Root Growth and Oxygen Relations at Low Water Potentials. Impact of Oxygen Availability in Polyethylene Glycol Solutions

Paul E. Verslues, Eric S. Ober, and Robert E. Sharp*

Department of Agronomy, Plant Science Unit, 1-87 Agriculture Building, University of Missouri, Columbia, Missouri 65211

Polyethylene glycol (PEG), which is often used to impose low water potentials ($\psi_w$) in solution culture, decreases O$_2$ movement by increasing solution viscosity. We investigated whether this property causes O$_2$ deficiency that affects the elongation or metabolism of maize (Zea mays L.) primary roots. Seedlings grown in vigorously aerated PEG solutions at ambient solution O$_2$ partial pressure ($pO_2$) had decreased steady-state root elongation rates, increased root-tip alanine concentrations, and decreased root-tip proline concentrations relative to seedlings grown in PEG solutions of above-ambient $pO_2$. (alanine and proline accumulation are responses to hypoxia and low $\psi_w$, respectively). Measurements of root $pO_2$ were made using an O$_2$ microsensor to ensure that increased solution $pO_2$ did not increase root $pO_2$ above physiological levels. In oxygenated PEG solutions that gave maximal root elongation rates, root $pO_2$ was similar to or less than (depending on depth in the tissue) $pO_2$ of roots growing in vermiculite at the same $\psi_w$. Even without PEG, high solution $pO_2$ was necessary to raise root $pO_2$ to the levels found in vermiculite-grown roots. Vermiculite was used for comparison because it has large air spaces that allow free movement of O$_2$ to the root surface. The results show that supplemental oxygenation is required to avoid hypoxia in PEG solutions. Also, the data suggest that the O$_2$ demand of the root elongation zone may be greater at low relative to high $\psi_w$, compounding the effect of PEG on O$_2$ supply. Under O$_2$-sufficient conditions root elongation was substantially less sensitive to the low $\psi_w$ imposed by PEG than that imposed by dry vermiculite.

In studies of plant responses to water deficit, low $\psi_w$ is often imposed by decreasing the supply of water in the soil or other solid media in which the plants are grown. Our previous studies of maize (Zea mays L.) primary root growth at low $\psi_w$ were conducted by transplanting seedlings to vermiculite containing limited amounts of water (e.g. Sharp et al., 1988). However, in certain types of experiments there are advantages to imposing low $\psi_w$ using osmotica in solution culture, e.g. when radiolabeled compounds must be supplied to the roots in a controlled manner. Despite its convenience, a liquid medium could potentially complicate the results because terrestrial plants such as maize do not normally grow in an environment in which the roots are surrounded by water. Solution culture has been used extensively at both high and low $\psi_w$, but there have been few attempts to verify that plants grown under such conditions are physiologically similar to those grown in solid media.

When studying the behavior of roots at low $\psi_w$ in solution culture, two factors are centrally important: the osmotica used and aeration of the solution. It is desirable to use a compound that does not interact with plants in any way other than lowering the $\psi_w$ of the medium. Thus, slowly penetrating osmotica such as mannitol or sorbitol (Hohl and Schopfer, 1991) or inorganic salts (Termaat and Munns, 1986) are not ideal, especially for experiments extending beyond a few hours. Polymers of PEG have been used for many years, principally because PEG molecules with a $M_w \geq 6000$ cannot penetrate the cell wall pores (Carpita et al., 1979). Because PEG does not enter the apoplast, water is withdrawn not only from the cell but also from the cell wall. Therefore, PEG solutions mimic dry soil more closely than solutions of low-$M_w$ osmotica, which infiltrate the cell wall with solute. Although some studies indicate that PEG could contain toxic contaminants that inhibit plant growth (e.g. Plaut and Federman, 1985), other studies have found that deleterious effects occurred only if PEG entered the tissue; for instance, if roots were damaged (Lawlor, 1970).

A potential disadvantage is that the high viscosity of PEG solutions limits the movement of O$_2$, thereby increasing the likelihood of root O$_2$ deficiency. Even in pure water, O$_2$ transport to the root surface is limited by its low mobility ($10^4$ times less than that in air [Nye and Tinker, 1977]) and by the presence of an unstirred boundary layer at the root surface (Drew, 1990). Outside of the boundary layer O$_2$ is carried largely by bulk movement of the solution, but within the layer molecular diffusion is the dominant transport component. The thickness of the boundary layer is determined by several factors, including the degree of stirring and solution viscosity. Therefore, viscous solutions of

---

1 Supported by National Science Foundation grant no. IBN-9306935 to R.E.S. and E.S.O. P.E.V. was supported by a fellowship from the University of Missouri Maize Biology Training Program, a unit of the Department of Energy/National Science Foundation/U.S. Department of Agriculture Collaborative Research in Plant Biology Program (grant no. BIR-9420688). This is journal series no. 12,710 from the Missouri Agricultural Experiment Station.

* Corresponding author; e-mail robert_e_sharp@muccmail.missouri.edu; fax 1–573–882–1469.

