

# Stomatal and photosynthetic responses of olive (*Olea europaea* L.) leaves to water deficits

A. MORIANA,<sup>1</sup> F. J. VILLALOBOS<sup>1,2</sup> & E. FERERES<sup>1,2</sup>

<sup>1</sup>Instituto de Agricultura Sostenible, CSIC, Apartado 4084, 14080 Cordoba, Spain and <sup>2</sup>Departamento de Agronomía, Universidad de Córdoba, Apartado 3048, 14080 Cordoba, Spain

## ABSTRACT

The leaf gas exchange of mature olive trees (*Olea europaea* L.) was characterized over a wide range of water deficits in the field during 1998, in Cordoba, Spain. Leaf photosynthesis ( $A$ ) and stomatal conductance ( $g_s$ ) responded diurnally and seasonally to variations in tree water status and evaporative demand. In the absence of water stress,  $A$  and  $g_s$  were generally high during autumn and low in days of high vapour pressure deficits (VPD). Leaf  $A$  varied between 0 and 2  $\mu\text{mol m}^{-2} \text{s}^{-1}$  under severe water deficits that lowered the stem water potential ( $\Psi_x$ ) to  $-8.0$  MPa, but recovered rapidly following rehydration. Transpiration efficiency (TE) was curvilinearly related to VPD and not influenced by water deficits except in cases of severe water stress, where low TE values were observed at  $\Psi_x$  below  $-4$  MPa. Three models of leaf conductance were calibrated and validated with the experimental data; two were based on the model proposed by Leuning (L) and the other was derived from the widely used Jarvis (J) model. The L models performed better than the J model in two validation tests. The scatter of the predictions and the limited accuracy of all three models suggest that, in addition to the physiological and environmental variables considered, there are additional endogenous factors influencing the  $g_s$  of olive leaves.

**Key-words:** *Olea europaea* L.; CO<sub>2</sub> assimilation; conductance models; drought; leaf gas exchange; transpiration efficiency; water relations.

## INTRODUCTION

Olive (*Olea europaea* L.) is one of the most characteristic tree crops from the Mediterranean. Traditionally, it was cultivated under rain-fed conditions, but the demand for its products has led to the application of irrigation in many areas, albeit with very limited amounts of water given the scarcity and competition for water in the region. The olive tree has a reputation of being drought tolerant from very early reports (Spiegel 1955) but few studies have been conducted in the field that quantified its responses to water deficits. Among such responses, gas exchange is of particular importance in determining the efficiency of water use in response to the limited water resources.

In potted olive trees, Angelopoulos, Dichio & Xiloyannis (1996) determined that leaf conductance ( $g_s$ ) was limiting photosynthesis ( $A$ ) in trees subjected to mild and moderate water stress, whereas non-stomatal factors influenced  $A$  only under severe stress conditions. The diurnal course of  $A$  and  $g_s$  in potted trees exposed to the natural environment exhibited a maximum value in the morning, declined towards midday, and was more or less constant throughout the afternoon (Angelopoulos *et al.* 1996); a pattern that is common in Mediterranean woody vegetation (Tenhunen, Pearcy & Lange 1987). Such patterns are normally attributed to the concomitant diurnal increase in vapour pressure deficit (Tenhunen *et al.* 1987) but recent field studies with olive trees do not provide conclusive evidence on the relation between  $g_s$  and vapour pressure deficit (VPD) (Fernández *et al.* 1997; Giorio, Sorrentino & d'Andria 1999). Some of the information collected suggests that soil water status plays an important role in controlling  $g_s$  in olive trees (Bongi & Palliotti 1994; Giorio *et al.* 1999).

Approaches to determine water use by tree canopies require knowledge of canopy conductance, which in turn should be based on leaf conductance models (Monteith 1995). Given the degree of coupling between trees and the atmosphere, one would expect that the modulation of leaf  $g_s$  is important in determining tree transpiration (Jarvis & McNaughton 1986). Thus, models designed to estimate tree transpiration require  $g_s$  models as input. There are a number of approaches to modeling  $g_s$  but they are either based on empirical relationships between  $g_s$  and environmental factors (Stewart 1988) or on more fundamental relationships between  $A$  and  $g_s$  (Leuning 1995). The empirical approach has been tested successfully in a number of models (Stewart 1988; Baille, Romero-Aranda & Baille 1996; Moreno *et al.* 1996; Van Wijk *et al.* 2000) but the uses of response functions have limitations, as the parameter calibration varies with the addition of environmental variables. The more physiologically based models have the prerequisite of determining or estimating  $A$  to predict  $g_s$ , and that poses limitations to the applicability of this type of model to species such as olive, where very few reports exist on the gas exchange responses to the environment.

This work was undertaken to characterize the gas exchange responses of olive trees in the field to various levels of water stress, from moderate to very severe. Such responses were quantified to determine the transpiration efficiency of olive leaves as affected by water deficit, and were then used to calibrate and validate three models of

Correspondence: E. Fereres. E-mail: ag1fecae@uco.es

stomatal conductance; one based on response functions to environmental variables (Stewart 1988) and two others that utilized the  $A$  measurements to predict stomatal conductance.

## MATERIALS AND METHODS

Measurements of leaf gas exchange and water relations were performed in 1998 within an irrigation experiment conducted in an 18-year-old olive (*Olea europaea* L., cv Picual) orchard located at the CIFA Experimental Station, Cordoba, Spain (38°N, 4·8°W, 110 m altitude). Tree arrangement was 6 m × 6 m and the four differential irrigation treatments, which started in 1997, were:

- T1: a control treatment, where irrigation fully replenished soil water extraction and no water deficits were allowed throughout the season.
- T2: a deficit irrigation treatment that applied 75% of the crop evapotranspiration ( $ET_c$ ) needs (rainfall plus irrigation) with a period in midsummer (15 July to 15 September) without irrigation.
- T3: deficit irrigation that supplied the same amount as T2 (75% of  $ET_c$ ) but without the midsummer drought period.
- T4: a rain-fed treatment that received over 900 mm of rainfall between September, 1997 and May, 1998, no rainfall in July and August and just 102·5 mm between September and December, 1998.

Orchard  $ET_c$  was calculated as the product of crop coefficients for mature olive trees (Orgaz & Fereres 1997) and the Penman–Monteith mean monthly reference evapotranspiration ( $ET_0$ ) for Cordoba. Irrigation was applied three times a week.

