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Leaf and root control of stomatal closure during drying in soybean

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The stomatal conductance of an illuminated 2.5 cm^2 area of an intact soybean leaflet was the same whether the rest of the shoot was in light or darkness. This was true throughout soil drying cycles. Water potential of tissue immediately outside the illuminated area consistently decreased about 0.3 MPa upon illumination of the shoot. This erroneously suggested that stomatal conductance during soil drying did not respond to diurnal reductions in leaf water potential, but was controlled by root or soil water status. Tests showed that the water potential of tissue in the illuminated area did not change in the steady-state upon illumination of the rest of the shoot. Water potentials of shaded sections of leaves were not different from predawn water potentials, and were higher than leaf xylem pressure potentials as determined with a pressure chamber. These steep local gradients of leaf water potential suggest that there is minimal interchange of water among xylem elements leading from roots to different sections of leaves. The relationship between stomatal conductance and leaf water potential was the same whether leaf water potential was reduced by soil drying, application of polyethylene glycol (PEG) to the root system, lowering root temperature, or leaf excision. In the root cooling experiment, there was no soil drying, and with leaf excision, there was no root drying. The similarity of stomatal responses to leaf water potential in all cases strongly suggests control of conductance by a signal produced by local leaf water potential rather than root or soil water status in these experiments.

Introduction

There are several different types of evidence that suggest that signals from roots in drying soil can reduce stomatal conductance of leaves without a reduction in leaf water potential. These include lack of change in leaf water potential with stomatal closure in drying soil (e.g., Bates and Hall 1981), stomatal closure in dry soil despite controlled maintenance of leaf water potential by root pressurization (e.g., Gollan et al. 1986), and detection of abscisic acid in the xylem stream as a signal produced by roots in drying soil that is transported to the leaves and causing stomatal closure (e.g., Zhang et al. 1987). This raises the question of whether diurnal reductions in leaf water potential below the predawn potential have any controlling influence on stomatal conductance in either wet or dry soil. In some cases, it appears that low leaf water potentials may increase the sensitivity to signals from drying roots (Tardieu and Davies 1992), but there remains the question of whether or not there exists a more direct role for leaf water potential. Stomatal conductance of leaves excised from well-watered plants decreases as the leaves dry, so a signal from dry roots is not required for stomatal closure. The relative importance of root and leaf signals in causing stomatal closure in intact plants as they dry remains uncertain.

Recent studies in woody species have indicated that changes in leaf water potential can affect stomatal conductance independently of soil or root conditions (Saliendra et al. 1995, Fuchs and Livingston 1996, Whitehead et al. 1996, Fort et al. 1997). The suggestion has been made that woody species may differ from herbaceous species in this regard, and hence in the location of the control of stomatal closure (Saliendra et al. 1995). On the other hand, measurements of canopy evapotranspiration of herbaceous crops during drying cycles often indicate that dry soil does not necessarily decrease early morning values of canopy conductance on days when midday values are reduced (e.g., Olioso et al. 1996). This suggests control of conductance by leaf water potential in a threshold fashion even in herbaceous species (Olioso et al. 1996).

However, these situations are ambiguous in the sense that lower midday leaf water potentials presumably also reduce water potentials in the roots, possibly causing them to produce more 'signal', which would be carried to the leaves

in the transpiration stream. It is unclear why this would not also happen in cases where diurnal reductions in leaf water potential appear not to affect stomatal conductance. It could be that the rate of drying, rather than root water potential, is important to root signaling, or that there is a threshold value of root potential above which no signal is produced (Tenhunen et al. 1994).

Relationships between leaf water potential and stomatal conductance during independent manipulations of the environment around test leaves, the rest of the shoot, and the root have been used in numerous experiments to separate leaf and root control of stomatal conductance. In an experiment of this type, I found that steep local gradients of water potential can exist in leaves, which, if not recognized, may lead to incorrect conclusions. In this report, I have compared relationships between leaf and soil water potential and stomatal conductance for leaf drying induced by either withholding soil water, application of polyethylene glycol (PEG), reduction in root temperature, or leaf excision.

