

## Stomatal control of water use in olive tree leaves

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### Abstract

Little is known about the strategies used by olive trees to overcome the long dry periods typical of the areas where they are cropped. This makes it difficult to optimize the water supply in orchards. To study the control of water consumption by olive trees, measurements of leaf water potential ( $\Psi$ ) and stomatal conductance to  $H_2O$  ( $g$ ) were made on 26-year-old *Manzanillo* olive trees under three irrigation treatments. The first treatment provided enough water to cover the crop water demand, the next treatment supplied one third of that rate, and the final treatment was no irrigation at all, typical of dry-farming conditions. Under conditions of high vapour pressure deficit of the air ( $D_a$ ), the olive trees prevented excessive water loss by closing their stomata. Leaves of the current year showed better stomatal control than did the 1-year-old leaves. The upper-bound functional relationships between  $g$  and  $D_a$  and photon flux density ( $I_P$ ) were obtained by boundary-line analysis, based on a technique of non-linear least squares. Maximum values of  $g$  were observed at relatively low levels of  $I_P$ , from about  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and a proportional decrease in  $g$  with increasing  $D_a$  was also found, at least for values of up to approximately 3.5 kPa. Higher values of  $g$  were observed in the morning than in the afternoon, for similar levels of  $I_P$  and  $D_a$ . Unirrigated olive trees recovered quickly after the dry season, showing values of  $\Psi$  and  $g$  similar to those of irrigated trees after just two days.

### Introduction

Many mediterranean fruit tree species are known to adopt drought tolerance, or drought avoidance, strategies to overcome the long dry periods that are typical of the region (Larsen et al., 1989; Lo Gullo and Salleo, 1988; Rieger, 1995). In most cases, however, current information is insufficient for a complete understanding of how the mechanisms involved actually work. This applies mainly to the short-term water-use dynamics, from hours to days - the time scale appropriate for micro-irrigation systems, which are widely used for the water supply of fruit trees in these areas. Therefore, more information is needed to optimize water supply in orchards.

In the case of the olive tree (*Olea europaea* L.), several aspects concerning its capacity to withstand arid environments have already been studied. Abd-

El-Rahman et al. (1966) measured the water content of olive leaves at saturation, finding a value,  $1.59 \text{ g water g}^{-1}$  dry weight, extremely low compared with other species growing in the same environment ( $5.77 \text{ g g}^{-1}$  for fig,  $5.85 \text{ g g}^{-1}$  for grape). Olive leaves contain a high amount of cuticular wax, which should significantly increase the diffusion resistance of the cuticular membrane (Leon and Bukovac, 1978). Specialized cells at the base of the peltate stalks are also effective at limiting water loss (Bongi et al., 1987a). The dense packing of the mesophyll layer in olive leaves of the commercial variety *Ascolana* leads to a low cellular wall conductance thereby providing an efficient system to limit cellular water loss under stress (Bongi et al., 1987b). Lo Gullo and Salleo (1988), however, observed that despite all this protection against water loss, leaves of the wild olive tree *Olea oleaster* underwent a substantial water loss under conditions of water stress. Regarding changes in the

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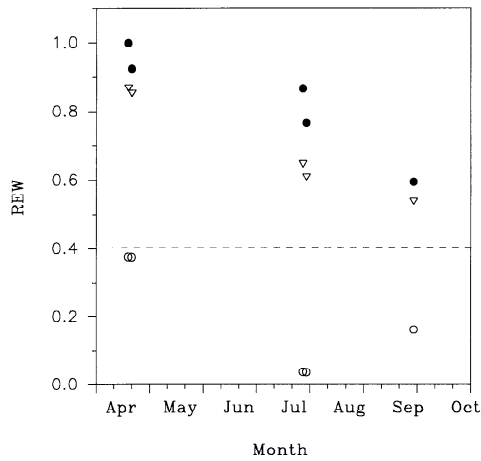


Figure 1. Relative extractable water (REW, see Equation 2 in Materials and methods) measured in the soil of the three treatments on the measurement days of April, July and September (● treatment I: weekly irrigation to cover the crop water demand - see Materials and methods for details; ▽ treatment 1/3: the same as in treatment I, but with about 1/3 of the water applied; ○ treatment D: unirrigated trees, with rainfall as the only water supply). The dotted line indicates the REW value considered to be the water deficit threshold (see Figure 2).

sap osmotic potential, Abd-El-Rahman et al. (1966) observed a marked rise in the osmotic pressure of the olive sap throughout the dry season, exceeding the values observed in other xerophytes. Rieger (1995) also observed osmotic adjustment in expanding olive leaves, finding values of osmotic potential similar to those reported by Abd-El-Rahman and El-Sharkawi (1974). The increase in sap osmotic pressure enables the roots to extract more water when the soil dries (Abd-El-Rahman et al., 1966).

The xerophytic nature of olive roots has been observed in anatomical studies (Fernández et al., 1994) and by the analysis of their hydraulic functioning (Moreno et al., 1996). Salleo et al. (1985) observed that the vessel lumina, when expressed as percentage of the total xylem cross-sectional area, was half that measured in other xerophytic Mediterranean species such as *Vitis vinifera*. The low hydraulic conductivity of olive xylem is a feature that seems to play an important role in the tree's water relations. For instance, Lo Gullo and Salleo (1988) considered that the water-saving feature of the wild olive tree (*Olea oleaster*) is due mainly to the low hydraulic conductance of the xylem, which allows the tree to avoid water loss on days of high atmospheric demand. Larsen et al. (1989) recognized the existence of this feature in the commer-

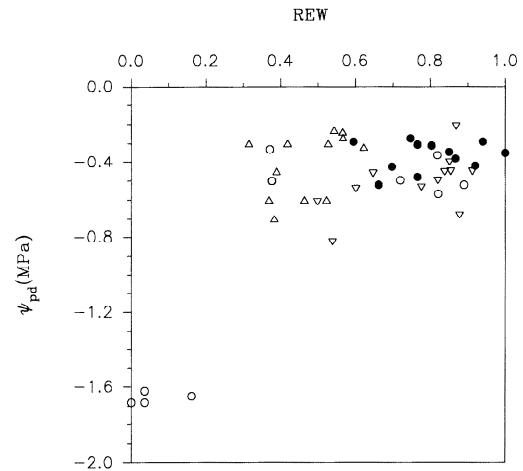


Figure 2. Relationship between relative extractable water (REW) and predawn leaf water potential ( $\Psi_{pd}$ ) measured on trees under different irrigation treatments. See Figure 1 for definitions of treatments. Data from this experiment and from the experiment by Fernández (1989) carried out in the same orchard (△ unirrigated trees), have been used. Each point represents the average of six  $\Psi_{pd}$  measurements per treatment. The mean value of  $\Psi_{pd}$  is -0.46 MPa for  $REW \geq 0.4$ .

cial olive tree *Nabali*, but they also observed a stomatal closure early in the day. The hydraulic conductivity of olive xylem can become even lower under certain circumstances. Salleo and Lo Gullo (1993) observed losses of about 10% of hydraulic conductivity in one-year-old twigs of young *Olea oleaster* trees, when these became stressed, due to xylem cavitation. Rieger (1995) observed that drought stress caused a reduction in root hydraulic conductivity. Moreno et al. (1996) observed cavitation in the xylem vessels of the outer annuli of a main near-surface root belonging to a tree resident in dry soil for over three months. After recovery by a single irrigation, the xylem vessels nonetheless remained cavitated, and water continued to be drawn from deep in the velocity profile.

