Cell wall water content has a direct effect on extensibility in growing hypocotyls of sunflower (*Helianthus annuus* L.)

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Abstract

It has been proposed that spacing between cellulose microfibrils within plant cell walls may be an important determinant of their mechanical properties. A consequence of this hypothesis is that the water content of cell walls may alter their extensibility and that low water potentials may directly reduce growth rates by reducing cell wall spacing. This paper describes a number of experiments in which the water potential of frozen and thawed growing hypocotyls of sunflower (*Helianthus annuus* L.) were altered using solutions of high molecular weight polyethylene glycol (PEG) or Dextran while their extension under constant stress was monitored using a creep extensiometer (frozen and thawed tissue was used to avoid confounding effects of turgor or active responses to the treatments). Clear reductions in extensibility were observed using both PEG and Dextran, with effects observed in hypocotyl segments treated with PEG 35 000 solutions with osmotic pressures of > 0.21 MPa suggesting that the relatively mild stresses required to reduce water potentials of plants *in vivo* by 0.21 MPa may be sufficient to reduce growth rates via a direct effect on wall extensibility. It is noted, therefore, that the water binding capacity of plant cell walls may be of ecophysiological importance. Measurements of cell walls of sunflower hypocotyls using scanning electron microscopy confirmed that treatment of hypocotyls with PEG solutions reduced wall thickness, supporting the hypothesis that the spatial constraint of movement of cellulose microfibrils affects the mechanical properties of the cell wall.

Key words: Cell wall extensibility, extensiometry, PEG, plant growth, sunflower, water relations.

Introduction

It is well known that reduced water availability can lead to a reduction in plant growth. This has generally been interpreted using a theoretical framework in which growth of a plant cell is a result of irreversible extension of the cell wall by stresses generated by the cell’s internal turgor pressure (Lockhart, 1965). It follows from this model that the duration and rate of growth are determined by the interaction between cell turgor and the mechanical properties of the cell walls (Nonami and Boyer, 1990a, b). Reduced growth rates in response to reduced water potentials have been thought to be due either to reduced turgor pressures (and therefore reduced cell wall stresses) or to cell-mediated regulation of cell wall mechanical properties (e.g. in response to ‘drought’ signals such as ABA; Gowing *et al.*, 1993).

This paper examines the hypothesis that the hydration status of the cell wall itself may have an effect upon its rheological behaviour, and therefore have a direct effect on growth. This was suggested by the behaviour of synthetic polymers, where it has been established that the spacing within polymer structures can have a substantial effect upon their properties because macromolecules are only able to move or flow (allowing the material to stretch reversibly or irreversibly) if the component molecules are not constrained by immediately adjacent large molecules. This effect is used in producing plastics with a range of properties using the same polymer by the addition of lower molecular weight compounds known as plasticisers to introduce space to the structure (Ward and Hadley, 1993). It has been noted that spacing between cellulose microfibrils may be of similar importance in determining the properties of plant cell walls (Thompson, 2005). If this were the case, then alteration of the water content of plant cell walls would be expected to have a direct effect on the...
Materials and methods

Plant material for extensiometry

Etiolated sunflower hypocotyls were prepared by sowing Helianthus annuus seeds (‘Giant Yellow’, Suttons) in water-saturated perlite (Silvaperl, Gainsborough, UK) and growing them in covered pots for 5 d at 30 °C. Segments, 20 mm, in length were cut from the top, the fastest growing part of the hypocotyl, longitudinally split using a hand-held razor blade, and immediately frozen using a freezing spray (RA Lamb, Eastbourne, UK). After 60 s, the segments were thawed by dropping them into the most concentrated solution used in the experiment. Segments were then pressed between microscope slides covered in absorbent paper using a 2 kg weight for 60 s and then returned to the solution for 10 min.

SEM

2 cm sections from the top of growing hypocotyls were immersed in control buffer or buffer containing PEG 6000 with \( \pi = 0.62 \) MPa for approximately 2 h (for concentration see Table 1). Then the segments were rinsed and blotted and 4 mm segments from the top of the hypocotyls excised using a hand-held razor blade. The pretreated sample segments from the top of the hypocotyls were immediately attached to the stub with carbon paste with the freshly cut surface uppermost, cryo-fixed, and gold coated on an Emscope SP2000 cryo stage and viewed in the frozen hydrated state on a Cambridge S200 SEM. Images were captured using a JEOL Semafore digital capture system.

