Cell expansion rate, temperature and turgor pressure in growing leaves of *Lolium temulentum* L.

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**Summary**

Leaf growth rate and the turgor pressure of cells in the expanding zone of leaves of *Lolium temulentum* were measured simultaneously. Growth rate was reduced over a range from 30 μm min⁻¹ to zero by reducing the temperature of the expanding zone from 20 to 2 °C. Turgor pressure remained constant at 0.5 MPa. This implies that growth reduction by low temperature is due to changes in cell wall rheology. Cell membrane hydraulic conductivity (Lₚ) was reduced with temperature as expected, but this was not sufficient to influence growth rate detectably.

Key words: *Lolium temulentum*, growth rate, turgor pressure, wall rheology, pressure probe, chill stress.

**Introduction**

The annual growth cycle of perennial temperate grasses involves substantial exposure to sub-optimal temperatures. These plants do not possess a true winter dormancy mechanism, but respond directly to prevailing temperature conditions. An understanding of the nature of these responses is important in improving performance at low temperatures and thence overall annual productivity (Pollock & Eagles, 1988). Grasses also provide an ideal system for studying cell expansion since, in common with other monocots, they possess a localized intercalary meristem and extension zone (Pollock & Eagles, 1988) allowing unequivocal identification of the position of the expanding cells. Stoddart et al., (1986) have described an instrument that allows the continuous and direct measurement of growth of graminaceous seedlings whilst the temperature of the leaf meristem is altered. Using seedlings of *Lolium temulentum* it was found that temperature perception and response occur within the extension zone over a time scale that precludes effects on the supply of nascent cells. It has been suggested that the sensitivity of extension growth to chilling may represent some physical limitation to growth based on changes in the water relations properties of the plants (Kleinendorst & Brouwer, 1970; Watts, 1971; Barlow, Boersma & Young, 1977). Current models of the water relations of growing cells are based on the Lockhart equation (Lockhart, 1965; Tomos, 1985).

\[
\frac{dV}{dt} = \frac{\phi L}{\phi + L} (\sigma \Delta \pi - Y + P_{\text{osm}})
\]  \hspace{1cm} (1)

This equation relates relative growth rate \((dV/dt)\) (dV/dt); where \(V\) = cell volume and \(t\) = time) to the wall rheological properties \(\phi\) (wall extensibility) and \(Y\) (wall yield stress threshold), to the hydraulic conductivity of the pathway for water transport to the expanding cell (L) and to the osmotic pressure difference across that pathway (\(\Delta \pi\)). This last parameter may be modified by the path reflection coefficient (\(\sigma\)) (Stedile, 1985). In tissues influenced by transpiration tension the resulting hydrostatic pressure of the wall enters the equation as \(P_{\text{osm}}\).

Although a term for turgor pressure \((P)\) does not appear explicitly in equation (1), relative growth rate is a linear function of \(P\) according to the equation

\[
\frac{dV}{dt} = \phi (P - Y)
\]  \hspace{1cm} (2)

(Lockhart, 1965). Equation (2) predicts that no growth would be observed at turgor pressures below
a yield threshold, but that, above this pressure, growth rate is a linear function of turgor pressure with $\phi$ as the constant of proportionality. [Equation (1) reduces to equation (2) when tissue hydraulic conductivity is not rate limiting (Ray, Green & Cleland, 1972).] Using the pressure probe (Hüsken, Steudle & Zimmermann, 1978), analysis of the water relations of the expanding cells of tissues permits the testing of the hypothesis that growth limitation at lowered temperatures may be mediated by altered water relations. The simultaneous use of a position transducer and pressure probe was pioneered by Cosgrove & Cleland (1983) who showed that growth rate of pea internodes was independent of turgor pressure under various treatments. This paper describes studies on *Lolium temulentum* seedlings using the temperature-profiled position transducer and pressure probe simultaneously.

A paper with similar conclusions but using psychrometric water relations techniques for other grass species has recently appeared (Woodward & Friend, 1988).

**MATERIALS AND METHODS**

**Plant material and growth measurements**

*Lolium temulentum* L. (Ba 3081) seedlings were grown from seed on a liquid medium (Pollock, 1982) in controlled environment cabinets (Fison’s model 600G3 THTLT). The cabinet was maintained at 20 °C on a 16 h light/8 h dark cycle at a relative humidity of 70%. Photon flux density was measured with a 550 Crump Quantum Photometer (T. & J. Crump, Rayleigh, Essex) as 80 $\mu$mol m$^{-2}$ s$^{-1}$.

