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**IMPROVEMENT OF REGULATED DEFICIT
IRRIGATION IN PISTACHIO (*PISTACIA VERA L.*);
Water relations and rootstocks influence.**

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- **Impacts of water stress, environment and rootstock on the diurnal behaviour of stem water potential and leaf conductance in pistachio (*Pistacia vera L.*).**
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RESUMEN

El objetivo general de esta tesis doctoral fue optimizar la gestión del riego en el pistachero en base a la aplicación de estrategias de riego deficitario controlado (RDC) y el uso de indicadores del estado hídrico de la planta. Este propósito implicaba la evaluación de la aplicabilidad y utilidad del potencial hídrico del tronco (Ψ_s) como herramienta para la programación del riego, así como la evaluación de las respuestas agronómicas y fisiológicas del pistacho a diferentes regímenes de riego y porta-injertos.

Para este propósito, pistacheros del cultivar "Kerman", injertados sobre tres patrones diferentes (*Pistacia Terebinthus*, *Pistacia atlantica* y *Pistacia integerrima*) fueron sometidos a tres regímenes de riego. Una estrategia de riego óptimo satisfaciendo el 100 % de las necesidades hídricas de los árboles, ajustando estas dotaciones con medidas de potencial hídrico del tronco, y dos estrategias de riego deficitario controlado. El éxito de una estrategia de RDC depende de la precisión del manejo del estrés hídrico siendo el tiempo, la duración y la severidad del estrés aplicado sus principales componentes. Por lo tanto, la aplicación de las estrategias de estrés hídrico se llevó a cabo intentando definir con precisión las fases de crecimiento del fruto y basándose en un umbral establecido del potencial hídrico del tronco.

Los tratamientos de RDC se regaron como el control durante toda la temporada, excepto durante la fase II de crecimiento del fruto en el que un nivel de estrés hídrico de -1.5 MPa se impuso a la primera estrategia (T1) y -2.0 MPa a la segunda (T2). El estudio se llevó a cabo durante tres años (2012, 2013 y 2014), en una parcela de pistacho situada en Ciudad Real (Castilla-La Mancha, España).

Este trabajo se ha dividido en dos partes principales; la primera parte trata con la respuesta

fisiológica del pistacho estudiando sus relaciones hídricas y ajustando la programación del riego a las necesidades del cultivo. En la segunda parte se propuso estudiar su respuesta agronómica comparando el crecimiento vegetativo y el potencial productivo en función de cada tratamiento y porta-injerto.

El resultado principal de este estudio fue la posibilidad de programar con precisión el riego en una plantación de pistachos cultivados en suelos poco profundos a base de medidas del potencial hídrico del tronco. La utilización del potencial hídrico para la programación de riego se ha traducido en una economía significativa de agua ya sea bajo condiciones de riego óptimas o bajo condiciones de estrés. En los árboles bajo riego óptimo, el ahorro de agua osciló entre 46 y 205 mm, dependiendo del año. En cuanto a los tratamientos de RDC, el ahorro de agua osciló entre 297 a 385 mm por año en el T1 y 347 a 445 mm en el T2. Esta gestión del recurso hídrico dio lugar a la generación de una línea base para pistacho que relaciona el Ψ_s a la DPV y que podría ser utilizada para la programación de riego. El curso del Ψ_s mostró un comportamiento diferente en función del grado de estrés, pero no se vio afectado por las fases fenológicas. Sin embargo, el comportamiento de la conductancia estomática de la hoja (g_l) mostró una dependencia a las fases fenológicas que se tradujo en un cambio de la relación g_l -DPV a lo largo de la temporada.

Las reducciones de las dotaciones hídricas han afectado el crecimiento vegetativo, pero no el rendimiento o su calidad, excepto en el primer año donde las reducciones del rendimiento se han estimado alrededor del 25 % en comparación con el control. Sin embargo, no se ha visto una reducción del rendimiento promedio en los tres años. Como resultado de este estudio, se sugirió un umbral de Ψ_s de -1.5 MPa durante la fase II de crecimiento del fruto (T1) para programaciones de RDC.

En cuanto a los porta-injertos, *P. integerrima* mostró poca adaptación a la escasez de agua en comparación con los otros dos patrones en las condiciones estudiadas.

ABSTRACT

The overall aim of this PhD thesis was to optimize irrigation management of pistachio according to Regulated Deficit Irrigation (RDI) strategies implementation and the use of plant-based water status indicators. This purpose involved the evaluation of the applicability and usefulness of stem water potential (Ψ_s) as a tool for irrigation scheduling as well as the assessment of the agronomic and physiological responses of pistachio trees to different irrigation regimes and rootstocks.

For this purpose, pistachio trees of the cultivar “Kerman”, grown on three different rootstocks (*Pistacia terebinthus*, *Pistacia atlantica* and *Pistacia integerrima*) were submitted to three irrigation regimes. A full irrigated strategy supplied with 100 % of its water needs, adjusted with stem water potential measurements, and two regulated deficit irrigation strategies.

The success of an RDI strategy depends on the precision of water stress management being the timing, duration and severity of the water stress applied its main components. So, the implementation of the water stress strategies was carried out attempting to define precisely fruit growth phases and according to an established stem water potential threshold.

RDI treatments were irrigated as control during the season except during stage II of fruit growth in which a water stress level of -1.5 MPa was imposed to the first strategy (T1) and -2.0 MPa to the second one (T2). The study was conducted for three years (2012, 2013 and 2014), in a pistachio orchard located in Ciudad Real (Castilla- La Mancha, Spain).

This work has been divided in two major parts; the first one treats with the physiological response of pistachio studying irrigation scheduling and water relations and the second part

deals with its agronomic response comparing the vegetative growth and the productive potential of each treatment and rootstock.

The main result in this study was the ability to schedule accurately irrigation in pistachio trees growing on shallow soils based on stem water potential measurements. The accuracy of the use of water status indicator for irrigation scheduling was translated in a significant economy of water whether under full irrigated or stressed regimes. In the full irrigated trees, water saving ranged between 46 and 205 mm depending on the year. Regarding RDI treatments, water saving ranged between 297 to 385 mm per year in T1 and 347 to 445 mm in T2. This management led to the generation of a baseline for pistachio relating Ψ_s to VPD that could be used for irrigation scheduling. Ψ_s course showed a different behaviour according to the degree of water stress but was not affected by the phenological stage. Nevertheless, g_l behaviour showed a reliance to the phenological stage which was translated in a change of the g_l -VPD relation along the season.

Water reductions affected vegetative growth but not the yield or its quality except for the first year where yield reductions were around 25 % compared to Control. Nonetheless, it does not result in a reduction of the mean yield for the three years.

As a result of this study, a Ψ_s threshold of -1.5 MPa during stage II (T1) was suggested for RDI scheduling.

Regarding rootstocks, *P. integerrima* showed little adaptation to water shortage compared to the two other rootstocks under the studied conditions.

LIST OF ABBREVIATIONS

CH:	Required chill hours
DOY:	Day Of the Year
ET:	Evapotranspiration
ETc:	Crop evapotranspiration
ETa:	Actual evapotranspiration
ETo:	Reference evapotranspiration
FAO:	Food and Agriculture Organization of the United Nations
g _l :	Leaf conductance
HU:	Heat units
I:	Irrigation
Kc:	Crop coefficient
Kr:	Reduction/corrector coefficient
ON year:	Term used in pistachio in reference to high bearing year
OFF year:	Term used in pistachio in reference to low bearing year
P:	Rainfall
P.:	Pistacia
PT:	<i>Pistacia terebinthus L.</i>
PA:	<i>Pistacia atlántica Desf.</i>
PI:	<i>Pistacia integerrima L.</i>
RCVG:	Relative canopy volume growth

RDI: Regulated Deficit Irrigation

RH: Relative humidity

RWC: Relative water content

SWC: soil water content

Tm: Mean temperature

Tmax: Maximum temperature

Tmin: Minimum temperature

VPD: Air vapor pressure deficit

Ψ_s : Stem water potential

Ψ_l : Leaf water potential

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CHAPTER 1: INTRODUCTION

1.1. ORIGIN, TAXONOMY AND MORPHOLOGICAL CHARACTERISTICS

The origin of the genus *Pistacia* L. is still controversial but there is a general belief to have originated in central Asia, from where the genus extended its distribution to West Asia, the Mediterranean basin, East Africa and the new world.

Pistacia L. belongs to the family Anacardiaceae related to the cashew, mango, poison ivy and oak, pepper tree and sumac (Ferguson et al., 2005), descending from the order Sapindales (Zohary, 1952; Pell, 2004). It contains nine species and five subspecies according to the most recent classification of Al-saghir (2012). Species are xerophytic being trees or shrubs, of a height varying from 1-5 m to 20 m and tending to an open canopy growth habit. Species are dioecious, deciduous or evergreen. Leaves are always pinnately-compound. In both male and female trees, flowers are apetalous. Being a dioecious genus, male and female flowers grow on different trees; they are unisexual and born in panicles. They consist of one to several hundred of flowers and are produced by lateral buds formed the previous year. *Pistacia vera* L. is the most primitive species in the genus according to the study of Al-saghir (2010). The leaves of *Pistacia vera* L. are deciduous, randomly oriented and isobilateral with a wingless rachis. *Pistacia vera* L. is the unique species in *Pistacia* genus that the leaflet internal anatomy are heterogeneous; the abaxial palisade consists of two layers of cells that appear slightly longer than other species when the adaxial palisade cells are shorter than those of other species.

Pistacia vera L. flowers are wind-pollinated. Male inflorescences are composed of 450 to 500 flowers arranged on the branches of one year (Mlika, 1980). The female inflorescences of *Pistacia vera* L. are larger than male inflorescences and are around 190-260 flowers

(Mlika, 1980). The feminine flowers lack of nectarine thus does not attract bees and are wind-pollinated.

Botanically, pistachio nuts are drupes, the same classification for almonds, peaches, apricots, cherries and plums. All drupes consist of three parts; an exocarp, a fleshy mesocarp and an endocarp that encloses a seed. The difference lies in the edible portion. In pistachios and almonds the seed is consumed, rather than the mesocarp as in stone fruits.

The rooting habit of the tree is classified as a phreatophyte. Phreatophytes have extensive root systems allowing them to mine the soil deeply. Thus, pistachios are adapted to survive long periods of drought (Ferguson et al., 2005).

1.2.PISTACHIO TRADE AND WORLD MARKET

The economic importance of the genus *Pistacia* L. has its roots in the species *Pistacia vera* L. for its edible seeds, currently known as pistachio. The pistachio has been considered a delicacy since the beginning of recorded history, and has been cultivated for centuries throughout its native range.

The total pistachio cultivated area worldwide is over 600.000 ha. Iran is the country with the greatest number of hectares devoted to this crop and account for around 300.000 ha followed by the USA with around 90.000 ha, Turkey with 54.000 ha, Syria with 40.000 ha and Tunisia with 30.000 ha (FAOSTAT, 2012). Pistachio is the third nut produced around the world after almonds and walnuts with 638.000 metric tons in 2014. The USA, Iran and Turkey are the main pistachio producers in the world accounting for 92 % of total pistachios production. Currently, USA led the world production with 235.000 metric tons, followed by Iran with 230.000 metric tons and Turkey with 130.000 metric tons. In the European Union, pistachio surface is around 14.000 ha divided into the following

countries: Spain (6000 ha), Greece (5400 ha), Italy (3200 ha) and Cyprus (150 ha). Greece led the European production with 8000 t, followed by Italy 2000 t, Spain 500 t and Cyprus 20 t.

Globally, Spain ranks tenth with the prospect to equalize the productions of Greece and Italy within a short period since the current low production of Spain is due to the fact that the majority of orchards still being young. Of the 6000 hectares of Spain, about 4000 ha are managed under rainfed regimes. Castilla-La-Mancha holds about 80 % of the total surface followed by Catalonia and Andalusia.

With 80 % of total, USA and Iran led pistachio exports with 139.000 metric tons and 128.000 metric tons respectively in 2013 (United States International Trade Commission, 2013). The largest pistachio import markets are Hong Kong (Peoples Republic of China), Canada, Germany, Belgium, the Netherlands, and Russia (Figure 1.1). Regarding Spain, imports rose 8.450 metric tons in 2013. 90 % of these imports came from USA, Iran and Germany. In parallel, Spain exports about 600 tons, being Poland (138 tons), Italy (124 tons), Germany (94 tons), Portugal (73 tons) and France (65 tons) the major recipients of these pistachios.

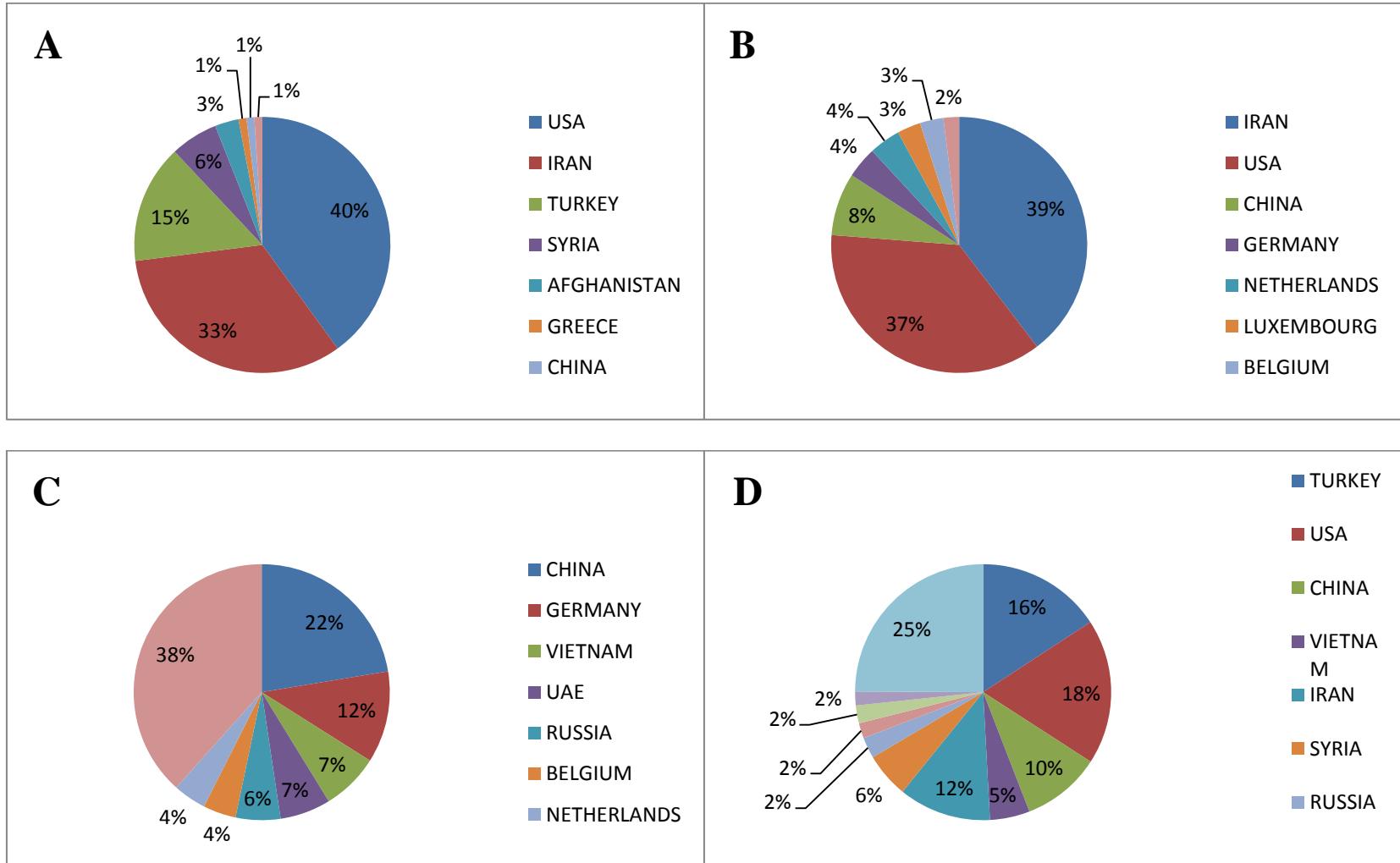


Figure 1.1. Percentage of Production (A), Exports (B), Imports (C) and consumption (D) of pistachio around the world per country.

1.3.SEASONAL PHENOLOGY

The annual phenological cycle of pistachio can be divided into two processes: blooming and the development of the fruit on one hand and the vegetative growth on the other; the two processes are physiologically closely related (Figure 1.2).

1.3.1. Blooming and the development of the fruit

From late March to April (central Spain conditions), depending on cultivars, biotic and abiotic conditions, flower buds start their movement. The lateral axillary male and female inflorescence buds on one year-old wood begin to swell in late March. The pollination and fertilization occurs with a significant fall of feminine flowers from the panicle simultaneously to fruits set. Once blooming and fruits set completed, a longitudinal and transversal enlargement of the pericarp takes place from May to early June. During the month of June and part of July, fruit growth slow down. Endocarp lignification is the predominating process during this period. At the end of July/early August, fruit show little growth prioritizing seed development. Simultaneously, based on physiological circumstances, a process of buds abscission ensues. In late August to September, the seed reaches its maximum size, the nut ripens, the radial suture around the shell's long circumference splits, the hull degrades resulting in an easy removal of the exocarp and mesocarp from the endocarp and abscission of the individual nut from the rachis commences. From the fall of leaves and the beginning of dormancy period until flowering, the activity of the plant is apparently paralyzed.

1.3.2. Vegetative growth

From late March to the beginning of April, vegetative buds initiate their movement showing a swelling and a bracts opening in apical buds. Shoot growth begins usually a month after flowering is over extending its development approximately from the second half of April to the ends of August. Throughout this period, pistachio tree produces two basic types of vegetative growth: short-shoots composed entirely of preformed units, and long-shoots composed of both preformed and neo-formed units. The terms preformed and neo-formed denote the interval of time between the moment when leaves are formed in their embryonic state in a bud and the moment of their expansion in the tree (Spann *et al.*, 2007). In preformed growth, leaves are formed in a bud during one season and expand the next season. The majority of buds that will bear fruits the following year are located in preformed shoots. The elongation of these shoots is very fast and occurs entirely during the spring flush beginning approximatively at the finale of April and ending in early June. Later flushes arises from the simultaneous initiation and extension of new metamers without bud formation or an intervening rest period (Pollard and Logan 1974) and are referred to as neo-formed shoots. This growth begins usually in late July or early august until the end of this month or early September. During the month of June to a part of July, the vegetative growth is negligible taking place the hardening of the new shoots and the culmination of the differentiation of reproductive and vegetative buds of the next year. Nonetheless, under optimal conditions, growth could show a continuous and uninterrupted development during the season.

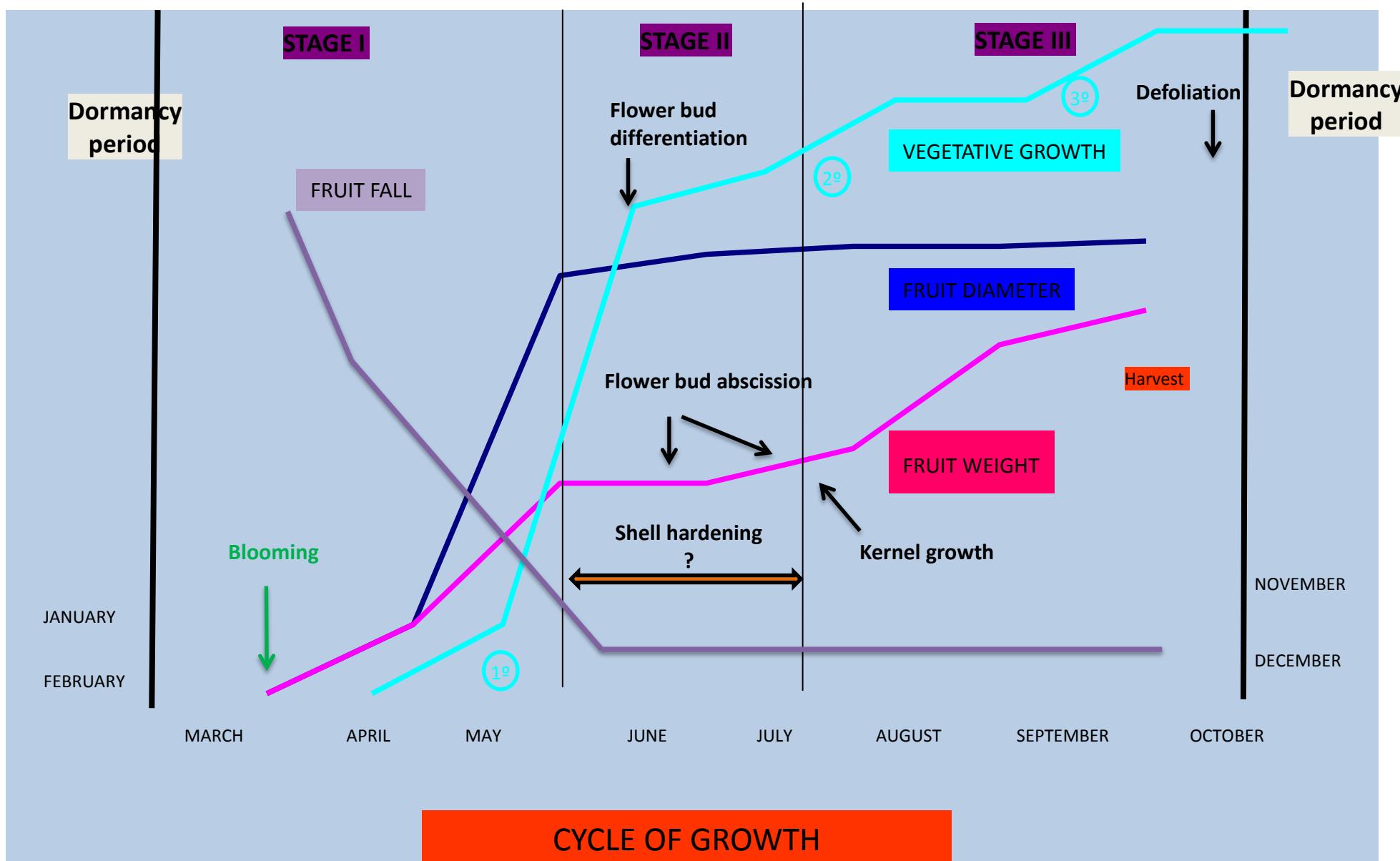


Figure 10.2. Cycle of growth of pistachio. Modified with permission. (Guerrero, 2011).

1.4.CROP ECOLOGY

1.4.1. Climatic requirements

In a General way, pistachio is a species that requires hot, dry summers and winters with moderately low temperatures. To have an optimal and homogeneous sprouting, it requires an accumulation of a determined number of chill hours which varies according to cultivars. Required chill hours (CH) range from 450 CH in early cultivars to 1100-1200 CH in late cultivars of temperatures between 0 and 7°C (Crane and Iwakiri, 1981). The Heat units (HU) to reach optimal ripening from fruit set for the region of Castilla-La-Mancha have been estimated at about 3000 °day for early cultivars and 3400° day for late cultivars (Kerman for example) taking a threshold of 2.6° C as a base (Guerrero, 2011).

Pistachio trees are extremely sensitive to high humidity levels causing an easy and extremely quick expansion of fungal diseases resulting in an additional boundary to surpass for maintaining a satisfactory healthy and a rentable orchard (Couceiro et al., 2013). Additionally, factors such as frost, very dry air waves and late spring rains could drastically affect the pollination process.

1.4.2. Soil requirements

Pistachio may be grown in a multitude of soil types enduring remarkably alkalinity and salinity. However, it grows better on well-drained soils since it is particularly intolerant of saturated conditions. It is also reported that shallow soils limits tree growth and productivity (Ferguson et al., 2005).

1.5.ROOTSTOCKS

The rootstock is the subterranean parts on which buds or scion of pistachio are inserted in grafting. It can belong to different species but all classified under the genus *Pistacia*: *P. terebinthus*, *P. atlantica*, *P. integerrima*, *P. palaestina B.*, etc., including also the comestible pistachio specie *P. vera* could be suitable for this purpose. Rootstocks are usually used to enhance; disease and pest resistance, cold and salinity tolerance, adaptability to edaphoclimatic conditions, yields and fruit quality, etc. In addition, being pistachio a dioecious tree, grafting is required to ensure a differentiation between male and female trees but also to ensure the obtainment of an identical offspring to the desired. The choice of the rootstock depends on each growing area and such decision is crucial for the good development and management of the orchard. *P. terebinthus* is the most used rootstock in Australia, Italy, Spain and Greece. *P. atlantica*, native of the north of Africa, west Asia and southeast of Europe was traditionally used in the USA before the infestation of the Californian orchards with the fungal disease verticillium wilt which affects the root system and kills the tree. Nowadays, *P. integerrima* and a hybrid between *P. atlantica* and *P. integerrima* (UCB-I), are the main rootstocks used in California. *P. vera* is frequently used in Turkey and Iran besides other rootstocks such as *P. mutica* and *P. khinjuk* (Guerrero, 2011). Then, to form a tree on a specific rootstock requires the knowledge of a number of characteristics that define the potential of each rootstock. Generally, and unlike other species, studies on Pistachio rootstocks are few and have usually focused on comparing tolerance to diseases or the productive potential that transmits.