Extensiometry

Bisected hypocotyls were clamped into the bottom of a reservoir tube by inserting the bottom 6–8 mm into a slot machined into 10 mm diameter nylon rod and tightening a screw applied to an epoxy-glass spacer so that the hypocotyl was firmly held between the spacer and the side of the slot. The top end of the hypocotyl was held in a similar clamp which was hooked onto the end of a cantilever arm using kevlar thread. The reservoir tube was then filled with the appropriate experimental buffer. Approximately 8 mm of the hypocotyl was exposed between the two clips, but the exact initial length was determined using a magnifying eyepiece with a graticule. An LVDT core was balanced on the opposite end of the cantilever to the tissue piece allowing measurement of any change in length using the LVDT (Schlumberger DFG 2.5 from RS Components Ltd., Corby, Northants, UK) and recorded by a personal computer via a Bede PC-ADH24 analogue input card (Bede Technology, Jarrow, Tyne and Wear, UK; computer program written by the authors). Every 30 s, 1000 individual LVDT readings were averaged and recorded for each segment. The cantilever arms were arranged to amplify length change by a factor of three and the whole arrangement was mounted on a heavy steel plate on balance benching giving resolution of ±0.2 μm for each averaged reading. The maximum deviation of the cantilever from horizontal was ±0.06 radians, and so no mathematical or mechanical correction for rotation of the cantilever was implemented.

The cantilever was counterweighted so that hypocotyl pieces were slack until the LVDT core was in place so that the initial weight was less than that of the LVDT core (equivalent to a force of 0.004 N). Step increases in force were applied by sliding counterweights along the cantilever arms to marked positions. The data presented is for a step increase in force exerted on the segments from 0.10 N to 0.20 N after 90 min.

<table>
<thead>
<tr>
<th>Table 1. Table of osmotica used in the experiments and their osmotic pressures determined using vapour pressure osmometry</th>
</tr>
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<tbody>
<tr>
<td>[PEG] (g g⁻¹ water)</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>PEG 6000 0.18</td>
</tr>
<tr>
<td>0.23</td>
</tr>
<tr>
<td>0.27</td>
</tr>
<tr>
<td>0.50</td>
</tr>
<tr>
<td>PEG 35 000 0.17</td>
</tr>
<tr>
<td>0.26</td>
</tr>
<tr>
<td>Dextran 10 000 Saturated</td>
</tr>
</tbody>
</table>

Note that values are given as ‘g solute g⁻¹ water’ and not ‘g solute g⁻¹ total’ and that these osmotic pressures are additional to solutes in the buffer.
Control buffer was 10 mM MES containing 5 mM KCl and 1 mM CaCl$_2$ titrated to the experimental pH with 1 M NaOH. PEG and Dextran solutions of known osmotic pressure were prepared using a standard curve generated using a Wescor Vapro 5520 vapour pressure osmometer (Wescor Inc., Utah, USA) with correction for the effect of the PEG on the molar volume of water. The concentrations of PEG and Dextran are given in Table 1. PEG and Dextran solutions were prepared by addition to control buffer, after which buffer pH was again corrected. All PEG was Fluka Biochemika Ultra with batch numbers 1198336 (PEG 6000, product no. 81255) and 1256950 (PEG 35 000, product no. 94646). Dextran was from *Leuconostoc mesenteroides* supplied by Sigma (product no. D9260, lot no. 115K0686). The manufacturer’s certificates of analysis including impurities for the PEG batches can be referred to on their web pages, but no heavy metal contaminants exceeded 10 mg kg$^{-1}$ (bismuth in the PEG 6000) giving a concentration of 24 µmolal in the most concentrated PEG 6000 solution (the 0.5 g g$^{-1}$ water) and 13 µmolal for the next most concentrated solution (the 0.27 g g$^{-1}$ water). All other heavy metals were ≤5 mg kg$^{-1}$. The densities of buffers containing PEG were all in the range between 1.0 g ml$^{-1}$ and 1.08 g ml$^{-1}$ (although the Dextran solution density was slightly higher, at 1.13 g ml$^{-1}$) and so although salt and MES concentrations will have been slightly reduced by addition of osmotica, the reductions were not substantial.