### Gas exchange measurements

Net photosynthesis ( $A$ ) and leaf conductance ( $g_l$ ) were measured using a portable open infrared gas analyser (CIRAS-1; PP System, Hitchin, Herts, UK) and fitted to a Parkinson leaf chamber operated at 250 mL min<sup>-1</sup>. The leaf chamber was specially designed for measurements in olive leaves and covered an exposed area of 1·75 cm<sup>2</sup>. Readings were taken after steady-state conditions in gas exchange were achieved (around 1 min). The CO<sub>2</sub> concentration inside the chamber was automatically controlled by the CIRAS-1 porometer at 350 μmol mol<sup>-1</sup>, whereas the radiation, temperature and evaporative demand were those of ambient conditions. Carbon dioxide and water vapour concentration differences between the inlet and outlet gas circulating through the leaf chamber, as well as leaf temperatures obtained from energy balance equations, were used to calculate leaf area based rates of CO<sub>2</sub> assimilation ( $A$ ), transpiration ( $T$ ) and stomatal conductance ( $g_l$ ) using von Caemmerer & Farquhar's equation (von Caemmerer & Farquhar 1981), taking into account that olive is a hypostomatous species. Transpiration efficiency (TE) was

calculated as the ratio of leaf photosynthesis to transpiration as measured instantaneously with the CIRAS-1 porometer. The VPD values used in modelling  $g_l$  were calculated using the air humidity and temperature measured with the CIRAS-1. Such VPD values exceeded those measured in a weather station nearby (300 m away from the experimental orchard) by about 0·8 kPa.

For gas exchange, three fully expanded, sunlit leaves in four trees per treatment were randomly selected and measurements of  $A$  and  $g_l$  started in mid-June, 1998. Diurnal cycles were performed with the porometer on 25 June, 7 July, 29 July, 20 August, 14 September, 21 September, 2 October and 14 October 1998. In addition, gas exchange measurements were routinely performed in the morning (0700–0800 h GMT) and at midday (1300–1600 h GMT), at least once a week, from June until mid-October.

### Tree water status measurements

Stem water potential ( $\Psi_s$ ) measurements were used to evaluate tree water status. Fully expanded leaves located on branches near the main trunk were covered with aluminium foil at least 1 h before excision and the water potential was measured with a pressure chamber (Soil Moisture Equip., Santa Barbara, CA, USA). Stem water potential was measured in one leaf per tree of the same four trees per treatment where gas exchange measurements were performed, and at about the same time.

### Soil water content measurements

Volumetric soil water content was measured with a neutron probe calibrated for the experimental soil with separate calibrations performed for the upper (0–0·3 m) and lower (below 0·3 m) layers. In the three irrigated treatments eight access tubes, 2·5 m long, were placed in two trees per replicate plot. Only six tubes per tree were placed in the rain-fed treatment plots, at representative locations around each tree. Measurements were taken every 15 d at various depths, starting at 0·075 m down to 2·25 m. The upper limit (field capacity) of available water was estimated to be 396 mm of H<sub>2</sub>O for the whole 2·4 m soil profile. The lower limit of available water was obtained from extrapolation of the observations in the rain-fed treatment and was equivalent to 123 mm of H<sub>2</sub>O for the profile.

### Leaf conductance models

Two models were evaluated to compute leaf conductance in olive trees; the model proposed by Jarvis (1976) hereafter termed the J model, and the model of Leuning (1995) hereafter named the L model.

The J model is defined in general form as:

$$g_l = g_{\max} * f(1) * f(2) * f(3) \dots f(i) \quad (1)$$

where  $g_l$  is the leaf conductance and  $g_{\max}$  is the maximum leaf conductance under optimal conditions of temperature and ambient humidity and also, under maximum  $A$ .

Jarvis (1976) defined  $f(i)$  as empirical functions to characterize the influence of various environmental factors on  $g_1$ . Each function provides a value between 0 and 1.

In our J model, the effects of specific humidity deficit ( $H_S$ ) are defined as:

$$f(D) = 1 - K_D * H_S \text{ if } H_S < D_C \quad (2)$$

$$f(D) = 1 - K_D * D_C \text{ if } H_S \geq D_C \quad (3)$$

where  $K_D$  and  $D_C$  are constants that are optimized using the experimental data.

The effect of radiation are computed as:

$$f(R) = \frac{\frac{1000 + K_R}{1000} + I}{I + K_R} \quad (4)$$

where  $K_R$  is a constant and  $I$  is the photosynthetically active radiation (PAR;  $W m^{-2}$ )

The temperature function is:

$$f(T) = \frac{(T - T_L) * (T_H - T)^a}{(K_T - T_L) * (T_R - K_T)^a} \quad (5)$$

$$a = \frac{T_H - K_T}{K_T - T_L} \quad (6)$$

where  $K_T$  is a constant that the model optimizes;  $T$  is the leaf temperature;  $T_H$  is an upper temperature limit (50 °C) and  $T_L$  is a lower temperature limit (0 °C)

The effect of water deficits are defined as:

$$f(w) = e^{K_p(\Psi - \Psi_{max})} \quad (7)$$

where  $K_p$  is a constant;  $\Psi$  is the midday stem water potential and  $\Psi_{max}$  is the maximum value of  $\Psi$ , assumed to be -0.5 MPa based on our observations.

The L model is described by Leuning (1995) as:

$$g_1 = \frac{a_1 * A}{(c_s - \Gamma) * (1 - D/D_0)} + g_0 \quad (8)$$

where,  $a$ ,  $g_0$  and  $D_0$  are constants,  $D$  is the VPD (kPa);  $c_s$  is the  $CO_2$  concentration at the leaf surface (p.p.m) and  $\Gamma$  is the  $CO_2$  compensation point. The first two variables ( $D$  and  $c_s$ ) were measured with the CIRAS-1 porometer at the same time as  $g_1$  and  $A$ . The value of  $\Gamma$  was taken as  $46 \mu mol mol^{-1}$ , obtained by Bongji & Palliotti (1994) in potted olive trees. We observed that the  $D_0$  values varied with the optimization interval used. Leuning, Dunin & Wang (1998) and Van Wijk *et al.* (2000) reported that the L model had low sensitivity to variations in  $D_0$ . Therefore, we used a constant value of 3.5 kPa for  $D_0$  (Leuning *et al.* 1995) and then obtained the values for the other constants.