---

Abbreviations: $pO_2$, O$_2$ partial pressure(s); $\psi_w$, water potential(s).
PEG tend to diminish the contribution of mass transport and increase the importance of diffusion to overall O₂ transport. Based on these principles and on measurements of O₂ transport in PEG solutions, Mexal et al. (1975) warned that roots growing in stirred, air-saturated solutions of PEG (M<sub>r</sub> ≈ 4000 and ψ<sub>w</sub> ≤ −0.7 MPa) could be severely O₂ limited. However, despite large numbers of studies using PEG, to our knowledge the effects of PEG on root O₂ status have never been quantified.

Our objectives were to determine whether O₂ deficiency limits growth or alters the metabolism of maize primary roots growing at low ψ<sub>w</sub> in PEG solutions, and, if O₂ is limiting, to determine the conditions necessary to ensure adequate oxygenation. To address these questions we used three approaches. First, the effects of elevated solution pO₂ on root elongation were measured at various ψ<sub>w</sub>. Second, in the same experiments we measured the root-tip concentrations of two metabolites: Ala, which accumulates under O₂ deficiency (Ricard et al., 1994), and Pro, which accumulates at low ψ<sub>w</sub> (Stewart and Hanson, 1980). Third, we directly measured tissue pO₂ in the tips of intact, growing roots using an O₂ microsensor (Ober and Sharp, 1996) to quantify the effects of PEG and supplemental O₂ on root O₂ status. The results establish that above-ambient solution pO₂ is required to avoid alterations in root growth and metabolism caused by low O₂ availability in PEG solutions.

**MATERIALS AND METHODS**

**Plant Culture Conditions and Root Elongation Measurements**

Seedlings were grown in the dark in a chamber maintained at 29°C and near-saturation humidity; when necessary, illumination was provided by a dim-green safelight (Saab et al., 1990). Kernels of maize (Zea mays L. cv FR27 × FMRo17) were germinated for 40 h in moist vermiculite. Seedlings with primary roots 20 to 25 mm long were then transferred to solution (5 mM Mes, 0.5 mM CaSO<sub>4</sub>, 6 μM H<sub>3</sub>BO<sub>4</sub>, adjusted to pH 6.0 with NaOH); root elongation rates were nearly identical to those in a complete nutrient solution. The solution was contained in Plexiglas boxes that were 20 cm long, 1.2 cm wide, and 18 or 25 cm tall. The taller boxes were used to accommodate greater root elongation. Twenty seedlings were arranged on a Plexiglas holder at the top of the box so that the caryopses were suspended above the solution. A Plexiglas cover enclosed the shoots. The primary roots grew downward through transparent root guides fashioned from plastic drinking straws (i.d. 6 mm), which facilitated measurements of root elongation rate. The solution was vigorously aerated through a perforated plastic tube extending along the bottom of the box. O₂ and air were mixed in various proportions before entering the box to give a solution pO₂ of 20.4 to 67 kPa. In most experiments one of three solution pO₂ treatments was used: 20.4 kPa (ambient), 28 ± 2.4 kPa, or 43 ± 4.3 kPa (means ± sd). The total flow rate was always 1100 mL min<sup>−1</sup>, and solution pO₂, measured with an O₂ probe (ISO2, World Precision Instruments, Sarasota, FL), was constant throughout each experiment.

The root guides were perforated with holes (diameter approximately 0.5 mm) large enough to allow exchange of solution, yet small enough to prevent most roots from growing through them. Preliminary experiments showed that the guides minimally affected root elongation at high ψ<sub>w</sub>, but substantially increased root elongation in PEG (M<sub>r</sub> 8000; Sigma) solution at a ψ<sub>w</sub> of −0.8 MPa (Fig. 1). This beneficial effect may be explained by prevention of damage to the roots from the vigorous aeration. Lawlor (1970) reported that root damage caused PEG uptake and growth inhibition. Likewise, in our experiments PEG could have entered roots that were grown without guides. To check that the guides did not excessively hinder solution mixing, food-coloring dye was injected into the PEG solution (−1.6 MPa) in one of the guides. The dye dispersed evenly throughout the box and within the guides in less than 1 min. Root elongation rates were quantified by marking the positions of the root apices on the side of the box at various times. In the preliminary experiments without guides, seedlings were periodically removed (without replacement) from the box to measure root length.

In all treatments, seedlings were grown without PEG for the first 2 h after transfer to solution culture (ψ<sub>w</sub> = −0.02 MPa). Imposition of low ψ<sub>w</sub> was then begun by pumping a solution of PEG (dissolved in growth solution) into the bottom of the box. Aeration mixed the contents and the excess solution drained from a tube near the top of the box. Solutions having ψ<sub>w</sub> of −0.3, −0.8, or −1.6 MPa were used. The rate of ψ<sub>w</sub> decline for each treatment was adjusted so that the final ψ<sub>w</sub> was reached 8 h after imposition of low ψ<sub>w</sub>.

**Figure 1.** Effect of root guides on increase of primary root length in vigorously aerated solutions at ψ<sub>w</sub> of −0.02 (no PEG) and −0.8 MPa (imposed by PEG). All treatments were at ambient (20.4 kPa) solution pO₂. Without the guides, marking the positions of the root apex was only possible at the first four time points. Data for the last two time points were obtained by destructively harvesting a portion of the roots in the box. Data points are means ± sd (n = 20–40) combined from two experiments. Error bars are not shown where they are smaller than the symbols.
was begun (unless otherwise noted). To convert PEG concentrations to $\psi_w$, the $\psi_w$ of a series of PEG solutions were measured using isopiestic thermocouple psychrometry (Boyer and Knippling, 1965). A time course of $\psi_w$ decline was then calculated for each $\psi_w$ treatment; measurements of samples of the growth media that were withdrawn periodically from the box confirmed the accuracy of the predicted $\psi_w$.