Some of the solutions were quite viscous, but not to a degree that hindered free movement of the upper clip (at least over the time periods from which data were recorded). The viscosity could have reduced diffusion of oxygen, but aeration would have substantially affected the accuracy of creep measurements and in tissue that had been killed by freezing it was assumed that there would be no respiratory oxygen requirement. However, some inhibition of scission of cell wall polysaccharides by free radicals generated from oxidative reactions could not be excluded (Fry, 1998; Schopfer, 2001).

Treatments with impermeating osmotic treatments did cause a visible (and in some cases substantial) reduction in the width and thickness of the explants. Although this would increase stress were reactions could not be excluded (Fry, 1998; Schopfer, 2001).

A direct effect of cell wall water content on extensibility

Relative length after a step increase in stress was calculated based upon ‘true strain’, that is:

$$L_{\text{rel}} = \frac{L_t}{L_0} + 1$$

where $L_{\text{rel}}$ is relative length, $L_t$ is the measured length at time $t$ and $L_0$ is the length immediately prior to the stress increase.

Extension was parameterized by fitting experimental data time series recorded over 240 min to equation 2 using non-linear regression by minimization of squares of residuals in Sigmaplot.

$$L_t = L_0 + N_{\text{creep}} \log \left( t + k + f_{111} \left(1 - \exp(-t/\tau_1)\right) + f_{122} \left(1 - \exp(-t/\tau_2)\right) + f_{3} t \right)$$

where $L_0$ and $L_t$ are, respectively, the initial length and length at time $t$, $f_{111}$, $f_{122}$ and $f_{3}$ are flow rates, $\tau_1$ and $\tau_2$ are Kelvin element retardation times, $N_{\text{creep}}$ is the constant associated with a log-time function (as described by Büntemeyer *et al.*, 1998) and $k$ is instantaneous elasticity combined with a correction because it is impossible to extrapolate the log-time function to $t = 0$. Equation 2 was previously found to describe creep of plant material after a step increase in stress with $r^2$ generally >0.999 (Thompson, 2001). Previously, ‘engineering strain’ has been used rather than true strain, but other than slight differences in the values obtained (in accordance with the slight changes in relative length values) there were no differences between model solutions using the alternate formulae for strain.

**Gel filtration**

Molecular weight and size distributions for PEG 6000 and PEG 35 000 were examined by gel filtration using a **SuperoseTMD** 12 size-exclusion FPLC column (HR 10/30, Amersham Pharmacia Biotech, St Albans, UK). The column was equilibrated with 0.1 M sodium acetate buffer at pH 5.5 containing 0.1 M NaCl and 0.01% (w/v) sodium azide. 100 µl of PEG solution was applied to the column at a flow rate of 1 ml min$^{-1}$ and the eluate was monitored at 280 nm, with measurements recorded every second by a PC fitted with a National Instruments CB-27 connector block (National Instruments Corporation, Austin, Texas, USA).

**Tissue weight changes**

For each solution, two 2 cm hypocotyl segments were bisected longitudinally, frozen using freezing spray, and thawed in control MES buffer at pH 5.0. At the start of the series of measurements the tissue pieces were gently blotted to remove surface liquid using tissue paper, weighed, and transferred to buffers containing PEG or glucose. At intervals, the pieces were again blotted and reweighed and then returned to the solution.

**Statistical analysis**

All statistical comparisons used Student’s *t* test. Values were assumed to be unpaired for cell wall thicknesses and paired for creep rates (as extension rates at two water potentials for the same segment are considered) and segment weights (as the same segments were reweighed at intervals).

**Results**

**The effect of PEG 6000 solutions**

In order to establish whether hydration status of the cell wall could alter mechanical properties in principle, initial experiments were carried out using PEG solutions with an osmotic pressure of 2.3 MPa. In these experiments creep of frozen and thawed hypocotyls bathed in MES buffers at pH 5.0 and pH 6.0, with and without PEG were examined. As can be seen from the representative examples shown in Fig. 1a, at pH 5.0 an osmotic pressure of 2.3 MPa PEG did not have a substantial effect on the initial extension, but caused a considerable reduction in the longer-term creep rate.