1.6. IRRIGATION SCHEDULING

The concept of irrigation scheduling is usually used to describe the management whereby the frequency and amount of water to apply in each irrigation recurrence are determined. Therefore, irrigation scheduling is a procedure targeted to resolve two basic questions: When is the auspicious moment to irrigate and how much water should be applied at this moment. Historically, water management has generally been based on previous experience and depended on measuring the time since the last rain or irrigation or occasionally on the extent of wilt of the leaves (Boyer, 1995). These methods had the advantage of simplicity but were too crude to detect early losses in growth, too irregular to repeat conditions for scientific investigations and too much inaccurate contrasting with the imperative need for conserving water. All these points led to the necessity of recompiling precise information allowing overcoming these issues to schedule irrigation.

Several methods of irrigation scheduling was proposed over time for this purpose and have traditionally been grouped into climatic methodologies, soil water content approaches or tree water status physiological response techniques as a way of decision.

Climatic methodologies introduced the concept of the establishment of the consumptive use requirements of the crop and are commonly coupled to a soil water balance for irrigation scheduling, estimating the consumed and lost water amounts. Soil water content and tree water status approaches are more based on the checking of the water status in one of the two first parts of the Soil-Plant-Atmosphere continuum. Traditionally, they have been used as a way of control to fine-tune the requirements estimated by the climatic methodologies since the output of these methodologies is properly an indicator of a given water status at soil level in the first case and tree level in the second one and not a defined quantity of

water. However, some protocols could be proposed to overcome the dependence of these methodologies to the climatic estimations.

1.6.1. Methods for determining trees water requirement

Water consumption of a field, corresponds to its evapotranspiration (ET) in a given environment and under a specific management. Evapotranspiration is figured as the combination of two separate processes whereby water is lost from the soil surface by evaporation and from the crop by transpiration. The amount of water required to compensate the evapotranspiration loss from a cropped field is defined as crop water requirement (Allen et al. 1998). The evapotranspiration of a crop can be measured directly by weighing the soil-plant system by the means of lysimeters; a device of a high expense and lacking a high technical knowledge for its full exploitation, or indirectly, by the means of several methodologies as the energy balance and microclimatological methods (Nichols, 1992; Laczniak *et al.* 1999).

1.6.1.1.Methods related to Agro-climatic data and energy balance

The most widespread method in the practice of irrigation nowadays to estimate ETc is based on periodic climatic records by specific existing models. ETc is calculated as the product of reference evapotranspiration (ETo) by a crop coefficient (Kc);

$$\text{ETc} = \text{ETo} \times \text{Kc} \quad (1)$$

Reference evapotranspiration (ETo) reflects the influence of the climate on the crop reference and the crop coefficient represents the difference in the energetic and aerodynamic behaviour between the crop reference and the crop under study. The reference evapotranspiration can be calculated in various ways, although until the present moment,

FAO Penman-Monteith method is the one gathering most agreement between researchers and is currently recommended as the standard equation for the definition and computation of the reference evapotranspiration under different locations;

$$ETo = \frac{0,408\Delta(Rn-G) + \gamma \frac{900}{T+273} u_2(e_s - e_a)}{\Delta + \gamma(1 + 0,34u_2)} \quad (2)$$

where

ETo	reference	evapotranspiration	[mm]	day^{-1}
R_n	net radiation	at the crop surface	[MJ m^{-2}]	day^{-1}
G	soil heat flux	density	[MJ m^{-2}]	day^{-1}
T	mean daily air temperature	at 2 m height	[$^{\circ}C$]	
u_2	wind speed	at 2 m height	[m s^{-1}]	
e_s	saturation vapour	pressure	[kPa]	
e_a	actual vapour	pressure	[kPa]	
Δ	slope vapour pressure curve		[kPa $^{\circ}C^{-1}$]	
γ	psychrometric constant [kPa $^{\circ}C^{-1}$].			

The meteorological measurements for the determination of ETo are normalized and should be performed over an extensive surface of green grass, free of diseases, completely shading the ground and without limitations of water or nutrients. An unambiguous definition was proposed by FAO for the surface reference assuming a crop height of 0.12 m, a fixed surface resistance of 70 s m^{-1} and an albedo of 0.23.

The crop coefficient (K_c) of pistachio was not the subject of many studies and the most used K_c nowadays is the proposed by Goldhamer (1995). From a general view, for

deciduous orchards on bare soil, Kc increases sharply in spring in response to bud break and leaf out until it reaches a maximum in early summer. The maximum Kc is more or less maintained until harvest, after which it declines gradually until leaf fall (Fereres et al., 2012). The crop coefficient act as a converter between the ETo of the crop reference and the studied crop, contemplating both crop characteristics and averaged effects of evaporation from the soil. However, it specifically applies to mature trees that have reached their maximum size and developing orchards are not considered in this conversion.

For this purpose, in the case of immature orchards, it is necessary to relate the ET of a young orchard to that of a mature orchard for which the Kc values were developed and a corrector coefficient (Kr) determined in young almonds orchard, was incorporated by Fereres (1982) to equation (1) to compute ET and was used for pistachios in this study;

$$\mathbf{ETc = ETo \times Kc \times Kr} \quad (3)$$

Kr relates the crop ground cover (G) to the ET and could be calculated by the following equations:

$$\mathbf{Kr = \frac{2G}{100}} \quad (4)$$

$$\mathbf{G = \frac{\pi \times D^2 \times N}{400}} \quad (5)$$

where,

D is canopy diameter (m); N: Number of trees per hectare (Trees ha⁻¹).

Also noteworthy is the existence of other micrometeorological methods for measuring evapotranspiration for crops which are related to energy balance:

- Eddy covariance (EC) method

The eddy covariance method (Swinbank, 1951) is the commonly used micrometeorological technique providing direct calculation of latent heat flux (or evapotranspiration). It allows for absolute point, measurements of the sensible heat (H) and latent energy (LE) at a defined height above canopy (Jarmain *et al.* 2008). EC adopts a sonic anemometer to measure high-frequency vertical wind speed fluctuations about the mean and an infrared gas analyzer to measure high frequency water concentration fluctuations. These fluctuations are paired to determine the mean covariance of the wind speed and humidity fluctuations about the mean to directly estimate latent heat flux. Alternatively, LE may be estimated as a residual from the simplified energy balance by measuring H , Rn and G . In the EC method, the sensible heat flux is also estimated using the covariance of the fluctuation in vertical wind speed and variations in temperature about their means (Consoli, 2011).

- Bowen ratio (BR) method

Unlike the Eddy Covariance method, the BR method is more difficult to set up and it requires more maintenance. The Bowen ratio (Bowen, 1926; Sverdrup, 1943) assumes that the proportionality between sensible and latent heat can be defined by the ratio between the temperature and vapor-pressure gradient. Because temperature and vapor pressure can be measured directly, the Bowen ratio can be substituted into the energy budget to determine

latent energy (evapotranspiration) by directly using measurable parameters. Two different types of BR systems have commonly been used (Jarmain *et al.* 2005). The single-sensor method involves using one hygrometer and two sensors for air temperature, with air being pumped alternately from the one level and then from the other (Tanner *et al.*, 1987; Cellier and Olioso, 1993). The other type involves an oscillating system in which two sensors, one at each measurement level, are used for air temperature and water vapour pressure determinations (Gay and Greenberg, 1985; Fritsch and Fritsch, 2005).

- Scintillometer method

A scintillometer is used to measure path-weighted H with a transmitter and a receiver at each end of the path of a radiation beam. This methodology consists in the measurement of the variations of refractive index of air (Thiermann, 1992), caused by temperature, humidity and pressure variations by the means of the intensity fluctuations of a visible or infrared radiation beam after it has been propagated above the plant canopy. H is derived from these measurements and LE may be estimated as a residual from the simplified energy balance equation.

- Surface renewal (SR)

The SR method (Paw U and Brunet, 1991) is based on the idea that an air parcel near a surface is renewed by an air parcel from above and consider its movement to and away the canopy surface. This method is relatively recent compared to EC and BR methodologies for the estimation of sensible heat flux (H). H is obtained from the measurement of the temperature of the air above the canopy at very short intervals since the air temperature fluctuations exhibit organized coherent structures which resemble ramp events (Paw U *et*

al., 1992) allowing the estimation of a scalar flux. The evapotranspiration is also estimated by the means of the shortened energy balance.

1.6.1.2. Methods related to mass balance

- Weighting lysimeters

The evapotranspiration of a crop can be measured directly by weighing the soil-plant system by the means of lysimeters; they quantify water loss from a control volume by measuring the change in mass and allow determining carefully the actual crop evapotranspiration at short intervals of time, often hourly but more frequently at daily time intervals for operational commitments. Consequently, they also allow the computation of crop coefficient (K_c) by comparing measured ET of a well-watered, actively growing healthy crop, to computed ET_o :

$$K_c = \frac{ETc}{ETo}$$

- Soil water balance

Evapotranspiration can also be determined by measuring the various components of the soil water balance. This method includes inputs and outputs of water in the cultivated plot root zone over a determined period, which directly affects the process of crop ET. Irrigation (I) and rainfall (P) add water to the root zone. Part of I and P might be lost by surface runoff (RO) and by deep percolation (DP) that will eventually recharge the water table. Water might also be transported upward by capillary rise (CR) from a shallow water table towards the root zone or even transferred horizontally by subsurface flow in (SF_{in}) or out of (SF_{out}) the root zone. Generally, SF_{in} and SF_{out} are negligible and can be ignored. Soil evaporation and crop transpiration deplete water from the root zone. If all fluxes other

than evapotranspiration (ET) can be assessed, the evapotranspiration can be deduced from the change in soil water content (D SW) over the time period (Allen et al. 1998):

$$ET = I + P - RO - DP + CR \pm D SF \pm D SW \quad (6)$$

1.6.1.3. Methods related to soil water status

These methods include both; techniques measuring soil water content (SWC) and those determining the soil water potential.

Soil water content could be measured directly by the means of thermogravimetric measurement, which is based in the simple determination of the mass of water lost on drying a volume of soil, weighting a wet sample before and after oven drying at 105° C until mass remain constant (Evett, 2008). This technique represents the reference method for SWC measurements.

Concerning the indirect techniques, a multitude of devices have been used for this purpose mentioning the neutron probe, the dielectric measurements by the means of time domain reflectometry systems (TDR) or frequency domain reflectometry systems (FDR). The device used in this work (Diviner 2000) belongs to the dielectric measurements group being a frequency domain reflectometry system (FDR). FDR device composition is based on the employment of an electronic circuit composed by a capacitor and an oscillator producing a repetitive oscillating current. Varying in soil moisture can be detected by changes in the circuit operating frequency of oscillation. The latter decreases as soil bulk electrical permittivity (and water content) increases. FDR are considered relatively inexpensive, accurate and allow the logging of data. They are minimally affected by

salinity however they need a specific soil calibration and a close contact between the sensor (or tube) and the soil for a good measurement.

1.6.1.4. Methods related to plant water status: plant “stress” sensing

- Water potential

Which make an indicator better than other is its ability to detect with more precision and faster small difference in tree water status.

Leaf water potential (ψ_l) is certainly the most commonly used parameter as indicator of water status and irrigation timing in fruit trees (Klepper, 1968, Syvertsen, 1985) both for its physiological relevance in the control of the water pathway in the tree and the speed and easiness of its measurement by the means of the pressure bomb (Ritchie and Hinkley, 1975). Leaf water potential involves the combination of soil moisture status, environment and endogenous tree factors which makes it a variable parameter along the day, the season, depending on the age of the leaf sample and its position within the tree (Sánchez-Blanco, 1983). However, leaf water potential was not always well correlated with soil water status showing a lack of precision to differentiate irrigation treatments. This factor makes the use and interpretation of leaf water potential as an indicator for irrigation scheduling an unsatisfactory tool. However, in spite of this concern, the measure of leaf water potential is universally accepted as sensitive index for irrigation when corrected for diurnal and environmental variation as the most rapid and economical way to evaluate the water status of plants (Peretz et al., 1984; Ruiz-Sánchez and Girona, 1995).

To overcome the lack of reliability of leaf water potential to soil moisture, many researchers proposed the predawn leaf water potential as a better indicator of tree water

status since it is supposed that water potential of the tree should express equilibrium with the soil water status at dawn when transpiration is negligible. However, predawn leaf water potential was often found to be insensitive to variation in soil moisture (Garnier and Berger, 1987) and that an imbalance between predawn leaf water potential and soil water potential still existing. This behaviour was explained by the fact that predawn leaf water potential seems to equilibrate with the water potential of the wettest fraction of the soil (Aussenac et al., 1984; Breda et al., 1995).

The works of Garnier and Berger (1985) founding that, the water potential of an attached leaf that has been prevented from transpiring by enclosure in a darkened plastic bag, was consistently related to irrigation treatment differences, whereas transpiring leaf water potential was not, opened the way to the use of another indicator called stem water potential. The basis of this measure is that the coverage of the leaf preventing transpiration would represent the pressure at which the leaf was submitted at the stem level. McCutchan and Shackel (1992) observed lower variability of stem water potential compared to leaf water potential in plum trees allowing the detection of small water status differences among treatments. Moreover, they found a good and better relationship between stem water potential and VPD than leaf water potential. A lower ability of leaf water potential compared to stem water potential to detect significant differences was also reported by Choné et al., (2001) in vine and Naor et al., (2006) in apple trees. Selles and Berger (1990) reported also an earlier detection of water stress differences between irrigation treatments by the means of stem water potential compared to predawn leaf water potential and midday leaf water potential. In addition, stem water potential has been shown to be a linear function of applied water (Lampinen et al., 1995) and soil water availability (Stevens et al., 1995).

Lastly, stem water potential has been also highly correlated with tree (Olien and Lakso, 1986) and fruit (Naor et al., 1995) size in apple.

- Xylem cavitation

Xylem cavitation can be defined as the interruption of water flow due to a fracture in the water column. This water column materialized in the xylem vessels is submitted to a physical tension when trees are transpiring. The increase of water stress lead to an increase in this tension as a consequence of the increasing imbalance between water loss and gain resulting in a rupture in the xylem vessel. This rupture known as cavitation lead to formation of gas bubbles in the column (embolism) preventing increasingly the flow of water as water stress increases (Tyree and Sperry, 1989). This cavitation happenings can be detected by the means of acoustic ultrasonic frequency detectors (Tyree and Dixon, 1983) being therefore a possible indicator of water stress (Jones et al., 1989). However, many doubts still be existing about the relationship between acoustic emissions and xylem embolisms (Sandford and Grace, 1985; Jones and Peña, 1986) making the adoption of this technique for irrigation scheduling very little developed. Moreover, xylem cavitation starts occurring at a more or less advanced degree of water stress with an apparent cumulative behaviour resulting in different relationship between cavitation and acoustic emission in each cycle of stress, additionally to an incomplete explanation of the reversing process during re-watering. All these factors represented a barrier to can use efficiently this parameter for irrigation scheduling.

- Stomatal conductance

Stomata are apertures in the epidermis of leaves, each bounded by two guard cells and are responsible of the variation of gas exchange between the plant and the atmosphere, regulating water loss and CO₂ uptake by the means of a mechanism of aperture and closure variation. This mechanism seems to be directed by turgor changes in guard cells (Esau, 1965) operated by the regulation of potassium ions transport. A wide variety of environmental conditions induce a response on guard cell turgor including CO₂ concentration, light, temperature, endogenous rhythms, atmospheric vapor pressure deficit, and soil/plant water potential (Heath and Mansfield, 1969). The fact that the degree of stomatal conductance is sensitive to developing water deficits in many plants provides potentially a good indicator for irrigation scheduling. However, the response of stomatal behaviour to other environmental and physiological factors imposes the need of a continuous sampling to separate the effect of water stress from other factors which make stomatal conductance a weak parameter for irrigation scheduling (Jones, 1990). However, the close relationship between stomatal conductance and photosynthesis make this parameter very useful to evaluate the recovery of plants after a cycle of water stress (Gebre and Kuhns, 1992; Ruiz-Sánchez et al., 1997). Nevertheless, the different comportment between species imposes the characterization of stomatal behaviour for the specie under study to allow its use for such purpose.

Despite there are different methodologies to determine stomatal conductance, currently, the most used devices are porometers and the most spread ones are the dynamic diffusion porometers or steady state porometers.

- Sap flow

Founded on the fact that sap flow should be sensitive to stomatal closure and consequently to water deficit, several researchers experienced the suitability of this method for irrigation scheduling (Eastham and Gray, 1998; Fernandez et al., 2001; Giorio and Giorio, 2003; Nicolás et al., 2005). The quantification of this parameter is based on the measure of sap flow velocity by monitoring the changes in sap temperature when heat is applied to the stem. Currently there are three main types of sap flow sensors; heat balance (Valancogne and Nasr, 1989), thermal dissipation (Granier, 1985) and heat pulse (Green and Clothier, 1988). The election of one type depends fundamentally in the species under work and the size of its stem. This methodology allows the estimation of transpiration with a continuous recording of data and is showing a promising approach for the estimation of water consume and the detection of water stress.

1.7. REGULATED DEFICIT IRRIGATION (RDI)

There is no doubt today that any action targeted to a more efficient use of water is a necessity tending to an obligation in many arid or semi-arid areas. The fact coming to the assumption that crops respond differently to the implementation of a water deficit according to the phenological stage, transformed the way of irrigation from an uncontrolled weather directive to a scientific controlled technique. Therein lays the difference between deficit irrigation and regulated deficit irrigation where the physiological response of the tree represents a tool allowing executing an irrigation strategy. Regulated deficit irrigation, by definition, consists in limiting water inputs in those phases in which a water limitation does not affect or would affect minimally the crop yield and its quality and to cover the water

requirements of the crop along the whole other parts of the growth cycle. Based on the definition of this concept, the accurate knowledge of critical sensitive periods, the precise characterization of the vegetative and reproductive cycle and their overlapping in time, soil characteristics, irrigation system, the weather, the water stress level and its duration, the plant material and its resistance capacity in front of drought are crucial for the appropriate management of RDI. The integration of all these factors in the assessment of RDI is a big challenge, and is normally carried out based on experience.

1.7.1. Identification of critical periods

It is difficult to pinpoint the critical and tolerant water stress periods in a common way for all crops. However, the general approach to impose a strategy of RDI starting from a lack of knowledge concerning the crop water stress response according to its physiology, is to fulfill water tree's requirement when fruit growth is fast and to impose a water stress during the vegetative growth. So, the asynchrony in the active growth of fruits and shoots provides a basis for the application of RDI.

There is a general statement that blooming represents a sensitive period for a majority of fruit crops. Water stress during this period could affect ovule fertilization (Hsiao, 1993), disturbing consequently fruit set process which may reduce the final number of fruits in the tree. This fact takes more importance during "OFF" years in alternate bearing species. In pistachio, this period is generally excepted of water stress since transpiration is low and soil water reserve remains sufficient to fulfill its demand.

Fleshy drupes are a good example to explain when, at a phenological level, the application of RDI could succeed. Following a double sigmoidal curve, their fresh fruit

growth pattern is characterized by a clear differentiation of three stages and a clear separation between shoot and fruit growth. Stages I and III are separated by a phase of decreasing growth rate (Stage II) known as the lag phase (Chalmers and van den Ende, 1975; 1977). Theoretically, water stress during the latter mentioned phase has little effect on fruit growth and could be applied.

Represented in fresh fruit weight, pistachio fruit development is similar to fleshy drupes pattern. The first works studying pistachio response to an implemented water deficit has been developed in California between 1983 and 1985, imposing a water stress during summer to Kerman cultivar budded on *Pistacia atlantica* rootstock (Goldhamer et al. 1983, 1985, 1987). In 1989, following the works of Chalmers *et al.* (1981) and Mitchell and Chalmers (1982) carried out in peach, the first researches testing the success of regulated deficit irrigation in pistachio took place in California (Goldhamer et al, 1995). These experiments evaluated numerous stress levels and showed different responses of the crop to water stress conditions depending on the fruit growth stage (Goldhamer, 1995; Goldhamer and Beede, 2004).

Stage I: Following fruit set, a high rate of fruit growth occurs excluding the kernel growth. During this period, fruit almost attains its final dimensions (Figure 1.3). As soon as the maximum diameter of the fruit is reached, stage II takes place. Stage I is considered as a sensitive phase in pistachio leading to induce smaller nuts (Goldhamer, 1995). However, it has been also reported that water stress during this stage can increase shell splitting.



Figure 1.3. Stage I of pistachio fruit growth.

Stage II: This stage is generally described as the period of shell hardening (Figure 1.4), characterized by a slowdown of fruit growth. This phase is considered to be less sensitive to water stress as has been found for other double sigmoid development fruit crops, such as late peach and nectarine.

Goldhamer and Beede (2004) advanced as a result of a series of experiments in California in pistachio that none; holding irrigation at, 25 % ETc and 50 % ETc during stage II have negative effects on final yield. However, they mentioned that irrigation at 25 % ETc during stage II on a very shallow soil reduced both shell size and splitting, recommending a minimal rate of 50 % ETc during this stage. Girona et al. (2005) mentioned that soil conditions and concretely depth can influence the productive response of peach trees in front of an RDI strategy.



Figure 1.4. Stage II of pistachio fruit growth.

Stage III: This stage starts with the beginning of kernel growth, resulting in a sharp increase of fruit weight and finishes with fruit maturation and harvest (Figure 1.5). This period is considered as the most sensitive to water stress (Goldhamer, 1995) affecting almost all yield components.



Figure 1.5. Stage III of pistachio fruit growth.

1.7.2. Effect of water deficit on tree physiology

A situation of water deficit occurs when the plant cannot uptake water at a sufficient rate to replenish the losses as a result of the atmospheric evaporative demand or/and water scarcity; such deficit is generally followed by the creation of a situation of water stress (English and Raja., 1996). The response of fruit trees to this situation is certainly the result of a large number of processes affected over time by evolutive stress situations.

The effects of water stress on different plant morphological and physiological processes are complex and interrelated (Fereres et al., 2012). Basically, any process of the plant can be altered by a water stress that is severe and long enough (Hsiao, 1973). The main consequences of drought in crop plants are reduced rate of cell division and expansion leading to an abridged leaf size, stem elongation and root proliferation. At a physiological

level, it results in a disturbed stomatal pattern, plant water and nutrient relations, limiting water use efficiency and crop productivity (Farooq et al. 2009).

Growth is reported to be the most immediate process affected by water stress being the rate of cell division and expansion highly sensitives including any process linked to the development of a vegetative or a reproductive organ (Hsiao, 1973).

Cell expansive growth is previously affected by water stress than cell division (Hsiao, 1973) leading to a direct effect on reducing leaf size and stem elongation (hence canopy growth). Limiting growth and leaf area has been considered as a mechanism employed to avoid excessive water loss (reducing transpiration) by limiting energy interception.

Another process affected by water stress is roots proliferation. Nevertheless, root growth is considerably less sensitive to water stress than leaves certainly due to a lag of time to experience the same level of stress than to a higher intrinsic resistance of root cells. This difference of sensitivity to water stress between roots and areal parts leads to an increase of root to shoot ratio. This morphological change ensures at a certain degree an easier transport of water through the tree under water stress.

The only way by which an environmental factor such as water can affect plant growth is by influencing physiological process and conditions (Kramer, 1983). Water relations have certainly a decisive impact as a promotor of such response. Relative water contents (RWC), leaf water potential, osmotic potential, pressure potential, and transpiration rate are the major attributes of plant water relations (Kirkham 2005) which are significantly affected under water deficit. Inter alia, water deficit leads to a reduction of leaf water potential and stomatal conductance (Castel and Fereres, 1982; Girona, 1993). The decrease of stomatal conductance is generally correlated with a decrease of transpiration rate, CO₂ absorption

and therefore photosynthesis. In spite of the intervention of several factors and dynamics in controlling photosynthesis (Schulze, 1986), it is widely assumed that stomatal conductance plays a significant role in the control of this process and is often taken as an indicator of the latter (Schulze and Hall, 1982). Even so, it should be mentioned that several reports stated that the reduced photosynthesis during water stress is not related to stomatal conductance closure but to the decrease of the intrinsic ability of photosynthesis (Azcón-Bieto, 1983; Farquhar and Sharkey, 1982; Osmond et al., 1980; Schulze, 1986). So, the prediction of photosynthesis response to water stress only according to stomatal conductance should be cautiously taken.

CHAPTER 2: BACKGROUND AND OBJECTIVES

Pistachio production registered one of the fastest growths in the last 13 years in the world with 8.5 %. This tendency was due to an extension of its surface around the world and to an increase of its productivity mainly by transforming its management from a rain-fed crop to an irrigated one.

In Spain, particularly in the Region of Castilla-La-Mancha, pistachio growing becomes relevant as an alternative to more traditional tree crops. However, this growth was linked to a decrease of water availability combined to a lack of knowledge of pistachio responses in front of water stress situations. This fact made crucial the need to improve water use of pistachio and to investigate the agronomic and physiological behaviour of this crop under irrigated and water stress situations.