To include the effects of water deficits in the L model, we tested another model (hereafter model L $\Psi$ ) by incorporating Eqn 7 into Eqn 8 as:

$$g_1 = \left( \frac{a_1 * A}{(c_s - \Gamma) * (1 - D/D_0)} + g_0 \right) * e^{K_p(\Psi - \Psi_{max})} \quad (9)$$

The  $A$  and  $g_1$  data described above were used in the calibration and verifications of the models. Experimental  $A$  and  $g_1$  values for T1 and T2 were used for model calibrations.  $A$  and  $g_1$  data from T3 and T4 were used for model verification under conditions of moderate (T3) and severe (T4) water stress. Data from the diurnal cycles were also used to verify the model under a different set of environmental conditions.

## Statistical analyses

The model parameters were optimized with a non-linear multiparameter optimization routine designed by F. Villalobos. All the linear regressions were calculated with Excel and paired comparisons of the linear regressions were performed following Steel & Torrie (1985).

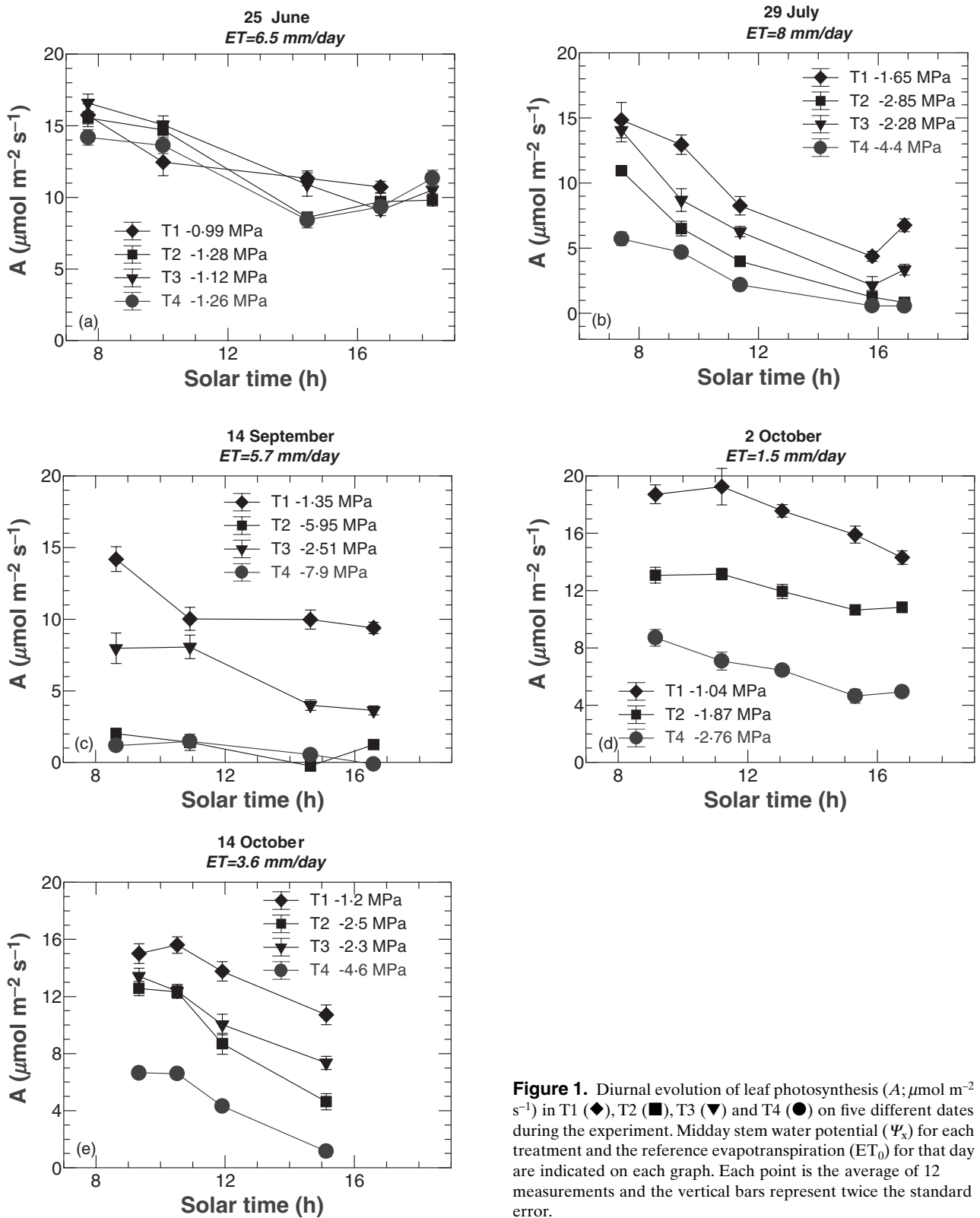
## RESULTS

### Water stress effects on the diurnal patterns of leaf gas exchange

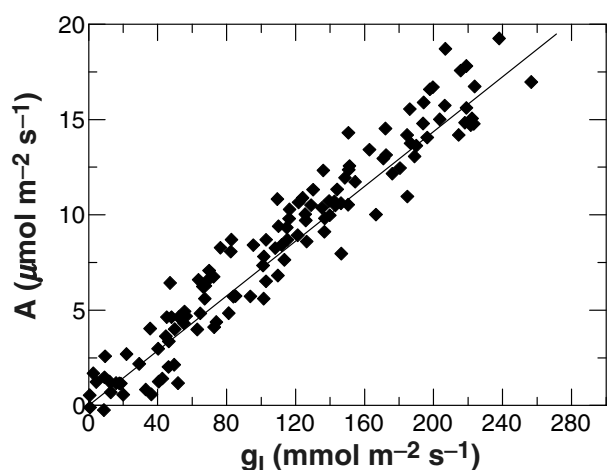
Leaf photosynthesis  $A$  was affected by time of day, day of the year, and the level of water stress. Figure 1 depicts the diurnal cycles of leaf  $A$  at representative days in 1998, for the four treatments. On 25 June, when water stress was very mild in T2 to T4,  $A$  followed a similar pattern, characteristic of woody Mediterranean vegetation (Tenhunen *et al.* 1987) with a maximum value in the morning that declines towards midday. By 29 July, evaporative demand and soil water deficits had increased, and there were obvious treatment differences in  $A$  throughout the day (Fig. 1b). Later on, the summer drought period caused tree  $\Psi_x$  in T2 and T4 to decline to very low values and, consequently,  $A$  was very low in both treatments (14 September; Fig. 1c). The lower VPD and reference evapotranspiration ( $ET_0$ ) characteristic of the Mediterranean autumn, led to very high  $A$  in T1 on 2 October, whereas the  $A$  of T2 and T4 recovered substantially from values in Fig. 1c following re-irrigation and rainfall, respectively (Fig. 1d). By 14 October, water deficits had developed again in T2 to T4 and  $A$  was substantially less in the water-stressed treatments than in T1 (Fig. 1e).