Materials and methods

Experiments were conducted on soybean, *Glycine max* [Merr.] L. cv. Clark, grown in a controlled environment chamber at 25 ± 0.2°C air temperature, 18 ± 1°C dew point temperature, 350–380 μmol mol^{-1} [CO_2], with 12 h per day of light from high pressure sodium and metal halide lamps at a photosynthetic photon flux (PPF) of 0.9 mmol m^{-2} s^{-1}. Plants were grown in 15 cm diameter plastic pots filled with vermiculite and flushed daily with a complete nutrient solution that had an osmotic potential of -0.03 MPa.

Stomatal conductance was measured on recently fully expanded leaves using an open gas exchange system (CIRAS-1; PP Systems, Haverhill, MA, USA) incorporating a broad-leaf cuvette with a window of 2.5 cm^2 area. Measurements were made on terminal leaflets of second mainstem trifoliate leaves of soybeans within a few days after maximum area expansion. The total area of the terminal leaflet was about 50 cm^2. A halogen lamp clamped over the cuvette window exposed just the measured section of leaf to light at a PPF of 0.9 mmol m^{-2} s^{-1}. The instrument and plants were in a controlled environment chamber so that temperature and humidity could be kept constant for all measurements. To maintain a constant temperature of the section of leaf in the cuvette, it was necessary to lower the chamber air temperature by 2°C when the chamber lights were on, and to lower the chamber air temperature slightly as conductance decreased with drying. All measurements were made at a leaf temperature of 25 ± 1°C, and a leaf to air vapor pressure difference of 1.2 ± 0.2 kPa.

Stomatal conductance was measured initially while the rest of the shoot was in the dark in a controlled environment chamber, and then the chamber lights were switched on and stomatal conductance monitored for periods of up to 2 h until conductance had stabilized. Before the chamber lights were turned on, leaf water potential was determined on leaf discs excised from tissue immediately outside the leaf cuvette after stable stomatal conductance values were recorded. After the chamber lights were on and stomatal conductance had re-stabilized, water potential was determined for leaf discs excised from the area within the leaf cuvette. Water potential was determined using dew point hygrometry with insulated C-52 sample chambers and an HR-33T microvoltmeter (Wescor Inc., Logan, UT, USA). The chambers were regularly calibrated with salt solutions, and leaf discs were assigned to chambers randomly.

This sequence of measurements was made in a set of experiments comparing methods of reducing leaf water potentials. In one method of reducing water potentials, stomatal conductance and leaf water potential measurements were made on several days during drying cycles that lowered stomatal conductances to about 30% of the initial values in about seven days. Soil water content was not determined, but predawn leaf water potentials reached values as low as -1.4 MPa. Another method was flushing the pots with solutions of varying concentrations of PEG while the plants were in darkness. The PEG had an average molecular weight of 6000, and the highest concentration used had a water potential of -1.5 MPa. Tests indicated that stomatal responses to water potentials were the same, whether the PEG solutions were applied 12 or 2 h before the initial measurements of stomatal conductance, suggesting that there were no toxic effects of the PEG. The shorter time was routinely used. A third method of reducing leaf water potential was by cooling the roots. Twelve hours before the normal light-on time (at the beginning of the dark period), pots were placed inside another plastic pot lined with copper tubing conforming closely to the exterior of the pot containing the plant. Water at temperatures of 12–20°C was circulated through the copper tubing to reduce the temperature of the rooting medium. The temperature in the center of the pots was about 3°C higher than the temperature of water in the tubing. This root cooling produced a range of leaf water potentials when leaves were illuminated. The final method of reducing leaf water potential was by excision of the leaf in the light. The side leaflets were removed at the same time, to reduce the rate of water loss. Water potential decreased gradually, and leaves were sampled for stomatal conductance and leaf water potential at various times up to 1 h after excision.

