Water uptake by roots of *Hordeum marinum*: formation of a barrier to radial O$_2$ loss does not affect root hydraulic conductivity

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Abstract

The adventitious roots of *Hordeum marinum* grown in stagnant deoxygenated solution contain a barrier to radial O$_2$ loss (ROL) in basal zones, whereas roots of plants grown in aerated solution do not. The present experiments assessed whether induction of the barrier to ROL influences root hydraulic conductivity ($L_p$). Wheat (*Triticum aestivum*) was also studied since, like *H. marinum*, this species forms aerenchyma in stagnant conditions, but does not form a barrier to ROL. Plants were grown in either aerated or stagnant, deoxygenated nutrient solution for 21–28 d. Root-sleeving O$_2$ electrodes were used to assess patterns of ROL along adventitious roots, and a root-pressure probe and a pressure chamber to measure $L_p$ for individual adventitious roots and whole root systems, respectively. $L_p$, measured under a hydrostatic pressure gradient, was 1.8-fold higher for individual roots, and 5.6-fold higher for whole roots systems, in *T. aestivum* than *H. marinum*. However, there was no difference in $L_p$ between the two species when measured under an osmotic driving force, when water moved from cell to cell rather than apoplastically. Root-zone O$_2$ treatments during growth had no effect on $L_p$ for either species (measured in aerobic solution). It is concluded that induction of the barrier to ROL in *H. marinum* did not significantly affect the hydraulic conductivity of either individual adventitious roots or of the whole root system.

Key words: Adventitious roots, aerenchyma, exodermis, hydraulic conductivity, hypodermis, O$_2$-deficiency, pressure chamber, radial O$_2$ loss, root pressure probe, *Triticum aestivum*, wheat.

Introduction

The external cell layers in the sub-apical regions of adventitious roots of many wetland plants develop a very low permeability to O$_2$, i.e. a barrier to restrict radial O$_2$ loss (ROL) (Armstrong, 1979; Armstrong *et al.*, 2000; Colmer, 2003). The barrier to ROL enhances longitudinal O$_2$ diffusion within aerenchymatous roots towards the root tip, and thus root growth into anaerobic waterlogged soils (Armstrong, 1979; Jackson and Armstrong, 1999). A barrier to ROL is induced by growth in stagnant, deoxygenated conditions in a number of wild *Hordeum* species that inhabit wetland areas; for example, *Hordeum marinum* (Garthwaite *et al.*, 2003). In contrast to these wild species, their cultivated, dryland relatives (barley, *Hordeum vulgare*, and wheat, *Triticum aestivum*) do not form a barrier to ROL (McDonald *et al.*, 2001). Wheat, barley, and *H. marinum* all form adventitious roots containing aerenchyma, but wheat and barley are much less tolerant of waterlogging than *H. marinum* (McDonald *et al.*, 2001).

In *H. marinum* grown in stagnant, deoxygenated nutrient solution, the apparent O$_2$ diffusivity across the cell layers external to the root aerenchyma was 27-fold lower in the basal regions (which have very low rates of ROL) compared with near the root tip (a location with high rates of ROL) (AJ Garthwaite *et al.*, unpublished data). The low O$_2$ permeability near the base of the root was associated with
secondary thickening, putatively lignin or suberin deposits, in the two outermost layers of the hypodermis (AJ Garthwaite et al., unpublished data). Oxygen microelectrode studies of roots of Phragmites australis, which also forms a barrier to ROL, showed high resistance to O2 movement across the epidermal/hypodermal layers (Armstrong et al., 2000). Other structures that have been associated with a barrier to ROL include layers of sclerenchymatous fibres with thickened secondary walls (Oryza sativa; Clark and Harris, 1981), or tight, multilayered hexagonal cell packing that eliminates gas spaces in the outer root tissues (numerous species, Justin and Armstrong, 1987; Armstrong et al., 1991).

Radial variation in the structure of root tissues can affect water uptake through roots, for a given ‘driving force’ (Steudle and Peterson, 1998; Lee et al., 2005; Schreiber et al., 2005). The effect on water uptake of changes in structure of the hypodermal layers in roots, albeit changes not associated with a barrier to ROL, have previously been evaluated for Agave desertii, Zea mays (maize), and Allium cepa (onion) (Melchior and Steudle, 1993). For example, development of an exodermis in Z. mays roots coincided with a 4-fold reduction in hydraulic conductivity (Zimmermann and Steudle, 1998). In roots of A. cepa, radial hydraulic conductivity decreased by a factor of five in the basal regions of the root where Casparian bands were present in the exodermis, and suberin lamellae had formed in the endodermis and exodermis (Melchior and Steudle, 1993). In Z. mays roots having an exodermis, there were significantly higher levels of aliphatic suberin and, to a lesser degree, lignin, in the basal half (Zimmermann et al., 2000). The presence of these compounds in the external cell layers has been related to a reduction in apoplastic water flow (Zimmermann et al., 2000). On the other hand, an increase in the amounts of suberin deposited in the exo- and endodermis of Z. mays and O. sativa did not necessarily reduce the resistance to radial water flow (Schreiber et al., 2005).