It seems evident that the olive tree has certain structural features for drought tolerance and it also possesses active mechanisms which allow it some degree of control over water loss. However, more research is needed to establish the degree to which these features and active mechanisms act to play a role in the parsimonious character of the olive tree as a water user. For instance, (i) as yet there is no clear evidence of stomatal control in *Manzanillo* olive trees (the main commercial variety for table consumption) as a way of avoiding losses of water (ii) the stomatal response to atmospheric vapor pressure deficit and radiation has

yet to be quantified, and (iii) the influence of leaf age on the stomatal behaviour has also not been studied yet. The objective of this work was to identify water use strategies of *Manzanillo* olive trees by studying how leaf water relations are affected by changes in environmental water status, radiation, and leaf age. The results we present here contribute to answer the three main points mentioned above. The recovery of unirrigated olive trees after a lengthy drought period was also studied.

## Material and methods

### *Orchard site and irrigation treatments*

The experiments were carried out at the experimental farm of the Instituto de Recursos Naturales y Agrobiología, at Coria del Río near Seville in Spain (37° 17' N, 6° 3' W, elevation 30 m). The 1 ha experimental orchard was planted in 26-year-old olive trees (*Olea europaea* L., var. *Manzanillo*) at a spacing of 7×7 m. The soil is a sandy loam of about 2 m depth, with 27.5% coarse sand, 36.5% fine sand, 13.4% silt and 22.6% clay. The volumetric soil water content is 0.33 m<sup>3</sup> m<sup>-3</sup> for a soil matric potential of 0 MPa, and 0.10 m<sup>3</sup> m<sup>-3</sup> for -1.5 MPa. More details on the soil characteristics are shown in Moreno et al. (1983, 1988).

Three water regimes were imposed during the experiment: (a) Treatment I involved a weekly irrigation to replace the crop water demand ( $ET_c$ , mm) as calculated by the equation

$$ET_c = K_r K_c ET_o \quad (1)$$

where  $K_r$  is the coefficient relating the degree of orchard floor plant cover with the evapotranspiration (Feres and Castel, 1981). In our case,  $K_r = 0.69\%$ , since the trees covered 34% of the orchard floor.  $K_c$  is the crop coefficient obtained by Pastor and Orgaz (1994) for olive trees in an area close to our experimental orchard (0.6 in April; 0.55 in May and September; 0.5 in June; 0.45 in July and August), and  $ET_o$  (mm) is the potential evapotranspiration as calculated by the FAO-Penman equation, which Mantovani (1994) evaluated as the best for the area. (b) Treatment I/3 was similar to treatment I, but with only about 1/3 of the water applied (Table 1). (c) Treatment D used some unirrigated trees, which had rainfall as their only source of water supply (Table 1).

Table 1. Water supplied during the experimental period for the three irrigation treatments (a) irrigation during the dry season (b) irrigation during the recovery experiment (c) rainfall events

Date	Water (litres per tree)		
	I	I/3	D
(a) Weekly average from 18 March to 19 September	815	312	0
(b) 26 September	1418	2269	9500
3 October	1513	1513	2424
Date	Rainfall (mm)		
(c) 22 April	3.0		
27 April	14.0		
5 May	4.5		
6 June	16.0		
26 June	10.0		
23 August	29.0		
5 September	2.5		

The two irrigation treatments were applied to trees in a part of the orchard which had been irrigated regularly since they were young. The unirrigated trees were in a different part of the orchard which had been maintained under dry-farming conditions from the beginning. Three representative trees were chosen for each treatment. Irrigation treatments I and I/3 were carried out during the dry season of 1995, from the middle of March to the middle of September (Table 1). The water was applied in a 2.5 m radius pond around each tree that was contained by a small earthen dyke. Near the end of the experiment, all the trees were abundantly irrigated and their recovery after the long drought period was subsequently studied. Two recovery irrigations were made: the first was on the 26th of September and the second was on the 3rd of October. Enough water was applied to return the rootzone back to field capacity (Table 1, Figure 9). For the irrigation of the D trees, a 3.5 radius pond was built around each tree. With this big pond we were trying to avoid dry roots in the rootzone, since dry roots may send a signal for the tree to limit its water use (Moreno et al., 1996; Tardieu and Davies, 1993). In the irrigated trees, the 2.5 m radius pond was deemed to be large enough, due to the more restricted root system of the trees (Fernández et al., 1991).