The initial results confirmed that water potential can directly alter long-term cell wall biomechanical behaviour, but the water potentials employed in these experiments would only be experienced in a handful of circumstances (probably only plants growing in extremely arid environments or tissues near the top of extremely tall trees). Therefore, in order to evaluate whether these effects are of more than theoretical interest, further experiments were conducted using solutions with water potentials comparable to those that might be experienced by plants growing under more usual conditions.
In order to facilitate detection of minor changes, the effects of increasing or decreasing water potential on explants already extending under an applied stress of 0.2 N were examined. Figure 2 shows the effect of replacing control buffer (pH 5.0) with equivalent buffer containing PEG with an osmotic pressure of 0.62 MPa together with the effect of the opposite exchange (i.e. decreasing the putative osmotic pressure by 0.62 MPa). Explants were initially bathed in PEG solution or control buffer while the applied force was increased in 0.1 N increments to 0.2 N. When a force of 0.2 N had been applied to the hypocotyl segments for long enough for the long-term extension rate to be broadly apparent (>180 min), the initial bathing solution was drained from the extensiometer tubes and gently replaced via a drainage tube in the base of the tube using a syringe. Typical examples are illustrated in Fig. 2.

It was found that when the water potential was increased there was a consistent increase in the rate of extension, which became apparent within a few minutes and although the extension rate decreased with time, it did so slowly and longer-term extension took place at a greater rate than had preceded the exchange. When the osmotic pressure was increased there followed a short rapid period of extension, after which elongation took place at a slower rate than that before the exchange.

Comparison of extension rates before and after exchange was performed by using the period before exchange to obtain the parameters required to model extension using equation 2 (proposed and described in detail in Thompson, 2001). Equation 2 was then used to predict the extension rates had the segments remained in their original solution. The average mean actual extension rates and predicted unaltered rates 2 h after exchange are shown in Fig. 3. The creep rates are compared in this way because the creep rate was still declining at the time that changes occurred and both increases and decreases in osmotic pressure cause transient effects which it was desirable to exclude, because it has been argued that longer term extension is of greatest relevance to growth (Thompson, 2001). The results in Fig. 3 show that increasing water potential by 0.62 MPa caused an increase in long-term creep rate by 50–60% and an equivalent reduction caused a decrease by approximately 35% (both changes were statistically significant at a level of >0.95). Figure 3 also includes the effects of solutions of PEG 6000 with osmotic pressures of 0.40 MPa and 0.26 MPa. 0.40 MPa exerted by PEG 6000 reduced creep rates by about 30% for both exchanges. There was no significant effect on extension of a solution of PEG with an osmotic pressure of 0.26 MPa. In addition, although the effect was not statistically significant, the changes were in the opposite direction to those observed at higher osmotic pressures (i.e. on average, growth rates slightly increased when segments were treated with PEG and slightly decreased when PEG solutions were replaced with control buffer). It therefore appears that the threshold of water potential change caused by PEG 6000 that reduces cell wall extensibility in this tissue is between 0.26 MPa and 0.40 MPa.
In order to confirm that PEG solutions do actually alter spacing within the cell walls, cell wall thicknesses of cortical cells of sunflower hypocotyls were measured in scanning electron micrographs of cryo-fixed sections. Although some dehydration may have occurred after fixing, PEG-treated and control segments were examined alternately and so both should have been affected equally. In addition, there was no downward trend in cell wall thicknesses. Although it was not possible to resolve individual components within the cell wall and directly examine spacing, a reduction in cell wall thickness must logically be associated with a reduction in spacing. Figure 4a and b show a number of cortical cells from a control segment and equivalent cells from a segment pre-treated with PEG 6000 to give an osmotic pressure of 0.62 MPa. Note that the PEG-treated segments were removed from the solutions and briefly rinsed before sectioning and so there was no direct contact between the solutions and the walls examined.

From Fig. 4a and b there seems to have been a particular change in the structure of the walls at the corners of cells of segments treated with PEG. An overall impression of the micrographs suggests that walls are thinner in the PEG-treated tissue, but any reduction in wall thickness logically be associated with a reduction in spacing. Figure 4a and b show a number of cortical cells from a control segment and equivalent cells from a segment pre-treated with PEG 6000 to give an osmotic pressure of 0.62 MPa. Note that the PEG-treated segments were removed from the solutions and briefly rinsed before sectioning and so there was no direct contact between the solutions and the walls examined.

