The contrasting influence of short-term hypoxia on the hydraulic properties of cells and roots of wheat and lupin

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Abstract. Little is known about water flow across intact root cells and roots in response to hypoxia. Responses may be rapid if regulated by aquaporin activity, but only if water crosses membranes. We measured the transport properties of roots and cortical cells of three important crop species in response to hypoxia (0.05 mol O$_2$ m$^{-3}$): wheat (Triticum aestivum L.), narrow-leaved lupin (Lupinus angustifolius L.) and yellow lupin (Lupinus luteus L.). Hypoxia influenced solute transport within minutes of exposure as indicated by increases in root pressure ($P_r$) and decreases in turgor pressure ($P_t$), but these effects were only significant in lupins. Re-aeration returned $P_r$ to original levels in yellow lupin, but in narrow-leaved lupin, $P_r$ declined to zero or lower values without recovery even when re-aerated. Hypoxia inhibited hydraulic conductivity of root cortical cells ($L_p$) in all three species, but only inhibited hydraulic conductivity of roots ($L_p$) in wheat, indicating different pathways for radial water flow across lupin and wheat roots. The inhibition of $L_p$ of wheat depended on the length of the root, and inhibition of $L_p$ in the endodermis could account for the changes in $L_p$. During re-aeration, aquaporin activity increased in wheat roots causing an overshoot in $L_p$. The results of this study demonstrate that the roots of these species not only vary in hydraulic properties but also vary in their sensitivity to the same external O$_2$ concentration.

Additional keywords: hydraulic conductivity, oxygen deficiency, pressure probe, root pressure, turgor pressure.

Introduction

Research investigating the tolerance of different species to waterlogging has predominantly focussed on the long-term effects on growth (days to weeks), yet many physiological processes are affected within much shorter time frames. Waterlogging leads to a depletion of oxygen in the rhizosphere and whilst this depletion can happen quickly, no plant experiences anoxia (zero O$_2$) immediately upon its root system becoming submerged (Blackwell 1983). Examining responses that occur when a plant senses the changing environment will help reveal mechanisms involved in acclimation to O$_2$ deficiency. For example, pre-exposure to hypoxia (low O$_2$) increases the tolerance of a tissue to subsequent anoxia through improved cytosolic pH regulation and respiration, higher rates of alcoholic fermentation and changes in protein production and gene expression (Saglio et al. 1988; Waters et al. 1991; Xia and Roberts 1996; Dennis et al. 2000; Kato-Noguchi 2000). Pre-exposure to hypoxia is also required to stimulate development of lysigenous aerenchyma (Drew et al. 2000).

Waterlogging and low O$_2$ in the rhizosphere tend to reduce the hydraulic conductivity of roots ($L_p$), but responses vary between species and type of treatment imposed (see review in Bramley et al. 2007a). Root death or anatomical changes may reduce $L_p$ during long-term exposure to O$_2$ deficiency by creating physical barriers to water flow. Anatomical changes that influence $L_p$ are usually associated with water and nutrient deficiencies and have rarely been characterised for waterlogging (Enstone and Peterson 2005). Even less is known about responses in $L_p$ to short-term exposure to O$_2$ deficiency, but $L_p$ can change rapidly in response to transpiration and other abiotic perturbations (Steudle 2001). Rapid changes in $L_p$ due to O$_2$ deficiency may be caused, indirectly or directly, by reduced respiration rates or pH-induced gating of aquaporins (Tournaire-Roux et al. 2003). However, these effects require bulk water flow to cross membranes somewhere along its flow-path between the root surface and vascular tissue. If water flows entirely through the apoplast (cell walls and intercellular spaces), then aquaporins will have little influence on $L_p$. 
This study examined the effects of short-term (0.5 h) hypoxia on water flow in wheat (*Triticum aestivum* L.), narrow-leaved lupin (*Lupinus angustifolius* L.) and yellow lupin (*Lupinus luteus* L.) roots when all three species were exposed to the same external oxygen concentration (0.05 mol O$_2$ m$^{-3}$). Wheat and lupins are important crops grown in winter in southern Australia. When sown on duplex soils, where a sandy/loam soil overlies clay, these crops often experience transient waterlogging during periods of high rainfall (Belford et al. 1992). Wheat is thought to tolerate waterlogging better than lupins, although direct comparisons under the same field conditions have not been undertaken (Dracup et al. 1992). In a glasshouse experiment comparing root growth in root chambers flooded for 1 week, wheat roots had superior survival and recovery than narrow-leaved lupin or yellow lupin (Bramley 2006). Roots of both lupin species died when submerged and only yellow lupin grew new roots when the chambers were dried. In a study on whole plants, Davies et al. (2000a) found that yellow lupin was more tolerant to waterlogging than narrow-leaved lupin due to properties of their root system, as indicated by cross-grafting roots and shoots, although it is not yet known what properties confer that response.