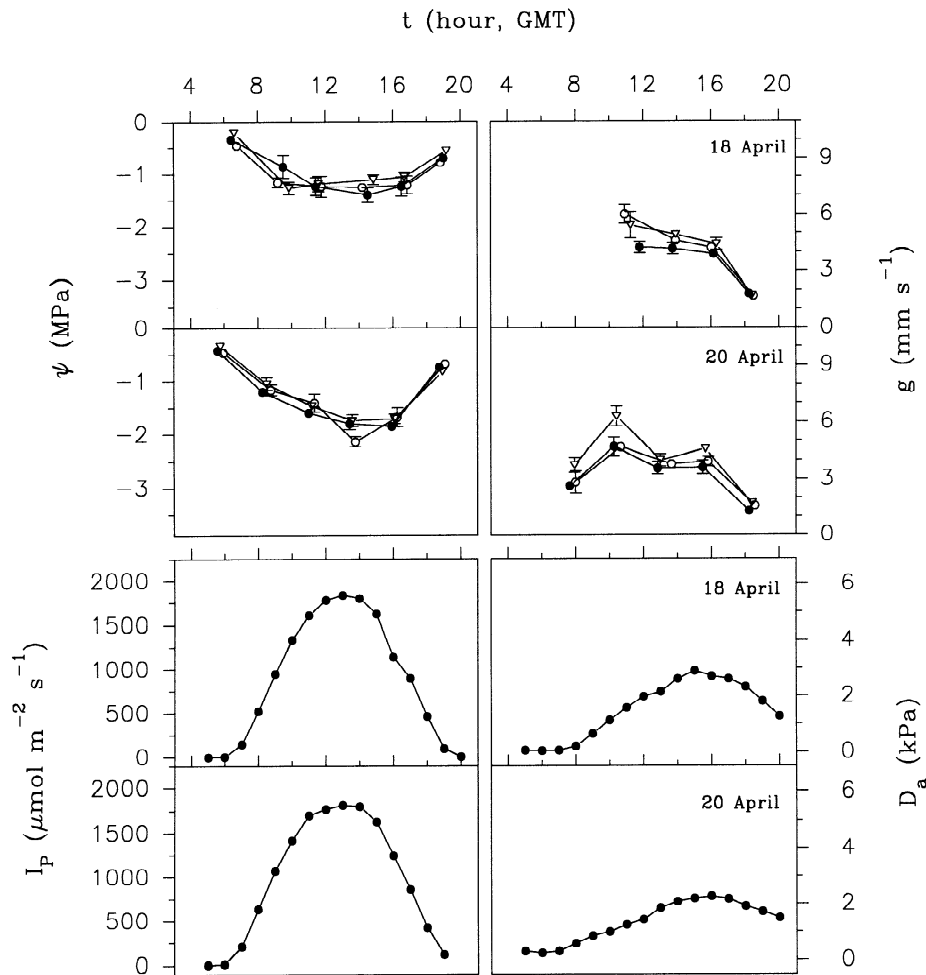


Figure 3. Diurnal time course of leaf water potential ( $\Psi$ ) and stomatal conductance ( $g$ ) measured in April in trees of the three treatments. See Figure 1 for definitions of treatments. Each point represents the average of six values per treatment. Vertical bars indicate twice the standard error. Values of photon flux density ( $I_p$ ) and vapour pressure deficit of the air ( $D_a$ ) recorded on the measurement days are also plotted.  $t$  = time of day.

#### Soil water and plant measurements

Soil water measurements were carried out around one tree in each treatment. Measurements were taken 10–15 days apart, and on the same day as plant measurements were made. The volumetric water content of the soil ( $\theta$ ,  $\text{m}^3 \text{m}^{-3}$ ) from 0.2 m down to 1.5 m was measured every 0.1 m using a neutron probe (Troxler 3300, Research Triangle Park, North Carolina, USA) in access tubes installed at 0.5, 1.5 and 2.5 m away from the tree trunk. For the top layer of the soil,  $\theta$  was measured by time-domain-reflectometry (TDR) using a Tektronix cable tester (Model 1502C, Beaverton, Oregon, USA). The TDR waveguides comprised

three parallel stainless steel rods, 2 mm in diameter and 0.15 m long. A portable computer was used to record and analyse the TDR wave-forms using an analysis similar to that of Baker and Allmaras (1990). The measurement points were at the same distance from the trunk as were the access tubes, and at 0.2 m on each side of the tube. Soil moisture profiles were used to calculate a depth equivalent of water, expressed here as the level of relative extractable water (REW, mm) defined by the equation (Granier, 1987):

$$REW = (R - R_{min}) / (R_{max} - R_{min}) \quad (2)$$

where  $R$  is the actual soil water content (mm),  $R_{min}$  the minimum soil water content measured during the

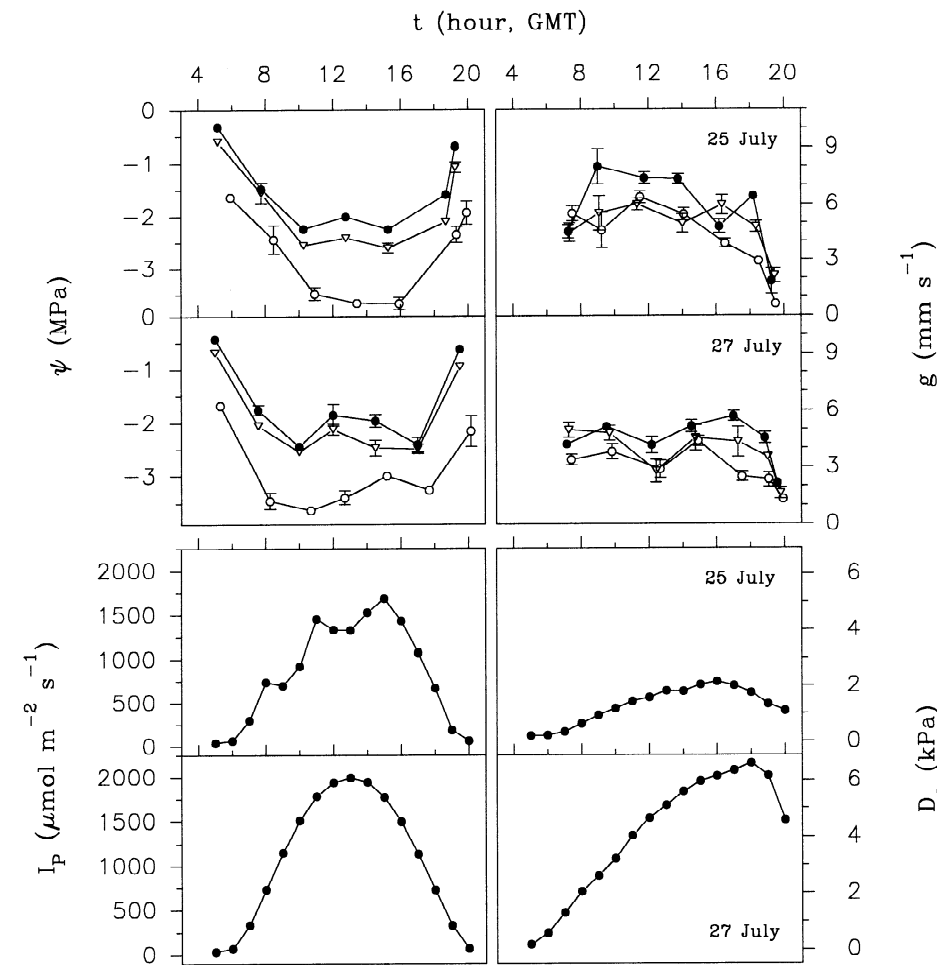


Figure 4. Diurnal time course of leaf water potential ( $\Psi$ ) and stomatal conductance ( $g$ ) measured in July in trees of the three treatments. See Figure 1 for definitions of treatments. Each point represents the average of six values per treatment. Vertical bars indicate twice the standard error. Values of photon flux density ( $I_p$ ) and vapour pressure deficit of the air ( $D_a$ ) recorded on the measurement days are also plotted.  $t$  = time of day.

experiments (mm), and  $R_{max}$  is the soil water content at field capacity (mm). Total soil extractable water calculated from  $R_{max} - R_{min}$  was 306 mm.

The soil matric potential head ( $h$ , MPa) was measured by a set of two mercury tensiometers installed at depths of 0.45 and 0.90 m, and a distance of 1.5 m from the trunk. When tensiometers were out of range,  $h$  was obtained from the  $\theta$  measurements and the soil water retention curve determined by Moreno et al. (1996) in the same orchard. In the soil of treatment I which was well watered, the  $h$  values were always higher than -0.05 MPa throughout the experimental period, typical values being around -0.03 MPa most of the time. For treatment I/3, the values of  $h$  were usually higher than -0.09 MPa. In the unirrigated treatment, values of  $h$

decreased throughout the experimental period, due to plant uptake and the scarce rainfall (Table 1). Values as low as -0.5 MPa were already observed by May, while values lower than -1.5 MPa were reached by the end of the dry season.