Effects of PEG-induced changes in water potential on cell wall thickness

In order to confirm that PEG solutions do actually alter spacing within the cell walls, cell wall thicknesses of cortical cells of sunflower hypocotyls were measured in scanning electron micrographs of cryo-fixed sections. Although some dehydration may have occurred after fixing, PEG-treated and control segments were examined alternately and so both should have been affected equally. In addition, there was no downward trend in cell wall thicknesses. Although it was not possible to resolve individual components within the cell wall and directly examine spacing, a reduction in cell wall thickness must
was not sufficient to eliminate substantial overlap between wall thicknesses in the control and treated segments. Therefore, the thicknesses of all cell walls visible in 12 micrographs of control segments and eight micrographs of PEG-treated segments were measured at their thinnest visible point and the distributions of thicknesses compared. In order to avoid selection bias, all visible walls were measured (247 from controls and 180 from treated segments) including walls intercepted at an angle, those towards the top or bottom of the cell where the ends of the corners were visible, and walls at the edge of micrographs so that the thinnest point was not observable, and so the values are to be expected to be higher than the true values in many cases. However, this should be equally true for measurements of the controls and the PEG-treated tissue. Figure 4c is a histogram showing the distribution of thicknesses measured for 247 cell walls from control hypocotyls and 180 cell walls from treated hypocotyls.

0.62 MPa by treatment with PEG 6000. From Fig. 4c, it seems clear that there was a shift in the distribution of wall thicknesses in treated segments. The mean measurement of cell wall thicknesses in PEG-treated hypocotyls was 1287 nm (SD 508 nm) and that for controls was 1479 nm (SD 463 nm). The difference was significant at a level of >0.9999.

Other osmotica

In order to confirm that the changes in creep rates observed were not due to osmotic pressure per se the effect of buffer solution containing 410 mM glucose was measured. In addition, in order to exclude the possibilities that PEG 6000 solutions affected creep because of contaminants or because they penetrated the cell wall and reduced creep by direct interaction, buffer solutions containing Dextran 10 000, and PEG 35 000 were tested. Dextran 10 000 should behave as an impermeant
osmoticum (Carpita et al., 1979), but does not share contaminants with PEG solutions. PEG 35 000 solutions should be excluded from the tissue and cell wall to a greater degree than PEG 6000 and therefore should cause a lesser effect than PEG 6000 if penetration of PEG is the reason for inhibition of creep. The effects of replacing control buffer with buffer containing 410 mM glucose, Dextran 10 000 with an osmotic pressure of 0.83 MPa, and PEG 35 000 with an osmotic pressure of 0.59 MPa are shown in Fig. 5a and replacement of the higher osmotic pressure solutions with control buffer are illustrated in Fig. 5b. The average mean observed extension rates and predicted unaltered rates 2 h after exchange are shown in Fig. 6. Neither exchanging control buffer for buffer containing 410 mM glucose nor the opposite change caused any more than a slight disturbance, showing that the changes in creep are not a non-specific effect of osmotic pressure or overall water potential.

Exchange of a saturated solution of Dextran 10 000 caused similar responses to PEG 6000, that is a transient increase in extension followed by a reduction in the creep rate to a level lower than before the exchange when control buffer was replaced with buffer containing Dextran and a long-term increase in creep rate when the buffer containing Dextran was exchanged for control buffer.

The size distributions of PEG 6000 and PEG 35 000 were examined by gel filtration and Fig. 7 shows data from separate elutions of 2.1 mg of PEG 35 000 and of PEG 6000 from the column (each was tested at least twice with no important differences between the data). The elution profiles show no overlap between the distributions of PEG 35 000 and PEG 6000. The molecular weight ranges and estimates of the corresponding Stokes diameters (based upon Khayet et al., 2002) are shown in Table 2 (although it should be noted that the first elution of PEG 35 000 was very close to the void volume for the column and so the upper limit of molecular weight and size for the PEG 35 000 are not likely to be reliable).

As can be seen from Figs 5a, b and 6, PEG 35 000 caused similar effects to PEG 6000 and indeed inhibition of extension by 0.59 MPa of PEG 35 000 appeared greater than that caused by 0.62 MPa of PEG 6000. If the effect of high molecular PEG is due to an effect of water potential on the cell wall, this may indicate that PEG 6000 was not completely excluded from the cell walls of the hypocotyls and this is supported by the weight changes observed in hypocotyl segments bathed in buffer containing PEG 6000 and PEG 35 000 shown in Fig. 8, as there was a slight but consistent increase in weight of tissue pieces in PEG 6000 solution from 30 min (the increase was statistically significant at >0.95 by 1 h and >0.99 by 24 h). Although weight loss in PEG 35 000 solution was slower, the minimum weight was lower and no recovery was observed. For this reason, the effects of buffer containing PEG 35 000 with an osmotic pressure of 0.21 MPa were also tested and are illustrated in Fig. 6. Promotion of extension after replacement of this concentration of PEG 35 000 with control buffer was limited but statistically significant and inhibition of extension when control buffer was replaced with PEG 35 000 with an osmotic pressure of 0.21 MPa was by more than 30% (also statistically significant). These results suggest that

