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# Turgor and cell wall yielding in dicot leaf growth in response to changes in relative humidity

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*Abstract.* Epidermal cell turgor ($P$) and leaf growth in *Begonia argenteo-guttata* L. were monitored simultaneously following changes in air humidity in order to evaluate $P$–growth relations. A decrease in air humidity from 70 to 5% caused a decrease in $P$ of 0.05 MPa. This small decrease in $P$ resulted in cessation of growth. Subsequently, growth recovered partially at constant $P$, indicating an increase in wall yielding to $P$. Notwithstanding this increase in wall yielding, the steady growth rates showed a marked dependence on $P$. Decreases in $P$ of 0.05 MPa caused a 30–40% reduction in the steady rate of elongation. These results were reversible. Upon a step increase in air humidity from 5 to 70%, $P$ and growth rate rapidly increased. Subsequently, growth declined without a corresponding decrease in $P$, although the rate of growth remained higher than at low humidity. The partial self-stabilization of growth following $P$ changes and the positive relationship between steady growth rate and $P$ are consistent with the notion that wall yielding is controlled by interactions between $P$ and metabolism. Results are discussed in relation to biophysical factors that control growth and to present theories that accommodate variable wall yielding.

## Introduction

Expansive growth of plant cells requires both cell wall expansion and water uptake. The conceptual framework with which these processes have been commonly analysed was formalized by Lockhart (1965) and augmented by Ortega to account for elastic changes and transpiration (Ortega 1985, 1990; Ortega *et al*. 1988, 1992). Therein, expansion is the simultaneous solution of expressions describing both cell wall yielding to turgor,

$$[(\mathrm{d}V/\mathrm{d}t)/V]_{\text{tissue}} = m\,(P - Y) + (1/\varepsilon)\,\mathrm{d}P/\mathrm{d}t\,; \qquad (1)$$

and water uptake down a gradient in water potential,

$$[(\mathrm{d}V/\mathrm{d}t)/V]_{\text{tissue}} = (L/V)\,(\Psi_o - \Psi_s - P) - [(\mathrm{d}V/\mathrm{d}t)/V]_{\text{transpiration}}. \qquad (2)$$

In equation 1, $V$ represents tissue volume, $[(\mathrm{d}V/\mathrm{d}t)/V]_{\text{tissue}}$ represents the relative volumetric rate of tissue expansion, $m$ the extensibility of the cell wall, $P$ the turgor pressure, $Y$ the critical turgor pressure or yield threshold below which growth does not occur, and $\varepsilon$ the volumetric elastic modulus. Similarly, in equation 2, $[(\mathrm{d}V/\mathrm{d}t)/\ V]_{\text{tissue}}$ represents the relative volumetric rate of tissue expansion, $L$ the hydraulic conductance of the water transport pathway, $(\Psi_o–\Psi_s – P)$ the difference in water potential between the source and the cell, and $[(\mathrm{d}V/\mathrm{d}t)/V]_{\text{transpiration}}$ the relative volumetric rate of transpiration.

Water deficits generally develop as a decrease in source water potential, and this factor has been the most amenable to experimental manipulation. Although growth inhibition at low $\Psi_o$ was often attributed to a loss of $P$, evidence has accumulated that growth regulation differs among species and/or tissues. For example, in soybean hypocotyls, a decrease in $\Psi_o$ inhibited growth without changes in $P$ (Nonami and Boyer 1989, 1993). According to the Lockhart model, these results are expected when the hydraulic conductivity of the tissue is much smaller than the wall extensibility, and growth is limited by water transport. In most experiments, however, changes in $\Psi_o$ have altered both growth and $P$ (Pritchard *et al.* 1991; Spollen and Sharp

Abbreviations used: $b$, cell wall hardening due to displacement of hemicellulose tethers; $c$, cell wall loosening due to enzymatic cutting of load-bearing tethers or breaking of hydrogen bonds; $C_{\text{wvair}}$, concentration of water vapor in the air; $C_{\text{wvleaf}}$, concentration of water vapor in the leaf; $d$, midvein diameter; $g^c$, measured cuticular conductance; $l$, length of the midvein segment; $L$, hydraulic conductivity of the water transport pathway; $m$, wall extensibility; $m_i$, wall extensibility estimated from the relation between steady-state growth and the effective turgor for growth; $m_{ss}$, wall extensibility estimated from the steady-state conditions of turgor and leaf elongation; $P$, cell turgor; PPFD, photosynthetic photon flux density; $r$, midvein radius; $rgr$, relative growth rate; RH, relative humidity; $V$, tissue volume; $Y$, yield threshold; $Y_i$, yield threshold estimated from the decline in turgor in response to a decrease in humidity; $Y_{ss}$, yield threshold estimated from steady-state conditions of turgor and leaf elongation; $\varepsilon$, volumetric elastic modulus; $\Psi_o$, source water potential; $\Psi_c$, cell water potential; $\Psi_s$, cell osmotic potential.


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1991). Furthermore, the initial change in *P* may parallel the change in $\Psi_o$ and/or the change in *P* of mature, non-growing cells (Serpe and Matthews 1992, 1994*a*; Frensch and Hsiao 1994, 1995). Such results are expected when the hydraulic conductivity is much larger than the wall extensibility, and thus when growth is limited by wall yielding (Cosgrove 1981).

Wall yielding varies in response to changes in plant water status (Green *et al.* 1971; Shackel *et al.* 1987; Zhu and Boyer 1992; Bogoslavsky and Neumann 1998; Proseus *et al.* 1999). This response too, seems to differ between systems or experimental conditions. In wheat roots (Pritchard *et al.* 1991), soybean stems (Nonami and Boyer 1990), and leaves from several species (Matthews *et al.* 1984; Passioura 1988; Saab and Sharp 1989; Schultz and Matthews 1993), wall yielding to *P* decreased under water deficits, thus contributing to a reduction in growth rates. In contrast, in maize roots (Spollen and Sharp 1991; Frensch and Hsiao 1994, 1995), and leaves of *Begonia* (Serpe and Matthews 1992, 1994*a*) and maize (Hsiao *et al.* 1998), water deficits increased wall yielding to *P*, resulting in recovery of growth at low *P* values.