Leaf water potential ( $\Psi$ , MPa) and stomatal conductance to  $H_2O$  ( $g$ ,  $mm\ s^{-1}$ ) were measured in leaves of the current year, which were sunny and healthy. The olive is an evergreen tree in which leaves abscise when they are two or three years old. Development of new leaves was noted at the end of February, and they were fully expanded after about four weeks. Leaf water potential was assumed to be equal to the xylem pressure potential at the petiole, as measured with a pressure chamber (Soilmoisture Equipment Corp,

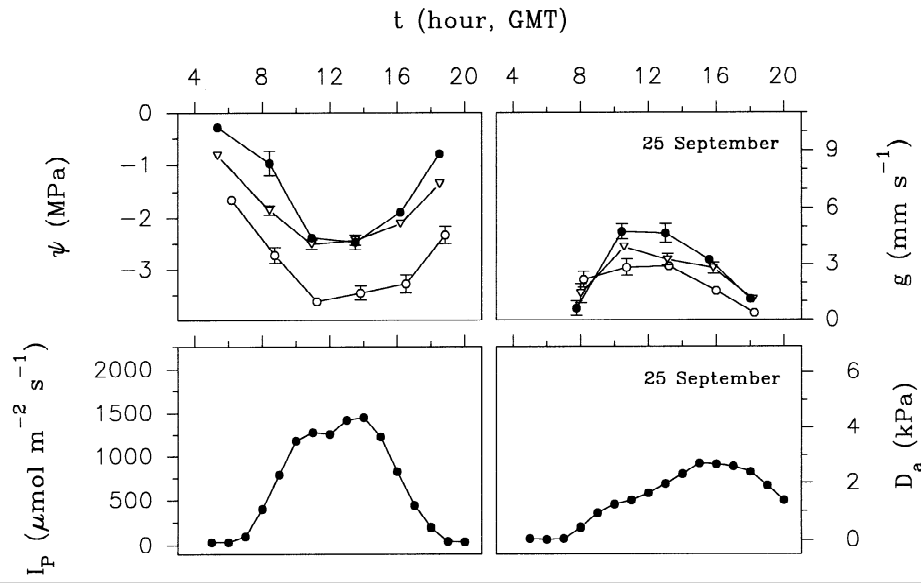


Figure 5. Diurnal time course of leaf water potential ( $\Psi$ ) and stomatal conductance ( $g$ ) measured in September in trees of the three treatments. See Figure 1 for definitions of treatments. Each point represents the average of six values per treatment. Vertical bars indicate twice the standard error. Values of photon flux density ( $I_P$ ) and vapour pressure deficit of the air ( $D_a$ ) recorded on the measurement day are also plotted.  $t$  = time of day.

Santa Bárbara, California, USA). Measurements of  $g$  were made with a steady-state porometer (LI-1600, LI-COR, Lincoln, Nebraska, USA) on the abaxial surface of leaves. The *Manzanillo* variety has stomata on the abaxial surface only, and the adaxial surface is covered by a thick, waterproof cuticle, which prevented water losses (Leon and Bukovac, 1978). Hence, no vapour diffusion was detected on the adaxial surface.

During the three irrigation treatments, leaf measurements were made twice in April and July, and three times in September, every 2.5 hours from dawn to sunset. Two leaves were sampled from each experimental tree, at 1.6-1.9 m above soil level. After analysing the data from all the experimental period, we found that the coefficients of variation were 15.2 for  $\Psi$  and 19.4 for  $g$ , which shows that six leaves per treatment were enough to calculate the averages of both parameters. In April and July, leaf measurements were made the first and third days after irrigation, to check the influence of soil drying. In September, measurements were made six days after irrigation, which was one day before the recovery experiment began. After the first recovery irrigation,  $\Psi$  and  $g$  were measured early in the morning, just before dawn for  $\Psi$ , and about 8:00 h for  $g$  - when the relative humidity of the air decreased below the operating value of the porometer. Subsequent leaf measurements were made at midday

(about 12:00 h) on days 1, 2, 3, 6 and 9 after irrigation. Functional relationships between  $g$  and photon flux density ( $I_P$ ) and vapour pressure deficit of the air ( $D_a$ ) were determined by boundary-line analysis based on field data and a technique of non-linear least squares (Jarvis, 1976).

On the 30th of April 1996, measurements of  $\Psi$ ,  $g$  and photosynthesis rate ( $P_N$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) were made in 1-year-old leaves and in leaves of the current year, to study the influence of leaf age on stomatal behaviour. Age-related changes in the stomatal behaviour have been observed in different tree species (Benecke et al., 1981; Reich 1984a, b; Reich and Borchert, 1988). Measurements were made in three trees of treatment I, every 2 hours from 8:00 to 14:00 h. Three young and three old leaves per tree were sampled each time. Measurements of  $P_N$  were made with a portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebraska, USA). In addition, ten 1-year-old leaves and ten leaves from the current year were detached from the trees. The subsequent water loss of these leaves was monitored during bench-drying, by frequent weight measurements with a  $10^{-4}$  g balance.

Table 2. Potential evapotranspiration ( $ET_o$ ) for the experimental period calculated by the FAO-Penman equation (Doorenbos and Pruitt, 1977). Data from the weather station located some 50 m away from the experimental trees were used to calculate  $ET_o$

10-day averages			Daily values	
Month	10-day period	$ET_o$ (mm)	Date	$ET_o$ (mm)
April	1	5.5	18 April	6.0
	2	5.7	20	6.1
	3	4.5		
July			25 July	6.9
	1	6.0	27	8.3
	2	7.9		
September	3	8.1	25 September	5.2
			27	4.8
	1	5.6	28	5.4
October	2	5.0	29	4.7
	3	4.8		
			2 October	1.7
	1	3.7	5	4.2
	2	3.8		
	3	2.8		

### Weather measurements

Weather variables were measured with an automatic weather station located some 50 m away from the experimental trees. Thirty-minute averages of global solar radiation, photosynthetically active radiation, wind speed, rainfall, air temperature, and relative humidity were recorded. These data were used to calculate  $ET_o$  by the FAO-Penman equation (Doorenbos and Pruitt, 1977) (Table 2), and for the calculation of  $D_a$ . Values of  $I_P$  used in the analysis of the stomatal conductance response were measured by the photon sensor on the porometer, so as to record the radiation normal to the leaf surface.