In the absence of a reliable plant-based tool to measure water stress, irrigation was historically scheduled using estimation of soil water availability and/or crop evapotranspiration. Though, the response of trees to water stress may have distinct consequences according to the moment of application, the intensity of stress and its duration (Ruiz-Sánchez et al., 2010) and the use of the traditional methodologies led to high variability and uncertainties to provide an accurate water supply in time and quantities since tree water use results being a complex process integrating the effect of all these factors.

Regulated deficit irrigation strategies have a physiological focus being based on the phenology of the crop and its ability to endure water stress conditions at specific periods. Thus, the inaccurate management of water stress intensity (level and duration) could be detrimental affecting adversely crop productivity and sustainment. Plant water status integrates the effect of soil, plant and atmospheric conditions. So, the simple scheduling of

water inputs according to an ETc fraction would be translated in unreliable water stress levels to water inputs. The ignorance of critical crop water stress thresholds made the implementation of a water deficient strategy subjective, difficultly reproducible and undoubtedly deleterious for orchard economic incomes. Thus, a reliable plant-based tool such as water potential would be decisive to apply precisely a regulated deficit irrigation strategy.

So, this work has been developed starting from the idea of combining two profitable techniques for irrigation scheduling; the use of stem water potential as a tool that has proven its efficiency in detecting water stress in fruit trees but that has been used in non-deficit irrigated conditions and the use of RDI strategy as a technique that has proven its efficiency in saving water but that mainly has been implemented based on a percentage of the ETc and not as a threshold of a particular water potential.

Thus, the main purpose of this work focuses on the optimization and saving of water use in pistachio and the study of its response in front of water stress. This starting point has been addressed in several steps and carried out through specific objectives that could be presented as following:

- The study of the viability of the use of stem water potential as a tool for irrigation scheduling following a specific protocol.
- The characterization of pistachio fruit growth patterns with the aim of improving their stages delimitation.
- The characterization of the physiological response of pistachio under different degrees of water stress.

- To compare the agronomic and physiological behaviour of different rootstocks when subjected to the imposed water stress with the aim of suggesting the most suitable rootstock for the area of study.

CHAPTER 3: MATERIALS AND METHODS

3.1. SITE DESCRIPTION AND CHARACTERISTICS OF THE EXPERIMENTAL PLOT

3.1.1. Duration and location of the study

The study was conducted for three years, from 2012 to 2014, in a pistachio orchard of 0.84 ha heading north-west to south-east. The plot was a part of the experimental farm “La Entresierra” research station belonging to the research centre “El Chaparrillo” pertaining to the regional government of Castilla-La-Mancha and located in Ciudad Real (Spain). The geographical coordinates of the orchard are; longitude: 3°56' W; Latitude: 39°0' N; altitude: 640 m above the Mediterranean Sea. The total area of the farm is about 39 ha (Figure 3.1).



Figure 3.1. Aerial photography of the experimental farm “La Entresierra”, the plot of study and its situation.

3.1.2. Soil characteristics

The soil at the experimental site is an alkaline (pH 8.1) shallow soil with a discontinuous petrocalcic horizon located at 0.50 m (Petrocalcic Paleixeralfs), with a clay loam texture, low electrical conductivity (0.2 dS m^{-1}), 1.05 % of organic matter, 0.12 % of nitrogen, $17 \times 10^{-4} \text{ mol kg}^{-1}$ potassium levels and high cationic exchange capacity (0.186 mol kg^{-1}). The volumetric water content of the soil for the first 0.3 m of depth was 22.8 % at field capacity (soil matric potential -0.03 MPa) and 12.1 % at permanent wilting point (soil matric potential -1.5 MPa); from 0.3 m to 0.5 m, it is 43.0 % and 21.1 %, respectively.

3.1.3. Local meteorological conditions

The climatic characterization of the area of study has been done based on data taken from the Spanish Meteorological Agency, of the weather station located in Ciudad Real. Its geographical coordinates are: Latitude: $38^{\circ} 59' 21''$ N - Longitude: $3^{\circ} 55' 13''$ O and Altitude: 628 m over the Mediterranean Sea.

Data are the historical series of 1981-2010 (Agencia Estatal de Meteorología, 2016). In Figure 3.2, monthly averages of temperatures (average, maximum and minimum), precipitation and relative humidity are presented.

The average annual temperature in the area of study is 15.6°C . During the 14 years extending from the installation of the orchard to the end of the study, the average temperature has been 2°C lower than the historical average. The warmest month is July with an average temperature of 26.7°C and the coldest one is January with an average temperature of 6°C . The highest average temperature is 34.5°C and the lowest average temperature is 1.1°C .

According to the Iberian Climate Atlas and following the classification of Köppen-Geiger (Köppen, 1936), the local area belongs to a climate of a type “Csa”. This classification distinguishes three levels. The type “C” refers to temperate climates where the average temperature in the coldest month is between 0 and 18 °C. “Cs” refers to a particularly dry period in summer and the “a” to an average temperature in the hottest month above 22 °C. So the local climate of this region could be defined as a temperate with dry or hot summer. This type of climate covers approximately 40 % of the Iberian Peninsula. It covers the majority of the southern central plateau region, and the Mediterranean coastal regions, with the exception of the arid zones of the southeast.

Precipitations occur mainly in autumn, winter and spring and are characterized by a high variability. The average annual rainfall is about 402 mm (series 1981-2010), mostly distributed outside a four-month summer drought period.

Relative humidity has an average of 61 % being the maximum during the month of December with 81 % and the minimum during the month of July with 40 %.

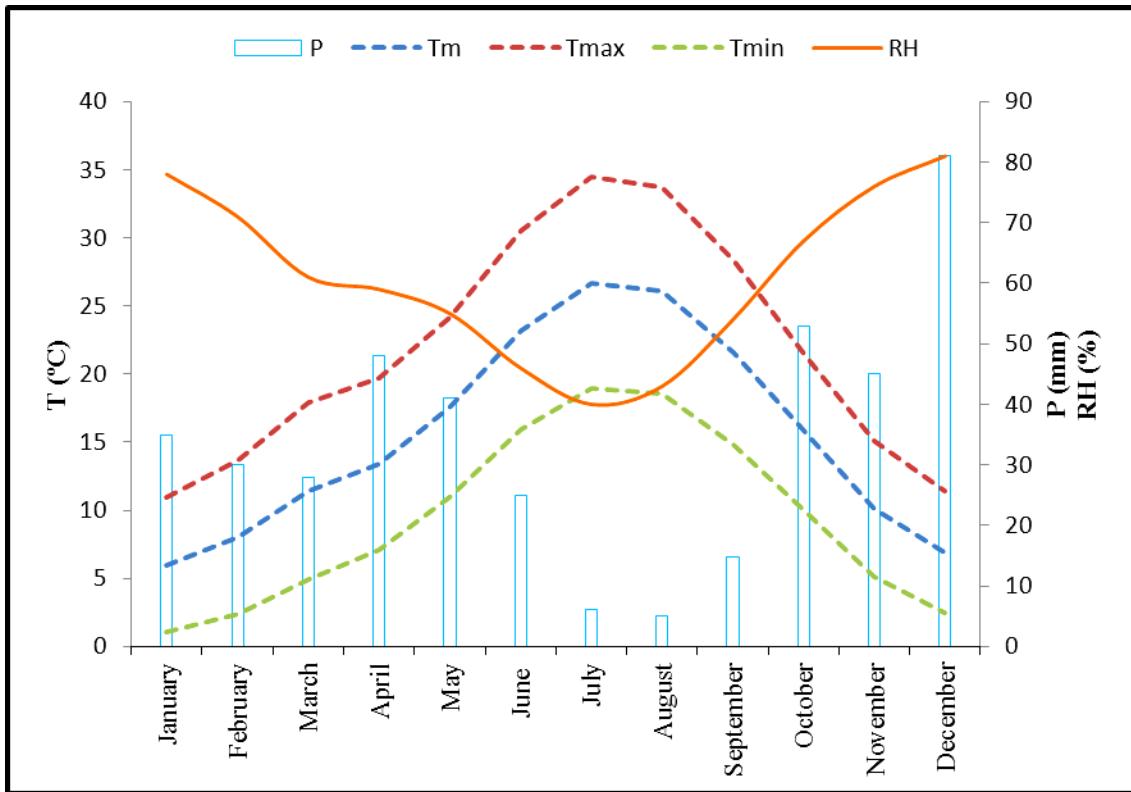


Figure 3.2. Monthly maximum, average and minimum temperature, relative humidity and precipitation of an ordinary year (1981-2010) of the weather station of Ciudad Real.

3.1.4. Plant material

Pistacia vera L. cv. Kerman was budded on three different rootstocks: *P. terebinthus* L., *P. atlantica* Desf. and *P. integerrima* L.. Tree spacing was set at 7 x 6 m (238 trees ha⁻¹). Peter cv. was used as male tree and was distributed evenly throughout the field, in a proportion of 10 %. Additionally, a field of several male varieties is located nearby the experimental plot, so the availability of pollen is mostly ensured.

- **Kerman**

Kerman is a selection carried out in the USA in 1929 from seeds coming from Iran. It is a scion with a moderate vigor, very productive with a high alternate bearing index and

high chilling requirements (> 1000 CH) compared to other cultivars. Its percentage of split nuts ranges between 40 and 80 % and blanks between 15 and 25 %. Its average yield under irrigated conditions in California ranges between 2200 and 3500 Kg/ha/year. Kerman nuts have a round shape with a clean unstained shell. In shell dry fruit (7 % of humidity) has an average weight of 1.5 g and the kernel of about 1.3 g (Figure 3.3).



Figure 3.3. Fruits of the pistachio cultivar Kerman.

- ***Pistacia terebinthus* L. (PT)**

Being autochthonous of the Mediterranean region, *Pistacia terebinthus* is the most used rootstock in Spain and is vulgarly known as “Cornicabra” (Figure 3.4). This species is less vigorous than the other commonly used rootstocks and shows a high variability being less uniform. Nevertheless, it is reported that it matches perfectly the edaphic properties of the Spanish pistachio cultivation area. This rootstock showed an excellent productive

potential under Spanish shallow sandy loam soils and is often advised for rain-fed conditions. However, in California, it showed a slow bearing entrance and a low capacity to produce high yields.

Pistacia terebinthus is known for its great tolerance to cold. It is also the most efficient absorbing nutrients specially zinc and copper (Couceiro et al., 2013).

This species is described to be susceptible to Verticillium wilt, nonetheless, compared to *P. atlantica*, *P. integerrima* and UCB-I, it has been reported to have a good resistance to nematodes and phytophtora and to be the most resistant to the Armillaria root rot disease.



Figure 3.4. Tree (A), leaves and fruits (B) of *Pistacia terebinthus* rootstock.

- ***Pistacia atlantica* Desf. (PA)**

Due to its good productive behaviour, *P. atlantica* is one of the most used rootstocks in certain production areas mainly the north of Africa (Figure 3.5). Its vegetative and productive behaviour was also classified as good under rain-fed conditions in Spain. This species started being the common rootstock for Californian orchards before the discovery of its sensitivity to Verticillium and was replaced by *P. integerrima* which is tolerant to this disease. *P. atlantica* is more vigorous than *P. terebinthus* but less than *P. integerrima* and UCB-I resulting in a slightly slower entrance into production and yields. Orchards grafted on *P. atlantica* show high homogeneity compared to *P. terebinthus* but less tolerance to cold. This species is highly resistant to salinity and Phytophtora but little resistant to Armillaria and Verticillium. At a nutritional level, it is also less efficient than *P. terebinthus* at uptake of zinc and copper but more efficient than *P. integerrima* and UCB-I.



Figure 3.5. Tree (A) and leaves (B) of *Pistacia atlantica* rootstock.

- ***Pistacia integerrima* L. (PI)**

P. integerrima (Figure 3.6) grows naturally in Asian countries like China and India. Although not native, it became the most widely planted rootstock in California under the denomination of PGI. Trees grafted on *P. integerrima* show high vigor and good uniformity. However, it is remarkably sensitive to cold and little resistant to salinity. This species is the most tolerant to Verticillium but little resistant to Armillaria and Phytophtora. *P. integerrima* was reported to come early into production with superior yields than *P. atlantica* and *P. terebinthus*. Nonetheless, it is reported to have low to medium yields in the dry lands of Spain (Couceiro et al., 2013) and was advised as a unique option in Spain, only

for warm areas with problems of Verticillium under deep soils and irrigated conditions. *P. integerrima* has less efficiency than *P. atlantica* for boron, zinc and copper uptake.



Figure 3.6. Tree (A), leaves and fruits (B) of *Pistacia integerrima* rootstock and vigor difference between *Pistacia integerrima* and *Pistacia atlantica*.

3.1.5. Experimental design

The experimental design was factorial and completely randomized. Two factors were involved: rootstocks and irrigation; with three levels for each factor.

The three rootstocks used were *P. terebinthus* L., *P. atlantica* Desf. and *P. integerrima* L.. These rootstocks were irrigated with three different treatments: a Control (C) without water stress, and two RDI treatments, T1 and T2.

The phenological stages taken into account in the implementation of RDI treatments were those suggested by Goldhamer (1995): stage I, from sprouting until the end of rapid nut growth (when pericarp reaches its maximum volume); stage II, from maximum nut size until the beginning of kernel growth (commonly known as the period of shell hardening); and stage III, from the beginning of kernel growth until harvest (this period starts after a fresh weight lag phase behaviour). Contrary to stone fruits, the growth of the pericarp and the growth of the seed (kernel) in pistachio fruits are separated in time. The period separating these two processes (stage II of fruit growth) has been reported to be the most tolerant phase to water stress and make the subject of a water deficit phase for water stress treatments. From each experimental plot, two trees were used for measurements, surrounded by trees under the same conditions (rootstock and irrigation). In total, eighteen plots were established in the field. Six plots for each irrigation treatment and six for each rootstock.

3.1.6. Irrigation regimes

During the irrigation season, control plants (C treatment) were irrigated at 100 % of Crop irrigation requirements (ET_c) of the previous week. ET_c was estimated according to daily reference evapotranspiration (ET_o), calculated using the Penman–Monteith equation (Allen *et al.*, 1998), a crop factor (K_c) based on each period of the growth cycle (Goldahmer, 1995) and (K_r) considering the canopy size and trees density (Fereres and Goldhamer, 1990). Midday stem water potential (Ψ_s) and soil water balance were

considered to modify the ET_c , for example, when Ψ_s values of that week were below -1.0 MPa, a water stress threshold used for several woody crops (Shackel *et al.*, 1997), irrigation amounts were increased a 10 %, as Goldhamer and Fereres (2001) suggested in order to ensure the presence of no irrigation-related stress. In addition to C, two RDI strategies (T1 and T2) were applied, in which water deficit during Stage I and III of fruit growth was avoided. This strategy was achieved maintaining the Ψ_s values around the threshold value indicated for C plants. During Stage II, the non-critical period, the water deficit was increased and the threshold values were -1.5 MPa for T1 and -2.0 MPa for T2 (Figure 3.7).

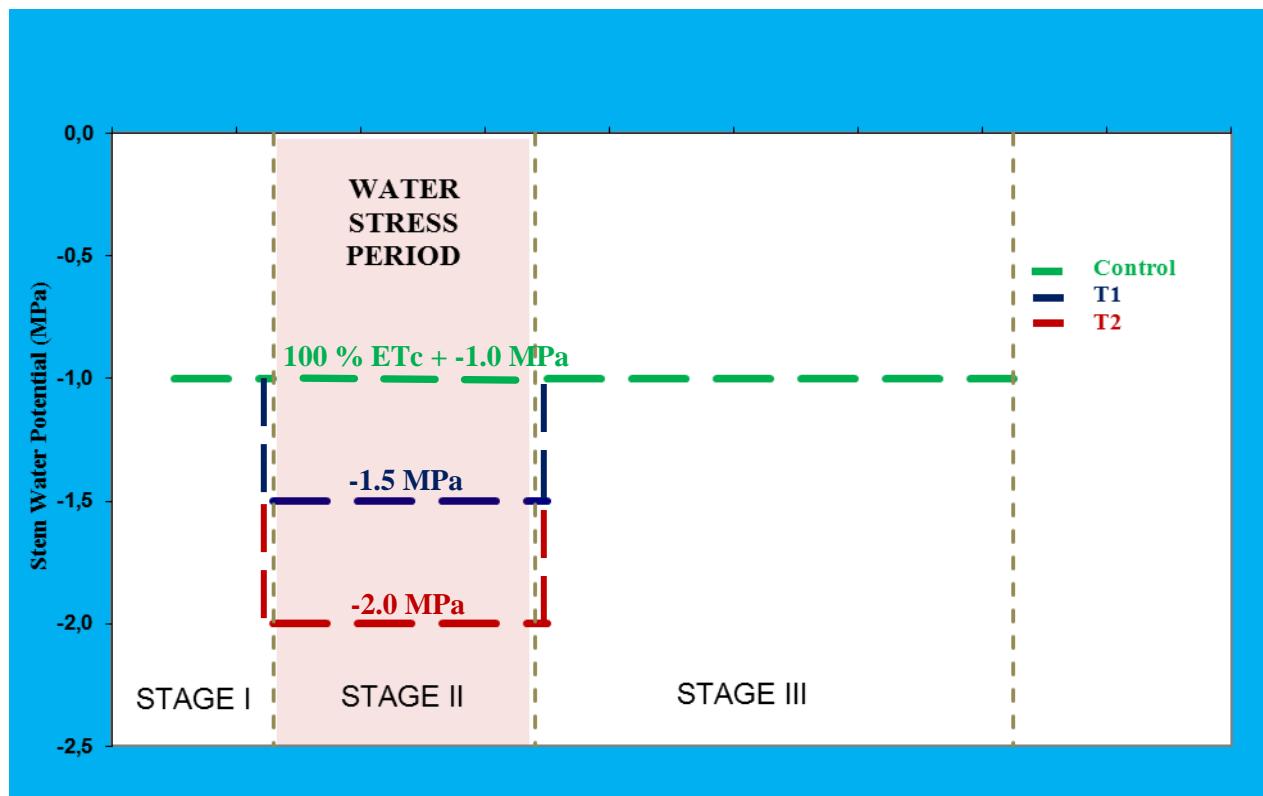


Figure 3.7. Seasonal irrigation regimes for Control, T1 and T2.

The irrigation protocol for RDI treatments was derived from the methodology proposed by Moriana *et al.* (2012) for olive trees and was adapted to pistachio trees

considering a Ψ_s threshold of -1.00 MPa (Shackel *et al.*, 1997) as an index for no water stress conditions.

Irrigation started when the measured Ψ_s values were lower than the threshold values considered. The first irrigation event was always 1 mm. Then, each increase or decrease of irrigation water supply was scheduled according to the percentage of the difference derived from the relationship between measured Ψ_s and the settled threshold for that stage:

- If the deviation rate was lower than 10 %, the variation in the irrigation was 0.25 mm day^{-1} .
- If the deviation rate was between 10 and 20 %, the variation in the irrigation was 0.5 mm day^{-1} .
- If the deviation rate was between 20 and 30 %, the variation in the irrigation was 1 mm day^{-1} .
- If the deviation rate was higher than 30 %, the variation in the irrigation was 2 mm day^{-1} .

Irrigation was stopped if this approach indicated a negative application.

3.2. STATISTICAL ANALYSES

The results were subjected to ANOVA. Means were compared using the Tukey test. The significance was set at $p < 0.05$. The arc sine transformation was applied to the percentage data.

Data were also subjected to a regression analysis. To check the regression model hypothesis (linearity, homoscedasticity, normality and independency), Kolmogorov–Smirnov was used with the Lilliefors correction, the Shapiro–Wilk tests for normality and

Bartlett's test for homoscedasticity. The linearity was observed in the graphics and independency was assumed due to the way that data were obtained. Statistical analysis were performed with SPSS 18 statistics program (Analytical Software, Tallahassee, FL, USA).

**CHAPTER 4: WATER RELATIONS IN
PISTACHIO: WATER STRESS AND ROOTSTOCKS
INFLUENCE**

4.1. ABSTRACT

Irrigation scheduling for fully-irrigated and water-stressed trees was managed by means of midday stem water potential (Ψ_s) measurements for three successive years. Trees were grown on three different rootstocks and water regimes. The use of direct measurements of the water status allowed estimating accurately the irrigation requirements for pistachio trees, with water reductions ranging from 46 to 205 mm in fully-irrigated trees. The combination of Ψ_s use and RDI regimes saved 43 to 70 % in T1 and 48 to 73 % in T2 of water compared to the calculated Crop Evapotranspiration (ET_c) for fully irrigated treatment (C).

Thus, the results indicate that full irrigation scheduling and RDI strategies can be achieved successfully using Ψ_s tool on pistachio trees growing in shallow soils. A Ψ_s threshold of -1.5 MPa during stage II (T1) was suggested for RDI. However a Ψ_s threshold of -2.0 MPa (T2) resulted in a significant reduction and an extensive delay in the recovery of stomatal conductance (g_l).

Little information is available on the diurnal behaviour of water potential and leaf conductance on pistachio trees despite their relevance to fine tune irrigation strategies. Mature pistachio trees were also subject to simultaneous measurements of stem water potential (Ψ_s) and leaf conductance (g_l) during the day, at three important periods of the irrigation season. A baseline relating Ψ_s to VPD is presented for irrigation scheduling in pistachio. Ψ_s was closely correlated with air vapor pressure deficit (VPD) but with a different fit according to the degree of water stress. No evidence of the variation of Ψ_s in relation to the phenology of the tree was observed. Furthermore, midday Ψ_s showed more

accuracy to indicate a situation of water stress than predawn water potential. Under well irrigated conditions, g_l was positively correlated with VPD during stage II of growth reaching its peak when VPD reached its maximum value (around 4 kPa). This behaviour changed during stage III of fruit growth suggesting a reliance of stomatal behaviour to the phenological stage independently to the tree water status.

Regarding rootstocks, *P. integerrima* showed little adaptation to water shortage compared to the two other rootstocks under the studied conditions.

4.2. INTRODUCTION

Cultivated for a long time in Mediterranean semi-arid areas, pistachio (*Pistacia vera* L.) has been considered as a drought-tolerant species (Spiegel-Roy *et al.*, 1977; Behboudian *et al.*, 1986; Rieger, 1995) mainly for its ability to survive under extreme conditions of water stress (Kanber *et al.*, 1990; Goldhamer, 1995). Nonetheless, the expansion of pistachio cultivation in countries like the USA has been linked to a change in the type of orchard management, with the aim of increasing yield mainly by transforming pistachio from a traditionally rain-fed cultivated crop to an irrigated crop. This evolution lifted the curtain about the fact that pistachio tree water demand is not particularly low (Goldhammer *et al.*, 1985) and that the potential evapotranspiration rate at the maximum seasonal evaporative demand is equal or exceeds that of many other deciduous species (Goldhammer *et al.*, 1983). Kanber *et al.* (1993) estimated a K_c for pistachio between 0.49 and 0.80 from May to August, but suspected that these values underestimated the real water consumption of pistachio trees. Goldhammer *et al.* (1995) presented a K_c much higher, estimating a maximum of 1.19 from the first fortnight of July to the first fortnight of August. This assumption was in discordance with the negative balance between water needs and its availability since the majority of pistachio producing regions were suffering an alarming continuous depletion of their water reserves and could not always sustain the tree water requirements due to the scarcity of rain and the increasing competition for fresh water. This situation led to the need of improving the efficiency of water use and provoked the claim of deficit irrigation strategies to maximize the rate of returns in front of water availability. Consequently, precision in irrigation has taken another level and the recourse to the study of the different mechanisms connecting the tree water relations to water deficit

strategies and the search of effectiveness in detecting stress become henceforth indispensable for a precise application of irrigation.

RDI works in pistachio trees were generally scheduled imposing a water stress that was quantified as a fraction of ET_c during a specific stage. However, the different agronomical conditions (mainly soil characteristics), when different locations are used for experimentation, produce a lack of similarity between results and make them little transferable. So, several authors suggested using plant water status measurements as an efficient tool for irrigation scheduling (Turner, 1990; Fereres and Goldhamer, 1990) rather than indirect estimations. Shackel *et al.* (1997) carried out probably the first study that suggested using plant water status measurements (leaf water potential) as a tool for irrigation scheduling.

Theoretically, the water status of a tree or a tree organ embodies a dynamic property controlled by the relative rate of water loss and water uptake. Thus, it would be expected to change with variation in soil moisture, environment and endogenous tree factors, making it a variable component along the day and the season (Sánchez-Blanco, 1983).

Plant water potential is certainly the most commonly used parameter as indicator of water status and irrigation timing in fruit trees (Klepper, 1968), mainly for its physiological relevance in the control of the water pathway in the tree (Ritchie & Hinkley, 1975).

Stem water potential (water potential of a covered leaf) has been reported to be the most representative index of tree water status compared to the water potential of an exposed leaf. Nevertheless, the time of taking this measure has made the subject of several studies and disagreement between researchers, where the effectiveness of detecting water stress was compared between measures at predawn and midday in many species.

In any case, theoretical predictions are that water potential will show a marked diurnal pattern where the degree of depression is closely related to variation in water availability and evaporative demand of the air along the day. These factors of variation limit the use and interpretation of Ψ_s to the role of checking (“How well are we doing”?) and make it an unsatisfactory tool for an independent use in irrigation scheduling. To overcome this concern, the development of references by the correction of this parameter for diurnal, seasonal and environmental variations could develop predictions of the behaviour of plants under specific water conditions and climate. Such references allow the establishment of specific protocols for irrigation by the only means of water potential. To reach this objective, sampling at different water availabilities under the same environment during different times of the day and season make possible to segregate the effect of each factor from the other and to determine the connection between fact and cause.