The effect of O$_2$ deficiency on water flow in lupin roots has not been previously investigated, but Zhang and Tyerman (1991) found that 0.5 h of hypoxia reduced the hydraulic conductivity of root cortical cells ($L_{pc}$) of wheat by 85%. Water uptake in wheat roots occurs predominantly in a region close to the root tip and under ambient conditions, radial water flow is influenced by aquaporins, probably located in the endodermis (Bramley et al. 2009). Therefore, hypoxia may inhibit $L_{pc}$ of wheat roots and the extent of the inhibition should be related to the inhibition at the cell level. In comparison, radial water flow in lupin roots occurs predominantly through the apoplast, despite significant aquaporin activity in cortical cells (Bramley et al. 2009). Any inhibitory effects of O$_2$ deficiency on aquaporin activity should therefore have less influence on $L_{pc}$, unless O$_2$ deficiency causes water flow to switch to the cell-to-cell pathway.

Water flow was measured across individual roots before, during and after a mild hypoxic treatment. Because wheat roots resume growth after waterlogging (Bramley 2006), water uptake must also resume and therefore, $L_{pc}$ should recover when wheat roots have sufficient oxygen. To determine whether effects on $L_{pc}$ were due to transport across cell membranes, the effects of hypoxia on $L_{pc}$ of root cortical cells were also measured. In comparison to the majority of studies that simulate waterlogging or O$_2$ deficient conditions by imposing sudden anoxia (zero oxygen), 0.05 mol O$_2$ m$^{-3}$ is a mild hypoxic treatment. However, it is similar to the concentration that reduced $L_{pc}$ of wheat root cells (Zhang and Tyerman 1991) through closure of aquaporins (Zhang and Tyerman 1999). This O$_2$ concentration can also induce changes in aquaporin expression within 0.5 h of treatment (Klok et al. 2002).

**Materials and methods**

**Plant material**

Seeds of wheat (*Triticum aestivum* L. cv. Kulin), narrow-leaved lupin (*Lupinus angustifolius* L. cv. Merritt) and yellow lupin (*L. luteus* L. cv. Wodjil) were germinated and grown as described by Bramley et al. (2007b). Plants were grown in individual sand-filled pots that allowed access to the roots without injury. Roots were carefully washed from the sand 10–14 days after sowing. The taproot of lupin or the longest seminal root of wheat was excised below emerging lateral roots as lateral roots prevented the root scaling to the root pressure probe. Roots were 70–180 mm long for measurements with the root pressure probe and roots were excised 80 mm from the tip for measurements on intact cells. Root diameters in the region punctured with the cell pressure probe were 0.61 ± 0.02 mm for wheat, 0.89 ± 0.05 mm for yellow lupin and 1.2 ± 0.20 mm for narrow-leaved lupin.

Roots had the same anatomy as those reported in Bramley et al. (2009), with none of the species developing an exodermis and the endodermis matured closer to the root tip in wheat than in either lupin species (Fig. 1).

**Hydraulic conductivity of roots ($L_{pc}$)**

The excised end of the root was connected to a root pressure probe (Steudle 1993) via a seal made from silicon impression material (Exaflex, Halas Dental Supplies, Adelaide, SA, Australia). The root was supported inside a glass tube (5 mm internal diameter), which was connected to a reservoir (maintained at 25°C by a water bath) of the same nutrient solution used to water the plants, supplemented with 5 mM glucose. Glucose was included as a surrogate carbohydrate (Gibbs et al. 1998a) supply because measurements on excised roots took several hours. The solution circulated past the root at a rate of 10–15 mm s$^{-1}$. The nutrient solution was bubbled with air or a combination of N$_2$ plus air (hypoxic treatment), through an air-stone, whilst a Rank oxygen electrode continuously monitored the O$_2$ concentration just before the solution passed the root. When the nutrient solution was bubbled with N$_2$ plus air, it took 120–600 s for the O$_2$ concentration to decrease to 0.05 mol O$_2$ m$^{-3}$. The circulation system was totally sealed apart from where the root entered the glass tube.

Measurements of $L_{pc}$ commenced when root pressure ($P_L$) was stable, which took a minimum of 2 h depending on the individual root. Water flow through the root was induced by applying a series of successive step changes in root pressure by rotating the rod inside the probe (Bramley et al. 2007b). Pressure was clamped for 60–120 s to ensure that pressures and flows were measured under steady-state conditions (Bramley et al. 2007b). The hydraulic conductance of the root ($L_c$) was calculated from the slope of the linear regression of volume flow rate against applied pressure. $L_c$ was normalised by the surface area of the root to give $L_{pc}$. $L_{pc}$ was measured on the same root three times; during perfusion with aerated solution (0.25 mol O$_2$ m$^{-3}$), after 0.5 h of hypoxia (0.05 mol O$_2$ m$^{-3}$) and after 1 h of re-aeration (0.25 mol O$_2$ m$^{-3}$).

