

Tree water status and gas exchange in walnut under drought, high temperature and vapour pressure deficit

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SUMMARY

Drought reduces photosynthesis in walnut (*Juglans regia* L.), but it is not known whether this is due mainly to the closure of stomata, or to possible effects on leaf biochemistry. In an attempt to answer this question we studied diurnal changes in the water status and gas exchange in droughted [50% crop evapotranspiration (ET_c)] and fully irrigated (100% ET_c) walnut trees, over 2 d. Stem water potential (Ψ_s) ranged from -0.5 MPa in the morning to -1.2 MPa in the afternoon under drought, and from -0.1 MPa to -0.4 MPa under full watering. Net CO_2 assimilation (A_{max}) ranged from $15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the morning to $3 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the afternoon under drought, and from $25 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the morning to $10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the afternoon under full watering. At these times, stomatal conductance (g_s) varied from 0.2 to $0.02 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and from 0.7 to $0.2 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, respectively. Drought reduced the internal CO_2 concentration (C_i) by about $55 \mu\text{mol mol}^{-1}$ on day-1, and by about $100 \mu\text{mol mol}^{-1}$ on day-2 and increased leaf temperature (T_l) by about $2^\circ\text{--}5^\circ\text{C}$. The reductions in g_s and C_i with drought suggest that lower photosynthesis was associated with stomatal closure. However, in each treatment, A_{max} decreased during the day, while C_i was stable, suggesting that photosynthesis was also reduced by a direct effect of heat on leaf biochemistry. Both A_{max} and g_s correlated with T_l and with the leaf-to-air vapour pressure deficit (VPD_l), but with different relationships for droughted and control trees. However, when stomatal limitations to photosynthesis were accounted for (i.e., based on the assumption that, under stomatal limitation, photosynthesis is proportional to C_i , a single relationship between A_{max} and T_l described all the data ($R^2 = 0.81$). Thus, photosynthesis was limited by the closing of stomata under drought, and by a direct effect of heat on leaf biochemistry. These results suggest that hot and dry weather reduces photosynthesis and potential productivity in walnut in the absence of soil water deficit.

Given the World's increasing water shortage and rising demands, the effects of drought on the physiology and productivity of tree crops is of growing interest and is not completely understood. Different species have different ways of adapting to drought. Walnuts close stomata under high leaf-to-air vapour pressure deficit (VPD_l) or low leaf water potential (Ψ_l) (Lucier and Hinckley, 1982), preventing the stem water potential (Ψ_s) from becoming lower than -1.4 MPa, when cavitation occurs in the xylem (Tyree *et al.*, 1993; Cochard *et al.*, 2002). Hence walnut has been defined as a "drought avoider" (Lucier and Hinckley, 1982).

Light-saturated net CO_2 assimilation rate (A_{max}) and stomatal conductance (g_s) are closely related in many species (Castel and Fereres, 1982; Lakso, 1994; Romero *et al.*, 2004). However it is not clear whether the reduction in carbon fixation is due to closing of stomata or changes in leaf biochemistry. In walnut, A_{max} decreases at high temperatures (Foott and Heinicke, 1967; Lucier and Hinckley, 1982), but it is not clear whether

temperature has a direct effect on photosynthesis, or just affects g_s . Another hypothesis is that A_{max} and g_s are co-regulated under water stress (Bunce, 1977; Escalona *et al.*, 1999).

While, at times, g_s is correlated with VPD_l (Schulze *et al.*, 1972; Jarvis, 1976; Watson *et al.*, 1978; Thorpe *et al.*, 1980; Fanjul and Jones, 1982; Grantz, 1990), an increasing body of literature suggests that g_s depends on leaf water status (Cowan, 1977; Fuchs and Livingston, 1996; Comstok and Mencuccini, 1998), possibly leaf or turgor pressure potentials (Jarvis, 1976; Garnier and Berger, 1987; Lakso, 1994; Franks *et al.*, 1995). Thus, while both water status and VPD_l affect g_s , the mechanisms of such responses are not clear.

The objective of this work was to test whether the decline in photosynthesis in walnut under drought and/or high temperature and VPD_l was due to lower internal CO_2 concentration (C_i) as the stomata close, or to a direct effect on leaf biochemistry. We also investigated the relative impact on photosynthesis of soil water deficit vs. atmospheric stress (i.e., high temperature and VPD_l). Additionally, we attempted to investigate possible mechanisms of stomatal closure.

