

# In Situ Measurement of Epidermal Cell Turgor, Leaf Water Potential, and Gas Exchange in *Tradescantia virginiana* L.<sup>1</sup>

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## ABSTRACT

A combined system has been developed in which epidermal cell turgor, leaf water potential, and gas exchange were determined for transpiring leaves of *Tradescantia virginiana* L. Uniform and stable values of turgor were observed in epidermal cells (stomatal complex cells were not studied) under stable environmental conditions for both upper and lower epidermises. The changes in epidermal cell turgor that were associated with changes in leaf transpiration were larger than the changes in leaf water potential, indicating the presence of transpirationally induced within-leaf water potential gradients. Estimates of 3 to 5 millimoles per square meter per second per megapascal were obtained for the value of within-leaf hydraulic conductivity. Step changes in atmospheric humidity caused rapid changes in epidermal cell turgor with little or no initial change in stomatal conductance, indicating little direct relation between stomatal humidity response and epidermal water status. The significance of within-leaf water potential gradients to measurements of plant water potential and to current hypotheses regarding stomatal response to humidity is discussed.

Since its development by Hüsken *et al.* (2), the miniaturized pressure probe has for the most part been used to estimate the water relations parameters (hydraulic conductivity and volumetric elastic modulus) of individual plant cells (*e.g.* 11). However, the value of cellular turgor itself is also considered to be a fundamentally important physiological parameter in plants (*e.g.* 9), particularly for current hypotheses regarding stomatal sensitivity to atmospheric humidity (*e.g.* as reviewed by Maier-Maercker [3]). These hypotheses ascribe a central role to within-leaf water potential gradients and the consequences of these gradients to epidermal and stomatal cell turgor. *In situ* measurement of epidermal cell turgor is possible on attached leaves of *Tradescantia virginiana* L. (11), and in principle may be combined with *in situ* measurements of leaf gas exchange and water potential under contrasting levels of leaf transpiration. This measurement should then provide direct evidence for the occurrence of transpirationally induced within-leaf water potential gradients (10) if the differences in leaf epidermal cell turgor that are associated with differences in transpiration are larger than the corresponding changes in leaf water potential. In addition it should provide information regarding the proposed relationship between epidermal cell turgor and level of stomatal conductance (*e.g.* 8). The following paper reports the development of a combined system for measuring epidermal cell turgor and leaf water relations parameters under controlled environmental conditions.

Results are also presented concerning within-leaf water potential gradients and relations between epidermal cell turgor and stomatal responses.

## MATERIALS AND METHODS

**Plant Material, Experimental Conditions, and Gas Exchange Measurements.** Potted plants of *Tradescantia virginiana* L. were grown under low humidity glasshouse conditions (air temperature, 20–25°C; approximate VPD<sup>2</sup>, 20 hPa) at Bayreuth, West Germany, and grew as well as the laboratory-grown plants of Brinckmann *et al.* (1). These were thinned to one shoot per pot before installation into a controlled environment plexiglas cuvette (Heinz Walz, Effeltrich, W.G.; air temperature, 23°C; 13-h photoperiod supplied by white and Osram 'Fluora' fluorescent tubes giving  $8.3 \text{ w m}^{-2} = 38 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). Control of cuvette humidity and measurement of leaf gas exchange were combined into a single system (Fig. 1) in which the airflow used to control cuvette humidity also passed through a secondary cuvette in which the gas exchange of a 15-cm length of test leaf was determined. Test leaves were approximately 1.8 cm wide  $\times$  30 to 40 cm (tip to base) with parallel veination and a prominent midvein. The test leaf was held magnetically to a fixed support in the secondary cuvette and its gas exchange was determined by