The seal connecting the root to the pressure probe was tested at the end of the measurements to ensure that it had not restricted water flow during the experiment (Steudle 1993).

**Water relations of root cortical cells**

Effects of hypoxia on cell water relations were determined using a cell pressure probe mounted on a micromanipulator with 1-μm increments (MX1, Narishige, Tokyo, Japan). The root segment was secured inside a small perspex chamber, as described by
Zhang and Tyerman (1991), and positioned on the stage of a microscope. A transparent cover was sealed to the top of the chamber with vacuum grease so that the circulatory system was entirely closed, apart from a 5-mm opening for entry of the microcapillary of the pressure probe. The same nutrient solution as that used in measurements of \( L_p \) was circulated through the chamber at a rate of 420 mL h\(^{-1} \) using a peristaltic pump (Exatech, Melbourne, Vic, Australia). The \( \text{O}_2 \) concentration of the nutrient solution was adjusted and monitored as described above.

Cortical cells were punctured by the microcapillary of the pressure probe in the second to sixth cell layer from the root surface (20–200 \( \mu \)m depth from root surface), 30–50 mm from the root tip. The methodology for measuring the water relations of cortical cells and the size of the cells were reported in Bramley et al. (2009). Turgor pressure (\( P_c \)), half-time of the rate of water exchange (\( T_{1/2} \)) and volumetric elastic modulus (\( \varepsilon \)) were measured and used to calculate the hydraulic conductivity of cells (\( L_p \)). Where water flow across membranes was rapid (\( T_{1/2} < 1 \) s), \( \varepsilon \) was corrected for the curvilinear relationship between volume and pressure change (Steudle et al. 1980). Because of the difficulty in maintaining constant turgor pressure and an unblocked probe capillary for long periods of time, measurements were conducted on roots within 0.2–0.5 h, after bathing roots for 0.5 h with aerated solution (0.25 mol \( \text{O}_2 \) m\(^{-3} \)) or hypoxic solution (0.05 mol \( \text{O}_2 \) m\(^{-3} \)).

**Results**

**Root pressure**

When connected to the root pressure probe, wheat roots generated significantly higher \( P_t \) than either species of lupin (\( P < 0.0001; \) Table 1). Roots responded to reduced \( \text{O}_2 \) concentration by increased \( P_t \) (Table 1, Fig. 2). \( P_t \) of both lupin species significantly increased in response to hypoxia (\( P = 0.001 \) for narrow-leafed lupin and \( P = 0.021 \) for yellow lupin), but the response in wheat was not statistically significant (\( P = 0.295 \)). \( P_t \) of yellow lupin began to increase soon after the commencement of bubbling with \( \text{N}_2 \) plus air when the \( \text{O}_2 \) concentration of the solution was between 0.06 and 0.07 mol \( \text{O}_2 \) m\(^{-3} \), and then returned to pre-hypoxia values when roots were re-aerated (Fig. 2b). In comparison, \( P_t \) of narrow-leafed lupin took 161 (\( \pm 32 \)) s at 0.05 mol \( \text{O}_2 \) m\(^{-3} \) before increasing, but the increase in \( P_t \) was only transient, declining during the hypoxic treatment (Fig. 2c). Re-aeration did not arrest the decline in 6 of the 11 narrow-leafed lupin roots, but in those roots, \( P_t \) continued to decline to zero without subsequent recovery (Fig. 2c).

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**Statistical analyses**

All data were analysed with SPSS ver. 11.0 (SPSS, Chicago, IL, USA) and GraphPad Prism ver. 5.01 (GraphPad Software, La Jolla, CA, USA). One-way ANOVA compared the initial root pressure (\( P_r \)) between species. To test whether hypoxia significantly changed \( P_r \) of each species, \( P_r \) before and during hypoxia were compared in a paired \( t \)-test. Repeated-measures ANOVA tested the effects of the \( \text{O}_2 \) treatments on \( L_p \) with Bonferroni post-test. Two-way ANOVA with Bonferroni post-tests compared the effects of \( \text{O}_2 \) treatment on the water transport parameters of cells.