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MATERIALS AND METHODS

Plant material

The experiment was carried out during Summer 2003 in a 10 year-old walnut (*Juglans regia* L. 'Chandler') orchard in Tehama County, California. Trees were spaced 4.7 m apart in rows 7.3 m apart. Trees were hedged on alternate sides each year and received standard commercial agronomy.

The orchard was split into fully-irrigated control and droughted plots that were irrigated every 2–3 d with micro-sprinklers providing about 100% of crop evapotranspiration (ET_c) or 50% ET_c , respectively. ET_c was calculated each week based on an estimated crop coefficient and modified Penman reference crop water use (ET_0) obtained from a nearby weather station (California Irrigation Management Information System, CA, USA). On 18 and 19 August, water status and gas exchange were measured approx. every 2 h on four trees per treatment.

Water status

Stem water potential (Ψ_s) was measured using the bagged leaf technique (McCutchan and Shackel, 1992) in a Scholander-type pressure chamber, using two shaded leaves per tree, near the trunk (i.e., eight readings per treatment). Since single leaflets were bagged, our Ψ_s corresponded to what Cochard *et al.* (2002) called Ψ_{rachs} .

Gas exchange

Light-saturated net CO_2 assimilation (A_{max}), stomatal conductance (g_s), transpiration (E), and internal CO_2 concentration (C_i) were determined using a portable computerised open-system IRGA (LI-6400; LI-COR Inc., Lincoln, NE, USA). Leaf temperature (T_l) and VPD_l in the chamber were also recorded. A computer-controlled cool light source (6400-02 LED) mounted on the chamber provided photosynthetically-active radiation (PAR) of $2,000 \mu mol m^{-2} s^{-1}$. Leaves were kept in the chamber for 1–3 min until photosynthesis was constant.

Measurements were taken on four, fully-exposed, upper-canopy leaves per tree (i.e., 16 per treatment). Air flow was kept constant and the humidity not controlled, so that leaves with lower E reached equilibrium at higher T_l and VPD_l than leaves with higher E , as would happen outside the chamber.

 C_i correction of A_{max}

In order to separate stomatal and non-stomatal limitations to CO_2 assimilation, we estimated what A_{max} would have been in the absence of stomatal limitations, that is at non-limiting C_i values. To do this, we assumed that, at low C_i , assimilation was limited by low CO_2 concentration and approximately linearly related to it as we found in peach (Rosati *et al.*, 1999). Based on this linearity, we calculated a corrected A_{max} ($A_{maxCorr}$) as:

$$A_{maxCorr} = A_{max} (C_{imax} - \Gamma^*) / (C_i - \Gamma^*) \quad (1)$$

where C_{imax} ($280 \mu mol CO_2 mol^{-1}$) is the average of the top 10% of the C_i values in our dataset, and Γ^* is the CO_2 concentration at which net assimilation is zero (we assumed $\Gamma^* = 50 \mu mol CO_2 mol^{-1}$). To avoid large errors in estimating $A_{maxCorr}$, we omitted data with $C_i < 175 \mu mol$

$CO_2 mol^{-1}$. A sensitivity analysis revealed that using 40 or $60 \mu mol CO_2 mol^{-1}$ for Γ^* , and using 290 or $270 \mu mol CO_2 mol^{-1}$ for C_{imax} did not affect the R^2 of the A_{max} vs. T_l relationship.

RESULTS

Daily course of Ψ_s , gas exchange and T_l

Stem water potential (Ψ_s) decreased during the day and was lower in droughted than in control trees (Figure 1A, B). The lowest average Ψ_s values were -1.2 MPa in droughted trees and -0.4 MPa in control trees.