Experiments to test the effect of supplemental O$_2$ on roots growing in vermiculite were conducted in Plexiglas boxes as described previously (Sharp et al., 1988). Humidified mixtures of air and O$_2$ passed through a perforated tube along the bottom of the box and into the vermiculite at a rate of 500 mL min$^{-1}$. The pO$_2$ within the vermiculite was monitored by inserting the O$_2$ probe so that its tip was at the same depth as the root apices. Various vermiculite $\psi_w$ were obtained by thorough mixing with different amounts of water (Sharp et al., 1988), and were measured by isopiestic psychrometry.

HPLC Analysis of Amino Acids

At the end of some experiments, the apical 10 mm of two to five roots growing at approximately the mean elongation rate for that particular treatment were collected in preweighed microcentrifuge tubes. The sampled region encompassed most or all of the root elongation zone, which extends 10 to 12 mm from the apex at high $\psi_w$ and is shortened at low $\psi_w$ in both solution culture and vermiculite (Sharp et al., 1988; E.S. Ober and R.E. Sharp, unpublished data). Samples were immediately frozen in liquid N$_2$ and stored at $-20^\circ$C. At the time of analysis, samples were weighed, freeze-dried, and reweighed to obtain the mass of water. Samples were then ground and weighed, freeze-dried, and reweighed to obtain the mass of water (Sharp et al., 1988), and were measured by isopiestic psychrometry.

O$_2$ Microsensor Measurements

Commercially available O$_2$ sensors are too large for direct measurement of pO$_2$ within root tissue, and previous studies using various types of bare O$_2$ microelectrodes have been problematic (for review, see Baumgärtl and Lubbers, 1983). Therefore, root pO$_2$ was measured with a newly developed Clark-type O$_2$ microsensor with a tip diameter of 1 to 5 $\upmu$m (Ober and Sharp, 1996). The microsensor was calibrated before and immediately after use using a two-point calibration method: first in N$_2$; then in either air-saturated water or air-saturated PEG ($\psi_w = -1.6$ MPa). If pre- and postmeasurement calibrations differed by more than 10% the data were discarded. The calibrations were linear from 0 to 100 kPa. Data were corrected for a small offset (approximately 3% of signal) that occurred in PEG solutions relative to water, perhaps as a result of an effect of osmotic pressure on the microsensor membrane. This correction did not affect interpretations or conclusions drawn from the data. Measurements of pO$_2$ in solution-cultured roots were made at various solution pO$_2$ and $\psi_w$ of $-0.02$ and $-1.6$ MPa in a small (30 mL) Plexiglas chamber. Conditions were identical to those described above for the root elongation measurements except that solutions were aerated in an adjacent chamber and pumped through the chamber housing the root at a rate of 3 mL min$^{-1}$. (During low-$\psi_w$ imposition the root chamber was aerated directly at the same ratio of gas-flow rate to chamber volume used in the growth experiments.) These conditions resulted in steady-state elongation rates identical to those obtained in the larger-volume root boxes and permitted vibration-free measurements to be made. Measurements of vermiculite-grown roots were made at ambient pO$_2$ and $\psi_w$ of $-0.02$ and $-1.6$ MPa in Plexiglas cylinders described by Spollen and Sharp (1991). Root lengths at the time of measurement were 80 to 100 mm.

The microsensor was attached to a micromanipulator (MO-203, Narishige Ltd., Tokyo, Japan) and impaled perpendicularly between 4 and 10 mm from the apex of vertically oriented primary roots elongating at approximately the mean rate for each treatment as observed in the growth experiments. No longitudinal gradients in pO$_2$ were observed along the root tip, so at each depth of impalement, measurements from all positions were averaged. The root and microsensor were viewed through a microscope mounted horizontally in front of the apparatus, and the depth of impalement was measured with the micromanipulator, subtracting any movement of the root itself as measured with an eyepiece reticle. Measurements were made across the outer 150 $\upmu$m of the roots; the maximum depth of impalement remained within the cortex in all treatments (based on micrographs of fresh cross-sections taken at 7 mm from the apex). Measurements were not made at greater depths to minimize tissue damage caused by the taper of the microsensor. Root pO$_2$ values at particular depths cannot be strictly compared between treatments because of differences in root diameter. Roots at low $\psi_w$, either in PEG solution with supplemental O$_2$ (but not at ambient pO$_2$) or in vermiculite, were thinner than roots at high $\psi_w$ in either medium (for a detailed analysis of the thinning response to low $\psi_w$ in vermiculite-grown roots, see Liang et al. [1997]).

