Growth Dynamics of Mechanically Impeded Lupin Roots: does Altered Morphology Induce Hypoxia?

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• *Background and Aims* Root axes elongate slowly and swell radially under mechanical impedance. However, temporal and spatial changes to impeded root apices have only been described qualitatively. This paper aims (*a*) to quantify morphological changes to root apices and (*b*) assess whether these changes pre-dispose young root tissues to hypoxia.

• *Methods* Lupin (*Lupinus angustifolius*) seedlings were grown into coarse sand that was pressurized through a diaphragm to generate mechanical impedance on growing root axes. *In situ* observations yielded growth rates and root response to hypoxia. Roots were then removed to assess morphology, cell lengths and local growth velocities. Oxygen uptake into excised segments was measured.

• *Key Results* An applied pressure of 15 kPa slowed root extension by 75 % after 10–20 h while the same axes thickened by about 50 %. The most terminal 2–3 mm of axes did not respond morphologically to impedance, in spite of the slower flux of cells out of this region. The basal boundary of root extension encroached to within 4 mm of the apex (cf. 10 mm in unimpeded roots), while radial swelling extended 10 mm behind the apex in impeded roots. Oxygen demand by segments of these short, thick, impeded roots was significantly different from segments of unimpeded roots when the zones of elongation in each treatment were compared. Specifically, impeded roots consumed O_2 faster and O_2 consumption was more likely to be O_2-limited over a substantial proportion of the elongation zone, making these roots more susceptible to O_2 deficit. Impeded roots used more O_2 per unit growth (measured as either unit of elongation or unit of volumetric expansion) than unimpeded roots. Extension of impeded roots *in situ* was O_2-limited at sub-atmospheric O_2 levels (21 % O_2), while unimpeded roots were only limited below 11 % O_2.

• *Conclusion*s The shift in the zone of extension towards the apex in impeded roots coincided with greater vulnerability to hypoxia even after soil was removed. Roots still encased in impeded soil are likely to suffer from marked O_2 deficits.

Key words: *Lupinus angustifolius*, lupin, mechanical impedance, hypoxia, root, apex, oxygen.

INTRODUCTION

The mechanism by which roots penetrate impeded matrices has been studied extensively since the early experiments of Pfeffer (1893) on maize and broad bean roots opposed by gypsum blocks. Early analyses of the forces that roots exert produced many estimates of axial pressures well above 1 MPa (Barley and Greacen, 1967; Taylor and Ratliff, 1969) but growth pressures in the range 0·5–0·6 MPa are more credible (Clark *et al.*, 2003). Radial pressures exerted by roots on the surrounding soil are also believed to be a critical feature in penetration of hard soils (Abdalla *et al.*, 1969; Richards and Greacen, 1986). In spite of an extensive literature on root–soil interactions (Cockroft *et al.*, 1969; Whiteley *et al.*, 1981; Bengough *et al.*, 1997), there is scant information on the biological processes that underpin adaptations to mechanical impedance. Eavis (1969) made measurements on roots of peas in hard soils, showing that cell production and expansion was perturbed in response to the matrix. Atwell and Newsome (1990), Clark *et al.* (1996) and Croser *et al.* (1999, 2000) further addressed the connection between water relations and the generation of root force but conclusive evidence as to what other cellular processes limit root growth in hard soils *in situ* is still not available. For

example, the allusions to O_2 limitations in impeded root axes in early studies (Gill and Miller, 1956; Eavis, 1972) lead to the hypothesis that root morphological changes might indirectly perturb ATP regeneration by inhibiting respiration. A further hypothesis that ethylene mediates growth of impeded roots is unresolved (Moss *et al.*, 1988; Sarquis *et al.*, 1991) but deserves attention particularly as ethylene-induced swelling could exacerbate the effects of hypoxia reported in this paper. Better knowledge of the precise location of radial swelling of root axes in relation to inhibition of elongation rates should offer clues as to the regulation of cell growth in impeded conditions.