A second set of measurements was made for a soil drying series, in which leaf discs for water potential measurement of plants in darkness were taken from within the illuminated leaf cuvette, as well as from tissue just outside the cuvette. These were destructive measurements, so the cuvette was then moved to an adjacent section of the same leaflet and conductance allowed to stabilize before the chamber lights were switched on.

Localized differences in water potential within leaves were further examined by shading a 2.5 cm^2 section of leaf on both surfaces with a clamp consisting of closed-cell foam covered with white plastic. The clamp was placed on a leaflet before the chamber lights were on and left in place. Two hours after the chamber lights came on, leaf discs were excised from the area shaded by the clamp and from an adjacent unshaded section of the same leaflet. The leaf was then excised, placed in a plastic bag, and xylem pressure potential immediately determined with a pressure chamber. These leaf disc water potential and pressure chamber mea-

surements were compared with similar measurements made on replicate plants before the lights came on. Water potential measurements of illuminated 2.5 cm^2 areas of these plants in the dark were made as described previously. The chamber was maintained at 25°C air temperature, 18°C dew point temperature, and the PPF was 0.9 mmol m^{-2} s^{-1}. Whole-plant transpiration rates expressed per unit leaf area were determined gravimetrically over the last 30 min before water potential measurements were made in the light.

Results

Stomatal conductance of the area illuminated in the leaf cuvette required about 30 min to increase to a steady value from darkness. When the chamber lights were then switched on to illuminate the rest of the shoot, stomatal conductance of the area under constant illumination within the cuvette initially increased by 20–40% within about 10 min, and then decreased. Sometimes there were additional oscillations before a steady value of stomatal conductance occurred. The stomatal conductances and leaf water potentials presented in this paper refer to steady-state values after such oscillations in conductance had dampened.

In the soil drying experiments, the steady-state values of stomatal conductance were the same, whether the rest of the shoot was in darkness or illuminated (Fig. 1). The leaf water potential of tissue just outside the leaf cuvette dropped by about 0.3 MPa when the whole shoot was illuminated (Fig. 2). However, the steady-state value of leaf water potential of the tissue within the leaf cuvette did not change upon illumination of the whole shoot. This was indicated by the same 0.3 MPa difference in water potential between tissue outside and inside the cuvette in the darkened chamber (Fig. 2), and the 1:1 relationship between leaf water potential inside and outside the cuvette when the shoot was illuminated (Fig. 3).

A 1:1 relationship also existed between stomatal conductance measured while the rest of the shoot was in darkness

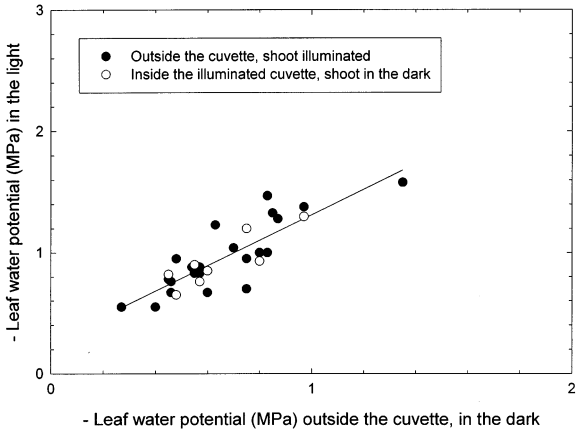

Fig. 2. Leaf water potential during soil drying of discs excised from outside of a leaf cuvette with the whole shoot illuminated (solid symbols), or from within the cuvette (open symbols), with the area in the cuvette illuminated and the area outside in the dark, as a function of the leaf water potential of discs excised from leaves outside the cuvette in the dark. Points represent measurements on different plants. The overall regression line is shown, and has the equation: $y = 1.053x + 0.265$, with $r^2 = 0.724$.

or in light when water potential was reduced by PEG (Fig. 4). In contrast, reductions in water potential caused by low root temperature reduced stomatal conductance when the whole shoot was illuminated, but did not reduce stomatal conductance when the shoot was in darkness (Fig. 4). While the relationship between leaf water potentials in the dark and in the light was the same for soil drying and for PEG treatments, low root temperature reduced leaf water potential in the daytime, but not in darkness (Fig. 5).