Presently, it is uncertain how these different responses are brought about. One factor that may affect plant response is the manner in which water deficits develop. Soil water depletion tends to occur gradually and affects the water potential of the whole plant. In contrast, fluctuations in evaporative demand can produce short-term and localized water deficits that principally affect leaves (Frensch 1997; Shackel *et al.* 1987). Leaf responses to whole plant water deficits may differ from leaf responses to localized water deficits. For expanding dicot leaves, the growing region is not localized and growth takes place in the presence of a relatively dry environment in contrast to the relatively protected environment of roots and monocot leaves.

Soil water depletion alters root metabolism (Saab and Sharp 1989), causing changes in the composition of the xylem sap that include decreased cytokinin (Itai and Vaadia 1971), increased abscisic acid (Schurr *et al.* 1992), and an increase in pH (Wilkinson and Davies 1997). All are factors that can affect the yielding properties of leaf cell walls (Huff and Ross 1975; Rayle *et al.* 1982; Saab and Sharp 1989). Such metabolic responses may be diminished or absent during transient or localized water deficits that mainly affect leaves, thus leading to different mechanisms of growth adjustment.

In previous studies, we investigated the wall yielding behavior of *Begonia* leaves following changes in soil water potential that were caused by addition of mannitol to the rooting media, withholding irrigation, and rewatering (Serpe and Matthews 1992, 1994*a*). These studies indicated that changes in plant water status led to rapid adjustment in the wall yielding properties that partially compensated for the initial effects of changes in *P* on leaf elongation. This is not consistent with observations of complete self-stabilization in some other systems. Our previous work involved the use of osmoticum and rather large changes in turgor, both of which could lead to possible artefacts. Also, it is feasible that the propensity for self-stabilization is limited and could be overwhelmed by large changes in turgor. Therefore, this study was conducted to test whether small changes in *P* invoked by changes in evaporative demand cause a similar response. This is an important question because presently the dominant view is that steady growth has little dependence on turgor, but rather that there is complete self-stabilization of growth rates (Passioura and Fry 1992; Zhu and Boyer 1992; Tomos and Pritchard 1994; Van Volkenburgh 1999).

## Materials and methods

### *Growing conditions*

*Begonia argenteo-guttata* L. plants were grown from rooted cuttings in 550-mL pots in a mixture of sphagnum peat moss : vermiculite : perlite (2:1:1) in a growth chamber under the conditions described by Serpe and Matthews (1992). A plant having seven or eight fully expanded leaves was used for each experiment. Measurements were made on leaves that were expanding rapidly (midvein length of 67–73 mm), expanding slowly (midvein length of 102–108 mm), or were fully expanded (midvein length of 138–145 mm).

### *Experimental conditions*

Six h after the initiation of the light period, plants were transported to the laboratory [air temperature 27 ± 3°C and relative humidity (RH) 35 ± 7%]. The pot was fixed to a custom-built vibration-free table, and the experimental leaf was positioned in a Plexiglas chamber (115 mL). The chamber was supplied with 300 μmol photons $m^{-2}$ $s^{-1}$ photosynthetic photon flux density (PPFD) from Vita lights (Duro-Test Corp., Fairfield, NJ) and conditioned air with a flow rate of 150 mL $min^{-1}$.

### *Growth and turgor measurements*

Elongation and epidermal cell turgor were measured simultaneously as previously described (Serpe and Matthews 1992). Measurements of growth were made along the leaf midvein using a linear variable differential transformer (Schaevitz Engineering, Pennsauken, NJ). *In situ* measurements of *P* were made using the pressure microprobe technique (Zimmermann *et al.* 1989). All *P* measurements were made in the abaxial epidermis near the center (± 5 mm) of the midvein region along the axis in which growth was measured. In growing leaves, these cells were approximately 60 μm in length and 33 μm in width and depth, with the longer axis parallel to the leaf midvein. In fully expanded leaves, the cells were approximately 135 μm in length and 35 μm in width and depth.

### *Treatments*

Steady conditions, including the rate of leaf elongation, were established for at least 30 min before imposing step changes in air humidity. To obtain 5 (± 2) % RH within the leaf chamber, the chamber was supplied with air coming directly from a compressed air cylinder. To obtain 70 (± 2) % RH within the chamber, air coming from the air cylinder was bubbled through water before entering the chamber. At the flow rate used, 150 mL $min^{-1}$, humidity changes were 90% complete within 3 min of introducing new air.

### *Transpiration*

The rate of transpiration was measured on the adaxial side of the leaf, which lacks stomata, with a porometer (Li-Cor, Lincoln, NE). Measurements were made under experimental light and temperature conditions at 70% RH.

## Results

Under our growth chamber conditions, young leaves separated from the stem after attaining a length of about 60 mm. The growth rate of these leaves was 20% $d^{-1}$. Thereafter, growth rates declined steadily until growth ceased at a leaf length of about 140 mm after 14 d (Serpe and Matthews 1994*b*). The rapidly growing leaves used in the experiments were 70 (± 3) mm long and they showed uniform elongation along the midvein (Serpe and Matthews 1994*b*). In planar view, bulges of tissue between midvein and secondary veins suggest that the axial load is not borne by the intermediary tissue and, therefore, that the midvein tissue is limiting axial expansion.

Air temperature in the chamber was maintained at 28°C (Fig. 1). The nominal 70% RH was maintained at 68 ± 2% RH. Upon introduction of dry air, RH decreased to 5–7% within 10 min, and the transition was 90% complete within 5 min. Leaf temperature decreased about 1°C when dry air was introduced. For a 70 mm leaf growing at 0.6% $h^{-1}$, a step decrease in RH caused leaf expansion to cease immediately and remain at zero for approximately 20 min (Fig. 2). The coincident decrease in epidermal *P*, determined in this case within 2 min of the change in RH, was about 0.05 MPa. Growth gradually recovered during the second 20 min following changed humidity, attaining a new steady-state rate that was greater than 50% of the initial control rate. The recovery of growth was accompanied by no recovery of turgor.