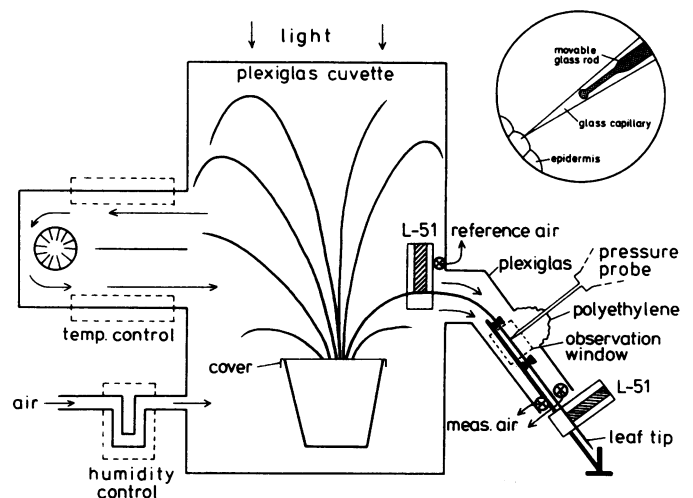


FIG. 1. System design combining environmental control with measurements of leaf gas exchange (reference and measurement air), leaf water potential (L-51 psychrometers), and epidermal cell turgor (pressure probe) using potted plants of *T. virginiana*. Insert shows a detail of the movable glass rod used both to seal the pressure probe during prepressurization and as a source of illumination.

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<sup>2</sup> Abbreviation: VPD, vapor pressure deficit.

measuring the difference in  $H_2O$  and  $CO_2$  concentrations between the two ends of the secondary cuvette (reference and measurement air in Fig. 1) with a Binos 1 gas analyzer (Leybold-Heraeus, Hanau, W.G.). All gas flows were measured using individually calibrated rotameters (Krohne, Durisburg, W.G.), cuvette humidity (reference air) was measured with a dewpoint mirror (Heinz Walz), and test leaf temperature was measured with a 0.05-mm copper-constantan thermocouple. Leaf transpiration and conductance were calculated on a total leaf area basis (two sides). Cuvette air was well stirred, and the rate of airflow through the system was sufficient to give an air speed across the test leaf of approximately  $0.5 \text{ m s}^{-1}$ . After installation into the cuvette, plants were allowed at least 1 week to stabilize under the new environmental conditions before experiments were performed. By this time very little differences could be observed between successive diurnal patterns of test leaf gas exchange.

**Measurement of Epidermal Cell Turgor.** The principles of operation for the miniaturized pressure probe have already been extensively described (2, 11, 12) although some improvements in technique were required in order for it to be used within a gas exchange system. Since epidermal cells in the measurement area were not directly supported by a fixed substrate, sharply pointed microcapillary tips which could easily penetrate the cell wall were required in order to avoid excessive flexing of the leaf tissue during probe insertion. These tips were produced by cutting the microcapillary between a broken glass surface and a section of razor blade at a 50 to 60° slant. Tips were inspected at  $\times 1250$  and only sharply pointed tips of 3 to 4  $\mu\text{m}$  in size (measured at the widest point of the cut portion) were selected for use. To avoid substantial discharge of cell sap into the probe and the resulting complete loss of cellular turgor normally caused by probe insertion, the silicone oil within the probe was pressurized before insertion, to a value close to that expected for cellular turgor. Loss of oil through the open microcapillary tip was prevented by plugging the end of the microcapillary during pressurization with the tip of a movable glass rod (Fig. 1, insert). This glass rod (approximately 400  $\mu\text{m}$  diameter) ran through the center of the probe assembly (replacing the silver electrode described in Ref. 2) and was reduced to approximately 100  $\mu\text{m}$  near its end, which was fire-polished to a hemispherical shape of approximately 200  $\mu\text{m}$  in diameter. After plugging, the probe was pressurized and positioned above the cell to be measured. The plug was then slowly withdrawn (drawing air into the microcapillary) until forward movement of the oil within the microcapillary indicated that the plug had at least partially opened. The cell was then penetrated to a depth of 50  $\mu\text{m}$  (approximately one-half of the average cell dimension) just at the moment that the advancing oil front reached the microcapillary tip, preventing any substantial quantity of cell sap from discharging into the microcapillary. If necessary, the plug was then fully opened (carefully by hand) and the pressure of the oil within the probe adjusted using an electromotor to maintain the oil/cell sap meniscus in a position approximately 80  $\mu\text{m}$  from the cell surface, at which the volume of sap outside the cell was equivalent to the displacement volume of the glass microcapillary within the cell (approximately 1 pl). The position of the oil/cell sap meniscus was measured with an ocular micrometer at  $\times 300$  through a fixed microscope fitted with  $\times 20$  long distance optics (Leitz, Wetzlar, W.G.) with a line of sight parallel to the leaf plane and perpendicular to the microcapillary axis (through the cover glass of the observation window; Fig. 1). The glass rod used to seal the pressure probe during pre-pressurization was also used as a fiber optic to illuminate both the meniscus and cells in the measurement area. This method of illumination increased the contrast between the meniscus and its surroundings, thus minimizing the quantity of light needed for meniscus observation. Cells chosen for measurement were from a  $1.5 \times 0.4 \text{ cm}$

area of epidermis (beginning from near the leaf margin) approximately 14 cm from the leaf tip on the half leaf closest to the observation window.