The behaviour of leaf  $g_1$  followed very closely that of  $A$  as shown by the close correlation between the two parameters, depicted in Fig. 2. Nevertheless,  $A$  was relatively more affected than  $g_1$  under severe water stress, at  $\Psi_x$  values below -4 MPa, as discussed below.

The diurnal evolution of TE followed a pattern similar to that of  $A$ , with a maximum value in the morning hours and a minimum at midday. Figure 3 presents the TE for all treatments in three days representative of the patterns observed. The evaporative demand determined the magnitude of TE; on 25 June (Fig. 3a), the minimum TE was about half that of 2 October (Fig. 3b), but higher than on 29 July, the hottest day, that also presented the lowest maximum TE values. There were no consistent differences in TE among treatments except when severe stress conditions ( $\Psi_x < -4.0$  MPa) affected T2 and T4 (e.g. T4 on 29 July).



**Figure 1.** Diurnal evolution of leaf photosynthesis ( $A$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in T1 ( $\blacklozenge$ ), T2 ( $\blacksquare$ ), T3 ( $\blacktriangledown$ ) and T4 ( $\bullet$ ) on five different dates during the experiment. Midday stem water potential ( $\Psi_x$ ) for each treatment and the reference evapotranspiration ( $ET_0$ ) for that day are indicated on each graph. Each point is the average of 12 measurements and the vertical bars represent twice the standard error.



**Figure 2.** Regression of photosynthesis ( $A$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) on leaf conductance ( $g_l$ ;  $\text{mmol m}^{-2} \text{s}^{-1}$ ). Each point represents the average of 12 measurements taken during the diurnal cycles on trees where midday  $\Psi_x$  was higher than  $-4.5$  MPa. ( $y = 0.074x$ ;  $R^2 = 0.92^{***}$ ;  $\text{RMSE} = 1.31$ ;  $n = 95$ )

### Seasonal effects of water stress on leaf gas exchange

Leaf gas exchange was monitored around midday at frequent intervals during the season. Figure 4 presents the seasonal evolution of  $A$  and  $g_l$  in the four treatments. Treatment differences were greatest around midsummer, although the rain-fed trees differed significantly from the others throughout the season. In the absence of water stress (T1), both  $A$  and  $g_l$  varied among days, being generally high during the autumn and low during days of high evaporative demand (Fig. 4). Rain-fed trees (T4) never recovered completely from severe stress with the autumn rains, whereas T2 trees had  $A$  and  $g_l$  values similar to those of T1, 2–3 weeks after water was applied following the summer drought period (Fig. 4).

Leaf conductance was associated with VPD but the relationship varied with the level of water stress, as shown in Fig. 5. The slope of the regression line of  $g_l$  on VPD decreased as water stress increased to the point that there was no relationship between the two for  $\Psi_x$  levels below  $-4$  MPa (Fig. 5).

The variation of TE throughout the season was also related to variations in VPD (Fig. 6a) and, generally, it did not differ among treatments. However, there was evidence that TE was decreased by severe water stress, as shown in Fig. 6b. In cases where trees rehydrated following the summer drought, there were indications that TE values departed from the generalized relationship of Fig. 6a (Fig. 6b).

### Models of leaf conductance

The three models were calibrated with experimental data of T1 and T2 and the parameters obtained are presented in Table 1. Two validation analyses were performed. Table 2

presents the results of the validation performed with data collected at different times of the day in T3, under moderate water stress and in T4 with severe stress. All three models tended to underestimate  $g_l$  but their predictive ability depended on the level of water stress. The L model was the best of the three under moderate water deficits, whereas the J model was the poorest (Table 2). However, when the severe stress data (T4) was used for the validation, both the L $\Psi$  and the J models performed well, whereas the L was the least accurate (Table 2). Additional verification tests were performed with the data of the diurnal cycles of Fig. 1, which were not included in the calibration and the validation analysis of Table 2. Table 3 presents the performance features of the three models and Fig. 7 depicts an example where the model predictions are shown against  $g_l$  measurements in 29 July for T1 and T4.

Plant responses to water deficits are often quantified in relation to available soil water (Stewart 1988). To provide relationship for modelling  $g_l$  of olives as a function of soil water levels we developed the relation between stem  $\Psi$  and available soil water in the four treatments (Fig. 8). This relationship was introduced into the equations of the J and L $\Psi$  models, accounting for the effects of water potential (Eqn 7) yielding the following relationships:

$$\text{J Model } f(w) = e^{0.35(-8e^{-0.03\text{ASW}} + 0.5)} \quad (10)$$

$$\text{J Model } f(w) = e^{0.18(-8e^{-0.63 \cdot \text{ASW}} + 0.5)} \quad (11)$$