Narrow-leaved lupin (*Lupinus angustifolius*) is a species that appears to penetrate hard soils with greater efficacy than most agricultural species tested (Materechera *et al.*, 1991). Lupin was chosen as a good candidate to study root expansion rates and O_2 demand in a mechanically impeded sand because it offers a substantial primary root axis (approx. 1 mm diameter) and grows quickly through hard soils. Materechera *et al.* (1991) ascribed this rapid growth to the capacity of lupin roots to swell radially. In the pressure cell used in the present study (similar to that used by Goss and Scott Russell, 1980), root growth was monitored closely as mechanical impedance was varied *in situ*, while roots

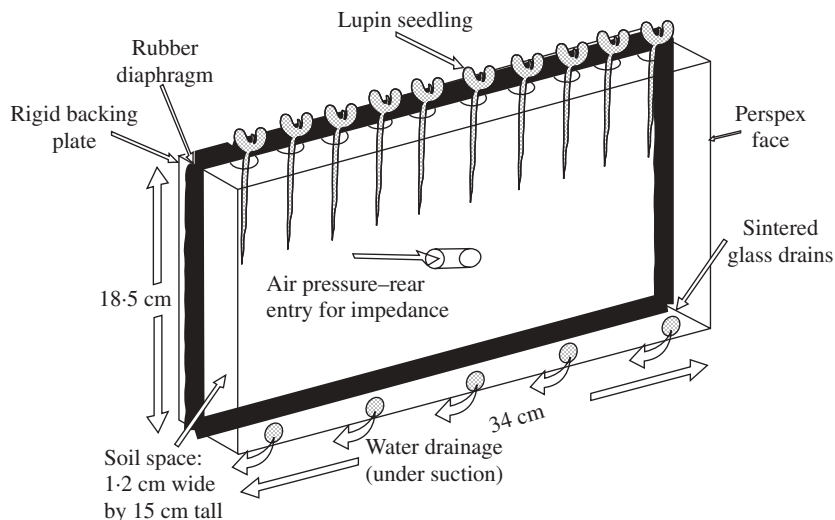* For correspondence. E-mail batwell@rna.bio.mq.edu.au

Fɪɢ. 1. The apparatus in which seedlings were grown and roots were pressurized. For further details of use, see Materials and Methods.

could be removed rapidly for other cell and physiological measurements. Quantifying dynamic properties such as respiration and water relations in impeded roots in a steady state has always been problematic (Atwell and Newsome, 1990; Croser *et al.*, 2000). In this study the compromise was to estimate O_2 uptake rates into excised root segments and infer that these reflected rates *in situ*. However, in the growth studies, O_2 was varied at will in the pressure chambers, allowing the response of root growth to hypoxia to be confirmed and the hypothesis tested that mechanical impedance leads directly to changes in morphology which inhibit respiration and growth of the root tip.

The aims were to measure (*a*) precise axial and radial growth rates of mechanically impeded roots; (*b*) the response of mechanically impeded roots to hypoxia; and (*c*) the effect of morphological changes induced by mechanical impedance on O_2 uptake characteristics by these roots.

MATERIALS AND METHODS

Growth conditions

Seeds of *Lupinus angustifolius* L. 'Danja' were rinsed in 2·5 % sodium hypochlorite solution for 5 min, followed by deionized water and placed on tissue dampened with 1 mM $CaSO_4$ to germinate in the dark at 20 °C. After radicle emergence 24 h later, seedlings were placed in the growth apparatus (Fig. 1). One emerging radicle was inserted into each ≈3-mm opening at the top of the diaphragm apparatus, sealed off with dampened cotton wool and allowed to grow down the layer of soil against the transparent Perspex face (inclined 15° to the vertical). There were ten plants per apparatus, which was kept in darkness at 20 ± 0·5 °C or illuminated during measurements with green light (wavelength 530 nm). Shoots were maintained at 100 % relative humidity. The cotyledons were retained by plants throughout the experiments with no sign of dehydration: only in

the longest experiments did first leaves emerge. No lateral roots emerged during experiments; roots were grown for <7 d in all cases.

