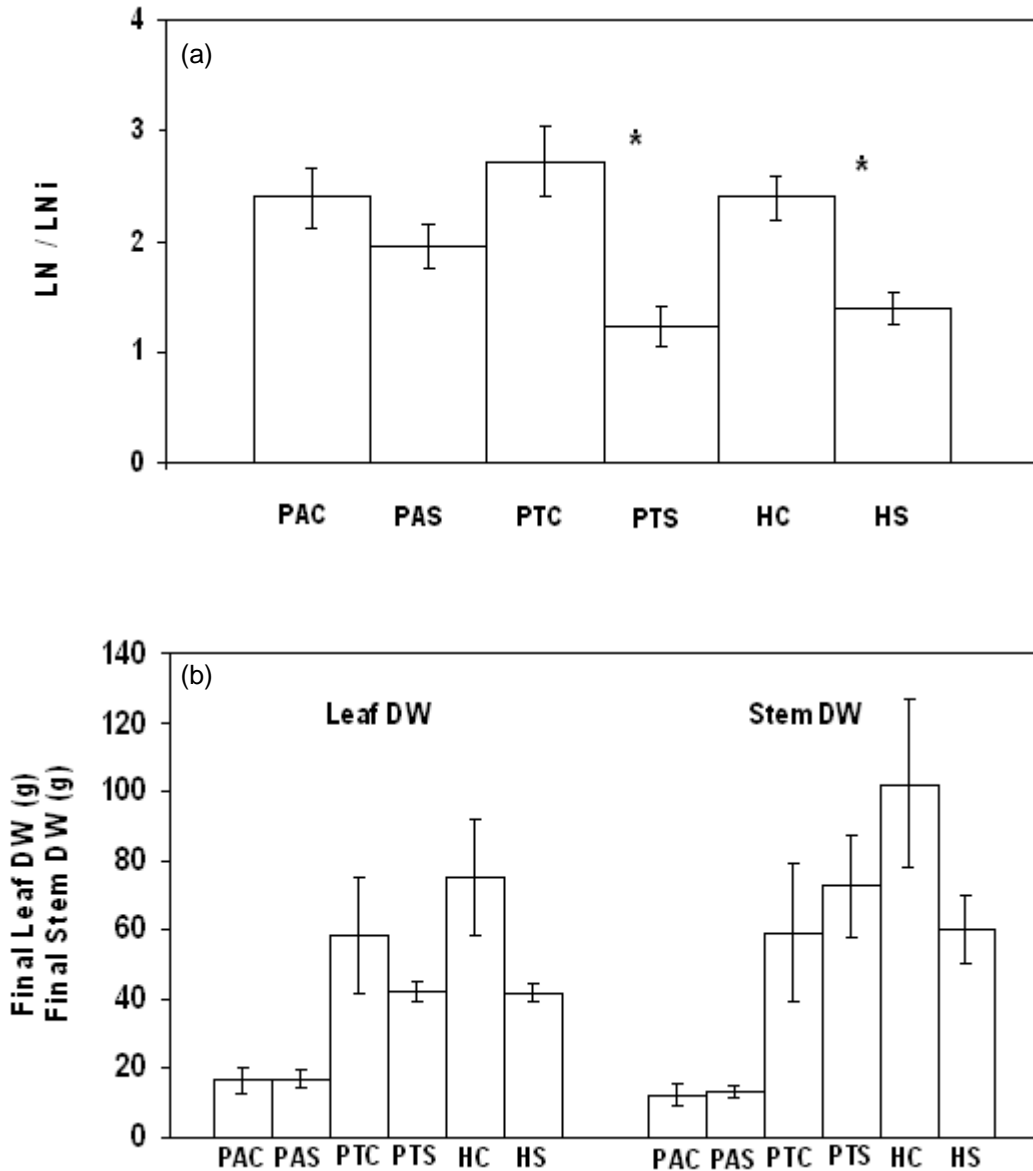


Fig. 5. (a) Leaf number as a fraction of the value at the beginning of the experiment (LN/LNi) and (b) leaf and stem dry weight (Final leaf DW and Final stem DW, g/plant) at the end of the experiment for all the rootstock and water treatments. Data are the average of 5 replicates. Bars represent the standard error. Symbols are indicated in the figure. Different letters indicated significant differences between control and stress treatment in each rootstock ( $P < 0.05$ , Tukey Test).

There were no significant differences among rootstocks in visual wilting symptoms of stressed plants, although it seemed that PTS was more affected than PAS and HS, with mean values of 2.5, 1.8 and 1.6 respectively (Fig. 6). Surprisingly, for each rootstock, although stressed plants showed higher wilting damage values than control plants, differences were not significant according to the scale chosen, as high variability was found among plants of the same treatment.

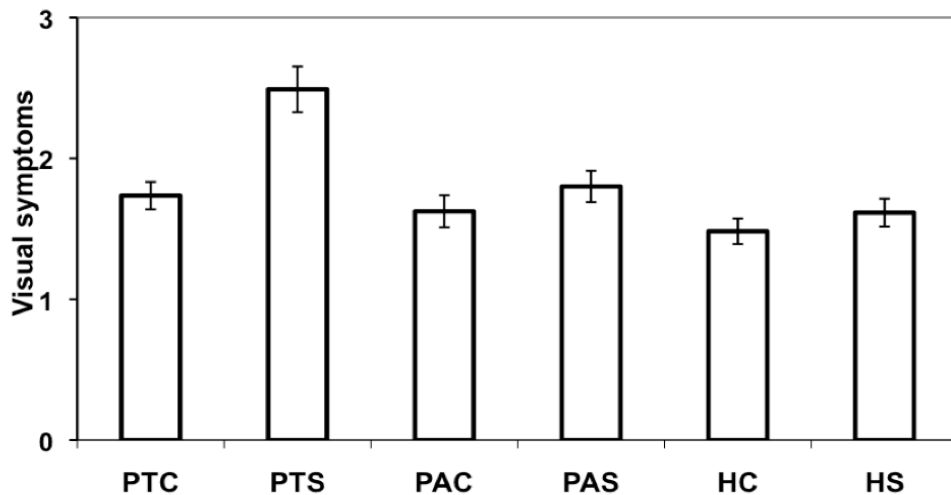


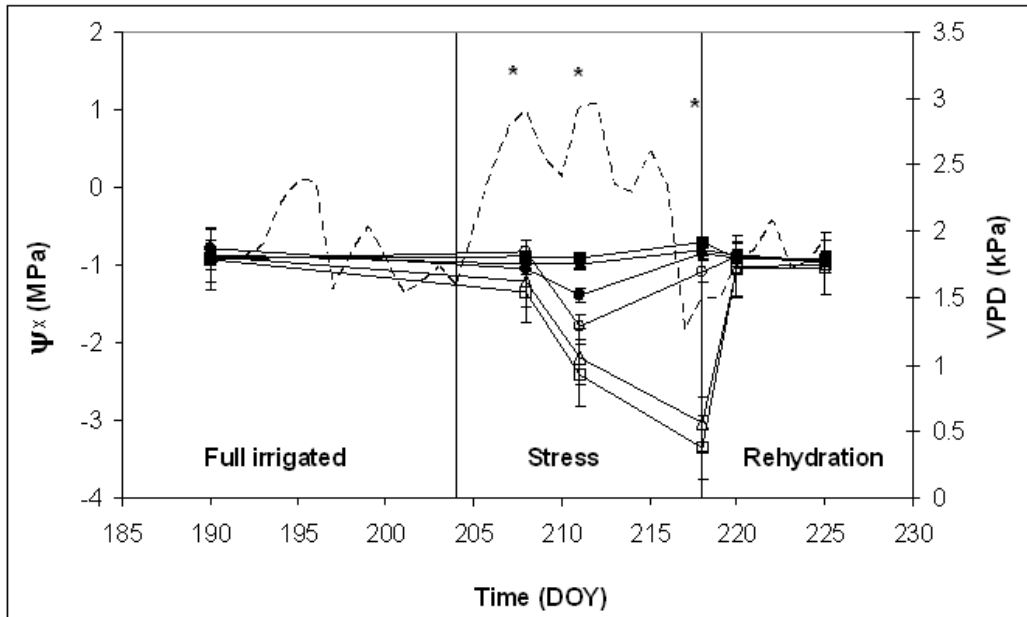
Fig. 6. Values of the visual symptoms of water stress (1 = no symptoms, 5 = 100% wilting surface) for all rootstocks and water treatments. Data are the average of 5 replicates. Bars represent the standard error.

### **Water relations**

Fig. 7 shows the time course of  $\Psi_x$  throughout the experiment for all the rootstocks.  $\Psi_x$  ranged from -0.6 to -1.45 MPa in control plants, and from -0.87 to -3.8 MPa in stressed plants. The effect of water stress on  $\Psi_x$  was not detected until DOY 211 as significant differences were not found neither between water treatments nor rootstocks on day 208, 4 days after the beginning of the drying cycle. However, water treatment-rootstock interaction was significant on day 208 showing lower  $\Psi_x$  values for HS and PTS than for PAS. The minimum values of  $\Psi_x$  were reached on DOY 218 (10 days after the onset of water stress), PTS and HS rootstocks showing

significantly lower  $\Psi_x$  than PAS, being respectively -3.35 and -3.04 MPa, and following the same pattern as in previous dates. Following, the final measurements on DOY 218, plants were fully irrigated and the recovery began. Only two days later (DOY 220) PAS was fully recovered from the stress, while PTS and HS had recovered 90 and 81% of the  $\Psi_x$  measured in the respective control plants that day. At the end of the experiment (DOY 225),  $\Psi_x$  values in PTS and HS were 93% and 91% of that of their controls, respectively. These differences between values of recovered and stressed plants were not statistically significant.

Stomatal conductance ( $g_s$ ) in control plants ranged from 149 to 326  $\text{mmol m}^{-2}\text{s}^{-1}$ , showing no differences between rootstocks for each date of measurement (Fig. 8). Water stress affected  $g_s$  in all rootstocks, with PAS once again being the rootstock less affected, followed by HS and finally PTS. Differences in  $g_s$  were significant on days 211, 213 and 215, with values for PAS being higher than those of PTS and HS. The minimum  $g_s$  values during the stress period were observed on DOY 215, 7 days after the beginning of the drying cycle, being 136.8, 33.9 and 62.8  $\text{mmol m}^{-2}\text{s}^{-1}$  in PAS, PTS and HS, respectively. Two days after rehydration (DOY 220), stomatal conductance was fully recovered in PAS at levels even higher than the control plants in some cases. However,  $g_s$  in PTS and HS was only 67.7 and 68.6% of that in control plants. On DOY 225, one week after the beginning of the recovery,  $g_s$  in PTS and HS was already 96% and 93.5% of the values measured in control plants.



**Fig. 7. Seasonal pattern of the stem water potential ( $\Psi_x$ ) and VPD (dotted line) during the experiment. Each point is the mean of 5 measurements. The two vertical lines represent the water stress period. Bars denote the standard error. Asteriks "\*" represent significant differences between rootstock in the stress treatment ( $P < 0.05$ , Tukey Test). ● = PAC; ○ = PAS; ■ = PTC; □ = PTS; ▲ = HC △ = HS.**

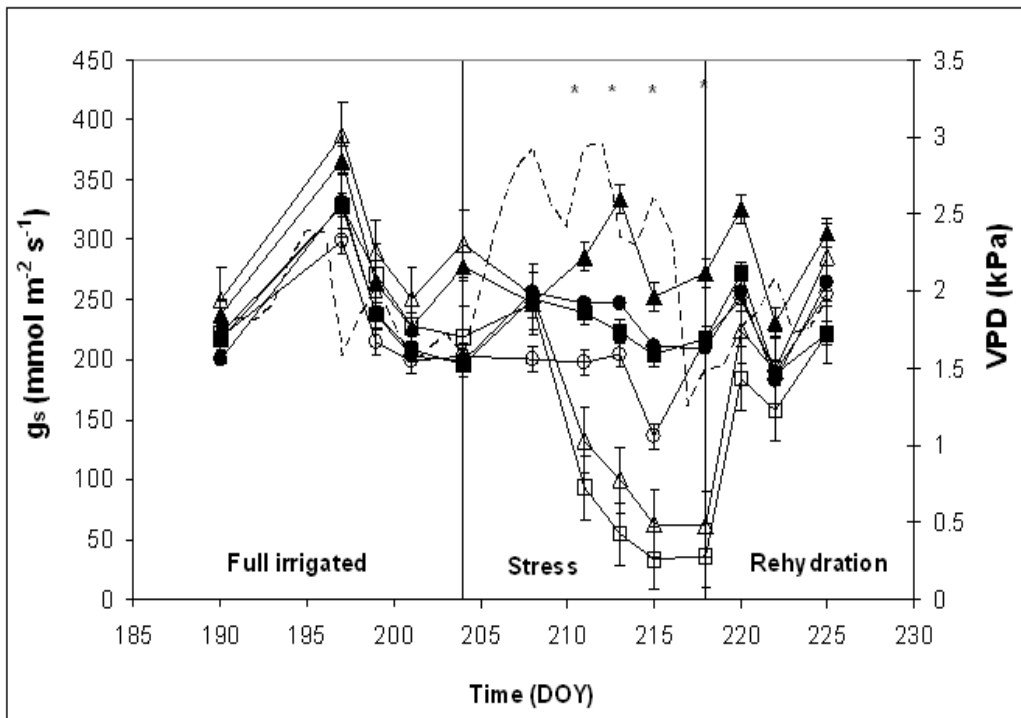


Fig. 8. Seasonal pattern of the stomatal conductance and VPD (dotted line) during the experiment. Each point is the mean of 10 measurements. The two vertical lines represent the water stress period. Bars denote the standard error. Asterisks "\*" represent significant differences between rootstock in the stress treatment ( $P < 0.05$ , Tukey Test). ● = PAC; ○ = PAS; ■ = PTC; □ = PTS; ▲ = HC △ = HS.

## Discussion

The three different rootstocks studied in this work induced a differential response of growth and water relations in the cultivar Kerman, both under well watered (control) and water stressed conditions. In the absence of water stress, the hybrid rootstock promoted higher vegetative growth in the shoot variety than the other two rootstocks, especially *P. atlantica*, with a larger leaf area and total biomass (Figs. 4 and 5). This greater growth during the initial stages, which was most marked with the hybrid, resulted in different plant size at the beginning of the stress period for the three rootstocks studied. Fig. 2 characterizes these differences very well, showing a much higher water use (DOY) during the drying cycle for both *P. terebinthus* and the hybrid compared to *P. atlantica*. These results are the

opposite of those obtained by Ferguson et al. (2005), who suggested that *P. terebinthus* is the least vigorous of all the rootstocks used in California. Guerrero et al. (2007) have shown significant differences in vigour among different but near populations of *P. terebinthus*. In our study, we could have used a particularly vigorous population of *P. terebinthus*, since *P. atlantica* is usually considered to be a rootstock of moderate vigour (Ferguson et al. 2005; Spann et al. 2007). The vigour of fruit trees may be important because it is likely to be related to yield. In pistachio, Spann et al. (2007) suggested that more vigorous rootstocks can also involve higher pruning costs because they promote more vegetative growth in the shoot cultivar. These authors also suggested that a more moderate vigour could be compensated by a higher plant density. Vigour has been also related to successful field grafting. Guerrero et al. (2007) found that under field conditions, grafting success in pistachio is closely related to the trunk diameter, and therefore greater vigour would probably reduce grafting failures.

Rootstock changes the response of the pistachio tree to water stress conditions. *P. atlantica* showed slight but not significant reductions in leaf number. In contrast, in *P. terebinthus* and the hybrid, growth was most affected by water stress conditions (Fig. 4 and 5). This response is generally found in all species, since expansive growth is the most sensitive process to water stress in plants and is affected even at relatively high leaf water potentials (Hsiao, 1973). Trunk diameter data showed that growth didn't stop absolutely under water stress but that it slowed down (Fig. 3). This agrees with the data shown by Behboudian et al. (1986) in which slight turgor loss under severe stress still allows some tree growth. In our study, the reduction of trunk diameter in response to water stress took place earlier in the cultivar grafted onto the hybrid and *P. terebinthus* than on *P. atlantica*. This response to water stress seems in accordance to the response measures in other parameters such as  $\Psi_x$  and  $g_s$  which will be discussed later. In contrast, the recovery of growth was slower in the hybrid than in the other two rootstocks (Fig. 3). Visual symptoms of water

stress in leaves and the decrease of green leaf area are indicators of leaf senescence caused by water stress. Apparently, *P. terebinthus* induced a stress avoidance response in Kerman cultivar with a greater leaf number reduction (Fig. 5.a) and visual damage (Fig. 6) than the other two rootstocks. However, hybrid rootstock showed a relatively greater loss of leaf area (Fig. 4). Unless the leaf area can be increased after the stress period, this response would reduce crop yield.

Water stress, characterises by stem water potential measurements, was more severe in plants grafted on *P. terebinthus* than the hybrid (Fig. 7). The level of induced water stress was not as great as described in other studies in which minimum  $\Psi_x$  values of -5 MPa have been reported (Behboudian et al., 1986). The higher  $\Psi_x$  values in *P. atlantica* were likely related to the smaller transpiring plant leaf area and biomass (Fig. 4 and 5). In relation to these leaf area values, differences in stem water potential between rootstocks (Fig. 7) were smaller than expected in the drought stress. In addition, *P. atlantica* also showed very little stomatal control of transpiration (Fig. 8), which could have contributed to reduced  $\Psi_x$  differences between this rootstock and *P. terebinthus* and the hybrid. These results are in agreement with the delayed response of trunk diameter to water stress in *P. atlantica* compared to *P. terebinthus* and the hybrid (Fig. 3). Considering the smaller leaf area of *P. atlantica*, the degree of water stress imposed in this experiment may not have not been strong enough to affect parameters such as  $\Psi_x$  and  $g_s$ , in this rootstock, as quickly as occurred in *P. terebinthus* and the hybrid.

Leaf conductance of well-watered control plants and stressed plants was slightly higher in the hybrid than in the other two rootstocks, especially during the water stress period (Fig. 8). This high  $g_s$  of control plants in the hybrid could have contributed to their higher growth through higher assimilation rates. The hybrid induced in the shoot cultivar a high degree of stomatal control in response to water stress. This behaviour is characteristic of drought resistant species (Loomis and Connor, 1992).

## Conclusions

The behaviour of the Kerman cultivar in terms of growth and water relations, under irrigated and water stress conditions, depended on the rootstock it was grafted onto. The rootstock that induced a higher sensitivity to water stress was *Pistacia atlantica* Desf, with low stomatal control of transpiration, although these results should be taken with caution as the degree of water stress imposed to that variety was milder as result of its low leaf area development. By contrast, *Pistacia terebinthus* L. rootstock showed a high degree of stomatal control, and slightly higher levels of water stress than the hybrid, which resulted in smaller reductions of leaf area but not in leaf number, probably due to differences in leaf size. Apparently, the hybrid rootstock showed slightly lower stomatal closure under stress conditions than *P. terebinthus*. This response produced a greater leaf area reduction in the hybrid than in *P. terebinthus*. Even though it was the most vigorous, it lost more leaf area through senescence. Although the reduction in leaf area allowed a quicker recovery of some parameters after water stress was over, it could result in a reduction in the assimilation capacity of the tree under field conditions, which could affect crop yield.

This hypothesis should be checked in field experiments. The responses found in this work should be taken into account when selecting the rootstock for the establishment of a new orchard, knowing the irrigation management that will be used. Under irrigation, the hybrid may be the best rootstock since it induces the biggest leaf conductance and vigour, which will be likely to be accompanied by a more productive response. In the case of dryland conditions or regulated deficit irrigation in which water stress periods are induced, *P. terebinthus* might be a good choice for their drought tolerance, as it is able to maintain greater leaf area relative to that of non-stressed plants with lower  $\Psi_x$  and  $g_s$  values.



All these results suggest the need to evaluate the effect of these rootstocks on the productive response of this variety under variable water stress conditions in the field.

### **Acknowledgments**

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# CAPÍTULO III





## CAPÍTULO III

**Influencia de los estados fenológicos y las dotaciones de agua en las relaciones hídricas en el cultivo del pistachero (*Pistacia vera* L.).**

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## Resumen

El pistachero es un frutal tolerante a la sequía que puede ser cultivado en condiciones de secano y regadío. Sin embargo, las necesidades de agua del árbol son considerables, de tal forma que en la mayoría de las plantaciones comerciales el riego deficitario es una práctica común. Esta técnica de riego deficitario controlado se ha descrito en varios trabajos, que muestran que el estado fenológico en el que ocurre el endurecimiento del endocarpio (fase II), es el más tolerante a la sequía. Este trabajo propone que tal resistencia a la sequía está relacionada con cambios en las relaciones hídricas ligados a las etapas fenológicas, incluso en condiciones de no estrés hídrico. Con el fin de evaluar dichos cambios, se midió el patrón diario de potencial hídrico xilemático y el intercambio gaseoso (fotosíntesis neta,  $P_n$ , y la conductancia de la hoja,  $g_s$ ), determinando también curvas presión-volumen en las tres diferentes etapas fenológicas de los árboles maduros de pistachero (*P. vera* L. cv Kerman sobre el portainjerto *P. terebinthus* L.). El patrón diario de potencial hídrico xilemático y el intercambio gaseoso fueron analizados en tres tratamientos de riego diferentes: control, riego deficitario y secano. Las curvas presión-volumen se realizaron sólo en los tratamientos control y secano. Se encontraron diferencias significativas en el patrón diario de potencial hídrico xilemático en todas las etapas fenológicas consideradas, mientras que sólo en la última se vio afectada por el estrés hídrico la fotosíntesis neta. El patrón diario de intercambio gaseoso al comienzo de la estación no se vio afectado por la demanda evaporativa, con un valor constante cuando la radiación no era limitante. Los niveles moderados de estrés hídrico durante la última fecha de medida redujeron los valores máximos de  $g_s$  y  $P_n$ , resultando también en un claro cambio en el patrón diario de la curva, con valores máximos sólo al comienzo del día. Las relaciones entre el potencial hídrico xilemático y los parámetros de intercambio gaseoso fueron diferentes durante la fase II, y casi las mismas en la etapa I y III. Los parámetros extraídos de las curvas presión-volumen también indican un cambio en el módulo de elasticidad de las

células de las hojas en la etapa II. Además, las diferencias en el índice de ajuste osmótico (AO) sugieren diferente grado de ajuste osmótico de las distintas etapas fenológicas en respuesta al estrés hídrico. Los resultados muestran que existen diferentes mecanismos de resistencia a la sequía que operan en las diferentes etapas fenológicas de los árboles de pistachero.

**Palabras clave:** intercambio gaseoso, conductancia foliar, riego, fotosíntesis neta, potencial osmótico, curvas presión-volumen, riego deficitario controlado y potencial hídrico.

### **Abstract**

Pistachio is a drought tolerant fruit tree that can be cultivated in rainfed and irrigated conditions. The water requirements of the tree, however, are considerable so in most of the commercial orchards deficit irrigation is a common practice. Regulated deficit irrigation in pistachio trees has been described in several works, which reported that the phenological stage of shell hardening, so called stage II, is the most drought tolerant. This paper proposes that such drought resistance is related to changes in water relations linked to the phenological stages, even in conditions of no water stress. In order to evaluate such changes, the daily pattern of stem water potential and gas exchange (net photosynthesis,  $P_n$ , and leaf conductance,  $g_s$ ) was measured, determining also the pressure-volume curves, in three different phenological stages of mature pistachio trees (*P. vera* cv Kerman on *P. terebinthus* L rootstock.). The daily pattern of stem water potential and gas exchange were performed in three different irrigation treatments: control, regulated deficit irrigation and rainfed. The pressure-volume curves were made only in the control and rainfed treatments. Significant differences were found in the daily pattern of stem water potential in all the phenological stages considered, while only in the last one the net photosynthesis was affected by water stress. The daily pattern of gas exchange at the beginning of the

season was not affected by the evaporative demand, with a constant value when radiation was not limiting. Moderate levels of water stress during the last measurement date reduced the maximum values of  $g_s$  and  $P_n$  resulting also in a clear change in the pattern of the daily curve, with maximum values only at the beginning of the day. The relationships between stem water potential and gas exchange parameters were different during stage II and almost the same in stage I and III. The parameters drawn from the pressure-volume curves also indicated a change in the elastic modulus of the leaf cells in stage II. In addition, differences in the osmotic adjustment (OA) index suggested different degree of osmotic adjustment of the phenological stages in the response to water stress. The results showed that different mechanisms of drought resistance are operating in the different phenological stages in pistachio trees.

**Keywords:** Gas exchange, leaf conductance, irrigation, net photosynthesis, osmotic potential, pressure-volume curves, RDI, water potential.

## Introduction

Pistachio is a drought tolerant fruit tree species. As with other trees, irrigation increases the yield, but particularly in pistachio it also improves the nut quality and dampens the normal alternate bearing pattern (Kanber et al., 1993; Goldhamer, 1995). Regulated deficit irrigation (RDI) is a system of managing water supply by imposing some water deficits in specific phenological stages, which are found to be less sensitive, with no (or low) reduction in economic benefits (Behboudian and Mills, 1997). The nut development in pistachio is characterised by three different periods (Goldhamer, 1995): stage I starts at the beginning of the nut growth and ends when its maximum size is reached; during stage II the shell hardening takes place and finally, the stage III is the period of



kernel growth (Goldhamer, 1995). Phene et al. (1987) found that in mature pistachio trees growing in shallow soils, a reduction in irrigation of 50% of the crop evapotranspiration ( $ET_c$ ) during shell hardening (stage II) had no effect on final yield. And recently, Goldhamer et al. (2004) showed that the same reduction of the irrigation water during stages I (nut growth) and II (shell hardening) did not reduce the total amount of fruits and increased the percentage of shell splitting, although it also increased early splitting.

Pistachio is a saline-tolerant species (Behboudian et al., 1986; Goldhamer et al., 1984). Within *Pistacia* genus, some investigations have been carried out to evaluate seasonal changes of net carbon assimilation and chlorophyll content and to assess the rates of net carbon assimilation, stomatal conductance, transpiration and related parameters in *P. vera* L. (Vemmos, 1994; Novello and de Palma, 1995; de Palma and Novello, 1996). Also, the diurnal patterns of net photosynthesis and leaf conductance have been compared among several *Pistacia* species (Lin et al., 1984). There is no information about the response of the diurnal patterns of gas exchange to water stress, which might be different in different phenological stages of the trees. In pear Marsal and Girona (1997) described variations in the water relations in different phenological stages in no water stress conditions. Such variations during the growing season of the trees may explain the higher drought resistance during some periods.