Goldhamer *et al.* (2005) suggested an initial approach based on shaded leaf water potential measurements for a fully irrigated mature pistachio orchard, recommending a range going from -0.7 MPa to -1.2 MPa and founding a good correlation between midday shaded leaf water potential and stem water potential ($R^2=0.948$).in which the shaded leaf water potential decreases gradually along the season.

On the other hand, stem water potential has proven to be a useful index of stress in a number of fruit tree species and fluctuations of this parameter under full irrigated conditions was found to be closely linked to vapour pressure deficit (VPD) variations (McCutchan & Shackel, 1992; Shackel, 2011; Williams & Baeza, 2007) and the determination of such relation would be useful for irrigation scheduling. The determination

of a relation between Ψ_s and VPD for well irrigated and stressed pistachio trees represents one of the objectives of this work.

As for water potential, stomatal conductance responds to a multitude of factors. However, while diurnal water potential profile can be mostly generalized for the majority of fruit species, stomatal behaviour differs. In the absence of water stress, in every diurnal cycle, stomatal conductance profile can oscillate, according to the species, between two behaviours; some species “prioritizes” saving water loss through stomatal closure, however others are characterized by counteracting the negative effect of excess heat by the opposite mechanism (stomatal opening) (Fereres *et al.*, 2012). Pistachio is a deciduous species; the majority of deciduous trees are characterized by a morning peak of stomatal opening with a substantial midday depression to maximize water use efficiency. By this way, the rate of carbon uptake is maximized per unit of water loss. However, few other deciduous species maintain a continuous stomatal opening to soften the heat effect at the expense of water loss. Pistachio has been reported to follow the first group of species (Goldhamer, 2012), however, such comportment does not explain at all, the dual behaviour of this crop as drought-tolerant as well as a species with an elevated water consumption and keep its behaviour somewhat ambiguous. One reason for this discrepancy can be the change of stomatal behaviour according to the requirement of each phenological stage. Such theory requires the study of stomatal behaviour at different key stages of pistachio growth.

A possible cause for the response variations of pistachio to water stress could be the influence of the rootstock. Turner (1991) stated the fundamental role of roots in directing the stomatal pattern. Such founding would be traduced in a consistent difference of stomatal behaviour when different rootstocks are used. *Pistacia integerrima* L. (PI),

Pistacia atlantica Desf. (PA), *Pistacia terebinthus* L. (PT) and UCB-I (a cross between PI and PA) are the most widely used rootstocks today for pistachio cultivation. There are few studies comparing the water relation responses between pistachio rootstocks. Germana (1997) mentioned that potted PA induced a more intense transpiration and net photosynthetic activity when it was compared to PT, which may limit its use in water-deficient areas. Ferguson *et al.* (2005) considered UCB-I as the most preferable commercial rootstock under irrigated conditions, and PT under rain-fed conditions.

In this chapter, the intention is to clarify several points of the mentioned above by the means of the study of pistachio water relations. The presentation of the results of this chapter and their discussion will be divided in three sub-chapters for a better structure to improve explanation.

4.3. MATERIALS AND METHODS

4.3.1. Plant water relations measurements

Water relations were characterized along the irrigation season taking weekly measurements of midday stem water potential (Ψ_s) and abaxial leaf conductance (g_l).

Ψ_s was measured at midday using a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA) (Hsiao, 1990). One leaf was taken from each control tree in each experimental plot for measurements. The leaf subjected to measurement was selected from the middle and shaded side of the canopy to avoid a sharp change in its state. Then, it was covered with aluminium foil 1 hour before measurement, stopping transpiration and enabling the leaf to reach a balance with the water potential of the stem (Begg and Turner, 1970). Owing to the difficulty in the determination of stem water potential in pistachio tree

because of the turpentine exudation, a piece of blotting paper was placed above the petiole cut-off to facilitate the measuring, as that paper does not get wet with turpentine but it does with xylem water.

Measurements of g_l were taken in two sun-exposed and fully expanded leaves per control tree, using a steady state porometer (Model LICOR-1600, U.K.). Both parameters were measured at the same time in order to observe the relationship between them.

Along the 2012 season, water relations were also characterized with three diurnal cycles of Ψ_s and g_l . Both parameters were measured throughout the day, approximately every two hours from predawn until dusk.

The daily cycles were performed on: 2 July 2012, corresponding to phenological stage II and a water stress period; 24 July 2012, corresponding to the beginning of change from stage II to stage III and the initiation of rehydration (one week after the start of rewetting); during this day, cloudiness was the cause of stopping measurements earlier than expected; and 7 August 2012, corresponding to stage III and a period of no water stress.

4.3.2. Soil water content and evapotranspiration measurements

The soil moisture was measured using a portable capacitance probe (Diviner 2000 Sentek Pty. Ltd., Stepney South, Australia). Four access tubes were installed in each experimental plot, measuring 50 cm in length; readings were made each 10 cm. Two access tubes were placed in the middle, between the tree axis and the drip line, at 50 cm from each other. One access tube was installed in front of a dripper. The other was placed in front of the middle of two drippers, at 50 cm from the other access tube. The other two access tubes were placed in the same perpendicular line from the tree axis as the previous access tube,

but this time in the middle, between the drip line and the centre of the row. In fact, they were at 1.75 m from the other two access tubes and 2.25 m from the tree axis (Figure 4.1).

The data thus obtained were used to calculate soil water balance by weighted average based on the area of each moisture zone.

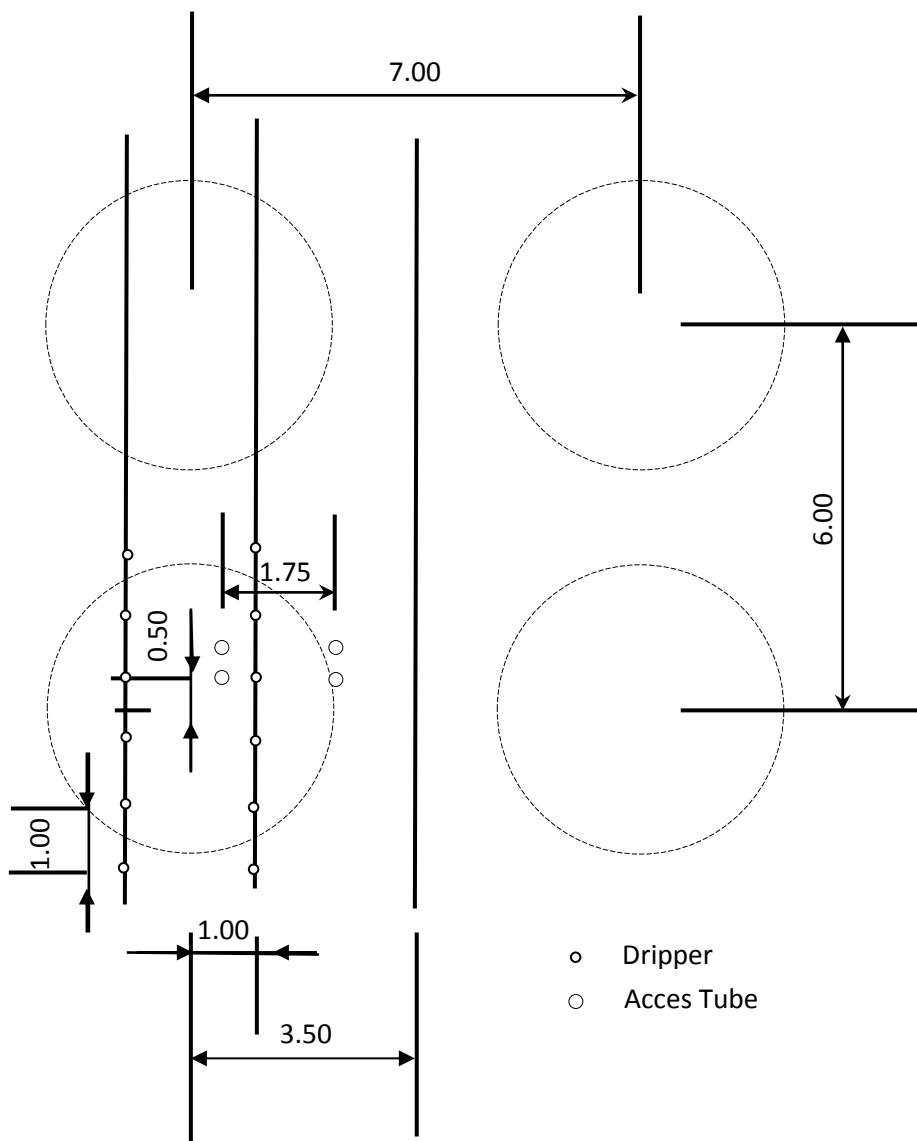


Figure 4.1. Distribution of the capacitance probes in the plot. Large circles represent the trees, the medium ones the access tubes, and the small ones the drippers. Distances are expressed in meter (m).

Actual crop evapotranspiration (ET_a) for each treatment and period was derived from the soil water balance of the root zone using the principles of conservation of mass, the expression used was:

$$ET_a = I + P + CR - RO - DP \pm \Delta SW$$

Where I is the irrigation, P is the precipitation, CR , is the capillary rise, RO is the surface runoff, DP is the deep percolation and ΔSW is the soil water balance. Irrigation and precipitation were considered as soil water inputs, while actual crop evapotranspiration (ET_a) was established as soil water output. The soil water content balance was taken into account as input or output, depending on its sign. Deep percolation of irrigation water, runoff and capillary rise were assumed to be negligible due to the type of irrigation system and the soil characteristics.

**SUB-CHAPTER 1: SEASONAL BEHAVIOUR AND
WATER STRESS THERSHOLD FOR THE
APPLICATION OF REGULATED DEFICIT
IRRIGATION.**

4.4. RESULTS

4.4.1. Meteorological data and irrigation water applied

During the irrigation season, the average daily maximum and minimum air temperatures were 31.8 °C and 13.2 °C in 2012, 31.7 °C and 12.7 °C in 2013 and 30.6 °C and 12.6 °C in 2014 (Figure 4.2). VPD_m ranged from 0.37 to 3.50 kPa in 2012, from 0.51 to 2.85 kPa in 2013 and from 0.34 to 2.76 kPa in 2014 (Figure 4.2), and the accumulated ETo were 843, 711 and 728 mm in 2012, 2013 and 2014, respectively (Figure 4.2, Table 4.1). Important differences in rainfall were recorded between seasons. Before the beginning of the irrigation seasons, the rainfall was 110, 347 and 184 mm respectively (Figure 4.2), and during these irrigation seasons, the rainfall was 25.6, 59.2 and 18.9 mm, respectively (Figure 4.2, Table 4.1).

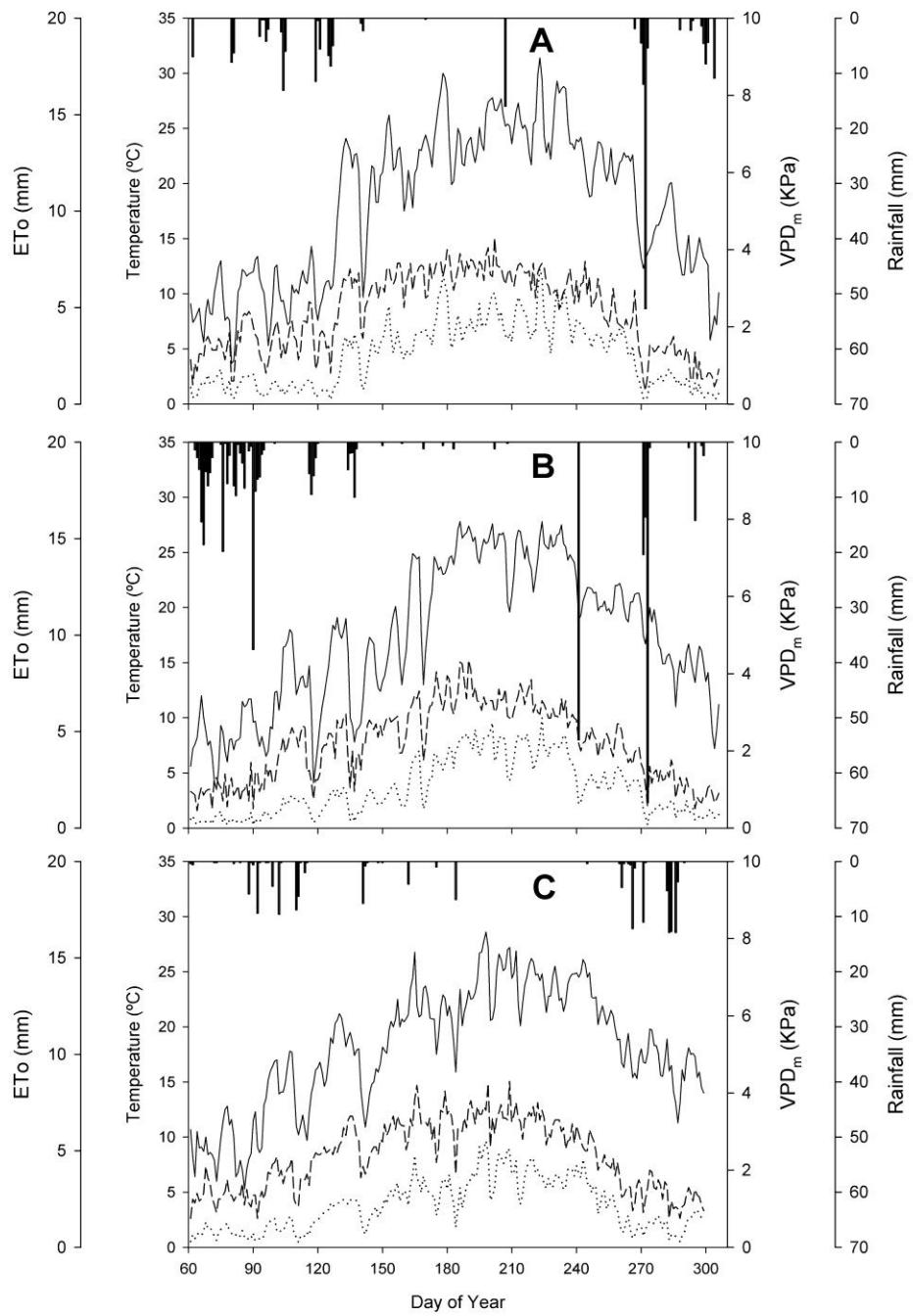


Figure 4.2. Daily mean temperature (T_m , solid line), crop reference evapotranspiration (ETo, short dash line), mean daily air vapour pressure deficit (VPD_m , dotted line) and daily rainfall (vertical bars) during years 2012 (A), 2013 (B) and 2014 (C).

The calculated crop evapotranspiration (ET_c) was 684, 607 and 796 mm in 2012, 2013 and 2014 respectively, while irrigation water in the C treatment was 489, 402 and 750 mm for the same years. Irrigation was just 71, 66 and 94 % of ET_c for this treatment. Irrigation water for T1 was 387, 179 and 411 mm in 2012, 2013 and 2014 respectively, and 337, 162 and 416 mm for T2 in the same years (Table 4.1).

The soil water balance in the C treatment was mainly positive, showing that the irrigation amount was higher than the water consumption. The period with the lowest value was during stage I in 2014, with a value of -11.3 mm, balanced in the next stage with a positive value of 15.2 mm.

T1 and T2 had negative values during stage I and II due to the effect of the irrigation strategy followed, while in stage III, they had positive values for the same reason.

The actual crop evapotranspiration (ET_a) in the C treatment ranged from 1.5 to 5.5 $mm\ day^{-1}$, with different means in each stage; 2.3, 4.8 and 4.6 $mm\ day^{-1}$ for stage I, II and III respectively. These results, ET_o and K_r were used to calculate the K_c of the control treatment (without water stress), which ranged from 0.31 to 1.18. The means of the Control K_c calculated were 0.51, 0.89 and 0.93 for stage I, II and III respectively (Table 4.1).

The ET_a in T1 and T2 treatments were always lower than C, especially in stage II.

Table 4.1. Crop reference evapotranspiration (ETo, mm), Theoretical Crop evapotranspiration (ETc, mm), Rainfall (mm), Irrigation per treatment (mm), Soil water balance per treatment (mm), Actual crop evapotranspiration per treatment (ETa, mm), Calculated reduction coefficient of control treatment (Kr) and Calculated crop coefficient of control treatment (Kc, mean value ± standard error) in the three irrigation stages in 2012, 2013 and 2014.

Year	Period	DOY	ETo (mm)	ETc (mm)	Rainfall (mm)	Irrigation (mm)			Soil Water Balance (mm)			ETa (mm day ⁻¹)			Kc	
						C	T1	T2	C	T1	T2	C	T1	T2		
2012	Stage I	135-156	131	69	3.2	43	9	8	-0.88	-14.06	-14.39	2.23	1.24	1.23	0.5934	0.60±0.05
	Stage II	157-200	305	246	0.3	187	100	45	14.51	-6.84	-13.74	4.02	2.49	1.37	0.7842	0.73±0.10
	Stage III	201-270	407	369	22.4	255	275	279	14.81	50.1	54.81	3.81	3.58	3.57	0.8520	0.77±0.15
	Total			684		489	387	337	28.44	33.20	26.68					
2013	Stage I	148-157	54	39	0.6	11			-2.15	-4.04	-3.14	1.50	0.52	0.42	0.707	0.31±0.004
	Stage II	158-211	349	309	4.6	246	30	5	0.23	-13.61	-21.9	4.72	0.91	0.59	0.7585	1.00±0.21
	Stage III	212-269	308	259	54	144	148	154	-9.29	9.5	10.17	3.63	3.37	3.48	0.7842	0.83±0.24
	Total			607		402	179	162	-11.21	-8.15	-14.87					
2014	Stage I	126-142	92	63	8.4	32			-11.26	-13.42	-15.42	3.25	1.36	1.49	0.9018	0.63±0.02
	Stage II	143-190	296	294	12.62	260	12	12	15.18	-11.32	-14.63	5.47	0.76	0.83	0.9268	0.94±0.02
	Stage III	191-261	429	439	5.5	458	400	404	5.61	38.20	41.72	6.54	5.24	5.25	0.9012	1.18±0.09
	Total			796		750	411	416	9.53	13.46	11.67					

4.4.2. Plant water relations

The midday stem water potential (Ψ_s) ranged in C from -0.65 MPa in 2013 to -1.39 MPa in 2014, with similar minimum values in 2012 (-1.36 MPa) and 2013 (-1.37 MPa). During stage I and due to the development of the leaf, only one measurement was done, showing a Ψ_s value of around -1.0 MPa for T1 and T2 in 2013 and 2014. In 2012, Ψ_s was -1.24 and -1.16 MPa for T1 and T2 respectively. During stage II, when a water stress was imposed, T1 ranged from -1.04 to -1.79 MPa in 2012, from -0.69 to -1.52 MPa in 2013 and from -0.99 to -1.43 MPa in 2014. During this stage, T2 ranged from -1.14 to -2.27 MPa in 2012, from -0.67 to -1.69 MPa in 2013 and from -0.94 to -1.72 MPa in 2014. Stage III started with the recovery and then maintained a good water situation. During this stage, T1 ranged from -0.72 MPa in 2013 to -1.60 MPa in 2012 and T2 ranged from -0.70 MPa in 2013 to -1.58 MPa in 2012 (Figure 4.3).

The Ψ_s in C was significantly higher than T1 and T2 from DOY 146 to 227 in 2012, from DOY 163 to 211 in 2013 and from DOY 161 to 217 in 2014, with some isolated days with no significant differences within periods (Figure 4.3). In addition, T1 had significantly higher Ψ_s than T2 from DOY 172 to 199 in 2012, on DOY 204 in 2013 and on DOY 182 and 188 in 2014.

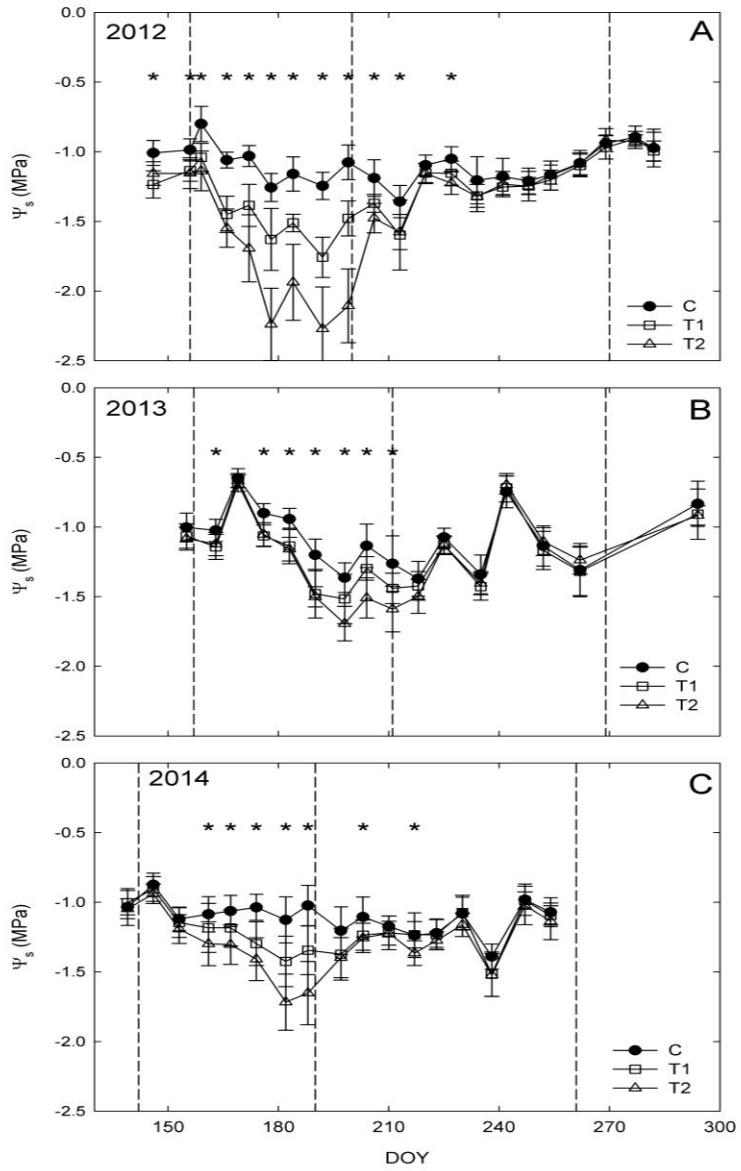


Figure 4.3. Stem water potential (Ψ_s) values (mean \pm SE, not shown when smaller than symbols, n=12) for pistachios in C (closed circles), T1 (open squares), T2 (open triangles up) treatment during 2012 (A), 2013 (B) and 2014 (C). Vertical dashed lines indicates the

stages I, II, III and post-harvesting. Asterisks indicate significant differences between treatments.

The abaxial leaf conductance (g_l) in the C treatment ranged from 213 to 363 mmol $m^{-2} s^{-1}$ in 2012, from 262 to 403 mmol $m^{-2} s^{-1}$ in 2013 and from 239 to 403 mmol $m^{-2} s^{-1}$ in 2014. The maximum values of g_l were slightly higher in stage II than in stage III in 2012 and 2013, 363 and 335 mmol $m^{-2} s^{-1}$ in 2012 and 403 and 347 mmol $m^{-2} s^{-1}$ in 2013 respectively. In 2014, the maximum values were similar in stage II and stage III, 392 to 403 mmol $m^{-2} s^{-1}$ (Figure 4.4).

In T1, g_l ranged from 173 to 258 mmol $m^{-2} s^{-1}$ in 2012, from 226 to 383 mmol $m^{-2} s^{-1}$ in 2013 and from 222 to 403 mmol $m^{-2} s^{-1}$ in 2014. For T2, g_l ranged from 159 to 261 mmol $m^{-2} s^{-1}$ in 2012, from 186 to 356 mmol $m^{-2} s^{-1}$ in 2013 and from 234 to 426 mmol $m^{-2} s^{-1}$ in 2014.

In the C treatment, g_l was higher than T2 from DOY 156 to 254 in 2012, on DOY 211 and 218 in 2013 and on DOY 188 in 2014 (Figure 4.4). G_l in T1 was lower than C on DOY 156, 166, 192 and 213 in 2012, on DOY 163 and 190 in 2013, and none in 2014.