Statistical Analysis

Steady-state root elongation rates and Ala and Pro concentrations were analyzed by analysis of variance using a
RESULTS

Effect of Elevated \( pO_2 \) on Root Elongation

Time-course measurements of root elongation rate after transfer to solution culture at ambient (20.4 kPa) and above-ambient (28 and 43 kPa) \( pO_2 \) showed that elevated solution \( pO_2 \) stimulated root elongation at high \( \psi_w \) (−0.02 MPa, no PEG used) as well as in PEG solutions at \( \psi_w \) of −0.3, −0.8, and −1.6 MPa (Fig. 2). In all treatments, root elongation rate was approximately 2 mm h\(^{-1}\) during the first 2 h after transfer to solution culture (before low-\( \psi_w \) imposition); steady-state rates were reached by 40 h after transfer and, except at −1.6 MPa, were greater than the initial rate. In the high-\( \psi_w \) treatment, roots reached maximum elongation rates sooner at elevated \( pO_2 \), indicating that during the first 30 h, \( O_2 \) supply limited root growth (Fig. 2A). The steady-state elongation rate was not significantly affected by \( pO_2 \), however (Fig. 3). After the addition of PEG in the three low-\( \psi_w \) treatments at ambient \( pO_2 \), root elongation rate first decreased and then recovered to varying extents (Fig. 2, B–D). When solution \( pO_2 \) was elevated, elongation increased more rapidly (Fig. 2, B–D) and steady-state elongation rates were significantly greater (Fig. 3). At all \( \psi_w \), steady-state elongation rates were not significantly different at solution \( pO_2 \) levels between 28 and 43 kPa (Fig. 3). To confirm that this range of solution \( pO_2 \) was optimal for root growth, elongation was examined when solution \( pO_2 \) was further elevated to 67 kPa. At \( \psi_w \) of −0.02, −0.3, and −0.8 MPa, root elongation at 67 kPa was inhibited relative to that at 43 kPa, both in terms of the steady-state elongation rate and the time required to reach steady state, and at −1.6 MPa there was no difference in elongation rate between 43 and 67 kPa (data not shown). Because the viscosity of the PEG solution increases with decreasing \( \psi_w \), one might expect that the greatest relative stimulation by \( O_2 \) (ratio of steady-state growth at 43 kPa to that at ambient \( pO_2 \)) would occur at −1.6 MPa. This was not the case; in fact, a slightly greater stimulation occurred at −0.8 MPa (Fig. 3, inset). This result indicates that at −1.6 MPa, the low \( \psi_w \) itself became the dominant limiting factor for root elongation.
Steady-state root elongation rates at \( \psi_w \) of 0.2 and 0.3 MPa were not significantly different at solution \( pO_2 \) of either 28 or 43 kPa (Fig. 3). This shows that elongation of solution-cultured roots can fully adapt to a \( \psi_w \) of 0.3 MPa if supplemental \( O_2 \) is supplied, and indicates that there were no toxic effects of PEG on root growth, at least at that concentration.

The growth data show that root elongation was \( O_2 \) limited in PEG solutions at ambient \( pO_2 \). To test whether supplemental \( O_2 \) could also stimulate root growth at low \( \psi_w \), in a solid medium, roots growing in vermiculite were supplied with an elevated \( pO_2 \) of 28 kPa. This treatment had a negligible effect on root growth at several \( \psi_w \) (data not shown). Thus, the stimulation of growth at elevated \( pO_2 \) in PEG was not a general feature of roots at low \( \psi_w \), and was probably attributable to alleviation of hypoxia.

**Amino Acid Measurements**

As an additional test for root hypoxia in PEG solutions without supplemental \( O_2 \), we measured the effects of solution \( pO_2 \) on root-tip Ala and Pro levels. Ala was measured because it often accumulates under hypoxic conditions (Thompson et al., 1966; Ricard et al., 1994; Xia and Roberts, 1994). Pro was measured because it generally increases in concentration in tissues at low \( \psi_w \); in the primary root tip of maize Pro accounts for as much as 50% of the osmotic adjustment (Voetberg and Sharp, 1991).

In PEG solution at a \( \psi_w \) of -1.6 MPa, Ala concentration was significantly higher at ambient solution \( pO_2 \) than at solution \( pO_2 \) of 28 or 43 kPa and was also significantly higher than at any other \( \psi_w \) (Fig. 4A). At higher \( \psi_w \), Ala concentration did not vary significantly as solution \( pO_2 \) decreased, suggesting that roots at -1.6 MPa were affected by \( O_2 \) limitation to a greater extent than roots at the other \( \psi_w \). To confirm that the trend in Ala accumulation observed at -1.6 MPa continued under more severe \( O_2 \) limitation, Ala was also measured at subambient solution \( pO_2 \) (12 kPa, achieved by mixing \( N_2 \) with the air flowing into the solution). The steady-state root elongation rate decreased to 0.22 mm h\(^{-1}\) and the Ala concentration increased further to 31.3 millimolal; which is similar to the values reported in maize roots exposed to \( pO_2 \) of 3 kPa for 4 h followed by 2.5 h of anoxia (Xia and Roberts, 1994).