The soil medium used was crushed quartz, sieved between 1·18 and 2·5 mm^2 mesh sizes. The soil medium was poured loosely into the apparatus and drained readily, thus maintaining large pores and a calculated Ψ of approx. −2 kPa near the bottom. Drainage was maintained by suction through sintered glass inlets (see Fig. 1). Prior to inserting seedlings, the apparatus was filled with nutrient solution and immediately allowed to drain freely. After this, 3 mL of nutrient solution was applied daily at the soil surface near each seedling. Nutrient solution pH was maintained within the range 6·3–6·5 during experiments. Observation of soil pores showed continuous air spaces adjacent to the Perspex face, except at the extreme bottom where water saturated the pore space; experiments were discontinued when roots approached the bottom of the apparatus.

Mechanical impedance

Mechanical impedance was applied to roots via the soil, by applying air pressure to a gas-tight rubber diaphragm at the back of the soil chamber (Fig. 1). Mechanical impedance in all experiments was achieved by an applied pressure of 15 kPa, while the controls had no applied pressure. Application of 15 kPa pressure did not produce any evidence of increased soil packing relative to the controls; pore sizes measured by Vernier microscope were similar across pressure treatments (diameter 0·40 ± 0·03 mm). The pressure was increased from 0 to 15 kPa over a period of at least 2 min.

Where possible, the diaphragm was not accompanied by a rigid backing plate in control treatments because this induced low levels of impedance. In cases where pressure was to be applied or relieved during the experiment, the backing plate had to be left in place during the control

period. Independent estimates showed that effects of this plate on root growth disappeared once roots were >30 mm long.

Gas supply

Air was flushed into the sand matrix through fine holes (0·5 mm diameter) in the Perspex wall of the apparatus (Fig. 1). Root extension rates were the same when humidified air was supplied at 60 mL min^{-1} or soil was left open to the atmosphere. Both techniques were employed in various experiments.

The shoots were contained in 340-mL boxes, each covering five plants. Air was supplied to the shoots by either venting the volume within the boxes to the atmosphere, while keeping each box humid with moistened cotton wool, or supplying pre-humidified air at a rate of 60 mL min^{-1}.

Root measurements

Root tip displacement was calculated using measurements of longitudinal (L_o) and lateral (L_a) displacement by a travelling Vernier microscope mounted parallel to the Perspex wall. Total displacement (d) was assumed to be in a straight line within any time interval, calculated using the Pythagoras Theorem thus:

$$d = \left[(L_o)^2 + (L_a)^2 \right]^{0.5}$$

Root extension rate (r) of the radicles was calculated from root tip displacement (d) and elapsed time (t):

$$r = d/t$$

Radii along extracted roots were measured under a light microscope with a calibrated eye-piece graticule and distance from the apex recorded.

Transverse sections of roots

Roots grown in impeded and loose soil were washed free of soil then fixed in 4 % paraformaldehyde (in 0·1 M phosphate buffer). Roots were then dehydrated in a graded ethanol series and embedded in LR White resin. Sections 1 μm thick were cut, stained with Toluidine Blue (0·1 % w/v) then mounted on slides for imaging. Sections were taken at prescribed displacements from the root apex as described in the legend to the light micrographs.

Root epidermal cell length measurement

Radicles grown as previously described were extracted from the soil and, within 5 min, washed with deionized water to remove adhering sand before being stained for 1 min with 1 % Toluidine Blue. Epidermal cell lengths were measured under a light microscope using a calibrated eye-piece graticule. Cells that had not yet emerged from underneath the root cap were observed by cutting longitudinal hand-sections through the apex and then stained for 1 min with 1 % Toluidine Blue. Each recorded cell length was a mean of 10–30 cells per root for those cells distal to the root cap and of four to eight cells per root for those underneath the root cap. The distance from the root apex (i.e. the farthest extremity of the root cap) was recorded.