Water deficits influence a wide variety of physiological processes in higher plants, including leaf expansion, stomatal opening, and photosynthetic carbon assimilation (Robichaux, 1984). Changes in turgor pressure may represent the principal effect by which small changes in plant water status are transduced into changes in metabolism (Hsiao et al., 1976, Zimmerman, 1978). As a result, mechanisms promoting the maintenance of high turgor pressure as the decrease in tissue water content would enhance growth and survival of plants under conditions of low moisture availability (Turner and Jones, 1980; Jones et al., 1981; Bradford and Hsiao, 1982). The hypothesis considered in this work was

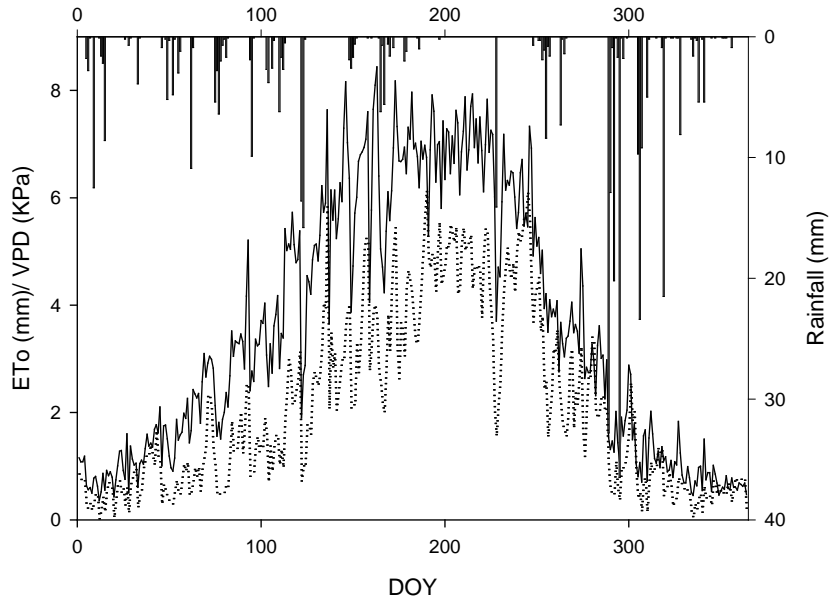
that some of these mechanisms may change throughout the phenological stages of the pistachio nut development.

The aim of this work was to characterise the response of water potential and gas exchange of pistachio trees to water stress during the three main periods of nut development, identifying the underlying mechanisms that operate at each stage. Also, information drawn from the pressure-volume curves was used to establish the degree of drought resistance of pistachio in each phenological stage.

## Materials and Methods

### *Experimental site and plant material*

The experiment was conducted during 2006 in an 11 year-old pistachio (*Pistacia vera* L. cv Kerman on *Pistacia terebinthus* L. rootstock) orchard at the *El Chaparrillo* Research Station, Ciudad Real, Spain (3° 56' W, 39° 0' N; altitude 640 m). The soil at the experimental site is a shallow clay-loam (Alfisol Xeralf Petrocalcic Palexeralfs) with a depth of 1.3 m and a discontinuous petrocalcic horizon between 0.75 and 0.85 m. The volumetric water content ( $\theta_v$ ) of the top 0.30 m is 22.8% at field capacity (soil matric potential -0.03 MPa) and 12.1% at wilting point (soil matric potential -1.5 MPa). For the layer 0.30-1.30 m,  $\theta_v$  is 43.0 and 21.1%, for field capacity and wilting point, respectively. The trees were 5 m apart giving a planting density of 400 trees ha<sup>-1</sup>. The time course of ETo, rainfall and vapour pressure deficit (VPD) is shown in Fig. 1. These patterns are the common in the area, with rainy autumns and winters and dry and hot summers.



**Fig. 1. Seasonal pattern of the rainfall (vertical bars), ET<sub>0</sub> (solid line) and VPD (dotted line), during 2006 season in the experimental site.**

The phenological stages taken into account in the experiment were those suggested by Goldhamer (1995): Stage I (shell expansion), from sprouting until the end of shell growth; Stage II (shell hardening), from maximum shell size until the beginning of kernel growth; and Stage III (kernel growth), from the beginning of kernel growth until harvest.

The crown volumes of the trees at the end of the season were  $6.5 \pm 0.9 \text{ m}^3 \text{ tree}^{-1}$  in C treatment,  $7.7 \pm 1.6 \text{ m}^3 \text{ tree}^{-1}$  in RDI treatment and  $8.8 \pm 2.1 \text{ m}^3 \text{ tree}^{-1}$  in RF treatment. The average total yield (dry nut) during this season was  $2447 \text{ Kg ha}^{-1}$  in control trees,  $1914 \text{ Kg ha}^{-1}$  in RDI and  $2004 \text{ Kg ha}^{-1}$  in rainfed treatment.

### ***Irrigation regimes***

The  $ET_c$  was calculated from values of reference evapotranspiration ( $ET_0$ ) and the crop coefficient ( $K_c$ ) (Doorenbos and Pruitt, 1974)]. The mean  $K_c$  values used were 0.39, 1.06 and 1.14 for stages I, II and III,

respectively (Goldhamer, 1995) after correcting for canopy size (Feres and Goldhamer, 1990).  $ET_o$  was estimated by the Penman-Monteith equation (Allen et al., 1998) with daily data from a nearby automatic weather station (1.5 km away from the experimental orchard).

The irrigation treatments were as follows:

- Control (C): irrigation was applied to cover the estimated crop evapotranspiration ( $ET_c$ ). In order to ensure non-stress conditions, it was increased by 20% of the  $ET_c$  rate if the midday stem water potential ( $\Psi_x$ ) fell below -1.0 MPa.
- Regulated deficit irrigation (RDI): irrigation was applied as 65 % of the control treatment during the stages I (shell expansion) and II (shell hardening), and 100% of the control treatment during stage III (kernel growth).
- Rainfed conditions (RF): no irrigation was applied.

Irrigation was performed four days per week with a drip irrigation system. Each tree had five drippers providing a total flow of  $20 \text{ l h}^{-1}$ . The irrigation water used was saline ( $EC = 3.4 \text{ dS m}^{-1}$ ) and had a high nitrate concentration ( $\text{NO}_3^- = 66 \text{ mg l}^{-1}$ ). A complete randomised design was used, with 20 trees per treatment. The total volume of applied water was 365.4 and 290.7 mm for the C and RDI treatments, respectively, throughout the experiment. No irrigation was provided during the post-harvest period in any of the treatments.

### ***Plant water relations***

In order to compare the response of plant water relations to phenological changes and to water stress a daily curve of leaf gas exchange and stem water potential ( $\Psi_x$ ) was performed on each of the three phenological stages considered (24<sup>th</sup> of May, 29<sup>th</sup> of June and 24<sup>th</sup> of August, 2006). Also, 4-6 pressure-volume (P-V) curves per treatment

were performed in C and RF trees, two to four days after the daily cycles of gas exchange and  $\Psi_x$ .

In the daily curves, gas exchange parameters and  $\Psi_x$  were determined from predawn until late afternoon. Fully expanded leaves on branches near the main trunk were covered with aluminium foil for at least 1 h before each  $\Psi_x$  measurement. These were made on one leaf per tree and six trees per treatment using a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA). Because pistachio leaves exude turpentine, a piece of blotting paper was used to determine the end point as turpentine cannot moisten blotting paper but the xylem sap can.

Leaf conductance ( $g_s$ ) and leaf net photosynthesis (Pn) were measured on the central foliole of the compound leaf approximately at the same time than  $\Psi_x$ , by using an infrared gas analyser (IRGA, CIRAS-2, PP system, U.K.). The measurements were made in sunlight and with fully expanded leaves from the same trees in which  $\Psi_x$  was measured.

The pressure-volume (PV) curves were performed only in the C and RDI treatments. Five leaves per treatment were collected at predawn, placed immediately in plastic bags and taken to the laboratory. There they were rehydrated for 1 h by placing the cut end of the petiole under water. A pressure chamber was used to measure leaf  $\Psi$ . Before placing them in the pressure chamber, each leaf was wrapped in moist cheesecloth, placed in a black plastic bag and weighed (Hsiao, 1990). The actual fresh weight of each  $\Psi$  determination was assumed to be the leaf weight measured immediately before the insertion of the leaf in the pressure chamber. Periodic measurements of fresh weight and  $\Psi$  were taken until a  $\Psi$  value around -3.5 MPa. Pressure-volume curves were generated by the free transpiration method (Hinckley et al. 1980) and  $1/\Psi$  was plotted versus relative water content (RWC).

Zero turgor point was determined through a graphical analysis, considering the lineal portion of the curve. It was calculated with the experimental points that resulted in the maximum determination coefficient ( $R^2$ ) of the lineal regression. The parameters derived from each curve were: osmotic potential at full turgor ( $\Psi_{s,100}$ ), osmotic potential at

zero turgor ( $\Psi_{s,0}$ ), relative water content at zero turgor ( $RWC_0$ ) and percentage of the symplastic water content (R). The tissue elasticity ( $E_0$ ) was calculated considering the two highest turgor data of the P-V curve (Dichio et al., 1997). Also, the average tissue elasticity ( $E_{0av}$ ) integrated over the full range of positive turgor was estimated assuming a linear relationship between turgor potential and RWC (Wilson et al., 1979) as proposed by Marsal and Girona (1997).

The osmotic adjustment index (OA index) and the breaking point (BP) (Turner, 2006) were used to compare the degree of osmotic adjustment at different phenological stages. The OA index is 1 minus the slope of the lineal relationship between the natural logarithms of osmotic potential (independent variable) and the RWC. OA index varies between 0 and 1 and it is an estimation of the degree of osmotic adjustment. The second parameter derived by Turner (2006) is the breaking point (BP). The relationship between the natural logarithms of osmotic potential and the RWC may be constant with no decrease in RWC until a threshold value (BP) is reached, from which that relationship is lineal. Lower values of BP mean higher capacity of drought resistance.

### ***Statistical analysis***

Statistical analyses of variance and Tukey test (SX 8.0, Analytical software) were performed for treatment comparison. Treatment differences were considered statistically significant at  $P < 0.05$

### **Results**

Fig. 2 shows the daily pattern of midday stem water potential ( $\Psi_x$ ) in three different phenological stages of the trees.  $\Psi_x$  ranged from -0.5 MPa to -1.4 MPa during Stage I (Fig. 2a). Significant differences were found between the irrigated (C and RDI) and the RF treatments in all the sampling times except the last one. RF data showed the fastest initial decrease and final increase of  $\Psi_x$  throughout the day. In the Stage II (Fig.

2b) the dehydration of the trees was higher in all the treatments, especially in rainfed conditions. The values of  $\Psi_x$  ranged from -0.7 to -2.1 MPa, being those of the RF treatment significantly the lowest at all sampling times. In addition, RDI treatment  $\Psi_x$  was significantly lower than the control at the beginning of the day (at 6, 8, 10 GMT). During Stage III (Fig. 2c) the range of  $\Psi_x$  was similar to that measured at Stage II (values from -0.8 MPa until -2.1 MPa). But in RF treatment  $\Psi_x$  decreased earlier in the day than in Stage II with significant differences with the irrigated treatments. RDI and C treatments showed similar  $\Psi_x$  values during all the daily cycle, being significantly different only at 9 and 15 GMT.

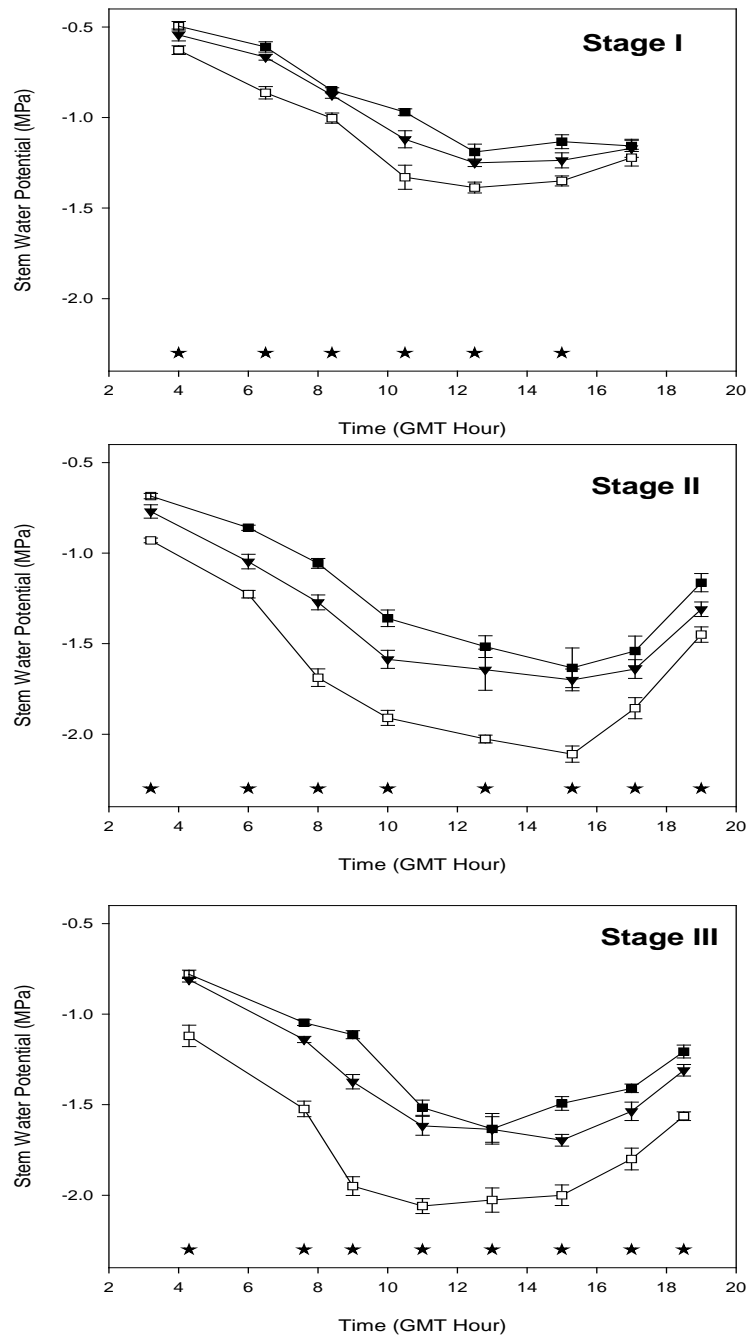
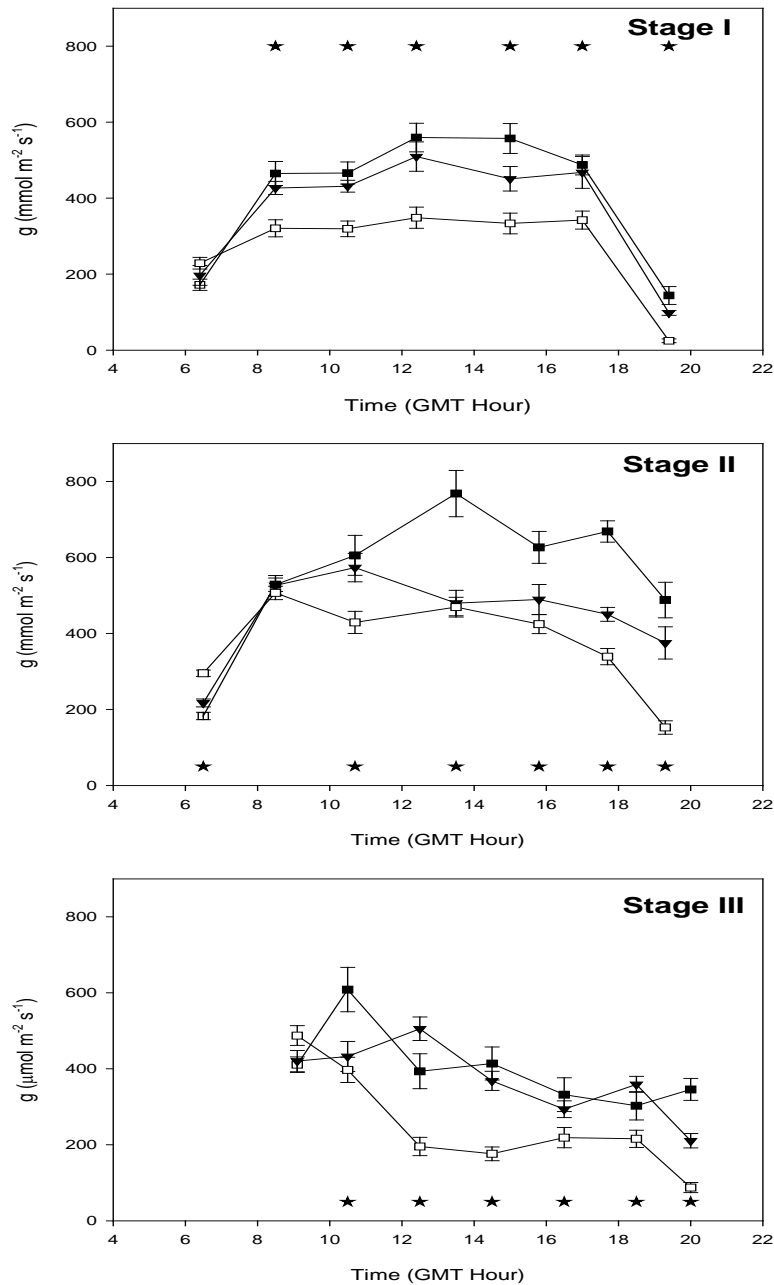


Fig. 2. Daily pattern of stem water potential ( $\Psi_x$ ) during the stages I, II, and III. Each point is the average of 5 measurements. ■ C treatment □ RF treatment ▼ RDI treatment. Asterisks denote the dates when significant differences were found among treatments (see text for explanation).



The gas exchange of the trees were characterised by measuring the leaf conductance ( $g_s$ ) throughout the day on the same dates that those selected for  $\Psi_x$  measurements (Fig.3). During the Stage I (Fig. 3a) the  $g_s$  of RF trees increased early in the morning until 8:00 GMT, reaching the maximum values at that time. In C and RDI trees  $g_s$  was maximum at midday. From the time that maximum  $g_s$  was reached it was roughly constant until the sunset, around 18:00 GMT, then decreasing in response to darkness. Stomatal conductance of RF trees was significantly lower than the control and RDI treatments, except in the first point early in the morning. The maximum values of  $g_s$  were around  $340 \text{ mmol m}^{-2}\text{s}^{-1}$  in RF trees, while in control and RDI trees were around  $500 \text{ mmol m}^{-2}\text{s}^{-1}$ . The daily patterns of  $g_s$  during Stage II were similar to those of the Stage I with a faster increase at the beginning of the day and higher maximum values.  $g_s$  ranged from 200 to  $750 \text{ mmol m}^{-2}\text{s}^{-1}$ , being those of the control treatment significantly higher than the other two treatments during all the day. The maximum  $g_s$  of rainfed trees was around  $450 \text{ mmol m}^{-2}\text{s}^{-1}$ , while in control trees was around  $700 \text{ mmol m}^{-2}\text{s}^{-1}$ .  $g_s$  in RDI treatment was significantly lower, around  $500 \text{ mmol m}^{-2}\text{s}^{-1}$ , than in control trees only from 13:30 to 17:30, when the maximum values were measured. In the daily cycle of Stage III the first data could not be taken due a technical problem (Fig. 3c). The daily pattern of stomatal conductance was different to those reported previously for Stages I and II in control and RDI treatment, showing a decrease throughout the day from before or around midday in C and RDI treatments, respectively. Also, the maximum  $g_s$  values in these two treatments were slightly lower than those showed in Stages I and II for the same treatments. In RF trees there was a higher and earlier stomata closure, showing a minimum  $g_s$  around midday, and staying roughly constant until the sunset. The leaf conductance in all the treatments in this date was clearly lower than that measured during Stage II but only slightly lower than that reported in Stage I. Maximum  $g_s$  values of different treatments occurred at different times, first in RF treatment ( $487 \text{ mmol m}^{-2}\text{s}^{-1}$ ), after that in C treatment ( $608 \text{ mmol m}^{-2}\text{s}^{-1}$ ) and finally in RDI treatment ( $505 \text{ mmol m}^{-2}\text{s}^{-1}$ ). There were very small

differences between C and RDI treatments, some of them not statistically significant. However  $g_s$  was significantly lower in RF treatment.



**Fig. 3.** Daily pattern of leaf conductance ( $g_s$ ) during the stages I, II, and III. Each point is the average of 12 measurements. ■ C treatment □ RF treatment ▼ RDI treatment. Asterisks denote the dates when significant differences were found among treatments (see text for explanation).

Leaf net photosynthesis ( $P_n$ ) was also measured on the same dates than  $\Psi_x$  and  $g_s$  (Fig. 4). In the Stage I the daily pattern of  $P_n$  was almost identical to the leaf conductance. Only at the end of the day  $P_n$  decreased faster than  $g_s$  in response to decreasing levels of radiation (Fig. 4a). The  $P_n$  values were similar between treatments and only slightly differences were found in the first three data taken. RF trees reached the maximum values earlier than control and RDI but such differences were likely related to the time lag from the first (C) and the last (RF) trees measured, as radiation increased very fast. In the next two measuring times, with no limitation of radiation,  $P_n$  of RF trees was significantly lower than in the C and RDI trees, but from midday no significantly differences were found among treatments. The daily patterns of  $P_n$  during Stage II were similar to those of Stage I (Fig. 4b), but the maximum values in Stage II were a 50% higher than in Stage I,  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$  compared to  $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ . The differences between irrigated treatments (C and RDI) and RF treatment were again small but significant at 10:00 GMT and in the last two measuring times.  $P_n$  of RDI and C trees were similar except at the beginning and at the end of the day. These differences were attributed to the same time-lag effect explained above. At the beginning and at the end of the day radiation varied very fast, increasing the variability of  $P_n$  measurements as the leaves were receiving different light intensity. Maximum values in Stage III were similar to those of the Stage I (Fig. 4c). The differences between treatments were short but significant between RF and the irrigated trees, both C and RDI, throughout the day.

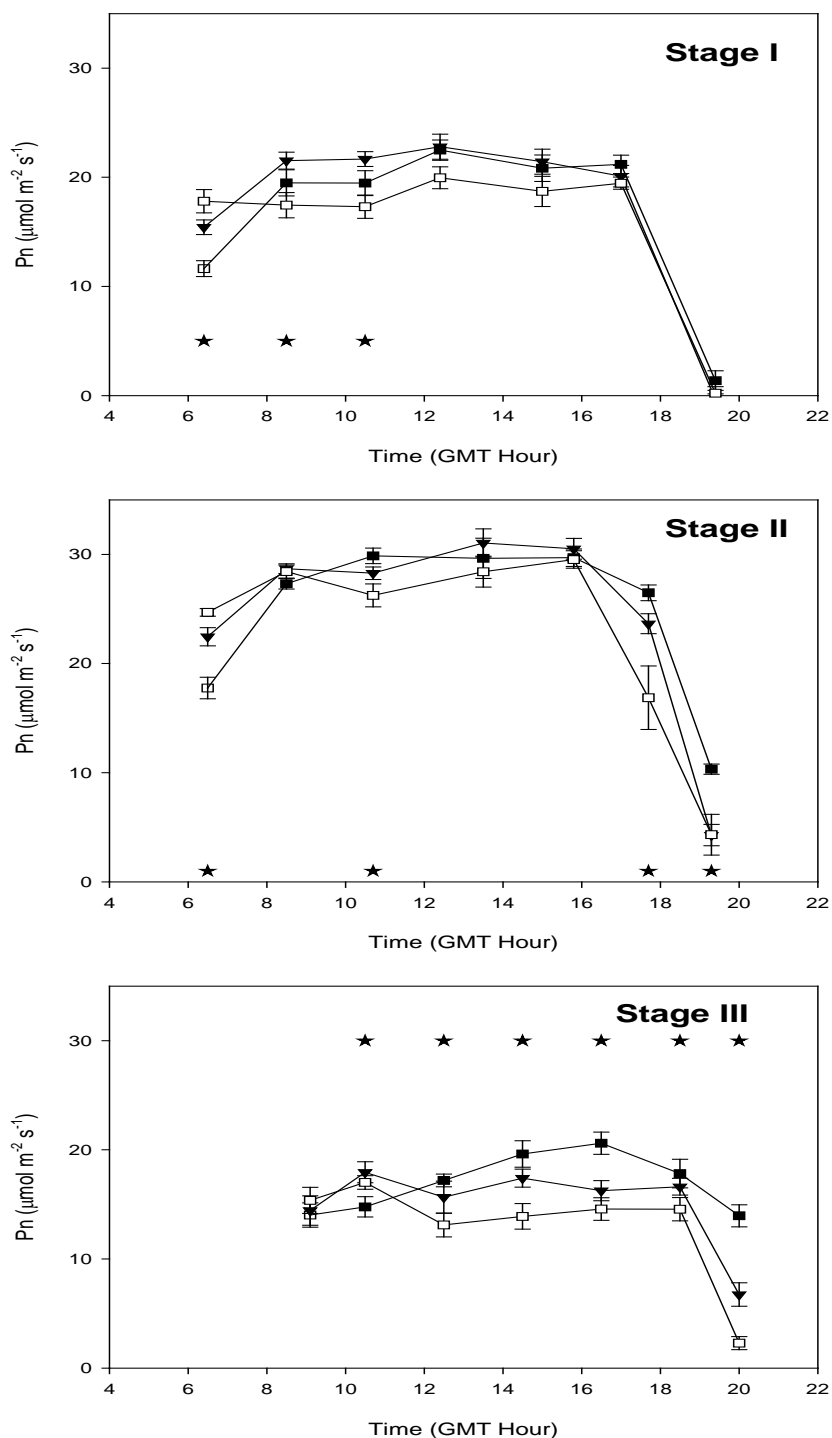
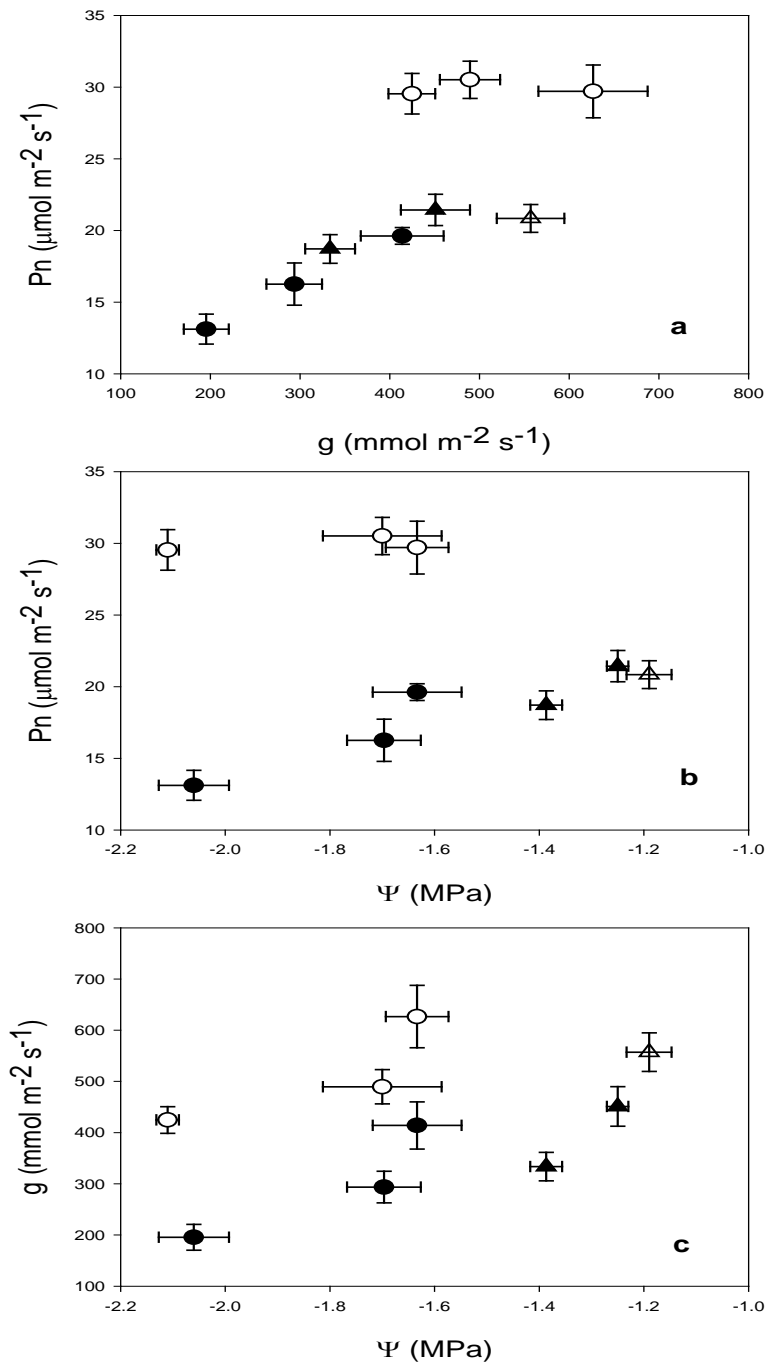


Fig. 4. Daily pattern of leaf photosynthesis (Pn) during the stages I, II, and III. Each point is the average of 12 measurements. ■ C treatment □ RF treatment ▼ RDI treatment. Asterisks denote the dates when significant differences were found among treatments (see text for explanation).