These responses, immediate cessation of growth, small decrease in turgor, partial recovery of growth with no recovery of turgor, were observed repeatedly (Fig. 3). The recovered growth rate was 0.40 ± 0.08% $h^{-1}$, and the mean decrease in *P* was less than 0.06 MPa (0.056 ± 0.016 MPa). Although no statistically significant recovery of turgor occurred, variation may have obscured a recovery of less than 0.02 MPa in 75 min after the humidity change, and this may have arisen as a consequence of stomatal closure in the low humidity air. Similar results were obtained for leaves at different stages of development. When humidity was decreased around leaves that were 3/4 fully expanded (105-mm leaves), growth ceased immediately and turgor decreased about 0.03 MPa within 5 min (data not shown).

In non-growing leaves (140 mm) exposed to a drop in RH, the decrease in *P* and the elastic contraction were complete in about 10 min. Furthermore, no recovery of *P* or elongation

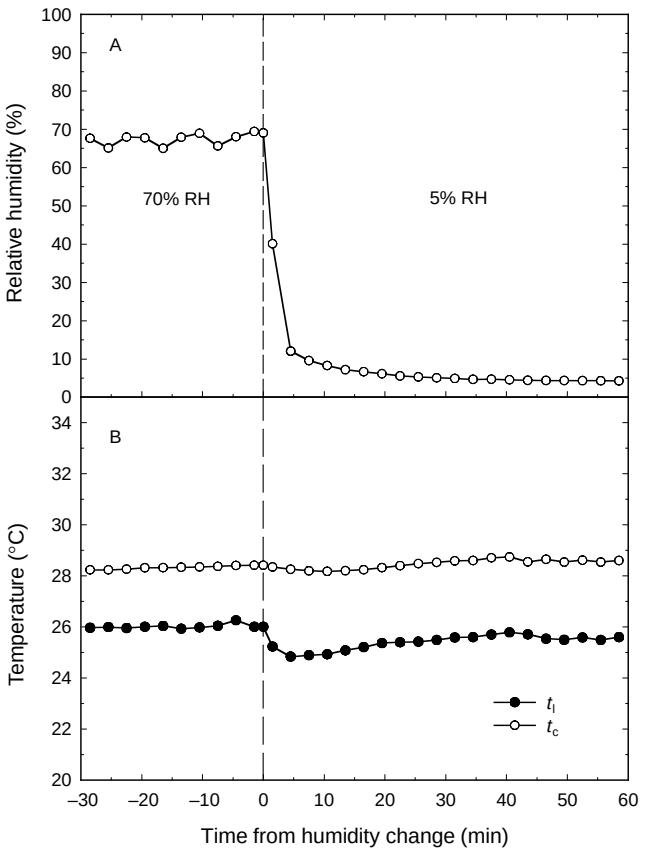


**Fig. 1.** Relative humidity (*A*) and temperature of chamber air ($t_c$) and leaf ($t_l$) (*B*) under experimental conditions.

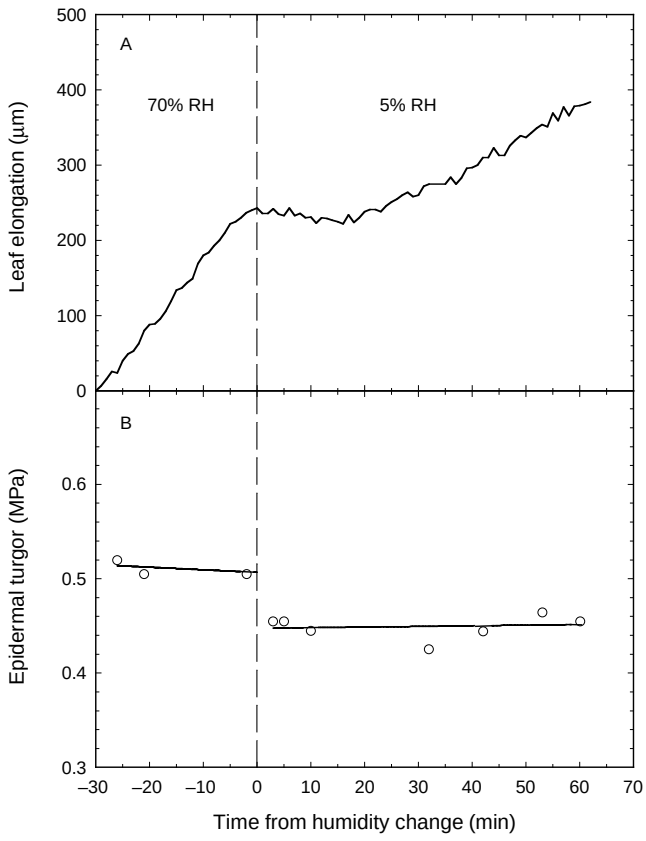


**Fig. 2.** Leaf elongation (*A*) and epidermal cell turgor (*B*) of a 70-mm-long *Begonia* leaf before and after decrease in air humidity. Each datum in *B* represents the turgor of a single and different cell.

occurred after the decrease in humidity. Elastic changes in growing cells may differ from those in non-growing cells, especially in magnitude due to differences in the value of ε (Proseus *et al.* 1999). Notwithstanding these differences, one common feature of elastic changes is that they seem to occur without a significant delay to alterations in *P* (Fig. 4; Serpe and Matthews 1994*a*; Proseus *et al.* 1999). Under this scenario, the growth responses observed in young leaves cannot be attributed to elastic contraction on top of growth, since elongation was zero for over 15 min after the completion of the decrease in *P* (Fig. 2).