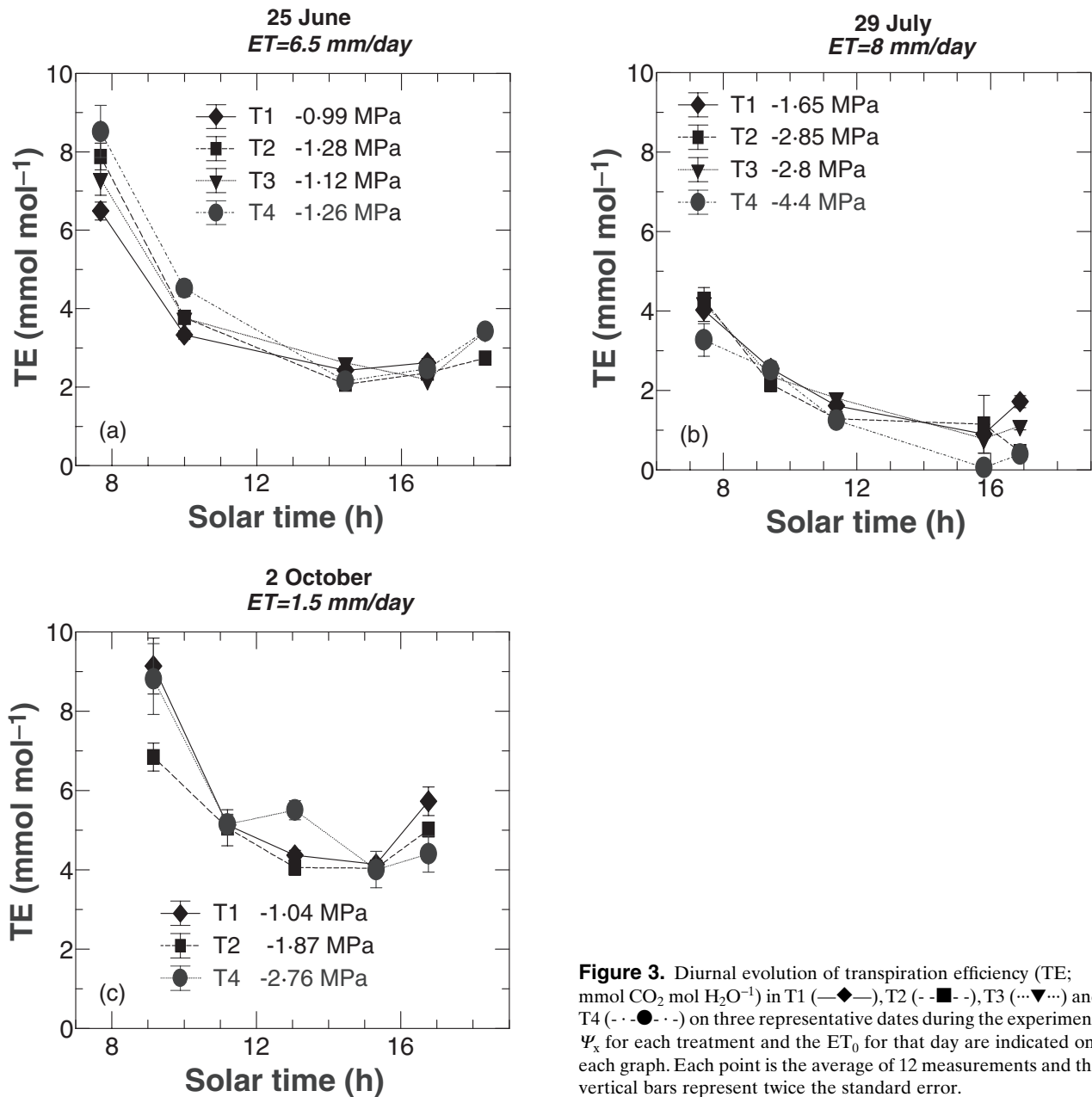
where ASW is the available soil water (%).

## DISCUSSION

### Leaf photosynthesis and stomatal conductance as affected by water deficits

The diurnal trends of  $A$  in olive, followed typical patterns described for woody Mediterranean vegetation (Tenhunen *et al.* 1987), with a maximum in the morning, which declined continuously towards the afternoon. The water deficits experienced by the trees in the T2, T3 and T4 had a significant effect on the diurnal course of  $A$  (Fig. 1). As water stress increased, both the maximum and minimum  $A$  values declined, although they were above zero for most of the day, even at a  $\Psi_x$  of  $-8$  MPa (Fig. 1c). Evaporative demand affected the minimum  $A$  level attained by the control trees; on 2 October, a low evaporative demand day, minimum  $A$  was  $15 \mu\text{mol m}^{-2} \text{s}^{-1}$ , whereas it varied between 6 and  $11 \mu\text{mol m}^{-2} \text{s}^{-1}$  when evaporative demand was high (Figs 1a, b, c).

Leaf conductance followed diurnal patterns, which were very similar to those of  $A$  depicted in Fig. 1. The close correlation between  $A$  and  $g_l$  that we found (Fig. 2) is common, but it does not aid in elucidating the role of stomata in controlling photosynthesis in olive (Jones 1998). However, water deficits in olive affected both  $A$  and  $g_l$  in a similar way, as shown by the parallel decline in both parameters as the tree water status decreased from  $\Psi_x$  of  $-1.0$  MPa down to less than  $-4$  MPa.