## Results and discussion

### Influence of drought stress on $\Psi$ and $g$

In April, at the beginning of the dry season, relative extractable water was still high enough in the soil of unirrigated trees to prevent significant water stress, as indicated by Figure 1. We assumed a REW threshold for soil water deficit of around 0.4, after plotting REW against predawn leaf water potential ( $\Psi_{pd}$ ), which was used as an indicator of water stress (Figure 2). Data

obtained by Fernández (1989) on unirrigated trees of the experimental orchard have also been included, to increase the number of datapoints. Figure 2 shows that  $\Psi_{pd}$  remains constant for values of REW higher than 0.4. A REW threshold of 0.3-0.4 seems to be a general feature for many tree species, as reported by Bréda et al. (1995). The daily curves of  $\Psi$  and  $g$  observed on the measurement days of April are shown in Figure 3. Some significant differences between treatments were found, especially in the  $g$  values measured before noon. Smaller differences were observed in  $\Psi$ . Measurements of  $\Psi$  and  $g$  made in July (Figure 4) show that the olive tree is able to restrict water loss by closing its stomata. The 25th of July was somewhat fresh, and partially cloudy day, as can be seen from the records of  $D_a$  and  $I_P$ . The 27th, however, was a clear, dry, hot day, with a very high  $D_a$ . On the 25th, the values of  $\Psi$  decreased quickly in the morning, showing the lowest values around the middle of the day, and late in the afternoon. The maximum values of  $g$  were reached before midday, in general, decreasing afterwards throughout the day. On the 27th, however, the high levels of radiation and atmospheric water demand caused a different  $\Psi$  and  $g$  response. Thus,  $\Psi$  decreased very quickly early in the morning, for all treatments, reaching minimum values before midday. It remained more or less constant throughout the day until late in the

afternoon, when a sharp increase was observed. The values of  $g$  remained fairly constant throughout most of the day, and they were lower than on the 25th for all treatments. This shows that the olive trees restricted water loss on a day of high atmospheric water demand by closing the stomata early in the morning, preventing an excessive drop in  $\Psi$ . This agrees with the findings of Larsen et al. (1989) in *Nabali* olive trees, though the diurnal patterns of  $g$  reported by them are different from those found in *Manzanillo* variety (Figures 3, 4 and 5). Larsen et al. (1989) found maximum values of  $g$  relatively late in the day, whereas Figures 3 to 5 show maximum values of  $g$  before noon, but in the case of the 27th of July already commented. The peak values of  $g$  found by Larsen et al. (1989) are also lower than those shown in this work. Thus, Larsen et al. (1989) reported maximum average values of about  $1.8 \text{ mm s}^{-1}$  in watered olive trees on the 20th of June 1987, a cloudless day with a maximum  $D_a$  of 2.3 kPa. A maximum average value for  $g$  of  $8 \text{ mm s}^{-1}$  was measured in the *Manzanillo* trees of treatment I on the 25th of July (Figure 4), a day of similar weather characteristics to those on the 20th of June 1987. Lo Gullo and Salleo (1988) observed large diurnal changes in the leaf relative water content of wild olives (*Olea oleaster*), in spite of their low  $g$ . They mentioned that a rapid recovery from water loss was probably impossible because of the very low hydraulic conductance of the xylem. This feature may be responsible for the tree to prevent water loss on days of high atmospheric demand. A high resistance to water flow has also been observed in the commercial olive (Rieger, 1995; Salleo et al. 1985). If this were the only mechanism involved in the prevention of water losses shown in Figure 4, however, there would be no reason for the  $g$  values measured on the 27th to be lower than on the 25th, at least for the case of unirrigated trees where no appreciable change in REW was detected between the two measurement days (Figure 1). This decrease in  $g$  must be due, therefore, to partial stomatal closure.

The daily curves of  $\Psi$  and  $g$  recorded in July and September (Figures 4 and 5) show the influence of the soil water supply on both parameters. The values of  $\Psi$  were much lower in the unirrigated trees than in the irrigated ones. This is in agreement with the values of REW measured in each treatment (Figure 1). The REW value of 0.87 measured in July in treatment I one day after irrigation shows that the supplied water was slightly lower than  $ET_c$ . This may be due to the fact that soil evaporation was greater in our case than when Pastor and Orgaz (1994) did their experiments to

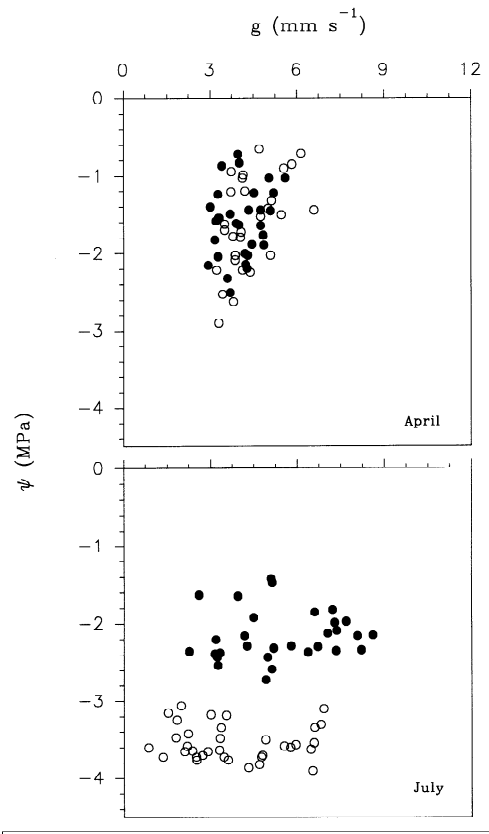


Figure 6. Stomatal conductance ( $g$ ) in relation to leaf water potential ( $\Psi$ ). Points represent single observations measured in April and July, between 11:00 and 13:00 h, and in trees of treatments I and D. See Figure 1 for definitions of treatments.

obtain the  $K_c$  values used in Equation (1). As explained in Materials and methods, we used irrigation ponds of  $19.6 \text{ m}^2$ . The low REW recorded in September in treatment I is due to the fact that measurements were made six days after irrigation, as explained in Materials and methods. Values of  $\Psi_{pd}$  never went below  $-0.50 \text{ MPa}$  in the I trees. In treatment I/3, the lowest average  $\Psi_{pd}$  value was  $-0.82 \text{ MPa}$ , observed in September (Figure 5). In the unirrigated trees, however, the average value of  $\Psi_{pd}$  was below  $-1.60 \text{ MPa}$  in both July and September. At midday, the lowest average values of  $\Psi$  were above  $-2.47 \text{ MPa}$  for the irrigated trees, observed in September, but the unirrigated trees reached average values as low as  $-3.63 \text{ MPa}$  in July and  $-3.62$  in September. Considerable differences were also observed in  $g$  values between treatments I and D, throughout most of the day. Values of  $g$  for treatment I/3 were intermediate. The highest average value of  $g$  was  $0.86 \text{ cm s}^{-1}$ , recorded in July in trees of treatment I.