The results caused by step decreases in RH were reversible. A steady-state rate of expansion of 0.38% $h^{-1}$ was established at 5% RH (Fig. 5). Upon increasing air humidity, the rate of expansion increased dramatically for about 5 min. Thereafter, the rate of expansion decreased for 40 min, until a new stable rate was established that was about 2-fold greater than the initial rate. The increase in steady-state *P* was about 0.04 MPa, and this increase was complete within about 10 min. These results are essentially the inverse of those obtained from a step decrease in humidity, although there was a less clear period of a sustained, high rate of growth that was followed by a decrease to a new stable rate of growth. This probably represents a rapid adjustment of *Y* by strain hardening upon a step increase in *P* (Green *et al.* 1971). Upon increasing RH, the extension that cannot be attributed to the final rate of elongation was 0.23 mm. This extension cannot be entirely attributed to an elastic effect, since a similar decrease in *P* (*cf.* Fig. 2) caused negligible leaf contraction. Furthermore, changes in *P* of 0.28 MPa, 6-fold larger that those caused by changes in RH, resulted in an elastic contraction of only 0.15 mm (Serpe and Matthews 1994*a*). Thus, the effect of elastic deformation on leaf elongation is too small to account for the rapid rates of growth upon the increase in RH.

The rapid cessation of growth upon a step decrease in RH reflects that *P* decreased below the yield threshold for irreversible growth. The yield threshold was not constant; however, it decreased during the period of low humidity as evidenced by the recovery of growth at constant *P*. The yield threshold and wall extensibility at a particular time will referred to as the instantaneous yield threshold ($Y_i$) and the instantaneous extensibility ($m_i$), respectively. Values for $Y_i$ and $m_i$ were estimated from the short-term growth responses

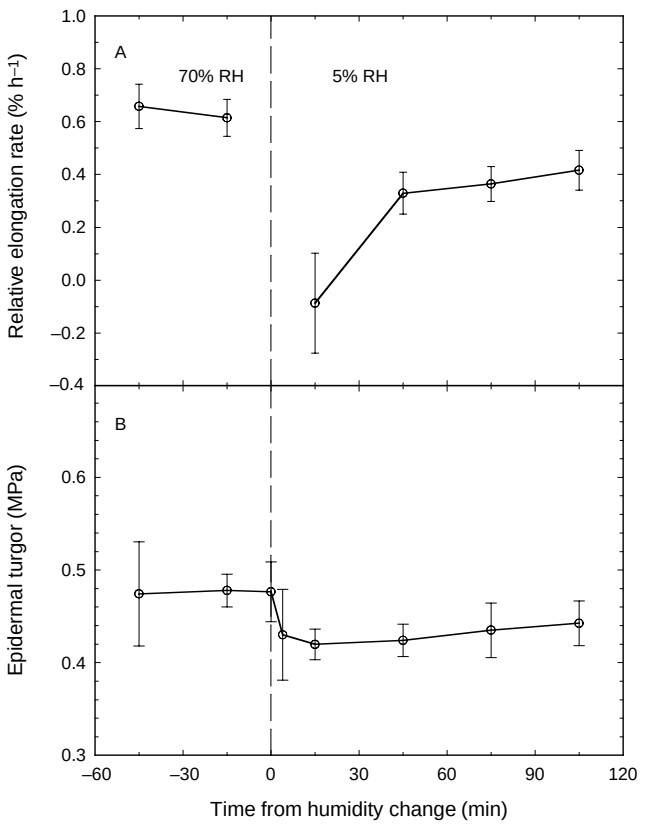


**Fig. 3.** Mean relative leaf elongation rate (*A*) and epidermal cell turgor (*B*) of 70-mm-long *Begonia* leaves before and after decrease in air humidity. Error bars indicate standard deviation of the mean of five experiments.

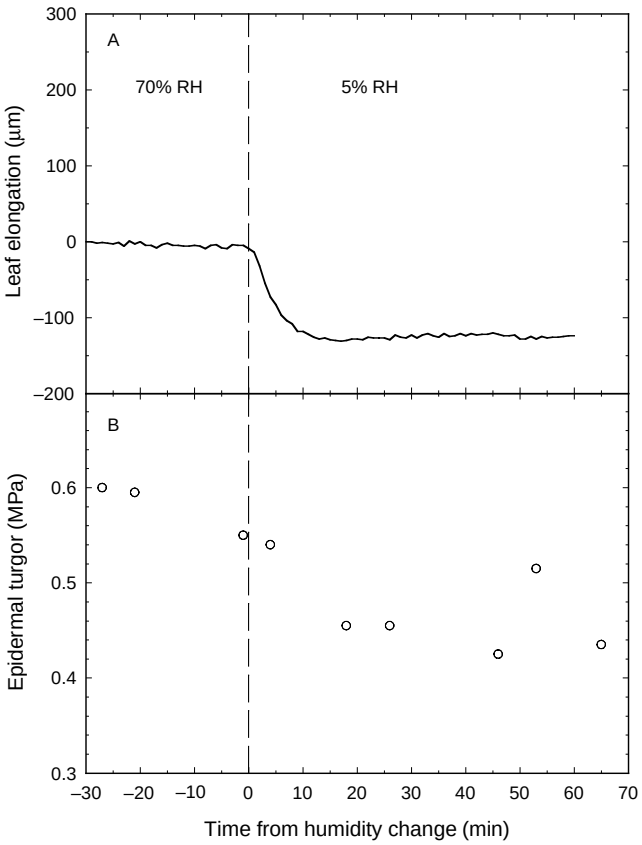


**Fig. 4.** Leaf elongation (*A*) and epidermal cell turgor (*B*) of a 140-mm-long *Begonia* (mature, non-growing) leaf before and after decrease in air humidity. Each datum in *B* represents the turgor of a single and different cell.

to changes in $P$. For example, for the young leaf under high RH, a decrease in $P$ of about 0.05 MPa caused the cessation of growth at a $P$ value of 0.43 Pa. Thus for young leaves at high RH, $Y_i$ was about 0.43 MPa. Similarly for the 105 mm leaf, $Y_i$ was 0.53 MPa or 0.030 MPa less than the $P$ required to sustain high rates of expansion. Both estimates of $Y_i$ indicate that the decrease in $P$ required to stop growth was not larger than 0.05 MPa. Assuming that under high humidity conditions the effective turgor for growth ($P - Y_i$) is equal to 0.05 MPa for the 70 mm leaves and 0.03 MPa for the 105 mm leaves, $m_i$ can be estimated using equation 1 and the growth rate. For the 70 and 105 mm leaves these estimates were 13.0 and 8.3% $h^{-1}$ $MPa^{-1}$, respectively.