**Measurement of Leaf Water Potential.** *In situ* leaf water potential was determined psychrometrically using the materials and methods described by Shackel (7). Values of water potential were calculated from psychrometric outputs (7 s after 3 s cooling) which had been calibrated against dewpoints obtained by the psychrometric interrupt technique (*i.e.* corrected for depletion errors caused by tissue resistance to water vapor exchange). Two L-51 psychrometers in standard commercial blocks (Wescor, Inc., Logan, UT) were mounted to apical ('tip') and basal ('base') positions on the same leaf half as used for turgor measurements. Heavily insulated psychrometer blocks were not used due to space limitations; however, no effect of psychrometer insulation on measured values of leaf water potential had been found in previous experiments (7) for the low light conditions used in this study.

## RESULTS

Throughout the diurnal course of transpiration exhibited by a test leaf of *T. virginiana*, reproducible values of turgor were measured in different epidermal cells, with individual cells exhibiting stable values of turgor for extended periods of time (Fig. 2). Similarly, stable and reproducible values of turgor were obtained using cells from both upper and lower epidermises. No consistent differences were found within either epidermis for cells situated above vascular bundles *versus* cells in interveinal areas, nor for cells at contrasting distances from stomata. Because the VPD was constant during this experiment ( $13 \pm 1 \text{ hPa}$ ), the diurnal pattern of transpiration reflected diurnal changes in stomatal conductance. The changes in epidermal cell turgor that were associated with diurnal changes in leaf transpiration were larger than the corresponding changes in leaf total water potential. This was also apparent in the relationship of epidermal cell turgor and leaf water potential to instantaneous values of leaf transpiration (Fig. 3). In the case of the leaf water potential data, the hysteresis which was exhibited in this relationship may have been caused by errors associated with diurnal fluctuations in psychrometer null offset (7); however, since a similar hysteresis

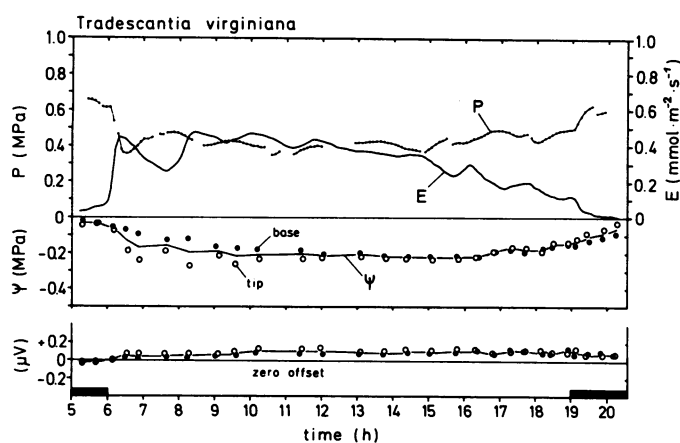


FIG. 2. Diurnal course of transpiration (E, expressed per unit of total leaf surface area), epidermal cell turgor (P), and water potential ( $\Psi$ ) of a test leaf of *T. virginiana*, including zero offsets exhibited by leaf psychrometers. The pressure measurements connected by a single line represent continuously measured turgor values from an individual epidermal cell, and a solid line connects the average values of water potential obtained from psychrometers at two positions along the test leaf. Photoperiod was from 6 to 19 h, and air vapor pressure deficit (VPD) was constant ( $13 \pm 1 \text{ hPa}$ ).