Figure 6 shows  $g$  in relation to  $\Psi$  for treatments I and D and for the months of April and July. Data of September have not been plotted because we made the measurements six days after irrigation, as explained in Materials and methods. A similar relationship between  $g$  and  $\Psi$  could be established for both treatments in April, though treatments were not well established by that time of the year, as has already been mentioned. In July, data of the two treatments appeared clearly separated, but the scatter of the data and the narrow range of  $\Psi$  in both treatments make it difficult to establish any relationship. The trend of  $g$  to decrease as  $\Psi$  becomes more negative has also been observed in orange trees by Fereres et al. (1979), and in almond trees by Castel and Fereres (1982) and Torrecillas et al. (1988). All those authors also found a wide scatter when plotting  $g$  versus  $\Psi$ . The stomatal response to other environmental factors (Jarvis, 1976) can be responsible for this scatter.

Higher values of  $g$  were observed in the morning, during the opening phase, than in the afternoon, for a similar level of  $D_a$ . This is clearly shown in Figure 7, where the relationships between  $g$  and  $D_a$  found in treatments I and D during the course of the 20th of April and the 25th of July have been plotted. The same tendency has been observed in the relationships between  $g$  and  $I_P$  (not shown) though not so clearly, perhaps because  $D_a$  is the main driving variable for stomatal closure. This behaviour could be due to the fact that the maximum values of  $I_P$  occurs earlier in the day than the maximum values of  $D_a$  (Jarvis, 1976). This sort of response seems to be a common feature in trees. It has also been observed in oak by Hinckley et al. (1975), in apple by Jarvis (1976), in peach by Punthakey et al. (1984), and by other authors working with other species.

#### $\Psi$ and $g$ following rewatering

The evolution of the  $\Psi$  and  $g$  recorded in the morning and at midday throughout the recovery experiment is shown in Figure 8. The corresponding climatic conditions and REW values for the measurement days are shown in Figure 9. After only two days we observed little difference between treatments in the values of  $\Psi_{pd}$  (Figure 8). Six days after irrigation any differences in  $\Psi_{pd}$  were not significant. At midday, similar values of  $\Psi$  were observed just one day after rewatering for all three treatments. Also, and strikingly, we observed higher values of  $\Psi$  in the D trees throughout the rest of the experiment. The  $g$  values (Figure 8) become simi-

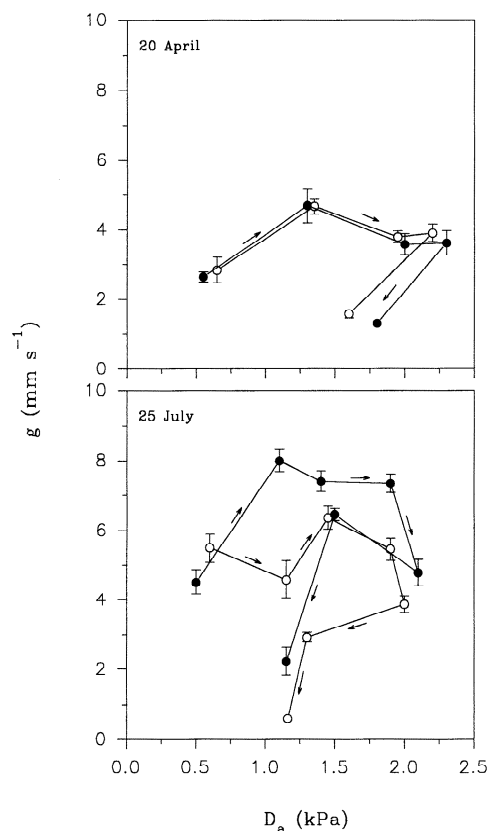


Figure 7. The relation between the stomatal conductance ( $g$ ) of olive trees of treatments I and D and the vapour pressure deficit of the air ( $D_a$ ) during the course of two experimental days. See Figure 1 for definitions of treatments. The arrows show the course of time from dawn to dusk. Each point represents the average of six values per treatment. Vertical bars indicate twice the standard error.

lar to those of I trees only two days after irrigation, and remained so afterwards. The low values of  $g$  measured in the morning of the 25th of September were due to difficulties in reaching the null point on the porometer, because of high air humidity. The drop in the midday values of  $g$  observed on the second and third day after irrigation (Figure 8) was due to the high  $D_a$  values of those days (Figure 9).

These responses of  $\Psi$  and  $g$  in stressed trees after rewatering show an remarkable capacity of the olive tree for a quick use of water when this is eventually available, even when the tree has been for a long period under very dry conditions. Salleo and Lo Gullo (1993) found that stressed *Olea oleaster* plants lost only about 10% of hydraulic conductivity of 1-year-old twigs, and that the plants were able to recover from xylem embolization in a short period. Some features

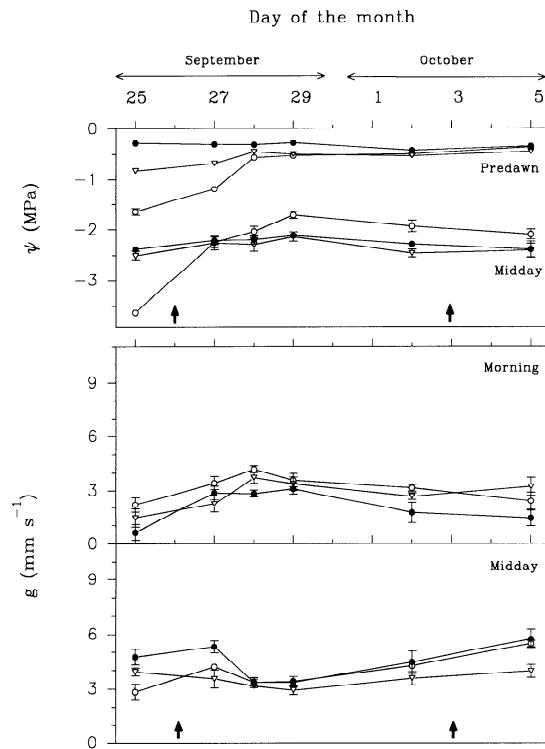


Figure 8. Evolution of leaf water potential ( $\Psi$ ) and stomatal conductance ( $g$ ) measured in trees of all the treatments during the recovery experiment. See Figure 1 for definitions of treatments. Each point represents the average of six values per treatment. Vertical bars indicate twice the standard error. The arrows indicate the recovery irrigations (see Table 1). Corresponding traces of  $I_P$  and  $D_a$  are shown in Figure 9.