The steady-state growth/turgor relation developed as a consequence of partial growth recovery for leaves of different stages of development is shown in Fig. 6. This relation has been used to estimate the values of $m$ and $Y$, assuming that the wall yielding properties remain constant during changes in plant water status. This and other studies (see Discussion) have questioned this assumption. Nevertheless, the values of $m$ and $Y$ calculated from steady-state conditions may provide a low estimate for these parameters. The $Y$ for steady-state conditions ($Y_{ss}$) can be estimated by extrapolation of these steady-state curves to zero growth. For the 70 and 105 mm leaves, $Y_{ss}$ was 0.36 and 0.51 MPa, respectively. These estimates of $Y$ are also quite close to the $P$ at high water status and growth rates, between 0.12 and 0.05 MPa less than the $P$ of controls at high RH. The $m$ for steady-state conditions ($m_{ss}$) can be estimated from the slopes of the plots of steady-state growth and $P$. For 70 and 105 mm leaves, $m_{ss}$ was 5.4% $h^{-1}$ $MPa^{-1}$ and 5.74% $h^{-1}$ $MPa^{-1}$, respectively. This simple 2-turgor analysis reveals no significant change in $m_{ss}$ and an increase in $Y_{ss}$ as leaf ontogeny progressed and the rate of growth declined.

To complete the Lockhart analysis, we calculated the rate of water transport required for growth and transpiration at 70 and 5% RH. This estimate was made for a rapidly growing leaf on a unit midvein segment. For the calculations, we assumed that this segment was a cylinder 10 mm in length and 1 mm in diameter, which is the approximate diameter of the midvein in the region where $P$ was measured. Based on the assumption that the midvein is a cylinder, the water required for growth was estimated as the increase in volume of this cylinder due to elongation. Similarly, the water required for transpiration was estimated as the water lost through the surface area of the cylinder considered. Thus, the water required for growth was calculated as follows:

$$\text{Water for growth} = (\mathrm{d}V/\mathrm{d}t)_{\text{tissue}} = l\,\pi\,r^2\,rgr, \qquad (3)$$

where $l$ is the length of the midvein segment considered (10 mm), $r$ is midvein radius (0.5 mm) and $rgr$ is the relative growth rate (0.65 and 0.40% $h^{-1}$ for 70 and 5% RH, respectively). Similarly, the water required for transpiration, all cuticular in the midvein, was estimated using the following equation:

$$\text{Water for transpiration} = (\mathrm{d}V/\mathrm{d}t)_{\text{transpiration}} = g^c\,(C_{\text{wvleaf}} - C_{\text{wvair}})\,\pi\,d\,l\,, \qquad (4)$$

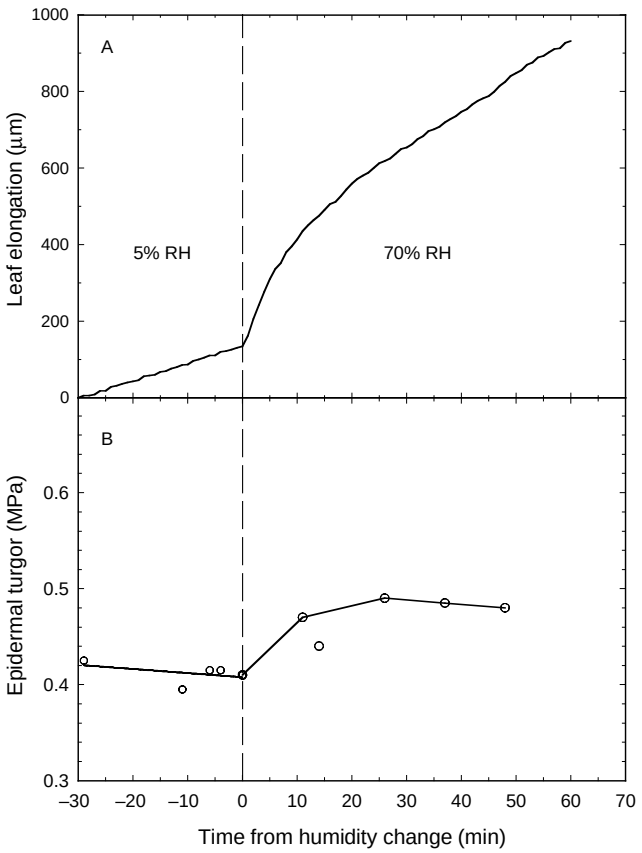


**Fig. 5.** Leaf elongation (*A*) and epidermal cell turgor (*B*) of a 70-mm-long *Begonia* leaf before and after increase in air humidity. Each datum in *B* represents the turgor of a single and different cell.

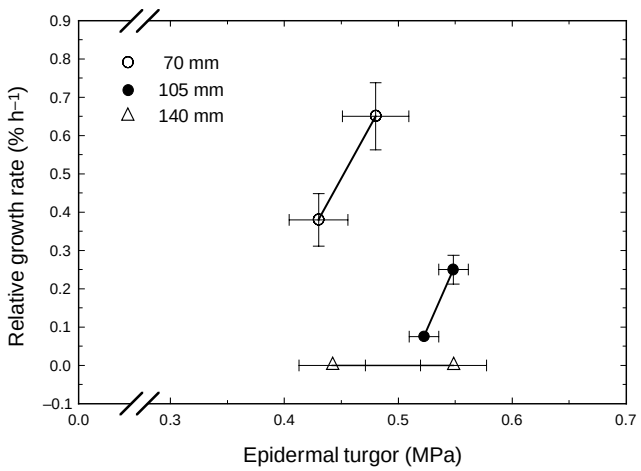


**Fig. 6.** Mean rate of elongation and epidermal cell turgor for 70, 105, and 140-mm-long *Begonia* leaves before and approximately 50 min after decreases in air humidity.

where $g^c$ is the measured cuticular conductance (0.1575 mm $s^{-1}$), $C_{wvleaf}$ is the concentration of water vapor in the leaf (estimated from the leaf temperature and assuming 100% RH within the leaf), $C_{wvair}$ is the concentration of water vapor in the air, $d$ is the midvein diameter (1 mm), and $l$ is the length of the midvein segment considered. The estimates obtained with equations 3 and 4 (Table 1) indicate that water transport at low RH was about 3-fold higher than at high RH.