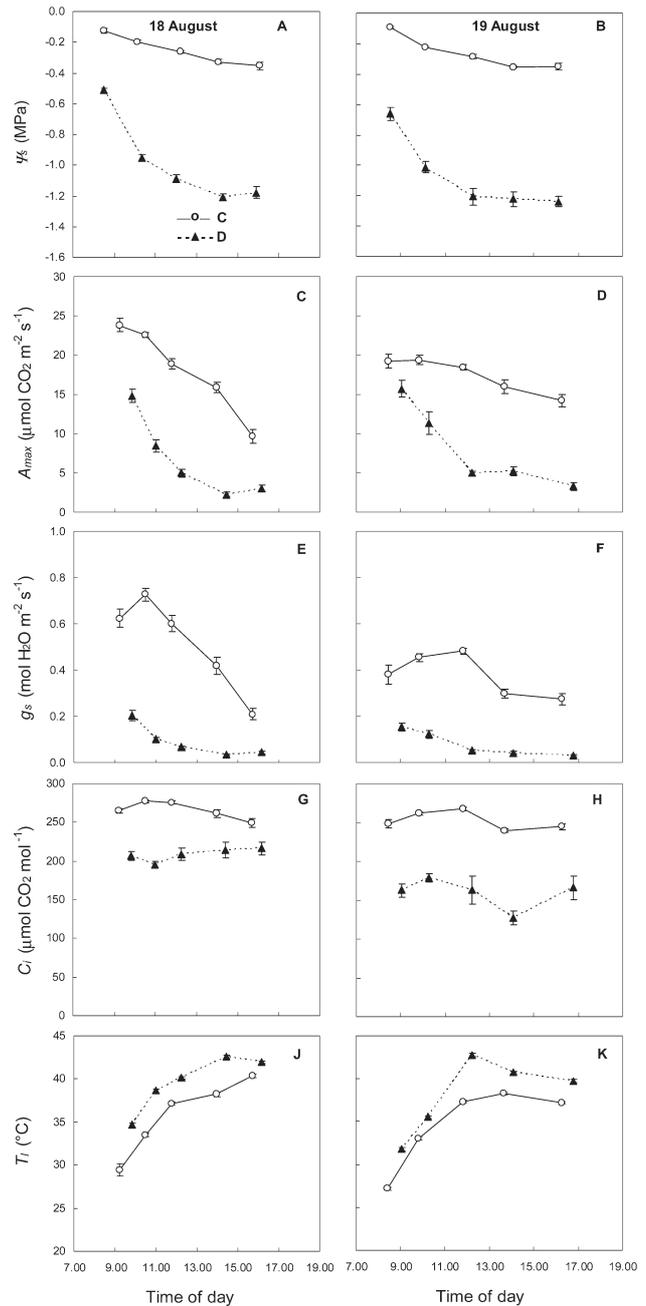


FIG. 1.

Daily course of stem water potential (Ψ_s ; Panels A, B), light-saturated net CO_2 assimilation (A_{max} ; Panels C, D), stomatal conductance (g_s ; Panels E, F), intercellular CO_2 concentration (C_i ; Panels G, H), and leaf temperature (T_l ; Panels J, K) in droughted (D; closed triangles) and control (C; open circles) trees on 18 August 2006 (left Panels) and on 19 August 2003 (right Panels). Each point is an average of 16 measurements. Bars indicate standard errors.

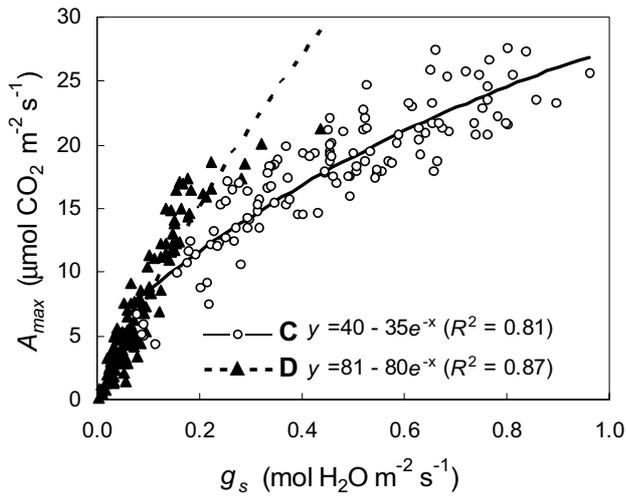


FIG. 2. Relationship between light-saturated net CO₂ assimilation (A_{max}) and stomatal conductance (g_s), in droughted (D) and control (C) trees.

Light-saturated net CO₂ assimilation (A_{max}) was highest in the early morning and decreased during the day (Figure 1C, D). However, while in the controls A_{max} decreased from 24 to 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on 18 August, and from 19 to 14 $\mu\text{mol m}^{-2} \text{s}^{-1}$ on 19 August, it decreased from 15 to 2 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the droughted treatment.