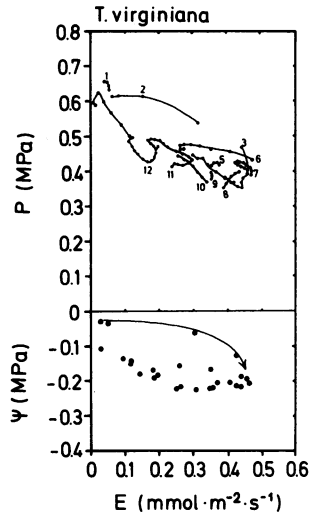


FIG. 3. Relationship of epidermal cell turgor ( $P$ ) and leaf water potential ( $\Psi$ ) to leaf transpiration ( $E$ ) for the data presented Figure 2. Turgor measurements for an individual cell are connected by a solid line with numbers indicating the sequence in which the cells were measured during the day. The solid line and arrow in the leaf water potential data also indicate the direction of change at the beginning of the day. Units are as in Figure 2.

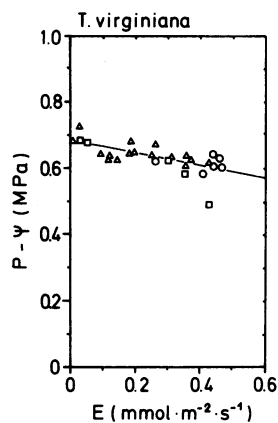


FIG. 4. Relationship of leaf transpiration ( $E$ ) to the difference between epidermal cell turgor and leaf water potential ( $P-\Psi$ ) for the measurements presented in Figure 2 collected from 5 to 7 h ( $\square$ ), from 7 to 12 h ( $\circ$ ), and from 12 to 20 h ( $\Delta$ ). Solid line is the fitted linear regression to all data (regression highly significant, slope =  $-0.2 \text{ MPa mmol}^{-1} \text{ m}^{-2} \text{ s}^{-1}$ ,  $r^2 = 0.46$ ) and units are as in Figure 2.

also occurred in the relationship between epidermal cell turgor and leaf transpiration, the simplest explanation is that the former hysteresis was real. If the osmotic potential of the epidermal cells did not change during this experiment, then the observed changes in epidermal cell turgor were caused by changes in total water potential at the epidermal level of a transpiring leaf. Since the *in situ* psychrometer measures the water potential of a nontranspiring portion of leaf (presumably in equilibrium with the vascular tissue that runs through it), the arithmetic difference between epidermal cell turgor and psychrometrically measured leaf water potential should provide a measure of the gradient in water potential between the vascular tissue and the leaf epidermis. The relationship between this difference and leaf transpiration rate for this experiment (Fig. 4) showed no hysteresis, and exhibited a negative slope, indicating that with increases in transpiration, more turgor was lost at the epidermal cell level than could be accounted for by decreases in the water potential of the vascular tissue. The inverse of this slope is an estimate of within-leaf

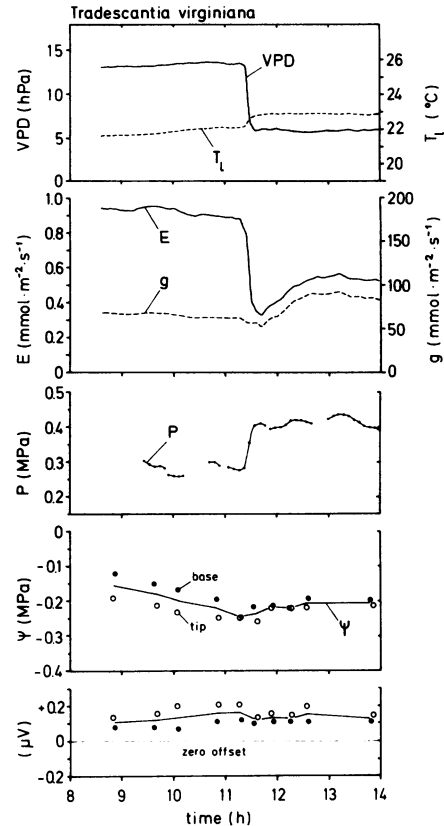


FIG. 5. Response of test leaf gas exchange (transpiration,  $E$ , and conductance,  $g$ , expressed per unit of total leaf surface area), epidermal cell turgor ( $P$ ), and leaf water potential ( $\Psi$ ) to a step decrease in air vapor pressure deficit (VPD). Test leaf temperature ( $T_1$ ) is also shown, with other symbols as in Figure 2.