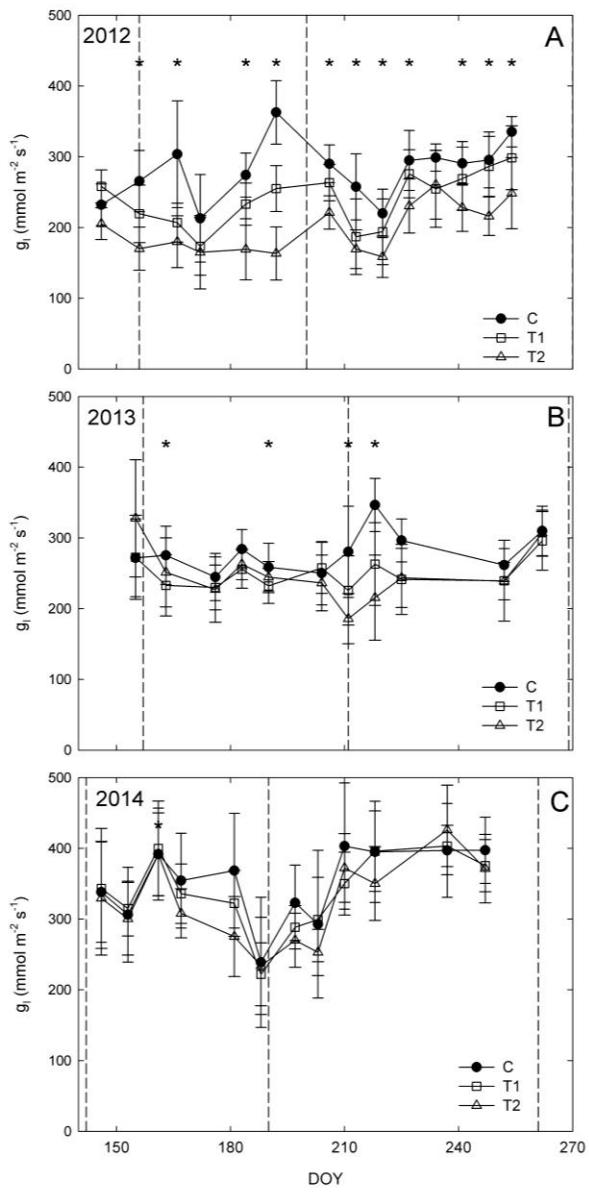


Figure 4.4. Abaxial leaf conductance (g_l) values (mean \pm SE, not shown when smaller than symbols, n=12) for pistachios in C (closed circles), T1 (open squares), T2 (open triangles up) treatment during 2012 (A), 2013 (B) and 2014 (C). Vertical dashed lines indicates the stages I, II, III and post-harvesting. Asterisks indicate significant differences between treatments.

4.5. DISCUSSION

4.5.1. Plant water relations

4.5.1.1. Irrigation scheduling in fully irrigated conditions

The fact that Ψ_s and g_l values in C plants were high and almost constant during the three years (Figures 4.3 and 4. 4) suggested that the irrigation applied to this treatment was sufficient to prevent any water deficit during the measurement period. The variations of Ψ_s were lineal in relation to changes in VPD (Figure 4.5) and allowed the generation of a baseline that could support irrigation scheduling decisions. This baseline was not affected by the phenological stage of the trees which could explain the clear reduction of Ψ_s in mid-summer. A similar decrease in Ψ_s has been reported in pistachio trees (Goldhamer et al., 2005; Gijón *et al.*, 2008) and in olive trees (Moriana *et al.*, 2003 and 2012). Similar baselines have been found in other crops, such as prunes (McCutchan and Shackel, 1992) or grapevines (Williams and Baeza, 2007) and they can be a useful tool for irrigation scheduling based on the plant water status. The water potential has been used as a scheduling irrigation tool for deciduous fruit trees (Shackel *et al.*, 1997), grapevines (Girona *et al.*, 2006; Williams *et al.*, 2012) and olive trees (Moriana *et al.* 2012).

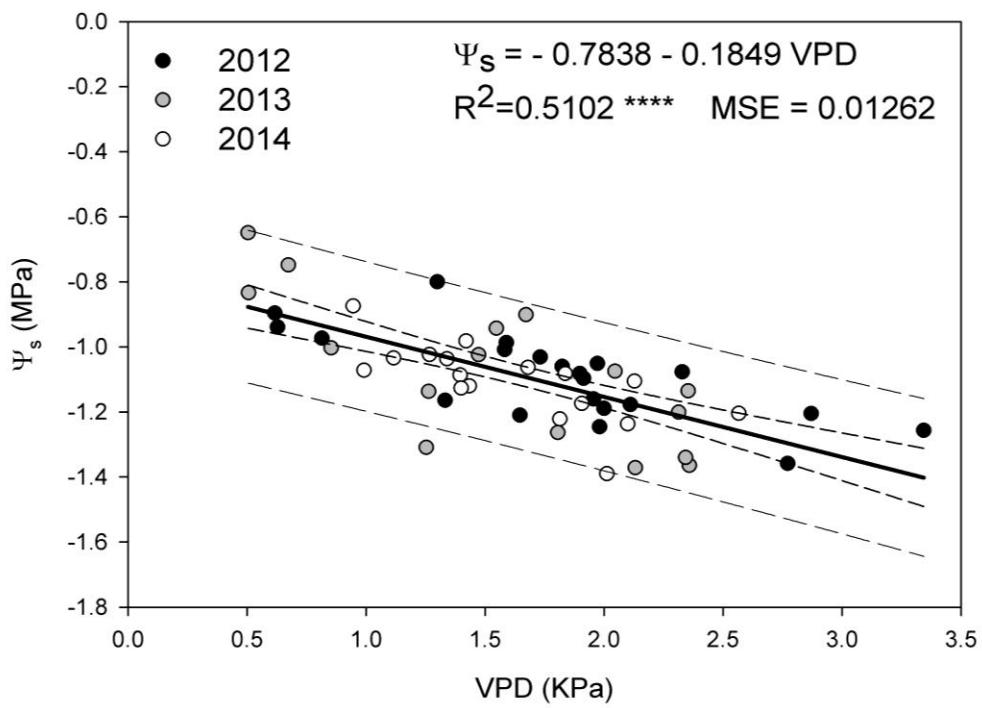


Figure 4.5. Relationship between stem water potential (Ψ_s , n=12) and vapour pressure deficit in C treatment during 2012 (closed circles), 2013 (grey circles) and 2014 (open circles). Adjusted line in continuous thick line, confidence intervals at $p<0.05$ in short dash line, prediction intervals at $p<0.05$ in medium dash line. Asterisks indicate $p \leq 0.0001$.

The water deficit in the C treatment was prevented with irrigation water amounts lower than those calculated using just the Penman–Monteith equation. Water was not extracted from soil in two of the three years of the study, seasonal soil water balance in the C treatment was positive (Table 4.1), therefore water was accumulated in the soil those years. The water saving achieved each season ranged from 46 to 205 mm. The average crop coefficients (K_c) calculated from the water balance (0.51, 0.89 and 0.93 for stage I, II and III respectively) were around 18 % lower in stage II and III than literature values

(Goldahmer, 1995). These K_{cs} were in the same range than the data obtained by Kanber *et al.*, (1993) but lower than those obtained by Iniesta *et al.* (2008), who reported even greater values than Goldhamer (1995). Maximum differences between K_{cs} calculated in the present work and K_{cs} estimated by Iniesta *et al.* (2008) were 26 % in stage II and 40 % in stage III. The irrigation technique can explain partially these differences, while Goldhamer (1995) and Iniesta *et al.*, (2008) used micro-sprinkler with high frequency irrigations, Kanber *et al.*, (1993) applied the irrigation with a hose every 20 days. Iniesta *et al.*, (2008) assumed evaporation losses of 7.5 % of water applied, but Koumanov *et al.*, (1997), in an almond orchard irrigated by micro-sprinkler, reported water losses ranging from 21 to 27 % of the applied water . Therefore, the irrigation scheduling based on the Ψ_s adjusted the water needs to calculate accurate K_c values. Although K_c data of the present work were calculated during three years only, they must be considered in drip irrigation scheduling to obtain important water savings.

4.5.1.2. Effect of water stress

Water stress in T1 and T2 was increased at the same rate in 2012 and 2013; both treatments had different water stress only when irrigation was used in T1 to maintain the water stress around the threshold of the treatment. However, in 2014, the decrease in Ψ_s in T2 was faster than T1. Such response could be related to an increase of embolism and it could produce a negative response to RDI scheduling in the long term (such as in almond, Fereres and Goldhamer, 1990). There are no data in the literature for pistachio trees about the threshold of the xylem pressure where the loss of conductivity begins. In olive trees, a similar species in terms of drought resistance, the loss of conductivity increased rapidly

from -1.3 MPa (Torres-Ruiz *et al.*, 2013). This pressure is quite near the water stress threshold of T1, so embolism could be even much more frequent in T2 than in T1. In addition, some species presented a cavitation fatigue, which produces an increase of the embolism for each new cycle of water stress (Hacke *et al.*, 2001).

The effect of the fruit load could reduce the accuracy of Ψ_s in the detection of water stress. In theory, Ψ_s integrates the effects of irrigation and crop load (Naor, 2001). In the present work, no clear trends of lower values of Ψ_s were observed in the ON year compared to the OFF year in C trees (Figure 4.3), as it was found in olive trees (Martín-Vertedor *et al.*, 2011) or peach trees (Alcobendas *et al.*, 2012). Therefore the same baseline could be used. Although no influence of fruit load was detected in full irrigated conditions, Ψ_s values in T1 and T2 were nearer to C during OFF than during ON seasons.

The pattern of leaf conductance (g_l) could suggest a threshold of the water stress level. Significant reductions in g_l were measured when the Ψ_s was lower than -1.5 MPa. Such threshold could indicate the beginning of severe water stress conditions. Significant decreases in g_l values were also related to the recovery period, which was the greater the g_l reduction was. The slow recovery of pistachio trees shortened the period of permissible water stress that should be taken into consideration to ensure the tree full water requirements were met in the sensitive stage. Pérez-Lopez *et al.*, (2008) suggested that a greater increase in the soil water content accelerated the rehydration in olive trees.

4.6. CONCLUSIONS

The relation between ψ_s and VPD was linear and allowed the estimation of a baseline that could be used to improve irrigation scheduling accuracy under similar

conditions. The course of ψ_s along the season was not particularly affected by phenological stages or crop load. So, the same baseline could be used for “ON” and “OFF” years. However, a higher influence of water stress on tree water status seems to take place during high bearing year. The study of the seasonal g_l pattern led to suggest a ψ_s threshold of -1.5 to avoid severe water stress conditions, a level from which g_l shows a consistent decrease and a long delay for recovery.

In summary, the use of ψ_s as a tool for irrigation scheduling allowed the accurate adjustment of water needs and showed the ability of the use of this indicator as an independent way of decision for irrigation. The use of this technique for irrigation permitted to maintain trees under an optimal water status with only 77 % of ETc and generated for this zone, a lower Kc than the reported for pistachio.

**SUB-CHAPTER 2: IMPACTS OF WATER STRESS
AND ENVIRONMENT ON THE DIURNAL
BEHAVIOUR OF STEM WATER POTENTIAL AND
LEAF CONDUCTANCE IN PISTACHIO**

4.7. RESULTS

4.7.1. Climatic parameters and amounts of water applied

The meteorological conditions during the diurnal cycles carried out in 2012 are presented in Figure 4.6. The minimum temperature was respectively, 11.82 °C, 16.91 °C and 13.51 °C and the maximum was 31.41 °C, 34.27 °C and 33.67 °C. From the first to the last day of the study, the maximum values of VPD were 4.04 kPa, 4.24 kPa and 4.42 kPa respectively and it was registered approximately in the early afternoon.

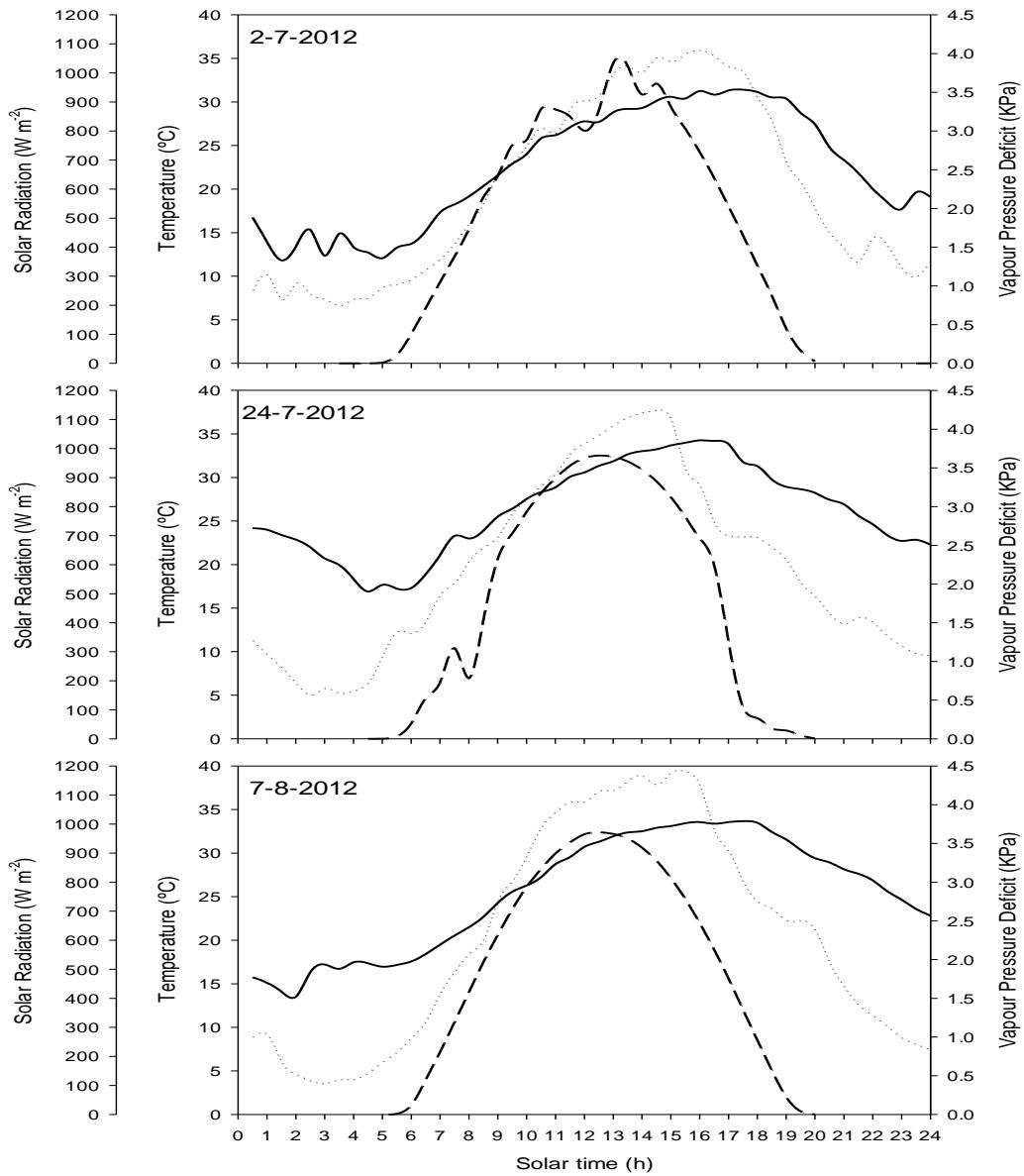


Figure 4.6. Diurnal courses of environmental factors: solar radiation (black dashed line), air temperature (black line) and air vapour pressure deficit (dotted line), on 02/07/2012 (period of water stress), 24/07/2012 (initiation of rehydration period), and 07/08/2012 (period of no water stress).

The water balance along the study is presented in Table 4.2. During stage I, there was no water stress in any of treatments. From the commencement of stage II until 02/07/2012, Control treatment was irrigated with 114.41 mm, which means 81 % of ETc, although there was a soil water accumulation positive balance of 9 mm. T1 was irrigated only with 33.46 % and T2 with only 6.45 % of ETc. The reduction in irrigation produced a consumption of the soil water reserve, which was reflected by a negative value of soil water balance (Table 4.2). In the following two weeks, irrigation in Control was 62 mm, 40 mm in T1 and 24 mm in T2, but with a soil water balance near zero, although negative in both deficit treatments, minor reductions of water consumption (based on ETa) was produced compared to Control.

When initiating the rewatering, irrigation was the same for all treatments, around 40 mm; this quantity was sufficient to cover crop water needs for all treatments without any additional depletion in soil water reserve. Water consumption was around 5 mm/day. Similar results were obtained in the period from 23/07/2012 to 06/08/2012 (Table 4.2).

Table 4.2. Reference evapotranspiration (ETo), precipitation, irrigation amount, soil water content balance and actual consumed water (ETa) for each treatment: Control (C), T1 and T2.

	Water regimes		Stage II	Rewatering initiation	Stage III	
Period			05/06/2012	02/07/2012	16/07/2012	23/07/2012
			02/07/2012	16/07/2012	23/07/2012	06/08/2012
ETo (mm)			189.4	100.3	51.4	101.1
ETo (mm/day)			6.7	7.2	7.3	6.7
Precipitation (mm)			0.2	0.1	0	16
Irrigation (mm)	C	114	61.7	37.3	66	
	T1	47.1	40.1	42.2	69.8	
	T2	9.1	24	39.9	70.2	
Soil water content balance (mm)	C	9.7	4.7	2.5	4.5	
	T1	-6.1	-0.7	5.1	11	
	T2	-12	-1.5	4.1	8.9	
ETa (mm)	C	105	57.1	34.8	77.5	
	T1	53.4	40.9	37.1	74.7	
	T2	21.4	25.7	35.7	77.3	
ETa (mm/day)	C	3.7	4.1	5	5.2	
	T1	1.9	2.9	5.3	5	
	T2	0.7	1.8	5.1	5.2	

4.7.2. Stem water potential and abaxial leaf conductance diurnal course throughout rehydration

4.7.2.1. Stem water potential diurnal pattern

On 2 July 2012 (in the middle of the water stress period), Ψ_s decreased in all treatments from predawn until a minimum was reached at around 14:00 h when a gradual recovery began (Figure 4.7A). Control reached a maximum of -0.64 MPa and a minimum of -1.33 MPa. T1 treatment reached a maximum of -0.90 MPa and a minimum of -1.65 MPa while T2 reached a maximum of -1.18 MPa and a minimum of -2.11 MPa. Statistical differences between all treatments were found during the whole day.

On 24 July 2012 (one week after initiating rewatering), diurnal pattern of Ψ_s followed a similar curve shape than the first diurnal cycle, and significant differences in Ψ_s values were measured (Figure 4.7B). The minimum of Ψ_s was observed at around 16:00 h for all treatments. Control treatment reached a maximum of -0.72 MPa at predawn and a minimum of -1.37 MPa. T1 treatment reached a maximum of -0.88 MPa at about 06:30 h and a minimum of -1.59 MPa. T2 treatment reached a maximum of -1.00 MPa between predawn and 06:30 h and a minimum of -1.64 MPa. Statistical differences were found between Control and the other two treatments throughout the day and between all treatments only at predawn, at 06:30 h and at 13:38 h. The extent of differences of Ψ_s between treatments was clearly smaller than the previous day of measurement (Figure 4.7B). On 7 August 2012, statistical differences between all treatments were found at approximately 08:30 h and 13:30 h, with T2 being the treatment with the lowest values, and between Control and the other treatments before noon (at 10:40 h) and late afternoon (17:50 h) (Figure 4.7C). Control reached a maximum of -0.71 MPa and a minimum of -1.33 MPa.

T1 reached a maximum of -0.85 MPa and a minimum of -1.41 MPa and T2 reached a maximum of -0.87 MPa and a minimum of -1.47 MP.

The relationship between VPD and Ψ_s for each irrigation treatment is presented in Figure 4.9. Data were grouped when there were no significant differences between daily cycles. In the Control trees, all the data of the three different dates were included in the same equation. The regression between Ψ_s and VPD was strong and significant (Figure 4.9A). In the T1 treatment, the first two diurnal cycles have been included in the same equation regression, which was significantly different from the Control ones (Figure 4.9B). Data measured during the drought cycle presented a significantly different fit in which the slope of the equation was significantly lower (-0.23 vs -0.16), while no significant differences were found between y-interception. Data in T2 trees were not grouped and each date had a significantly different fit (Figure 4.9C). The regression equation of the last date was near to Control though significantly different from it. The slopes of the daily cycles were lower with the water stress.

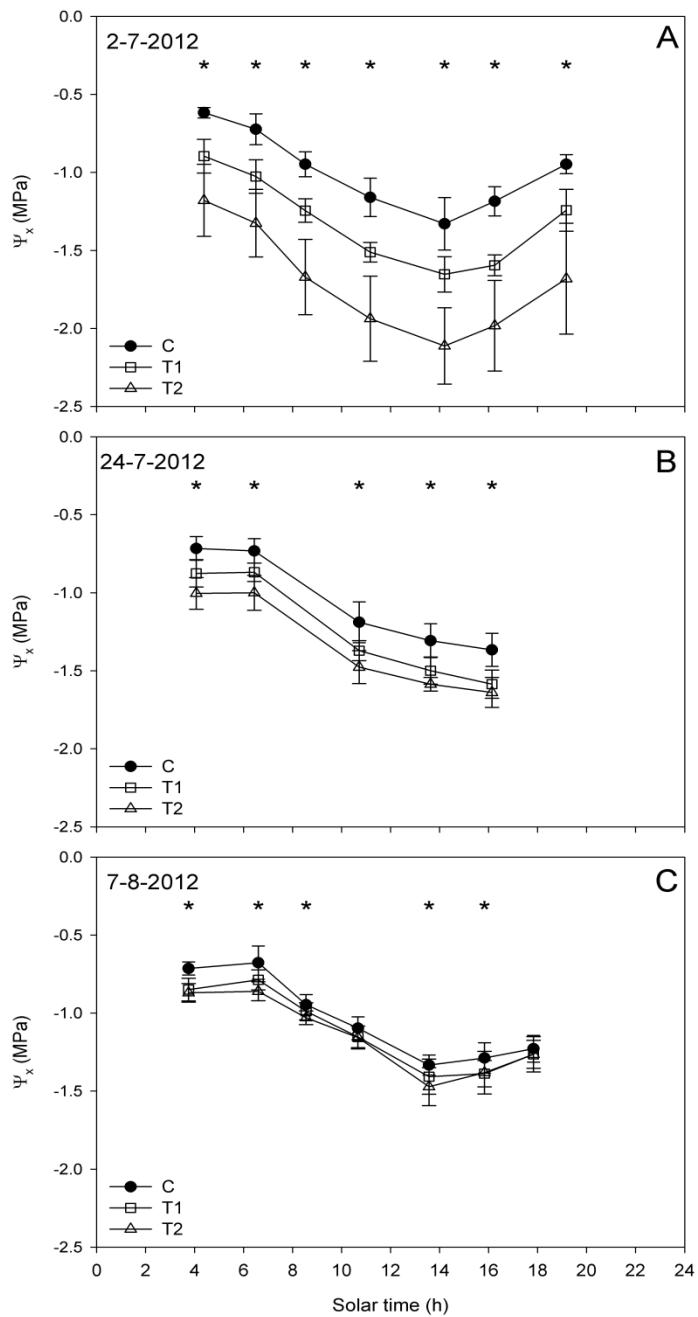


Figure 4.7. Diurnal pattern of stem water potential (Ψ_s) of pistachio leaves under different water regimes on three dates. Each point is the mean of 12 measurements. Vertical bars indicate the standard error of the mean. Stars denote that significant differences were found among treatments ($p < 0.05$).

4.7.2.2. Abaxial leaf conductance diurnal pattern

On 02/07/2012 (corresponding to stage II, drought period), abaxial leaf conductance (g_l) in fully irrigated Control trees increased throughout the day, reaching a maximum g_l value of $315 \text{ mmol m}^{-2} \text{ s}^{-1}$ at around 16:00 h, followed by a gradual decline during the rest of the day (Figure 4.8A). T1 and T2 reached a g_l peak earlier than Control. T1 and T2 peaked at 08:30 h, reaching a value of 242 and $220 \text{ mmol m}^{-2} \text{ s}^{-1}$ respectively and then declined gradually throughout the day. Statistical differences have been observed between Control and the other treatments from 11:00 h until sunset. Statistical differences between T1 and T2 were only observed at 14:00 h when VPD was near its maximum (3.91 kPa).

On 24/07/2012, the general behaviour of g_l diurnal pattern was similar for all treatments, characterized by an increase of g_l early in the morning reaching a maximum peak at 10:00 h when VPD reached 3.27 kPa and a gradual decrease throughout the day. Control treatment has been characterized by a different pattern compared to the previous diurnal cycle. There were statistical differences between all treatments at approximately 11:00 h and between Control and the other treatments at 13:30 h (Figure 4.8B).

On 07/08/2012, the diurnal progress of g_l showed a similar behaviour for all treatments. It was characterized by an increase of g_l values early in the morning reaching a maximum peak at about 08:30 h and a continuous decrease during the rest of the day. Statistical differences were found between Control and the other treatments at 08:30 h, 13:15 h, and 15:30 h, and a difference only with T2 at approximately 11:00 h.

The relationship between g_l and VPD is presented in Figure 4.10. Relationships were weaker than the ones obtained for Ψ_s . In all treatments, there were significant differences between data obtained during the two first cycle (stage II) and the last date

(stage III). In fact, there was no significant relationship between g_1 and VPD during stage III in any of treatments. The regression in all treatments suggests lower values for the same VPD during this last diurnal cycle than in the other two. The relationships during stage II were significantly different between treatments with a greater slope in Control (Control>T1>T2).