To discern whether low \( O_2 \) availability also had an effect on metabolic changes that normally occur in response to low \( \psi_w \), changes in the level of Pro in the root tip were analyzed. In -1.6 MPa PEG, Pro concentration was significantly decreased at ambient solution \( pO_2 \) compared with solution \( pO_2 \) of 28 or 43 kPa (Fig. 4B). Levels of Gln, which may provide substrate for Pro synthesis (through conversion to Glu), were also decreased (data not shown). As with Ala, no significant differences in Pro concentration between different solution \( pO_2 \) levels were observed at higher \( \psi_w \). When the \( pO_2 \) of the -1.6-MPa solution was reduced to 12 kPa, Pro accumulation was further inhibited to 19.1 millimolal. The inhibition of Pro accumulation at lower \( pO_2 \) is consistent with the results of a previous study that...
showed that Pro accumulation in wilted turnip leaves was inhibited by O₂-limited conditions (Thompson et al., 1966).

The amino acid data show that roots in PEG at a ψₕ of -1.6 MPa and ambient solution pO₂ exhibited metabolic signs of O₂ deficiency in addition to inhibition of elongation. However, root elongation was more sensitive than Ala or Pro accumulation to low O₂ supply because only elongation was affected by ambient solution pO₂ at ψₕ of -0.3 and -0.8 MPa.

**Root pO₂**

To directly assess the effects of PEG and solution pO₂ on the O₂ status of the root elongation zone, pO₂ was measured across the outer 150 µm of the cortex with an O₂ microsensor in roots grown at -0.02 and -1.6 MPa. Measurement of root O₂ status was essential to ensure that supplemental O₂ did not increase tissue pO₂ above normal physiological levels. To aid in interpreting the data for solution-cultured roots, the pO₂ of roots growing in vermiculite at ambient pO₂ and the same ψₕ were measured for comparison. Vermiculite was used because the large air spaces allow much more rapid movement of O₂ to the root surface than is possible in liquid media. Given the lack of root-growth stimulation with supplemental O₂, as noted above, the pO₂ of vermiculite-grown roots appears to be optimal for growth and metabolism.

At high ψₕ (-0.02 MPa), root pO₂ was much lower in solution culture at ambient pO₂ (20.4 kPa) than in vermiculite (Fig. 5A). This difference was attributable to a lower root-surface pO₂, which was associated with a pO₂ gradient extending from the bulk solution through the boundary layer next to the root. When the solution pO₂ was increased by 6.4 kPa to 26.8 kPa, root-surface and internal pO₂ increased by approximately the same extent. Unexpectedly, when the solution pO₂ was further increased by 20.8 kPa to 47.6 kPa, root-surface and internal pO₂ increased by only 5 kPa, which gave values similar to those in vermiculite-grown roots. This result was associated with an increase in boundary-layer thickness from approximately 500 µm at solution pO₂ of 20.4 and 26.8 kPa to 1000 µm at solution pO₂ of 47.6 kPa (Fig. 5A). (Boundary-layer thickness was measured as the distance from the root surface at which pO₂ began to decrease from the bulk solution pO₂.)

The increase in boundary-layer thickness at high solution pO₂ was associated with an increased thickness of the mucilage layer. The mucilage was distinctly visible as a layer coating the surface of the root when viewed through the microscope, and the thickness of the layer could be measured with the eyepiece reticle. The thickness varied greatly with distance from the root apex and among different roots within each treatment, but was generally less than 400 µm at solution pO₂ of 20.4 and 26.8 kPa and in the range of 400 to 800 µm at solution pO₂ of 47.6 kPa. The importance of the mucilage layer for determining the thickness of the boundary layer is illustrated for the 26.8-kPa treatment by the dashed line in Figure 5A, which shows the effect of removing most of the mucilage from the measured side of a root. This resulted in a substantial decrease in boundary-layer thickness and, accordingly, an increase of approximately 3.5 kPa in root-surface pO₂.

An additional factor that may have contributed to the thicker boundary layer at solution pO₂ of 47.6 kPa was the

---

**Figure 5.** Effect of solution pO₂ on the pO₂ profile across the boundary layer and into roots at high ψₕ (-0.02 MPa) (A) and low ψₕ (-1.6 MPa, imposed by PEG) (B). The indicated bulk solution pO₂ are means from these specific experiments. Profiles obtained with roots grown in vermiculite at ambient pO₂ and the same ψₕ are also shown. Data were collected with a microsensor on a perpendicular approach to the root surface between 4 and 10 mm from the root apex. Values are means ± se (n = 3–10). The inset in B shows a comparison of the pO₂ profiles across the cortex of roots growing at ambient pO₂ in high- or low-ψₕ solution. The dashed line in A shows the effect of removing most of the mucilage (by gently sliding the root tip past a portion of the root guide) from the measured side of a root at a solution pO₂ of 26.8 kPa. These measurements were made at 4 mm from the apex, and the experiment was repeated with similar results.
presence of root hairs, which in this treatment were generally observed starting at 10 mm from the root apex. Root hairs were not observed in this region at lower solution pO₂. Root hairs increase the thickness of the boundary layer by decreasing fluid velocity near the root surface.

Within the roots at high ψw, the pO₂ decreased steeply from the surface to a depth of 100 μm, with a similar slope at all solution pO₂ and in vermiculite, and then exhibited little additional decrease from 100 to 150 μm. A similar plateau of pO₂ within the cortex of maize primary roots was reported by Armstrong et al. (1994).