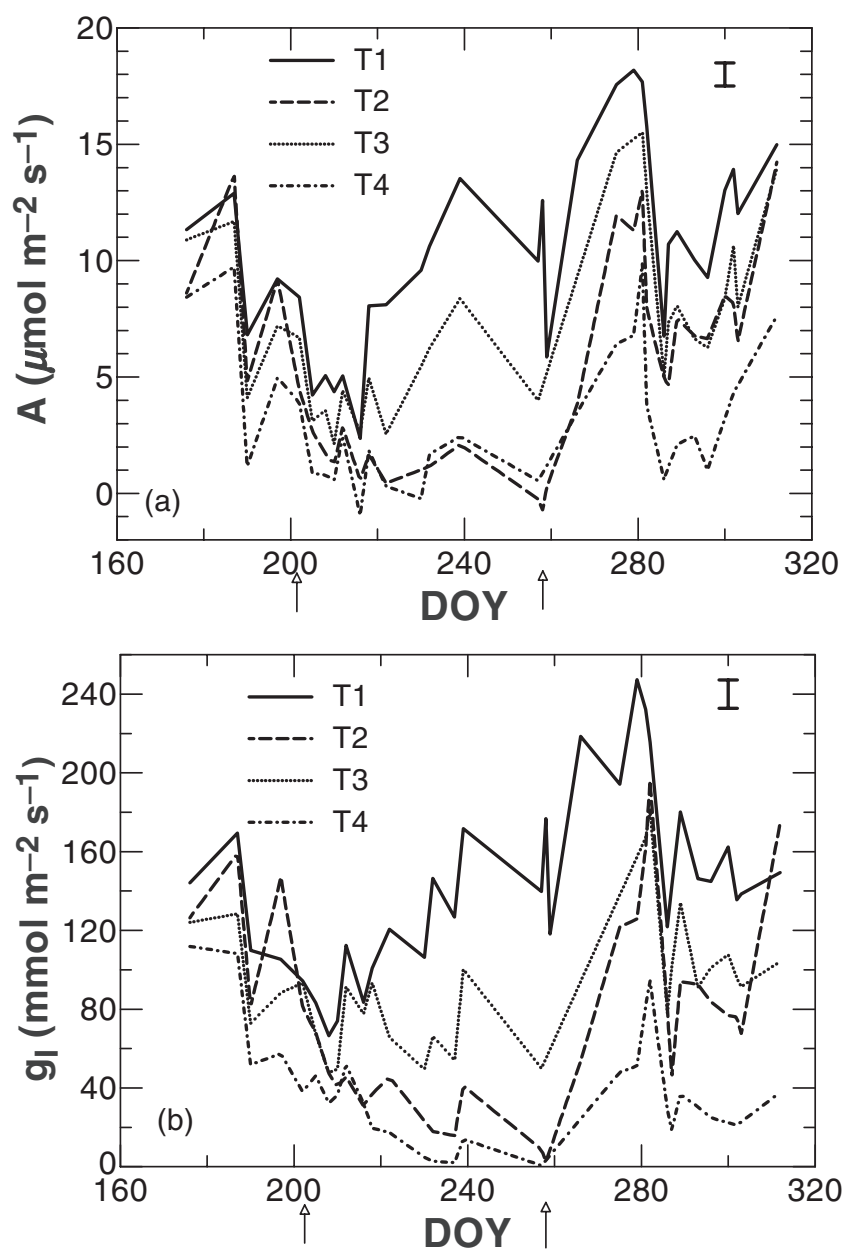


**Figure 3.** Diurnal evolution of transpiration efficiency (TE; mmol CO<sub>2</sub> mol H<sub>2</sub>O<sup>-1</sup>) in T1 (—◆—), T2 (- -■- -), T3 (···▼···) and T4 (- · · · ● · · ·) on three representative dates during the experiment.  $\Psi_x$  for each treatment and the  $ET_0$  for that day are indicated on each graph. Each point is the average of 12 measurements and the vertical bars represent twice the standard error.

It is often assumed that the diurnal increase in VPD is the environmental variable driving stomatal behaviour and causing the midday depression in  $A$  in Mediterranean woody vegetation (Tenhunen *et al.* 1987; Tognetti *et al.* 1998). There are diverse observations on the response of olive trees to VPD under field conditions. Giorio *et al.* (1999) did not observe a correlation between  $g_1$  and VPD on young trees in the field, whereas Fernández *et al.* (1997) had a number of field observations that suggested an upper-bound relationship between  $g_1$  and VPD. In both of these cases, trees were either well watered or under moderate water stress (leaf  $\Psi$  of about -3 MPa). The apparent lack of sensitivity of  $g_1$  to VPD in trees under severe stress ( $\Psi_x <$

-6 MPa) reported in Fig. 5 is obviously related to the overriding effect of water deficits on most of the tree functions, including leaf gas exchange. Hypotheses that take into account the effect of soil water deficits on stomatal behaviour via hydraulic and chemical signals (Jones 1998) are useful in explaining the response depicted in Fig. 5.

The modulation of seasonal  $A$  and  $g_1$  by water deficits was apparent through the changes observed in T2, T3 and T4. The summer drought period caused  $A$  and  $g_1$  of T2 to drop to values very similar to those of the rain-fed treatment (Fig. 4). However, recovery of  $A$  and  $g_1$  following rehydration was faster in T2 than in T4, perhaps reflecting more conservative internal controls in T4 trees which had



**Figure 4.** (a) Seasonal evolution of midday leaf photosynthesis ( $A$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ); and (b) midday leaf conductance ( $g_l$ ;  $\text{mmol m}^{-2} \text{s}^{-1}$ ) for treatments T1, T2, T3 and T4. Arrows in the graph indicate the drought period of T2. Vertical bars on each graph indicate a representative value of twice the standard error.

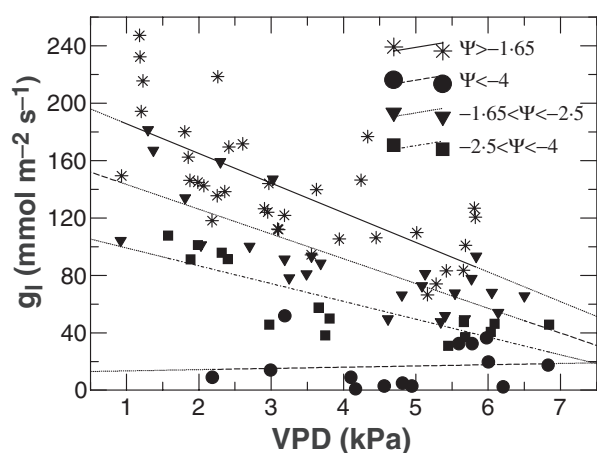
been subjected to more prolonged water deficits. The speed and degree of  $A$  and  $g_l$  recovery in T2 trees is remarkable, even though they had reached  $\Psi_x$  values close to  $-6$  MPa at the end of the drought period. Fereres *et al.* (1979) observed that citrus trees, which had been subjected to similar levels of water stress, did not fully recover their  $g_l$  even after 2 months of full rehydration.

#### Transpiration efficiency as affected by water deficits

Transpiration efficiency followed a diurnal course similar to those of  $A$  and  $g_l$  (Fig. 3). All TE observations in trees that had  $\Psi_x$  higher than  $-4.0$  MPa fitted very well the expected

inverse hyperbolic relationship with VPD (Fig. 6a). The observed, average TE values for olive at VPD's of 1.0 and 2.5 kPa (5.2 and 2.7  $\text{mmol CO}_2 \text{ mol H}_2\text{O}^{-1}$ ) are very similar to those reported by Turner (1986) for almond and pistachio trees (3.9–4.8 and 2.7–2.5  $\text{mmol CO}_2 \text{ mol H}_2\text{O}^{-1}$ , respectively). Water deficits did not affect TE until very severe water stress had reduced TE (Fig. 6b).

The present results contrast with reports indicating that water stress increases TE in woody plants (*Pinus ponderosa* Dougl. ex Laws, Zhang *et al.* 1997; *Fraxinus americana* L., Premachandra, Chaney & Holt 1997; *Vitis vinifera* L., Iacono, Buccella & Peterlunger 1998; *Pinus radiata* D. Don, Korol *et al.* 1999). Theoretically, reduced  $g_l$  induced by water deficits lead to increased TE by affecting  $T$  propor-