of the olive root system, as observed by Fernández et al. (1991, 1994), which give it a high adaptability to water stress conditions, may be also partly responsible for the quick recovery of  $\Psi$  and  $g$ . Fernández et al. (1991) found, in our experimental orchard, that the root system of olive trees growing under dry-farming conditions was well developed, exploring a greater soil volume than those of drip-irrigated trees. Fernández et al. (1994) observed a more rapid maturation of the tissues close to the apex in unirrigated trees than in irrigated ones. This may increase water movement through the root system due to the increased water flux associated with secondary vascular development. They also found that the metaxylem vessel and central cylinder size were not reduced under drought. Jorba et al. (1985) mentioned the possibility of hormones playing some role in the stomatal control of stressed trees after rewatering. They found that  $\Psi$  values of previously-wet trees were similar to those of irrigated trees three days

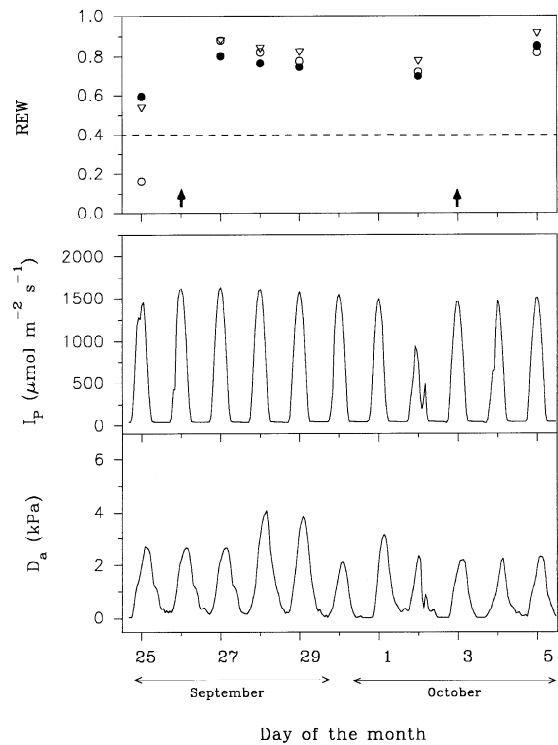


Figure 9. Relative extractable water (REW) measured in the soil of the three treatments during the recovery experiment. See Figure 1 for definitions of treatments. The dotted line indicates the REW value considered to be the water deficit threshold. Values of vapour pressure deficit ( $D_a$ ) and photon flux density ( $I_P$ ) are also shown. The arrows indicate the recovery irrigations (see Table 1).

after rewatering. Fernández et al. (1993) found the same in trees of the same orchard where the present work has been carried out. No measurements of  $g$  were made by any of those authors. Kaufmann and Levy (1976) studied the recovery of  $\Psi$  and  $g$  in lemon trees. They found that maximum conductances did not occur until two or three days after rewatering, although recovery from leaf water stress was complete after one day. Fereres et al. (1979) observed in orange trees that rehydration began immediately after irrigation, normal  $\Psi$  values being observed in less than a week. The recovery of  $g$  was much slower. On trees which had leaf water potentials of less than  $-5$  MPa before dawn, prior to irrigation,  $g$  did not fully recover in two months.

#### Relationships between $g$ and $I_P$ and $D_a$

The values of  $g$  measured in April and July have been plotted in Figure 10 together with their counterparts of  $I_P$ . The upper-bound functional relationships between

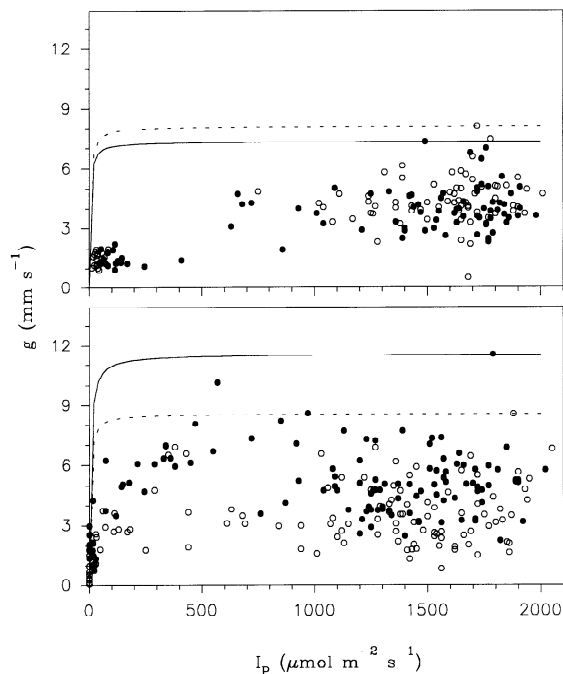


Figure 10. Stomatal conductance ( $g$ ) plotted against photon flux density ( $I_P$ ) measured normal to the leaf surface, in trees of treatments I and D. See Figure 1 for definitions of treatments. The upper-bound relationship between  $g$  and  $I_P$ , as given by Equation (3) (see Results and Discussion) has been drawn in the figure. The points represent single measurements made in April (top) and July (bottom).

$g$  and  $I_P$  shown in the figure have been obtained by fitting the equation of Jarvis (1976) to our field data, as explained in Materials and methods. The curve corresponds to the equation

$$g = \frac{b_1 b_2 I_P}{b_1 + b_2 I_P} \quad (3)$$

where  $b_1$  is the asymptotic value of  $g$  at infinite  $I_P$ , and  $b_2$  is  $dg/dI_P$  at  $I_P = 0$ . We measured  $g = 0$  in the dark, so the parameter  $b_{10}$  mentioned by Jarvis (1976) is not included in Equation (3). When the maximum values of  $g$  were measured in April in treatments I and S,  $D_a$  was 0.8 kPa and 1.6 kPa respectively. In July,  $D_a$  was 1.1 kPa when maximum values of  $g$  were measured in both treatments. The curves show a marked response of  $g$  to the increase in  $I_P$ , from 0 to about  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Few data of  $g$  were obtained in April for low values of  $I_P$ , but in July maximum values of  $g$  were obtained at a relatively low photon flux density, from about  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ . This is in accord with the high  $g$  values measured early in the morning (Figures 3, 4 and 5).