The examination of the relationships between water potential, leaf conductance and leaf net photosynthesis could clarify the response of the trees to water deficits in each phenological stage (Fig. 5). The data used to obtain such relationships were those measured at midday. The relationship between leaf P<sub>n</sub> and g<sub>s</sub> was poor when all the data were considered (Fig. 5a). For the same g<sub>s</sub>, midday P<sub>n</sub> was much higher in Stage II than in the other two phenological stages. However, when only Stage I and III were considered there was no significant relationship. Also the relationship between leaf P<sub>n</sub> and Ψ<sub>x</sub> was poor when the Stage II data were considered (Fig. 5b). The values of midday leaf P<sub>n</sub> during this phenological stage were the highest at low water potential. When only Stages I and III were considered a significant but weak relationship between P<sub>n</sub> and Ψ<sub>x</sub> was found (R<sup>2</sup>=0.50\*). Finally, the relationship between leaf conductance and water potential (Fig. 5c) was similar to the P<sub>n</sub>-Ψ<sub>x</sub> (Fig. 5b). The g<sub>s</sub> values of Stage II were closer to the other treatments but again higher for similar levels of dehydration. In this case, when Stage II data were considered alone a significant but weak linear relationship was found (R<sup>2</sup>=0.53\*\*\*).

**Table 1. Pressure-volume curves parameters obtained in each phenological stage in the rainfed and control treatments. Each value is the average of 5 data. For the same stage, different subscript mean significant differences between treatments (Tukey Test; P<0.05). For the same treatments, different superscripts letters mean significant differences among phenological stages (Tukey Test; P<0.05). RWC<sub>0</sub>: Relative water content at zero turgor; Ψ<sub>s,0</sub>: osmotic water potential at zero turgor; Eo: elastic modulus; Ψ<sub>s,100</sub>: osmotic water potential at full turgor; Eo<sub>av</sub>: average elastic modulus; R: symplastic water content.**

	Stage I		Stage II		Stage III	
	Rainfed	Control	Rainfed	Control	Rainfed	Control
<b>RWC<sub>0</sub> (%)</b>	86	85 <sup>b</sup>	90	92 <sup>a</sup>	85	88 <sup>ab</sup>
<b>Ψ<sub>s,0</sub> (MPa)</b>	-2.3	-2.5	-2.4	-2.0	-2.7	-2.6
<b>Eo (MPa)</b>	15.7 <sup>b</sup>	15.3	32.2 <sup>a</sup>	29.5	9.8 <sup>ab</sup>	26.6 <sup>a</sup>
<b>Ψ<sub>s,100</sub> (MPa)</b>	-1.8	-2.0	-1.6	-1.5	-2.0	-1.5
<b>Eo<sub>av</sub> (MPa)</b>	13.3	13.4 <sup>b</sup>	16.5	19.9 <sup>a</sup>	13.8	12.4 <sup>b</sup>
<b>R (%)</b>	65 <sup>a</sup>	63 <sup>a</sup>	34 <sup>b</sup>	30 <sup>b</sup>	55 <sup>ab</sup>	47 <sup>ab</sup>



**Fig. 5. Relationship between the midday leaf conductance and leaf photosynthesis (a); midday leaf photosynthesis and midday stem water potential (b); and midday leaf conductance and midday stem water potential (c). Each point is the value obtained in Figs 2 to 4. ▲ Stage I ; ○ Stage II ; ● Stage III. SE bars are depicted for both parameters in each average value.**

One of the plant responses to water stress is the osmotic adjustment. The P-V curve technique can give information about these mechanisms of response. The P-V curves were performed only with data of RF and C treatments (Table 1). There were no significant differences between treatments in any of the parameters estimated due to the high coefficient of variation. The relative water content at zero turgor ( $RWC_0$ ) showed no differences among treatments and phenological stages (occurred at around 90% RWC). The water potential at zero turgor ( $\Psi_{s,0}$ ) varied from -2.0 MPa (Stage II - Control) to -2.7 MPa (Stage III - Rainfed), but again differences were not statistically significant. The average  $\Psi_{s,0}$  value was similar and around -2.3 MPa. The elasticity modulus ( $E_o$ ) presented a seasonal trend, with higher values in Stage II (around 33 MPa) than in Stage I (around 15 MPa).  $E_o$  values in Stage III were opposite to expected as RF trees showed significantly lower  $E_o$  than control, while they were similar in stages I and II. The variability of this parameter decreased when the calculation was made between zero and full turgor point ( $E_{o_{av}}$ ).  $E_{o_{av}}$  values were similar in Stages I and III (around 13 MPa), being slightly higher in Stage II (around 1.7 MPa), but with no significant differences between treatments. The osmotic potential at full turgor ( $\Psi_{s,100}$ ) was not significantly different among treatments. The mean values of  $\Psi_{s,100}$  were around -1.9 MPa, being only in stage II slightly lower in both treatments (around -1.6 MPa). The symplastic water content (R) was again similar in both irrigation treatments but differed between different phenological stages. Maximum values of symplastic water content were around 65% in Stage I, then it sharply decreased in Stage II reaching values around 35% and finally it increased to 50% during Stage III.

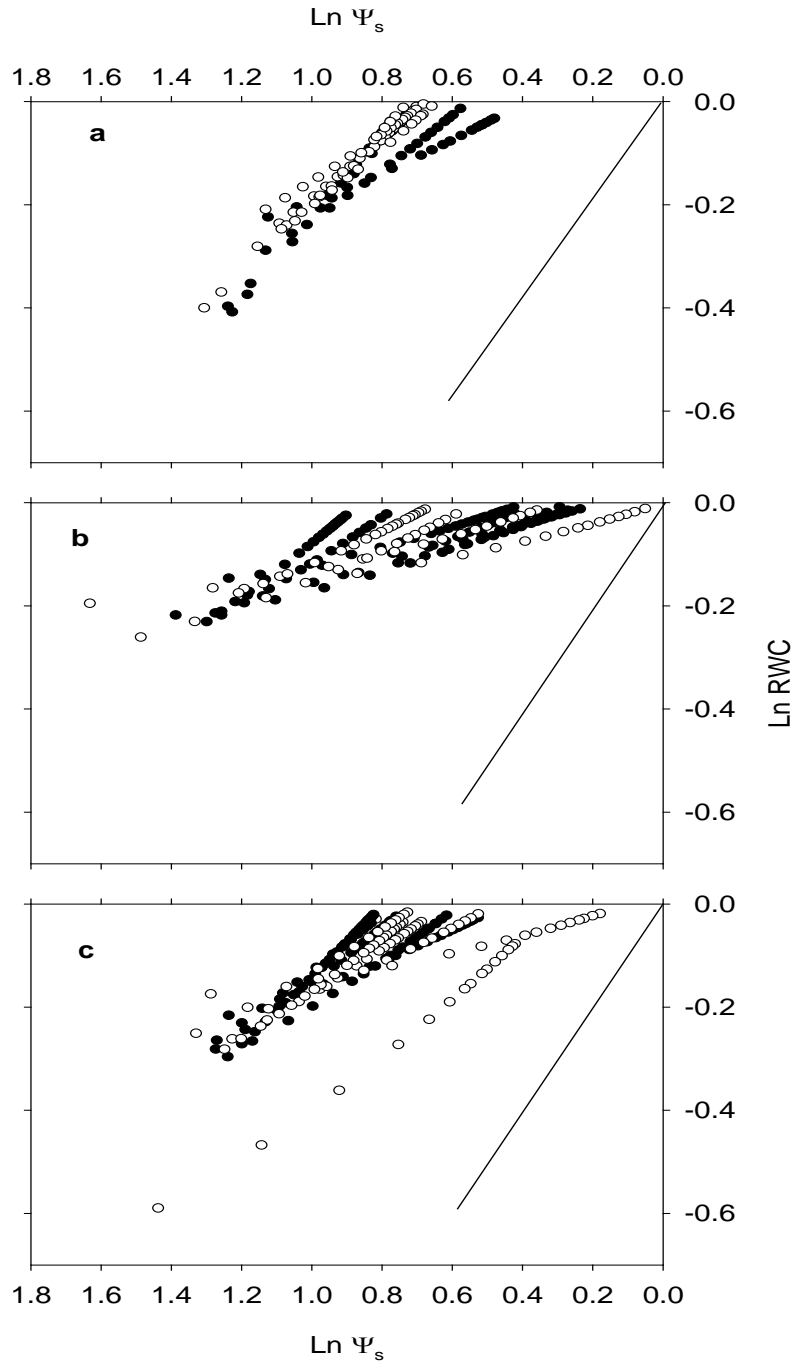
Turner (2006) suggested a methodology to compare the magnitude of osmotic adjustment between species and their degree of adaptation to drought. In this work, this methodology has been used to compare the degree of osmotic adjustment of different phenology stages for a given irrigation treatment. The osmotic potential data have been calculated from the P-V curves. Turner (2006) derived two indexes (OA index and BP, as

described in Material and Methods section) from the relationship between the natural logarithms of osmotic potential and relative water content. Fig. 6 depicts such relationships and the regression equations are shown in Table 2. There were no significant differences between RF and C trees in BP (Table 2). However, for the control treatment BP was significantly lower at Stage I than Stage II and III showing values around -1.50, -1.10 and -1.30 MPa, respectively. There were neither significant differences in OA index between C and RF treatments for the same phenological stage. However, the seasonal pattern changed with phenology. The higher degree of osmotic adjustment was found in Stage II, showing OA values of 0.78, while in Stages I and III it was 0.42 and 0.62, respectively.

**Table 2. Regression equations of the relationship between natural logarithms of osmotic potential ( $\Psi_s$ ) and the relative water content (RWC) grouped in the three phenological phases. Each equation corresponds with the pressure volume curves performed during the experiment. The data of osmotic potential was calculated with the pressure volume curves. Different letters mean significant differences (Tukey Test;  $P < 0.05$ ) between phenological stages in the same column and treatment. There were no significant differences between irrigation treatments. BP: breaking point. OA index: osmotic adjustment index.**

Phenological Phase	Control				Rainfed			
	$\text{Ln } \Psi_s = a + b * \text{LnRWC}$				$\text{Ln } \Psi_s = a + b * \text{LnRWC}$			
	a	b	BP(MPa)	OA Index	a	b	BP(MPa)	OA Index
Stage I	0.39	-0.58	-1.48 b	0.42 b	0.37	-0.59	-1.45	0.41b
Stage II	0.14	-0.27	-1.15 a	0.78 a	0.09	-0.22	-1.09	0.73a
Stage III	0.19	-0.38	-1.21 a	0.62 a	0.33	-0.48	-1.39	0.52ab





**Fig. 6. Relationship between  $\text{Ln RWC}$  and  $\text{Ln } \Psi_s$  during stages I, II and III. Points were calculated from the P-V curves of the  $\circ$  C treatment and  $\bullet$  RF treatment. The line represents the same variations between the RWC and the osmotic potential. Points in this line indicate that the increase in the osmotic pressure is related with the decrease in the water content.**

## Discussion

The daily pattern of stem water potential (Fig. 2), leaf conductance (Fig. 3) and net photosynthesis (Fig. 4) indicated that pistachio trees are tolerant to mild dehydration conditions. The leaf conductance was maximum at midday, even in conditions of high vapour pressure deficit (VPD) (Fig. 3). Leaf conductance was very high, especially during stage II in control trees. The values reported in literature usually are lower than those obtained in this experiment. However, Goldhamer et al. (1985) and Vemmos (1994) give similar  $g_s$  values to those presented in this work. In these two works the leaf conductance was measured with an infrared gas analyser and includes both, abaxial and adaxial conductances, as in the values presented here. In this work, when only abaxial leaf conductance was measured by using a steady state porometer maximum values around 450-500  $\text{mmol m}^{-2} \text{s}^{-1}$  were found (Gijón et al., 2008). The daily pattern of net photosynthesis was similar to that of leaf conductance but differences among treatments were lower. Maximum values of Pn changed throughout the season with higher values during stage II than in the other two (Fig. 4). Similar changes in net photosynthesis have been described in pistachio under non-stress conditions and were related to leaf age (Li et al., 1984; Vemmos, 1994; Novello, 1998). In addition, net photosynthesis at midday was linearly related to leaf conductance up to a  $g_s$  value of 450  $\text{mmol m}^{-2} \text{s}^{-1}$  in this experiment (Fig. 5a) that may indicate a partial stomatal control as it has been reported in other drought tolerant species as olive trees (Moriana et al., 2002).

The daily pattern of  $g_s$  was clearly affected by water stress, especially at Stage III when the degree of water stress was higher (Fig. 3). In that stage, not only the maximum  $g_s$  value but the pattern itself was different in RF from control trees. The RF tree pattern showed a reduction of leaf conductance at midday, while maximum values were still measured in the irrigated treatments (Fig. 3c). However, assimilation was less sensitive to water stress, as Pn differences between C and RF trees

were lower than those found in  $g_s$  as the stress progressed (Fig. 4). In Stage III, when the lowest values of  $\Psi_x$  were measured, the stomata started to close before midday in RF trees but assimilation stayed fairly constant until the sunset. These results support that in pistachio the assimilation processes other than the diffusion component are less sensitive to water stress and operate at lower levels of leaf water potential. Therefore, this species can be considered as tolerant to mild dehydration as it maximizes the assimilation rate under these stress conditions. This has been described as a common response in arid habitats in species like *Quercus* (Tenhunen et al., 1983) and may partially explain the high drought resistance of pistachio trees (Behboudian et al., 1986; Ferguson, 2005). There are several physiological mechanisms in plants to survive in drought-prone environments (Feres, 1984) which are shown by different species and depend on the water stress level. The stomatal control is usually linked to a threshold level of leaf water potential, below that the leaf resistance increases progressively (Tenhunen et al., 1983; Feres, 1984).

The relationships between the midday values of stem water potential, net photosynthesis and leaf conductance were different depending on the phenological stage. Stages I and III presented similar relationships (Fig. 5) with a good lineal fit (Fig. 5a:  $R^2=0.84$ ; Fig. 5b:  $R^2=0.86$ ; Fig. 5c:  $R^2=0.76$ ). However, in stage II those relationships were clearly different. These variations indicate that some physiological changes are occurring during this phenological stage. The net photosynthesis during Stage II was almost constant with variations of leaf conductance and stem water potential that indicated a drought adaptation during this period of time. Therefore, the stomatal or hydraulic control of assimilation starts at a lower threshold in the phenological Stage II than in Stages I and III. Sajjadinia et al. (2010) also reported an increase in transpiration during the endocarp hardening in different cultivars of pistachio subjected to water deficit.

There were no significant differences in the pressure-volume curves data (Table 1). These results are likely related to the water stress imposed

which was probably too fast to find significant differences between treatments (Turner and Jones, 1980). However, these results also show clear variations of some parameters depending on the phenological stage of the trees. During stage II, the elastic modulus ( $E_o$  and  $E_{o_{av}}$ ) was clearly higher than in the rest of the season, while the percentage of symplastic water was reduced in the same period of time. An increase of the elastic modulus has been described as an adaptation to drought in other species like olive (Dichio et al., 1997) and pear (Marsal and Girona, 1997). The decrease of symplastic water has been also reported as a drought response in pear (Marsal and Girona, 1997), carob (Nunes et al., 1989), *Pseudotsuga menziesii* (Mirb) Franco (Joly and Zaerr, 1987), *Acer rubrum* L (Nash and Graves, 1993) but no in olive trees which show the opposite effect (Dichio et al., 1997). In addition, Marsal and Girona (1997) have also described differences in some of these parameters throughout the season in full-irrigated pear trees. Such variations indicated that during Stage II, even under non-stress conditions, the trees modified several water-relations parameters. These modifications would confer higher drought tolerance to the trees during this stage. In pistachio the shell wall is thickening during Stage II, which is considered the less sensitive period to water stress (Goldhamer, 2005). The wall thickening is considered as the greatest metabolic cost in stone fruit trees and variations in carbon balance and water relations related to different fruit loads have been reported in peach (DeJong, 1986) and in olive (Rallo and Suarez, 1989).

The osmotic adjustment index and the break point parameter proposed by Turner (2006) allow the comparison between different species in relation to the response to drought conditions, and in this study they will be used to compare different phenological stages. The higher OA index in Stages II and III (Table 2) indicated a higher degree of osmotic adjustment in these periods than in Stage I. The variations in elastic modulus and percentage of symplastic water during Stages II and III may be related to this higher osmotic adjustment than in Stage I. These values of OA index are similar to those reported for high osmotic adjusting lines of wheat (Turner, 2006). The BP estimated the osmotic potential at which

the RWC starts to decrease and it is an estimation of drought resistance. In pistachio, BP values presented in the Stage I were lower than Stages II and III in control trees (Table 2) and were similar to those reported for drought-sensitive sorghum lines and higher than those reported for wheat (around -3 MPa, Turner 2006). All these results suggest that different mechanisms of drought resistance are operating in pistachio at different phenological stages. In Stage I the pistachio trees tolerate low water potentials before osmotic adjustment takes place, showing lower BP values. In Stages II and III the degree of osmotic adjustment increases resulting in higher values of OA index. This would be the most important mechanism of drought resistance in this species.

## **Conclusions**

The water relations in pistachio trees are dynamics and change throughout the season. In mild water stress conditions, like those occurring during stage I, the differences in  $g_s$  between stressed non-stressed plants are small enough to allow similar values of net photosynthesis. As water stress progresses, also variations in the daily pattern of  $g_s$  were found. Instead of a roughly constant maximum  $g_s$  when light is not limiting as occurred in stages I and II, there was a progressive stomatal closure from before the midday. However, net photosynthesis was less sensitive to water stress than stomata, maximizing plant assimilation in conditions of mild water stress. In addition, during Stage II the leaves showed some physiological changes like variations in the elastic modulus and percentage of simplastic water. Such variations seem to be related to a higher degree of osmotic adjustment during Stages II and III. These differential changes in the water relations of pistachio trees in different phenological stages support the recommendation of reducing the irrigation during stage II with no or slight decrease in crop yield.

## **Acknowledgments**

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# DISCUSIÓN GENERAL







La eficiencia del riego en el presente trabajo (Capítulo I) varió dependiendo del componente de la cosecha considerado. La cosecha total se incrementó de forma lineal hasta aproximadamente los 250 mm de evapotranspiración del cultivo (ETc). A partir de estos valores de ETc la relación tiende a saturarse y a mantenerse en torno a valores máximos de 160 kg ha<sup>-1</sup> m<sup>-3</sup> de volumen de copa (Fig. 9a, Capítulo I). Esto supone que en determinados estados fenológicos la restricción de la ETc no da lugar a una disminución en cosecha. Resultados similares se han sugerido en otros trabajos de pistachero (Phene et al., 1989; Monastra, 1995) aunque no se había cuantificado este tipo de respuesta. Esta función de producción no es la habitual, especialmente en especies herbáceas (Vaux y Pruitt, 1983) aunque diferentes estudios de riego deficitario en frutales (Behboudian y Mills, 1997; Behboudian et al., 2011) sugieren que al menos en las especies más resistentes a sequía se podría esperar una respuesta similar a la obtenida, como por ejemplo la descrita en olivo (Moriana et al., 2003).

La producción de frutos abiertos también se correlacionó positivamente con la ETc, pero esta vez de forma lineal (Fig. 9 b, Capítulo I), lo cual sugiere que este parámetro en pistachero es muy sensible al estrés hídrico. Varios autores han encontrado, sin embargo, que el porcentaje de frutos abiertos no se ve afectado cuando la restricción del riego se realiza durante la fase II del cultivo (Phene et al., 1987; Goldhamer et al., 2004; Goldhamer y Beede, 2004) o que incluso se incrementa si se realiza en la fase I (Goldhamer y Beede, 2004), lo que debería de dar lugar a una respuesta similar a la de la cosecha total. Puede que en los ensayos experimentales de este trabajo el nivel de estrés hídrico en los árboles en fase I fuese insuficiente o no se mantuviera el tiempo necesario para tener un incremento significativo de frutos abiertos. Hsiao (1990) sugiere que el efecto real de un periodo de estrés hídrico en un estado fenológico concreto es la suma de la duración y del nivel alcanzado. Por otro lado, teniendo en cuenta que el portainjerto empleado en los árboles de este trabajo difiere del de los experimentos desarrollados en California, se podría esperar un comportamiento diferente de *Pistacia terebinthus* L. en condiciones de estrés hídrico. Guerrero et al. (2006)

trabajando en las mismas condiciones edafoclimáticas en un ensayo de patrones concluye que *P. terebinthus* es más adecuado para condiciones de secano que *Pistacia atlantica*. Kanber et al. (1993), trabajando con cultivares y patrones diferentes a los ensayos californianos y a los de este trabajo, tampoco encontraron diferencias significativas en el porcentaje de frutos abiertos entre secano y regadío.

La respuesta al estrés hídrico del cultivar está, por tanto, influenciada de manera muy importante por el portainjerto. Los niveles de estrés hídrico a los que fueron sometidos los árboles en el ensayo en macetas (Capítulo II) fueron mucho mayores que los obtenidos en campo, lo que permitió comparar los portainjertos y describir la respuesta fisiológica a condiciones de estrés hídrico severo. El estrés hídrico, incluso a niveles moderados (alrededor de -2 MPa) produjo una parada drástica del crecimiento y un cierre estomático en todos los portainjertos (Figs. 3, 4, 5 y 8, Capítulo II). En ambos casos se trata de respuestas ampliamente descritas (Hsiao, 1990). Sin embargo, teniendo en cuenta los ciclos diarios de conductancia y fotosíntesis foliar presentados en el Capítulo III (Figs. 3 y 4) es probable que la carencia de agua de lugar a una disminución mayor de la transpiración que en especies consideradas resistentes a sequía. El ciclo diario de intercambio gaseoso en ausencia de estrés hídrico en pistachero no varió por efecto del déficit de presión de vapor (Figs. 3 y 4, Capítulo III). Esta respuesta es similar a la descrita, por ejemplo, en especies de *Quercus* (Tenhunen et al., 1987) pero diferentes a las descritas en especies como el olivo en la que hay una disminución de la conductancia al mediodía (Xiloyannis et al., 1988). En condiciones de estrés hídrico durante la fase III, la disminución de la conductancia en pistachero llevó consigo un drástico cambio del ciclo diario dando lugar a un cierre estomático al mediodía (Fig. 3, Capítulo III). Este cambio en el intercambio gaseoso, posiblemente, de lugar a cambios más acentuados en la transpiración que los que se producen, por ejemplo, en olivar. Sin embargo, los datos del Capítulo III (Fig. 5) sugieren que la fotosíntesis precisa de niveles de deshidratación más elevados que la conductancia para que se produzca una disminución del mismo grado. Estos resultados

irían en la línea de los sugeridos en otras especies en las que el estrés hídrico da lugar a la acumulación de sustancias procedentes de las raíces (especialmente el ABA) que inducen un cierre estomático pero no afecta con la misma intensidad a la asimilación (Zhang y Davies, 1990).

Cuando el nivel de deshidratación se incrementa hasta niveles severos (Fig. 7, Capítulo II) la respuesta obtenida es diferente según el portainjerto considerado. En el patrón híbrido (*Pistacia atlantica* x *Pistacia vera*) hay una reducción severa del área foliar en relación a *P. terebinthus* aunque no en el número de hojas (Figs. 4 y 5a, Capítulo II). Por otro lado, el peso seco del tallo y la hoja fue significativamente menor en el híbrido por efecto del déficit hídrico, mientras que en *P. terebinthus* el estrés hídrico redujo menos el peso seco de la hoja no afectando al del tallo (Fig 5b, Capítulo III). Esto sugiere mecanismos de respuesta de la variedad claramente diferenciados según el portainjerto (Fig. 9b, Capítulo I). El mayor vigor del híbrido lo hace más aconsejable como portainjerto en condiciones de moderado déficit hídrico, mientras que en condiciones de estrés severo *P. terebinthus* podría tener una respuesta más favorable al reducir menos su área foliar en condiciones de estrés.

Es evidente, por lo tanto, que la estrategia de riego nos va a condicionar las decisiones a tomar. Estadísticamente hablando, no hubo diferencias significativas en la cosecha total, el porcentaje de frutos abiertos y el de vacíos entre las distintas estrategias de riego planteadas en el Capítulo I (Tabla 3). Sin embargo, de la relación de estos componentes de la cosecha calculados bianualmente y la ETc (Fig. 9, Capítulo I), se deduce que las restricciones de agua, especialmente las del tratamiento de secano y el deficitario al 50%, afectan a la cosecha total y a la cantidad de frutos abiertos. La estrategia deficitaria al 65% parece tener un comportamiento intermedio entre éstas y el control, seguramente muy relacionado con el nivel y el momento de estrés hídrico que podrían variar mucho entre estaciones. Los riegos deficitarios progresivos son recomendados en algunas especies, como el olivar, por su sencillez a la hora de la aplicación y sus buenos resultados sin diferencias aparentes con el riego deficitario controlado (RDC) (p.ej. Iniesta et al., 2009). Sin

embargo, en este trabajo los datos del tratamiento al 65% ponen en evidencia que en este tipo de manejo son de esperar grandes variaciones en la cosecha dependiendo del año.