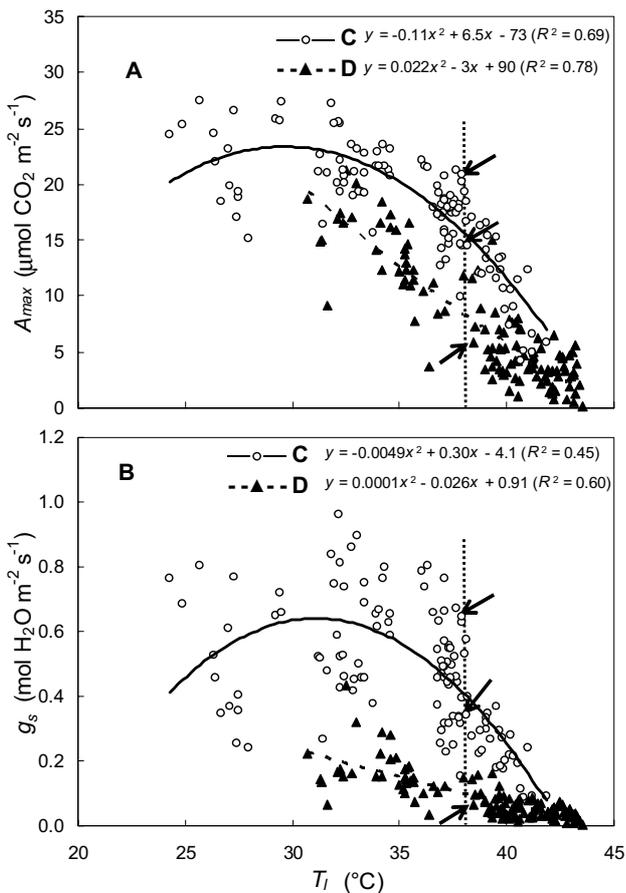


FIG. 3. Relationship between light-saturated net CO₂ assimilation (A_{max} ; Panel A) or stomatal conductance (g_s ; Panel B), and leaf temperature (T_l) in droughted (D) and control (C) trees. The three arrows indicate three leaves (i.e., one in the droughted and two in the control treatments) at similar temperature (38°C), as indicated by the vertical dotted lines, but different A_{max} and g_s .

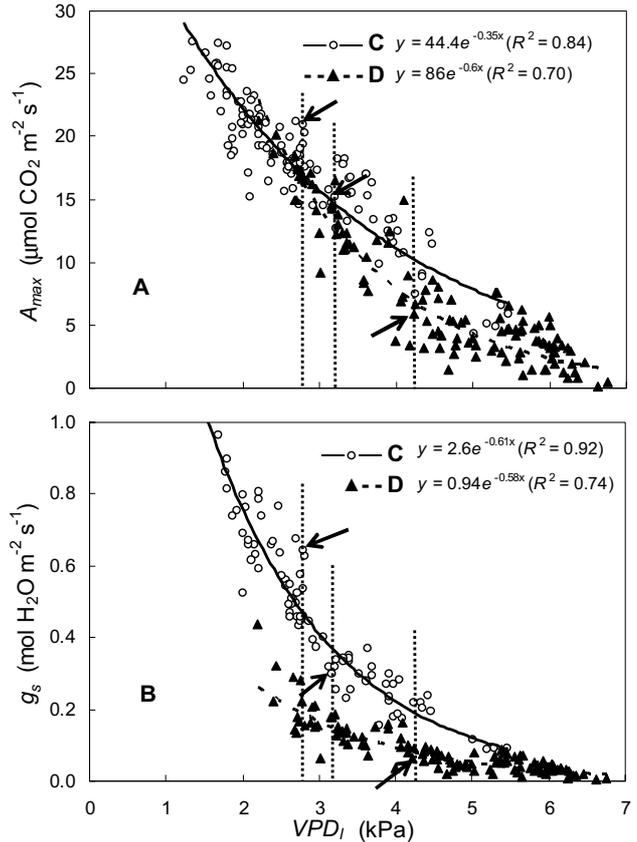


FIG. 4. Relationship between light-saturated net CO₂ assimilation (A_{max} ; Panel A) or stomatal conductance (g_s ; Panel B), and the leaf-to-air vapour pressure deficit (VPD_l) in droughted (D) and control (C) trees. The three arrows indicate the same three leaves as in Figure 3. In Panel B, early morning data, before g_s had reached its daily maximum, were omitted.

Stomatal conductance (g_s) followed A_{max} , except for increasing in the early morning before decreasing for the rest of the day in the controls (Figure 1E, F).