Nine roots that had been extracted 3–6 d after germination were measured from each treatment. In this way, only cells formed after a uniform growth rate had been established were measured. The distribution of cell lengths along the mature axis did not vary significantly with time at this age and local root extension rates were approximately constant. This enabled calculation of the local velocity of growth (v_z) (Scott *et al.*, 1968; Silk and Erickson, 1979) where:

$$v_z = l_z (v_f / l_f)$$

where z is distance from the root apex (mm), l_z is epidermal cell length (mm) at z, v_f is the final measured longitudinal extension rate of the root (mm h^{-1}) and l_f is the mature (final) epidermal cell length (mm).

The ratio v_f/l_f is an expression of the rate of addition of cells to a cell file (cells h^{-1}). The local velocity of growth (v_z) was expressed as a function of the distance from the root apex.

Establishment of impedance conditions

Reduction in growth rate with pressure. Four diaphragm apparatuses were initially kept at 0 kPa and root extension rates recorded. Pressure (15 kPa) was applied to two of the apparatuses when roots were ≈30 mm deep into the soil (0 h), while no pressure was applied to the other apparatuses for the duration of the experiment. Root extension immediately following pressure application was monitored frequently. It was generally observed that roots responded to applied pressure more rapidly if they had first become established in unpressurized medium for 36 h. Thus this initial period of establishment was used in all experiments.

Recovery of growth rate. No pressure was applied to two diaphragm apparatuses and root extension rates were measured. Pressure (15 kPa) was applied to two of the apparatuses when roots were ≈30 mm deep into the matrix (termed 0 h) then replaced by 0 kPa after 47 h in one apparatus (recovery treatment). For the duration of the experiment, 15 kPa was maintained in the other apparatus (impeded treatment).

Oxygen measurements

Root growth and critical O_2 pressure of extension. The root environment was isolated from the shoots by sealing plants with a beeswax–lanolin mixture into the pressure apparatus at the root–shoot junction, thus allowing the gaseous environment of the roots and shoots to be varied independently. Plants were initially supplied with air to both shoot and root environments. Then, in separate experiments, the concentration of O_2 around the roots was altered, while the shoots were aerated and isolated from the root

environment. Altered O_2 treatments were applied with a gas stream at a rate of $60\,mL\,min^{-1}$ per apparatus and allowed to continue for at least 24 h. Root extension rates were measured in control plants supplied continuously with 21 % O_2 (air) and a range of other O_2 concentrations after extension rates had stabilized. Preliminary experiments not reported here showed that by 24 h the new O_2 regime was established, as indicated by stable root extension rates.

Concentrations of O_2 from 0–30 % in the soil atmosphere were used to determine that concentration at which root extension was first inhibited by O_2 deficits (termed the critical O_2 pressure of extension, or COP_e).

Oxygen consumption and critical O_2 pressure of respiration. Plants were grown as described previously. Roots subjected to 0 kPa and 15 kPa pressure were extracted from the soil and washed in deionized water to remove adhering sand.

Roots were immediately cut into sections of three separate series depending upon the experiment: series I, 0–2, 2–5, 5–10, 20–25 mm; series II, 0–1, 1–3, 3–5, 5–7, 20–25 mm; series III, 0–1, 1–2, 2–3, 3–4 mm. All measurements are quoted in millimetres from the root apex. Root sections from eight to ten plants were placed in 1·5 mL of nutrient solution in the cuvette of a Clark O_2 electrode. Nutrient solution was saturated with air prior to addition of the root segments. The cuvette was sealed and O_2 consumption was calculated from the rate of change in O_2 concentration in the cuvette. Rates of O_2 consumption by root tips were measured for 30–90 min during which maximal O_2 consumption rate was stable, suggesting no time-dependent loss of respiratory activity. The rate of stirring within the cuvette was raised to a point where O_2 consumption did not respond, thus minimizing boundary layer effects on O_2 uptake rates. Contents of the cuvette were maintained at 20 °C.