El RDC, por el contrario, redujo la cantidad de agua aplicada en un 20% (Tabla 2, Capítulo I), sin apreciarse diferencias en la cosecha con el control (Tabla 3 y Fig. 9, Capítulo I), aunque el estado hídrico del árbol se vio afectado en la fase II (Fig. 3 y 4, Capítulo I). Estos datos están en consonancia a lo publicado en trabajos anteriores (Phene et al., 1987; Goldhamer y Beede, 2004). El manejo RDC además presentó la menor alternancia de todos, incluso por debajo del tratamiento control. Aunque el mecanismo de vecería no ha sido completamente aclarado en pistachero, distintos estudios muestran que probablemente es el resultado de la competencia por los carbohidratos entre los frutos de una temporada y las yemas de flor que se están formando para la temporada siguiente (Barone et al., 1995; Stevenson y Shackel, 1998; Stevenson et al., 2000; Ferguson et al., 2005; Marra et al. 2010). A mayor producción, mayor será la competencia por los hidratos de carbono, y por tanto, mayor caída de yemas y vecería. Sin embargo, esta tendencia puede verse atenuada si se logra un equilibrio entre la fuente de carbohidratos (fotosíntesis) y el sumidero (número de yemas de flor). Los resultados obtenidos en este ensayo sugieren que un estrés hídrico moderado durante las fases I y II puede reducir la inducción y diferenciación de yemas de flor, con lo que disminuye el tamaño del sumidero. La recuperación durante la fase III en el tratamiento RDC provoca que la acumulación de reservas sea suficiente, de manera que la abscisión sea la menor de todas con este tipo de programación, incluso menor que la del control.

La resistencia al estrés hídrico durante la fase II de endurecimiento de la cáscara está relacionada con cambios fisiológicos que realiza el árbol en este periodo. En ausencia de estrés hídrico la conductancia estomática y la fotosíntesis neta se incrementan durante la fase II, respecto a las fases I y III ( Figs. 3 y 4, Capítulo III). Esta respuesta podría estar relacionada con un porcentaje mayor de hojas totalmente expandidas en la fase II que en la fase I (que aún estarían creciendo) o en la fase III

(donde empezaría la senescencia). El árbol además presenta un patrón estacional de evolución fisiológica muy característico. Durante la fase II, la conductancia estomática y en menor grado la fotosíntesis están menos afectadas por la deshidratación foliar que en las otras dos fases (Fig. 5, Capítulo III). Por otro lado, el potencial hídrico al que tendría lugar el comienzo del ajuste osmótico es menor en la fase I que en las fases II y III (punto de rotura (BP), Tabla 2, Capítulo III). Todo ello sugiere la existencia de mecanismos distintos al ajuste osmótico para evitar la deshidratación en la fase inicial (fase I). El retraso en el comienzo del ajuste osmótico podría estar relacionado con una estrategia de ahorro de metabolitos que son posiblemente más necesarios durante la brotación (fase I) que posteriormente. Una vez que una gran parte de la masa foliar está expandida, a partir del comienzo de la fase II, el intercambio gaseoso se hace menos sensible a las variaciones de potencial hídrico foliar y es a partir de aquí cuando el ajuste osmótico comienza antes que en la fase I (punto de rotura (BP) según la metodología de Turner (2006)). A partir de este momento (fase II) también se incrementan significativamente los valores del índice de ajuste osmótico (OA index). Esto coincide con módulos de elasticidad mayores y contenidos de agua simplástica menores en fase II que en las otras dos fases. Por lo tanto, el mecanismo de respuesta al déficit hídrico varía respecto a la fase I, lo que explicaría la mayor capacidad de restricción del agua de riego en esta fase II. El aumento en el módulo de elasticidad ha sido descrito como una adaptación a la sequía en otras especies como el olivo (Dichio et al., 1997) y peral (Marsal y Girona, 1997). La disminución de agua simplástica también ha sido considerada como una respuesta a la sequía en peral (Marsal y Girona, 1997), algarrobo (Nunes et al., 1989) y *Acer rubrum* L (Nash y Graves, 1993), pero no en olivo donde se ha encontrado el efecto contrario (Dichio et al., 1997). Marsal y Girona (1997) también han descrito diferencias en algunos de estos parámetros a lo largo del ciclo de cultivo en perales regados al 100% de sus necesidades. Estos cambios fisiológicos durante la fase II (mayor rapidez en realizar el ajuste

osmótico, mayor módulo de elasticidad y menor agua simplástica) explicaría la mayor resistencia al déficit hídrico en este estado fenológico.

# CONCLUSIONES







**En cuanto a las relaciones hídricas según la fase fenológica y las distintas dotaciones de riego (Capítulo III) podemos concluir que:**

1. Las relaciones hídricas en pistachero se comportan de una forma dinámica, cambiando a lo largo de toda la estación de crecimiento dependiendo del estado fenológico de la planta.
2. A medida que va aumentando el estrés hídrico, también se observan variaciones en el patrón diario de conductancia estomática, disminuyendo ésta desde antes del mediodía. Sin embargo la asimilación de carbono es menos sensible que la conductancia estomática en condiciones de estrés hídrico moderado.
3. Durante la fase II, las hojas mostraron algunos cambios fisiológicos, tales como variaciones en el módulo de elasticidad y en el porcentaje de agua simplástica. Esto parece estar asociado con un alto grado de ajuste osmótico durante la fase II, que sigue existiendo en la fase III.
4. Los diferentes cambios en las relaciones hídricas apoyan la idea de reducir el riego durante la fase II sin reducciones significativas en el rendimiento del cultivo.

**En cuanto a la respuesta productiva al riego deficitario controlado (Capítulo I), se concluye que:**

5. No hubo una relación lineal entre la producción total y evapotranspiración del cultivo (ETc). El riego deficitario controlado (RDC) supuso un ahorro de agua de un 20% sin encontrarse variaciones significativas en la productividad entre este tratamiento y los árboles regados al 100% de sus necesidades. Además, el

tratamiento RDC amortiguó de forma significativa el ciclo vecero respecto al resto de tratamientos.

6. El rendimiento total de los árboles corregido para el volumen de copa se correlacionó positivamente con la ETc sólo hasta valores aproximados de 250 mm.
7. El riego incrementa tanto la cantidad como la calidad de la cosecha, aunque el aumento en el porcentaje de frutos abiertos está mucho más relacionado con el aumento de la producción que con el estado hídrico del árbol.
8. Los árboles injertados sobre el portainjerto *Pistacia terebinthus* L. mostraron, en los tratamientos de riego deficitario, condiciones de estrés hídrico inferiores a las esperadas. Esto podría indicar que induce una mayor resistencia al estrés hídrico que otros portainjertos y que los recortes de riego en las fases I y II (del 50% en el ensayo) podrían, incluso haber sido mayores.
9. La reducción de la vecería producida en los tratamientos deficitarios respecto del control es probablemente producto de un mejor balance de carbono en el árbol. Posiblemente el estrés hídrico en las fases I y II redujo el número de las yemas de flor inducidas lo que se ajustó mejor a la capacidad de asimilación durante la caída de yemas propia de la etapa III.
10. El porcentaje de frutos rajados mostró un patrón similar en todas las estaciones de crecimiento con un incremento drástico al comienzo del mes de Septiembre. Esto sugiere que en el presente ensayo esta variable no estuvo claramente relacionada con el estrés hídrico. Sin embargo, la disminución de la temperatura mínima por debajo de 13 °C aumentó el porcentaje de frutos

rajados, lo que sugiere este factor como uno de los relacionados con este proceso.

**En cuanto a la influencia del portainjerto en el crecimiento y las relaciones hídricas del cultivar Kerman tanto en la etapa de estrés hídrico como en la de posterior recuperación (Capítulo II), se concluye que:**

11. El comportamiento del cultivar Kerman en lo que se refiere al crecimiento y las relaciones hídricas depende del portainjerto sobre el que ha sido injertada.
12. El portainjerto en el que se observó una mayor sensibilidad al déficit hídrico fue *P. atlantica*, con un bajo control estomático de la transpiración. Sin embargo estos resultados se deben tomar con cuidado, dado que el grado de estrés hídrico impuesto fue más ligero en relación a las plantas injertadas sobre los otros portainjertos, como resultado del bajo desarrollo foliar.
13. Las plantas injertadas sobre *P. terebinthus* mostraron un alto grado de control estomático, y ligeramente mayores niveles de estrés hídrico que el híbrido.
14. Las plantas injertadas sobre el patrón híbrido sufrieron un estrés hídrico ligeramente menor y mostraron en términos absolutos un cierre estomático también ligeramente menor que las injertadas sobre *P. terebinthus* bajo condiciones de estrés hídrico. Aunque las plantas injertadas sobre el híbrido mostraron ser las más vigorosas, el estrés hídrico provocó una senescencia de las hojas más precoz que en las plantas injertadas sobre los otros portainjertos. Esta reducción de masa foliar le permitió una recuperación del estrés hídrico más rápida que en el resto de portainjertos. Sin embargo esto podría resultar en una disminución de la capacidad de

asimilación de la planta en condiciones de campo, lo cual podría afectar a la producción. Esta hipótesis debería ser comprobada en condiciones de campo.

14. Los resultados previos nos llevan a concluir las condiciones en las que cada portainjerto podría tener un mejor comportamiento. En parcelas con una alta dotación de agua, el híbrido podría ser el mejor candidato ya que induciría un mayor vigor y una conductancia estomática más alta, lo cual podría ir acompañado de una mayor respuesta productiva. En secano o riego deficitario, *P. terebinthus* podría ser una mejor elección por su resistencia a la sequía, ya que es capaz de mantener una mayor superficie foliar relativa en condiciones de déficit hídrico.

15. Todos estos resultados sugieren la necesidad de evaluar los efectos de estos patrones en la respuesta productiva de este cultivar bajo diferentes condiciones de estrés hídrico en campo.

Los 3 capítulos de este documento tratan sobre ensayos que se realizaron en campo, en un cultivo leñoso y en un número muy limitado de años. Por tanto todos estos resultados obtenidos deberían ser comprobados durante varios años para ver el patrón de comportamiento de este cultivo ante el riego y el estrés hídrico a largo plazo.

# CONCLUSIONS





**Regarding the influence of phenological stage and irrigation regime (Chapter III) on the water relations response in pistachio, the following conclusions can be drawn:**

1. Water relations in pistachio show a dynamic behavior, changing over the growing season depending on the phenological stage of the plant.
2. As water stress increases, the daily pattern of stomatal conductance changes, becoming reduced from before midday. Carbon assimilation is less sensitive than stomatal conductance under moderate water stress conditions.
3. During stage II, the leaves show a number of physiological changes, such as a change in elastic modulus and the percentage of symplastic water. This would seem to be associated with the strong osmotic adjustment that takes place during stage II and indeed stage III.
4. The changes in the water relations response support the idea that reducing irrigation during stage II would lead to no significant reduction in crop yield.

**Regarding the response of productivity under conditions of deficit irrigation (Chapter I), the following conclusions can be drawn:**

5. No linear relationship exists between total yield and ETC. RDI is associated with a 20% water saving with no significant loss of productivity compared to covering 100% of the trees' needs. RDI also clearly dampens alternate bearing.



6. The total yield of the trees, corrected for canopy volume, correlates positively with ETc only up to approximate values of 250 mm.
7. Irrigation increases both yield and crop quality. The increase in the percentage of split fruits is more strongly related to the increase in yield than to tree water status.
8. In deficit irrigation treatments, plants grown on *Pistacia terebinthus* L. rootstocks showed less water stress than expected. This rootstock may therefore offer greater resistance to water stress than the others. During stages I and II, greater reductions in irrigation (50% in the present experiment) may even have been possible.
9. The dampening of alternate bearing observed in the RDI treatments with respect to the controls is probably due to the establishment of a better carbon balance. Possibly, the water stress experienced in stages I and II reduced the number of flower buds induced; this might reduce the loss of flower buds during stage III.
10. The percentage of early split fruits showed a similar pattern in all growing seasons, but with a dramatic increase at the beginning of September. This suggests that, in the present tests, this variable was not clearly related to water stress. The reduction in minimum temperatures to below 13°C increased the percentage of early split fruits, suggesting this factor to be influential in this respect.

**Regarding the influence of rootstock type on growth and water relations in cv. Kerman, both during water stress and subsequent recovery (Chapter II), the following conclusions can be drawn:**

11. The behavior of cv. Kerman with regard to growth and water relations depends on the rootstock onto which it has been grafted, both under rainfed and irrigated conditions.
12. The rootstock showing the greatest sensitivity to water deficit was *P. atlantica*; with this rootstock the control of stomatal transpiration was poor. However, these results should be taken with care since the degree of water stress imposed was not as great as in plants grafted onto the other rootstocks as a result of relatively poor leaf development.
13. The plants grafted onto *P. terebinthus* rootstocks showed a high degree of stomatal control and slightly higher levels of water stress than those grafted onto the hybrid rootstock.
14. Under water stress conditions, the plants grafted onto the hybrid rootstock suffered a slightly less water stress and showed in absolute terms an stomatal closure also slightly less than those grafted onto the *P. terebinthus* rootstock. Although the plants on the hybrid rootstock were the most vigorous, water stress caused leaf senescence earlier than in the plants on the other rootstocks. This reduction in leaf mass allowed for the recovery from water stress more quickly than in plants on other rootstocks. However, this could result in reduced assimilative capacity in the field, and therefore reduce production. This hypothesis should be tested under field conditions.
15. The present results suggest that, when establishing a pistachio orchard, the rootstock should be chosen depending on whether growth will be under rainfed or irrigated conditions. Under irrigation, the hybrid rootstock would be the best candidate since it would induce greater vigour and higher stomatal conductance, which might be accompanied by increased productivity. Under

rainfed or deficit irrigation conditions, *P. terebinthus* rootstocks would be a better choice given the resistance they afford to drought; plants with these rootstocks are able to maintain a relatively high leaf area under water stress conditions.

16. The effects of these rootstocks on production in this cultivar under different water stress conditions should be examined in the field.

The three chapters of this thesis deal with experiments performed in the field crop over a limited number of years. The results obtained should be checked over a longer period to confirm the behavior of this crop with respect to irrigation and water stress.

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**ANEXO**







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## Deficit irrigation without reducing yield or nut splitting in pistachio (*Pistacia vera* cv Kerman on *Pistacia terebinthus* L.)

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### ABSTRACT

An irrigation experiment involving pistachio (*Pistacia vera* cv Kerman on *Pistacia terebinthus* L. rootstocks) was performed over a four-year period in central Spain to determine the effect of regulated deficit irrigation (RDI) on nut yield and quality. The growth season was divided into three phenological stages: stage I – from sprouting until the end of rapid nut growth; stage II – from maximum nut size until the beginning of kernel growth; and stage III – from the beginning of kernel growth until harvest. Control trees were irrigated to supply their full water needs throughout the growth season, except for the post-harvest period. Sustained deficit irrigation at 65% (DI<sub>65</sub>) and 50% (DI<sub>50</sub>) of control irrigation was provided to two other groups of trees. The RDI provided to a further group was designed to provide a stress period during stages I and II but no water stress during stage III; the aim was to reduce water use and increase the percentage of split nuts. A fifth group of trees was maintained under rain fed conditions. Water potential and leaf conductance were affected in the DI<sub>65</sub>, DI<sub>50</sub> and rain fed treatments mainly during stages II and III, with midday water potentials below –2.0 MPa. The RDI trees were only significantly water stressed during stage II, showing midday water potentials of around –1.4 MPa. On most days, leaf conductance was not significantly affected in any of the irrigation treatments. The nuts of the DI<sub>65</sub> and DI<sub>50</sub> trees were smaller in diameter and their total yield was reduced compared to the controls. However, no significant differences in kernel dry weight were observed. The RDI trees showed a total yield and percentage of split nuts similar to those of the controls, even though they received around 20% less water. The split nut yield showed a linear relationship with crop evapotranspiration. However, since the percentage of split nuts was similar in all treatments this variation was likely related to the total yield. The RDI trees did not show the normal alternate bearing pattern (which was clearly maintained in the control trees). Early splitting, a process that decreases the quality of the yield, was not related to water status but to temperatures lower than 13 °C. The results suggest that *P. terebinthus* L. rootstocks confer *P. vera* scions a degree of drought-resistance, reducing the likelihood of water stress and, therefore, allowing more severe RDI scheduling.

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### 1. Introduction

Pistachio nuts are an important product of Iran and the USA (the world's first and second producers, respectively), but interest in

this species as an alternative to traditional fruit crops is growing in other countries. In the European Union, the uncertainty about financial support for traditional Mediterranean crops such as olives and grapes has led to an increase in the area planted with

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pistachio trees. Over the last 15 years production in Turkey (the world's third producer) has been steady, but in Greece it increased from 5000 t in the early 1990s to 9000 t at the beginning of the present century (FAOSTAT, 2006). In Spain the total area now planted with pistachio trees is around 4000 ha, yet at the beginning of the 1990s it was almost negligible.

Pistachio is considered a drought- and saline-resistant species (Behboudian et al., 1986; Rieger, 1995), and in the Mediterranean basin it is mainly grown in rain fed conditions. However, it is an exceptional candidate for growth under irrigation. As with other trees, irrigation increases the yield, but with pistachio it also improves the quality of the nuts (the percentage of split nuts is higher) and dampens the normal alternate bearing pattern (Kanber et al., 1993; Goldhamer, 1995). The irrigation efficiency of this crop may, therefore, be better than that of others (although this needs to be properly quantified). This is of great interest since increasing the amount of irrigated land is difficult; water is scarce and only the most efficient agricultural systems are likely to receive inputs of irrigation water (Ferreles et al., 2003).

A basic tool for assessing irrigation efficiency is the crop-water production function (Vaux and Pruitt, 1983). Crop biomass and yield are linearly related to crop evapotranspiration ( $ET_c$ ). Sometimes, however, non-linear relationships between yield and  $ET_c$  are observed, e.g., in cotton (Orgaz et al., 1992) and olive (Moriani et al., 2003). This may be related to the increase in drought resistance shown by most fruit trees during one or more of their phenological stages; during these periods, a reduction in  $ET_c$  does not significantly reduce yield (Behboudian and Mills, 1997). If the response to variation in  $ET_c$  is non-linear, optimal  $ET_c$  levels need to be defined for different fruits and water prices so that deficit irrigation can be programmed.

The crop coefficient ( $K_c$ ) of pistachio is one of the highest of all deciduous fruit trees, exceeding 1.0 for the bulk of the growing season (Ferreles and Goldhamer, 1990). Therefore, in most countries where this crop is planted, irrigation has to be in deficit. Regulated deficit irrigation (RDI) is a system of managing the soil water supply (mainly for fruit crops) by imposing periods of water deficit that cause no (or only small) reductions in economic benefits (Behboudian and Mills, 1997). At the end of the 1980s RDI schedules were designed for pistachio in California (Goldhamer et al., 1987; Phene et al., 1987). These experiments showed different responses of nut yield to water stress conditions depending on the phenological stage of the fruit during which they occurred, e.g., water stress had little impact during stage II (nut hardening) (Goldhamer et al., 1987; Phene et al., 1987). A further three-year-long experiment with a controlled reduction of irrigation during stage II, and no irrigation during post-harvest (thus providing a water saving of around 20%) confirmed these results (Gold-

hamer and Beede, 2004). In addition, it was hypothesized that mild water stress during stage I (a period of rapid fruit growth) would increase the percentage of split nuts and therefore the quality of the yield (Goldhamer and Beede, 2004). In more recent experiments, Goldhamer reported an increase in split nut yield but also in early split nut yield in some years for some – but not all – trees undergoing RDI during stage I (Goldhamer et al., 2004, 2005, 2006). Early splitting is a rupture of the hull that leads to mould infections of the kernel and a reduced marketable yield. Early splitting occurs before harvest and reduces the quality and the value of the yield. Nut splitting is a process that occurs in the endocarp (pit); this also occurs before harvest, but increases the quality and value of the yield. Increases in early split nuts have been associated with a delay in the harvest period (Doster and Michailides, 1997) but not with the influence of water stress at the beginning of the growth season. All the above investigations were performed with trees grafted onto commercial Californian rootstocks of *Pistacia atlantica* Desf, UCB and Pioneer Gold I (*Pistacia integerrima* L.). Oddly, *Pistacia terebinthus* L., which is thought to be even more resistant to drought and frost (Ferguson et al., 2005), has been less commonly studied.

The aim of this work was to study, over three experimental growth seasons, the influence of RDI scheduling (imposing water stress conditions during rapid nut growth and nut hardening i.e., stages I and II), sustained deficit irrigation and rain fed conditions on nut yield and quality. A further objective was to investigate the relationship between early splitting and environmental/water stress conditions. All the trees in the present experiment were *Pistacia vera* cv Kerman on *P. terebinthus* L. rootstocks – a rootstock that has been less frequently studied.

## 2. Methods and materials

### 2.1. Experimental site and plant material

This experiment was conducted between 2003 and 2006 in an eight-year-old pistachio (*P. vera* L. cv Kerman on *P. terebinthus* L. rootstock) orchard at the El Chaparrillo Research Station, Ciudad Real, Spain (3°56'W, 39°0'N; altitude 640 m). The results of the first year of the experimental period (2003) were not taken into the account because pistachio has a biannual fruit bearing pattern. The soil at the experimental site is a shallow clay-loam (Alfisol Xeralf Petrocalcic Palexeralfs) with a depth of 1.3 m and a discontinuous petrocalcic horizon between 0.75 and 0.85 m. The volumetric water content of the first 0.3 m is 22.8% at field capacity (soil matric potential –0.03 MPa), 12.1% at wilting point (soil matric potential –1.5 MPa), and for the layer from 0.3 to 1.3 m it is 43.0 and 21.1%, respectively. The trees were spaced

**Table 1 – Environmental conditions, rainfall and reference evapotranspiration ( $ET_0$ ) for the three experimental growth seasons**

	Rainfall irrigated period (mm)	$ET_0$ irrigated period (mm)	Annual $ET_0$ (mm)	Annual Rainfall (mm)
2004	24.8	677.9	1172.9	296.6
2005	17.9	727.5	1326.7	225.0
2006	40.8	714.7	1293.7	417.0

The annual values and the values for the irrigated periods are provided. The irrigated period was from day of the year 155–273 in all years.

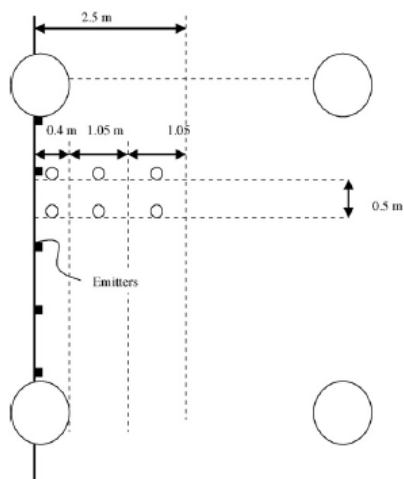


Fig. 1 – Distribution of the capacitance probes in the plot. Large circles represent the trees, the small ones the probes, and the shaded squares the emitters.

5 m × 5 m apart (400 trees ha<sup>-1</sup>). Table 1 shows the annual reference evapotranspiration (ET<sub>o</sub>; the evapotranspiration rate from a hypothetical grass reference crop with specific characteristics, without water shortage), the ET<sub>o</sub> during the irrigation period, and the rainfall during the year and the irrigated period alone. The ET<sub>o</sub> expresses the evaporating power of the atmosphere for a specific location and time of the year without taking into account crop characteristics or soil factors. The FAO Penman–Monteith method is recommended as the sole method for determining ET<sub>o</sub> (Allen et al., 1998). Rain fell mainly outside of the irrigation period and was below the historic average (397 mm) during 2004 and 2005. The absolute minimum temperatures were -7 °C (2004), -8.1 °C (2005) and -9.2 °C (2006), occurring in March in 2004 and 2005, and in January in 2006. The absolute maximum temperatures were 40.4 °C (2004), 41.4 °C (2005), 39.9 °C (2006), occurring in June, August and July, respectively.

The treatments consisted of five different irrigation and rain fed strategies. The phenological stages taken into the account in the RDI treatment were those suggested by Goldhamer and Beede (2004):

- stage I – from sprouting until the end of rapid nut growth;
- stage II – from maximum nut size until the beginning of kernel growth;
- stage III – from the beginning of kernel growth until harvest.

A complete randomised design was used, involving 20 trees per experimental plot with the following treatments:

- Control trees: these received water to cover estimated evapotranspiration ET<sub>c</sub> losses by fully replenishing all soil water extracted. In order to maintain no-water-stress conditions, irrigation was increased by 20% of the evapo-

transpiration rate if the midday stem water potential ( $\psi$ ) fell below -1.0 MPa.

- DI<sub>50</sub>: throughout the growing season these trees received water to replace 50% of the ET<sub>c</sub> calculated for the control treatment.
- DI<sub>65</sub>: throughout the growing season these trees received water to replace 65% of the evapotranspiration calculated for the control treatment.
- Regulated deficit irrigation (RDI): these trees received 50% of the water received by the control trees during stages I (rapid nut growth) and II (nut hardening), and the same amount of water as the control trees during stage III (kernel growth). In each of the three growth seasons of the experiment the water deficit recovery period was started slightly before stage III in order to reduce any delay in the recovery of  $\psi$  (Goldhamer and Beede, 2004; Guerrero et al., 2006b).
- Rain fed conditions.

The orchard was maintained under rain fed conditions until the end of 2002. No irrigation was provided during the post-harvest period in any of the treatments. The irrigation water used was saline and had high concentrations of nitrate (EC = 3.4 dS m<sup>-2</sup>; SAR = 1.4 mmol<sup>1/2</sup> l<sup>-1/2</sup>; NO<sub>3</sub><sup>-</sup> = 66 mg l<sup>-1</sup>; pH 7.2; HCO<sub>3</sub><sup>-</sup> = 362 mg l<sup>-1</sup>). Drip irrigation (five emitters per tree providing 4 l h<sup>-1</sup>) was provided 4 days/week.

## 2.2. Irrigation regimes

The amount of water provided was calculated on the basis of the crop evapotranspiration (ET<sub>c</sub>) and the crop coefficient (K<sub>c</sub>) [according to the FAO method (Doorenbos and Pruitt, 1974)]. The mean K<sub>c</sub> values provided by Goldhamer (1995) for pistachio trees growing under Californian conditions were used -0.39, 1.06 and 1.14 for stages I, II and III, respectively - after correction for canopy size (Feres and Goldhamer, 1990). The reference evapotranspiration, ET<sub>o</sub>, was estimated using the Penman–Monteith equation and employing daily data from a nearby automatic weather station (1.5 km from the experimental orchard).

## 2.3. Soil water and evapotranspiration measurements

The soil water content was measured using a portable capacitance probe (Divinier, 2000, Sentek Pty. Ltd., Australia) employing the default calibration supplied by the manufacturer. The data, therefore, reflect the relative changes in soil water content. It was assumed that, in a 5 m × 5 m square around each tree, the same soil conditions reigned. In addition, it was assumed that the distribution of the water from the emitters was the same. To measure the soil moisture each 5 m × 5 m square was divided into small rectangles of 2.5 m × 0.5 m (Fig. 1). These were further divided into three moisture zones depending on the distance from the emitters. A 1.5 m-long access tube was then placed at the centre of each moisture zone of two of the rectangles (one close to the emitters, one between the emitters) (Fig. 1). A weighted average of the water content based on the area of each moisture zone (Fig. 1) was then calculated. Weekly measurements were taken every 10 cm between soil depths of 0.1 and 1 m.