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**Fig. 1.** Freehand cross-sections of (a) wheat, (b) yellow lupin and (c) narrow-leafed lupin roots, 10 cm from the root tip. Examples are typical of 14-day-old roots grown in sand. Sections were stained for 5 min with 0.05% toluidine blue. Bars = 50 \( \mu \)m.
Table 1. Root pressure ($P_r$) of roots bathed with aerated solution (0.25 mol O$_2$ m$^{-3}$) and the maximum increase in $P_r$ ($\Delta P_r$) when bathed with hypoxic solution (0.05 mol O$_2$ m$^{-3}$)

$P_r$ was measured with the root pressure probe. Values are means ± s.e.m. and values in brackets = n. Different superscript letters represent significant difference between the species ($P<0.05$)

<table>
<thead>
<tr>
<th>Species</th>
<th>$P_r$ (MPa × 10$^{-4}$)</th>
<th>$\Delta P_r$ (MPa × 10$^{-4}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>96 ± 6 (10)$^a$</td>
<td>5 ± 6 (10)$^a$</td>
</tr>
<tr>
<td>Yellow lupin</td>
<td>52 ± 7 (9)$^b$</td>
<td>14 ± 4 (9)$^{ab}$</td>
</tr>
<tr>
<td>Narrow-leaved lupin</td>
<td>40 ± 5 (11)$^b$</td>
<td>22 ± 4 (11)$^b$</td>
</tr>
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</table>

Hydraulic conductivity of roots

$Lp_r$ of aerated wheat roots was almost twice that of both lupin species (Fig. 3; $P=0.008$). Aeration treatment significantly affected $Lp_r$ of wheat roots ($P=0.0007$; Fig. 3), but did not significantly affect $Lp_r$ of either lupin species when roots with stable root pressure at the end of the experiment were analysed ($P=0.294$ for narrow-leafed lupin and 0.331 for yellow lupin; Fig. 3). The effect of O$_2$ treatment on $Lp_r$ was not analysed in lupin roots where $P_r$ decreased to zero without recovery, as this ‘leakiness’ creates artificially high water flows. For wheat roots, hypoxia reduced $Lp_r$ by 2–45%, but due to this variability, the effect was not significant ($P>0.05$; Fig. 3). However, after re-aeration, $Lp_r$ was on average 1.4 and 1.6 times greater than $Lp_r$ before and during hypoxia, respectively ($P<0.05$; Fig. 3). Because $Lp_r$ of individual wheat roots is inversely related to root length (Bramley et al. 2009), the relative change in $L_r$ ($Lp_r \times A$) due to O$_2$ treatment (normalised to the value before hypoxia) was examined in relation to root length (Fig. 4). The relative change in $L_r$ due to hypoxia tended to be greater in shorter roots (Fig. 4a), but was independent of root length after re-aeration (Fig. 4a).

Because changes in root anatomy could be discounted during such short-term aeration treatments, changes in $Lp_r$ of wheat roots may have been mediated by varying aquaporin activity. Aquaporin activity was tested before hypoxia and after re-aeration using mercuric chloride to inhibit water flow across cell membranes, the assumption being that if more aquaporins were active there would be greater inhibition of water flow. The influence of aquaporins on $Lp_r$ was not tested during hypoxic treatment as the combined treatment of mercury and hypoxia was toxic to roots, causing loss of root pressure without recovery. Wheat roots were incubated for 0.5 h with 50 μM HgCl$_2$ before hypoxia or after 0.5 h hypoxia plus 1 h re-aeration. Aquaporin activity was also tested in lupin roots, but only for pre-hypoxia treatment as $Lp_r$ was not significantly affected by O$_2$ treatments.

Mercuric chloride inhibited water flow of wheat roots more after re-aeration (60%) than before hypoxia (40%), reducing $Lp_r$ to the same level (Fig. 5a). In comparison, mercury did not significantly affect $Lp_r$ of either lupin species ($P>0.05$; Fig. 5b).

Cell water relations

Cortical cells of wheat had significantly higher turgor pressure than cells of either lupin species (Table 2; $P<0.001$). Hypoxia decreased the turgor pressure of yellow lupin cells by 0.12 MPa ($P<0.001$; Table 2), but did not affect turgor pressure of cells in wheat roots ($P>0.05$; Table 2). Cells of narrow-leaved lupin roots bathed with hypoxic solution were less stable than cells in aerated roots (Fig. 6). Cells often lost turgor pressure almost immediately after puncturing and the consistency of the cell sap appeared...

![Fig. 2. Examples of the response in root pressure ($P_r$) when individual roots of (a) wheat, (b) yellow lupin and (c) narrow-leaved lupin were bathed with hypoxic solution (0.05 mol O$_2$ m$^{-3}$) and then re-aerated (0.25 mol O$_2$ m$^{-3}$). Arrows indicate the switch from aerated to hypoxic solution and then back to aerated solution. The steps in pressure are pressure-clamps for measuring $Lp_r$. The example shown for narrow-leaved lupin (c) was a typical response for six roots, with the remaining roots ($n=5$) behaving similar to yellow lupin (b).](image-url)
to be altered by hypoxia, causing frequent blockages of the microcapillary.