Intercellular CO₂ concentration (C_i) was about 55 $\mu\text{mol CO}_2 \text{mol}^{-1}$ lower in the droughted than in the control trees on 18 August, and about 100 $\mu\text{mol CO}_2 \text{mol}^{-1}$ lower on 19 August, with little variation during the day (Figure 1G, H).

Leaf temperature (T_l) increased from early morning to mid-afternoon (on 18 August) or to mid-morning (on 19 August) and was 2°–5°C higher in droughted than in control trees (Figure 1J, K).

Gas exchange vs. T_l and VPD_l

Light-saturated net CO₂ assimilation and stomatal conductance were related in both treatments, but with greater A_{max} at any g_s under drought (Figure 2).

Both A_{max} and g_s were related to T_l , but with greater A_{max} and g_s at any T_l in the controls (Figure 3). The three arrows in Figure 3A, B indicate two leaves for the control and one for the droughted treatment with similar T_l (38°C) but different A_{max} and g_s .

Light-saturated net CO₂ assimilation and stomatal conductance were closely related to VPD_l (Figure 4). The three arrows in Figure 4A, B indicate the same leaves as those in Figure 3A, B.

Corrected assimilation was closely related to T_l , across both treatments and dates (Figure 5).

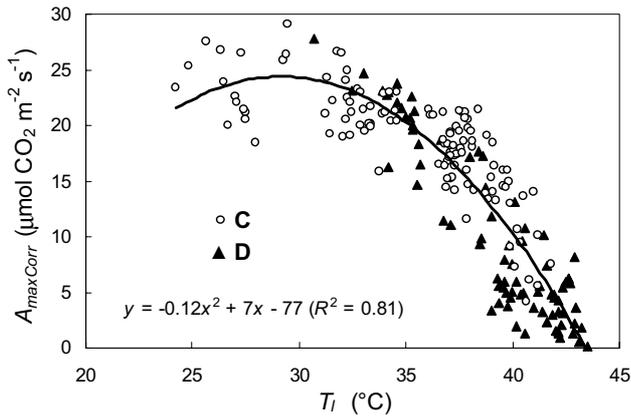


FIG. 5.

Relationship between light-saturated net CO₂ assimilation corrected for stomatal limitation ($A_{maxCorr}$) and leaf temperature (T_l), in droughted (D) and control (C) trees. The line and the equation represents a fit to all data.

Gas exchange vs. Ψ_s

Light-saturated net CO₂ assimilation decreased with Ψ_s , but with different relationships in the two groups (Figure 6). Stomatal conductance also decreased with Ψ_s , but with a closer relationship under drought (Figure 6).

Stem water potential decreased linearly with increasing VPD_l , with droughted trees having lower Ψ_s at any VPD_l than the controls (Figure 7).

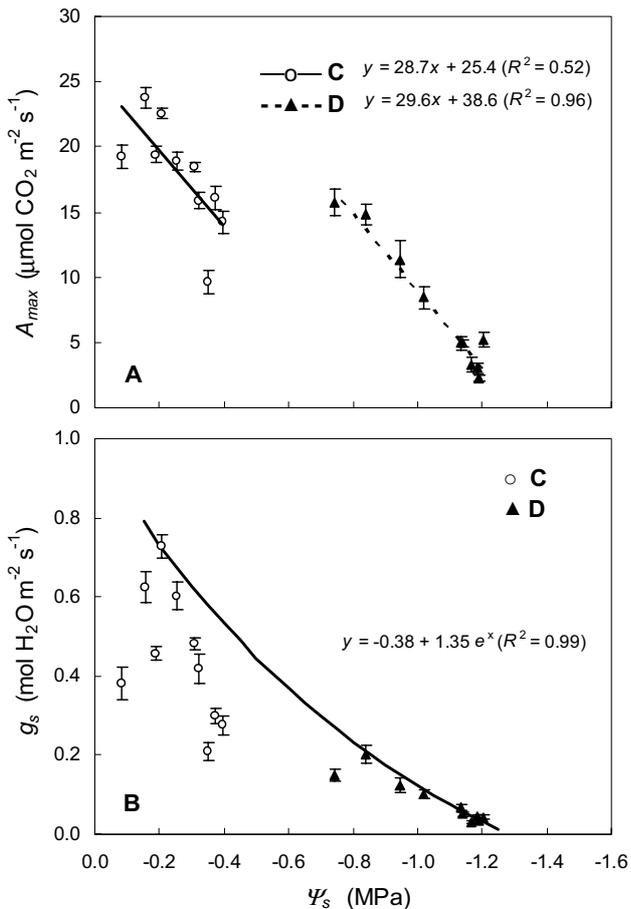


FIG. 6.