| Table 2. Molecular distributions for PEG 6000 and PEG 35 000 based on the gel filtration data in Fig. 5 and calculated Stokes diameters for the modal molecular weight and for the last and first eluting PEG for each peak |
|------------------|------------------|------------------|
| PEG 6000         | Mₘ range (kDa)   | Modal size (nm)  | Size range (nm) |
| PEG 35 000       | 3.0–9.9          | 4.3              | 2.9–5.6          |
| PEG 35 000       | 29.3–89.1        | 11.4             | 10.1–18.7        |

Fig. 5. Illustration of the effects of (a) increasing osmotic pressure by replacing control buffer with buffer containing 410 mM glucose (open inverted triangles), Dextran 10 000 with π=0.83 MPa (open circles) or PEG 35 000 with π=0.59 MPa (open squares) or (b) decreasing the osmotic pressure by the opposite exchange (symbols as for a). For clarity some data have been offset by +0.03 (asterisk) or –0.03 (two asterisks).
reduction of tissue water potential by as little as 0.2 MPa may be sufficient to reduce cell wall extensibility in sunflower directly. In addition, if it is critical that PEG treatments are completely excluded from plant tissue (perhaps particularly if cut surfaces are exposed to the solution), it may be necessary to use PEG with an average molecular weight greater than 6000.

Discussion

The hypothesis that polymer mobility in the plant cell wall might be an important factor in determining the extensibility of plant cell walls (Thompson, 2005) in turn suggested that the water content of the cell wall might affect mechanical properties and, therefore, that limitations in water availability may reduce growth rates via a direct reduction in cell wall extensibility (in addition to the already accepted effect caused by decreases in turgor).

In these experiments it has been found that reducing the water potential of frozen and thawed growing sunflower hypocotyls using solutions of high molecular weight PEG or Dextran can cause a measurable reduction in long-term extensibility. As osmoticum most likely to be excluded from the wall affect extensibility to the greatest degree and excluded Dextran and PEG cause similar effects, it seems

Fig. 6. The effects of glucose, Dextran, and PEG 35 000 solutions on mean relative (a, b) and actual (c, d) extension rates. The actual measured values (shaded) are compared with the predicted extension rates had the solutions not been changed (unshaded). (a, c) Control buffer was replaced with higher osmotic pressure solutions and if extensibility was reduced by the osmoticum then the predicted values should be greater than the measured values. In (b, d) Higher osmotic pressure solutions were replaced with control buffer and predicted values should be less than those measured if the osmoticum reduced extensibility. The ±values in the columns are 1 SD.

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reasonable to suggest that the changes observed are due to the water potential exerted on the cell wall. It has also been established that treatment of sunflower hypocotyls with PEG 6000 causes a significant reduction in cell wall thickness, logically suggesting that there must have been a reduction in the space separating wall components. Therefore, it seems at least possible that this underlies the decrease in wall extensibility. Similar effects of PEG-generated osmotic pressures of 0.6 MPa have been reported in rye coleoptiles by Edelmann (1995), but this study also found mannitol (a permeating osmoticum) to affect extensibility. This may indicate an additional effect or be because the mannitol was not able to permeate the tissue (certainly the material seems to have required extensive abrasion and pre-incubation, presumably to release apoplastic solutes).

Changes in wall properties due to reduced water potential would often be masked by confounding effects of water potential on cell turgor in vivo, but Chazen and Neumann (1994) have shown extensibility to decrease in growing maize leaves within 2 min of a reduction in water potential by measuring the effects of applying additional stress on extending leaves so that reductions in turgor and cell wall behaviour could be distinguished. Effects on wall properties may also be of particular importance in instances where partial or complete recovery of turgor pressure occurs following a reduction in water potential as reported in wheat roots by Pritchard et al. (1991) and in observations by Nonami and Boyer (1990a, b) of a rapid reduction in growth of soybean stems after transplantation to dry vermiculite despite maintenance of turgor in the growing tissues.