$Lp_c$ of cortical cells in aerated roots was not significantly different between the species ($P = 0.333$), but was significantly reduced by hypoxia ($P < 0.0001$; Table 2). $Lp_c$ of hypoxic treated cells was lower than aerated cells, by an average of 46% for wheat, 63% for yellow lupin and 74% for narrow-leafed lupin (Table 2). Bramley et al. (2009) demonstrated that $Lp_c$ of cells in aerated wheat and lupin roots tends to increase with depth from the root surface, but the effect of hypoxia on $Lp_c$ was independent of cell location within the cortex.

$Lp_r$ influences the rate of water flow across the membrane and hence influences $T_{1/2}$. $T_{1/2}$ for cells in roots bathed in aerated solution ranged between 0.3 and 1.3 s, and was not significantly different between the species ($P = 0.678$; Table 2). For all species, mean $T_{1/2}$ of cells from hypoxic roots was significantly greater than cells from aerated roots ($P < 0.0001$; Table 2; Fig. 6). Increase in $T_{1/2}$ due to hypoxia was greater for narrow-leafed lupin root cells than for the other species (Table 2), but only five narrow-leafed lupin cells were stable for sufficient time to obtain measurements. Mean $T_{1/2}$ of wheat and yellow lupin root cells doubled in response to hypoxia (Table 2), however, the effect on individual cells was variable as $T_{1/2}$ ranged between 0.6–3.0 s and 0.7–4.6 s, respectively.

The elastic modulus of cortical cells from aerated roots ranged from 3.8 to 9.0 MPa for wheat and 1.8 to 16.7 MPa for lupins, and the average was not significantly different between the species ($P = 0.705$; Table 2). The variability in $\varepsilon$ was due to the dependence of $\varepsilon$ on cell volume (Fig. 7). Hypoxia had no effect on $\varepsilon$ of wheat or yellow lupin ($P > 0.05$; Table 2, Fig. 7). However, hypoxia apparently reduced $\varepsilon$ of narrow-leafed lupin cells, so that there was no longer a relationship between cell volume and $\varepsilon$ ($P = 0.0005$, Fig. 7). The rate of water exchange across the cell is dependent on $\varepsilon$ and therefore, a lower $\varepsilon$ would result in an increase in $T_{1/2}$. However, the apparent reduction in $\varepsilon$ may be an artefact of the measurement process since cells with a lower $\varepsilon$ may have been the only ones that were measurable due to greater membrane instability during hypoxia. When the pressure probe microcapillary is pushed against the cell wall just before puncturing the cell, it could cause a surge in turgor pressure,
disrupting cells with unstable membranes. Cells with a lower ε would buffer against sudden changes in turgor pressure.

To test aquaporin activity, incubating roots with mercury and low O2 caused membranes to become leaky when punctured with the cell pressure probe; therefore, \( L_p \) with HgCl2 during hypoxia was not measured.

**Discussion**

Short-term hypoxia rapidly affected transport properties in the roots of wheat and both lupin species. Root pressure changed within minutes of bathing roots with hypoxic solution, particularly lupin roots. This is a rapid response in \( P_r \) and demonstrates how sensitive roots are to their changing environment and especially how sensitive solute transport is to oxygen deficiency. The reported effects of O2 deficiency or waterlogging on water transport in roots are diverse, ranging from zero effect to more than 70% inhibition (reviewed in Bramley et al. 2007a). The source of this variability relates not only to the different species, but also to the nature of the treatment imposed. There have been few direct comparisons between species treated with the same external low O2. In this study, hypoxia affected different components of water transport in the three species, which is related to their contrasting hydraulic properties (Bramley et al. 2009) and may relate to their tolerance to waterlogging. In addition, measurements during re-aeration identified an important regulation of water transport in wheat roots, which may be beneficial for recovery in growth and the transport of water and nutrients to the shoot when flooded soils have drained. The results of this short-term study are consistent with earlier speculations (Dracup et al. 1992) based on anecdotal field observations that wheat roots are more tolerant of waterlogging than the roots of lupins. Lupin roots are more sensitive to low O2 than wheat roots, especially narrow-leafed lupin roots that become leaky.