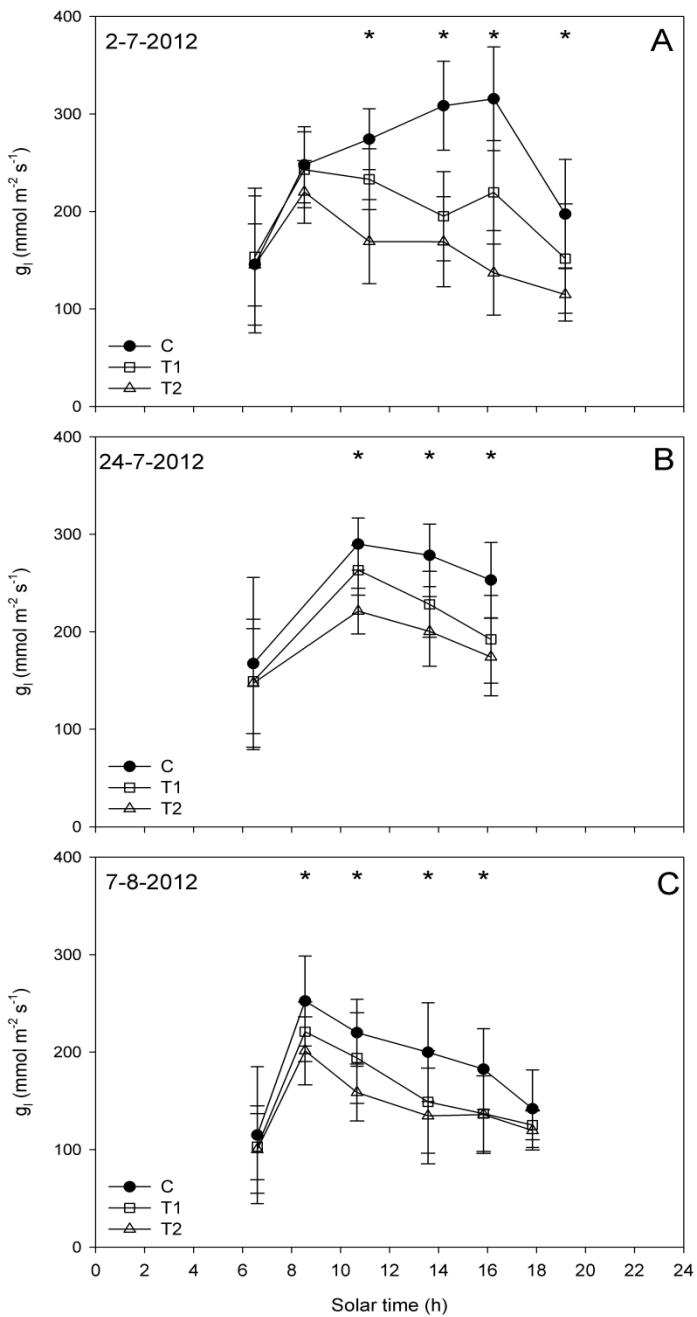


Figure 4.8. Diurnal pattern of abaxial leaf conductance (g_l) of pistachio leaves under different water regimes on three dates. Each point is the mean of 12 measurements. Vertical bars indicate the standard error of the mean. Stars denote significant differences were found among treatments ($p < 0.05$).

4.8. DISCUSSION

4.8.1. Ψ_s as water stress indicator for pistachio

The measurement of water potential at predawn has usually been taken as a reference to detect water stress in plants in several studies because it is assumed that the potential before dawn depicts the moment of equilibrium between tree and soil water potential (Shultz, 1996; Williams & Araujo, 2002). In this study, measurement of Ψ_s before dawn had showed its limitation even when statistical water stress differences were detected between treatments from the fact that it was not representative of a steady state; the maximum of Ψ_s did not take place only at predawn but a few hours later, when trees were in a rehydration process from stage II to stage III (Figures 4.7B and 4.7C). In fact, the soil-plant water potential unbalance just before sunrise is quite common and has been mentioned in several studies (Sellin, 1996; Schmidhalter, 1997; Schmidhalter *et al.*, 1998). The fact that predawn water potential is biased by the spatial distribution of soil moisture makes it a weak recommended indicator for tree water status determination (Ameglio *et al.*, 1999). Even when the soil humidity is uniform, Donovan *et al.* (2001) found disequilibrium between predawn water potential and soil water potential for well irrigated plants and attributed a great part of the unbalance to a putative mechanism of high concentration of leaf apoplastic solutes and/or to night time transpiration. Therefore, the use of a more specific indicator related to the current status of plant could be more useful. Unlike predawn Ψ_s measurements, significant differences have been found at midday or mid-afternoon with considerably smaller coefficients of variation. During this time interval, the detection of water stress in pistachio trees could be the most adequate. In fact, measurement at midday showed its efficiency in several deciduous trees such as peach, almond and prune

(Shackel *et al.*, 1997) due to their high sensitivity to water deficit (McCutchan & Shackel, 1992). In pistachio, at a practical level, measurement of Ψ_s has the advantage of not being limited to midday time as with many other fruit trees for a better sensing of water stress, and a measurement at mid-afternoon might be more representative of water status than midday. In fact, differences between treatments were more evident at mid-afternoon than at midday. This practical specificity in pistachio is mainly due to its particular stomatal behaviour since the lower values of Ψ_s were obtained approximately at the moment of maximum evaporative demand producing the temporary imbalance, which occurs between the variant rate of roots water absorption, the xylem water transport and the stomata water loss (Medrano & Flexas, 2003). The use of plant water status measurements has been suggested previously as a tool for irrigation scheduling (Shackel *et al.*, 1997; Goldhamer *et al.*, 1999; Moriana & Fereres, 2002), given the fact that the Ψ_s measurement results in an accurate state of the dynamic relation integrated by water stress and environmental conditions (Morianá *et al.*, 2012). The reference to a baseline under well-watered conditions and varying atmospheric situations is required for a correct use of midday/middle-afternoon Ψ_s measurements as indicators in pistachio irrigation scheduling. The fact that this baseline is not affected by the phenological stage of the tree is very useful. As a primarily approximation, the relation represented in Figure 4.9A, obtained between Ψ_s and VPD could be a useful tool for this purpose.

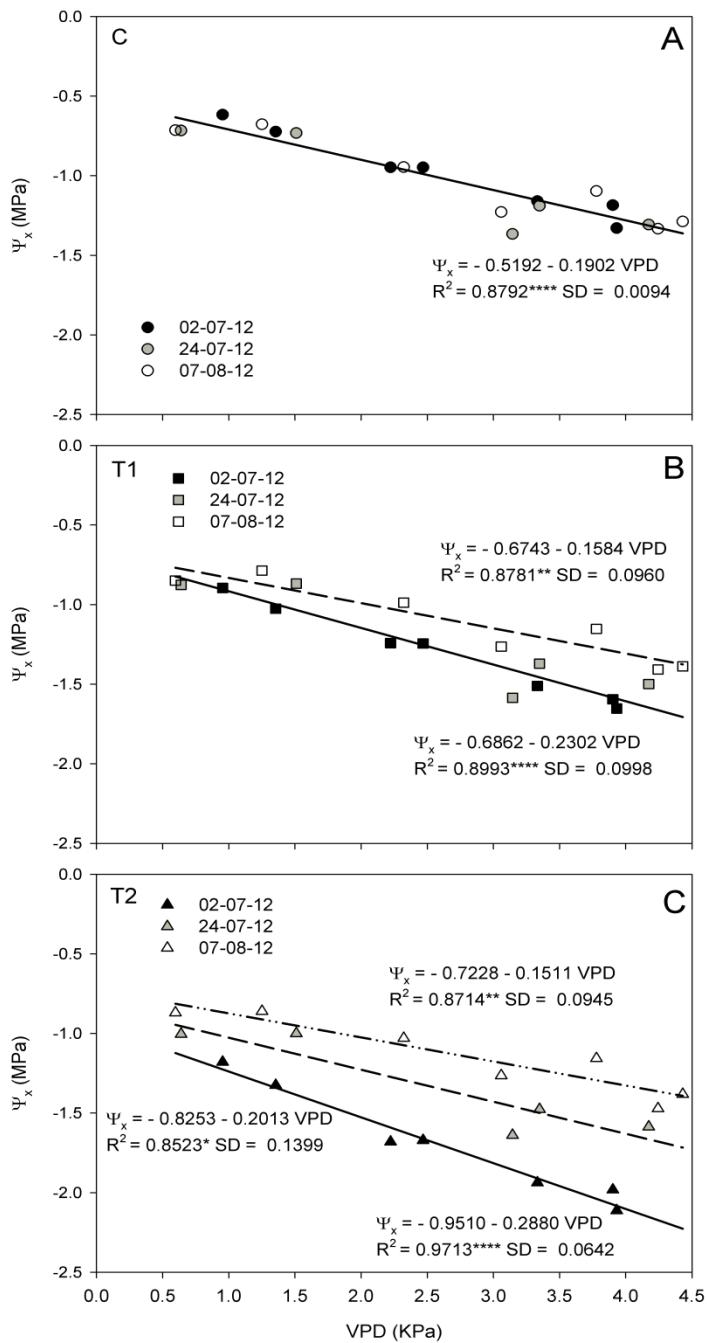


Figure 4.9. Relationship between air vapour pressure deficit (VPD) and stem water potential (Ψ_s) for Control (A), T1 (B) and T2 (C) treatments. All data of the three dates of the experimental period are included.

4.8.2. Stomatal response to increasing atmospheric VPD, water availability and phenological stage

According to several studies of stomatal behaviour in fruit trees, it was found that stomatal response throughout the day is characteristic of each species (Acherar & Rambal, 1992). During stage II, in fully irrigated trees, no closing response of g_l was observed, reaching its peak approximately the same time VPD reached its maximum. A strong relationship was found between g_l in fully irrigated trees and VPD ($R^2=0.96$), indicating that there is a low stomatal closure regulation for pistachio trees when water is not a limiting factor in this stage (Figure 4.10A). This correlation presented a lower fit as water stress increased (Figures 4.10B and 4.10C), which suggests that the association between g_l and VPD is mainly governed by the water stress level in the tree. In this case, these results advocate that under water stress, tree gas exchange was more affected by factors related to water soil availability than by atmospheric components in pistachio.

During stage III, gas exchange was clearly affected in Control trees with a decrease by 20 % in g_l levels between stage II and stage III, and maximum g_l peak was observed approximately 7 hours earlier (Figures 4.8A and 4.8C). In addition, the correlation between VPD and g_l on the third day was weaker compared to the first two days of the experiment, taking into account there were no significant changes in VPD levels throughout the experiment. Therefore, this change in g_l pattern can be regarded as physiological changes affecting tree water relations from a stage to another, rather than VPD or soil moisture conditions. Several authors reported that changes in water relation parameters in pistachio during stage II is characterized by an increase in g_l values (Sajjadinia *et al.*, 2010; Gijon *et al.*, 2011). In almonds, several authors suggested that the seasonal decrease in gas exchange

rates were related to changes in the hormonal balance or development of leaves (Sanchez-Blanco *et al.*, 1991; Klein *et al.*, 2001).

Depending on the phenological stage, the stomatal response of a full irrigated pistachio trees oscillates between two different behavioural "strategies": (i) a strategy where the stomatal opening is substantial and prolonged during the day, approaching to an apple tree behaviour or (ii) a more conservative strategy which avoids high water loss traduced by a partial stomata closure at the middle of the morning which is more similar to almonds or olives trees behaviour. The first strategy is observed during shell hardening, a process of high energy consumption which could be the cause of little noticeable water stress effect at the final yield according to Goldhamer & Beede (2004). The second strategy, a more conservative approach, is recorded during the kernel filling stage which is considered imperative to the survival of the species.

On the other hand, water stress reduced the maximum g_l in deficit treatments (T1 and T2). Under these conditions, the g_l pattern followed by T1 and T2 treatments showed the ability of pistachio trees to regulate stomatal activity under water stress conditions by closing stomata early in the morning, anticipating an excessive decline in water potentials. This control from roots to shoots could be assigned to a hydraulic or/and a biochemical signal, which prevents excessive water loss as in other fruit trees: orange, Fereres *et al.* (1979); almond, Castel & Fereres (1982), Torrecillas *et al.* (1988); olive: Bongi & Loreto (1989), Giorio *et al.* (1999).

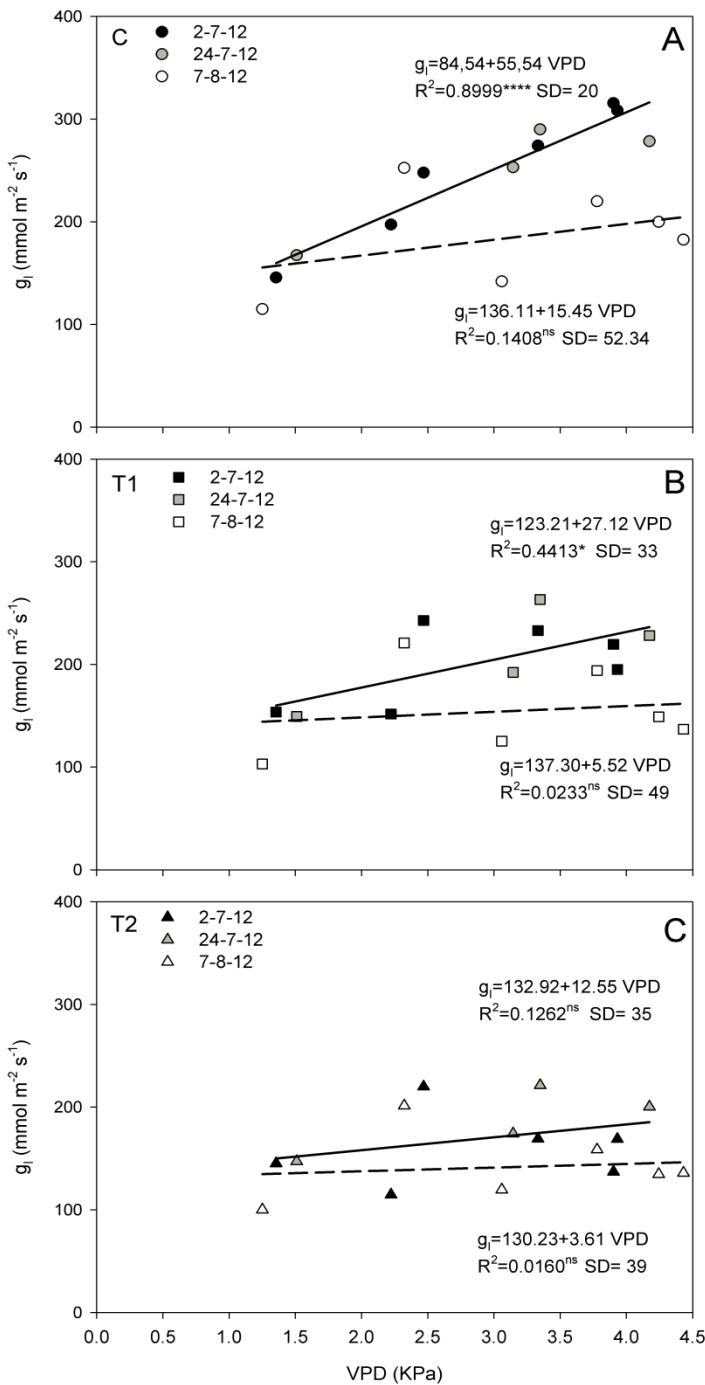


Figure 4.10. Relationship between air vapour pressure deficit (VPD) and leaf conductance (g_l) for Control, T1 and T2 treatments. All data of the three dates of the experimental period are included.

4.8.3. Pistachio water recovery course

Three weeks after the beginning of rehydration, a maintained positive soil water balance did not allow stressed trees to recover completely from stress levels of -1.5 MPa and -2.0 MPa maintained approximately for 40 days. Torrecillas *et al.* (1996) mentioned that a rapid recovery after a stress condition can be related to a greater physiological tolerance to drought, so the delay observed in the recovery of pistachio trees was not expected due to the great reputation of pistachio as a drought-tolerant species. Hence, the faster or slower response to water renewal may be related to the extent of drought (level of stress and duration) and its possible progressive effects on different plant organs (short and long term effects).

Guerrero *et al.* (2005) imposed a similar extent of water stress as T1 and observed a rapid recovery for g_l in pistachio trees, however, they concluded that more than three weeks are required for a complete recovery of water status in pistachio because of lagging trunk growth. Moreover, Rieger (1995) mentioned for potted pistachio plants the possibility that stomatal conductance could recover before hydraulic conductivity. In this study, a partial recovery was observed 3 weeks after the beginning of rewetting but a total recovery could not be confirmed when Ψ_s drops to values around -2.2 MPa during stress period (T2 treatment).

Several studies involving acoustic sensing of woody tissues in other species showed that cavitation often occurred when the water potential falls below -1.5 MPa (Dixon *et al.*, 1984; Peña & Grace, 1986; Tyree & Sperry, 1989). Then the recovery delay observed in this study may also be related to the complexity of the process for the refilling of xylem cavitation. The variant time necessary for each individual mechanism involved in the

recovery to come to pre-stress conditions translates the complexity of the recovery process initiated by re-watering.

The unexpected delay for the recovery of pistachio from water stress should be taken into account and exceeding rewatering should start early for RDI major benefits specially under shallow soil conditions combined with drip irrigation system.

4.9. CONCLUSIONS

Midday stem water potential seems to be more precise to detect water stress than predawn water potential in pistachio and the interval of time for the measure could be extended to mid-afternoon. The relation between VPD and stem water potential was affected by the degree of water stress but not by the phenological stage. However, the relation between gl and VPD was altered by water stress and the phenological stage. During stage II, this relation was more affected by soil water availability than by VPD increase. However, during stage III, independently of the irrigation treatment, a change in gl behaviour was observed. So, depending on the phenological stage, pistachio stomatal behaviour was characterized by a substantial stomatal opening during the day (stage II) or a partial closure at the first hours of the morning (stage III).

The complete recovery of pistachio from stress levels of -1.5 MPa and -2.0 MPa maintained approximately for 40 days was slow taking at least more than three weeks.

SUB-CHAPTER 3: COMPARISON BETWEEN ROOTSTOCKS BEHAVIOUR.



4.10. RESULTS

4.10.1. Seasonal course

The Ψ_s was higher in trees on *P. terebinthus* rootstocks than those on *P. integerrima* rootstocks on DOY 248, 254 and 269 in 2012, on DOY 204 and from 235 to 252 in 2013 and on DOY 203 and 230 in 2014. The Ψ_s was also higher in trees on *P. terebinthus* rootstocks than in trees on *P. atlantica* rootstocks on DOY 242 in 2013. The Ψ_s was higher in trees on *P. atlantica* rootstocks than in trees on *P. integerrima* rootstocks on DOY 217 in 2013 (Figure 4.11-left).

The Ψ_s was higher in trees on *P. integerrima* rootstocks than in trees on *P. terebinthus* rootstocks on DOY 163 and 183 in 2013, on DOY 139 and 146 in 2014. The Ψ_s was also higher in trees on *P. integerrima* rootstocks than in trees on *P. atlantica* rootstocks on DOY 169 in 2013 and on DOY 146 in 2014.

There were no statistical differences in g_l among trees using the three roostocks, except on DOY 166 in 2012 and on DOY 153 in 2014. In both cases *P. integerrima* showed higher values than *P. atlantica* (Figure 4.11-right).

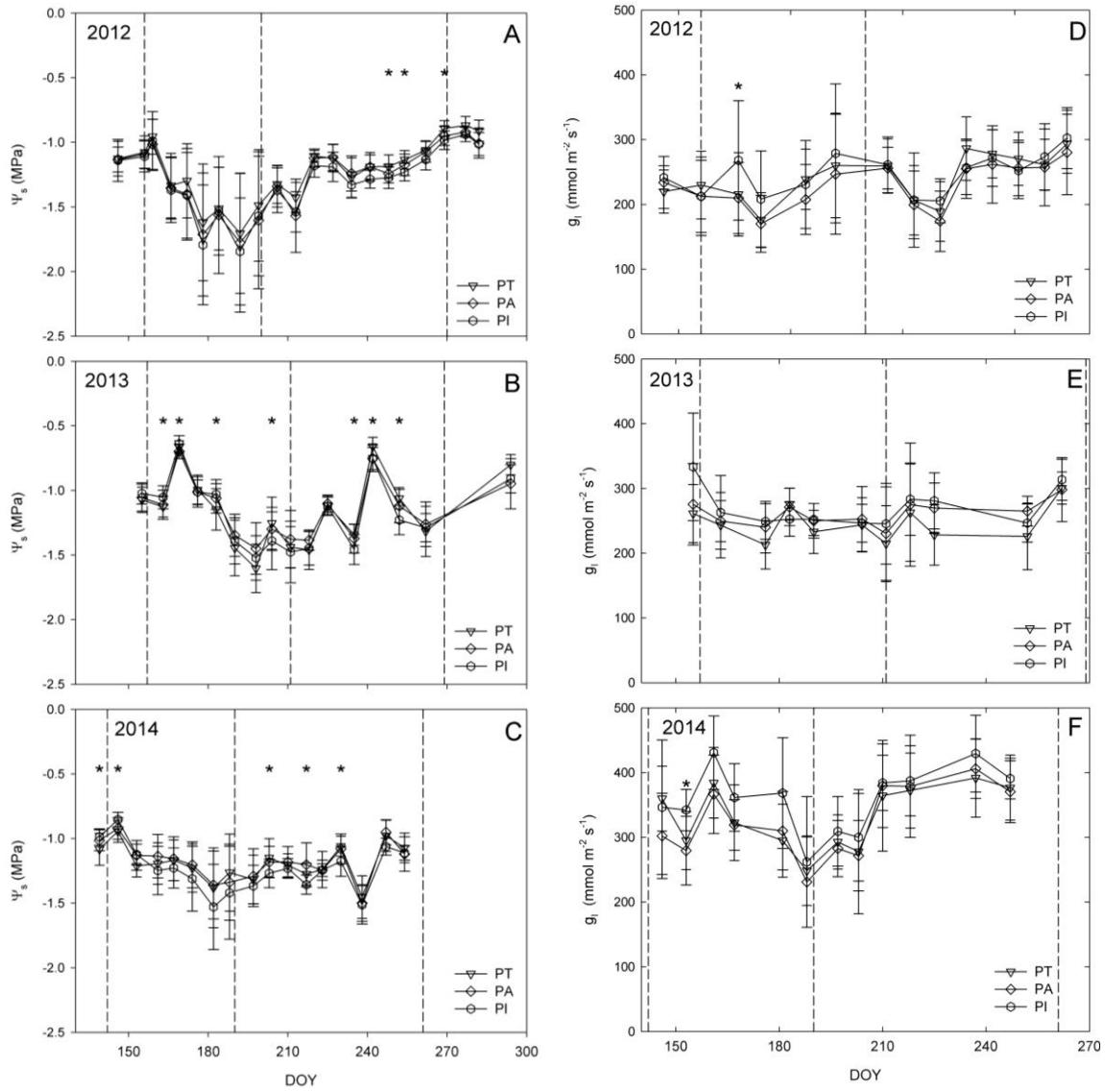


Figure 4.11. Stem water potential (Ψ_s ; left) and Abaxial leaf conductance (g_l ; right) values (mean \pm SE, not shown when smaller than symbols, n=12) for pistachios on *Pistacia terebinthus* (open triangles down), *Pistacia atlantica* (open diamonds) and *Pistacia integerrima* (open hexagons) rootstocks during 2012 (A and D), 2013 (B and E) and 2014 (C and F). Vertical dashed lines indicates the stages I, II, III and post-harvesting. Asterisks indicate significant differences between treatments.

4.10.2. Diurnal course

During stage II, PT, PA and PI reached respectively a maximum of -0.88 MPa, -0.97 MPa and -0.85 MPa, and a minimum of -1.68 MPa, -1.72 MPa and -1.69 MPa during the first diurnal cycle. A significant difference was found between rootstocks only at 06:30 h although PA maintained lower values than the other rootstocks during the whole day (Figure 4.12A-left). At the beginning of the rehydration process, significant differences were found between rootstocks at predawn (04:00 h), early morning (06:30 h) and during the afternoon, approximately at 13:30 h and 16:00 h (Figure 4.12B-left). Trees budded on PT manifested a better general water status than PA and PI during almost all the day and PA a better water status than PI specially at the second half of the day which corresponds to the time of VPD maximum values. The values ranged from -0.80 to -1.46 MPa for PT, -0.92 to -1.51 MPa for PA and -0.87 to -1.62 MPa for PI between predawn and mid-afternoon. Two weeks after rewetting, significant differences between PI and the other rootstocks were found from late morning until the last measurement. Statistical differences between all rootstocks were perceived at 10:40 h and 17:50 h where PT reached a value of -1.20 MPa, PA a value of -1.23 MPa and PI a value of -1.32 MPa at this time (Figure 4.12C). In this interval of time, VPD had a maximum of 4.42 kPa.