The rates of total water transport were used in combination with equation 2 to estimate the hydraulic conductance of the path from source xylem to expanding epidermal cells. Following multiplication of equation 2 by $V$ and rearrangements the total rate of water transport ($dV/dt$) can be expressed as follows:

$$dV/dt = (dV/dt)_{tissue} + (dV/dt)_{transpiration} = L\,(\Psi_o - \Psi_c), \quad (5)$$

where $\Psi_c$ is the cell water potential, which is equal to $\Psi_s$ plus $P$ in equation 2 . All other symbols are those used in equation 2.

Equation 5 can be used to represent the total water transport for high and low RH conditions:

$$dV/dt^1 = L\,(\Psi_o - \Psi_c^1) = L\,(\Delta\Psi^1) \quad (6)$$

$$dV/dt^2 = L\,(\Psi_o - \Psi_c^2) \quad (7)$$

$$dV/dt^2 = L\,(\Psi_o - (\Psi_c^1 + \Delta P)) = L\,(\Delta\Psi^1 - \Delta P)\,, \quad (8)$$

where $dV/dt^1$ is total water transport under high RH and $dV/dt^2$ is total water transport under low RH. If we assume that the change in the water potential gradient required for the two rates of water transport was the change in $P$ of epidermal cells, then equation 7 can be set equal to equation 8, which produces two equations with two unknowns. Solving these for $L$ produces an estimate of 303 μmol $h^{-1}$ $MPa^{-1}$. For 70 mm leaves and the unit segment considered, the value of $m_i$ expressed in the same units is 56.6 μmol $h^{-1}$ $MPa^{-1}$ (= 13% $h^{-1}$ $MPa^{-1}$ × π (0.5 mm)$^2$ 10 mm × 1000 μg $mm^{-3}$ × 5.556 $10^{-2}$ μmol $\mu g^{-1}$). Thus despite the steep growth rate *vs* $P$ curve, $L$ is several times greater than the estimated wall extensibility.

**Table 1. Estimates of water transport for different humidity conditions**

Calculations were made for midvein segments, 10 mm long and 1 mm in diameter, of rapidly growing leaves according to equations 3 and 4

| RH (%) | Water for growth (μmol $h^{-1}$) | Water for transpiration (μmol $h^{-1}$) | Total water transport (μmol $h^{-1}$) |
|---|---|---|---|
| 70 | 2.83 | 5.27 | 8.10 |
| 5 | 1.74 | 21.51 | 23.35 |

## Discussion

In this study, small decreases in epidermal cell $P$ caused by a decrease in air humidity were sufficient to stop growth. Growth recovered to 70% of the initial rate without a corresponding increase in $P$. These responses were reversible. Upon a step increase in humidity, $P$ and growth rates rapidly increased. Subsequently, growth rates declined without a corresponding decrease in $P$. These growth responses to changes in $P$ are almost identical to those observed when $P$ was altered by changes in soil water potential (Serpe and Matthews 1992, 1994*a*). Thus, rapid changes in the water status of a single leaf had similar effects on growth and wall yielding as changes in the water potential of the whole plant propagated through the root system. However in our experiments involving decreases in soil water potential (Serpe and Matthews 1992, 1994*a*), water deficits developed over a short period; minutes in the case of experiments involving osmoticum, to 2 d in experiments where water was withheld. Also, we only investigated the effects of mild water deficits; those that develop more gradually or that last for a longer period may elicit a different response (Matthews *et al.* 1984; Schultz and Matthews 1993).

Factors controlling water transport or wall yielding can in principle be responsible for growth adjustments at constant $P$. To assess the role of these two processes in the control of growth, we estimated the hydraulic conductivity and wall extensibility of a unit midvein segment. The estimated value of $L$ was 5-fold larger than the wall extensibility. Based on these estimates, wall yielding is the main growth-limiting factor in *Begonia* leaf elongation.

The simulation of water transport that gave rise to our estimates of hydraulic conductivity and wall extensibility involves several assumptions that may be incorrect. Errors in our estimates of transpiration may have occurred because we used a steady-state gas exchange system to measure the low diffusive conductance through the cuticle. However, even if our estimates of cuticular conductance were 100% greater than the true value, the estimated $L$ would still be significantly greater than our estimates of wall extensibility.

Epidermal cells may have adjusted osmotically in response to lowered $P$. This would lower cell water potential and increase the water potential gradient. Consequently, our estimated hydraulic conductance would be erroneously high. However, the short time frame of the experiments makes significant osmotic adjustment unlikely (*cf.* days required in the soybean hypocotyl, Nonami and Boyer 1989), and $P$ did not recover as in rapidly osmotically adjusting systems (e.g. maize roots, Frensch and Hsiao 1995). We also have not found evidence of osmotic adjustment in *Begonia* leaves in longer-term experiments. Indeed, the lack of osmotic adjustment may be an important aspect of this system in that the inability to regulate $P$ simplifies the analysis of growth recovery.

Our estimates also assumed that the decrease in air humidity did not alter the source water potential. A transient decrease in the source water potential may have occurred due to the increased rate of transpiration, particularly over the lamina where stomata are present. However, in this system (Serpe and Matthews 1994*a*) and in wheat leaves (Passioura 1988), expanding leaf cell and source water potentials changed similarly, virtually simultaneously, and by the amount that expanding cell $P$ changed when altered by treating roots with osmoticum or pressure, respectively. Thus in those two cases, there was no net change in the water potential gradient from stem xylem to expanding leaf cells when plant water potential was perturbed at the roots. If a sustained decrease in the source water potential did occur, it would represent a decrease in the driving force for water transport where none was assumed. Thus, our estimate of $L$ would again be low.