**Solute transport**

The accumulation of solutes in the xylem creates an osmotic gradient that draws water into the xylem generating \( P_r \) (Steudle 1993). Solutes are able to accumulate in the apoplast of the stele because Casparian bands, located in the endodermis of all three species (Bramley et al. 2009), prevent backflow out of the stele (Steudle et al. 1993). When aerated, \( P_r \) of wheat was similar to a range of other crop species (Steudle and Jeschke 1983; Steudle and Brinckmann 1989; Azaizeh and Steudle 1991; Miyamoto et al. 2001; Lee et al. 2004). In comparison, lupins only generated low \( P_r \) when connected to the root pressure probe, similar to woody species and *Lotus japonicus* (Regel) K. Larsen (Hallgren et al. 1994; Steudle and Meshcheryakov 1996; Henzler et al. 1999). The difference in \( P_r \) between wheat and lupins may be related to greater active transport of ions or organic solutes in wheat roots or a lower effective reflection coefficient in lupin roots.Whilst increases in concentration of certain solutes in the

**Table 2. Water relation parameters of root cortical cells bathed with aerated (0.25 mol O2 m\(^{-3}\)) or hypoxic (0.05 mol O2 m\(^{-3}\)) solution**

<table>
<thead>
<tr>
<th>([O_2]) (mol O2 m(^{-3}))</th>
<th>(P_r) (MPa)</th>
<th>(T_{1/2}) (s)</th>
<th>(\varepsilon) (MPa)</th>
<th>(L_p) (m s(^{-1}) MPa(^{-1}) x 10(^{-8}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.55 ± 0.02 (18)</td>
<td>0.8 ± 0.1 (10)</td>
<td>7.5 ± 0.5 (10)</td>
<td>1.3 ± 0.2 (10)</td>
</tr>
<tr>
<td>0.05</td>
<td>0.60 ± 0.01 (12)</td>
<td>1.6 ± 0.3* (10)</td>
<td>7.7 ± 0.6 (10)</td>
<td>0.7 ± 0.1** (10)</td>
</tr>
<tr>
<td>Yellow lupin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.39 ± 0.01 (12)</td>
<td>0.7 ± 0.1 (12)</td>
<td>7.9 ± 1.0 (12)</td>
<td>1.6 ± 0.2 (12)</td>
</tr>
<tr>
<td>0.05</td>
<td>0.27 ± 0.01*** (22)</td>
<td>1.9 ± 0.4*** (12)</td>
<td>9.6 ± 1.2 (12)</td>
<td>0.6 ± 0.1*** (12)</td>
</tr>
<tr>
<td>Narrow-leafed lupin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>0.41 ± 0.01 (17)</td>
<td>0.6 ± 0.1 (12)</td>
<td>11.0 ± 1.2 (12)</td>
<td>1.4 ± 0.2 (12)</td>
</tr>
<tr>
<td>0.05</td>
<td>0.39 ± 0.04 (7)</td>
<td>4.3 ± 0.7*** (5)</td>
<td>5.0 ± 0.4*** (5)</td>
<td>0.4 ± 0.1*** (5)</td>
</tr>
</tbody>
</table>
xylem during flooding have been found earlier (Jackson et al. 1996), changes in $P_r$ in response to oxygen deficiency have only previously been reported for Zea mays (L.) (Birner and Steudle 1993; Gibbs et al. 1998a). However, the response depended on the oxygen concentration because $P_r$ of hypoxic Z. mays roots gradually recovered when re-aerated (Birner and Steudle 1993), but not roots treated for 5 h with anoxia (Gibbs et al. 1998a).

The effect of hypoxia on $P_r$ was related to root diameter with the greatest response in narrow-leafed lupin. Root diameter is an important factor in comparisons of waterlogging tolerance as greater diffusive distances from the external medium to the root centre could lead to greater internal oxygen deficiency. Therefore, the oxygen deficiency in the stele of narrow-leafed lupin could be twice as severe as wheat, assuming similar rates of respiration. Using microelectrodes, radial profiles of the O$_2$ partial pressure across roots have shown that the stele may be close to anoxic even with appreciable amounts of O$_2$ in the cortex (Armstrong and Beckett 1987; Armstrong et al. 1994).

The response in $P_r$ is likely to be due to leakage of ions and/or carbohydrates rather than an effect on energy-dependent processes because $P_r$ began to change immediately upon O$_2$ decreasing in the bathing medium of yellow lupin and within a few minutes of the lowered O$_2$ for narrow-leafed lupin. Whilst the effects of hypoxia on energy-dependent solute transport to hypoxia are not slow (Trought and Drew 1980; Buwalda et al. 1988; Thomson et al. 1989), the response has not been shown to occur so rapidly. In comparison, patch clamping techniques have shown that reversible leakage of ions due to membrane depolarisation/hyperpolarisation in response to changes in aeration can occur within seconds, as has been observed for cortical cells of wheat roots (Zhang and Tyerman 1997). The process in yellow lupin roots appears to be reversible as $P_r$ returned to original levels during re-aeration. However, the subsequent loss of $P_r$ in narrow-leafed lupin indicates that either the endodermis became leaky or solutes leaked from the

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**Fig. 6.** Examples of turgor pressure ($P_c$) traces recorded with the cell pressure probe, for cells in narrow-leafed lupin roots bathed with aerated solution (0.25 mol O$_2$ m$^{-3}$; a) or hypoxic solution (0.05 mol O$_2$ m$^{-3}$; b). Arrows indicate where new cells were stabbed by the microcapillary of the probe. The highlighted regions are expanded in the figure insets to show the differences in $T_{1/2}$ of pressure relaxations due to aeration treatment.