Reductions in extensibility were observed using PEG 35 000 solutions with an impermeating osmotic potential of −0.21 MPa, which would suggest that growing cell walls in sunflower could be affected by relatively mild water stresses, although it should be noted that PEG solution osmotic pressures were determined by vapour pressure osmometry (as is most commonly reported in the plant physiology literature) but measurement by freezing point depression gives higher values (Kiyosawa, 2003). Freezing point osmometry data would indicate direct effects on wall properties would only occur at lower water potentials and if the water potentials suggested by freezing point measurements (approximately −0.4 MPa) are a better reflection of the effect of water stress in an intact plant, reduced turgor would generally be of substantially greater importance than any effects on wall extensibility, but they may still be relevant if osmotic adjustment leads to recovery of turgor. However, Winzor (2004) has noted that osmotic pressures determined by vapour pressure osmometry are inversely related to temperature and that empirical models for this relationship suggest convergence with freezing point data at approximately 0 °C. This was attributed to an inverse effect of temperature on the second virial coefficient of the extended Van’t Hoff equation (Winzor, 2004). If this is correct, vapour pressure and osmotic pressure measurements can be reconciled, with vapour pressure values usually to be preferred as the measurement temperatures are generally closer to those at which the solutions are used (as was the case in these experiments).

In live tissues the water potential reduction affecting wall extensibility may be even less than these values as McClendon (1981) has noted that the cell wall at the cell surface will be compressed by the internal turgor pressure (a factor that will have been absent from the experiments using frozen and thawed tissue detailed above) and any reduction in polymer mobility would be enhanced by such
In the simplest possible scenario, in which the cell wall radial and longitudinal moduli are homogeneous across the wall and constant within the range of stresses experienced and assuming that pressure at the cell surface is equal to the turgor pressure and is zero at the outer edge of the wall or middle lamella, the threshold water potential required for a direct effect on wall properties would be increased by half of the cell turgor pressure. In this case extensibility could become reduced by direct modulation of cell wall properties by virtually any reduction in water potential if turgor was maintained by osmotic adjustment in this tissue (assuming the turgor to be 0.5 MPa; Kutschera and Köhler, 1993).

The observation that the behaviour of explants at reduced water potential and in control buffer were similar at pH 6.0 (Fig. 1b), and comparable to extension of explants at reduced water potential at pH 5.0 suggests that reduced water potential particularly suppresses the acid-induced extension thought to be caused by expansins (McQueen-Mason et al., 1992). This may be because both spatial freedom and other factors such as enzyme activity are required to promote extension or because acid growth is caused by increased space in the walls at low pH so that it can be suppressed by reduced water potential or increased pH without the effect being additive. The observation by Yennawar et al. (2006) that expansins caused pellets of centrifuged maize cell walls to swell is consistent with the latter possibility. This does raise the question of what effect endogenous active enzymes in the hypocotyls might be having during the experiments. Unfortunately, the creep measurements used in these experiments must take place over several hours and is suppressed by factors that inhibit acid growth so it is not easy to avoid possible changes due to ongoing activity. However, the same enzymes should act in control and treated segments and so the experiments should lead to the same conclusions [although Passioura (1994) has considered whether water content could affect cell wall behaviour because of reduced spacing limiting the mobility of growth-promoting enzymes rather than movement of structural components as proposed here].

If the fluidity of primary cell walls required for growth is affected by wall water content, then it is to be expected that plant cell walls are adapted to ensure that walls remain in an optimally hydrated state during growth and this may be reflected in the composition of primary plant cell walls. Indeed the roles of some wall components may include maintaining the correct relationship between cell wall water potential and cell wall water content. It is worth noting that the reversibility of effects of reduced water potential implies a source of free energy to drive re-expansion. In addition, evidence that changes to cell wall structure alter the relationship between water potential and water content [e.g. water binding and wall mechanical properties are affected by rhamnogalacturonan I structure in potato tubers and water binding by cell wall peroxidase activity in tobacco leaves, respectively, by Ulvskov et al. (2004) and Mercado et al. (2004)] suggest that increasing cell wall water content to maintain growth under conditions of water stress may be a feasible element of drought adaptation on a physiological or evolutionary scale.

In summary, it has been established that water potential directly alters extensibility of sunflower hypocotyls and that this may occur at reasonably common levels of water stress. Electron micrographs also show that these treatments alter wall thickness and so it seems possible that the effect is due to a reduction in spacing in the wall as proposed in Thompson (2005). These observations suggest that the water content of primary cell walls may be of physiological importance and that further investigation of how composition of plant cell walls affects their water content may shed light on plant water relations and cell wall biochemistry.

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