Relationship between light-saturated net CO₂ assimilation (A_{max} ; Panel A) or stomatal conductance (g_s ; Panel B), and stem water potential (Ψ_s) in droughted (D) and control (C) trees. Each point is an average of 16 measurements. Bars indicate standard errors. The line in Panel B is a fit to the ten outermost (i.e., boundary) data points (see text for explanation).

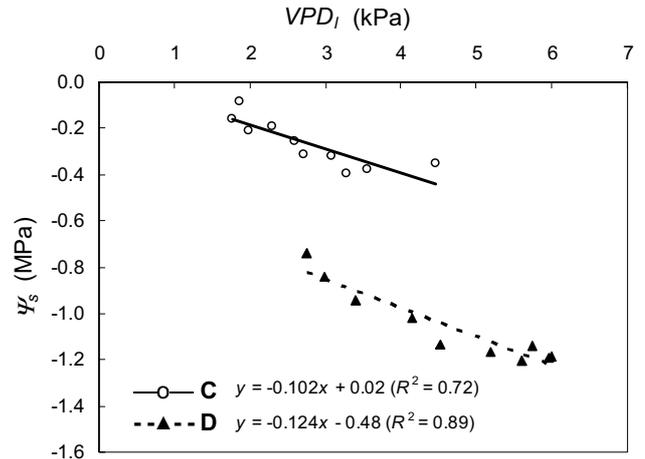


FIG. 7.

Relationship between stem water potential (Ψ_s) and the leaf-to-air vapour pressure deficit (VPD_l) in droughted (D) and control (C) trees. Each point is an average of 16 measurements.

DISCUSSION

T_l vs. g_s limitations to A_{max}

The lower C_i under drought (Figure 1G, H) suggests that A_{max} was limited, at least in part, by the closing stomata. The greater slope of the A_{max} vs. g_s relationship under drought (Figure 2) also supports this interpretation. However, in each treatment, A_{max} decreased during the day (Figure 1C, D), while C_i remained nearly constant (Figure 1G, H) suggesting that something else limited CO₂ assimilation.

The strong relationships between A_{max} or g_s and T_l (Figure 3) or VPD_l (Figure 4) suggest that T_l and VPD_l affect gas exchange. The generally better correlations with VPD_l rather than T_l seem to suggest that the leaves responded more to VPD_l . However, while it is well-documented that g_s responds to VPD_l (Schulze *et al.*, 1972; Jarvis, 1976; Watson *et al.*, 1978; Thorpe *et al.*, 1980; Fanjul and Jones, 1982; Grantz, 1990), it is also established that, when stomatal limitations are excluded, the A_{max} of C₃ plants declines at high temperature due to increased photorespiration (Jones, 1992). If A_{max} was controlled by T_l in our study, then it is unclear why A_{max} correlated better with VPD_l , at least in the controls. This was probably because A_{max} was limited both by high T_l and low g_s , and plotting A_{max} against VPD_l accounted, at least in part, for both limitations. To explain this, we selected three leaves at the same temperature (one in the droughted treatment and two in the control), but with different A_{max} and g_s (Figure 3). When A_{max} and g_s were plotted against VPD_l , the same leaves had different VPD_l , despite having the same T_l , thus reducing the scatter and improving the fits (Figure 4). This was because, at any T_l in the leaf chamber, leaves with lower g_s had lower E . Lower E at a given T_l resulted in greater VPD_l as we did not control the VPD_l in the chamber. Thus, better fits between A_{max} and VPD_l , compared with fits between A_{max} and T_l , did not necessarily imply a causal relationship.

To test the hypothesis that A_{max} was limited by both T_l and g_s , we corrected A_{max} for the g_s (i.e., C_i) limitation and plotted the corrected A_{max} ($A_{maxCorr}$) against T_l (Figure 5). A single fit described all the data, suggesting that CO₂ assimilation responded directly to T_l , and that the rest of the variation in A_{max} was due to additional g_s limitations

(i.e., low C_i), especially under drought. Given the close correlation between T_l and VPD_l , $A_{max,corr}$ was also closely correlated with VPD_l (data not shown). Our data do not allow us to determine which of these two variables affected stomata.