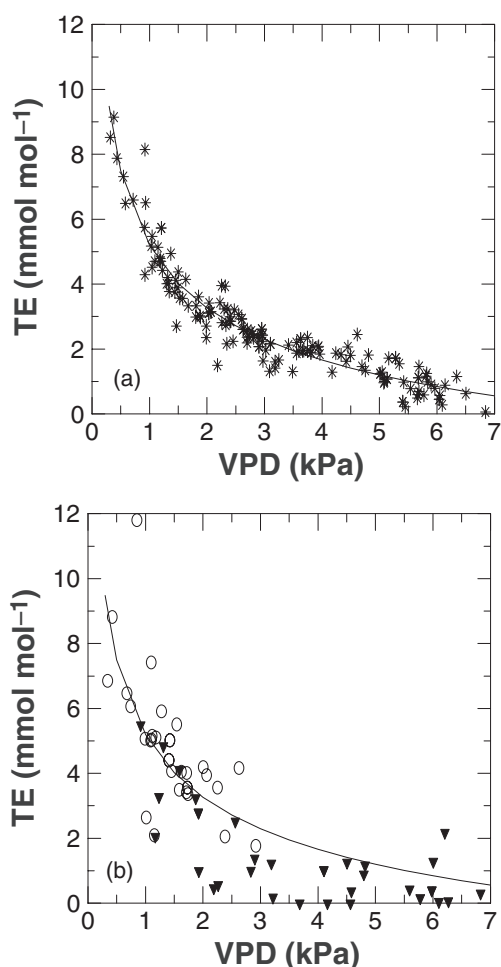


**Figure 5.** Relationships between midday leaf conductance ( $g_l$ ;  $\text{mmol m}^{-2} \text{s}^{-1}$ ) and the vapour pressure deficit (VPD; kPa) for four different levels of water stress. Each point is the mean of 12 observations at various levels of  $\Psi$  as follows: (—\*)  $\Psi > -1.65$  MPa;  $y = 206.4 - 20.7x$ ;  $R^2 = 0.5^{***}$ ;  $n = 36$ ; RMSE = 30.7; (-●-)  $-1.65 < \Psi < -2.5$  MPa;  $y = 160.6 - 17.3x$ ;  $R^2 = 0.6^{***}$ ;  $n = 26$ ; RMSE = 23.7; (—■)  $-2.5 < \Psi < -4$  MPa;  $y = 111.5 - 12.4x$ ;  $R^2 = 0.66^{***}$ ;  $n = 15$ ; RMSE = 15.9; (-●●-)  $\Psi < -4$  MPa;  $n = 14$ ; NS.  $^{***}P < 0.0001$ .

tionally more than  $A$  (Jones 1992). However, it has been observed in many cases that  $g_l$  and  $A$  change in proportion, so that there is little net change in TE with increasing water stress (Condon & Hall 1997). The underlying feedback mechanisms of  $g_l$  on  $A$  are subject to both hydraulic and chemical controls (Jones 1998). One response that would decrease TE as  $g_l$  is reduced, is the increase in leaf temperature leading to increased VPD and  $T$  (Condon & Hall 1997). The reduction of TE by severe water deficits observed in Fig. 6b must be caused by the direct effects of dehydration on chloroplast function (Brodribb 1996). At

**Table 1.** Calibration of the J model (Table 1a) and the L and  $L\Psi$  models (Table 1b). Only the data of Treatments 1 and 2 were used in the calibration ( $n = 1280$ ). The Residual Mean Square Error (RMSE) values of the model predictions were 41.8, 40.6 and 36.9  $\text{mmol m}^{-2} \text{s}^{-1}$  for the J, L and  $L\Psi$  models, respectively. For explanations of the symbols, see text

| (a) Parameters | J model | Units                              |                                    |
|----------------|---------|------------------------------------|------------------------------------|
| $G_{\max}$     | 341.53  | $\text{mmol m}^{-2} \text{s}^{-1}$ |                                    |
| $K_R$          | 19.70   | $\text{W m}^{-2}$                  |                                    |
| $K_D$          | 0.022   | $\text{kg g}^{-1}$                 |                                    |
| $D_c$          | 11.43   | $\text{g kg}^{-1}$                 |                                    |
| $K_T$          | 24.13   | $^{\circ}\text{C}$                 |                                    |
| $K_P$          | 0.35    | $\text{MPa}^{-1}$                  |                                    |
| (b) Parameters | L model | $L\Psi$ model                      | Units                              |
| $D_0$          | 3.5     | 3.5                                | kPa                                |
| $a_1$          | 4530    | 4320                               |                                    |
| $g_0$          | 45      | 84                                 | $\text{mmol m}^{-2} \text{s}^{-1}$ |
| $K_P$          |         | 0.18                               | $\text{MPa}^{-1}$                  |



**Figure 6.** Relationship between transpiration efficiency (TE;  $\text{mmol CO}_2 \text{ mol H}_2\text{O}^{-1}$ ) and the vapour pressure deficit (VPD; kPa). (a) Only data from trees with  $\Psi_x$  greater than  $-4$  MPa ( $y = -6.48 + 11.69x^{-0.26}$ ;  $R^2 = 0.91^{***}$ ;  $n = 154$ ) (b) Data from trees with  $\Psi_x$  lower than  $-4$  MPa (▼) and following rehydration after the summer drought period (○).

that time, the level of tree gas exchange is extremely low (Fig. 3b) and the impact of the reduced TE on the seasonal carbon and water balances of the orchard is probably insignificant. The present evidence that TE increases following rehydration is inconclusive (Fig. 6b) even though Larcher, De Moraes & Bauer (1981) observed increased TE in the potted olive trees after several cycles of water deficits. The significance of a consistent TE-VPD relationship over a wide range of water status, suggest that it may be possible to use it for estimation of the gross carbon assimilation of olive in a given climate, if the water available for transpiration is known. The achievement of such an objective will require scaling up the TE estimates, from the instantaneous, leaf level depicted in Fig. 6 up to the canopy level on a daily and seasonal basis.

Olive trees in T4 completely depleted the soil profile during the summer and reached very low  $\Psi_x$  values, much lower than those observed in most crop plants subjected to



| Models      | RMSE<br>(mmol m <sup>-2</sup> s <sup>-1</sup> ) | a                 | b                 | R <sup>2</sup> | b                   | R <sup>2</sup> |
|-------------|---|-------------------|-------------------|----------------|---------------------|----------------|
| T3 L        | 29.5  | 32.0 <sup>c</sup> | 0.76 <sup>a</sup> | 0.73***        | 0.99 <sup>NSa</sup> | 0.65***        |
| T3 L $\Psi$ | 31.6  | 47.6 <sup>b</sup> | 0.68 <sup>b</sup> | 0.70***        | 1.01 <sup>NSa</sup> | 0.48***        |
| T3 J        | 36.5  | 57.8 <sup>a</sup> | 0.50 <sup>c</sup> | 0.56***        | 0.91 <sup>b</sup>   | 0.09***        |
| T4 L        | 36.9  | 41.4 <sup>a</sup> | 0.71 <sup>b</sup> | 0.78***        | 1.06 <sup>a</sup>   | 0.42***        |
| T4 L $\Psi$ | 29.5  | 23.8 <sup>b</sup> | 0.88 <sup>a</sup> | 0.82***        | 1.08 <sup>a</sup>   | 0.74***        |
| T4 J        | 29.9  | 16.4 <sup>c</sup> | 0.88 <sup>a</sup> | 0.78***        | 1.01 <sup>NSb</sup> | 0.74***        |