An alternative possibility is that $L$ of membranes decreased with an increase in $P$. This was observed in *Elodea* cells (Steudle *et al.* 1982), but not in other species (Steudle 1989; Nonami and Boyer 1990; Lu and Neumann 1999). Also, none of the cloned aquaporins is directly gated by hydrostatic pressure (Maurel 1997). Given the small changes in $P$ involved in this study and the lack of evidence for a widespread role of $P$ on $L$, it seems unlikely that $L$ decreased at high humidity.

In *Begonia* leaves, the decrease in RH caused a $P$ reduction of 0.05 MPa and 30–40% reduction in the steady rate of elongation. These results agree with other studies showing that leaf expansion is very sensitive to changes in water potential (Boyer 1968; Acevedo *et al.* 1971; Dale 1988; Kramer and Boyer 1995). For *Begonia*, this sensitivity is due to a strong dependence of growth on $P$, rather than an effect of water deficits on decreasing wall yielding. Water deficits caused by increasing transpiration or decreasing soil water potential resulted in an increase in wall yielding, but growth was reduced due to the decrease in $P$. Hsiao *et al.* (1998) observed a similar phenomenon in maize leaves. Presently, there are few studies where growth and $P$ of expanding cells were measured simultaneously and directly in the leaves of intact plants (Serpe and Matthews 1992, 1994*a*). Other studies have provided evidence that water deficits tend to decrease wall yielding, but the evidence is largely based on observations that leaf growth is reduced although $P$ is maintained through osmotic adjustment (Matsuda and Riazi 1981; Michelena and Boyer 1982; Schultz and Matthews 1993). In those studies, however, $P$ was not measured directly with the pressure microprobe technique. Consequently, small decreases in $P$ of growing cells may have not been detected.

Probably since Michelena and Boyer (1982) reported evidence of growth inhibition in the presence of complete turgor maintenance, perception of growth regulation has been moving away from turgor. Recently the movement has progressed towards the conclusion that turgor plays little role in growth regulation, because both turgor and growth are self-regulated in the presence of changing water availability (Passioura and Fry 1992; Zhu and Boyer 1992; Tomos and Pritchard 1994; Van Volkenburgh 1999). Much of the data supporting this concept is from tissues in aqueous or non-transpiring environments. The data in this study provide a compelling contrast. In dicot *Begonia* leaves growing in air, direct and simultaneous measurements of growth and turgor showed that growth was highly dependent on turgor. Growth was only partially self-regulated, and $P$ was not self-regulated. The lack of self-regulation was observed even when the system was perturbed by only 0.05 MPa using non-invasive changes in air humidity. Thus, this *Begonia* leaf behavior is in contradiction with much present thinking. Consistent with present theory is the evidence of a regulated yield threshold. The factors that regulate the yield threshold are not clearly understood (Okamoto *et al.* 1990). However, the results of this and a recent study in internodal cells of *Chara*, (Proseus *et al.* 1999) provide evidence that turgor plays a role in the control of wall yielding. In both systems, small changes in $P$ markedly altered steady growth rates, notwithstanding adjustments in the wall yielding properties. The similarities in the results observed in these two distant species also suggest that a strong dependence of growth on turgor may be a common phenomenon-throughout the plant kingdom.

In several systems small reductions in $P$ result in immediate growth cessation, implying a $P$ close to $Y$. In *Nitella*, the steady-state $P - Y$ was estimated to be 0.02 or 0.03 MPa (Green *et al.* 1971). Frensch and Hsiao (1995) made similar observations in maize roots, and in expanding *Vitis* leaves, leaf water potential did not drop measurably upon excision from their water supply (Schultz and Matthews 1993). In the *Begonia* leaves, maize roots, and *Nitella* internodes, growth resumed within 30 min of a small decrease in $P$. The recovery of growth without recovery of $P$ in *Begonia* leaf showed that wall yielding was increased by or during low $P$ conditions. The rapid regulation renders the implementation of $Y$ as a fixed parameter problematic, as some have noted (Shackel *et al.* 1987; Okamoto *et al.* 1989, 1990; Passioura and Fry 1992; Frensch and Hsiao 1995).

This situation was anticipated in concept by Lockhart (1965) and soon addressed in theory by Green *et al.* (1971). Green *et al.* (1971, 1977) proposed a yield threshold that would vary positively with strain hardening and negatively with time, due to enzymatic wall softening. Passioura and Fry (1992) further refined this concept by introducing physicochemical definitions for wall yielding and using wall tension rather than pressure. The tension is borne by hemicellulose tethers between cellulose microfibrils and by the hydrogen bonds securing the tethers to the microfibrils. Wall loosening arises from enzymatic cutting of load-bearing tethers or breaking of hydrogen bonds. The ideas depicted in

this model are probably a simplification of the processes involved in wall yielding, but they are in agreement with our present understanding of cell wall structure and metabolism (Fry 1989; Carpita and Gibeaut 1993; Cosgrove 1999).

To account for variable wall yielding, Passioura and Fry (1992) related strain hardening to the number of new taut hemicellulose tethers created by a unit wall displacement (*b*). Wall softening is the number of taut tethers enzymatically cut per unit time (*c*). Under steady conditions, the rate of growth is equal to the ratio *c/b* (Passioura and Fry 1992).

If *c* and *b* are constants, the Passioura–Fry model (1992) predicts complete self-stabilization of growth following *P* perturbations. Complete self-stabilization has been observed (e.g. in wheat leaves, Passioura and Fry 1992) but often at high *P* and over a narrow *P* range (Green *et al.* 1971; Shackel *et al.* 1987; Hsiao *et al.* 1998). For example, in *Nitella*, complete self-stabilization was observed only for *P* between 0.5 and 0.6 MPa; for *P* below 0.5 MPa there was a significant growth dependence on *P* (Green *et al.* 1971, Fig. 2, top curve). We too, found that growth was completely self-stabilized when *Vitis* leaves at relatively high *P* were subjected to an increase in *P* (Shackel *et al.* 1987). Growth response to a decrease in *P* from well-watered conditions was not investigated.