**Table 2 – Water applied and estimated crop evapotranspiration (ET<sub>c</sub>) for the different treatments and years**

		Water applied (mm)	ET <sub>c</sub> (mm)
2004	Control	182.0	210.4
	RDI	141.8	146.3
	DI <sub>65</sub>	111.5	185.3
	DI <sub>50</sub>	90.1	171.3
	Rain fed	0	112.5
2005	Control	277.4	305.6
	RDI	232.5	267.9
	DI <sub>65</sub>	156.9	203.4
	DI <sub>50</sub>	120.3	149.6
	Rain fed	0	49.6
2006	Control	365.4	404.7
	RDI	290.7	295.2
	DI <sub>65</sub>	194.4	248.6
	DI <sub>50</sub>	153.6	213.6
	Rain fed	0	54.8

To calculate the crop evapotranspiration (ET<sub>c</sub>), the growing season [day of the year (DOY) 116–293] was divided into the rainy and dry period. During the dry period (DOY 155–273) rain was scarce (Table 2) and deep percolation was assumed to be negligible. The dry period was considered to cover the same period in each year of the experiment. The ET<sub>c</sub> during the dry period was calculated as:

$$ET_c = (SM_1 - SM_2) + I + R \quad (1)$$

where:

- SM<sub>1</sub> and SM<sub>2</sub> represent the soil moisture on the first and the last days of the period considered in 1 m depth (mm).
- I is the irrigation provided (mm).
- R is the rainfall (mm).

During the rainy season ET<sub>c</sub> could not be determined using Eq. (1) since deep percolation could not be estimated. The equation to use under such circumstances would be:

$$ET_c = ET_o \times K_c \quad (2)$$

where:

- K<sub>c</sub> is the crop coefficient (dimensionless).
- ET<sub>o</sub> is the reference evapotranspiration (mm).
- ET<sub>c</sub> is the crop evapotranspiration (mm).

However, since pistachio is a deciduous tree the values of K<sub>c</sub> in Eq. (2) would be very low during this part of the growth season, as would those of ET<sub>o</sub> and ET<sub>c</sub>. In addition, the values in K<sub>c</sub> should be the same for all the treatments though they may be different for water stress. Therefore, the different irrigation strategies were only compared during the dry season when water needs are significant.

The amounts of water provided in the DI<sub>50</sub> and DI<sub>65</sub> treatments were actually slightly lower than that suggested since the controls were allowed up to 120% ET<sub>c</sub> if the water potential fell to below -1.0 MPa (Table 2). Each year the maximum ET<sub>c</sub> values corresponded to the control trees and the minimum to the rain fed trees. The ET<sub>c</sub> of the control trees increased over the 3 years of the experiment due to canopy growth. This increase was also seen in all the other irrigation-treatment trees, but not in the rain fed trees: the

ET<sub>c</sub> of the rain fed trees in the last two seasons was half that of the first.

#### 2.4. Plant water relations

Midday stem water potential ( $\psi$ ) was determined to evaluate the water status of the trees. Fully expanded leaves on branches near the main trunk were covered with aluminium foil for at least 1 h before their removal between 12:00 and 14:00 h. Measurements were made for one leaf per tree from six trees per treatment using a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA). Because pistachio leaves exude turpentine, a piece of blotting paper was used to determine the end point; turpentine cannot moisten blotting paper but the contents of the xylem can.

Abaxial leaf conductance ( $g$ ) was measured with a steady-state porometer (Model LICOR-1600, U.K.) between 12:00–14:00 p.m. using the central foliole of the compound leaf. This measurement was made in sunlight and with fully expanded leaves from the same trees in which  $\psi$  was measured. The number of samples used was 18 (three leaves  $\times$  6 trees). Measurements were made in the years 2004 and 2006, but not during 2005 due to technical problems with the porometer.

#### 2.5. Nut development

Nut development was monitored via the measurement of kernel dry weight, nut diameter and the percentage of early splitting in all 3 years of the experiment. However, since the results for the three growth seasons were similar only the data for 2006 are presented. Sixty inflorescences per treatment were randomly selected at the beginning of each growth season. The diameters of their eventual nuts were recorded periodically from the beginning of nut formation, and the percentage of early split nuts was recorded periodically from the beginning of August until harvest. Finally, from the beginning of July, a further 60 nuts per treatment were sampled every two weeks and the kernels dried at 70 °C until a constant weight was achieved.

#### 2.6. Nut yield

The yield of each treatment was evaluated in terms of total nut, split nut, and total blank nut (including aborted and blank nut) yields [kilogram per hectare (always peeled and dried)].

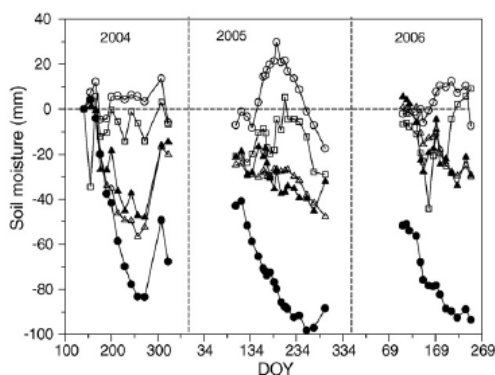
#### 2.7. Regressions and statistical analyses

The main treatment effects were examined by ANOVA. Differences between means were analysed using the Tukey test. Polynomial and linear regressions were performed to investigate the relationship between yield and ET<sub>c</sub>.

### 3. Results

#### 3.1. Plant water relations

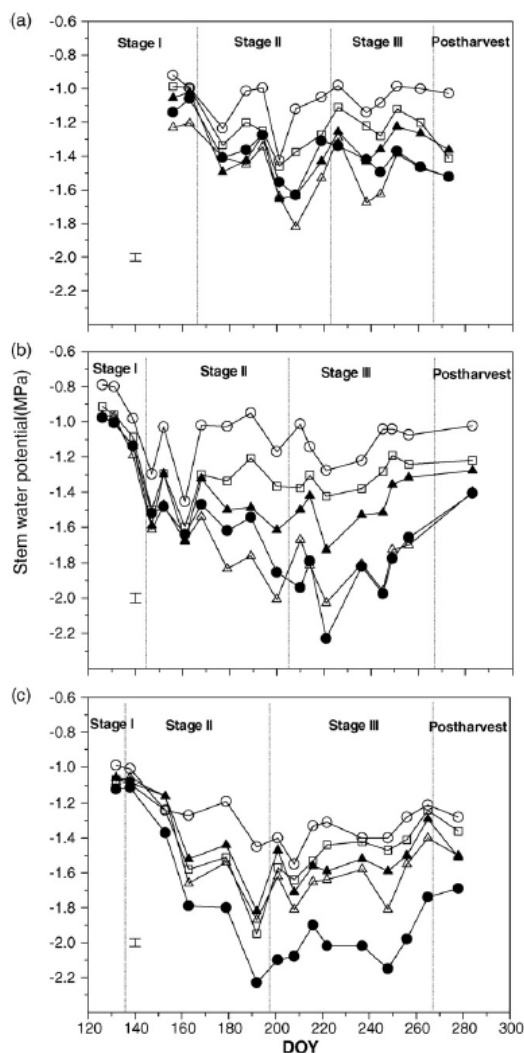
Fig. 2 shows the patterns of soil moisture for the three season of the experiment. Since the default calibration of the



**Fig. 2** – Variation in soil water content from the beginning of the experiment. Dashed lines separate the different seasons (DOY: day of the year). ○ = Control; □ = RDI; ● = rain fed; ▲ =  $DI_{65}$ ; △ =  $DI_{50}$ .

capacitance probe was used, all data are relative to the starting measurements recorded at the beginning of the first season (year 2004), which were tared. For all three seasons the soil moisture was similar in the control treatment, with a slight reduction at the end of each season and a recovery with the beginning of the autumn–winter rains. The increase in soil moisture during the second season (year 2005) indicated that the soil was probably not at field capacity in most seasons (see Fig. 2). The soil moisture content in the remaining treatments was clearly lower than in the control treatment. In the RDI treatment, soil moisture showed a seasonal pattern, with a reduction at the beginning of the season and a recovery at the end. Nevertheless, this increase did not always allow the soil moisture content to recover to that seen in the control treatment or even that recorded for the same RDI treatment at the beginning of the experiment. The soil moisture patterns in the  $DI_{65}$ ,  $DI_{50}$  and rain fed treatments followed the same trend but differed from that seen in the RDI treatment. A continuous reduction in soil moisture was seen in all three study seasons, and full recovery was not always achieved with the autumn–winter rains. The slope of the reduction in soil moisture was steepest in the rain fed treatment. The difference in the soil moisture content between the beginning of the experiment and the end in the rain fed treatment was around 80 mm, while in the  $DI_{65}$  and  $DI_{50}$  treatments it was around 30 mm, and in the control and RDI treatments it was 0 mm.

The patterns of midday stem water potential ( $\psi$ ) in each treatment were similar in all three seasons (Fig. 3). The value of  $\psi$  at the beginning of the season in the control trees was around  $-1$  MPa, with a slight reduction during stage II of nut development, although recoveries were seen in 2004 and 2005 (Fig. 3a and b). In 2006 the reduction in  $\psi$  was stronger and remained at  $-1.3$  MPa over stages II and III (Fig. 3c). Compared to the other treatments, significant differences were seen during stage II and early stage III (Fig. 3a and c). Only during 2005 there were significant differences in  $\psi$  between the controls and the different treatments during stage I (Fig. 3b). Minimum values of  $\psi$  below  $-2.0$  MPa were recorded during



**Fig. 3** – Seasonal pattern of midday stem water potential during the 3 years of the experiment: (a) 2004; (b) 2005; and (c) 2006. The periods corresponding to each phenological stage are shown. Each point represents the average of six measurements. The mean standard error is shown in each figure. Statistical differences are reported in the text ( $P < 0.05$ , Tukey test). ○ = Control; □ = RDI; ● = rain fed; ▲ =  $DI_{65}$ ; △ =  $DI_{50}$ .

stage III in 2005 and 2006 in the rain fed trees (Fig. 3b and c), and of  $-1.8$  MPa in 2004 in the  $DI_{50}$  trees (Fig. 3a). At the end of stage III in all seasons, the rain fed and  $DI_{50}$  trees showed a recovery of their  $\psi$  values, even though no rain fell. This recovery was, however, incomplete, with values never reaching those obtained in the control treatment. The  $\psi$  values for the RDI trees were significantly lower than those for

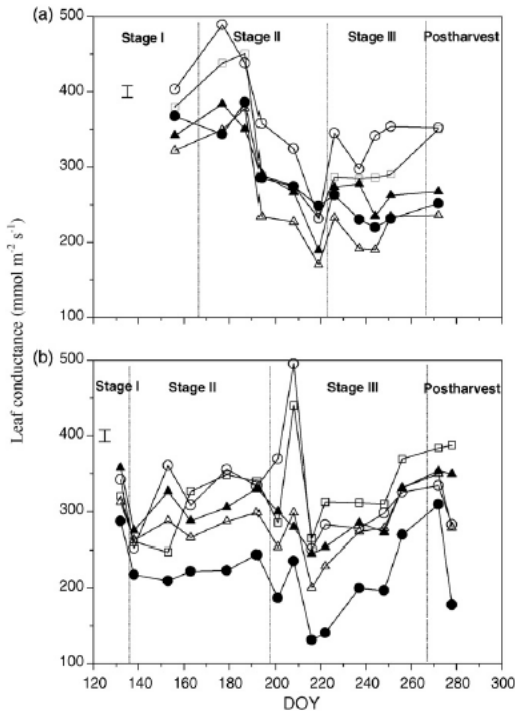


Fig. 4 – Seasonal pattern of midday leaf conductance during the 2004 (a) and 2006 (b) seasons. The periods corresponding to each phenological stage are shown. Each point represents the average of 12 measurements. The mean standard error is shown in each figure. Statistical differences are reported in the text ( $P < 0.05$ , Tukey test). ○ = Control; □ = RDI; ● = rain fed; ▲ = DI<sub>65</sub>; △ = DI<sub>50</sub>.

the control trees during stage II, but recovery during stage III was fast and no significant differences were seen between these trees and the controls in this phenological stage in any year.

Midday leaf conductance was significantly affected by the irrigation treatment (Fig. 4). During 2004, its seasonal pattern showed maximum values in all treatments at the beginning of the year, a slight reduction during stage II, and steady values during stage III (Fig. 4a). In the same year, the values of the DI<sub>65</sub>, DI<sub>50</sub> and rain fed trees were always significantly lower than those of the control trees, but no differences were seen between the RDI and control trees (Fig. 4a). The seasonal pattern in 2006 was different to that of 2004. Values were steady in all treatments throughout the season but lower than those recorded in 2004. During 2006, the values for the rain fed trees were significantly lower than those of the control trees for the entire season (Fig. 4b). The DI<sub>65</sub> and DI<sub>50</sub> trees had lower values than the control trees but these differences were often not significant. The values for the RDI trees were similar to those of the control trees throughout 2006.

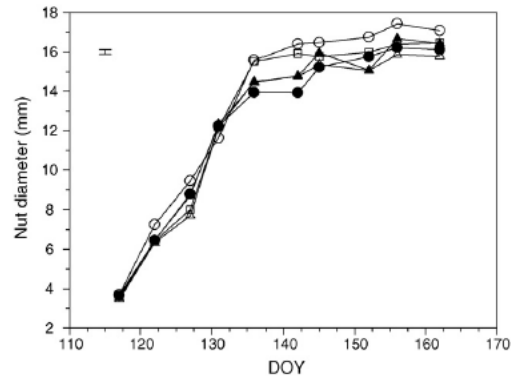


Fig. 5 – Nut diameter growth during stage I in the 2006 season. Each point represents the average of 60 measurements. The mean standard error is shown in each figure. Statistical differences are reported in the text ( $P < 0.05$ , Tukey test). ○ = Control; □ = RDI; ● = rain fed; ▲ = DI<sub>65</sub>; △ = DI<sub>50</sub>.

### 3.2. Nut development

The nut diameter results were similar for all 3 years of the experiment; therefore, only the data for 2006 are presented (Fig. 5). The period of rapid nut growth (stage I) varied from 22 days, from full bloom, in 2006 to 49 days in 2004. The maximum diameter was around 18 mm and the difference between the control and rain fed nuts was significant at the end of this stage (although differences greater than 2 mm were never seen). The control trees had the largest diameter nuts and the rain fed the smallest; the remaining treatments all produced nuts of a similar, intermediate size. The increase in kernel dry weight was also similar for all 3 years; thus again, only the data for 2006 are shown (Fig. 6). The accumulation of

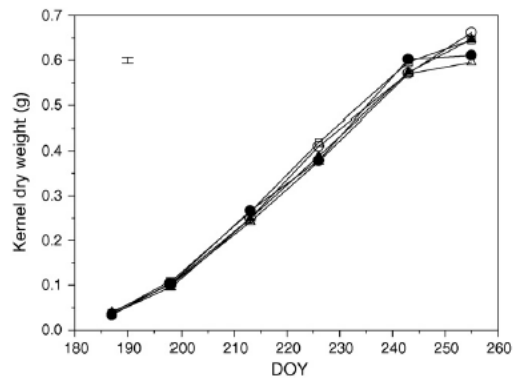


Fig. 6 – Kernel dry weight pattern during stage III in the 2006 season. Each point represents the average of 60 measurements. The mean standard error is shown in each figure. No significant differences exist (Tukey test). ○ = Control; □ = RDI; ● = rain fed; ▲ = DI<sub>65</sub>; △ = DI<sub>50</sub>.



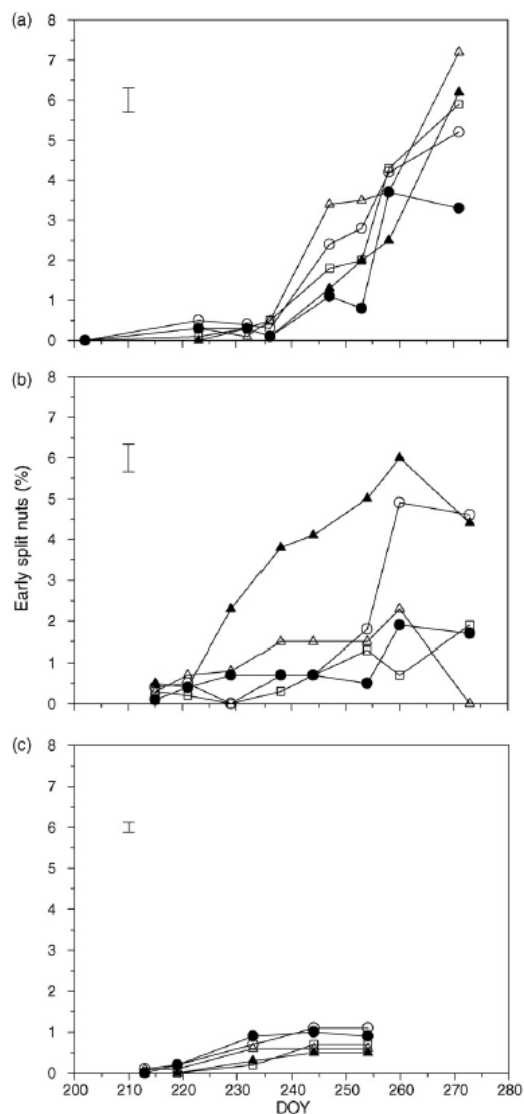


Fig. 7 – Percentage of early split nuts for 2004, 2005 and 2006. Each symbol represents the average of 60 measurements. The mean standard error is shown in each figure. Statistical differences are reported in the text ( $P < 0.05$ , Tukey test). ○ = Control; □ = RDI; ● = rain fed; ▲ =  $DI_{65}$ ; △ =  $DI_{50}$ .

dry matter was linear from the end of July or the beginning of August until harvest; no significant differences were observed between treatments.

The pattern of early splitting was different between years but the effect of irrigation was not always clear (Fig. 7). In all three growth seasons an increase in the percentage of early

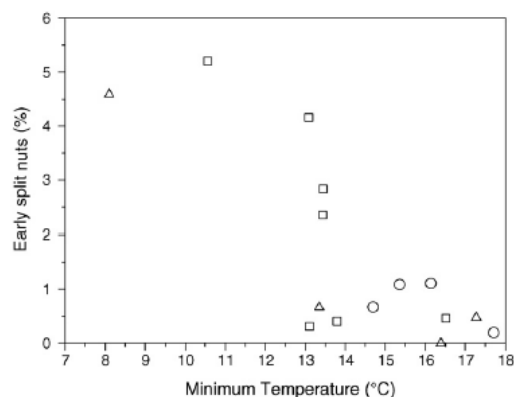


Fig. 8 – Increase in the percentage of early split nuts in the control treatment with respect to the average minimum temperature recorded between nut sampling points. Each point is the average for 60 inflorescences. (○) 2006; (△) 2005; (□) 2004.

splitting occurred at the end of August, but to very different extents. In 2004, the percentage of early split nuts in all treatments increased from mid August until the end of September, although this problem was significantly less serious in the rain fed trees (Fig. 7a). Similar results were obtained in 2005, although the increase in early split nuts peaked in mid-September (Fig. 7b). In 2005, the  $DI_{65}$  and control treatments produced the highest percentages of early split nuts (significantly greater than in the other treatments). In both years (2004 and 2005) the maximum yield of early split nuts reached around 6% of the total (in the RDI,  $DI_{65}$  and  $DI_{50}$  treatments in 2004, and in the control and  $DI_{65}$  treatments 2005). In 2006 the increase in early split nuts in all treatments took place mainly in mid August, with no increase during September (Fig. 7c). In addition, no significant differences were seen among the irrigation treatments and controls. The maximum percentage of early split nuts in the 2006 season was around 1% – six times lower than the maxima of the previous years. When the percentages early split nuts of the three seasons for the control trees were compared to the average minimum temperature in the period between sampling, a sharp increase was seen when this fell below 13 °C (Fig. 8). No relationship was seen with air humidity.

### 3.3. Nut yield

All trees showed the typical alternate bearing pattern except the RDI trees (Table 3). In the “off” year (2005) a reduction was seen in the yield of more than 50% with respect to the “on” years in the rain fed, control and  $DI_{65}$  trees, while in the  $DI_{50}$  treatment this reduction was around 30%. No significant differences were seen between the treatments during the “on” years though the total split nut yield, total yield and nut load of the  $DI_{50}$  trees were around 40% of the control. This may be related to the high coefficient of variation of the results (>40%). In the “off” season (2005), the total yield, blank yield and nut load of the RDI trees



**Table 3 – Yield and yield components for the three growth seasons**

	Total yield (Kg ha <sup>-1</sup> )	Split nut (Kg ha <sup>-1</sup> )	Blank nut (Kg ha <sup>-1</sup> )	Dry nut weight (g)	Nut load	WPTY (Kg ha <sup>-1</sup> mm <sup>-1</sup> )	WPSN (Kg ha <sup>-1</sup> mm <sup>-1</sup> )
<b>2004</b>							
Control	1766 ± 189.9	1242 ± 141.4	119 ± 25.2	0.7a	6112 ± 651	8.4ab ± 0.9	5.9ab ± 0.7
65%	1707 ± 268.3	1108 ± 190.3	119 ± 28.9	0.7ab	6296 ± 914	9.2ab ± 1.4	6.0ab ± 1.0
50%	887 ± 274.4	618 ± 159.7	58 ± 15.6	0.7bc	3341 ± 982	5.2b ± 1.6	3.6b ± 0.9
Rain fed	1380 ± 172.3	1043 ± 109.7	106 ± 25.3	0.6c	5751 ± 677	12.3a ± 1.5	9.3a ± 1.0
RDI	1435 ± 258.8	1161 ± 234.7	63 ± 16.8	0.7ab	5083 ± 824	9.8ab ± 1.8	7.9ab ± 1.6
<b>2005</b>							
Control	498 b ± 112.1	366 ± 107.4	26 b ± 9.4	0.8	1483b ± 326	8.0ab ± 0.8	5.2ab ± 0.8
65%	754 ab ± 230.0	295 ± 122.4	99 ab ± 29.2	0.9	2139ab ± 607	13.5a ± 1.4	8.2a ± 1.1
50%	677 ab ± 235.9	319 ± 64.1	85 ab ± 40.3	0.8	2148ab ± 777	4.5b ± 1.6	2.1b ± 0.4
Rain fed	538 b ± 189.0	293 ± 106.8	36 b ± 17.8	0.8	1614ab ± 543	10.8ab ± 3.8	5.9ab ± 2.2
RDI	1442 a ± 265.5	802 ± 240.3	170 a ± 37.0	0.9	4191a ± 766	5.4ab ± 1.0	3.0b ± 0.9
<b>2006</b>							
Control	2447 ± 253.1	1589 ± 230.1	78 ± 7.5	0.8	8082 ± 760	6.0b ± 0.6	3.9b ± 0.6
65%	2756 ± 282.9	1675 ± 218.5	103 ± 19.6	0.7	9167 ± 857	11.1b ± 1.1	6.7b ± 0.9
50%	1683 ± 370.2	1053 ± 178.2	84 ± 14.8	0.7	5798 ± 1249	7.9b ± 1.7	4.9b ± 0.8
Rain fed	2004 ± 345.8	1370 ± 293.8	72 ± 13.1	0.7	6803 ± 1175	36.6a ± 6.3	25.0a ± 5.4
RDI	1914 ± 393.3	1237 ± 238.6	124 ± 27.3	0.7	6273 ± 1146	6.5b ± 1.3	4.2b ± 0.8

Different letters, within column and in the same season, indicate significant differences ( $P < 0.05$ ; Tukey test). The results are presented as means (left) ± standard errors (right). The water productivity of the total yield (WPTY) and the water productivity of the split nut yield (WPSN) are the ratios between the total yield and split nut yield, respectively, and crop evapotranspiration ( $ET_c$ ).

were significantly greater than in the control and rain fed treatments. The water productivity (the ratio between yield and  $ET_c$ ) was significantly greater in the rain fed trees (especially in 2006), while no clear differences were found between the irrigated treatments in any year (although the  $DI_{50}$  trees showed a trend towards having the lowest).

The absence of significant yield losses in all the irrigation and rain fed treatments compared to the controls may be explained by the different crown volumes of the trees in the different treatments. Over the 3 years of the experiment these differences became smaller, even though the crown volumes of the  $DI_{65}$ , RDI and rain fed trees remained somewhat larger. The difference between the mean crown volumes of the rain fed and control trees for 2004/2005 was 2.5 m<sup>3</sup>/tree, while for 2005/2006 it was 1.9 m<sup>3</sup>.

Another factor that might have explained the absence of yield losses described above is the alternate bearing pattern. To better analyse the relationship between yield and  $ET_c$ , two different transformations of the yield data were performed. First, the mean yields obtained in each treatment for 2004/2005 and 2005/2006 were calculated and used instead of annual yields (thus reducing the influence of the alternate bearing pattern) to determine the relationship with  $ET_c$ . Secondly the yield was normalised with respect to the crown volume in each treatment.

Biannual total nut yield increased from 100–160 kg ha<sup>-1</sup> m<sup>-3</sup> as  $ET_c$  increased between 100 and 250 mm (Fig. 9a). When  $ET_c$  values were higher than 250 mm the total yield obtained remained almost constant at around 160 kg ha<sup>-1</sup> m<sup>-3</sup>. In all seasons the  $ET_c$  values obtained in the RDI treatment were around this 250 mm maximum, while those of the control treatment were slightly higher; the yield of these trees, however, was the same.

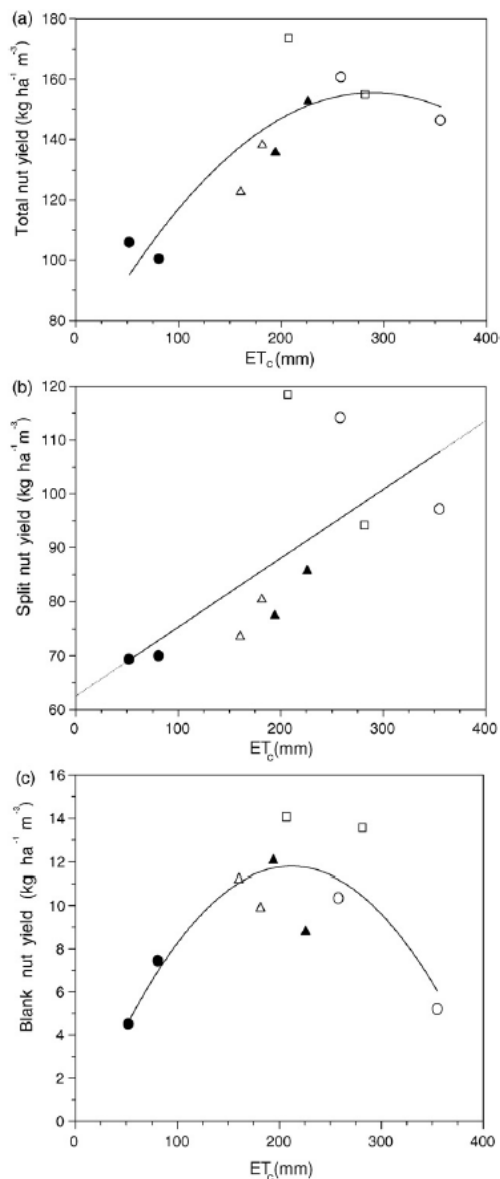
The relationship between the split nut yield and  $ET_c$  showed a linear pattern (Fig. 9b). The slope was smaller than

that between  $ET_c$  and the total nut yield (Fig. 9a). The split nut yield varied from around 70 kg ha<sup>-1</sup> m<sup>-3</sup> in the rain fed trees to 100 kg ha<sup>-1</sup> m<sup>-3</sup> in the control trees. However, the percentage of split nuts in the total yield varied (mainly between 60% and 70%) with no significant differences between treatments within years.