**Table 2.** Results of the linear regression ( $y = a + bx$ ) of observed on estimated leaf conductance for the L, L $\Psi$  and J models. The data used were collected in the treatments T3 and T4. Different letters in the same column and treatment represent significant differences ( $P < 0.05$ ) and NS indicates that the slope is not significantly different from 1 ( $P < 0.05$ ). (The numbers of individual measurements used in the validation were:  $n = 755$  for T3L;  $n = 710$  for T3L $\Psi$  and T3J;  $n = 787$  for T4L;  $n = 697$  for T4L $\Psi$  and T4J)

| Model          | RMSE<br>(mmol m <sup>-2</sup> s <sup>-1</sup> ) | a                 | b                 | R <sup>2</sup> | b                 | R <sup>2</sup> |
|----------------|---|-------------------|-------------------|----------------|-------------------|----------------|
| J model        | 35.7  | 33.2 <sup>c</sup> | 0.73 <sup>a</sup> | 0.75***        | 0.94 <sup>a</sup> | 0.66***        |
| L model        | 30.8  | 41.0 <sup>a</sup> | 0.72 <sup>a</sup> | 0.83***        | 0.97 <sup>a</sup> | 0.67***        |
| L $\Psi$ model | 32.0  | 36.9 <sup>b</sup> | 0.73 <sup>a</sup> | 0.82***        | 0.96 <sup>a</sup> | 0.7***         |

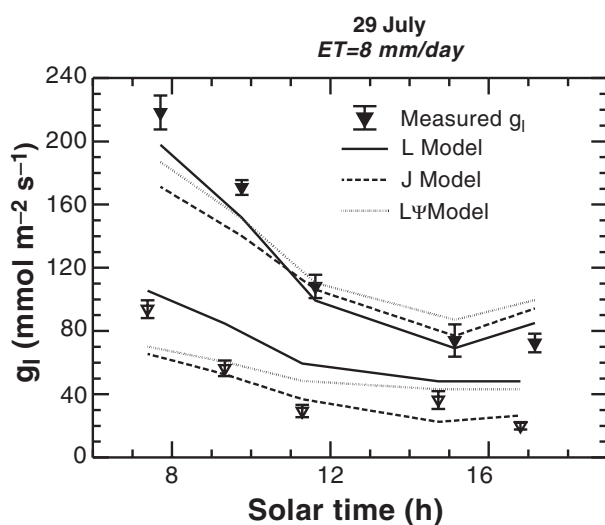
**Table 3.** Results of the linear regression ( $y = a + bx$ ) of observed on estimated leaf conductance for the L, L $\Psi$  and J models. The data correspond to individual measurements of the diurnal cycles. Different letters in the same column represent significant differences.  $P < 0.05$ .  $n = 886$

drought. By the time  $\Psi_x$  reached  $-8$  MPa, the soil water content in the 240 cm profile was 150 mm, whereas the available water at a soil matric potential of  $-1.5$  MPa, estimated from soil-based measurements, was 192 mm. Evidently, the low  $\Psi_x$  was the driving force leading to soil water extraction by the trees, well beyond the standard lower limit of water availability for crop plants (Cassel, Ratliff & Ritchie 1983).

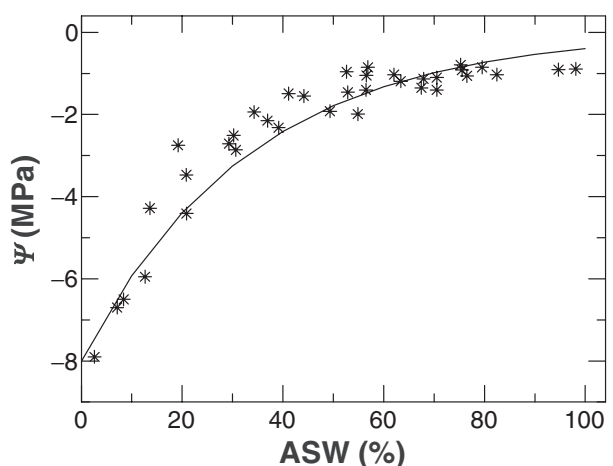
### Performance of leaf conductance models

Despite the large number of observations used in the calibration of the models, none of the three models developed

for predicting the  $g_l$  of olive leaves gave very accurate predictions (Tables 2 and 3). Of the three models, the two based on Leuning's (1995) model performed better in the two validation tests. When data from a moderate stress treatment (T3) was used for validation, the L and L $\Psi$  models were more precise than the J model, as indicated by a lower residual mean squared error (RMSE; Table 2). Similarly, when the data from the diurnal cycles was used for validation, the L and L $\Psi$  models had lower RMSE than the J model (Table 3). It was only when data from T4, the rainfed treatment, were used for validation that the J model outperformed the L model; however, when the L model was modified to include a water stress component (the L $\Psi$  model), its performance was as good as that of the J model (Table 2).



**Figure 7.** Comparison between measured leaf conductance and predictions of the three models for the 29 July. Only the data for T1 (closed symbols) and T4 (open symbols) are presented. Each point is the average of 12 measurements and the vertical bars represent twice the standard error.



**Figure 8.** Relationship between available soil water (ASW; %) in the 240 cm profile and midday stem water potential ( $\Psi$ ; MPa). ( $y = -8e^{-0.03x}$ ;  $n = 36$ ;  $R^2 = 0.93***$ ).

Baille *et al.* (1996) found that an L model gave better predictions of  $g_1$  of rose leaves than a J model, in the absence of water deficits. Van Wijk *et al.* (2000) compared the use of three models of stomatal conductance in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), including the Leuning and the Jarvis models. They found that both models performed satisfactorily as components of the simulation of carbon and water fluxes when their parameters were optimized. Previous modelling efforts of leaf  $g_1$  in olive were based on J type models; for instance, Moreno *et al.* (1996) used a J model with only two variables, radiation and VPD and failed to predict the  $g_1$  of rain-fed olive trees subjected to moderate stress after the model was calibrated with data from well-watered trees.

All three models tested here predicted  $g_1$  with limited accuracy (Tables 2 and 3). The degree of scattering and the variations observed from day to day and in different times of the year suggest that, in addition to the environmental variables considered, there are endogenous factors influencing the  $g_1$  of olive leaves. A number of recent reports indicate that factors such as time of day (Mencuccini, Mambelli & Comstock 2000) or crop load (Palmer, Giuliani & Adams 1997) seem to play a role in modulating the hydraulic and chemical signals that control  $g_1$ . It would be important to determine if the models developed here for olive at the leaf level, have sufficient accuracy to predict the carbon and water fluxes at the canopy level.

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