The optimal temperature for photosynthesis was approx. 29°C, with A_{max} zero at about 44°C (Figure 5). Foott and Heinicke (1967) found that A_{max} of walnut was highest between approx. 21°C and 27°C, and was greatly reduced above 34°C. Lucier and Hinckley (1982) found an optimum temperature from 22°C to 27°C and zero A_{max} at temperatures > 40°C or < 15°C in black walnut. However, in these studies, no attempt was made to separate stomatal limitations on assimilation from direct effects of temperature on leaf biochemistry.

Ψ_s and A_{max}

Light-saturated photosynthesis decreased with decreasing Ψ_s (Figure 6), but the relationship was probably not a causal one as the relationship differed between treatments and the A_{max} in the controls at the end of the day was lower than the A_{max} under drought at the beginning of the day, despite the higher Ψ_s . In previous work, A_{max} was linearly related to Ψ_s (Marsal *et al.*, 1997; Klein *et al.*, 2001). However, only pre-dawn or mid-day values of Ψ_s were measured.

Stomatal responses

The stomata seemed to respond to VPD_l (Figure 4B). However, on both days, g_s was lower in the early morning (Figure 1E, F) when VPD_l was lowest in the day. At this time, g_s was probably limited by light or low temperature (Schulze *et al.*, 1972; Jarvis, 1976; Lucier and Hinckley, 1982). For these reasons, the early morning data were omitted in the regression between g_s and VPD_l (Figure 4). The good fits of these regressions suggest that, once the stomata had reached their maximum aperture in the morning, then g_s was related to VPD_l , as found before (Schulze *et al.*, 1972; Jarvis, 1976; Watson *et al.*, 1978; Thorpe *et al.*, 1980; Lucier and Hinckley 1982; Fanjul and Jones, 1982; Grantz 1990).

Droughted trees had lower g_s values at any given VPD_l compared to the controls (Figure 4), suggesting a role for tree water status (Figure 6). These results can be interpreted with boundary line analysis, which indicates

the upper limits of a relationship or, in other words, the relationship that would be observed if other factors were not limiting (Webb, 1972; Jarvis, 1976). Since the g_s vs. Ψ_s relationship is typically exponential (Castel and Fereres, 1982; Cochard *et al.*, 2002; Romero *et al.*, 2004), we fitted an exponential curve to the ten outermost (i.e., boundary) points in the graph. The results suggest a role of water status in controlling g_s at low water potentials, as in the droughted trees, in agreement with previous literature (Roberts *et al.*, 1981; Castel and Fereres, 1982; Torrecillas *et al.*, 1988).

Stomatal conductance is probably more related to Ψ_l (Cowan, 1977; Fuchs and Livingston, 1996; Comstok and Mencuccini, 1998) and not Ψ_s , but these two parameters are closely related in droughted walnut (Cochard *et al.*, 2002).

If g_s was limited by water status at low Ψ_s , rather than by VPD_l , then it remains unclear why g_s was also closely related to VPD_l ($R^2 = 0.85$) under drought (i.e., low Ψ_s), although with a different relationship than for the controls (Figure 4). This was probably due to the strong link between Ψ_s and VPD_l (Figure 7). A strong relationship between Ψ_s and VPD_l or VPD (i.e., vapour pressure deficit in the air) has been found in several species, and is commonly used to explain variation in Ψ_s for fully-irrigated trees (Shackel *et al.*, 1998).

While the correlations between g_s and VPD_l , g_s and Ψ_s , and between Ψ_s and VPD_l were studied previously in separate work, in our study all these correlations were examined at once, providing a possible framework for the interpretation of the different findings and for the development of strategies for irrigation and orchard management in walnut.

CONCLUSIONS

We showed that walnut photosynthesis is reduced under drought by the closing of stomata, which impairs gas exchange and increases leaf temperature. However, even fully-irrigated trees experienced reductions in photosynthesis at high T_l and VPD_l , which affected photosynthesis not only *via* stomata, but also directly. Thus, at $T_l > 30^\circ\text{C}$ and/or $VPD_l > 2$ kPa, photosynthesis and the potential productivity of walnut trees began declining even in fully-irrigated trees, becoming severely impaired at $T_l > 40^\circ\text{C}$ and/or $VPD_l > 4$ kPa.

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