The blank nut yield was very low and its relationship with  $ET_c$  unclear (Fig. 9c). Values varied from 4 to 14 kg ha<sup>-1</sup> m<sup>-3</sup>, although most clustered around 10–14 kg ha<sup>-1</sup> m<sup>-3</sup>. The percentage of blank nuts varied from 4% to 11% of the total nut yield with no significant differences between treatments.

#### 4. Discussion

The total yield of the experimental trees was positively related to  $ET_c$  until values of around 250 mm (Fig. 9). Research into yield responses to decrease in  $ET_c$  have shown that most water production functions for row crops are linear (Vaux and Pruitt, 1983). In contrast, similar investigations with fruit trees strongly suggest that there are developmental periods during which water stress is not detrimental to yield – thus RDI can be used (Behboudian and Mills, 1997). Moriana et al. (2003), working with mature olive trees, reported a relationship between fruit yield and  $ET_c$  with an almost constant yield after 750 mm. In the present work, a mean crown volume of 10 m<sup>3</sup> per tree is related to a rate of variation in the water-productivity function (the slope of Fig. 9a) in the range of 4.0–0.7 kg ha<sup>-1</sup> mm<sup>-1</sup> – one-sixth of that reported for mature olive trees (Moriana et al., 2003). Olive trees are considered more drought-resistant than pistachio (Rieger, 1995); the latter therefore shows a lower water productivity. However the increase in the total and split nut pistachio yields seen in the present work (Fig. 9) may compensate for this.



**Fig. 9 – The influence of  $ET_c$  on yield differs according to the yield component considered. The yield data provided are biannual and relative to average crown volume – (a) total nut yield ( $\text{kg ha}^{-1} \text{m}^{-3}$ ), (b) split nut yield ( $\text{kg ha}^{-1} \text{m}^{-3}$ ) and (c) blank nut yield ( $\text{kg ha}^{-1} \text{m}^{-3}$ ) – and are plotted against crop evapotranspiration ( $ET_c$ , mm). ○ = Control; □ = RDI; ● = rain fed; ▲ =  $DI_{65}$ ; △ =  $DI_{50}$ . The best fit relationships are: (a) total nut yield;  $Y = 65.6 + 0.62ET_c - 1.1 \times 10^{-3}ET_c^2$ ;  $R^2 = 0.75$  ( $P < 0.01$ ); resid. Mean square = 177;  $n = 10$ , (b) split nut yield;  $Y = 62.5 + 0.13ET_c$ ;  $R^2 = 0.42$  ( $P < 0.05$ ); resid. Mean square = 201;  $n = 10$ , (c) blank nut**

The split nut yield was also positively related to  $ET_c$  (Fig. 9b). The best fit was linear, rather than polynomial as for the total nut yield, but the wide scatter of the data limits the conclusions that can be drawn. This linear relationship suggests high water efficiency in terms of split nut yield; this agrees with the results of Phene et al. (1987) who reported pistachio trees to show high sensitivity to water stress. However, this response has been reported to be strongly related to the period when water stress occurs. In the present work the increase in split nuts was mainly related to the increase in total yield since the percentage of splitting was similar in all treatments; certainly, no clear effect of water stress during stages I and III was seen. The Kerman cultivar is very sensitive to water stress in stage III, to which its response is a reduction in the percentage of split nuts (Goldhamer and Beede, 2004). Mild water stress during stage I, however, increases the percentage of split nuts (Doster and Michailides, 1995; Goldhamer and Beede, 2004; Goldhamer et al., 2004, 2005). The lack of agreement between the results of the former researchers and those of the present work suggests that the present rootstocks increase the drought-resistance of the scion compared with *Pistacia atlantica* or UCB rootstocks used in the former authors' work. *P. terebinthus* has been reported as the most drought-resistant pistachio rootstocks (Guerrero et al., 2006a; Ferguson et al., 2005). Therefore, to increase the yield of split nuts when using *P. terebinthus* rootstocks, greater water stress might be advisable during stage I. This agrees with the findings of Kanber et al. (1993), who worked with cultivars and rootstocks different to those used in the present and in Goldhamer's work; these authors also reported no significant differences in the percentage of split nuts between rain fed and irrigated trees.

Regulated deficit irrigation reduced the amount of water applied by some 20% (Table 2) without difference in nut yield (Table 3 and Fig. 9), although the water status of the trees was affected during stage II (Figs. 3 and 4). In addition, this treatment provided an almost steady total yield and no alternate bearing pattern was seen, unlike in the remaining treatments (Table 3). Though the mechanism of alternate bearing has not been completely elucidated, it may be the result of carbohydrate competition between the nuts of one season and the forming buds that will produce nuts in the following season (Ferguson et al., 2005; Stevenson and Shackel, 1998; Stevenson et al., 2000). The greater the nut yield, the greater the carbohydrate competition and, therefore the greater the bud fall for the same water status conditions. However, this pattern can be dampened if a balance between the carbohydrate source (photosynthesis) and sink (number of buds) is achieved. The present results suggest that moderate water stress during stages I and II may reduce the number of flower buds produced in RDI treatment, but the recovery during stage III of this treatment (Fig. 3) reduces bud fall due to improved assimilation.

These advantages of RDI in pistachio production could be limited, however, by the increase of an early splitting (Goldhamer et al., 2004). In the present work, early splitting was not clearly related to irrigation treatment, although the

yield;  $Y = -0.85 + 0.12ET_c - 3 \times 10^{-4}ET_c^2$ ;  $R^2 = 0.70$ ; resid. Mean square = 4;  $n = 10$ .

rain fed trees commonly showed the least early splitting (Fig. 7). For rainy seasons, Goldhamer et al. (2006) reported no significant differences between treatments in terms of early splitting. The results of these authors, plus the absence of an early splitting response to irrigation in our work, suggest that factors in addition to water status must influence this process. For Pistachio trees, harvesting begins when the hull can easily be removed. Doster and Michailides (1997, 1998) reported an increase in early split nuts in cv Kerman at a delayed harvest in September in two commercial orchards in California. This was probably related to environmental conditions more than water status. The present data strongly suggest that such environmental influence(s) may exist since an increase in the percentage of early split nuts was always seen by the beginning of September (Fig. 7), even though the phenological stages reached in the different seasons were different (Fig. 3). According to the present data, temperature may be involved in this (Fig. 8). Minimum temperatures below 13 °C are commonly recorded from the beginning of September in the area of the experimental orchard, the very time when the percentage of early split nuts increased sharply (Fig. 7).

## 5. Conclusions

The present study shows that irrigation increases the total nut and split nut yields of pistachio. However, the increase in split nuts is mainly related to the increase in the total yield more than to the splitting process, since the percentage of split nuts in all irrigation treatments was similar. These results suggest that *P. terebinthus* L rootstocks may increase the drought resistance of cv Kerman scions, and therefore reduce the effect of water stress during stage I.

The increase in total yield with the increase in  $ET_c$  was not linear and the RDI treatment allowed for a 20% water saving with no variations in yield and a very appreciable dampening of the alternate bearing pattern. The RDI strategy followed was designed to reduce the water supply during stages I and II by 50% of  $ET_c$  and to increase nut splitting. According to the conclusions above, when the rootstock *P. terebinthus* L. is used the water stress imposed (at least during stage I) should be more severe in order to increase the split nut yield. The reduction in the alternate bearing in the RDI treatment was probably a product of a balance between the flower buds induced during stages I and II (period of water stress) and the assimilation capacity during bud fall in stage III (period of water recovery).

Early split nut production showed a similar pattern in all the seasons with a sharp increase at the beginning of September that was not clearly related to water stress. When minimum temperatures were below 13 °C, the percentage of early splitting sharply increased. Thus, under the present conditions, temperature was the main factor affecting its onset.

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## Rootstock influences the response of pistachio (*Pistacia vera* L. cv. Kerman) to water stress and rehydration

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### ABSTRACT

Pistachio cultivation requires the use of rootstock because grafting is the only form of vegetative propagation. The main commercial rootstocks are *Pistacia integerrima* L., *Pistacia atlantica* Desf., *Pistacia terebinthus* L. and *Pistacia vera* L. Pistachio is considered to be a drought and saline-resistant crop; however, there is little information describing varietal responses of rootstocks to water stress. Some studies have suggested that *P. terebinthus* L. is the most drought and cold resistant rootstock. The effect of the rootstock on the water relations of the grafted plant is crucial for improving crop performance under water stress conditions and for developing the best irrigation strategy. This work studied the physiological response to water stress of pistachio plants (*P. vera* L. cv. Kerman) grafted onto three different rootstocks *P. terebinthus* L., *P. atlantica* Desf. and a hybrid from crossbreeding *P. atlantica* Desf. × *P. vera* L. Plant physiological responses were evaluated during a cycle of drought and subsequent recovery in potted plants. Parameters measured were soil moisture, trunk diameter, leaf area, leaf number, leaf and stem dry weight, stem water potential, leaf stomatal conductance. The results showed different responses of cv. Kerman depending on the rootstock onto which it had been grafted. The hybrid rootstock was associated with a higher degree of stomatal control and reduced leaf senescence compared to *P. atlantica* and *P. terebinthus*, despite being associated with the most vigorous shoot growth. *P. terebinthus* enabled very effective stomatal control but was also associated with the most rapid leaf senescence. *P. atlantica* was associated with less vigorous shoot growth and similar levels of water stress as occurred with the others rootstocks under conditions of high evaporative demand, which was associated with lower stomatal control. The selection of the most effective rootstock choice for different environmental conditions is discussed.

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### 1. Introduction

Pistachio is an important crop in Iran and USA, which are the major world producers, while in other countries it is becoming an interesting alternative to traditional crops. In the Mediterranean region, uncertainty about the future of economic support from the European Union to traditional crops, such as olive and vineyards, and the use of low fertility soils for agriculture has resulted in a large increase in the area planted with pistachio. In Spain, the total planted area was almost negligible in 1990, and currently is about 4000 ha. While the production in Turkey (third world producer) has been steady during the last 15 years, in Greece it has increased from 5000 t in the early 90s to 9000 t at the beginning of the twenty-first (FAOSTAT, 2007).

Pistachio is considered as a drought and saline-resistant crop (Behboudian et al., 1986; Rieger, 1995), and in the Mediterranean basin it is mainly grown under rain fed conditions. However, as with other tree species, irrigation increases yield. In pistachio, irrigation also improves nut quality (higher percentage of splitted nuts) and dampens the alternate bearing pattern (Kanber et al., 1993; Goldhamer, 1995). The benefits of irrigation in this crop may be higher than in others crops; however, there is a requirement properly quantify these responses. The good performance under dryland conditions and the favourable response to irrigation are very important considering that water is a scarce resource and in the future only the most efficient agricultural systems are likely to receive inputs of irrigation water (Feres et al., 2003).

Pistachio cultivation requires the use of rootstocks, and grafting is the only form of vegetative propagation. The main pistachio rootstocks are *Pistacia integerrima* L., *Pistacia atlantica* Desf., *Pistacia terebinthus* L. and *Pistacia vera* L. The most used rootstock in Iran is *P. vera* L., in the USA is *P. integerrima* L. and a hybrid between *P.*

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*integerrima* and *P. atlantica* Desf. (UCB), and in the Mediterranean basin is *P. terebinthus* L. Most scientific studies with pistachio trees have been done with varieties grafted onto Californian commercial rootstocks, such as *P. atlantica* Desf., UCB and *P. integerrima* L. There are few reported studies comparing different rootstocks. Ferguson et al. (2005) reported that *P. terebinthus* L. was the most drought and cold resistant rootstock, while *P. integerrima* L. was susceptible to frost but tolerant to verticillium. *P. atlantica*, which was once one of the most popular rootstocks in California, has been discarded in many places because of its high susceptibility to verticillium (Ferguson et al., 2005). Nowadays, UCB is considered the best commercial rootstock under irrigation (Ferguson et al., 2005). Germana (1997) comparing different rootstocks observed that *P. atlantica* has higher transpiration and photosynthetic activity than *P. terebinthus*, particularly in stressed plants, which could make it more susceptible to drought stress. Guerrero et al. (2003) found no rootstock effect on production when comparing *P. terebinthus* L., *P. integerrima* L., *P. atlantica* Desf. and *P. vera* L. under rain fed conditions.

In other woody crops, the use of rootstocks is optional; they may be grown without rootstock, and they are mainly used because of improving some of the cultivar characteristics as tree size, crop yield or yield quality. Different studies on apple (Olien and Lakso, 1986; Cohen and Naor, 2002) and peach (Weibel et al., 2003) showed that specific rootstocks had an important influence on vegetative growth rate. Solari et al. (2006) confirmed in peach that rootstock effects on the tree water relations and vegetative growth are derived, at least partially, from differences in the tree hydraulic conductance associated with specific rootstocks. In addition, several hypothesis reviewed by Rogers and Beakbane (1957), Lockard and Schneider (1981) and Webster (1995) suggest that rootstock can affect vegetative tree growth through hormonal effects (Kamboj et al., 1999), mineral nutrition (Jones, 1971) or water status (Olien and Lakso, 1986).

The pistachio response to water stress has not been adequately characterized. There are few works studying the water relations of this species. Behboudian et al. (1986) subjected potted pistachio plants (*P. vera* L. cv. Kerman grafted on *P. atlantica* Desf.) to water and saline stress and reported some photosynthetic activity even at midday stem water potential ( $\psi_x$ ) between  $-5$  and  $-6$  MPa. In this work, they concluded that the stress response of pistachio plants was better than some other fruit trees and other typical xerophytes species (Behboudian et al., 1986).

In recent years, a breeding program has been developed to improve pistachio rootstocks at El Chaparrillo Research Station, Ciudad Real, Spain. This program is being conducted using two techniques: first, crossings have been made between traditional rootstocks, and second, germplasm from different areas of Castilla-La Mancha (central Spain) and northern Andalucía (southern Spain) has been collected to be used in the breeding program (Guerrero et al., 2003). At present, the most promising individuals are being tested. One of the best rootstocks obtained in this program is the second generation, open-pollinated seed crossing *Pistacia atlantica* Desf.  $\times$  *Pistacia vera* L., which shows a high vigour (Guerrero et al., 2007). This is the rootstock referred to as 'hybrid' in current study. Initial assessments of collected germplasm have shown differences depending on the area they come from, the one from Calzada de Calatrava (Ciudad Real, Spain) showing the highest vigour. This germplasm has been used in our previous testing to establish the characteristics of *P. terebinthus* as a rootstock (Guerrero et al., 2007).

As pistachio is planted in Spain mainly in dryland areas, the effect of rootstock in the cultivar response to water stress under these conditions will be of major importance. Rootstock effects are important for both physiological responses of the shoot variety and its productivity. The objective of this work was to study the effect of three different rootstocks (*P. terebinthus* L., *P. atlantica* and a hybrid

from the crossbreeding of *P. atlantica*  $\times$  *P. vera*) on the physiological responses to water stress of pistachio plants (*P. vera* L. cv. Kerman). Plant responses were evaluated during cycle of drought and recovery.

## 2. Materials and methods

### 2.1. Site description and experimental design

The experiment was conducted during the summer of 2007 at La Entresierra Research Station, Ciudad Real, Spain ( $3^{\circ}56'W-39^{\circ}0'N$ ; altitude 640 m). One-year old pistachio plants (*P. vera* L. cv. Kerman) grafted onto three different rootstocks, *P. terebinthus* L., *P. atlantica* Desf. and a hybrid from the crossbreeding of *P. atlantica*  $\times$  *P. vera* (hereafter referred to as 'hybrid') were used. The hybrid was obtained in the breeding program at the El Chaparrillo Research Station. Thirty plants were planted in the spring of 2007 in 50 L pots filled with a mixture of gravel, sand and peat (5, 80 and 15% respectively), and placed outdoors. The experiment took place from "day of the year" (DOY) 190 until DOY 225. Reference evapotranspiration (E<sub>0</sub>) was calculated according to Allen et al. (1998), and rainfall data were obtained from a nearby (approximately 500 m) meteorological station.

The experimental design was a completely randomized split plot design with 5 replicates. The main factor was the rootstock and the secondary factor was irrigation. The irrigation treatments were full irrigation (Control) and no irrigation (Stress). The different combination of the two factors will be named as follows:

- *P. terebinthus*-Control (PTC)
- *P. terebinthus*-Stress (PTS)
- *P. atlantica*-Control (PAC)
- *P. atlantica*-Stress (PAS)
- Hybrid-Control (HC)
- Hybrid-Stress (HS)

From DOY 190, the control and stressed plants were drip irrigated until slight drainage occurred. Each pot had 4 drippers ( $4\text{ Lh}^{-1}$ ) and was irrigated every afternoon. To determine pot weight at field capacity, all pots were weighed early in the morning, always at the same time, at least three times per week (Monday, Wednesday and Friday). In plants subjected to water stress, irrigation was withdrawn from DOY 204 until 218 when they were re-watered to study re-hydration. Once the stress period was completed, pots were re-watered up to field capacity. The weight at field capacity at the end of the experiment was different to that at the beginning of the experiment, probably due to the decreasing water retention capacity of the peat as it dried out. Soil moisture measurements were taken at 10, 20 and 30 cm depth with a portable capacitance probe (Diviner, 2000, Sentek Pty. Ltd., Australia) placed approximately 15 cm away from the stem. As the results were similar to those obtained by the weighing method, only the latter will be shown in Section 3.

### 2.2. Measurements

Trunk diameter was measured in all plants once a week with a digital gauge, 1 cm above the grafting point. Measurements were taken early in the morning, from 183 to 225 DOY. At the end of the experiment, leaf area (LA) data were obtained using a leaf area meter (LI-3100C, Lincoln, Nebraska, USA), and leaf and stem biomass was determined drying the plant organs in an oven at  $70^{\circ}\text{C}$  until constant weight. Prior to each biomass determination, visual leaf damage caused by water stress was visually evaluated on a scale of 1–5. Value 1 corresponded to leaves without

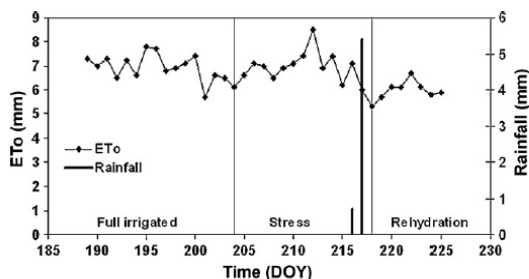


Fig. 1. Rainfall and reference evapotranspiration (ETo) during the experiment. The two vertical lines represent the water stress period.

wilting symptoms, value 3 to when 50% of leaf surface presented wilting symptoms and value 5 indicated leaves completely wilted.

Stem water potential ( $\Psi_x$ ) was periodically determined, six measurements were made throughout the experiment to evaluate plant water status. Fully expanded leaves were covered with aluminium foil at least 1 h before measurement, and  $\Psi_x$  determined at midday using a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA). Each time, one leaf per plant and per replicate was used. Abaxial leaf conductance ( $g_s$ ) was measured with a steady-state porometer (LICOR-1600, UK) between 12:00 and 14:00 local time, on the central leaflets of the composite leaf. Measurements were taken in all plants (two measurements per plant) on fully expanded leaves receiving direct sunlight, three times a week throughout the experiment.

Vapour pressure deficit (VPD) was calculated from data of a meteorological station around 500 m from the place where the experiment was performed.

### 2.3. Statistical analysis

The main effects of the two factors were examined by ANOVA and means were compared using the test of Tukey, with a significance  $P < 0.05$ . Significant differences are identified with different letters. The Statistix 8.0 (Analytical Software, USA) was used for the statistical analysis.

## 3. Results

### 3.1. Evapotranspiration and plant water requirements

During the experimental period (DOY 190–225), ETo ranged from 5.3 to 8.5 mm d<sup>-1</sup>. During the water stress period (DOY 204–218), except on day 212, ETo was more steady, with values around 7 mm d<sup>-1</sup> (Fig. 1). Precipitation was low, just two events were recorded on day 216 (0.7 mm) and day 217 (5 mm) at the end of the stress period (Fig. 1). The pots had an initial mean weight of 58 kg (100% in Fig. 2) when the soil was at field capacity, and those of the stressed plants lost on average 17.8, 17.0 and 10.5 kg (PTS, HS and PAS, respectively) from the beginning of the experiment to the time of maximum stress (DOY 218; Fig. 2). The recovery of the soil water content was very fast. Two days after the beginning of the rehydration, PAS pots recovered 94.7% of their initial weight, followed by the HS (91.6%) and PTS (90.4%) (Fig. 2). After a week, the 3 rootstocks had recovered almost their initial weight: 97.7, 96.3 and 95.7% (PAS, HS and PTS, respectively) (Fig. 2).

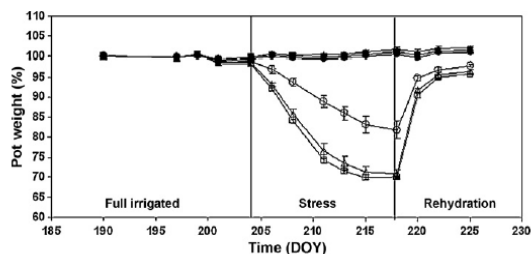


Fig. 2. Pot weight throughout the experiment as a percentage of the weight at field capacity, for all groups. Each point represents the mean of 5 measurements. The two vertical lines represent the water stress period. Bars represent the standard error. (●) PAC; (○) PAS; (■) PTC; (□) PTS; (▲) HC; (△) HS.

### 3.2. Plant biomass and trunk and leaf growth

Trunk diameter increased with time in all water treatments and rootstocks. Fig. 3 shows the values of the trunk diameter as a fraction of the diameter at the beginning (TD/TDi) of the experiment for each treatment and rootstock. When plants were well irrigated, there was no effect of the rootstock on the diameter trunk growth. Although *P. atlantica* and the hybrid showed higher final values (1.40) than *P. terebinthus* (1.25), differences were not significant.

Water stress affected trunk diameter earlier in PTS and HS than in PAS, showing a decrease in the slope of TD/TDi with time in relation to well-watered plants with the same rootstock. However, it was only at the end of the stress period (218 DOY) when signifi-

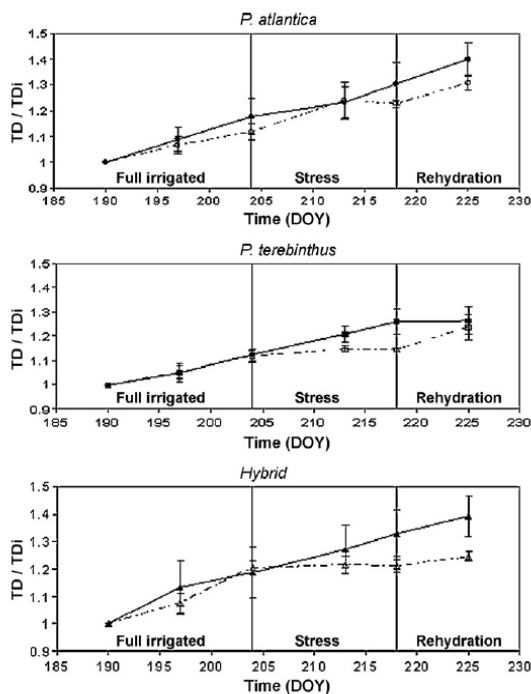


Fig. 3. Seasonal pattern of the trunk diameter as a fraction of the initial value (TD/TDi), for the three rootstocks and two water treatments. Each point is the average of 5 measurements. Bars represent the standard error. (●) PAC; (○) PAS; (■) PTC; (□) PTS; (▲) HC; (△) HS.



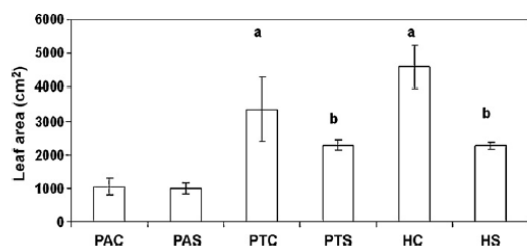


Fig. 4. Leaf area for all the rootstock and water treatments at the end of the experiment (DOY 225). Data are the averages of 5 replicates. Bars represent the standard error. Different letters indicated significant differences between control and stress treatment in each rootstock ( $P < 0.05$ ; Tukey test).

cant differences were found among rootstocks, with smaller TD/TDI values in PTS than in PAS and HS.

The response of plants to re-watering on DOY 218 was faster in PAS and PTS than in HS, although all the rootstocks showed no significant differences between control and stressed plants at the end of the rehydration period.

Another parameter that characterises growth is leaf area. In control conditions, PTC and HC showed a significantly higher leaf area than PAC. Differences between PTC and HC, even though the hybrid was approximately 30% higher, were not statistically significant (Fig. 4). Water stress mainly affected leaf area development of PTS and HS, producing significant reductions in leaf area of both these treatments. The largest leaf area decrease occurred in the HS, being about 50%, while in PTS it was 32%. On the contrary, there was no significant effect on leaf area development in PAS, with differences between the control and stress treatments being only 6%.

Fig. 5 shows the final leaf number as a fraction of that at the beginning of the experiment (LN/LNi, Fig. 5a) and the leaf dry weight and stem dry weight (Fig. 5b) at the end of the experiment

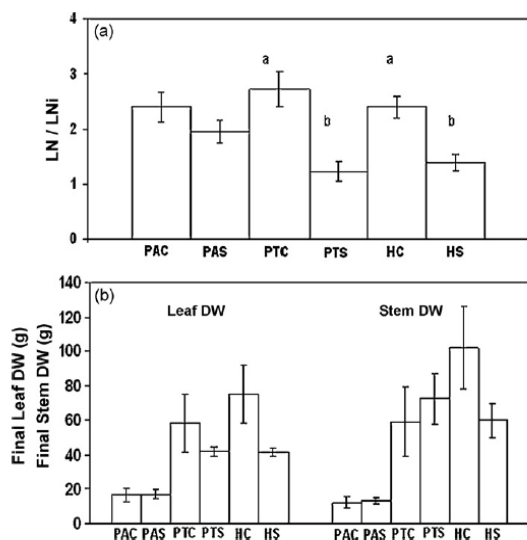


Fig. 5. (a) Leaf number as a fraction of the value at the beginning of the experiment (LN/LNi) and (b) leaf and stem dry weight (final leaf DW and final stem DW, g/plant) at the end of the experiment for all the rootstock and water treatments. Data are the average of 5 replicates. Bars represent the standard error. Symbols are indicated in the figure. Different letters indicated significant differences between control and stress treatment in each rootstock ( $P < 0.05$ ; Tukey test).