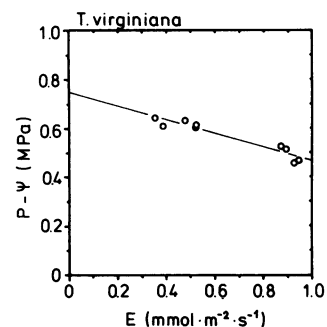


FIG. 6. Relationship of leaf transpiration ( $E$ ) to the difference between epidermal cell turgor and leaf water potential ( $P-\Psi$ ) for the data presented in Figure 5. Solid line is the fitted linear regression to all data (regression highly significant, slope =  $0.28 \text{ MPa mmol}^{-1} \text{ m}^{-2} \text{ s}^{-1}$ ,  $r^2 = 0.92$ ).

hydraulic conductivity (Ohms law analogy) which for this experiment was  $5 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ .

Differences in leaf transpiration were also obtained by experiments in which VPD was changed. For rapid VPD decreases (Fig. 5), marked increases in epidermal cell turgor accompanied the reduction in leaf transpiration, whereas little or no overall change occurred in either leaf water potential or psychrometer zero offset. The relationship of leaf transpiration to the difference between epidermal cell turgor and leaf water potential for this experiment was also linear (Fig. 6) and indicated a within-leaf hydraulic conductivity of  $3.5 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ . In additional experiments using both upper and lower leaf epidermises, values of 3 to  $5 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$  have been obtained for this

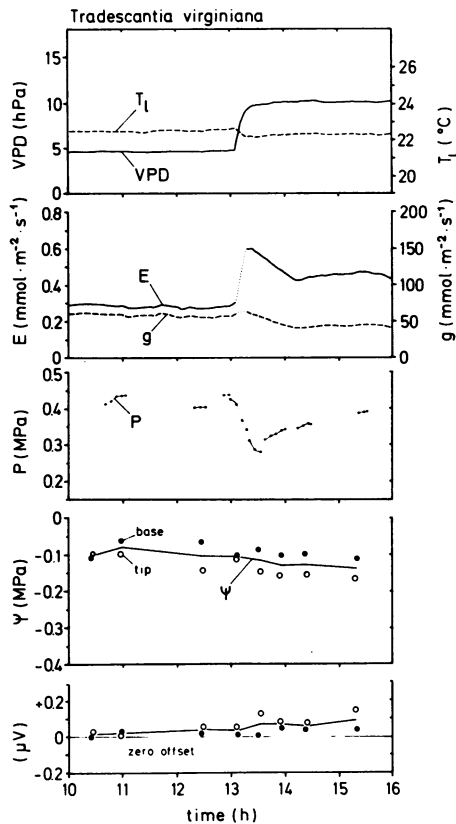


FIG. 7. Response of test leaf gas exchange and water potential parameters (as in Fig. 5) to a step increase in air VPD.

parameter. The time course of events presented in Figure 5 also indicated that the change in stomatal conductance associated with the VPD step was much slower than the recovery in epidermal cell turgor, and only occurred after the recovery in epidermal cell turgor was complete. The slight apparent reduction of stomatal conductance during the 12 to 15 min required to complete the VPD step represents in part an artifact caused by water vapor exchange of the gas exchange cuvette assembly itself. Similar initial reductions were also exhibited by filter paper replicates within the test leaf cuvette, but these replicates did not show the subsequent slow increases in conductance that were exhibited by leaves. In addition, whole plant transpiration (determined by monitoring the output air of the system humidity control; Fig. 1) also showed the same slow increase following the VPD step regardless of whether a leaf or filter paper replicate was present in the test leaf cuvette. Decreases in epidermal cell turgor that were independent of reductions in leaf water potential were also associated with increases in transpiration accompanying VPD increases (Fig. 7). Again the change in epidermal cell turgor was more rapid and began sooner than the stomatal response to the VPD step, but the two processes were not as clearly separated in time as was observed for VPD decreases.