At a ψw of −1.6 MPa, root-surface and internal pO₂ were again much lower in solution culture at ambient pO₂ than in vermiculite (Fig. 5B). However, root pO₂ at all measured depths were higher in the PEG solution than in the solution without PEG (Fig. 5B, inset). This result was unexpected because the roots in PEG exhibited signs of O₂ deficiency (Figs. 3 and 4). Also, when the PEG solution pO₂ was increased by 10.0 to 30.4 kPa, root-surface pO₂ increased only slightly and internal pO₂ were unaffected, even though this treatment increased root elongation rate and alleviated the metabolic signs of hypoxia. Thus, the root pO₂, at least across the cortex, was not a good indicator of O₂ limitations to growth and metabolism. The lack of effect of increasing solution pO₂ on root pO₂ was associated with a steeper decrease in pO₂ across the boundary layer and the outer region of the root, and probably reflected increasing O₂ influx and consumption as the O₂ supply increased. The gradient in pO₂ was even steeper when the solution pO₂ was further increased to 45.9 kPa, resulting in root-surface and internal pO₂ that were similar to, or still less than (depending on depth), those of roots in vermiculite. In contrast to the roots at high ψw, mucilage was usually barely detectable at all positions at low ψw, and was not observed in any of the roots studied at solution pO₂ of 45.9 kPa. Accordingly, there was no effect of increased O₂ supply on the thickness of the boundary layer, which was 300 to 400 μm. As expected, the viscosity of the PEG solution increased the thickness of the boundary layer, since at high ψw, the boundary layer was only around 200 μm thick after most of the mucilage was removed (Fig. 5A, dashed line).

Comparison of the results at high and low ψw (Fig. 5, A and B) shows that the pO₂ gradient across the boundary layer and outer region of the root was considerably steeper at low ψw and solution pO₂ of 45.9 kPa than at high ψw, at any solution pO₂. This resulted in a comparable decrease in pO₂ from the bulk solution to the root surface at high and low ψw despite the much greater thicknesses of the mucilage and boundary layers at high ψw. A possible explanation of these results is that the influx of O₂ and O₂ consumption were higher at low ψw (see "Discussion").

In summary, at both high and low ψw, raising the solution pO₂ to around 47 kPa increased the pO₂ in the root-elongation zone to values equal to or less than those of vermiculite-grown roots, and therefore did not oxygenate the tissues above normal physiological levels.

**Comparison of PEG with Vermiculite**

Because the vermiculite system for growing seedlings at low ψw has been used extensively in our laboratory, we compared the responses of vermiculite-grown roots with those obtained using PEG. To do this, steady-state elongation rates of O₂-sufficient roots (43 kPa) in PEG were plotted together with data from vermiculite-grown roots as a function of medium ψw (Fig. 6). The response of root elongation rate to decreasing ψw was strikingly different in vermiculite compared with PEG. At all ψw tested, elongation rate was less inhibited in PEG than in vermiculite. At −0.3 MPa, elongation rate decreased by roughly one-third in vermiculite but was not significantly affected in PEG. At −1.6 MPa, elongation rate was inhibited by 63% in PEG compared with 75% in vermiculite. Likewise, shoot growth was much less sensitive to low ψw in PEG than in vermiculite (data not shown). Consistent with the growth data, Pro concentration in the apical 1 cm of the primary root at −0.3 and −0.8 MPa PEG was considerably lower than that in vermiculite-grown roots at the same ψw (compare Fig. 4 with figure 1 of Voetberg and Sharp, 1991). Pro accumulation at −1.6 MPa, however, was similar in the two systems.

Because the rate of low-ψw imposition can affect Pro accumulation (Naidu et al., 1990) and could possibly affect steady-state root elongation, the time over which ψw was decreased in the PEG system was extended from 8 to 50 h to determine if this could account for the different responses of elongation rate to low ψw in PEG and vermiculite. A time of 50 h was chosen to exceed the time required by maize roots transplanted into dry vermiculite to reach steady-state root-tip osmotic potential (approximately 35 h; Sharp et al., 1990). Thus, low ψw was imposed with PEG over a period as long or longer than that required for roots to adapt to low ψw in the vermiculite system. The gradual

---

**Figure 6.** Response of root elongation rate to low ψw imposed by PEG or vermiculite. Steady-state elongation rates of roots grown in PEG at a pO₂ of 43 kPa (○, after low ψw was imposed over 8 h) are from Figure 3. The vermiculite response (dashed line) is from Sharp (1990), and has been consistently reproduced. Also shown is the steady-state elongation rate obtained after −1.6 MPa PEG was imposed over 50 h at a solution pO₂ of 43 kPa ( □, mean ± se, n = 22 combined from two experiments). Error bars are smaller than the symbols for all data points.
addition of PEG to attain −1.6 MPa over 50 h yielded a steady-state root elongation rate that was slightly higher than that obtained when the same \( \psi_w \) was reached after 8 h (Fig. 6). The concentration of Pro in the apical 1 cm of the root was the same after the two rates of low-\( \psi_w \) imposition \((83.1 \pm 4.8 \) mmol/l \([n = 3]\) after the slow rate versus \(82.5 \pm 3.5 \) mmol/l \([n = 5]\) after the rapid rate; means \( \pm \) se). Thus, the different responses of root elongation rate to low \( \psi_w \) in vermiculite and PEG were not explained by the rate at which low \( \psi_w \) was imposed.