The relationship between stomatal conductance and leaf water potential measured when the whole shoot was illuminated was the same, whether leaf water potential was reduced by soil drying, application of PEG, lowering root temperature, or drying of excised leaves (Fig. 6).

Water potentials of shaded portions of leaves were the same whether the rest of the leaf and shoot was illuminated

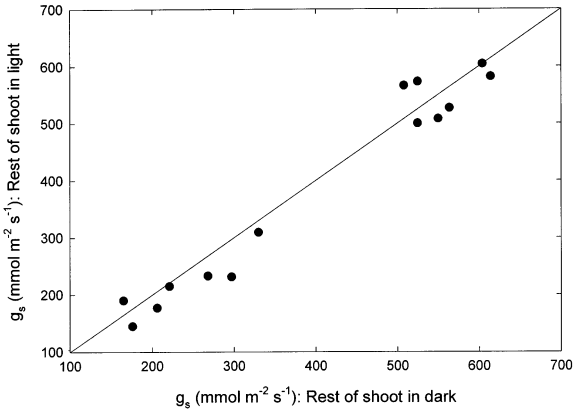

Fig. 1. Stomatal conductance (g_s) of an illuminated section of a leaf with the rest of the leaf and shoot in the dark or in the light during soil drying. Points represent measurements on different plants. The line illustrates a 1:1 relationship. The slope of the regression was not significantly different from 1, and the intercept was not significantly different from 0, at $P = 0.05$.

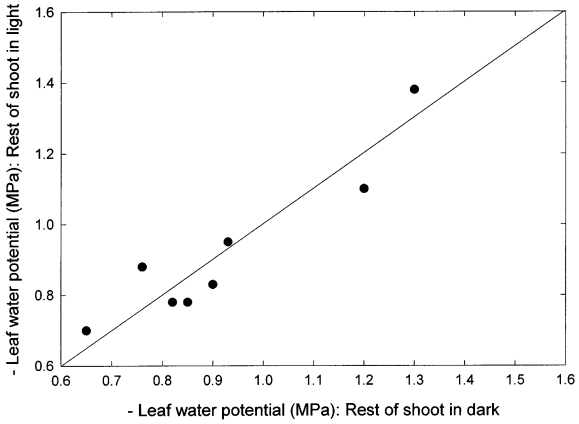

Fig. 3. Leaf water potential during soil drying of discs excised from inside an illuminated cuvette, with the rest of the leaf and shoot outside the cuvette in the dark or in light. Points represent measurements on different plants. The line illustrates a 1:1 relationship. The slope of the regression was not significantly different from 1, and the intercept was not significantly different from 0, at $P = 0.05$.

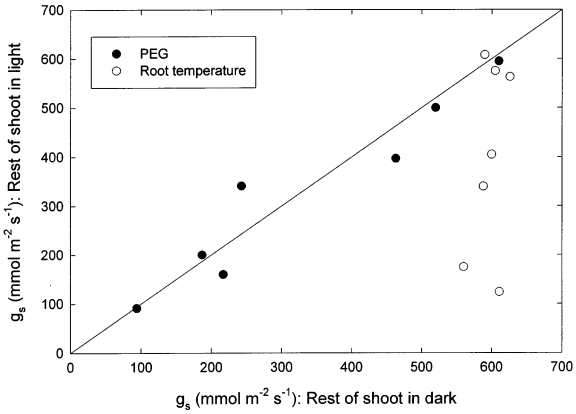

Fig. 4. Stomatal conductance (g_s) of an illuminated section of a leaf with the rest of the leaf and shoot in the dark or in the light, during drying caused by the application of PEG (solid symbols) to the root system or by lowering the root temperature (open symbols). Points represent measurements on different plants. The line illustrates a 1:1 relationship. For the data on PEG, the slope of the regression was not significantly different from 1, and the intercept was not significantly different from 0, at $P = 0.05$.

or not (Table 1). Water potentials of shaded portions of leaves were higher than leaf xylem pressure potentials of illuminated shoots as measured with a pressure chamber (Table 1). The water potentials of illuminated areas were lower than water potentials of areas of the same leaves in the dark or than xylem pressure potentials (Table 1). Mean transpiration rates in the light for whole plants were 4 mol H_2O m^{-2} s^{-1}.