During the water stress period, significant difference in g_l was found between rootstocks at mid-afternoon, PI being the rootstock which showed the highest level of leaf conductance ($247 \text{ mmol m}^{-2} \text{ s}^{-1}$) at about 16:00 h, and PA the lowest one ($205 \text{ mmol m}^{-2} \text{ s}^{-1}$) (Figure 4.11-right). The latter showed a more pronounced g_l decrease trend than PI and PT practically during the whole day (Figure 4.12D). On 24/07/2012, significant differences were found between rootstocks at only 06:30 h, being PI the highest one. On 07/08/2012, at

the first measurement of g_l (06:30 h), PT, PA and PI had respective values of about 87 mmol m⁻² s⁻¹, 107 mmol m⁻² s⁻¹ and 124 mmol m⁻² s⁻¹. Significant differences were found between the three rootstocks only at this time.

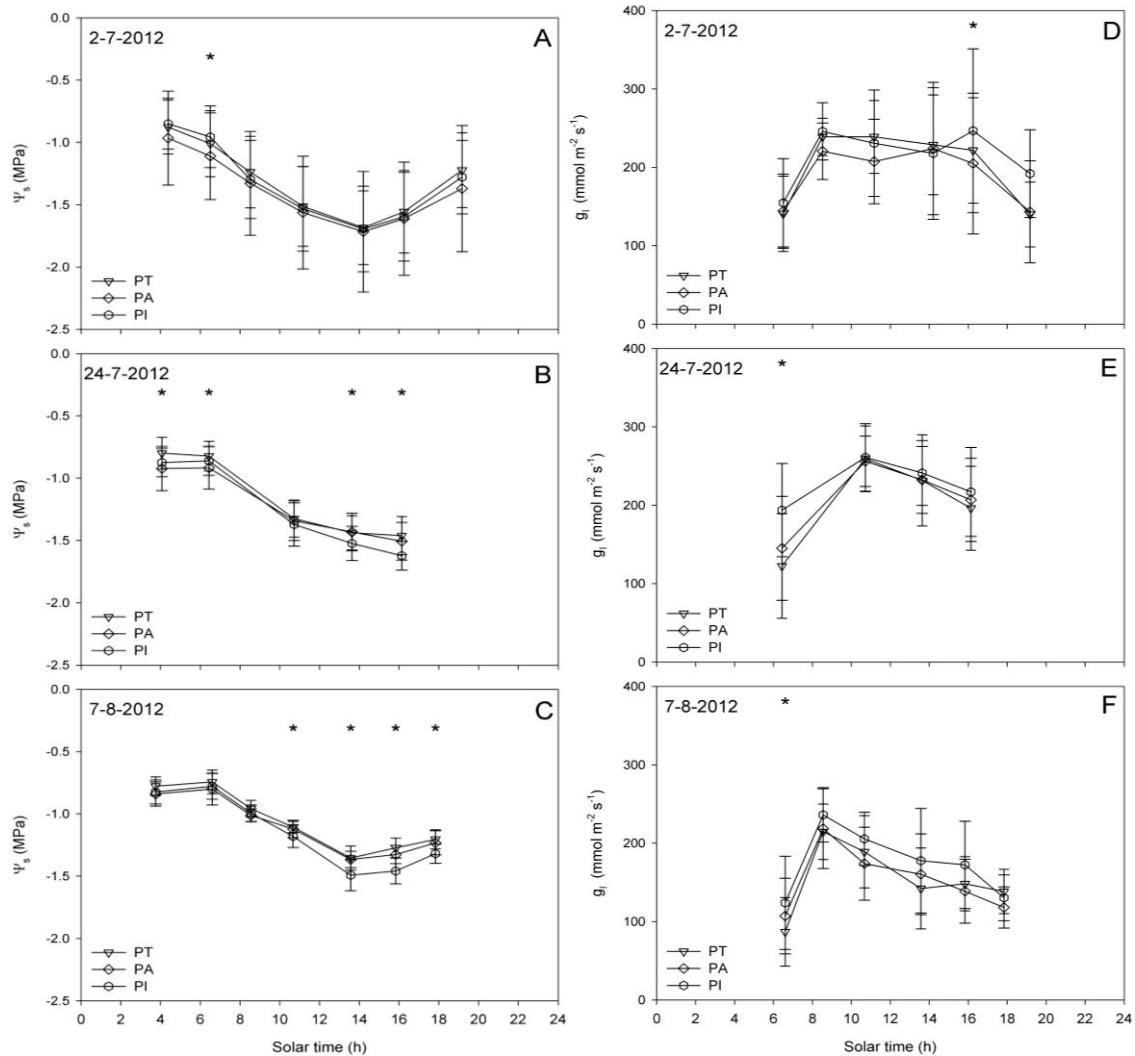


Figure 4.12. Diurnal pattern of stem water potential (Ψ_s ; left) and abaxial leaf conductance (g_l ; right) of pistachio leaves related to different rootstocks (PT, *Pistacia terebinthus*; PA, *Pistacia atlantica* and PI, *Pistacia integerrima*) on three dates. Each point is the mean of 12 measurements. Vertical bars indicate the standard error of the mean. Stars denote significant differences between rootstocks ($p < 0.05$; Tukey test).

4.11. DISCUSSION

4.11.1. Influence of rootstock on pistachio water relations

Over the three years, the effect of the rootstock on pistachio water relations was not significant and noticeably conclusive in any of the years. But water relations in *P. integerrima* showed clear changes in g_l and Ψ_s during the season. Trees with this rootstock tended to show greater g_l and lower Ψ_s at the end of stress period (Figure 4.11).

This result was more apparent when Ψ_s and g_l diurnal patterns of *P. integerrima* were characterized during 2012. Ψ_s of trees budded on PI was significantly lower than that of the two other rootstocks from the second diurnal cycle. Despite this, PI presented higher g_l than the other two rootstocks. This result suggests that PI has less stomatal control than PT and PA and no discernible threshold for water potential maintenance, which could give it a little anhydric tendency. Duhme & Hinckley (1992) reported that the values of leaf osmotic potential at full turgor for PI were only 0.5 to 1.0 MPa higher than osmotic potentials at the turgor loss point for PT. This confirms that PI is less adapted than the other rootstocks to conditions of water scarcity. PI has been described as the most trunk and canopy size vigorous rootstock amongst the three (Ferguson *et al.*, 2005), which could provide a higher capacity for energy interception and water transport, and can result in a major loss of water.

Unlike PI, PA showed the lowest g_l values during the day in stage II (Figure 4.12-right). Spiegel-Roy *et al.* (1977) and Lin *et al.* (1984) described PA as an adapted species for very severe conditions and dry areas where higher stomatal control is exerted by PA

compared to PI and PT. PT compared to other Mediterranean plants was considered by Sakcali & Ozturk (2004) as a water spender and described it as a partially water-tolerant species which cannot sustain long in harsh conditions. These results differ from those reported by Germana (1997) and Gijón *et al.* (2010) for potted pistachio plants, where PA is considered as a rootstock with little stomatal control compared to PT. In this study, PA maintained lower g_l than PT and PI during stage II, but during stage III, PA displayed a greater g_l than PT in early morning and similar activity during the rest of the day. This variance between results could be explained by the wide genetic diversity that affects the spectrum of potential responses, and by the different responses given by the rootstock depending on whether it is combined or not to a scion.

4.12. CONCLUSIONS

The effect of rootstocks on pistachio water relations was not conclusive. Nonetheless, *P. Integerrima* seems to be less adapted to water stress and local area conditions having less stomatal control than *P. atlantica* and *P. terebinthus* and no discernible threshold for water potential maintenance.

According to the general trend of g_l , the influence of rootstocks on the degree of leaf conductance control when they are combined with cv. Kerman could be classified in this order, from the greatest to the lowest: PA > PT > PI.

CHAPTER 5: EFFECT OF RDI ON GROWTH, YIELD AND INFLUENCE OF THE ROOTSTOCK ON THESE TRAITS

5.1. ABSTRACT

The vegetative and productive response of “Kerman” pistachio trees budded on three different rootstocks (*Pistacia terebinthus*, *Pistacia atlantica* and *Pistacia integerrima*) to regulated deficit irrigation (RDI) in shallow soils was studied for 3 years. The trees were either fully irrigated (C treatment) or subjected to deficit irrigation during Stage II of fruit growth with two water stress thresholds (T1: $\psi_s = -1.5$ MPa and T2: $\psi_s = -2.0$ MPa). Deficit irrigation during Stage II significantly reduced the vegetative growth of trees, concretely neo-formed shoots settling the possibility to apply RDI during ON and OFF years. Water stress also reduced the number of buds per shoot but not their retention percentage. Stage II seems to involve shell hardening at a first stage and shell hardening and kernel growth as a second phase. Yield and fruit quality were not affected by any irrigation regime, except during the first year of the study but uncontrolled water deficit during stage II could be detrimental for yield since dry matter accumulation appears to follow a linear trajectory.

Pistacia integerrima showed a weaker capacity of adaptation to the study conditions compared to *Pistacia atlantica* and *Pistacia terebinthus*, having a tendency to get more stressed and to produce a lower crop quality.

5.2. INTRODUCTION

The increase in pistachio consumption due to its high incomes, nutritious value and favourable taste has led to an intensification and expansion of its cultivation. Thus, planting pistachio trees outside its autochthonous area of cultivation has become more common and now it can be found in places such as the USA since 1960 and, more recently, Spain.

Pistachio is a drought tolerant fruit tree species (Behboudian et al., 1986; Rieger, 1995). Though, it is well known that yield is highly correlated with the level of applied irrigation water and as with other trees, irrigation increases yield, but particularly in pistachio it is also reported to improve the nut quality and dampens the normal alternate bearing pattern (Kanber et al., 1993; Goldhamer, 1995). Bilgen (1982) indicated that irrigation should be considered among the most important cultural practices to sustain high yields in pistachio orchards.

However, drought is the most limiting abiotic factor in the Mediterranean area and the majority of orchards are grown under rain-fed conditions or with limited water inputs. This situation led to the need of improving the efficiency of water use. Deficit irrigation is proposed as a tool for this purpose and it consists on imposing a continuous water deficit during the season (Sustained Deficit Irrigation) (Goldhamer et al., 1987) or during specific phenological stages (Regulated Deficit Irrigation). Regulated deficit irrigation (RDI) has been a common research line for most fruit trees (Behboudian and Mills, 1997). The concept of RDI was first suggested by Chalmers et al. (1981) and Mitchell and Chalmers (1982) to control vegetative growth in peach orchards, and they found that savings in irrigation water could be realized without reducing the yield. In order to determine the response of pistachio trees to sub-optimal water application at several periods of growth,

Goldhamer (1995) divided the pistachio fruit growth cycle into four phenological stages (stage I, II, III and postharvest). Stage I covers the whole period of shell expansion, stage II the period of shell hardening, and stage III the embryo growth. He observed that stage II and postharvest are the most tolerant stages to water stress. In contrast, stage III was reported to be the most sensitive stage to water deficit that can have a dramatically negative impact on all pistachio yield components. Goldhamer and Beede (2004) observed that the water stress during stage I increases shell splitting; however it could affect the final fruit size and increase undesirable premature shell splitting. Gijón *et al.* (2008) imposed a water stress during stage I and II (50% of ET_c) and observed no differences on yield, but no positive effects were reported either with regards to an increase of nuts splitting . The early nut splitting was more closely associated to a roof temperature than to the water status during stage I.

Increasing crop tolerance to water stress would be the most economical approach to improve productivity (Wang *et al.*, 2012) and grafting is a widespread technique used in fruticulture to achieve this objective. Rootstock affects numerous vegetative and reproductive parameters of the scion, such as canopy growth (Ezzahouani and Williams, 1995) and yield (Ezzahouani and Williams, 2005) which should exert significant physiological effects on scions. To facilitate adaptation to conditions of limited rainfall and low irrigation water, the study of vegetative and reproductive response of rootstocks could provide an alternative means to control pistachio response to irrigation with maximum water use efficiency. *Pistacia integerrima L.* (PI), *Pistacia atlantica Desf.* (PA), *Pistacia terebinthus L.* (PT) and UCB-I (a hybrid from *Pistacia integerrima L.* × *Pistacia atlantica Desf.*) are the most widely used rootstocks today for pistachio cultivation. *P. integerrima* is

recognized as the most vigorous rootstock, *P. atlantica* as having medium vigour, and *P. terebinthus* as the weakest one (Ferguson *et al.* 2005). UCB was considered as the most preferable commercial rootstock under irrigated conditions and PT under rainfed but there is limited knowledge underlying rootstocks effects.

5.3. MATERIALS AND METHODS

5.3.1. Vegetative growth

Trunk perimeter was tracked at 20 cm above graft. In order to carry out the measurement at the same place, a strip of the trunk was painted, leaving a narrow line without paint where the measurement was made.

The canopy volume was determined monthly with one vertical and two horizontal diameters of the crown, using a measuring pole with marks every 20 cm.

Each year, at the end of August, the shoot growth, the number of fruit buds and the fruit buds position, as well as the places where, at the moment of measurement, was or had been a bud, were measured. For this purpose, ten shoots around each control tree were used.

5.3.2. Fruit growth

In the years of high load, 2012 and 2014, fruit samples were taken weekly. Fruit samples in 2013 (low load) were not taken because sampling could disturb the natural pattern of fruit growth.

Fresh weight and transverse diameters were measured on 35 fruits per control tree. In ten of those fruits, kernel, shell and hull were separated to determine fresh and dry kernel

weight. Only in 2014, other ten fruits were used to determine the shell hardness using a device specifically developed for this purpose, described in detail in Rapoport *et al.*, (2013). The rest of fruits (15) were used to determine the dry weight. To determine dry weights, kernels and fruits were dried in a forced-air oven for 48 hrs at 80 °C.

5.3.3. Yield

When fruits reached maturity, a sample of a hundred of them was collected from each control tree. The fresh weight was calculated. Then, fruits were peeled and divided into split; fruits with seed and opened shell non-split; fruits with seed and closed shell and blank fruits; fruits without seed. The fresh weight of each group was determined. The transverse diameter was measured in split nuts. The shell was separated from the kernel and both were weighed for split and non-split fruits. All fractions were dried in a forced-air oven for 48 hrs at 80 °C. Finally they were weighed again to determine the dry weight.

The yield of each control tree was weighed individually.

5.4. RESULTS

5.4.1. Vegetative growth

Canopy volume and trunk perimeter growth have been related to the initial measurement of each year to offset the initial size difference among trees, so in its denominations were used relative.

The relative canopy volume growth (RCVG) ranged from 0.15 to 1.9 m³ m⁻³, with no differences between irrigation treatments or rootstocks (Table 5.1). The RCVG was decreasing from one year to the next while the canopy volume was increasing from one

year to the next one; therefore the yearly rate of canopy increase was inversely proportional to the canopy volume. When the soil cover was at its maximum ($K_r=0.9$), RCVG was at its minimum, with values around 0.24 in the C trees. Although there were no significant differences in the RCVG values, T2 tended to show lower values than C trees in all seasons. The effect of rootstock was not significant.

The relative trunk perimeter growth ranged from 0.008 to 0.095 cm cm^{-1} (Table 5.1). The years with high crop load, “ON years” (2012 and 2014) had lower values (0.04 and 0.05 cm cm^{-1} in the C treatment) than the year with low crop load “OFF year” (0.10 cm cm^{-1} in the C treatment). There were statistical differences between irrigation treatments in the years of high fruit load, being the C growth higher than the T1 in both years and higher than T2 in 2012. There were statistical differences between C and the other two treatments in mean data. The effect of rootstock was not significant.

Shoot growth ranged from 11 to 25 cm (Table 5.1), being higher in the C treatment than T1 in all years and higher than T2 in 2012 and 2014 (years “ON”). The number of buds per shoot ranged from 7 to 11 with the same statistical differences as the shoot growth. In fact, the number of buds per shoots was related to the shoot growth ($N = 4.11 + 0.25 \text{ Shoot-growth}; R^2=0.8049; p<0.0001; MSE=1.7494; N= 1082$: Data not shown). In 2013, there were fewer buds per shoot when the rootstock was *P. atlantica* than when the other two were used.

The percentage of dropped buds ranged from 30 to 86 % (Table 5.1). In years ON, the percentage of dropped buds was significantly higher (63 and 77 % in the C treatment) than in year OFF (30 % in the C treatment). The effect of the irrigation treatment was

significant only in 2013, when the T1 treatment dropped more buds than C. During the 2012 and 2014 seasons, though no significant differences were found, T1 and T2 tended to produce lower values than the C treatment. The effect of rootstock was not clear. Only in 2013, *P. atlantica* significantly dropped more buds than the other two rootstocks. However, the trends for the rest of the seasons were not always consistent with this.

Table 5.1. Growth parameters; relative canopy volumen ($\text{m}^3 \text{ m}^{-3}$), relative trunk perimeter growth (cm cm^{-1}), shoots growth (cm), nº of buds per shoot and percentage of dropped buds per irrigation treatment and rootstock (PT; *Pistacia terebinthus*. PA; *Pistacia atlantica*. PI; *Pistacia integerrima*). Different letters in the same column means significative difference between irrigation treatments or rootstocks in each year.

Year	Irrigation treatment /Rootstock	Relative Canopy volume growth ($\text{m}^3 \text{ m}^{-3}$)	Relative Trunk perimeter growth (cm cm^{-1})	Shoots Growth (cm)	Nº buds per shoot	Percentage of dropped buds
2012	C	1.6843	0.0440a	18.41a	8.89a	62.80
	T1	1.9048	0.0124b	11.02b	6.79b	67.51
	T2	1.4683	0.0084b	10.51b	6.73b	70.06
	PT	1.8384	0.0151	12.98	7.74	70.65
	PA	1.7059	0.0237	13.31	7.27	64.53
	PI	1.5130	0.0260	13.65	7.41	65.19
2013	C	0.4835	0.0946	24.68a	10.67a	30.08b
	T1	0.3953	0.0826	16.07b	8.01b	45.78a
	T2	0.3703	0.0818	18.46ab	8.75ab	41.95ab
	PT	0.4221	0.0896	19.88	9.51a	31.69b
	PA	0.3482	0.0893	18.25	8.57b	50.20a
	PI	0.4788	0.0801	21.09	9.36a	35.92b
2014	C	0.2392	0.0487a	23.07a	9.92a	76.89
	T1	0.2643	0.0326b	18.30b	8.56b	85.56
	T2	0.1543	0.0454ab	16.41b	8.72b	85.64
	PT	0.1502	0.0389	19.78	9.18	86.03
	PA	0.2967	0.0426	18.32	9.14	80.87
	PI	0.2110	0.0452	19.67	8.88	81.20
MEAN	C	0.8210	0.0621a	18.70a	8.10a	56.02
	T1	0.8548	0.0412b	13.89b	6.27b	66.10
	T2	0.6643	0.0447b	13.87b	6.47b	65.88
	PT	0.8223	0.0472	15.59	6.91	62.22
	PA	0.7836	0.0494	14.80	6.92	65.00
	PI	0.7343	0.0504	16.70	7.01	60.77

5.4.2. Fruit growth

The transverse diameter had a rapid growth in the last weeks of May and the first of June, reaching a maximum of around 14 mm, on Day of Year (DOY) 156 and 142 in 2012 and 2014 respectively. There were no differences between irrigation treatments or rootstocks.

The kernel fresh weight showed a sigmoid pattern (Figure 5.1A), without differences between irrigation treatments or rootstocks.

The shell hardness showed a sigmoid pattern, starting to increase when fruit reached its maximum volume and ending about 20 days after the beginning of the kernel growth (Figure 5.1A). There were no differences between irrigation treatments or rootstocks.

The fresh fruit weight showed a double sigmoidal pattern (Figure 5.1B). In 2012, there were statistical differences between treatments in the last week of May and in three weeks of June, with the C treatment being heavier than T1, and in two cases, heavier than T2. In 2014, there were statistical differences three times (Figure 5.1B), with differences between irrigation treatments without a regular pattern.

The dry fruit weight showed a linear pattern in 2012 (data not shown) and 2014 (Figure 5.1B). In 2012, there were no statistical differences. In 2014, T2 had a heavier dry weight than T1 until DOY 175 and C until DOY 204.

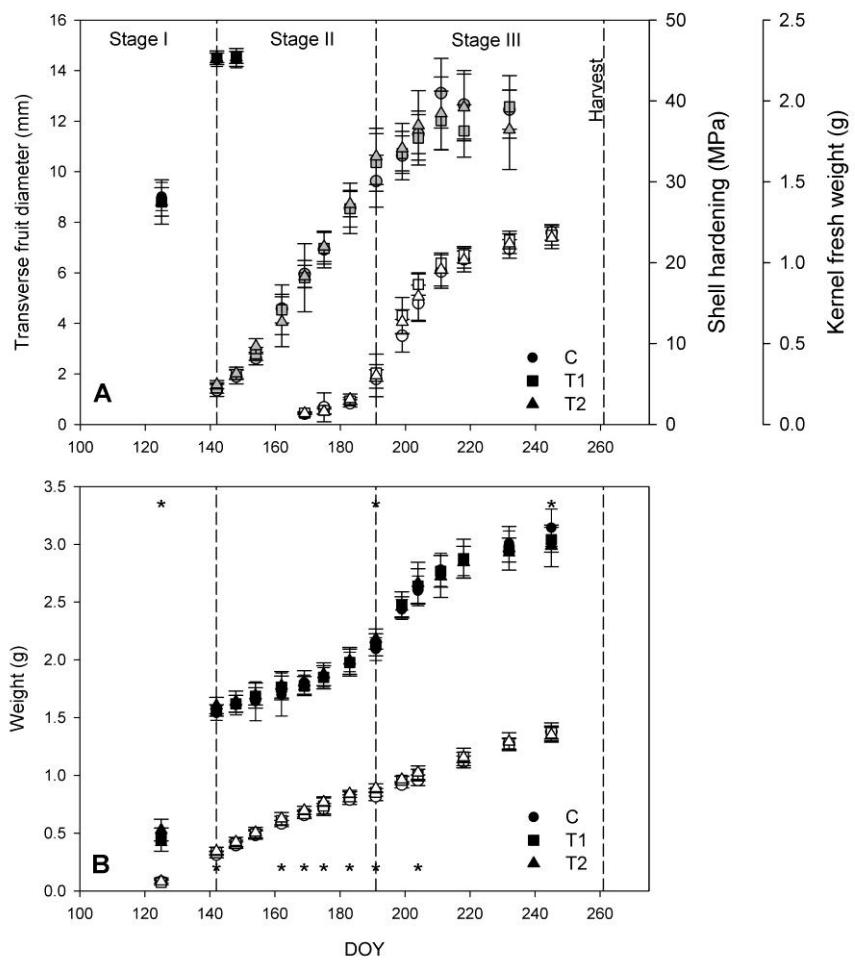


Figure 5.1. Transverse fruit diameter (closed symbols in A, mm), shell hardening (grey symbols in A, MPa), kernel fresh weight (open symbols in A, g), fresh fruit weight (closed symbols in B, g) and dry fruit weight (open symbols in B, g) values (mean \pm SE, not shown when smaller than symbols, n=12) in pistachio fruits during year 2014 by irrigation treatment (C, circle; T1, square; T2, triangle up). Vertical dashed lines indicates the stages I, II, III and post-harvesting. Asterisks indicate significant differences between treatments.

In 2012 there were no statistical differences between rootstocks. In 2014, fruits from trees on *P. atlantica* were heavier than on *P. integerrima* on several dates (Figure 5.2B) and also heavier than on *P. terebinthus*. These differences were both in fresh weigh and dry weight, but the differences in dry weight were noticeable in every sample, from the beginning of fruit growth to DOY 183, while in fresh weight, they were observed in the samples during the whole season without any difference.

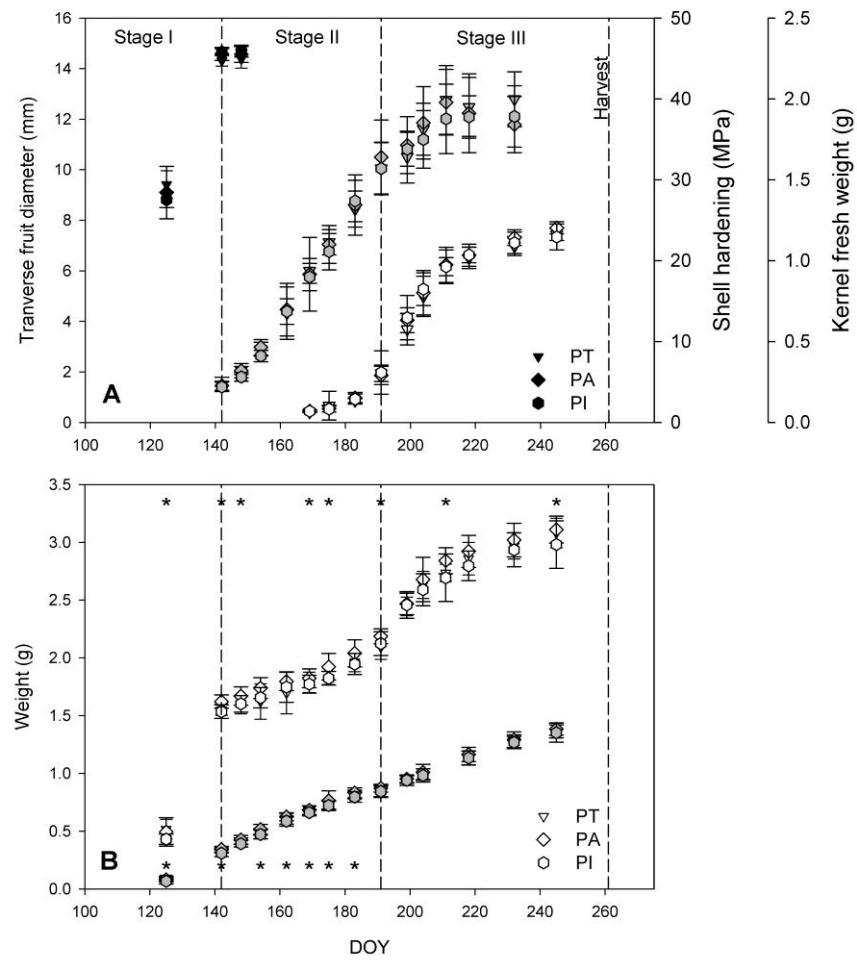


Figure 5.2. Transverse fruit diameter (closed symbols in A, mm), shell hardening (grey

symbols in A, MPa), kernel fresh weight (open symbols in A, g), fresh fruit weight (closed symbols in B, g) and dry fruit weight (open symbols in B, g) values (mean \pm SE, not shown when smaller than symbols, n=12) in pistachio fruits during year 2014 by rootstock (*Pistacia terebinthus*, PT, triangles down; *Pistacia atlantica*, PA, diamonds; *Pistacia integerrima*, PI, hexagons). Vertical dashed lines indicates the stages I, II, III and post-harvesting. Asterisks indicate significant differences between treatments.