Adventitious roots of O. sativa constitutively form an exodermis (hypodermis with Casparian bands), and a layer of sclerenchymatous fibres with thickened secondary walls at the outer cortex (Clark and Harris, 1981). These hypodermal structures (in conjunction with a well-developed endodermis), were hypothesized to contribute to the ‘low’ root hydraulic conductivity measured for O. sativa (compared with Z. mays) (Miyamoto et al., 2001). However, by means of pressure-perfusion of solution through the large cortical air spaces in O. sativa adventitious roots, it was possible to measure the hydraulic conductivity of the cell layers external to the aerenchyma. Hydraulic conductivity of the external cell layers was 30-fold higher than the overall root hydraulic conductivity, indicating that the ‘apoplastic barriers’ in the external cell layers of O. sativa roots do not limit water uptake (Ranathunge et al., 2003). The barrier to ROL in adventitious roots of O. sativa is induced by growth in stagnant conditions (Colmer et al., 1998; Colmer, 2003a), however, a comparative analysis of the hydraulic conductivity for O. sativa roots with and without the barrier to ROL has not yet been performed, so it is not possible to draw conclusions on the effect of the barrier to ROL per se on water uptake by roots of O. sativa.

To determine whether or not induction of a barrier to ROL influences water uptake, the present study compared the hydraulic properties for individual adventitious roots, and whole root systems, of H. marinum (a species that forms a barrier to ROL) and T. aestivum (a species without a barrier to ROL) grown in aerated or stagnant, deoxygenated nutrient solution.

Materials and methods

Plant materials and culture

Hordeum marinum ssp. gussoneanum (accession H 819, Nordic Gene Bank, Alnarp, Sweden) and Triticum aestivum cv. ‘Crocus’ were used. H. marinum forms a barrier to ROL when grown in stagnant, deoxygenated nutrient solution, whereas T. aestivum does not (see Introduction).

Seeds were placed on plastic mesh floating on 0.1-strength aerated nutrient solution in darkness in a 20/15°C (12 h day/night) controlled environment chamber (PAR 500 μmol m⁻² s⁻¹). The composition of the nutrient solution at full strength was (mol m⁻³): K⁺ 5.60, Ca²⁺ 1.50, Mg²⁺ 0.40, NH₄⁺ 0.625, NO₃⁻ 4.375, SO₄²⁻ 1.90, H₂PO₄⁻ 0.20, Na⁺ 0.20, H₂SiO₄⁻ 0.10; and the micronutrients (μmol mol⁻¹): Cl⁻ 50, B 2.5, Mn 2, Zn 2, Ni 1, Cu 0.5, Mo 0.5, Fe-Na-ethylenediaminetetra-acetic acid 50. The solution also contained 2.5 mol m⁻³ 2-(N-morpholino)ethanesulfonic acid (MES) and the pH was adjusted to 6.5 with KOH (the final K⁺ concentration as above). All chemicals used were analytical grade.

Seedlings were exposed to light 4 d after imbibition and given 0.25-strength nutrient solution for an additional 4 d, before being transferred to pots of aerated, full-strength nutrient solution. Each 4.5 dm³ plastic pot held four seedlings. Plants were held in the lids using polystyrene foam holders. The pots and lids were covered with aluminum foil to ensure the roots were in darkness.

Root-zone O₂ treatments

Eight replicate pots of each root-zone O₂ treatment x species combination were grown, with each complete replicate block germinated one week apart over a series of eight weeks to ensure a supply of plants of the correct age throughout the experimental period. Each pot contained four seedlings. Pots were arranged randomly within blocks. Seven days after the seedlings were transplanted into pots, root-zone O₂ treatments were initiated (i.e. on 15-d-old plants). In the pots designated for the aerated treatment, the nutrient solution continued to be bubbled with air. For the remaining pots, designated for the stagnant treatment, the nutrient solution was replaced with deoxygenated nutrient solution containing 0.1% (w/v) agar; the agar prevented convective movements in the solution (Wijngaard et al., 1997). Solutions in the aerated pots were also renewed at this time with aerated nutrient solution (without agar). All solutions were renewed every 7 d. Treatments were maintained for 21–28 d, with measurements taken during the final 7 d.

Radial O₂ loss (ROL) from intact adventitious roots in an O₂-free medium

ROL was measured on three replicates of each species x root-zone O₂ treatment combination to confirm the profiles of ROL were as
previously found for these species (McDonald et al., 2001; Garthwaite et al., 2003). Plants were sealed just above the root-shoot junction with rubber lids fitted in 100×100×170 mm Perspex chambers filled with an O2-free solution containing 0.1% (v/v) agar and (mol m\(^{-3}\)) Ca\(^{2+}\), SO\(_4^{2-}\), 0.5 and K\(^+\), Cl\(^-\), 5.0. The intact root system was immersed in O2-free solution while the shoot remained in air. A root-sleeving O2 electrode (height 5 mm; internal diameter 2.25 mm) (Armstrong and Wright, 1975; Armstrong, 1994) fitted with guides was placed around a selected adventitious root with a length in the range of 100–120 mm. The flux of O2 from the root to the electrode was measured with the centre of the electrode positioned at 80, 70, 60, 40, 20, and 5 mm behind the apex. The first measurements were taken at least 2 h after the plants were transferred into the system. All measurements were taken at 20 °C in a temperature-controlled room with photon-flux density at shoot height of 100 μmol m\(^{-2}\) s\(^{-1}\).

Measurements of ROL were also conducted to confirm that the barrier to ROL, induced in adventitious roots of *H. marinum* by growth in stagnant, deoxygenated nutrient solution, was still effective after the plant had been in aerated nutrient solution for 12 h. This enabled root pressure probe and pressure chamber experiments (described below) to be conducted in aerated nutrient solution.