**Fig. 7.** Relationship between volumetric elastic modulus ($\varepsilon$) and cell volume ($V$) of cortical cells in roots of (a) wheat, (b) yellow lupin and (c) narrow-leafed lupin bathed with aerated solution (open circles) or hypoxic solution (closed circles). Regression analysis was not relevant for wheat because of the small range of cell sizes. Error bars represent ± s.e.m.; $n$ = 5–12.
root tip. Root tips are usually the most sensitive region of roots to O₂ deficiency (Drew 1997) and when waterlogged, narrow-leaved lupin roots die first at the tip (Bramley 2006).

Changes in turgor pressure provide evidence that solute leakage occurred from cortical cells of lupin roots, but not wheat, in response to hypoxia. Turgor pressure decreased indicating a loss of osmotica. Leakage from epidermal cells may also occur, but cells were physically too small to measure with the pressure probe. Unlike the effect on $P_t$, which was related to root diameter, cortical cells measured with the cell pressure probe were within the same cell layers and depth from the root surface for all species so the distance for O₂ diffusion would have been the same. However, the instability of narrow-leaved lupin cortical cells suggests that this species is particularly sensitive to hypoxia.

Cell and root hydraulics

The influence of O₂ deficiency on $L_p$ has only been previously measured in wheat and $Z$. mays (Zhang and Tyerman 1991; Tyerman et al. 1992; Zhang and Tyerman 1999). In those studies, the impact on wheat (60–85%) may have been slightly greater than the present study (46%) because cells were closer to the root tip (10–20 mm) and exposed to a slightly lower O₂ concentration (0.04 mol O₂ m⁻³). Hypoxia dramatically reduced $L_p$ in all species, with the effect being greater in lupins than wheat. It is unlikely that the greater reduction of $L_p$ in lupins was due to greater internal oxygen deficiency due to thicker roots because $L_p$ was measured within the same range of depth from the root surface in all species. In addition, recent evidence by Armstrong et al. (2009) indicates that respiration rates may not decline in the cortex with increasing depth into the root even when oxygen concentrations decline. Instead, the hypoxia results are analogous to those of mercury inhibition of aerated roots (Bramley et al. 2009), indicating greater inhibition of aquaporin activity in lupins than wheat. Closure of aquaporins in membranes decreases the hydraulic conductivity of membranes and hence increases the half-time of the rate of water exchange ($T_{1/2}$) across the cell. Cytoplasmic pH, Ca²⁺ and respiration, processes that are affected by hypoxia, can regulate cell water permeability through aquaporin gating and expression (Gerbeau et al. 2002; Tournaire-Roux et al. 2003; Alleva et al. 2006; Verdoucq et al. 2008). However, although 0.05 mol O₂ m⁻³ can induce changes in aquaporin expression within 0.5 h of treatment (Klok et al. 2002), those aquaporins that are highly expressed in roots and facilitate water transport across membranes (e.g. PIP2s, tables 2 and 5 of Bramley et al. 2007a) tend to be downregulated after a few hours of hypoxia. Given the short-term exposure of wheat and lupin roots to hypoxia, it seems more probable that $L_p$ was inhibited through a closure of aquaporins, rather than a reduction in their expression. What mechanism closed aquaporins is not known, but 0.5 h of zero O₂ reduced cytoplasmic pH of Arabidopsis thaliana (L.) roots to 7.1 and $Z$. mays root tips to 6.9 (Xia and Roberts 1996; Tournaire-Roux et al. 2003). These values of pH are within the range of pH that can decrease the osmotic water permeability of plasma membranes by half due to aquaporin closure (Alleva et al. 2006; Verdoucq et al. 2008).

Despite the large reductions in $L_p$ in lupin roots due to hypoxia, the insignificant reduction in $L_p$ indicates that water flow across the cortex occurs predominantly through the apoplastic space. If flow occurred by the cell-to-cell pathway in the cortex, then $L_p$ would be proportionally reduced. This is consistent with Bramley et al. (2009) where $L_p$ of narrow-leaved lupin and yellow lupin was not affected by mercury treatment despite dramatic reductions in $L_p$. This is also consistent with a smaller inhibition in $L_p$ compared with $L_p$ for $Z$. mays (Tyerman et al. 2008).
et al. 1992), another species believed to have predominantly apoplastic flow (Steudle and Freensch 1989), although Gibbs et al. (1998b) argue that the symplast is important. Measurements by Armstrong et al. (2009) indicate that the critical oxygen pressure (COP) at the root surface is a function of respiratory decline in the stele and that roots with larger stelar diameters should tend to have higher COPs, confirming original predictions by Berry and Norris (1949). Narrow-leaved lupin may therefore experience greater respiratory decline in the stele in comparison to the other species, when exposed to the same external oxygen concentration. However, this does not change our conclusion that bulk water flow across lupin roots bypasses cells without crossing membranes.