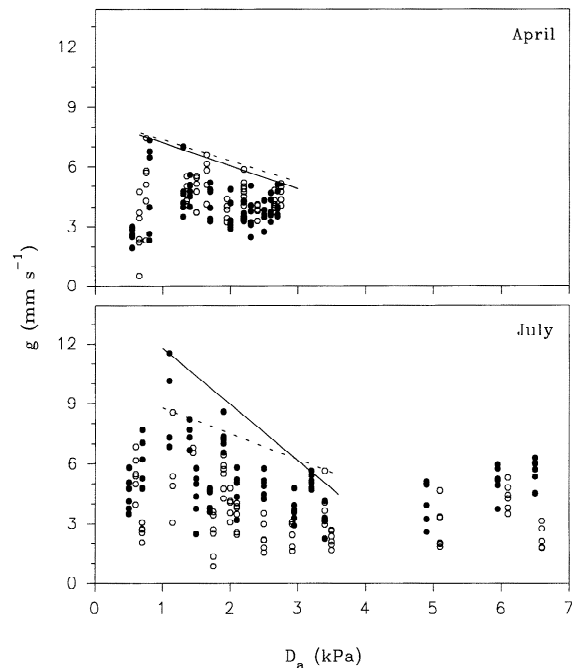


Figure 11. Stomatal conductance ( $g$ ) plotted against vapour pressure deficit of the air ( $D_a$ ), in trees of treatments I and D. See Figure 1 for definitions of treatments. The upper-bound relationship between  $g$  and  $D_a$ , as given by Equation (4) (see Results and Discussion) has been drawn in the figure. The points represent single measurements made in April and July, for  $I_P$  greater than  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

In Figure 11, the  $g$  values measured in April and July in trees I and trees D have been represented with their counterparts of  $D_a$ , after removing the data for  $I_P$  below  $500 \mu\text{mol m}^{-2} \text{s}^{-1}$  to be sure that radiation was not a limiting factor. The upper-bound functional relationships show a linear reduction of  $g$  versus  $D_a$ , similar to that observed by Jarvis (1976) and Thorpe et al. (1980), among others. The equation is

$$g = 1 - \alpha D_a \quad (4)$$

where  $\alpha$  is the slope of the relation. A linear reduction in  $g$  with increasing  $D_a$  is generally assumed, from its maximum value to  $g = 0$  (Jarvis, 1976). Equation (4) shows a proportional decrease in  $g$  with increasing  $D_a$ , for  $D_a$  values of up to approximately 3.5 kPa. The stomata, however, remained partly opened at higher  $D_a$ , as shown in Figure 11.

The curves shown in Figures 10 and 11 show the probable upper-limit of  $g$  versus  $I_P$  and  $D_a$ . It has to be taken into account, however, that  $I_P$  and  $D_a$  tend to be correlated, causing some bias in the estimated parameters of Equations (3) and (4) (Jarvis, 1976). It

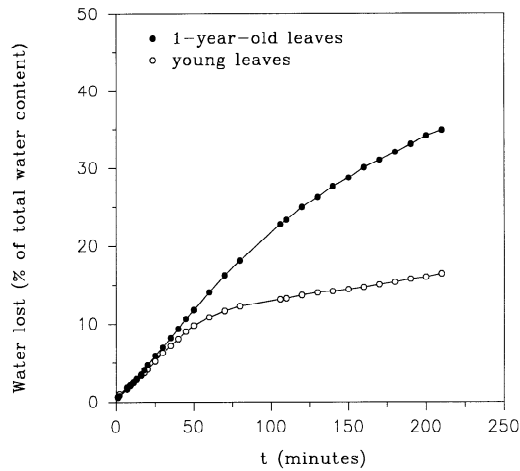


Figure 12. Water loss of detached, bench-dried 1-year-old leaves and the current season leaves. A group of 10 leaves was weighed in each case. Fresh weight of the 10 old leaves: 2.1706 g; fresh weight of the 10 young leaves: 1.8190 g. Water content of the 10 old leaves: 1.0256 g; water content of the 10 young leaves: 1.0898 g.

is also known that there are other variables influencing  $g$ , which cause the wide scatter shown in the figures. It seems clear, however, that higher values of  $g$  were observed in July than in April, for similar values of  $D_a$  and  $I_P$ . This may be due to a lower stomatal closure in aged leaves, which would agree with the results shown in the next Section. In July, when the water treatments are already well established, the upper-limit curves were lower for the unirrigated trees than for the irrigated ones, showing the effect of the soil moisture on  $g$ .

#### Changes in stomatal control with leaf age

Measurements of  $\Psi$ ,  $g$  and  $P_N$  and water loss of detached leaves showed marked differences between 1-year-old leaves and leaves of the current year. Averages of  $\Psi$  were  $-1.11$  MPa for young leaves and  $-1.35$  for old leaves. A Student's  $t$  test showed no significant differences between those averages at  $p = 0.05$ , but was significantly different by a narrow margin ( $p = 0.06$ ). The averages of  $g$  (young leaves:  $5.2$  mm  $s^{-1}$ ; old leaves:  $6.3$  mm  $s^{-1}$ ) and  $P_N$  (young leaves:  $14.57$   $\mu\text{mol CO}_2$   $\text{m}^{-2}$   $\text{s}^{-1}$ ; old leaves:  $20.66$   $\mu\text{mol CO}_2$   $\text{m}^{-2}$   $\text{s}^{-1}$ ) were found to be significantly different ( $p = 0.05$  for  $g$ ;  $p = 0.01$  for  $P_N$ ). Considering that no  $\text{H}_2\text{O}$  or  $\text{CO}_2$  diffusion takes place through the cuticle (Leon and Bukovac, 1978), these results seem to show

that stomatal closure is more marked in young than in old leaves.

Results from the measurement of water loss by detached leaves are shown in Figure 12. Young and old leaves lost water at the same rate soon after detachment. When the water lost exceeded about 10% of the total leaf water content, the rate of water loss in young leaves decreased, while it remained about the same in old leaves. 210 minutes after detachment, old leaves had lost more than twice the amount of water compared to the young leaves. It appears that 1-year-old leaves do not have the same capacity for stomatal control. They remain open under adverse conditions, while stomatal closure occurs in leaves of the current year.

In hybrid poplar leaves, stomatal conductance was observed to decline with leaf age (Reich, 1984a). Reich and Borchert (1988) measured changes with leaf age in stomatal conductance and xylem pressure potential in leaves of five tropical tree species, finding differences between species. In *Tabebuia rosea* older leaves had higher conductance and greater water deficits than younger leaves. In mature leaves of *Mangifera indica* and *Licania arborea*, however, midday values of conductance were found to decrease with leaf age. The experiments with excised leaves of *Tabebuia rosea* and *Cordia glabra* showed that transpiration was higher in older than in younger leaves. The older leaves of both species lost about 80% of their water content by 3 h after excision, while young leaves lost only about 40%. For the olive tree, a xerophytic species, the water lost by 3 h after excision was 35% for 1-year-old leaves and 15% for leaves of the year (Figure 12).

#### Conclusions

*Manzanillo* olive trees are able to prevent excessive water loss on days of high water demand by closing the stomata early in the morning. This capacity for stomatal control reduces with leaf age. When water was supplied after a long dry period, they were able to recover quickly from water stress. The trees showed a good response to the deficit irrigation. With the frequent but reduced water supplies of treatment I/3, values of leaf water potential and stomatal conductance were quite close to those measured in the trees of treatment I. Considerable differences in both parameters were observed between irrigated and unirrigated trees. Maximum values of stomatal conductance were observed at photon flux densities of about  $500$   $\mu\text{mol m}^{-2}$   $\text{s}^{-1}$  which equates to about 1/4 full sun. A proportional decrease

in conductance with increasing vapour pressure deficit was also observed, for values of up to approximately 3.5 kPa.

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