**Hydraulic conductivity of individual adventitious roots or whole root systems**

The hydraulic conductivity was compared for individual adventitious roots and whole root systems of *H. marinum* and *T. aestivum* grown in aerated or stagnant nutrient solution. In all cases, hydraulic conductivity was measured with individual roots, or whole root systems, in aerobic conditions as this enabled it to be determined whether or not induction of the barrier to ROL impacts on root hydraulic conductivity, while avoiding possible direct effects of O2 deficiency on hydraulic conductivity during the measurements.

**Using the root pressure probe to measure hydraulic conductivity of individual adventitious roots**

Root pressure probe measurements were conducted as previously described (Steudle et al., 1987; Steudle and Frensch, 1996). In brief, excised roots with tips (100–140 mm) were connected to a root pressure probe using silicon seals to ensure root pressure was maintained without blocking xylem vessels. Roots attached to the probe were bathed in aerated nutrient solution with 20 mol m\(^{-3}\) glucose. Following attachment of roots, the system was left for at least 5 h or until stable root pressures (*P\(_r\)*, MPa) were established. Hydrostatic relaxations were performed by either increasing or decreasing the xylem pressure (using the probe). Osmotic relaxations were performed by suddenly increasing or decreasing the osmotic pressure of the external solution which was running through 0.25 m pipe (diameter=6 mm) with the fixed root protruding into it at one end. The resulting transient responses in pressure allowed hydraulic conductivity (*L\(_p\)*) to be calculated from half-times of pressure relaxations (*T\(_{50}\)*) using the equation:

\[
L_p = \frac{\ln(2)}{T_{50}(\Delta P/\Delta V)}A_r
\]

where \(\Delta P/\Delta V\) is the elastic coefficient of the measuring system, determined by induced step changes in volume (\(\Delta V\)) using the pressure probe and recording the resulting changes in root pressure (\(\Delta P\)); \(A_r\) is the surface area of the root, calculated by measuring the root length and average diameter. Measured root pressure relaxations usually showed a slower phase at the end of the relaxation, occupying about 20% of the entire relaxation. This has been attributed to a reversible concentration polarization of solutes at the endodermis (Steudle and Frensch, 1989; Ye and Steudle, 2005). This part of the curve was omitted in the analysis of relaxations.

In osmotic experiments, the external unstirred layer around the root may have contributed to the measured values of *L\(_p\)* (‘gradient-dissipation effect’; Steudle and Frensch, 1989; Ye and Steudle, 2005). However, at flow rates along the root of usually between 0.3 and 0.5 m s\(^{-1}\), the effect should be negligible (see Results).

Osmotic experiments used additions of 25 mol m\(^{-3}\) NaCl to the bathing solution. The permeability coefficient for a given solute (*P\(_w\)*) is given by:

\[
P_w = \frac{V_r \ln(2)}{A_t T_{50}}
\]

where *T\(_{50}\)* is the half-time of solute exchange and *V\(_r\)* is the volume of functioning (mature) xylem, estimated by examining root cross-sections (data not shown); \(A_t\) as defined in equation 1.

Root reflection coefficients (\(\sigma_{sr}\)) were calculated from:

\[
\sigma_{sr} = (\frac{(P_{in} - P_{min})}{\Delta P_{o}})\exp(k_{sr}t_{min})
\]

where \(P_{in}\) is the original root pressure, \(P_{min}\) is the minimum root pressure due to water efflux, \(\Delta P_{o}\) denotes the change in osmotic pressure of the medium, \(k_{sr}\) is the rate constant of permeation of a given solute (NaCl), and \(t_{min}\) is the time required to reach the minimum root pressure in the presence of the permeating solute ‘s’.

As with the osmotic relaxations, measurements of permeability and reflection coefficients of roots can be influenced by effects of external unstirred layers. However, at the flow rates used in these experiments, 0.3–0.5 m s\(^{-1}\), these effects are likely to be small, as discussed in the Results section.

**Hydraulic conductivity of whole root systems: measurement of root exudation in the presence of gradients in osmotic or hydrostatic pressure**

Root exudation under an osmotic pressure gradient: Tillers were cut 40–70 mm above the shoot base. All tillers except the main stem were sealed using clamps. Xylem sap exuding from the cut surface of the main stem was collected using a syringe and then weighed. Water uptake by the root was driven by the difference in osmotic pressure (\(\Delta P\) in MPa) between the medium and the xylem sap:

\[
J_v = L_p \sigma_o \Delta P = L_p \sigma_o RT(C' - C^o)
\]

The symbol \(\sigma_o\) denotes the root’s reflection coefficient for the solutes in the external nutrient solution which was determined from experiments with the root pressure probe (described above). The osmotic pressure of the solution and exuded sap was measured using a freezing-point depression osmometer (Osmomat 030; Gonotec, Berlin, Germany).

Root exudation under a hydrostatic pressure gradient: The root systems used to measure osmotic water flow were also used to measure hydraulic conductivity of the root system in the presence of hydrostatic pressure gradients. The root system was enclosed in a steel chamber where pneumatic pressure was applied to the root medium. Silicon seals and flexible rubber was placed around the stem to ensure a tight seal. The pressure in the chamber was raised in steps of 0.05 MPa and typically measurements were taken between 0.05 and 0.3 MPa above atmospheric. Exuded xylem sap was collected as described above, and the volume (\(V\) in m\(^3\)) determined. The volume of exuded sap was plotted with time at a given applied pressure (*P\(_{gas}\)* in MPa). For the resulting linear relationship, the calculated slope was divided by the whole root surface area (m\(^2\), see below) to give the volume flow, *J\(_v\)* in m\(^3\) m\(^{-2}\) s\(^{-1}\). When plotting this against the applied pressure, the slope represented the hydraulic conductivity. The osmotic component was negligible for *P\(_{gas}\)*>0.05 MPa.
(Miyamoto et al., 2001). The technique resulted in an average value of the hydraulic resistance to water transport between all roots and the main stem when the tillers were clamped off. Root hydraulic conductivity ($L_p$) was calculated from linear ranges of $J_{Vs}$ ($P_{ev}$) curves.