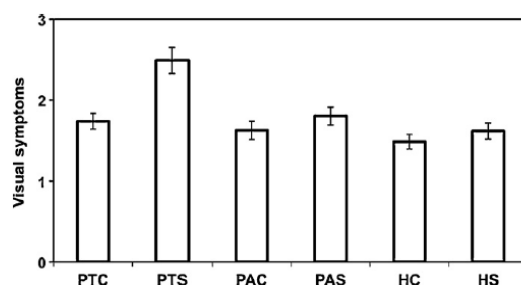


Fig. 6. Values of the visual symptoms of water stress (1 = no symptoms; 5 = 100% wilting surface) for all rootstocks and water treatments. Data are the average of 5 replicates. Bars represent the standard error.

for all the rootstocks and water treatments. LN/LNi was calculated in order to avoid the effect of the variability in leaf number among plants at the beginning of the experiment on the possible differences between water treatments at the end of the stress period. It was not possible to use the same approach for leaf and stem dry weight as it involved destructive measurements. As it was observed in leaf area, in the control treatment, HC and PTC showed significantly higher leaf and stem dry weight than PAC (Fig. 5b), while the ratio LN/LNi was similar for all the rootstocks. Water stress resulted in a significant reduction in LN/LNi in PTS and HS, but not in PAS (Fig. 5a). PAS also showed similar values of final leaf and stem dry weight than PAC. However, although leaf and stem DW of stressed plants of the hybrid were lower than those of control plants, the differences were not statistically significant, which was probably due to the initial variability of this parameter among plants. The response of PTS plants to water stress was intermediate, with leaf DW being affected but not stem DW, although once again the differences were not statistically significant (Fig. 5b).

There were no significant differences among rootstocks in visual wilting symptoms of stressed plants, although it seemed that PTS was more affected than PAS and HS, with mean values of 2.5, 1.8 and 1.6 respectively (Fig. 6). Surprisingly, for each rootstock, although stressed plants showed higher wilting damage values than control plants, differences were not significant according to the scale chosen, as high variability was found among plants of the same treatment.

### 3.3. Water relations

Fig. 7 shows the time course of  $\Psi_x$  throughout the experiment for all the rootstocks.  $\Psi_x$  ranged from  $-0.6$  to  $-1.45$  MPa in control plants, and from  $-0.87$  to  $-3.8$  MPa in stressed plants. The effect of water stress on  $\Psi_x$  was not detected until DOY 211 as significant differences were not found neither between water treatments nor rootstocks on day 208, 4 days after the beginning of the drying cycle. However, water treatment–rootstock interaction was significant on day 208 showing lower  $\Psi_x$  values for HS and PTS than for PAS. The minimum values of  $\Psi_x$  were reached on DOY 218 (10 days after the onset of water stress), PTS and HS rootstocks showing significantly lower  $\Psi_x$  than PAS, being respectively  $-3.35$  and  $-3.04$  MPa, and following the same pattern as in previous dates. Following, the final measurements on DOY 218, plants were fully irrigated and the recovery began. Only 2 days later (DOY 220) PAS was fully recovered from the stress, while PTS and HS had recovered 90% and 81% of the  $\Psi_x$  measured in the respective control plants that day. At the end of the experiment (DOY 225),  $\Psi_x$  values in PTS and HS were 93% and 91% of that of their controls, respectively. These differences between values of recovered and stressed plants were not statistically significant.



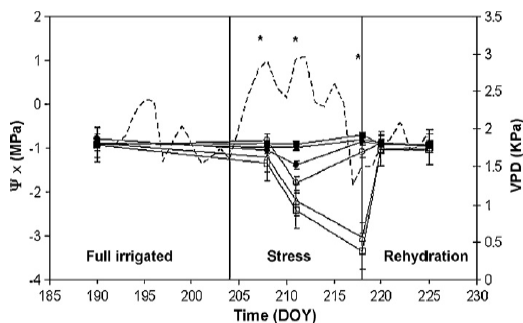


Fig. 7. Seasonal pattern of the stem water potential ( $\Psi_x$ ) and VPD (dotted line) during the experiment. Each point is the mean of 5 measurements. The two vertical lines represent the water stress period. Bars denote the standard error. Asterisks \*\*\*\* represent significant differences between rootstock in the stress treatment ( $P < 0.05$ ; Tukey test). (●) PAC; (○) PAS; (■) PTC; (□) PTS; (▲) HC; (△) HS.

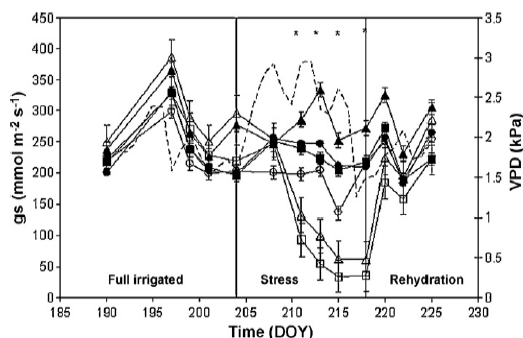


Fig. 8. Seasonal pattern of the stomatal conductance and VPD (dotted line) during the experiment. Each point is the mean of 10 measurements. The two vertical lines represent the water stress period. Bars denote the standard error. Asterisks \*\*\*\* represent significant differences between rootstock in the stress treatment ( $P < 0.05$ ; Tukey test). (●) PAC; (○) PAS; (■) PTC; (□) PTS; (▲) HC; (△) HS.

Stomatal conductance ( $g_s$ ) in control plants ranged from 149 to 326  $\text{mmol m}^{-2} \text{s}^{-1}$ , showing no differences between rootstocks for each date of measurement (Fig. 8). Water stress affected  $g_s$  in all rootstocks, with PAS once again being the rootstock less affected, followed by HS and finally PTS. Differences in  $g_s$  were significant on days 211, 213 and 215, with values for PAS being higher than those of PTS and HS. The minimum  $g_s$  values during the stress period were observed on DOY 215, 7 days after the beginning of the drying cycle, being 136.8, 33.9 and 62.8  $\text{mmol m}^{-2} \text{s}^{-1}$  in PAS, PTS and HS, respectively. Two days after rehydration (DOY 220), stomatal conductance was fully recovered in PAS at levels even higher than the control plants in some cases. However,  $g_s$  in PTS and HS was only 67.7% and 68.6% of that in control plants. On DOY 225, 1 week after the beginning of the recovery,  $g_s$  in PTS and HS was already 96% and 93.5% of the values measured in control plants.

#### 4. Discussion

The three different rootstocks studied in this work induced a differential response of growth and water relations in the cultivar Kerman, both under well watered (control) and water stressed conditions. In the absence of water stress, the hybrid rootstock promoted higher vegetative growth in the shoot variety than the other two rootstocks, especially *P. atlantica*, with a larger leaf area and

total biomass (Figs. 4 and 5). This greater growth during the initial stages, which was most marked with the hybrid, resulted in different plant size at the beginning of the stress period for the three rootstocks studied. Fig. 2 characterizes these differences very well, showing a much higher water use during the drying cycle for both *P. terebinthus* and the hybrid compared to *P. atlantica*. These results are the opposite of those obtained by Ferguson et al. (2005), who suggested that *P. terebinthus* is the least vigorous of all the rootstocks used in California. Guerrero et al. (2007) have shown significant differences in vigour among different but near populations of *P. terebinthus*. In our study, we could have used a particularly vigorous population of *P. terebinthus*, since *P. atlantica* is usually considered to be a rootstock of moderate vigour (Ferguson et al., 2005; Spann et al., 2007). The vigour of fruit trees may be important because it is likely to be related to yield. In pistachio, Spann et al. (2007) suggested that more vigorous rootstocks can also involve higher pruning costs because they promote more vegetative growth in the shoot cultivar. These authors also suggested that a more moderate vigour could be compensated by a higher plant density. Vigour has been also related to successful field grafting. Guerrero et al. (2007) found that under field conditions, grafting success in pistachio is closely related to the trunk diameter, and therefore greater vigour would probably reduce grafting failures.

Rootstock changes the response of the pistachio tree to water stress conditions. *P. atlantica* showed slight but not significant reductions in leaf number. In contrast, in *P. terebinthus* and the hybrid, growth was most affected by water stress conditions (Figs. 4 and 5). This response is generally found in all species, since expansive growth is the most sensitive process to water stress in plants and is affected even at relatively high leaf water potentials (Hsiao, 1973). Trunk diameter data showed that growth did not stop absolutely under water stress but that it slowed down (Fig. 3). This agrees with the data shown by Behboudian et al. (1986) in which slight turgor loss under severe stress still allows some tree growth. In our study, the reduction of trunk diameter in response to water stress took place earlier in the variety grafted onto the hybrid and *P. terebinthus* than on *P. atlantica*. This response to water stress seems in accordance to the response measures in other parameters such as  $\Psi_x$  and  $g_s$  which will be discussed later. In contrast, the recovery of growth was slower in the hybrid than in the other two rootstocks (Fig. 3). Visual symptoms of water stress in leaves and the decrease of green leaf area are indicators of leaf senescence caused by water stress. Apparently, *P. terebinthus* induced a stress avoidance response in Kerman variety with a greater leaf area reduction (lower number and higher damage) than the other two rootstocks. Unless the leaf area can be increased after the stress period, this response is considered as an undesirable feature if it reduces crop yield through a lower assimilation capacity.

Water stress, characterised by stem water potential measurements, was more severe in plants grafted on *P. terebinthus* than the hybrid (Fig. 7). The level of induced water stress was not as great as described in other studies in which minimum  $\Psi_x$  values of  $-5$  MPa have been reported (Behboudian et al., 1986). The higher  $\Psi_x$  values in *P. atlantica* were likely related to the smaller transpiring plant leaf area and biomass (Figs. 4 and 5). In relation to these leaf area values, differences in stem water potential between rootstocks (Fig. 7) were smaller than expected in the drought stress. In addition, *P. atlantica* also showed very little stomatal control of transpiration (Fig. 8), which could have contributed to reduced  $\Psi_x$  differences between this rootstock and *P. terebinthus* and the hybrid. These results are in agreement with the delayed response of trunk diameter to water stress in *P. atlantica* compared to *P. terebinthus* and the hybrid (Fig. 3). Considering the smaller leaf area of *P. atlantica*, the degree of water stress imposed in this experiment may not have been strong enough to affect parameters such as  $\Psi_x$  and  $g_s$ , in this rootstock, as quickly as occurred in *P. terebinthus* and the hybrid.

Leaf conductance of well-watered control plants was higher in the hybrid than in the other two rootstocks, especially during the water stress period (Fig. 8). Therefore, the hybrid induced in the shoot variety the highest degree of stomatal control in response to water stress, maintaining also the highest  $g_s$  under irrigation. This high  $g_s$  of control plants in the hybrid could have contributed to their higher growth through higher assimilation rates. This behaviour is characteristic of drought resistant species (Loomis and Connor, 1992).

## 5. Conclusions

The behaviour of the Kerman variety in terms of growth and water relations, under irrigated and water stress conditions, depended on the rootstock it was grafted onto. The rootstock that induced a higher sensitivity to water stress was *Pistacia atlantica* Desf., with low stomatal control of transpiration, although these results should be taken with caution as the degree of water stress imposed to that variety was milder as result of its low leaf area development. By contrast, the rootstock *P. terebinthus* L. showed a better stomatal control, and slightly higher levels of water stress than the hybrid which resulted in smaller reductions of leaf area but not in leaf number, probably due to differences in leaf size. Finally, the hybrid rootstock showed slightly less stomatal control under stress conditions than *P. terebinthus* and, even though it was the most vigorous, it lost more leaf area through senescence. Although the reduction in leaf area allowed a quicker recovery of some parameters after water stress was over, it could result in a reduction in the assimilation capacity of the tree under field conditions, which could affect crop yield. This hypothesis should be checked in field experiments. The responses found in this work should be taken into account when selecting the rootstock for the establishment of a new orchard, knowing the irrigation management that will be used. Under irrigation, the hybrid may be the best rootstock since it induces the biggest leaf conductance and vigour, which will be likely to be accompanied by a more productive response. In the case of dryland conditions or regulated deficit irrigation in which water stress periods are induced, *P. terebinthus* might be a good choice for their drought tolerance, as it is able to maintain greater leaf area relative to that of non-stressed plants with lower  $\psi_x$  and  $g_s$  values.

All these results suggest the need to evaluate the effect of these rootstocks on the productive response of this variety under variable water stress conditions in the field.

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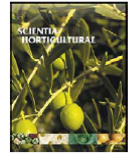
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## Water relations of pistachio (*Pistacia vera* L.) as affected by phenological stages and water regimes

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### ABSTRACT

Pistachio is a drought tolerant fruit tree that can be cultivated in rainfed and irrigated conditions. The water requirements of the tree, however, are considerable so in most of the commercial orchards deficit irrigation is a common practice. Regulated deficit irrigation in pistachio trees has been described in several works, which reported that the phenological stage of shell hardening, so called stage II, is the most drought tolerant. This paper proposes that such drought resistance is related to changes in water relations linked to the phenological stages, even in conditions of no water stress. In order to evaluate such changes, the daily pattern of stem water potential and gas exchange (net photosynthesis,  $P_n$ , and leaf conductance,  $g_s$ ) was measured, determining also the pressure–volume curves, in three different phenological stages of mature pistachio trees (*Pistacia vera* cv Kerman on *P. terebinthus* L. rootstock). The daily pattern of stem water potential and gas exchange were performed in three different irrigation treatments: control, regulated deficit irrigation and rainfed. The pressure–volume curves were made only in the control and rainfed treatments. Significant differences were found in the daily pattern of stem water potential in all the phenological stages considered, while only in the last one the net photosynthesis was affected by water stress. The daily pattern of gas exchange at the beginning of the season was not affected by the evaporative demand, with a constant value when radiation was not limiting. Moderate levels of water stress during the last measurement date reduced the maximum values of  $g_s$  and  $P_n$  resulting also in a clear change in the pattern of the daily curve, with maximum values only at the beginning of the day. The relationships between stem water potential and gas exchange parameters were different during stage II and almost the same in stages I and III. The parameters drawn from the pressure–volume curves also indicated a change in the elastic modulus of the leaf cells in stage II. In addition, differences in the osmotic adjustment (OA) index suggested different degree of osmotic adjustment of the phenological stages in the response to water stress. The results showed that different mechanisms of drought resistance are operating in the different phenological stages in pistachio trees.

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### 1. Introduction

Pistachio is a drought tolerant fruit tree species. As with other trees, irrigation increases the yield, but particularly in pistachio it also improves the nut quality and dampens the normal alternate bearing pattern (Kanber et al., 1993; Goldhamer, 1995). Regulated deficit irrigation (RDI) is a system of managing water supply by imposing some water deficits in specific phenological stages, which are found to be less sensitive, with no (or low) reduction in economic benefits (Behboudian and Mills, 1997). The nut development

in pistachio is characterised by three different periods (Goldhamer, 1995): stage I starts at the beginning of the nut growth and ends when its maximum size is reached; during stage II the shell hardening takes place and finally, the stage III is the period of kernel growth (Goldhamer, 1995). Phene et al. (1987) found that in mature pistachio trees growing in shallow soils, a reduction in irrigation of 50% of the crop evapotranspiration ( $ET_c$ ) during shell hardening (stage II) had no effect on final yield. And recently, Goldhamer et al. (2004) showed that the same reduction of the irrigation water during stages I (nut growth) and II (shell hardening) did not reduce the total amount of fruits and increased the percentage of shell splitting, although it also increased early splitting.

Pistachio is a saline-tolerant species (Behboudian et al., 1986; Goldhamer et al., 1987). Within *Pistacia* genus, some investiga-

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tions have been carried out to evaluate seasonal changes of net carbon assimilation and chlorophyll content and to assess the rates of net carbon assimilation, stomatal conductance, transpiration and related parameters in *Pistacia vera* L. (Vemmos, 1994; Novello and de Palma, 1995; De Palma and Novello, 1996). Also, the diurnal patterns of net photosynthesis and leaf conductance have been compared among several *Pistacia* species (Lin et al., 1984). There is no information about the response of the diurnal patterns of gas exchange to water stress, which might be different in different phenological stages of the trees. In pear Marsal and Girona (1997) described variations in the water relations in different phenological stages in no water stress conditions. Such variations during the growing season of the trees may explain the higher drought resistance during some periods.

Water deficits influence a wide variety of physiological processes in higher plants, including leaf expansion, stomatal opening, and photosynthetic carbon assimilation (Robichaux, 1984). Changes in turgor pressure may represent the principal effect by which small changes in plant water status are transduced into changes in metabolism (Hsiao et al., 1976; Zimmermann, 1978). As a result, mechanisms promoting the maintenance of high turgor pressure as the decrease in tissue water content would enhance growth and survival of plants under conditions of low moisture availability (Turner and Jones, 1980; Jones et al., 1981; Bradford and Hsiao, 1982). The hypothesis considered in this work was that some of these mechanisms may change throughout the phenological stages of the pistachio nut development.

The aim of this work was to characterise the response of water potential and gas exchange of pistachio trees to water stress during the three main periods of nut development, identifying the underlying mechanisms that operate at each stage. Also, information drawn from the pressure–volume curves was used to establish the degree of drought resistance of pistachio in each phenological stage.

## 2. Materials and methods

### 2.1. Experimental site and plant material

The experiment was conducted during 2006 in an 11-year-old pistachio (*Pistacia vera* L. cv Kerman on *Pistacia terebinthus* L. rootstock) orchard at the El Chaparrillo Research Station, Ciudad Real, Spain (3°56'W, 39°0'N; altitude 640 m). The soil at the experimental site is a shallow clay-loam (Alfisol Xeralf Petrocalcic Palaxeralfs) with a depth of 1.3 m and a discontinuous petrocalcic horizon between 0.75 and 0.85 m. The volumetric water content ( $\Theta_v$ ) of the top 0.30 m is 22.8% at field capacity (soil matric potential  $-0.03$  MPa) and 12.1% at wilting point (soil matric potential  $-1.5$  MPa). For the layer 0.30–1.30 m,  $\Theta_v$  is 43.0 and 21.1%, for field capacity and wilting point, respectively. The trees were 5 m apart giving a planting density of 400 trees ha<sup>-1</sup>. The time course of ET<sub>o</sub>, rainfall and vapour pressure deficit (VPD) is shown in Fig. 1. These patterns are the common in the area, with rainy autumns and winters and dry and hot summers.

The phenological stages taken into account in the experiment were those suggested by Goldhamer (1995): stage I (shell expansion), from sprouting until the end of shell growth; stage II (shell hardening), from maximum shell size until the beginning of kernel growth; and stage III (kernel growth), from the beginning of kernel growth until harvest.

The crown volumes of the trees at the end of the season were  $6.5 \pm 0.9$  m<sup>3</sup> tree<sup>-1</sup> in C treatment,  $7.7 \pm 1.6$  m<sup>3</sup> tree<sup>-1</sup> in RDI treatment and  $8.8 \pm 2.1$  m<sup>3</sup> tree<sup>-1</sup> in RF treatment. The average total yield (dry nut) during this season was 2447 kg ha<sup>-1</sup> in control trees, 1914 kg ha<sup>-1</sup> in RDI and 2004 kg ha<sup>-1</sup> in rainfed treatment.

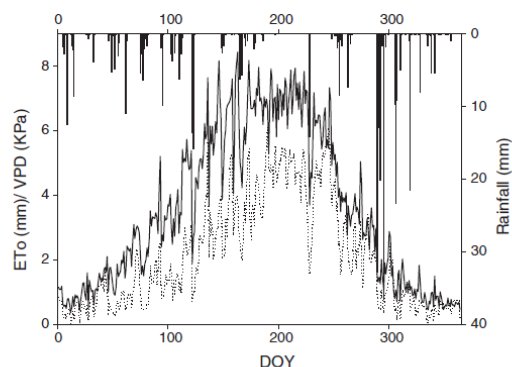


Fig. 1. Seasonal pattern of the rainfall (vertical bars), ET<sub>o</sub> (solid line) and VPD (dotted line), during 2006 season in the experimental site.

### 2.2. Irrigation regimes

The ET<sub>c</sub> was calculated from values of reference evapotranspiration (ET<sub>o</sub>) and the crop coefficient ( $K_c$ ) (Doorenbos and Pruitt, 1974). The mean  $K_c$  values used were 0.39, 1.06 and 1.14 for stages I, II and III, respectively (Goldhamer, 1995) after correcting for canopy size (Feres and Goldhamer, 1990). ET<sub>o</sub> was estimated by the Penman–Monteith equation (Allen et al., 1998) with daily data from a nearby automatic weather station (1.5 km away from the experimental orchard).

The irrigation treatments were as follows:

- Control (C): irrigation was applied to cover the estimated crop evapotranspiration (ET<sub>c</sub>). In order to ensure non-stress conditions, it was increased by 20% of the ET<sub>c</sub> rate if the midday stem water potential ( $\Psi_x$ ) fell below  $-1.0$  MPa.
- Regulated deficit irrigation (RDI): irrigation was applied as 65% of the control treatment during the stages I (shell expansion) and II (shell hardening), and 100% of the control treatment during stage III (kernel growth).
- Rainfed conditions (RF): no irrigation was applied.

Irrigation was performed four days per week with a drip irrigation system. Each tree had five drippers providing a total flow of 20 l h<sup>-1</sup>. The irrigation water used was saline (EC = 3.4 dS m<sup>-1</sup>) and had a high nitrate concentration (NO<sub>3</sub><sup>-</sup> = 66 mg l<sup>-1</sup>). A complete randomised design was used, with 20 trees per treatment. The total volume of applied water was 365.4 and 290.7 mm for the C and RDI treatments, respectively, throughout the experiment. No irrigation was provided during the post-harvest period in any of the treatments.

### 2.3. Plant water relations

In order to compare the response of plant water relations to phenological changes and to water stress a daily curve of leaf gas exchange and stem water potential ( $\Psi_x$ ) was performed on each of the three phenological stages considered (24th of May, 29th of June and 24th of August, 2006). Also, 4–6 pressure–volume ( $P$ – $V$ ) curves per treatment were performed in C and RF trees, two to four days after the daily cycles of gas exchange and  $\Psi_x$ .

In the daily curves, gas exchange parameters and  $\Psi_x$  were determined from predawn until late afternoon. Fully expanded leaves on branches near the main trunk were covered with aluminium foil for at least 1 h before each  $\Psi_x$  measurement. These were made on one

leaf per tree and six trees per treatment using a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA). Because pistachio leaves exude turpentine, a piece of blotting paper was used to determine the end point as turpentine cannot moisten blotting paper but the xylem sap can.

Leaf conductance ( $g_s$ ) and leaf net photosynthesis ( $P_n$ ) were measured on the central foliole of the compound leaf approximately at the same time than  $\Psi_x$ , by using an infrared gas analyser (IRGA, CIRAS-2, PP system, UK). The measurements were made in sunlight and with fully expanded leaves from the same trees in which  $\Psi_x$  was measured.

The pressure–volume ( $P$ – $V$ ) curves were performed only in the C and RDI treatments. Five leaves per treatment were collected at predawn, placed immediately in plastic bags and taken to the laboratory. There they were rehydrated for 1 h by placing the cut end of the petiole under water. A pressure chamber was used to measure leaf  $\Psi$ . Before placing them in the pressure chamber, each leaf was wrapped in moist cheesecloth, placed in a black plastic bag and weighed (Hsiao, 1990). The actual fresh weight of each  $\Psi$  determination was assumed to be the leaf weight measured immediately before the insertion of the leaf in the pressure chamber. Periodic measurements of fresh weight and  $\Psi$  were taken until a  $\Psi$  value around  $-3.5$  MPa. Pressure–volume curves were generated by the free transpiration method (Hinckley et al., 1980) and  $1/\Psi$  was plotted versus relative water content (RWC).

Zero turgor point was determined through a graphical analysis, considering the lineal portion of the curve. It was calculated with the experimental points that resulted in the maximum determination coefficient ( $R^2$ ) of the lineal regression. The parameters derived from each curve were: osmotic potential at full turgor ( $\Psi_{s,100}$ ), osmotic potential at zero turgor ( $\Psi_{s,0}$ ), relative water content at zero turgor (RWC<sub>0</sub>) and percentage of the symplastic water content ( $R$ ). The tissue elasticity ( $E_o$ ) was calculated considering the two highest turgor data of the  $P$ – $V$  curve (Dichio et al., 1997). Also, the average tissue elasticity ( $E_{o,av}$ ) integrated over the full range of positive turgor was estimated assuming a linear relationship between turgor potential and RWC (Wilson et al., 1979) as proposed by Marsal and Girona (1997).

The osmotic adjustment index (OA index) and the breaking point (BP) (Turner, 2006) were used to compare the degree of osmotic adjustment at different phenological stages. The OA index is 1 minus the slope of the lineal relationship between the natural logarithms of osmotic potential (independent variable) and the RWC. OA index varies between 0 and 1 and it is an estimation of the degree of osmotic adjustment. The second parameter derived by Turner (2006) is the breaking point (BP). The relationship between the natural logarithms of osmotic potential and the RWC may be constant with no decrease in RWC until a threshold value (BP) is reached, from which that relationship is lineal. Lower values of BP mean higher capacity of drought resistance.

2.4. Statistical analysis

Statistical analyses of variance and Tukey test (SX 8.0, Analytical software) were performed for treatment comparison. Treatment differences were considered statistically significant at  $P < 0.05$ .

3. Results

Fig. 2 shows the daily pattern of midday stem water potential ( $\Psi_x$ ) in three different phenological stages of the trees.  $\Psi_x$  ranged from  $-0.5$  MPa to  $-1.4$  MPa during stage I (Fig. 2a). Significant differences were found between the irrigated (C and RDI) and the RF treatments in all the sampling times except the last one. RF data showed the fastest initial decrease and final increase of  $\Psi_x$  through-

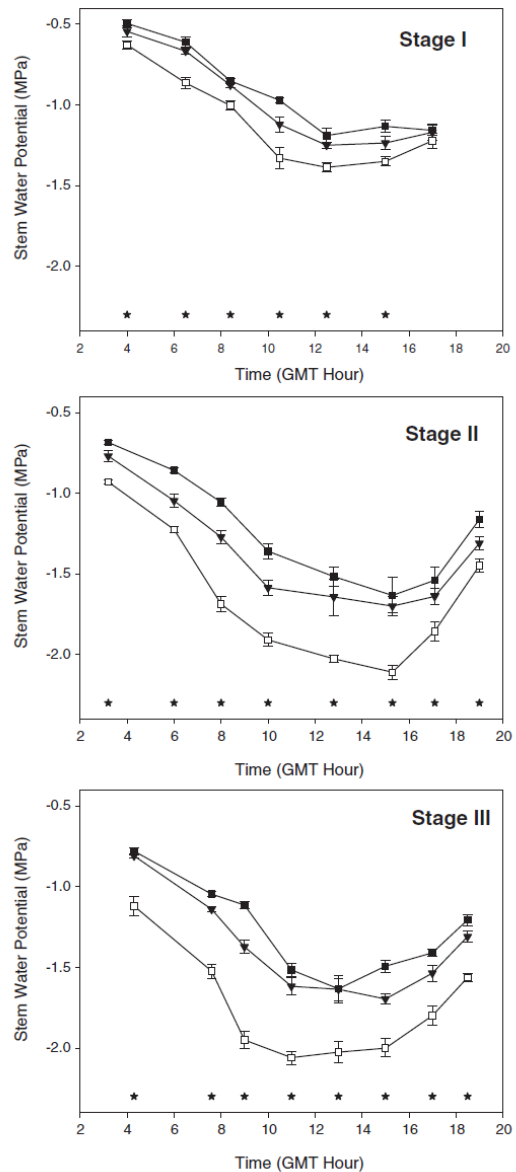


Fig. 2. Daily pattern of stem water potential ( $\Psi_x$ ) during the stages I, II, and III. Each point is the average of 5 measurements. (■) C treatment, (□) RF treatment and (▼) RDI treatment. Asterisks denote the dates when significant differences were found among treatments (see text for explanation).

out the day. In the stage II (Fig. 2b) the dehydration of the trees was higher in all the treatments, especially in rainfed conditions. The values of  $\Psi_x$  ranged from  $-0.7$  to  $-2.1$  MPa, being those of the RF treatment significantly the lowest at all sampling times. In addition, RDI treatment  $\Psi_x$  was significantly lower than the control at the beginning of the day (at 6, 8, 10 GMT). During stage III (Fig. 2c)

the range of  $\Psi_x$  was similar to that measured at stage II (values from  $-0.8$  MPa until  $-2.1$  MPa). But in RF treatment  $\Psi_x$  decreased earlier in the day than in stage II with significant differences with the irrigated treatments. RDI and C treatments showed similar  $\Psi_x$  values during all the daily cycle, being significantly different only at 9 and 15 GMT.