Using a model of a root comprising of concentric cylinders in conjunction with measurements of $L_p$, of each cortical cell layer and $L_n$, Bramley et al. (2009) identified which regions of the root contribute to $L_n$. In aerated wheat roots, $L_n$ is constant with root length because water uptake preferentially occurs within 40 mm of the root tip and is probably controlled by the endodermis (Bramley et al. 2009). Changes in $L_n$ due to aeration treatment can be predicted by altering $L_p$, the length of the absorbing region and the contribution of cortical cell layers. Applying the model here shows that increasing the length of the endodermis that transports water via aquaporins reduces the inhibition by hypoxia on root hydraulic conductance (Fig. 8). Despite hypoxia inhibiting $L_p$, increasing the length of the absorbing region creates more parallel pathways for flow, allowing longer roots to maintain their hydraulic conductance.

During re-aeration, water transport recovered in wheat roots, but with an overshoot. Overshoots in $L_p$ have also been observed during recovery from water deficit in *Agave deserti* Engelm., nutrient deprivation in wheat, hypoxia in *Z. mays*, and low temperature in *Cucurbita ficifolia* Bouché (Carvajal et al. 1996; Gibbs et al. 1998b; North et al. 2004; Lee et al. 2005), but the reason for this phenomenon has not been investigated. In this study, a greater reduction in $L_p$ by mercury after re-aeration, than before hypoxia, indicated that the overshoot in $L_p$ of wheat roots was due to greater aquaporin activity. Therefore, more aquaporins were gated open or more active aquaporins were embedded in plasma-membranes during re-aeration. Furthermore, $L_p$ was greater in wheat roots during re-aeration, irrespective of root length. If longer roots were absorbing water over a longer length than shorter roots, then $L_n$ would increase with length (Fig. 8). Either $L_p$ returned to its original values but the length of the absorbing region was slightly longer (55 mm predicted by the model) than before hypoxic treatment, or additional pathways were opened (Fig. 8). If $L_p$ increased 1.6-fold during re-aeration, but only in the endodermis (35 mm, not including 5 mm apex), then $L_n$ would be 60% greater than the value before hypoxia (Fig. 8). It would be difficult to measure such an increase in $L_p$ without automating the cell pressure probe, as half-times can be <1 s under ambient conditions. However, with care and a fine resolution micro-manipulator (Bramley et al. 2009), it may be possible to detect changes in $L_p$ in different regions of the endodermis.

The physiological significance for the effect of re-aeration could be related to the recovery of root growth when waterlogging has subsided. The rate of extension of previously waterlogged wheat roots during the recovery period was greater than control roots (Bramley 2006). An increased rate of water uptake into the cells in the zones of elongation, mediated by aquaporins, may increase the rate of root extension. In addition, an increase in the rate of water uptake after waterlogging would be needed to restore the water balance and transport of nutrients to the shoot. In comparison to wheat, lupin roots have inferior survival during waterlogging and recovery is dependent on the growth of new roots originating near the base of the stem (Bramley 2006). The hydraulics of lupin roots depends on changes in anatomy and morphology, but appears to have little ability to regulate water flow in the short term, under ambient or hypoxic conditions.

Our study focussed on the rapid responses to hypoxia using excised roots to examine their hydraulic properties. Water transport through roots is only part of the multifaceted soil–plant–atmosphere continuum that has many regulatory controls. Further research is required to link root hydraulic properties to those of shoots and functioning of whole plants. For example, although root systems of wheat and lupins are generally more sensitive to waterlogging than shoots (Trought and Drew 1980; Davies et al. 2000b), sensitivity is not solely dependent on the root system. In longer-term studies, waterlogging decreased stomatal conductance and transpiration in narrow-leaved lupin and yellow lupin, but despite closure of stomata shoot water potential of narrow-leaved lupin also declined (Davies et al. 2000c). Waterlogging also reduced leaf gas exchange in wheat plants, with a greater inhibition in a more sensitive genotype that also experienced decreased shoot water potential (Huang et al. 1994). Reductions in leaf gas exchange are believed to be caused by insufficient supply of water from the roots (Cannell and Jackson 1981), although correlations between the two processes are not clear.

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