**Surface area of the whole root system**: The surface area of the whole (seminal and adventitious) root system for *H. marinum* and *T. aestivum* plants grown in aerated or stagnant nutrient solution was measured to enable calculations of whole root hydraulic conductivity (as described above). Root system surface area was determined using a video camera and image analysing software (Skye Instruments, Llandrindod Wells, UK). For better contrast, roots were stained with toluidine blue (0.03% w/v) prior to scanning. The surface area of the root system was calculated from projected areas of roots, assuming the roots to be cylindrical. The system was calibrated using metal wires of known lengths and diameters.

**Statistics**

ANOVA was performed on the various data sets to evaluate species and root-zone O$_2$ treatment effects. Means were compared using the LSD ($P<0.05$), and are presented in the tables and figures accompanied by standard errors (GenStat, 6th edn, VSN International, www.vsn-intl.com).

**Results**

**Radial O$_2$ loss (ROL) from intact adventitious roots in an O$_2$-free medium**

Figure 1 shows the induction of a barrier to ROL in the adventitious roots of stastically treated *H. marinum*. ROL near the base of the root was low (resistance to ROL high) and ROL rose sharply towards the root tip. By contrast, for roots previously in aerated solution, ROL was highest at the base of the root and it declined towards the root tip. Adventitious roots of *T. aestivum* grown in aerated solution had very low rates of ROL, which increased for roots in stagnant solution, with most ROL from basal regions; i.e. the absence of a barrier to ROL. These results confirmed that the experiments on hydraulic conductivity were performed on roots with, or without, a barrier to ROL.

**Hydraulic conductivity of individual adventitious roots**

When measured using hydrostatic pressure gradients, the hydraulic conductivity ($L_p$) of adventitious roots of *T. aestivum* was 1.8-fold higher than values for roots of *H. marinum* ($P<0.05$; Table 1). Growth in the stagnant nutrient solution did not affect hydrostatic $L_p$ of roots of either species (Table 1).

$L_p$, when measured using an osmotic pressure gradient, did not differ significantly between *T. aestivum* and *H. marinum*, nor did the root-zone O$_2$ treatments affect $L_p$, which ranged from 1.0×10$^{-8}$ to 1.5×10$^{-8}$ m s$^{-1}$ MPa$^{-1}$ in roots of both species from the two treatments (Table 1). The ratio of hydrostatic/osmotic estimates of $L_p$ was lower in *H. marinum* roots, being 5.1 and 4.0 in plants grown in aerated and stagnant nutrient solution, respectively, compared with 6.2 and 8.3, respectively, for roots of *T. aestivum*.

There was no marked difference in solute permeability (i.e. $P_{sr}$ of NaCl) between roots of *T. aestivum* and *H. marinum*, however, in plants grown in the stagnant nutrient solution, $P_{sr}$ was significantly higher in both species ($P<0.05$; Table 1). $P_{sr}$ ranged from 1.5–1.6×10$^{-9}$ m s$^{-1}$ in the aerated nutrient solution, compared with 2.3–2.5×10$^{-9}$ m s$^{-1}$ in the plants grown in stagnant solution. The reflection coefficient ($\sigma_{sr}$) for individual adventitious roots for NaCl did not significantly differ between the two species, or root-zone O$_2$ treatments, ranging from 0.21 to 0.30 (Table 1).

The steady-state root pressures ($P_r$) varied between 1.0–1.6×10$^{-1}$ MPa (Table 1). *T. aestivum* had root pressures that were 20% higher, on average, than *H. marinum* ($P<0.05$; Table 1). Root pressures were between 18% and 40% higher in roots from plants grown in the stagnant solution ($P<0.05$; Table 1).

The half-time for hydrostatic pressure relaxations ($T_{1/2}$) ranged from 17 s to 24 s, while those for the osmotic pressure relaxations ($T_{1/2}^o$) ranged from 102 s to 152 s.
Table 1. (A) Hydraulic conductivity ($L_p$), solute (i.e. Na$^+$ and Cl$^-$) permeability ($P_s$), the reflection coefficient ($\sigma$), steady-state root pressure ($P_r$), and half-times from hydrostatic ($T_{wh}$) and osmotic ($T_{ws}$) pressure relaxations for individual adventitious roots of *Hordeum marinum* and *Triticum aestivum* grown in aerated or stagnant, deoxygenated nutrient solution for 21–28 d, and then measured in aerated solution; (B) shows, for comparison, values obtained for $L_p$, $P_s$, and $\sigma$ published for other species (references provided in the table).

Root pressure probes were used to measure $L_p$ via responses to changes in xylem pressure or changes in the osmotic pressure of the outer medium (osmoticum: 25 mol m$^{-3}$ NaCl). The values presented are means ± SE ($n$=8), where each replicate represents a single adventitious root (apical 100–140 mm) from an individual plant. Different letters within the same column indicate significant differences at $P$=0.05 level (data from this study only); n.d., not determined.