## DISCUSSION

The reproducibility of turgor values among leaf epidermal cells under steady environmental conditions, and the stability of these values for individual cells over long periods of time is good indirect evidence that reliable determination of cellular turgor is possible *in situ* using the miniaturized pressure probe. Since it is unlikely that changes in cellular osmotic potential could account for the rapid responses of epidermal cell turgor which occurred during changes in leaf transpiration rate, these changes in turgor represent the first direct evidence that significant within-leaf

water potential gradients can be associated with transpirational water fluxes. It may be argued that these turgor changes were due to changes in leaf water potential and that the apparent lack of change in leaf water potential was caused by the psychrometers inability to follow these changes. In previous studies (7) using the same plant material, however, these psychrometers exhibited a much wider range of measured leaf water potentials, and even for the most resistive samples an equilibration time of the order of minutes for water vapor exchange between the leaf surface and the psychrometer. Although the estimate of within leaf hydraulic conductivity presented in this study ( $3\text{--}5 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ ) must be considered tentative pending correction of psychrometric errors associated with zero offsets and direct determination of epidermal cell osmotic potential, it is of the same order of magnitude as estimates obtained using substantially different methods ( $1 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ , after conversion of units) both for the same (8) and different (10) plant species. It should be noted that although Tyree and Yianoulis' (10) criticism of Sheriff's (8) methods may be valid, the error due to water films in Sheriff's system would have been eliminated by the method used to estimate within-leaf hydraulic conductivity. The water potential gradients observed in this study were not large, but the rates of transpiration exhibited by *T. virginiana* under these experimental conditions were also low compared to the range of values obtainable for other plant species under natural environments. If the above estimates of within-leaf hydraulic conductivities are approximately correct, then substantial differences in water potential would be expected within leaves that were transpiring at even moderately high rates. This has important implications for the interpretation of leaf water potential values obtained using methods which may differ in the position along the gradient effectively being measured. Alternative methods, such as the pressure chamber and psychrometer, may measure equivalent values of water potential for leaves under low transpiration conditions such as may occur with dry soil and closed stomata, whereas they may not be equivalent when the same range of measured leaf water potentials are the result of high leaf transpiration rates. Thus, direct comparisons between such alternative methods may not be appropriate, particularly when one method is used to 'calibrate' the other.

The presence of within-leaf water potential gradients may also have important physiological implications for transpiring leaves, since a change of environmental conditions could have a substantially greater effect on the water potential at one position in the water flow pathway (*e.g.* the epidermis) than elsewhere (*e.g.* the vascular tissue). Even under steady state conditions, cells in different positions along a water potential gradient must experience differences in turgor potential, osmotic potential, or both, so that any differences in physiological activity related to turgor or osmotic potential differences between such cells would be correlated with the rate of leaf transpiration. Specific hypotheses concerning within-leaf water potential gradients and their relation to stomatal function, especially stomatal response to humidity (6), have been proposed both at the leaf to epidermis level (8) and the epidermis to guard cell complex level (3, 4). In general, these hypothesis suggest that stomata are situated at or near the end of the gradient and will experience greater changes in water potential for a given change in evaporative demand than the bulk leaf. This may enable stomata to respond to the change in evaporative demand, before a large change of water potential occurs in the rest of the leaf. If the stomata in *T. virginiana* are at the end of a water potential gradient, then the changes in epidermal cell turgor associated with transpiration observed in this study represent a conservative estimate of the water potential changes experienced by stomata. However, these studies indicated little direct relation between instantaneous epidermal turgor (and possibly stomatal water potential) and stomatal con-

ductance during stomatal response to humidity. For an intact leaf, such a direct relation may not be expected, however, since changes in guard and subsidiary cell turgor may have antagonistic effects on stomatal opening (5), which may be further modified by turgor changes in the surrounding epidermal cells. In addition, if a significant length of time is required for completion of stomatal movements, then the consequences of a change in humidity may depend not only on the level of transpiration which it causes but also on whether the humidity was increased or decreased. If humidity is increased, then the water potential increase (*i.e.* increase in turgor) of any cells near the end of the pathway will be limited by the highest water potential in the leaf (*e.g.* within the xylem); whereas, if the humidity is equivalently decreased, then the loss of turgor in these same cells may be substantially greater than their turgor increase in the previous case, since their water potential would continue to fall during the time it takes for stomata to respond. This may explain why turgor and stomatal responses were more clearly separated in time for VPD decreases than for VPD increases in this study. A more complete understanding of leaf and stomatal water relations will be possible if the spatial characteristics of within-leaf water potential gradients can be identified, namely whether the gradient is uniform between the vascular tissue and epidermis or whether it occurs mainly between specific tissues such as vascular tissue and mesophyll or mesophyll and epidermis.

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