We also investigated whether mannitol or melibiose, which are sometimes used to impose low \( \psi_w \) in solution culture, could reproduce the results obtained with PEG solutions. However, both mannitol (tested at −0.8 and −1.6 MPa) and melibiose (tested at −1.6 MPa) caused almost complete inhibition of root elongation by 50 h after transfer to solution culture (data not shown), and thus apparently had toxic effects.

**DISCUSSION**

Our results show that maize seedlings growing in PEG solutions at ambient \( pO_2 \) are \( O_2 \) deficient despite vigorous aeration. Because it proved necessary to grow roots in guides (presumably to prevent root damage and PEG uptake), it is not feasible to supply sufficient \( O_2 \) by increased solution mixing. Therefore, supplemental \( O_2 \) must be supplied to have confidence in experiments on responses to low \( \psi_w \) using PEG solutions.

**Assessment of Root \( O_2 \) Status**

At a \( \psi_w \) of −1.6 MPa and ambient solution \( pO_2 \), the roots exhibited signs of \( O_2 \) deficiency (decreased elongation rate, Ala accumulation, and decreased Pro accumulation) relative to roots grown with supplemental \( O_2 \). In view of this finding, it is paradoxical that at ambient solution \( pO_2 \) roots at low \( \psi_w \) had slightly greater \( pO_2 \) at all measured depths across the cortex than roots at high \( \psi_w \) (Fig. 5B, inset), which, in the longer term, did not exhibit signs of hypoxia. It could be that stelar \( pO_2 \), which was not measured, was in fact lower in the roots at low compared with high \( \psi_w \); this could have determined the root growth and metabolic responses regardless of cortical \( pO_2 \). Even at high \( \psi_w \) the roots appeared to be \( O_2 \) deficient early in the experiments, since their elongation rate was inhibited relative to that of roots at higher solution \( pO_2 \) during the first 30 h after transfer to solution culture. However, whereas root growth at high \( \psi_w \) acclimated to this condition, the roots at low \( \psi_w \) exhibited maximal elongation rates only at the higher tissue \( pO_2 \) levels that resulted from supplemental oxygenation. This suggests that the ability to acclimate to low tissue \( pO_2 \) may have been impaired at low \( \psi_w \).

At high \( \psi_w \), it was unexpected that a solution \( pO_2 \) as high as 47 kPa was required to raise cortical \( pO_2 \) levels to the levels of vermiculite-grown roots. It is important to note that because of the thick boundary layer at a solution \( pO_2 \) of 47 kPa, the root-surface \( pO_2 \) was similar to that in vermiculite. Thus, the root tips were exposed to the same local \( O_2 \) environment in the two conditions. Furthermore, the factors that contributed to the thickness of the boundary layer at high solution \( pO_2 \) (increased mucilage thickness and root hairs closer to the apex than at lower \( pO_2 \)) are not unique to this condition. First, extensive expansion of maize root mucilage can occur in soils at high \( \psi_w \) (Sealey et al., 1995), although, consistent with our PEG-grown roots, not at lower \( \psi_w \) (McCully and Boyer, 1997). Second, root hairs were observed in the same region in roots growing in vermiculite at high \( \psi_w \) as in roots at high solution \( pO_2 \). Arguably, therefore, even at high \( \psi_w \), the roots grown at a solution \( pO_2 \) of 47 kPa were physiologically more similar to roots grown in solid media than those grown at lower solution \( pO_2 \). Thus, supplemental oxygenation may be an important consideration in any solution-culture study in which normal oxygenation of root tissues is desired. Consistent with this suggestion, previous studies of maize roots at high \( \psi_w \) have reported that above-ambient solution \( pO_2 \) was needed for maximal \( O_2 \) consumption (Saglio et al., 1984; Atwell et al., 1985).

Taken together, our results confirm the view that bulk solution \( pO_2 \) is not a good indicator of root \( O_2 \) status (Drew, 1990), and, furthermore, a static measure of root \( pO_2 \) cannot provide unambiguous evaluation of sufficient oxygenation. Data on growth and metabolism are also required to assess \( O_2 \) status accurately.

**Low \( \psi_w \) May Increase Root \( O_2 \) Demand**

In addition to the expected effect of PEG viscosity on boundary-layer thickness, the results suggest that with adequate oxygenation the root elongation zone may have an increased demand for \( O_2 \) at low compared with high \( \psi_w \). This would necessitate an even higher solution \( pO_2 \) to provide adequate oxygenation than what could be predicted from consideration of \( O_2 \)-transport properties alone. Evidence for an increased influx of \( O_2 \) per unit surface area of the root tip at low relative to high \( \psi_w \), comes from the finding that the decrease in \( pO_2 \) from the bulk solution to the root surface at approximately 47 kPa was of similar magnitude at \( \psi_w \) of −0.02 and −1.6 MPa, despite the fact that the boundary layer was more than twice as thick at −0.02 MPa (Fig. 5, A and B). The greater thickness of the boundary layer at high \( \psi_w \), which was associated with a thick mucilage layer, presumably presented a greater overall resistance to \( O_2 \) transport from the bulk solution to the root surface than at low \( \psi_w \), under which mucilage was not observed. It is important to note that the diffusive resistance to \( O_2 \) movement within the boundary layer would have been minimally altered by the addition of PEG, since the diffusivity coefficient of \( O_2 \) is similar in water and PEG solutions (Mxal et al., 1975). Thus, to maintain the same decrease in \( pO_2 \) across the boundary layer, but with a lower resistance to \( O_2 \) movement, it seems likely that the \( O_2 \) flux was considerably greater at low compared with high \( \psi_w \). This could not be quantified from the \( pO_2 \) gradients, however, because exact knowledge of the extent of the unstirred boundary layer is required (Henriksen et al., 1992).