<table>
<thead>
<tr>
<th>(A) Species</th>
<th>Root O$_2$ treatment</th>
<th>Barrier to ROL</th>
<th>$L_p$</th>
<th>Ratio of hydrostatic/osmotic</th>
<th>$P_s$ (NaCl)</th>
<th>$\sigma$ (NaCl)</th>
<th>$P_r$ ($10^{-1}$ MPa)</th>
<th>$T_{wh}$ (s)</th>
<th>$T_{ws}$ (s)</th>
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<tbody>
<tr>
<td><em>Hordeum marinum</em></td>
<td></td>
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<tr>
<td></td>
<td>Aerated</td>
<td>No</td>
<td>5.2±0.5 a</td>
<td>1.0±0.2 a</td>
<td>5.1</td>
<td>1.6±0.2 a</td>
<td>0.22±0.02 a</td>
<td>1.0±0.13 a</td>
<td>24±3</td>
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<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>5.6±0.7 a</td>
<td>1.4±0.2 a</td>
<td>4.0</td>
<td>2.3±0.3 b</td>
<td>0.21±0.03 a</td>
<td>1.4±0.22 bc</td>
<td>21±4</td>
</tr>
<tr>
<td><em>Triticum aestivum</em></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aerated</td>
<td>No</td>
<td>9.3±1.6 b</td>
<td>1.5±0.4 a</td>
<td>6.2</td>
<td>1.5±0.3 a</td>
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<td>1.3±0.14 b</td>
<td>18±4</td>
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<td>No</td>
<td>8.6±0.9 b</td>
<td>1.0±0.2 a</td>
<td>8.3</td>
<td>2.5±0.5 b</td>
<td>0.30±0.04 a</td>
<td>1.6±0.12 c</td>
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</table>

<table>
<thead>
<tr>
<th>(B) Species</th>
<th>Root O$_2$ treatment</th>
<th>Barrier to ROL</th>
<th>$L_p$</th>
<th>Ratio of hydrostatic/osmotic</th>
<th>$P_s$ (NaCl)</th>
<th>$\sigma$ (NaCl)</th>
<th>Source</th>
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<tr>
<td>cv. ‘Azucena’ and ‘IR64’</td>
<td>Aerated</td>
<td>n.d.$^a$</td>
<td>4.7, 5.8</td>
<td>4.0, 9.2</td>
<td>0.7, 1.9</td>
<td>7.3–17</td>
<td>0.3</td>
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<td>cv. ‘Azucena’ and ‘IR64’</td>
<td>Aerated</td>
<td>n.d.$^a$</td>
<td>3.8, 4.0</td>
<td>1.1</td>
<td>3.5, 3.6</td>
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<td><em>Zea mays</em></td>
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<td>No$^b$</td>
<td>10</td>
<td>1.3</td>
<td>7.7</td>
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<td>16</td>
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<td>7.0</td>
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<tr>
<td></td>
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<td>27</td>
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<td>12</td>
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<td><em>Hordeum distichon</em> (syn. <em>H. vulgare</em>)</td>
<td>Aerated</td>
<td>No$^c$</td>
<td>0.3–0.8</td>
<td>0.5–4.3</td>
<td>~1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Allium cepa</em></td>
<td>Aerated</td>
<td>n.d.</td>
<td>14</td>
<td>0.02–0.2</td>
<td>70–700</td>
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<td>–</td>
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<tr>
<td><em>Phaseolus coccineus</em></td>
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<td>n.d.</td>
<td>2–8</td>
<td>3–7</td>
<td>~1</td>
<td>0.2</td>
<td>0.6</td>
</tr>
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$^a$ *Oryza sativa* forms a barrier to ROL (Armstrong, 1971; Colmer, 2003a), however, in almost all cultivars that have been studied the barrier to ROL is induced by growth in stagnant, deoxygenated root conditions (Colmer et al., 1998; Colmer, 2003a) and so is unlikely to have been formed in the plants used in these studies.

$^b$ Darwent et al., 2003.

$^c$ McDonald et al., 2001; Garthwaite et al., 2003.

$^d$ Mannitol was used as the osmoticum (Steudle and Jeschke, 1983).
(Table 1). Since these parameters were used to calculate \( L_p \), statistical analysis was not performed on the data.

For these experiments the external medium was flowing around the roots at rates between 0.3 and 0.5 m s\(^{-1}\). This caused a vigorous stirring of the internal solution as discussed in an earlier paper (Ye and Steudle, 2005). Hence, the thickness of unstimulated layers was estimated to be no bigger than around 50 \( \mu \)m. Assuming a slab geometry of the unstimulated layer, this refers to a half-time of complete exchange of osmylates of \( T_{2/3}=0.5 \) s; calculated as: \( T_{2/3}=0.281 \times (5 \times 10^{-3})^2/(1.5 \times 10^{-6}) \); where \( D_v \) of NaCl=1.5\( \times 10^{-9} \) m\(^2\) s\(^{-1}\) (Jost, 1960; Ye and Steudle, 2005). This was certainly not rate-limiting at half-times of water exchange of the root of 102–152 s during the osmotic experiments (Table 1).