The gas exchange of the trees was characterised by measuring the leaf conductance ( $g_s$ ) throughout the day on the same dates that those selected for  $\Psi_x$  measurements (Fig. 3). During the stage I (Fig. 3a) the  $g_s$  of RF trees increased early in the morning until 8:00 GMT, reaching the maximum values at that time. In C and RDI trees  $g_s$  was maximum at midday. From the time that maximum  $g_s$  was reached it was roughly constant until the sunset, around 18:00 GMT, then decreasing in response to darkness. Stomatal conductance of RF trees was significantly lower than the control and RDI treatments, except in the first point early in the morning. The maximum values of  $g_s$  were around  $340 \text{ mmol m}^{-2} \text{ s}^{-1}$  in RF trees, while in control and RDI trees were around  $500 \text{ mmol m}^{-2} \text{ s}^{-1}$ . The daily patterns of  $g_s$  during stage II were similar to those of the stage I with a faster increase at the beginning of the day and higher maximum values.  $g_s$  ranged from 200 to  $750 \text{ mmol m}^{-2} \text{ s}^{-1}$ , being those of the control treatment significantly higher than the other two treatments during all the day. The maximum  $g_s$  of rainfed trees was around  $450 \text{ mmol m}^{-2} \text{ s}^{-1}$ , while in control trees was around  $700 \text{ mmol m}^{-2} \text{ s}^{-1}$ .  $g_s$  in RDI treatment was significantly lower, around  $500 \text{ mmol m}^{-2} \text{ s}^{-1}$ , than in control trees only from 13:30 to 17:30, when the maximum values were measured. In the daily cycle of stage III the first data could not be taken due a technical problem (Fig. 3c). The daily pattern of stomatal conductance was different to those reported previously for stages I and II in control and RDI treatment, showing a decrease throughout the day from before or around midday in C and RDI treatments, respectively. Also, the maximum  $g_s$  values in these two treatments were slightly lower than those showed in stages I and II for the same treatments. In RF trees there was a higher and earlier stomata closure, showing a minimum  $g_s$  around midday, and staying roughly constant until the sunset. The leaf conductance in all the treatments in this date was clearly lower than that measured during stage II but only slightly lower than that reported in stage I. Maximum  $g_s$  values of different treatments occurred at different times, first in RF treatment ( $487 \text{ mmol m}^{-2} \text{ s}^{-1}$ ), after that in C treatment ( $608 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) and finally in RDI treatment ( $505 \text{ mmol m}^{-2} \text{ s}^{-1}$ ). There were very small differences between C and RDI treatments, some of them not statistically significant. However  $g_s$  was significantly lower in RF treatment.

Leaf net photosynthesis ( $P_n$ ) was also measured on the same dates than  $\Psi_x$  and  $g_s$  (Fig. 4). In the stage I the daily pattern of  $P_n$  was almost identical to the leaf conductance. Only at the end of the day  $P_n$  decreased faster than  $g_s$  in response to decreasing levels of radiation (Fig. 4a). The  $P_n$  values were similar between treatments and only slightly differences were found in the first three data taken. RF trees reached the maximum values earlier than control and RDI but such differences were likely related to the time lag from the first (C) and the last (RF) trees measured, as radiation increased very fast. In the next two measuring times, with no limitation of radiation,  $P_n$  of RF trees was significantly lower than in the C and RDI trees, but from midday no significant differences were found among treatments. The daily patterns of  $P_n$  during stage II were similar to those of stage I (Fig. 4b), but the maximum values in stage II were a 50% higher than in stage I,  $30 \mu\text{mol m}^{-2} \text{ s}^{-1}$  compared to  $20 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . The differences between irrigated treatments (C and RDI) and RF treatment were again small but significant at 10:00 GMT and in the last two measuring times.  $P_n$  of RDI and C trees were similar except at the beginning and at the end of the day. These differences were attributed to the same time-lag effect explained above. At the beginning and at the end of the day radiation var-

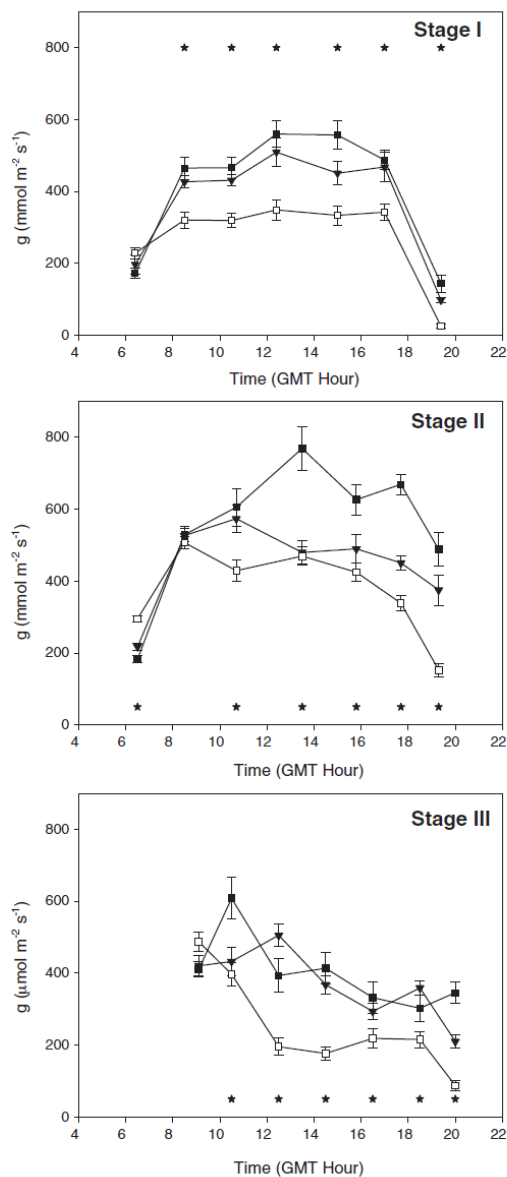


Fig. 3. Daily pattern of leaf conductance ( $g_s$ ) during the stages I, II, and III. Each point is the average of 12 measurements. (■) C treatment, (□) RF treatment and (▼) RDI treatment. Asterisks denote the dates when significant differences were found among treatments (see text for explanation).

ied very fast, increasing the variability of  $P_n$  measurements as the leaves were receiving different light intensity. Maximum values in stage III were similar to those of the stage I (Fig. 4c). The differences between treatments were short but significant between RF and the irrigated trees, both C and RDI, throughout the day.

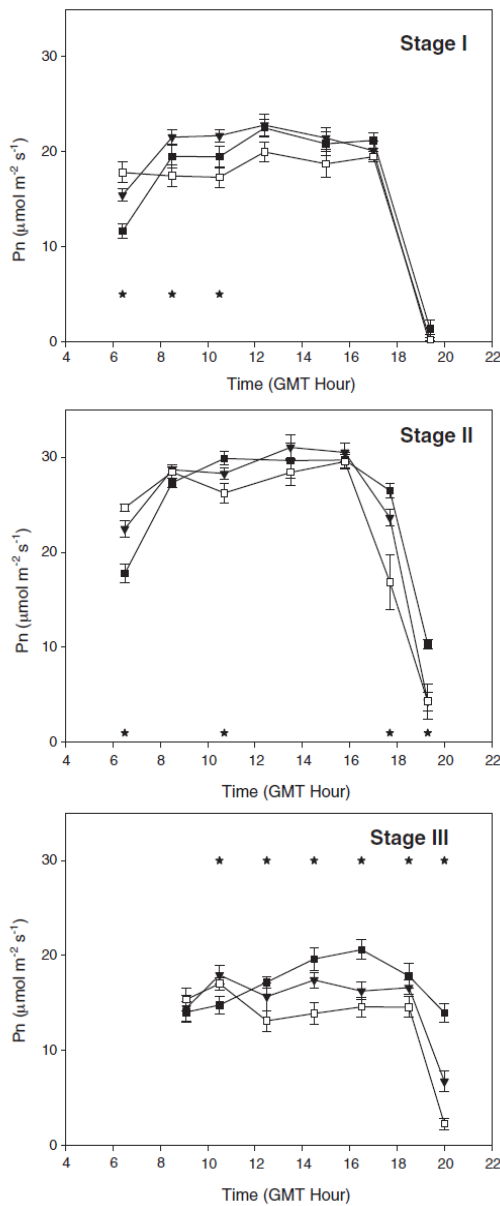


Fig. 4. Daily pattern of leaf photosynthesis ( $P_n$ ) during the stages I, II, and III. Each point is the average of 12 measurements. (■) C treatment, (□) RF treatment and (▼) RDI treatment. Asterisks denote the dates when significant differences were found among treatments (see text for explanation).

The examination of the relationships between water potential, leaf conductance and leaf net photosynthesis could clarify the response of the trees to water deficits in each phenological stage (Fig. 5). The data used to obtain such relationships were those measured at midday. The relationship between leaf  $P_n$  and  $g_s$  was poor when all the data were considered (Fig. 5a). For the same  $g_s$ , midday

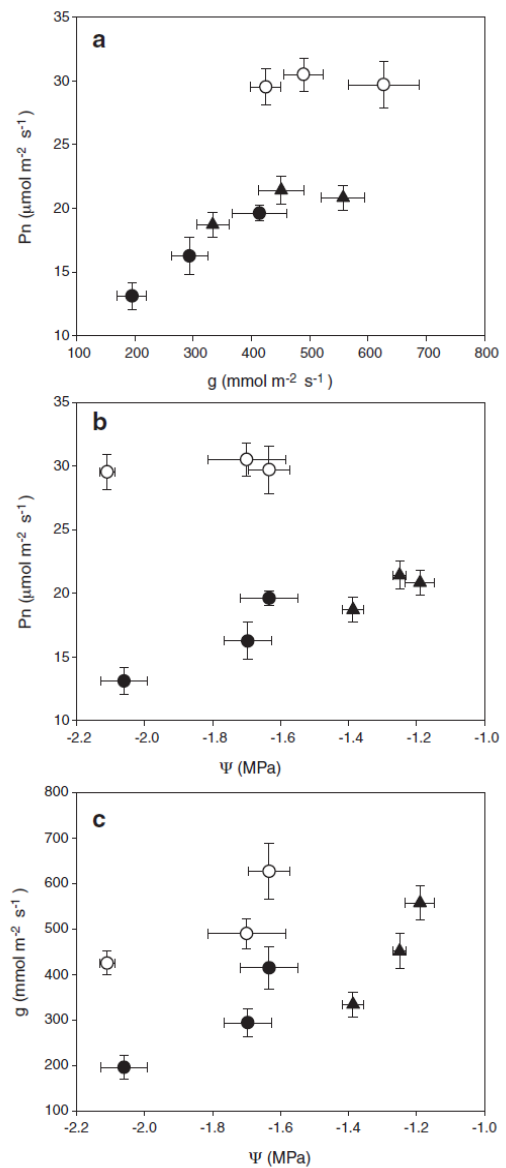


Fig. 5. Relationship between the midday leaf conductance and leaf photosynthesis (a); midday leaf photosynthesis and midday stem water potential (b); and midday leaf conductance and midday stem water potential (c). Each point is the value obtained in Figs. 2–4. (▲) Stage I; (○) stage II; and (●) stage III. SE bars are depicted for both parameters in each average value.

$P_n$  was much higher in stage II than in the other two phenological stages. However, when only stages I and III were considered there was no significant relationship. Also the relationship between leaf  $P_n$  and  $\Psi_x$  was poor when the stage II data were considered (Fig. 5b). The values of midday leaf  $P_n$  during this phenological stage were the highest at low water potential. When only stages I and III were



**Table 1**

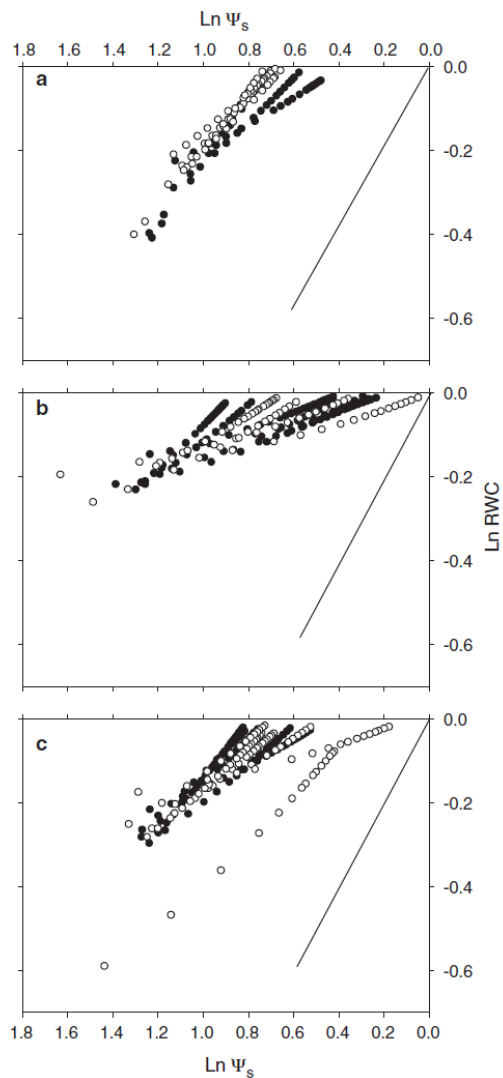
Pressure–volume curve parameters obtained in each phenological stage in the rainfed and control treatment. Each value is the average of 5 data. For the same stage, different subscript letters mean significant differences between treatments (Tukey test;  $P < 0.05$ ). For the same treatment, different superscript letters mean significant differences among phenological stages (Tukey test;  $P < 0.05$ ).  $RWC_0$ : Relative water content at zero turgor;  $\Psi_{s,0}$ : osmotic water potential at zero turgor;  $E_0$ : elastic modulus;  $\Psi_{s,100}$ : osmotic water potential at full turgor;  $E_{0av}$ : average elastic modulus;  $R$ : symplastic water content.

	Stage I		Stage II		Stage III	
	Rainfed	Control	Rainfed	Control	Rainfed	Control
$RWC_0$ (%)	86	85 <sup>b</sup>	90	92 <sup>a</sup>	85	88 <sup>ab</sup>
$\Psi_{s,0}$ (MPa)	-2.3	-2.5	-2.4	-2.0	-2.7	-2.6
$E_0$ (MPa)	15.7 <sup>b</sup>	15.3	32.2 <sup>a</sup>	29.5	9.8 <sup>ab</sup>	26.6 <sup>a</sup>
$\Psi_{s,100}$ (MPa)	-1.8	-2.0	-1.6	-1.5	-2.0	-1.5
$E_{0av}$ (MPa)	13.3	13.4 <sup>b</sup>	16.5	19.9 <sup>a</sup>	13.8	12.4 <sup>b</sup>
$R$ (%)	65 <sup>a</sup>	63 <sup>a</sup>	34 <sup>b</sup>	30 <sup>b</sup>	55 <sup>ab</sup>	47 <sup>ab</sup>

considered a significant but weak relationship between  $P_n$  and  $\Psi_x$  was found ( $R^2 = 0.50^*$ ). Finally, the relationship between leaf conductance and water potential (Fig. 5c) was similar to the  $P_n - \Psi_x$  (Fig. 5b). The  $g_s$  values of stage II were closer to the other treatments but again higher for similar levels of dehydration. In this case, when stage II data were considered alone a significant but weak lineal relationship was found ( $R^2 = 0.53^{***}$ ).

One of the plant responses to water stress is the osmotic adjustment. The  $P-V$  curve technique can give information about these mechanisms of response. The  $P-V$  curves were performed only with data of RF and C treatments (Table 1). There were no significant differences between treatments in any of the parameters estimated due to the high coefficient of variation. The relative water content at zero turgor ( $RWC_0$ ) showed no differences among treatments and phenological stages (occurred at around 90% RWC). The water potential at zero turgor ( $\Psi_{s,0}$ ) varied from  $-2.0$  MPa (stage II – control) to  $-2.7$  MPa (stage III – rainfed), but again differences were not statistically significant. The average  $\Psi_{s,0}$  value was similar and around  $-2.30$  MPa. The elasticity modulus ( $E_0$ ) presented a seasonal trend, with higher values in stage II (around 33 MPa) than in stage I (around 15 MPa).  $E_0$  values in stage III were opposite to expected as RF trees showed significantly lower  $E_0$  than control, while they were similar in stages I and II. The variability of this parameter decreased when the calculation was made between zero and full turgor point ( $E_{0av}$ ).  $E_{0av}$  values were similar in stages I and III (around 13 MPa), being slightly higher in stage II (around 17 MPa), but with no significant differences between treatments. The osmotic potential at full turgor ( $\Psi_{s,100}$ ) was not significantly different among treatments. The mean values of  $\Psi_{s,100}$  were around  $-1.9$  MPa, being only in stage II slightly lower in both treatments (around  $-1.6$  MPa). The symplastic water content ( $R$ ) was again similar in both irrigation treatments but differed between different phenological stages. Maximum values of symplastic water content were around 65% in stage I, then it sharply decreased in stage II reaching values around 35% and finally it increased to 50% during stage III.

Turner (2006) suggested a methodology to compare the magnitude of osmotic adjustment between species and their degree of adaptation to drought. In this work, this methodology has been used to compare the degree of osmotic adjustment of different phenology stages for a given irrigation treatment. The osmotic potential data have been calculated from the  $P-V$  curves. Turner (2006) derived two indexes (OA index and BP, as described in Section 2) from the relationship between the natural logarithms of osmotic potential and relative water content. Fig. 6 depicts such relationships and the regression equations are shown in Table 2. There were no significant differences between RF and C trees in BP (Table 2). However, for the control treatment BP was significantly



**Fig. 6.** Relationship between  $\ln RWC$  and  $\ln \Psi_s$  during stages I, II and III. Points were calculated from the  $P-V$  curves of the (○) C treatment and (●) RF treatment. The line represents the same variations between the RWC and the osmotic potential. Points in this line indicate that the increase in the osmotic pressure is related with the decrease in the water content.

lower at stage I than stages II and III showing values around  $-1.50$ ,  $-1.10$  and  $-1.30$  MPa, respectively. There were neither significant differences in OA index between C and RF treatments for the same phenological stage. However, the seasonal pattern changed with phenology. The higher degree of osmotic adjustment was found in stage II, showing OA values of 0.78, while in stages I and III it was 0.42 and 0.62, respectively.



Table 2

Regression equations of the relationship between natural logarithms of osmotic potential ( $\Psi_s$ ) and the relative water content (RWC) for the control and rainfed treatments in the three phenological phases. Each equation corresponds with the pressure–volume curves performed during the experiment. The data of osmotic potential was calculated with the pressure–volume curves. Different letters in the same column mean significant differences (Tukey test;  $P < 0.05$ ) between phenological stages. There were no significant differences between irrigation treatments. BP: breaking point, and OA index: osmotic adjustment index.

Phenological phase	Control				Rainfed			
	Ln $\Psi_s = a + b \times \text{Ln RWC}$				Ln $\Psi_s = a + b \times \text{Ln RWC}$			
	a	b	BP (MPa)	OA index	a	b	BP (MPa)	OA index
Stage I	0.39	–0.58	–1.48b	0.42b	0.37	–0.59	–1.45	0.41b
Stage II	0.14	–0.27	–1.15a	0.78a	0.09	–0.22	–1.09	0.73a
Stage III	0.19	–0.38	–1.21a	0.62a	0.33	–0.48	–1.39	0.52ab

#### 4. Discussion

The daily pattern of stem water potential (Fig. 2), leaf conductance (Fig. 3) and net photosynthesis (Fig. 4) indicated that pistachio trees are tolerant to mild dehydration conditions. The leaf conductance was maximum at midday, even in conditions of high vapour pressure deficit (VPD) (Fig. 3). Leaf conductance was very high, especially during stage II in control trees. The values reported in literature usually are lower than those obtained in this experiment. However, Goldhamer et al. (1985) and Vemmos (1994) give similar  $g_s$  values to those presented in this work. In these two works the leaf conductance was measured with an infrared gas analyser and includes both, abaxial and adaxial conductances, as in the values presented here. In this work, when only abaxial leaf conductance was measured by using a steady state porometer maximum values around 450–500  $\text{mmol m}^{-2} \text{s}^{-1}$  were found (Gijón et al., 2008). The daily pattern of net photosynthesis was similar to that of leaf conductance but differences among treatments were lower. Maximum values of  $P_n$  changed throughout the season with higher values during stage II than in the other two (Fig. 4). Similar changes in net photosynthesis have been described in pistachio under non-stress conditions and were related to leaf age (Lin et al., 1984; Vemmos, 1994; Novello, 1998). In addition, net photosynthesis at midday was linearly related to leaf conductance up to a  $g_s$  value of 450  $\text{mmol m}^{-2} \text{s}^{-1}$  in this experiment (Fig. 5a) that may indicate a partial stomatal control as it has been reported in other drought tolerant species as olive trees (Moriani et al., 2002).

The daily pattern of  $g_s$  was clearly affected by water stress, especially at stage III when the degree of water stress was higher (Fig. 3). In that stage, not only the maximum  $g_s$  value but also the pattern itself was different in RF from control trees. The RF tree pattern showed a reduction of leaf conductance at midday, while maximum values were still measured in the irrigated treatments (Fig. 3c). However, assimilation was less sensitive to water stress, as  $P_n$  differences between C and RF trees were lower than those found in  $g_s$  as the stress progressed (Fig. 4). In stage III, when the lowest values of  $\Psi_x$  were measured, the stomata started to close before midday in RF trees but assimilation stayed fairly constant until the sunset. These results support that in pistachio the assimilation processes other than the diffusion component are less sensitive to water stress and operate at lower levels of leaf water potential. Therefore, this species can be considered as tolerant to mild dehydration as it maximizes the assimilation rate under these stress conditions. This has been described as a common response in arid habitats in species like *Quercus* (Tenhunen et al., 1987) and may partially explain the high drought resistance of pistachio trees (Behboudian et al., 1986; Ferguson, 2005). There are several physiological mechanisms in plants to survive in drought-prone environments (Feres, 1984) which are shown by different species and depend on the water stress level. The stomatal control is usually linked to a threshold level of leaf water potential, below that the leaf resistance increases progressively (Tenhunen et al., 1987; Feres, 1984).

The relationships between the midday values of stem water potential, net photosynthesis and leaf conductance were different depending on the phenological stage. Stages I and III presented similar relationships (Fig. 5) with a good lineal fit (Fig. 5a:  $R^2 = 0.84$ ; Fig. 5b:  $R^2 = 0.86$ ; Fig. 5c:  $R^2 = 0.76$ ). However, in stage II those relationships were clearly different. These variations indicate that some physiological changes are occurring during this phenological stage. The net photosynthesis during stage II was almost constant with variations of leaf conductance and stem water potential that indicated a drought adaptation during this period of time. Therefore, the stomatal or hydraulic control of assimilation starts at a lower threshold in the phenological stage II than in stages I and III. Sajjadinia et al. (2010) also reported an increase in transpiration during the endocarp hardening in different cultivars of pistachio subjected to water deficit.

There were no significant differences in the pressure–volume curves data (Table 1). These results are likely related to the water stress imposed which was probably too fast to find significant differences between treatments (Turner and Jones, 1980). However, these results also show clear variations of some parameters depending on the phenological stage of the trees. During stage II, the elastic modulus ( $E_o$  and  $E_{oav}$ ) was clearly higher than in the rest of the season, while the percentage of symplastic water was reduced in the same period of time. An increase of the elastic modulus has been described as an adaptation to drought in other species like olive (Dichio et al., 1997) and pear (Marsal and Girona, 1997). The decrease of symplastic water has been also reported as a drought response in pear (Marsal and Girona, 1997), carob (Nunes et al., 1989), *Pseudotsuga menziesii* (Mirb) Franco (Joly and Zaerr, 1987), *Acer rubrum* L. (Nash and Graves, 1993) but no in olive trees which show the opposite effect (Dichio et al., 1997). In addition, Marsal and Girona (1997) have also described differences in some of these parameters throughout the season in full-irrigated pear trees. Such variations indicated that during stage II, even under non-stress conditions, the trees modified several water-relations parameters. These modifications would confer higher drought tolerance to the trees during this stage. In pistachio the shell wall is thickening during stage II, which is considered the less sensitive period to water stress (Goldhamer, 1995). The wall thickening is considered as the greatest metabolic cost in stone fruit trees and variations in carbon balance and water relations related to different fruit loads have been reported in peach (DeJong, 1986) and in olive (Rallo and Suárez, 1989).

The osmotic adjustment index and the break point parameter proposed by Turner (2006) allow the comparison between different species in relation to the response to drought conditions, and in this study they will be used to compare different phenological stages. The higher OA index in stages II and III (Table 2) indicated a higher degree of osmotic adjustment in these periods than in stage I. The variations in elastic modulus and percentage of symplastic water during stages II and III may be related to this higher osmotic adjustment than in stage I. These values of OA index are similar to those reported for high osmotic adjusting lines of wheat (Turner,

2006). The BP estimated the osmotic potential at which the RWC starts to decrease and it is an estimation of drought resistance. In pistachio, BP values presented in the stage I were lower than stages II and III in control trees (Table 2) and were similar to those reported for drought-sensitive sorghum lines and higher than those reported for wheat (around  $-3$  MPa, Turner, 2006). All these results suggest that different mechanisms of drought resistance are operating in pistachio at different phenological stages. In stage I the pistachio trees tolerate low water potentials before osmotic adjustment takes place, showing lower BP values. In stages II and III the degree of osmotic adjustment increases resulting in higher values of OA index. This would be the most important mechanism of drought resistance in this species.

## 5. Conclusions

The water relations in pistachio trees are dynamics and change throughout the season. In mild water stress conditions, like those occurring during stage I, the differences in  $g_s$  between stressed non-stressed plants are small enough to allow similar values of net photosynthesis. As water stress progresses, also variations in the daily pattern of  $g_s$  were found. Instead of a roughly constant maximum  $g_s$  when light is not limiting as occurred in stages I and II, there was a progressive stomatal closure from before the mid-day. However, net photosynthesis was less sensitive to water stress than stomata, maximizing plant assimilation in conditions of mild water stress. In addition, during stage II the leaves showed some physiological changes like variations in the elastic modulus and percentage of symplastic water. Such variations seem to be related to a higher degree of osmotic adjustment during stages II and III. These differential changes in the water relations of pistachio trees in different phenological stages support the recommendation of reducing the irrigation during stage II with no or slight decrease in crop yield.

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