In contrast, a dependence of growth upon *P* is a common observation in growth studies (Acevedo *et al.* 1971; Bunce 1977; Matthews *et al.* 1984; Serpe and Matthews 1992, 1994*a*; Hsiao *et al.* 1998; Proseus *et al.* 1999). When the steady-state rates of growth are plotted against the steady-state epidermal *P* for experiments from several studies with our system, a positive relationship between growth and *P* emerges (Fig. 7). The treatments to decrease *P* and growth rate included addition of mannitol to the roots, withholding water, and increasing evaporative demand. The approaches included time scales from days to about 2 min, and changes at both ends of the soil–plant–atmosphere continuum that altered *P*. The results of the various treatments were reversible. The steady rates (after adjustment periods) appear to fall on the same curve. The recovery of growth following *P* reductions in *Begonia* was never complete, but was in proportion to the *P* reduction (inset Fig. 7). Thus, the *Begonia* leaf exhibits a partial self-stabilization of growth in the short-term, and a clear (and approximately linear) relation to *P* in the recovered state.

These patterns can be predicted by the Passioura–Fry model if *c* and/or *b* is not constant. Passioura (1992) indicated that the rate at which load-bearing tethers are cut (*c*) might increase with their number. Similarly, an increase in load-bearing tethers may increase the rate of disruption of hydrogen bonds. From a biochemical perspective, this can be interpreted as an in increase in substrate (number of load-bearing tethers) available for enzymatic activity (Passioura 1992). Furthermore, in the Passioura–Fry model the number of load-bearing tethers increases with *P*. If *c* increases with the number of load-bearing tethers, then the model predicts an increase in the steady growth rate with increasing *P* and partial self-stabilization of growth following *P* perturbations (Passioura 1992). These were precisely the patterns of growth observed in *Begonia* leaves.

The curve in Fig. 7 extrapolates to a *P* intercept of about 0.3 MPa. When a –0.4 MPa mannitol treatment was applied to the root system, *P* decreased to 0.27 MPa and growth ceased without recovering (Serpe and Matthews 1994*a*). From this analysis, we conclude that there is a non-adjustable *Y* that exists as a minimum *P* for growth in addition to the yield threshold determining the instantaneous growth/turgor relation. In the *Begonia* leaf system, this minimum value appears to be near 0.3 MPa.

Passioura and Fry (1992) incorporate a 'putative minimum tension' (see their equation 5) that is explicitly a constant and apparently a minimum tension required to break hydrogen bonds between hemicellulose and cellulose microfibrils. However, if *c* and *b* are constants, growth would be maintained at any *P* greater than zero. This prediction is in contrast to the common observation that a *P* significantly above zero is required for growth. Again incorporation of a dependence of wall softening on the number of load-bearing tethers such that wall softening stops below a certain number of load-bearing tethers would result in predictions that better mimic experimental results.

The growth/P relations observed in *Begonia* leaves are consistent with the idea that wall yielding is controlled by

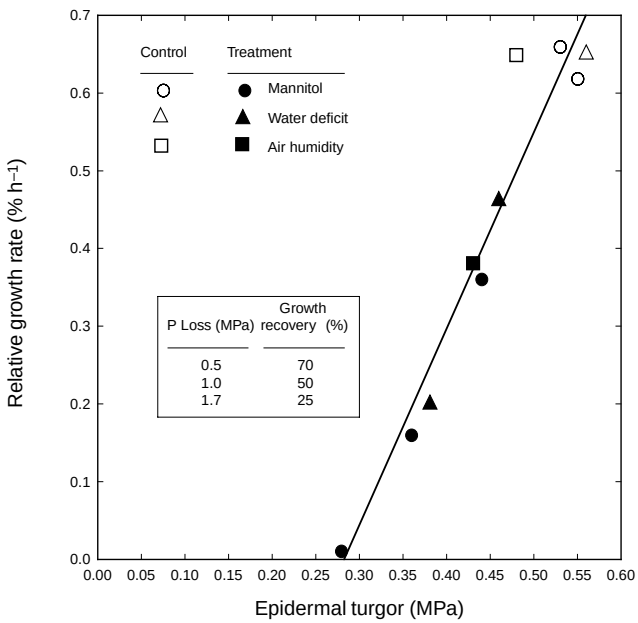


**Fig. 7.** Relative growth rate at various epidermal cell turgor values for 70-mm-long *Begonia* leaves before and after various treatments to reduce cell turgor. Treatments included addition of –0.2, –0.3, and –0.4 MPa mannitol solutions to root media, withholding water for several days, rewatering, and increasing and decreasing air humidity.

one or more interactions between metabolism and *P* (Green *et al.* 1971; Ray *et al.* 1972; Passioura 1992; Proseus *et al.* 1999). The nature of the interaction is, however, unclear. As proposed by Passioura (1992), one possibility is that tension increases enzyme access to wall substrates. Other scenarios are, however, equally possible. For example, tension and its effect on molecular displacement may counteract the tendency of cell wall polymers for self-assembly (Roland *et al.* 1977). If wall strength is primarily determined by weak bonds (Preston 1979; Cosgrove 1999), this role of *P* on wall yielding may be particularly important. As weak bonds are disrupted due to the activity of expansins (McQueen-Mason and Cosgrove 1994) or similar molecules, tension and molecular displacement will prevent their aggregation in the same arrangement. Less direct effects of *P* on wall yielding could also be considered as alternative hypotheses. Turgor causes appression of the plasma membrane against the wall (Nobel 1991). This may impact wall loosening via proton pumping (Robinson and Cummins 1976; Bogoslavsky and Neumann 1998) or vesicle secretion (Battey and Blackbourn 1993), although at present direct evidence of either is lacking. Independent of the mechanisms involved, taken together the results in *Begonia* indicate that leaf growth is strongly dependent on *P* and that wall yielding is continually controlled by an interaction between *P* and metabolism.

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