5.4.3. Yield

The yield in the C treatment was 49, 11 and 57 kg tree⁻¹ in 2012, 2013 and 2014 respectively. In T1, it was 36, 12 and 48 kg tree⁻¹ and in T2, 36, 13 and 49 kg tree⁻¹ in 2012, 2013 and 2014 respectively (Table 5.2).

Therefore, the yield in the C treatment was higher than that for the other treatments in the ON years, although this difference was significant only in 2012; while in 2013, the yield in C was lower without statistical significance. These facts made alternate bearing higher in the C treatment than in the other treatments.

The mean yield for the three years was 39, 33 and 33 kg tree⁻¹ in the C, T1 and T2 treatments respectively, without statistical differences (Table 5.2).

When the yield was adjusted based on the canopy to offset the tree size effect, there were differences between irrigation treatments only in 2014, then the T1 treatment had higher value (2.15 kg m⁻³ canopy tree⁻¹) than the C treatment (1.66 kg m⁻³ canopy tree⁻¹)

while the T2 treatment was 1.91 kg m^{-3} canopy tree $^{-1}$, with no statistical difference with the other treatments (Table 5.2).

The nut weight varied in relation to yield, with lower values (less than 3 g nut^{-1}) the year with highest yield (2014) and higher values (more than 3.3 g nut^{-1}) the year with the lowest yield (2013). However the variation in nut weight was lower than the variation in yield. There were no statistical differences in nut weigh between the irrigation treatments (Table 5.2).

The crop load (no. of nuts per tree) varied according to the yield, with the same statistical differences (Table 5.2).

There were statistical differences in yield between trees on different rootstocks in 2013, and trees on *P. atlantica* had a higher yield than trees on *P. terebinthus*. This effect was also observed in the crop load for the same year.

In 2014, there were statistical differences in yield adjusted according to the canopy size, with trees on *P. integerrima* showing the lowest values. The same differences between rootstocks were recorded in the nut weight.

Table 5.2. Yield (Kg tree⁻¹), yield per canopy volume (kg m⁻³), nut weight (g nut⁻¹) and crop load (nº nuts tree⁻¹) per irrigation treatment and rootstock (PT; *Pistacia terebinthus*. PA; *Pistacia atlantica*. PI; *Pistacia integerrima*) in 2012, 2013 and 2014 and mean data. Different letters in the same column means significative differences between irrigation treatment or rootstocks in the each year.

Year	Irrigation treatment / Rootstock	Total yield (Kg tree ⁻¹)	Total yield (Kg m ⁻³ canopy)	Nut weight (g nut ⁻¹)	Crop load (Nº nuts tree ⁻¹)
2012	C	48.90a	4.28	3.20	15,569a
	T1	35.79b	4.91	3.11	11,897b
	T2	35.56b	3.69	3.01	11,386b
	Pt	38.14	4.95	3.21	12.143
	Pa	42.00	4.26	3.07	13.584
	Pi	40.11	3.67	3.05	13.125
2013	C	10.52	0.49	3.45	2.838
	T1	12.17	0.77	3.33	3.615
	T2	13.34	0.79	3.38	3.922
	Pt	8.83b	0.51	3.44	2278b
	Pa	16.98a	0.96	3.36	5077a
	Pi	10.23ab	0.51	3.36	3020b
2014	C	57.23	1.66b	2.98	19.161
	T1	48.42	2.15a	2.95	16.400
	T2	48.86	1.91ab	2.93	16.685
	Pt	49.93	1.98a	3.00a	16.599
	Pa	57.57	2.12a	3.01a	19.156
	Pi	47.02	1.61b	2.85b	16.491
MEAN	C	38.94	2.19	3.22	12.330
	T1	32.67	2.61	3.13	10.467
	T2	32.05	2.13	3.11	10.835
	Pt	32.36	2.52	3.22	10.148
	Pa	38.85	2.45	3.15	12.605
	Pi	32.45	1.95	3.08	10.879

5.4.4. Yield quality

The kernels of split fruits were heavier than the kernels of non-split fruits in several cases in 2012 and 2013 (Table 5.3). In those years, the dry weight of the kernels of split fruits was always heavier than that of non-split fruits. The fresh weight of the kernels of split fruits was also heavier in all cases than that of non-split fruits, but a statistical significance was only found in the T1 treatment in 2012 and the C and T2 treatments in 2013. There were no statistical differences in 2014.

Neither fresh nor dry weight of kernel of split or non-split fruits showed statistical differences for different irrigation treatments, except for the kernel of split fruits in 2014, whose kernel dry weight of the T1 treatment was heavier than that of the C treatment.

The percentage of split fruits in the fresh weight ranged from 46 to 80 %, and it was related to the yield (Table 5.3). The percentage of non-split fruits in the fresh weight ranged from 16 % to 41 % and it was also related to the yield. For both cases, there were no statistical differences between irrigation treatments.

The relationship between percentages in fresh and dry weight did not follow any pattern. The percentage in dry weight in 2012 kept a more or less equal trend to the fresh weight and only the percentage of blank fruits was lower than in fresh weight. In 2013, the percentage of split fruits in dry weight decreased, and that of non-split fruits increased when compared to the fresh weight. The percentage of blank fruits kept a more or less equal trend. This trend was the opposite in 2014, when the percentage of split fruits increased in dry weight when compared to fresh weight and the percentage of non-split fruits decreased similarly to the percentage of blank fruits.

The diameter of split nuts was related to the yield and it ranged from 12.78 mm to 14.44 mm. There was a statistical significance in 2014, when the diameter of the T1 treatment was larger than that of the C treatment (Table 5.3).

The production from trees on *P. integerrima* had a lower percentage of split fruits than that from trees on *P. terebinthus* in fresh weight in 2012, 2013 and 2014, also lower than trees on *P. atlantica* in 2012 and 2013. These differences were observed in the dry weight as well, except in 2013, when the pattern was the same but differences were not significant enough (Table 5.3).

Split fruits from trees on *P. atlantica* had a larger diameter than those from trees on *P. integerrima*.

5.5. DISCUSSION

5.5.1. Growth

5.5.1.1. Vegetative growth

The water stress during stage II at the threshold of -1.5 MPa of Ψ_s reduced the neo-formed shoots growth. This response has been reported generally when water stress is applied during both stage I and II in pistachio tree but not during stage II only (Goldhamer, 2012). The neo-formed shoot growth was always higher in the C treatment than in stress treatments (Mean 3 years: 5 cm of difference between C and stress treatments). Additionally, the stress reduced the number of buds per shoot in T1 and T2 (Mean of 2 buds per shoot less than in C) with a similar bud retention percentage to the control treatment (Table 5.1). However, these differences were not translated into a greater crop load or yield in C, except the first year of the experiment and this could be probably related to the previous year's conditions.

These results indicate that the difference between the control and the stress treatments in the neo-formed shoot growth is mostly unproductive, confirming the previous works of Spann *et al.* (unpublished) mentioned by Goldhamer (2012) stating that fruit positions are found mostly on the short shoots (pre-formed shoots) and that these later reach a full elongation before the onset of stage II. The stress applied in the above mentioned work was around -1.6 MPa midday shaded leaf water potential (Goldhamer, 2012), which could be the most similar to that of the T1 treatment of the present work and showed no reduction in pre-formed shoot growth. It seems that a more severe stress (-2.0 MPa in T2) did not affect neither the initiation of short shoots in the short term, since yield was little affected by this level of stress in this study.

The pistachio shoot growth was not particularly dependent on the crop load (ON or OFF year) and the growth reached during the stress treatment was sufficient to reach the productivity that makes the application of RDI viable during ON and OFF years. The growth lost by water stress could imply a saving during the pruning practice, since the main part of production is initiated in stage I. Tree water needs during this stage are generally satisfied from the soil water reserve. Spiegel-Roy *et al.* (1977) found that 54 to 163 mm of annual precipitation were sufficient for dryland trees to differentiate enough flower buds and obtain appreciable yields.

5.5.1.2. Determination of the water stress tolerant period

The pistachio fresh fruit weight follows a double sigmoidal pattern (Figure 5.1B). This pattern is widely assumed to be similar to the fleshy drupes pattern when represented as fresh weight. However, the tissues of the pistachio fruit develop in time and space like those of drupe, in general, while others develop quite differently (Crane, 1986). The growth in diameter of pericarp fleshy drupaceous fruits, such as apricot, cherry and peach, occurs in three distinct periods with two rapid growth cycles separated by one of slow growth. In pistachios, the pericarp growth is fully completed in one period, since transverse diameter has a rapid growth in the last weeks of May and the first of June, reaching a maximum of around 14 mm (Figure 5.1). This period is defined as stage I of fruit growth for pistachio trees. Contrary to stone fruits, the growth of the pericarp and the growth of the seed in pistachio trees are separated in time. The seed growth in pistachio trees follows a simple sigmoidal pattern, starts after a fresh weight lag behaviour and defines the stage III.

The pit hardening has been used for irrigation scheduling in peach trees (Chalmers *et al.*, 1981; Domingo *et al.*, 2011), cherry trees (Papenfuss and Black, 2010), nectarine

trees (Thakur and Singh, 2012) and olive trees (Pérez-López *et al.*, 2008; Gucci *et al.*, 2009; Hidalgo *et al.*, 2011) to mark the period of tolerance to water stress.

There are no previous data about shell hardening in pistachio fruits, so by analogy with nut trees, the stage II and shell hardening are named interchangeably (Doster *et al.*, 2001; Sajjadinia *et al.*, 2010). Shell hardening is characterized by a simple sigmoid curve in which, the first point of inflection collapses with the maximum diameter of fruit growth. Approximately in the middle of shell hardening period, the kernel starts its accelerated growth. When the shell hardening reaches its maximum (around 40 MPa), the pistachio fruit accumulates approximately 80 % of its final dry weight (Figure 5.1A). Therefore, the period of shell hardening, associated traditionally to stage II in pistachios (Goldhamer, 1995) is, in fact, made up of two phases, one with only shell hardening and the other with both processes; shell hardening and kernel growth. Even the role that the shell hardening can play in pistachio water stress tolerance is still unknown. The fact that it could be related to water stress tolerance opens the way to the possibility of a longer water stress period during the growth cycle.

Chalmers *et al.* (1981) showed that the water stress during stage II in peaches has no effect on the final yield, as there is a stagnation in the dry matter accumulation. Goldhamer and Beede (2004) found that Stage II was also a stress tolerant period in pistachio trees, as it had been found for other double sigmoid development fruit crops, such as peach, plum, and nectarine. Contrary to Crane and Al-Shalan (1974), they found that dry matter accumulation decrease during stage II as in stone fruits. In Figure 5.1B, the dry matter accumulation seems to follow a linear trajectory, no lag phase was observed in dry weight accumulation and no difference between the growth slopes was found between stages (Data

not shown). The continuous dry matter increase pattern suggests that, although there was a good tolerance reported to stage II, an uncontrolled tree water deficit between stage I and III could affect yield and should be avoided.

5.5.2. Production

The mean yield for three years was not reduced in T1 and T2 compared to the C treatment. This was achieved with water savings of 40 % in T1 and 45 % in T2, and the water saving of the C treatment must be added. Only in 2012, the yield in the C treatment was higher than in the RDI treatments, caused by a greater number of fruits rather than greater fruits (Table 5.2). The number of fruits was related to the canopy size, since there was no difference in canopy productivity in this year. In fact, the canopy productivity was higher in the T1 treatment in 2014. According to Monastra *et al.*, 1995, the number of fruit per inflorescence could be negatively influenced by high volume irrigation. Therefore, for the same canopy size, the T1 treatment could even reach a higher yield than the control. This treatment had also higher intensities of characteristic sensory attributes and a greater level of satisfaction among international consumers (Carbonell-Barrachina *et al.*, 2014).

Management practices, especially the irrigation management, are reported to enhance shell splitting (Goldhamer *et al.* 1987; Kanber *et al.* 1993). Closed shell nuts can be as low as 5 % of the harvested nut load and as high as 60 % (Goldhamer, 2012). In this study, the percentage of split fruits in fresh weight ranged from 46 to 80 % and non-split fruits went from 16 to 41 %. Both percentages were related to yield and were not statistically different between irrigation treatments (Table 5.3).

The amount of water applied during stage II for T1 and T2 (Ψ_s threshold of -1.5 and -2.0 MPa) was equivalent to 40, 10 and 4 % of ET_c in 2012, 2013 and 2014 for T1 and 18, 1.6 and 4 % of ET_c for T2. Goldhamer and Beede (2004), imposed a stress of 0, 25 and 50 % of ET_c during the stage II of fruit growth and no effect of any water regime on kernel weight and split nut percentage was observed either. However, a trend toward lighter nuts when stage II irrigation was totally eliminated was mentioned. In the T2 treatment, the water inputs were practically eliminated during stage II; however no reduction of kernel weight was observed. The little amount of water supplied during stage II could have been sufficient to prevent this effect. Ψ_s thresholds reached by T1 and T2 during stage II seem to be tolerated by pistachio trees during this stage, since it does not affect this yield component.

The kernel weight of split fruits was heavier than the kernel weight of non-split fruits in many occasions (Table 5.3). These results support the hypothesis presented by Nevo *et al.* (1974) stating that there were no structural differences between the two types of nuts, suggesting the possibility that physical forces, exerted as the drying shell shrinks and encounters resistance from the kernel, may be responsible for inducing endocarp dehiscence. However, if physical forces were the only cause of nut dehiscence, this trend should be generalized in the study amongst all treatments and all years, since the splitting percentage was not enhanced by any water regime. Crane and Iwakiri (1982) and Crane (1986) presented a different approach considering the shell dehiscence as a result of biochemical factors produced by the growing embryo that activated a dehiscence response in the endocarp. On the other hand, Polito and Pinney (1999) based the dehiscence process on two distinct events: the first is driven by kernel growth and enlargement and is

responsible for the longitudinal dehiscence; the second is responsible for apical dehiscence and may result from the developmentally mediated cell-separation processes. Although the dehiscence process mechanisms are still little known, the physical forces seem to play a role in the dehiscence process, since heavier kernels generate a major percentage of split fruits (Table 5.3).

5.5.3. Rootstocks

Ferguson *et al.*, (2005) reported that in trees on *P. integerrima* the yield was a 20 % higher than on *P. atlantica* in fully irrigated conditions. The lower weight and diameter of split kernel on *P. integerrima* can be caused by the lower Ψ_s , since growth is the most sensitive parameter to water stress (Hsiao, 1973). The minor percentage of split fruits could be due to the lower weight of split kernel, since a physical effect may be partially responsible for inducing endocarp dehiscence.

P. atlantica fruits had a higher growth than others rootstocks, then when fruits reached its genetically regulated size, the size amongst rootstocks becomes more even. Although in the mean yield there were no significant differences, *P. atlantica* showed the highest yield with significant differences in 2013 and 2014 per unit volume. Due to the relationship between yield and percentage of dropped buds, the high yield in 2013 produced a high percentage of dropped buds.

The differences with Ferguson *et al.*, (2005) that recorded a higher yield in *P. integerrima* than *P. atlantica*, can be caused by the worst physiological status of *P. integerrima* during most of the season.

Table 5.3. Quality parameters of production; weight of split kernel (g kernel^{-1}), weight of non-split kernel (g kernel^{-1}), percentage of split, non-split and blank fruits in fresh and dry matter per irrigation treatment and rootstocks (PT; *Pistacia terebinthus*. PA; *Pistacia atlantica*. PI; *Pistacia integerrima*) in 2012, 2013 and 2014 and mean data. Different capital letters in the same row means significative differences between split and non-split weight kernels. Different lowercase letters in the same column means significative differences between irrigation treatments or rootstock in each year.

Year	Irrigation treatment /Rootstock	Fresh weight						Dry weight						Diameter of split nuts (mm)
		Split Kernel (g kernel^{-1})	Non split Kernel (g kernel^{-1})	Percentage			Split Kernel (g kernel^{-1})	Non split Kernel (g kernel^{-1})	Percentage					
				Splits	Non-splits	Blanks			Splits	Non-splits	Blanks			
2012	C	1.22	1.15	53,47	28.84	19.63	0.6748A	0.5775B	54.38	28.90	16.72	13.10		
	T1	1.19A	1.13B	58,27	23.07	21.99	0.6638A	0.6071B	57.32	24.61	18.07	12.93		
	T2	1.15	1.12	48,23	29.92	23.55	0.6300A	0.5790B	48.87	31.03	20.10	12.78		
	PT	1.22A	1.15B	60,26a	23,19b	18.94	0.6593A	0.5934B	59.31a	24.54b	16.15	12.98		
	PA	1.17	1.09	57,02a	24,31ab	20.65	0.6421A	0.5648B	57.06a	25.51ab	17.44	12.76		
	PI	1.17A	1.15B	42,70b	34,34a	25.58	0.6671A	0.6054B	44.20b	34.50a	21.30	13.07		
2013	C	1.32A	1.17B	74.46	20.23	9.00	0.7058A	0.5899B	57.32	34.65	9.24	14.44		
	T1	1.29	1.20	80.02	16.05	7.68	0.7145A	0.5766B	65.56	27.74	7.70	14.37		
	T2	1.31A	1.20B	74.73	16.75	9.16	0.7148A	0.6083B	61.30	30.00	8.71	14.37		
	PT	1.35A	1.17B	80,99a	10,76b	9.61	0.7185A	0.5589B	64.57	27.00	9.63	14.54		
	PA	1.28	1.16	83,51a	12,74b	8.09	0.7193A	0.5822B	65.41	27.92	6.67	14.30		
	PI	1.30A	1.23B	64,71b	29,54a	8.45	0.6973A	0.6338B	53.19	37.47	9.35	14.34		
2014	C	1.16	1.18	46.22	40.51	15.16	0.6216b	0.6270	56.88	32.80	10.32	13.10b		
	T1	1.17	1.19	57.88	31.99	12.39	0.6450a	0.6427	64.49	26.81	8.70	13.32a		

	T2	1.17	1.18	50.96	33.97	17.09	0.6341ab	0.6291	58.92	28.78	12.30	13.26 ab
	PT	1.20 a	1.18	68.57 a	21.37 b	12.18	0.6394a	0.6224	72.61 a	18.69 b	8.69	13.26 ab
	PA	1.20 a	1.18	50.91 ab	36.50 ab	14.47	0.6454a	0.6304	59.83 ab	30.02 ab	10.16	13.32 a
	PI	1.10 bB	1.18 A	35.58 b	48.60 a	17.99	0.6160bB	0.6459 A	47.85 b	39.69 a	12.47	13.09 b
MEAN	C	1.24 A	1.13 B	58.41	29.39	14.67	0.6694A	0.5816 B	56.42	31.47	12.10	13.56
	T1	1.22	1.17	65.39	23.71	14.02	0.6744A	0.6088 B	62.12	26.38	11.49	13.54
	T2	1.21	1.17	57.97	26.88	16.60	0.6596A	0.6055 B	56.36	29.94	13.70	13.47
	PT	1.26 aA	1.13 B	70.30 a	17.97 b	13.55	0.6744A	0.5751 aB	65.73 a	22.76 b	11.51	13.61
	PA	1.22 abA	1.15 B	63.81 a	24.51 b	14.41	0.6689A	0.5924 abB	60.77 a	27.81 b	11.42	13.46
	PI	1.19 b	1.19	47.66 b	37.49 a	17.34	0.6601A	0.6284 aB	48.41 b	37.22 a	14.37	13.50

5.6. CONCLUSIONS

- The application of RDI combined to stem water potential implicated a water save of 40 % and 45 % in T1 and T2 respectively. The application of these water rates was not associated with a reduction of the mean yield or the production quality.
- Water stress at -1.5 MPa and -2.0 MPa of stem water potential reduced neo-formed growth, the number of buds per shoot but not the percentage of bud retention. However, these differences were not translated into a greater crop load or yield suggesting that the difference in neo-formed growth seems to be mostly unproductive. The maintenance of productivity was not affected by water stress or crop load probably due to preformed shoots initiation which makes the application of RDI viable during “ON” and “OFF” years.
- The characterization of shell hardening pattern showed that it follows a simple sigmoid curve. Breaking pressure of the shell reached a maximum of around 40 MPa. At this time, pistachio fruit accumulates approximately 80 % of its final dry weight. Therefore, the period of shell hardening, associated traditionally to stage II in pistachios is, in fact, made up by two phases, one with only shell hardening and the other with both processes; shell hardening and kernel growth.
- Physical forces seem to play a role in the dehiscence process, since heavier kernels generate a major percentage of split fruits.
- Between the three rootstocks, *P. atlantica* seems to be the most suitable rootstock under these conditions since although in the mean yield no significant differences were observed, it showed the highest yield with significant differences in 2013 and 2014 per unit volume. On the other hand, *P. integerrima* seems to be the least

suitable rootstock for this area where it produced lower percentage, diameter and weight of split nuts.

CHAPTER 6: FINAL CONCLUSIONS

- 1) RDI application was successfully assessed in shallow soils with drip irrigation systems by means of ψ_s measurements satisfying the fundamental requirements of the technique by reducing the vegetative growth without affecting yield or yield quality.
- 2) Midday stem water potential measurement has higher capacity in the detection of water stress and lower variability than predawn water potential and consequently could be considered as a better indicator of the water status of pistachio.
- 3) A ψ_s threshold of around -1.5 MPa could be recommended as stress level during stage II for pistachio trees due to the fact that it does not reduce the yield or causes any undesirable effects. A stem water potential threshold of -2.0 MPa seems to be tolerated by pistachio trees during stage II, as the yield was not affected. The influence of this stress level on g_l indicates that a long term stress at this level could be detrimental for pistachio production.
- 4) Efficient irrigation management requires an accurate quantification of the tree water needs. When ψ_s measurements were used for irrigation scheduling under drip irrigation, the Kc decreased resulting in about 18 % of water saving compared to the typically used Kc for pistachio trees.
- 5) Under fully irrigated conditions, pistachio stem water potential seems to be mainly governed by VPD; the found relationship between ψ_s and VPD could be useful for pistachio irrigation.
- 6) Water stress conditions modified significantly the fit of the relationship between stem water potential and VPD. However, the phenological stage has no effect on this relationship. The relationship between leaf conductance and VPD has shown a

positive linear correlation. Such behaviour was recorded only during stage II of fruit growth where pistachio follows the tendency of a water spender species when all water requirements are fulfilled. Once water stress took place, pistachio showed an ability to reduce its water consumption by closing the stomata early in the morning.

- 7) The recovery from water stress levels reaching -1.5 MPa and -2.0 MPa prolonged for 40 days could be considered a slow process since it takes around 4 weeks. Therefore, it should be taken into account for deficit irrigation scheduling.
- 8) There is a generalized association between stage II in pistachio and shell hardening, the shell hardening process actually takes place until the middle of stage III.
- 9) The sum of the different histological process involved in the fruit growth showed that pistachio dry matter accumulation follows actually a linear behaviour which supports the fact that an uncontrolled water stress during stage II of growth, coupled to the slow recovery of pistachio could have drastic effects on tree productivity.
- 10) Pistachio tree seems to be more sensitive to water stress during high bearing years, having a more pronounced tendency to lower ψ_s and to delay the recovery. Also the kernel weight and percentage of split fruits were inferior in ON than OFF years. These two latter parameters seem to be strongly correlated, which led to suggest that fruit dehiscence is partially caused by mechanical effects.
- 11) Regarding rootstocks, in contrast with previous works and to the general belief and although the fact that no significant differences were found in the response of *P. terebinthus* and *P. atlantica*, the latter seems to have better potential to reach a higher productivity and showed that it could be, at least as *P. terebinthus*, a suitable rootstock for water deficit conditions. On the other hand, *P. integerrima* showed

less stomatal regulation, promoting water loss despite the major drop of water potential and showed less adaptation than *Pistacia atlantica* and *Pistacia terebinthus* to the edapho-climatic conditions of the zone and to water stress conditions.

CHAPTER 7: REFERENCES

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