Discussion

The initial stomatal opening induced by illuminating the rest of the shoot suggests that the increase in transpiration upon illumination caused at least a transient decrease in the turgor pressure of epidermal cells relative to guard cells

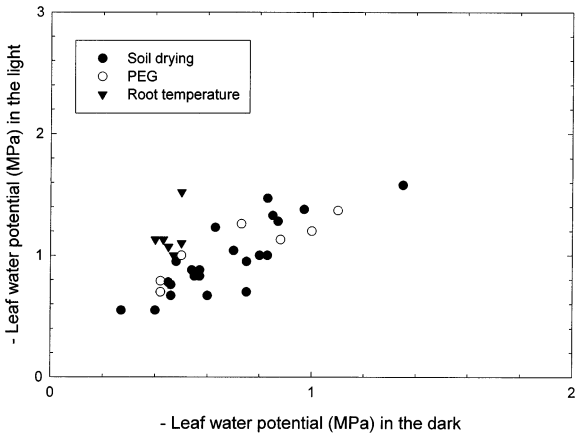

Fig. 5. Leaf water potential of discs excised from leaves in the dark or in the light during drying caused by soil drying (solid circles), the application of PEG to the root systems (open circles), or by lowering the root temperature (triangles). Points represent measurements on different plants. The data are from the same experiment illustrated in Fig. 4.

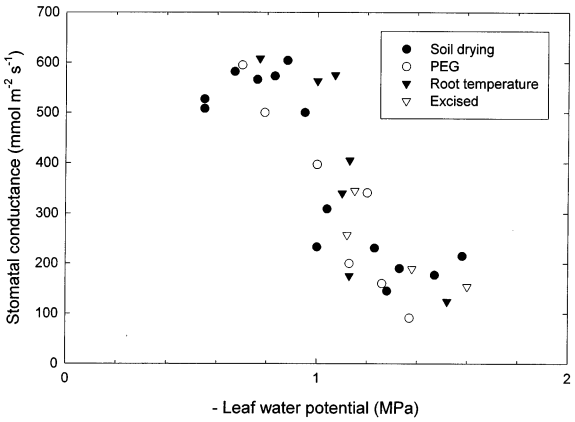

Fig. 6. Relationships between stomatal conductance and leaf water potential of leaves during drying caused by soil drying (solid circles), application of PEG to the root system (open circles), lowering root temperature (solid triangles), or leaf excision (open triangles). Points represent measurements on different plants.

within the area in which stomatal conductance was measured. However, bulk leaf water potentials within the measurement cuvette did not change in the steady-state upon illumination of the rest of the shoot, suggesting that some unknown adjustments in water relations were occurring during the period in which the oscillations in stomatal conductance dampened.

The lack of change in steady-state stomatal conductance of an illuminated portion of a leaf, despite a drop in water potential measured on adjacent tissue outside the cuvette when the chamber lights were switched on, could be taken as evidence that the drop in leaf water potential had no effect on stomatal conductance. However, measurements of water potential of the illuminated area indicated that switching on the chamber lights also had no effect on the steady-state value of water potential. This invalidates the conclusion that diurnal changes in leaf water potential are unimportant to stomatal conductance, and points to the need for stomatal conductance and water potential measurements to be made on the same areas of leaves.

When one includes the data for the low root temperature experiments, there was no unique relationship between stomatal conductance and soil water status. Low root temperatures probably reduced leaf water potential in plants in

Table 1. Leaf water potentials (LWP) and leaf xylem pressure potentials (XPP) measured with a pressure chamber for leaves either in darkness or after 2 h of exposure to a PPF of 0.9 mmol m^{-2} s^{-1}, and LWP of 2.5 cm^2 areas that had been either illuminated while the rest of the shoot was in the dark, or shaded continuously. Values followed by different letters were significantly different at $P = 0.05$, for n = 8.