An increased \( O_2 \) flux into the roots at low \( \psi_w \), most likely reflects increased \( O_2 \) consumption. The \( pO_2 \) gradient from the root surface to the interior was also steeper at low than
at high $\psi_w$ both in O$_2$-sufficient roots in solution culture and in vermiculite-grown roots. This is consistent with a higher rate of O$_2$ flux and consumption at low $\psi_w$, although possible effects of low $\psi_w$ on root permeability to O$_2$ could also be involved. There are reports of increased O$_2$ consumption by water-stressed relative to well-watered roots of maize (root tips; Greenway, 1970) and Arnica alpina (whole root systems; Collier and Cummins, 1992). Increased respiration at low $\psi_w$ may provide energy for adaptive processes such as osmolyte synthesis for osmotic adjustment. Direct measurement of root respiration rates is required to confirm that this occurs in our system because we do not know what contribution shoot-supplied O$_2$ may have made to root O$_2$ consumption (Saglio et al., 1984; Armstrong et al., 1994). Treatment differences in O$_2$ flux from the solution into the root could have been in response to differences in O$_2$ supply from the shoot.

**Comparison of PEG and Vermiculite**

By investigating the O$_2$ requirements of roots growing in PEG solutions, our results allow a straightforward comparison between PEG solutions and other methods of imposing low $\psi_w$. Under O$_2$-sufficient conditions, root elongation was less sensitive to low $\psi_w$ imposed by PEG than by vermiculite at all $\psi_w$ tested (Fig. 6). This finding emphasizes that demonstration of similar growth rates in the two media at a given $\psi_w$ cannot be interpreted as evidence against hypoxia in PEG, but would in fact suggest the opposite.

It is not surprising that such different environments have different effects on root growth. In another example, Reinhardt and Rost (1995) found that primary roots of cotton seedlings responded differently to salinity stress depending on whether they were grown in solution culture or vermiculite. One major difference between PEG solutions and vermiculite is the hydraulic contact between the root and the medium. In solution culture, the entire surface of the root is in contact with the medium, whereas in vermiculite only a portion of the root surface contacts the vermiculite particles. All water uptake must then occur through these limited areas of contact, which increases the resistance to water flow into the root. Growth itself generates a $\psi_w$ gradient between the expanding tissue and its water source (Nonami and Boyer, 1993), and any increase in resistance to water flow will increase the $\psi_w$ gradient between the root and the medium. Preliminary measurements indicate that under the nontranspiring conditions used in this study, root tips in vermiculite at a $\psi_w$ of $-0.3$ MPa had a $\psi_w$ that was $0.27$ MPa lower than that of the vermiculite, whereas the mature region had equilibrated with the vermiculite. In $-0.3$ MPa PEG, in contrast, root-tip $\psi_w$ was nearly the same as solution $\psi_w$ indicating that only a very small $\psi_w$ gradient was needed to drive water uptake (P.E. Verslues and R.E. Sharp, unpublished data). Root-tip $\psi_w$ substantially lower than that of the surrounding media have also been observed in soil-grown plants (Sharp and Davies, 1979; Westgate and Boyer, 1985). In our system, these data indicate that the root tips of seedlings in vermiculite at a given $\psi_w$ are more “stressed” than those in PEG solution of the same $\psi_w$. The extent to which the different responses of root-tip $\psi_w$ explain the difference observed between PEG and vermiculite in the response of root elongation to the $\psi_w$ of the medium is not known. Other factors may also be involved; for instance, the diffusion of gases out of the root differs between the media. The concentrations of ethylene and CO$_2$ in particular, can affect root elongation (Radin and Loomis, 1969).

In conclusion, our results show that PEG solutions with supplemental oxygenation can be used to conduct experiments at low $\psi_w$ without the confounding effects of root O$_2$ deficiency. However, caution must be used in comparing results obtained using PEG with those obtained using other methods of imposing low $\psi_w$.

**ACKNOWLEDGMENTS**

We thank Dr. Gary Krause for assistance with the statistical analysis, Dr. David Rhodes (Purdue University, West Lafayette, IN) for advice concerning the amino acid analysis, Steven Wells for technical assistance in constructing the root boxes, and Drs. Tobias Baskin and Stephen Pallardy for constructive comments on the manuscript.

Received September 5, 1997; accepted December 18, 1997.


**LITERATURE CITED**


Boyer JS, Knippling EB (1965) Isopistichi technique for measuring leaf water potentials with a thermocouple psychrometer. Proc Natl Acad Sci USA 54: 1044–1051