It may be argued that the diffusion of solutes within the root apoplast, from the root surface to the exodermis and endodermis, and the diffusion in the stele (from the endodermis) to mature vessels, were contributing to the overall osmotic \( T_{2/3} \). This may be true. However, model calculations of Steudle and Frensch (1989) for roots of \( Z \) mays have shown that the effects are rather small (<10\%). Moreover, the contributions of different internal pathways is equally interesting, depending on the type of experiments. If there are, for example, exchanges of water between the cell-to-cell and apoplastic path, this should affect the absolute value of \( L_p \), i.e. the apoplastic passage will be reflected in the overall measured parameter \( T_{2/3} \) besides the cell-to-cell component (see Discussion). The same is true for the measured values of \( P_{sr} \) and reflection coefficients. During the measurement of these latter parameters, effects of unstimulated layers should have been even smaller because of the longer half-times of the exchange of solutes (for discussion of effects of unstirred layers, the reader is referred to Steudle and Frensch, 1989; Ye and Steudle, 2005).

### Hydraulic conductivity of whole root systems: measurements of root exudation in the presence of an osmotic or a hydrostatic pressure gradient

Hydrostatic exudation (exudation in the presence of a hydrostatic pressure gradient) for the whole root system was significantly higher in \( T. \) aestivum than in \( H. \) marinum (\( P<0.05 \); Table 2). Hydrostatic conductivity of \( H. \) marinum was \( 0.8 \times 10^{-8} \) m s\(^{-1}\) MPa\(^{-1} \) in both the aerated and stagnantly treated plants. In \( T. \) aestivum, hydrostatic exudation was \( 2.6 \times 10^{-8} \) m s\(^{-1}\) MPa\(^{-1} \) in the plants raised in aerated solution, but was markedly higher at \( 6.4 \times 10^{-8} \) m s\(^{-1}\) MPa\(^{-1} \) in those from the stagnant solution.

Osmotic exudation (root exudation in the presence of an osmotic pressure gradient) for the whole root system did not differ significantly between \( T. \) aestivum and \( H. \) marinum, or for the plants from either root-zone \( O_2 \) treatment; values of the osmotic water permeability ranged from \( 0.8–1.9 \times 10^{-8} \) m s\(^{-1}\) MPa\(^{-1} \) (Table 2).

The ratio of hydrostatic/osmotic exudation for the whole root system was 1.0 for \( H. \) marinum grown in either aerated or stagnant nutrient solution. The same ratio was 2.4 for \( T. \) aestivum in the aerated solution, and it was 3.4 for plants in the stagnant solution.

### Surface area of the whole root system

In the aerated nutrient solution, whole (seminal and adventitious) root surface area was 2.6-fold higher in \( T. \) aestivum compared with \( H. \) marinum (Table 2). In the

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**Table 2.** (A) Hydraulic conductivity of whole root systems (\( L_p \)) for Hordeum marinum and Triticum aestivum grown in aerated or stagnant, deoxygenated nutrient solution for 21–28 d, and then measured in aerated solution; (B) shows, for comparison, values obtained for root system area and \( L_p \), published for other species (references provided in the table).

The values presented are means \( \pm SE \) (\( n=6 \)), where each replicate represents an individual plant. For \( L_p \) values, different letters within the same column indicate significant differences at \( P<0.05 \), for the species×root-zone \( O_2 \) treatment interaction (data from this study only); n.d., not determined.

<table>
<thead>
<tr>
<th>Species</th>
<th>Root ( O_2 ) treatment</th>
<th>Barrier to ROL</th>
<th>Root surface area (10(^{-2}) m(^2))</th>
<th>( L_p )</th>
<th>Hydrostatic (( P_{sr} )) (10(^{-8}) m s(^{-1}) MPa(^{-1}))</th>
<th>Osmotic (( P\Delta \pi \sigma_{ud} )) (10(^{-8}) m s(^{-1}) MPa(^{-1}))</th>
<th>Ratio of hydrostatic/osmotic</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(A) H. marinum</strong></td>
<td>Aerated</td>
<td>No</td>
<td>5±0.06 a</td>
<td>0.8±0.1 a</td>
<td>0.8±0.4 a</td>
<td>1.0</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stagnant Yes</td>
<td>9±0.13 ab</td>
<td>0.8±0.2 a</td>
<td>0.8±0.2 a</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T. aestivum</strong></td>
<td>Aerated</td>
<td>No</td>
<td>13±0.12 b</td>
<td>2.6±0.5 b</td>
<td>1.1±0.5 a</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stagnant No</td>
<td>6±0.09 a</td>
<td>6.4±1.41 c</td>
<td>1.9±0.6 a</td>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(B) Oryza sativa</strong></td>
<td>A aerated</td>
<td>n.d.(^a)</td>
<td></td>
<td>5.9</td>
<td>4.8</td>
<td>1.2</td>
<td>Miyamoto et al. (2001)</td>
<td></td>
</tr>
<tr>
<td>cv. ‘Azucena’</td>
<td>A aerated</td>
<td>n.d.(^a)</td>
<td></td>
<td>3.4</td>
<td>2.7</td>
<td>1.2</td>
<td>Ranathunge et al. (2003)</td>
<td></td>
</tr>
<tr>
<td>cv. ‘IR46’</td>
<td>A aerated</td>
<td>n.d.(^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Zea mays</strong></td>
<td>A aerated</td>
<td>No(^b)</td>
<td>1.2</td>
<td>26</td>
<td>4.6</td>
<td>5.6</td>
<td>Zimmermann and Steudle (1998)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) O. sativa forms a barrier to ROL (Armstrong, 1971; Colmer, 2003a), however in almost all cultivars that have been studied the barrier to ROL is induced by growth in stagnant, deoxygenated root conditions (Colmer et al., 1998; Colmer, 2003a) and so is unlikely to have been formed in the plants used in these studies.