Parameter	Water potential (MPa)
Shoot in the dark	
LWP	−0.56a
XPP	−0.50a
LWP of illuminated area	−0.81b
Shoot in the light	
LWP	−0.85b
XPP	−0.78b
LWP of shaded area	−0.57a

the light by increasing resistance to water flow through the root system, and presumably also reduced water potential in the roots despite wet soil. Root drying could have produced a signal affecting stomatal conductance. However, the importance of a presumed signal from water-stressed roots in controlling stomatal conductance is discounted by the data for excised leaves, where there was no signal from the roots. All of the drying treatments indicated a unique relationship between leaf water potential and stomatal conductance (Fig. 6), which would not have been the case if a signal from water stressed roots had an additional effect on conductance. It can also be concluded that the rate of drying had no effect on the relationship between leaf water potential and stomatal conductance over the range of drying rates including slow soil drying over several days, root drying for 2–12 h with PEG, and root or leaf drying for 1 h or less for the low root temperature and excised leaf treatments. The unique relationship between leaf water potential and stomatal conductance during drying was undoubtedly a function of the fact that other variables affecting stomatal conductance, such as light, temperature, humidity and carbon dioxide, were kept constant (Jones 1998). The consistent relationship between leaf water potential and stomatal conductance does not contradict the considerable evidence that abscisic acid is involved in mediating the response of conductance to leaf water potential. These experiments provide no information on the causal connection between reduced leaf water potential and stomatal closure, but indicate that the correlation is the same when the possibility of root signals is excluded.

Although it seems unexpected that water potential of a section of leaf would be independent of the transpiration rate or water potential of adjacent areas of the same leaf, similar results have been obtained by others. For example, Turner et al. (1984, 1985) found in herbaceous species that changing the water vapor pressure to change the transpiration rate of the leaf area outside the cuvette did not affect the water potential of the leaf area inside the cuvette, as measured with an in situ psychrometer. Similarly, Schulze and Kuppers (1979) found that the xylem pressure potential of leaves within a cuvette had no clear relationship to the xylem pressure potential of leaves outside the cuvette when the external water vapor pressure was changed to alter transpiration rate and water potential.

Two explanations for the substantial independence of water potential of different parts of a leaf are apparent, the resistance to water flow from the soil to the leaf could be dominated by the resistance within the leaf lamina, or there may be little exchange of water among xylem elements leading from roots to different sections of the same leaf. The difference between the leaf xylem pressure potential of a darkened leaf and the water potential of a small illuminated area of that leaf was the same as the difference between the predawn and daytime leaf water potentials (Table 1). This suggests that resistance to flow within the leaf dominated the total resistance to flow. While it is recognized that resistance to water flow within leaves can be substantial (e.g., Melcher et al. 1998), a dominant role of the leaf resistance in the total flow resistance is not consistent with many other observations of water potential gradients within plants (cf. Kramer 1983, Yang and Grantz 1996). The fact that the leaf water potential of a shaded section of a leaf was greater than the xylem pressure potential of the same leaf probably reflects the fact that the process of measuring leaf xylem pressure potential with a pressure chamber may allow xylem pressure potential to equilibrate with the mesophyll water potential and, therefore, xylem pressure potential may reflect a volume-weighted average water potential for the whole leaf (cf. Passioura 1982). If this explanation is accepted, then all of the data is consistent with the idea that the measured local gradients of water potential within leaves could result from local transpiration differences and little exchange of water among xylem elements connecting different sections of a leaf with the root system. This data re-emphasizes the fact that pressure chamber measurements of xylem pressure potential may not accurately reflect the water potential of all parts of the tissue downstream.