The position of the extension zone in the expanding fourth leaf at 24 days after sowing was determined by piercing fine holes through the stem of the intact plant at regular intervals above the base (Kemp, 1980). The plant was then allowed to grow for a further 24 h, when the outer leaves were removed and the position of the holes in the experimental leaf determined. Through the application of this method to 10 individual plants, the zone of maximum extension was found to be about 30 mm from the base of the stem.

At 24 days after germination, seedlings were transferred to nutrient solutions in individual 50 ml sample bottles and mounted in the cooling collar of a temperature profiled position transducer (Stoddart et al., 1986) which had been modified to allow simultaneous growth and turgor pressure measurement. A hollow brass collar was clamped around the extension zone. The temperature of the collar and hence the extension zone was varied by circulating coolant from a thermostatically controlled water bath. The modification in the collar involved forming a slot in the cooling jacket that exposed a small portion of the meristem to microscopic inspection.

Growth rate was continuously measured with a linear variable displacement transducer (model 1353, Penny & Giles, UK) attached to the tip of the growing leaf. The lightest counterweight that would overcome the friction of the apparatus was used (0.27 g).

The thermocouple of an electronic thermometer (model 1624; Comark, Sussex, UK) was placed within the collar between the second and third leaves of the seedlings to allow continuous monitoring of extension zone temperature *in situ*.

Growth rate was determined for a meristem temperature of 20 °C. This value was taken as the control growth rate. The plant was then removed from the apparatus and a ‘window’ (2 x 2.5 mm) carefully cut with a fresh scalpel blade through the leaf bases overlying the fourth leaf expansion zone some 25 mm from the base. The plant was returned to the cooling collar in such a way that the ‘window’ corresponded to the slot in the collar. The growth rate at 20 °C was again determined. Plants with a growth rate reduced by 20% or more by this treatment were discarded. (Detailed drawings of modifications to the collar and details of the ‘window’ are available from the authors.)

**Pressure probe technique**

Turgor pressure, volumetric elastic modulus and cell hydraulic conductivity were determined by the use of a pressure probe (Hüsken et al., 1978). Most turgor pressure measurements were performed on cortical cells. Fewer measurements were made on the epidermal cells, but the turgor pressure values of the two cell types were indistinguishable. Measurements of elasticity and conductivity were only performed on epidermal cells since the geometry of the experimental system made the required measurement of cortical cell dimensions very difficult.

![Figure 1](image-url). Relationship between temperature of meristem, leaf growth rate and expanding cell turgor pressure of cortical and epidermal cells (accumulated data).
RESULTS

The growth rate of *L. temulentum* drops with temperature from 20 μm min⁻¹ at 20 °C, ceasing altogether by 0 °C (Fig. 1). In contrast to the growth rate, turgor pressure remained constant at 0.5 ± 0.02 MPa over the entire range of temperature (Fig. 1). The data in Figure 1 represent pooled pressure results from 12 plants of uniform dimensions (turgor pressures of 59 cells). Growth rate appears to be quite variable between plants so attempts were made to measure growth rate and turgor pressure in a single cell over the whole temperature range. The results of the most successful experiment are illustrated in Figure 2.

![Graph showing relationship between temperature and turgor pressure](image)

**Figure 2.** Relationship between temperature of meristem, leaf growth rate and expanding cell turgor pressure of a single cortical cell.

Volumetric elastic modulus (εₐ) of the cells was found to be 1.25 ± 0.12 MPa (20 cells) and to be unaffected by temperature (data not shown) as previously found for *Tradesantia virginiana* (Tomas *et al.*, 1981).

Cell hydraulic conductivity (Lᵥ) decreased as temperature was lowered, from 3.7 × 10⁻⁹ m s⁻¹ MPa⁻¹ at 20 °C to 1.0 × 10⁻⁹ m s⁻¹ MPa⁻¹ at 1 °C (Fig. 3). Since any cell under investigation moves away from the probe tip as the plant grows, it has so far proved impossible to measure Lᵥ on a single cell over the whole temperature range and thus avoid variation due to imprecise volume determinations (Tomas *et al.*, 1981), but a value for the activation energy of water transport of 69 kJ mol⁻¹ (Tomas *et al.*, 1981) fitted to the 1 °C value appears to give a meaningful fit to the pooled data of Figure 3.