\(^b\) Darwent et al., 2003.

\(^c\) Osmotic \( L_p \) value given for a \( \Delta \pi \) range of 0.02–0.06 MPa.
Discussion

This study demonstrates that formation of a barrier to radial O_2 loss (ROL) in adventitious roots of *H. marinum* does not significantly affect root hydraulic conductivity. The barrier to ROL was induced in adventitious roots of *H. marinum* by growth in stagnant solution (Fig. 1); however, measurements of root hydraulic conductivity for both individual adventitious roots and whole root systems showed no difference between plants from aerated or stagnant treatments; i.e. with or without the barrier to ROL (Tables 1, 2). Thus, hypodermal structures putatively associated with the barrier to ROL may not necessarily impede other root functions, such as water uptake. Thus, although such trade-offs for root functioning had previously been hypothesized (reviewed by Colmer, 2003b), the few data available do not support this notion, although studies of root functioning of a wider range of wetland species are needed to evaluate this hypothesis fully.

For *H. marinum* and *T. aestivum*, the hydraulic conductivity for individual adventitious roots was on average, 4.5- and 7.2-fold higher, respectively, when measured using a hydrostatic pressure gradient compared with an osmotic gradient. Since effects of unstirred layers could be excluded (see Results), this indicates a porous apoplastic bypass (Steudle and Peterson, 1998). Root pressure probe studies using *Zea mays* (maize) (Steudle et al., 1987; Steudle and Frensch, 1989) and *Oryza sativa* (rice) (Ranathunge et al., 2003) have given similar ratios between hydraulic conductivity measured using hydrostatic- or osmotically-induced water flows (Table 1; Steudle et al., 1987; Steudle and Frensch, 1989). This phenomenon has also been reported for other herbaceous plants (Table 1) and has been interpreted using the composite transport model (Steudle and Peterson, 1998); i.e. that in the absence of a hydrostatic pressure gradient, the apoplastic path is inefficient due to its very low reflection coefficient (σ). As such, water will flow predominantly via the protoplastic (cell-to-cell) path in response to an osmotic driving force.

That root hydraulic conductivity was not reduced by the presence of a putative apoplastic barrier (the barrier to ROL), might indicate that the cell-to-cell pathway is the dominant pathway of water uptake in *H. marinum* as already suggested for *Hordeum distichon* (syn. *Hordeum vulgare*) by Steudle and Jeschke (1983). However, work on *O. sativa* (Ranathunge et al., 2004) indicates that the apoplast is the dominant pathway for water uptake in the external cell layers, even in the presence of Caspian bands and suberin lamellae in the hypodermis/exodermis. Moreover, Schreiber et al. (2005) showed that, although the external cell layers of *O. sativa* roots contained significantly more suberin (per tissue surface area) than those of *Z. mays*, this did not contribute to the lower overall hydraulic conductivity of *O. sativa* roots compared with those of *Z. mays* (Ranathunge et al., 2003), leaving the authors to suggest that suberin deposits in root cell walls do not necessarily equate to reduced water and ion transport.

Further evidence of a dominating apoplastic pathway for water flow in the external cell layers of roots of *O. sativa*, was revealed (Ranathunge et al., 2004) by a comparison of diffusional and osmotic (bulk) H_2O permeability. The diffusional H_2O permeability of the external cell layers of *O. sativa* roots was 600–1400 times lower than the osmotic H_2O permeability. The authors suggest that a high bulk versus diffusional H_2O flow in roots of *O. sativa* could enable water uptake (via bulk H_2O permeability), even when the external cell layers show low diffusional permeability to O_2 (although O_2 diffusion was not measured by Ranathunge et al., 2004). In the case of radial O_2 diffusion across the outer part of the roots, consumption of O_2 in respiration by the cells across the diffusion path would further diminish O_2 flux from the exterior of the root (cf. Armstrong et al., 2000). Nevertheless, diffusional H_2O permeabilities for *O. sativa* were 3-fold larger than the osmotic H_2O permeability between the base and tip of the roots of *O. sativa* (Ranathunge et al., 2004). However, since the plants used by Ranathunge et al. (2004) were not grown in stagnant conditions, a ‘tight’ barrier to ROL might not have been induced (Colmer et al., 1998; Colmer, 2003a) and actual differences in diffusional H_2O permeability between the base and tip of the roots of *O. sativa* might be significantly larger when the barrier to ROL is fully induced. By analogy with *O. sativa*, water flow in roots of *H. marinum* could also occur via both the apoplastic and cell-to-cell pathways, despite the presence of putative apoplastic barriers.

The absolute values of individual root hydraulic conductivity of *H. marinum* and *T. aestivum* (present study) are comparable to those measured for *O. sativa* (Miyamoto et al., 2001; Ranathunge et al., 2003). However, Miyamoto et al. (2001) considered the value for *O. sativa* to be ‘low’ compared with roots of *Z. mays*, and this suggestion has been taken up by others (Ranathunge et al., 2003; Schreiber et al., 2005). When measured using a hydrostatic pressure gradient, the root hydraulic conductivity of *O. sativa* (Miyamoto et al., 2001; Ranathunge et al., 2003) was between 16–45% of values for *Z. mays* (Steudle et al., 1987, 1993; Steudle and Frensch, 1989) (Table 1). However, when compared with values of root hydraulic conductivity for other herbaceous species, including *H. distichon* (syn. *H. vulgare*), *Phaseolus coccineus*, and *Allium cepa* (Table 1), the values of hydraulic conductivity...
presented here for *H. marinum* and *T. aestivum* (and previous measurements of *O. sativa*) are not particularly low. Similarly, when root hydraulic conductivity was measured using an osmotic pressure gradient, the values for *H. marinum* and *T. aestivum* were also comparable to those of the species listed above (Table 1). Thus, root hydraulic conductivity of *Z. mays* might be considered to be at a higher end of this range for herbaceous species, and that for *O. sativa* does not appear to be abnormally low.