While these data for soybeans provides no evidence of a signal from drying roots affecting stomatal conductance, and show that no root signal is necessary to account for stomatal closure during drying in this case, they do not exclude the possibility of such a signal being important in other circumstances. Experiments by Sadras et al. (1993) suggest that leaf water potential rather than root signals primarily affect leaf expansion in sunflower. In other species, leaf water potential has been shown to influence the sensitivity to abscisic acid (Tardieu and Davies 1992). Thus, there can be a significant and sometimes dominant role of leaf water potential in affecting physiology, even in herbaceous species.

References

Bates LM, Hall AE (1981) Stomatal closure with soil water depletion not associated with change in bulk leaf water status. Oecologia 50: 62–65

Fort C, Fauveau ML, Muller F, Label P, Granier A, Dreyer E (1997) Stomatal conductance, growth and root signaling in young oak seedlings subjected to partial soil drying. Tree Physiol 17: 281–289

Fuchs EE, Livingston NJ (1996) Hydraulic control of stomatal conductance in Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] and alder [*Alnus rubra* (Bong.)] seedlings. Plant Cell Environ 19: 1091–1098

Gollan T, Passioura JB, Munns R (1986) Soil water status affects the stomatal conductance of fully turgid wheat and sunflower leaves. Aust J Plant Physiol 13: 459–464

Jones HG (1998) Stomatal control of photosynthesis and transpiration. J Exp Bot 49: 387–398

Kramer PJ (1983) Water Relations of Plants. Academic Press, New York, NY, pp 489.

Melcher PJ, Meinzer FC, Yount DE, Goldstein G, Zimmermann U (1998) Comparative measurements of xylem pressure in transpiring and non-transpiring leaves by means of the pressure chamber and the xylem pressure probe. J Exp Bot 49: 1757–1760

Olioso A, Carlson TN, Brisson N (1996) Simulation of diurnal transpiration and photosynthesis of a water stressed soybean crop. Agric For Meteorol 81: 41–59

Passioura JB (1982) Water in the soil-plant-atmosphere continuum. In: Encyclopedia of Plant Physiology, New Series, Physiological Plant Ecology II. Water Relations and Carbon Assimilation, 12B. Springer-Verlag, New York, NY, pp 1–33.

Sadras VO, Villalobos FJ, Fereres E (1993) Leaf expansion in field-grown sunflower in response to soil and leaf water status. Agron J 85: 564–570

Saliendra NZ, Sperry JS, Comstock JP (1995) Influence of leaf water status on stomatal response to humidity, hydraulic con-

ductance, and soil drought in *Betula occidentalis*. Planta 196: 357–366
Schulze E-D, Kuppers M (1979) Short-term and long-term effects of plant water deficits on stomatal response to humidity in *Corylus avellana* L. Planta 146: 319–326
Tardieu F, Davies WJ (1992) Stomatal response to abscisic acid is a function of current plant water status. Plant Physiol 98: 540–545
Tenhunen JD, Hanano R, Abril M, Weiler E, Hartung W (1994) Above- and below-ground environmental influences on leaf conductance of *Ceanothus thryrsiflorus* growing in a chaparral environment: Drought response and the role of abscisic acid. Oecologia 99: 306–314
Turner NC, Schulze E-D, Gollan T (1984) The responses of stomata and leaf gas exchange to vapour pressure deficits and soil water content. I. Species comparisons at high soil water contents. Oecologia 63: 338–342
Turner NC, Schulze E-D, Gollan T (1985) The responses of stomata and leaf gas exchange to vapour pressure deficits and soil water content. II. In the mesophytic herbaceous species *Helianthus annuus*. Oecologia 65: 348–355
Whitehead D, Livingston NJ, Kelliher FM, Hogan KP, Pepin S, McSeveny TM, Beyers JN (1996) Response of transpiration and photosynthesis to a transient change in illuminated foliage area for a *Pinus radiata* D. Don tree. Plant Cell Environ 19: 949–957
Yang S, Grantz DA (1996) Root hydraulic conductance in pima cotton: Comparison of reverse flow, transpiration, and root pressurization. Crop Sci 36: 1580–1589
Zhang J, Schurr U, Davies WJ (1987) Control of stomatal behaviour by abscisic acid which apparently originates in the roots. J Exp Bot 192: 1174–1181

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