![Graph showing relationship between temperature and hydraulic conductivity](image)

**Figure 3.** Values of Lᵥ of a single expanding cell measured over a range of temperatures. Each point corresponds to a single relaxation experiment; the error bars indicate standard deviation when 3–5 relaxation experiments were performed at the same temperature. The fitted curve represents a linear Arrhenius plot for an activation energy of 69 kJ mol⁻¹ passing through the lowest temperature point.

DISCUSSION

From the Lockhart equation [equation (1)], growth rate is a function of osmotic, membrane and wall properties. Some predictions can be made regarding the situation in which each in turn is rate limiting. Table 1 (Tomas, 1985) illustrates the predicted behaviour of turgor pressure for different combinations of Lᵥ, φ and solute uptake as the rate-limiting component.

It appears (Figs 1 and 2) that turgor pressure is held constant over the entire range of temperatures and that the growth rate reduction in *L. temulentum* caused by decreasing temperature is not mediated via decreased turgor pressure within the resolution of the pressure probe technique (±0.02 MPa in this case). That (P′ - Y) [equation (2)] might be less than this cannot be ruled out although values are larger in systems analysed to date (Tomas, 1988).

From Table 1 it would appear that the only prediction satisfied by an observation of constant turgor pressure is that in which growth rate is limited by the wall extensibility, with changes in tissue hydraulic conductivity and solute supply (osmotic adjustment) not being involved to a significant degree. This independence occurs despite the dependence of Lᵥ and presumably osmotic adjustment on temperature (Lᵥ as in Fig 3 and osmotic adjustment because of the biochemical nature of the transport processes involved). These effects influence the relationship of growth rate and temperature only negligibly.

Table 1, however, does not consider yield threshold Y. From equation (2) it can be seen that an increase in the value of Y will result in decreased growth rates and it can be envisaged that a value of Y reaching the turgor pressure value at about 2–3 °C may be responsible for growth cessation at that point.

By elimination, therefore, it appears that growth
### Table 1. The influence of $L$, $\phi$, and solute flux on water potential equilibrium, turgor pressure and cellular osmotic potential in growing cells (Tomos, 1985)

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Water potential</th>
<th>Turgor pressure</th>
<th>Osmotic pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L \gg \phi$</td>
<td>Solute flux</td>
<td>Near equilibrium</td>
<td>Constant</td>
</tr>
<tr>
<td>(i) Non-limiting</td>
<td></td>
<td>Near equilibrium</td>
<td>Lowered</td>
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<tr>
<td>(ii) Limiting</td>
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<tr>
<td>$L \ll \phi$</td>
<td>Solute flux</td>
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diminution at lowered temperatures is a result of changes in the rheological properties (either extensibility or yield threshold) of the cell walls.

Although the plastic properties of the walls have not been investigated directly in this study, some suggestion that cell expansion is limited by changes in wall rheology as cells mature is provided by the plastic modulus values determined. The values of 1.25 ± 0.12 MPa for $\xi$ are not only at the lower end of the range for higher plants (Tomos, 1988) but are in order of magnitude lower than the values for mature cells of leaves of *L. temulentum* (approx. 8.0 MPa). In studies using mutant and normal barley seedlings changes in wall plasticity are qualitatively related to growth rate (Pollock et al., in preparation).

The conclusion that wall rheology is the key to the biophysical control of growth rate is consistent with previous conclusions from this and other laboratories (barley mutants, Pollock et al., in preparation; wheat roots, Jones et al., 1987; Pritchard, Tomos & Wyn Jones, 1987; phototropic mustard seedlings, Rich & Tomos, 1988; pea internodes, Cosgrove & Cleland, 1983; maize leaves, Michelen & Boyer, 1982; sunflower leaves, Matthews, Van Volkenburgh & Boyer, 1984; birch leaves, Taylor & Davies, 1986; Poa spp., Woodward & Friend, 1988) agreeing with Cosgrove (1987) that in general cell growth appears to be regulated and controlled through changes in the cell wall properties.

Finally, it is implicit in the constancy of turgor pressure with temperature change (despite considerable variation in growth rate) that complete osmotic adjustment of the expanding cells must occur. Decreased growth rate will result in a lower demand for osmotic solutes. If transport of osmotica into the cell, or generation of solute by hydrolysis of polymeric material, was not diminished at lower growth rates turgor pressure would increase. One could speculate that the diminution of such metabolic processes with temperature is unlikely to match precisely the diminution of growth rate and thus the constancy of turgor pressure with growth rate indicates an effect of growth rate upon solute transport.

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**References**


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