Whole root hydraulic conductivity of *T. aestivum* measured in the present study was 5.9-fold higher than in an earlier study (Gallardo et al., 1996). In the earlier study, hydraulic conductivity of the whole root system of 30–36-d-old *T. aestivum* growing in pots of soil was measured under a hydrostatic pressure gradient (Gallardo et al., 1996). The lower hydraulic conductivity of roots measured by Gallardo et al. (1996) compared with the present study presumably reflects, to a large degree, growth in soil compared with in hydroponics (cf. *Z. mays*, Zimmermann and Steudle, 1998).

The hydraulic conductivity measured in this study for individual roots of *H. marinum* and *T. aestivum* were generally higher than those measured for the whole root systems. In the case of *H. marinum*, the individual adventitious root hydraulic conductivities were 6.8- and 1.5-fold higher than those measured for the whole root systems, using hydrostatic or osmotic pressure gradients, respectively. Large differences between individual roots and whole root systems have not been observed in other species, i.e. *O. sativa* (values were essentially the same) (Miyamoto et al., 2001; Ranathunge et al., 2003) or *Z. mays*; individual roots 32% (hydrostatic gradients) to 58% (osmotic gradients) lower than whole systems (Steudle et al., 1987, 1993; Steudle and Frensch, 1989; Zimmermann and Steudle, 1998). This could be attributed to differences in root and shoot development between the plants of *O. sativa* and *Z. mays* used in the above studies, compared with the *H. marinum* and *T. aestivum* used in the present study. For example, the seedlings of *Z. mays* had small, simple root systems by comparison with the large seminal and adventitious root systems of *H. marinum* and *T. aestivum*. Furthermore, in *O. sativa* the adventitious root system dominates (only one seminal root is formed). Consequently, the individual root measurements made on *O. sativa* and *Z. mays* would better represent ‘whole’ root system measurements, than in the cases of *H. marinum* and *T. aestivum*. Moreover with *H. marinum* and *T. aestivum* having significantly more tillers (and adventitious roots originating from various tillers, resulting in flow pathways between vascular bundles of the tillers and main stem), the whole root system measurements will include any resistances to water movement between the tillers and main stem. Collectively, these differences in root and shoot development could contribute to the disparity in measured values of hydraulic conductivity of single adventitious roots, and whole root systems, for *H. marinum* and *T. aestivum*.

Roots of both *H. marinum* and *T. aestivum* grown in stagnant solution had higher values of $P_{sr}$ (NaCl) compared with plants grown in aerated solution. $P_{sr}$ (NaCl) in the aerated solution was $1.5–1.6 \times 10^{-9}$ m s$^{-1}$ for both species, whereas in the stagnant solution this increased to $2.3–2.5 \times 10^{-9}$ m s$^{-1}$. Waterlogging can reduce the capacity of roots to ‘exclude’ Na$^+$ and Cl$^-$ (Barrett-Lennard, 2003). For example, in *T. aestivum* in 60 mol m$^{-3}$ NaCl, 7 d of root-zone O$_2$ deficiency imposed by flushing pots with N$_2$, increased Na$^+$ and Cl$^-$ concentrations in leaves by 240–330% (Barrett-Lennard, 2003). This is thought to occur via two processes (Barrett-Lennard, 2003), (i) loss of membrane integrity such that transpiration results in the uptake of ions by mass flow, and (ii) waterlogging having more specific effects on ion influx and efflux, mediated by deficits of energy (ATP). In the present experiments, $P_{sr}$ (NaCl) was measured in aerated solution, so the higher values for roots previously in stagnant solution suggests that membrane integrity of at least a portion of the root might have been compromised (e.g. cells most distant from the source of O$_2$, such as the stele and root tips; Colmer and Greenway, 2005), and this did not recover within the first few hours following return to aeration. Furthermore, re-aeration following O$_2$-deprivation can result in damage to membranes if reactive oxygen species (ROS) accumulate (Blokhina et al., 2003).

In the present study, plants were grown in stagnant, deoxygenated nutrient solution for up to 28 d prior to measurement of hydraulic conductivity in aerobic conditions. This enabled it to be determined whether or not induction of the barrier to ROL impacts on root hydraulic conductivity, while avoiding the possible direct influence of O$_2$ deficiency on water uptake by roots. Short-term hypoxia, or anoxia, cause declines in root hydraulic conductivity, as shown for *Z. mays* (Birner and Steudle, 1993; Gibbs et al., 1998), *Musa* ssp. (banana) (Aguilar et al., 2003) and tomato (Jackson et al., 1996), although the effects may be transient (Jackson et al., 1996; Gibbs et al., 1998), indicating that the long-term effects of flooding on water uptake (see review by Kozlowski, 1984) may be an indirect, rather than a direct, effect of O$_2$ deficiency (Gibbs et al., 1998). However, results presented here indicate, at least for *H. marinum*, that the formation of a barrier to ROL in the basal regions of adventitious roots, induced by growth in stagnant conditions, does not significantly affect root hydraulic conductivity.

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