The sections closest to the root apex (i.e. 0–1 or 0–2 mm) were sometimes placed in nutrient solution equilibrated with an O_2 concentration greater than 0·284 mM (the O_2 concentration in equilibrium with air at 20 °C). Concentrations exceeding 0·35 mM often resulted in a decreased rate of O_2 consumption compared with rates observed up to 0·35 mM O_2. Plotting O_2 consumption rate against O_2 concentration in the cuvette shows a break of slope at the point where O_2 consumption becomes limited by O_2 diffusion to the tissue—this value was termed the critical O_2 pressure of respiration (COP_r). COP_r values and the maximal O_2 consumption were recorded.

Curve fitting

The experiments were carried out in four pressure chambers, each containing ten plants. Thus each observation was the result of ten replicate roots, except in cases such as Fig. 2 where 20 roots gave rise to each observation. Standard errors of means were quoted in the figures. Anatomical observations were made on four replicate roots of which one representative example is shown. Logistic curves were fitted to the root local velocity (v_z), O_2 consumption and
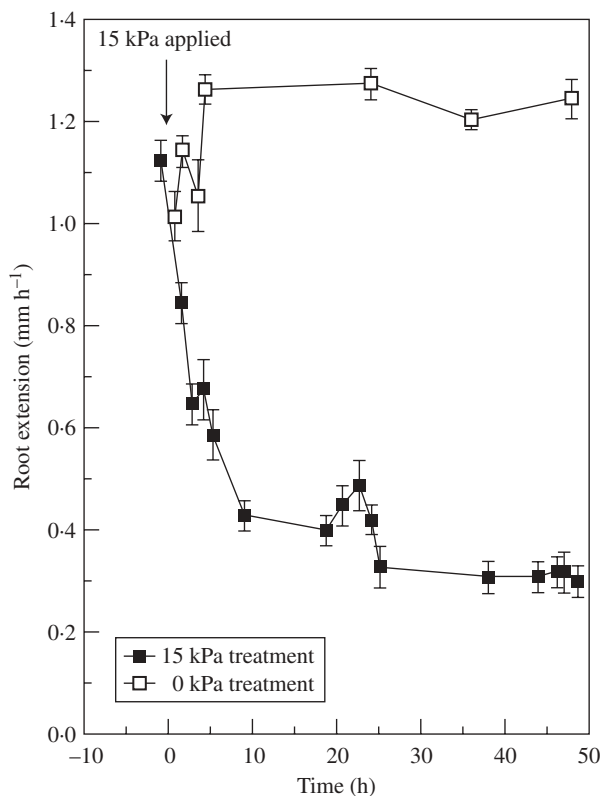

FIG. 2. Rate of extension of lupin roots in the first 47 h after coarse sand was pressurized to 15 kPa or left unpressurized (0 kPa). Observations are means ± s.e.

critical O_2 pressure data using non-linear regressions in the statistics package Genstat Version 6·1 (Genstat, 2002). Logistic curves were fitted due to the excellent fit to the data ($P < 0.001$ in all cases) and were of the form:

$$y = A + C/(1 + e^{-B^*(x-M)}).$$

Recent criticism of the use of logistic curves in representing root cell elongation suggests that in precise measurement of individual roots that growth can be separated into three zones, with abrupt changes in local velocity at the boundaries—rapid increase at the boundary of division/elongation and rapid decrease at the boundary of elongation/maturation. However, it was considered that the gradual changes in oxygen consumption observed at these boundaries justified the fitting of logistic curves, especially as observations were means of a number of roots (van der Weele *et al.*, 2003).

RESULTS

Root response to impedance

Figure 2 demonstrates a rapid but not instantaneous decrease in elongation of primary roots of lupin when pressure was applied to entire axes. Rates of elongation declined from $1·1–1·3\,mm\,h^{-1}$ to approx. $0·4\,mm\,h^{-1}$ within <10 h and settled to approx. $0·3\,mm\,h^{-1}$ after the
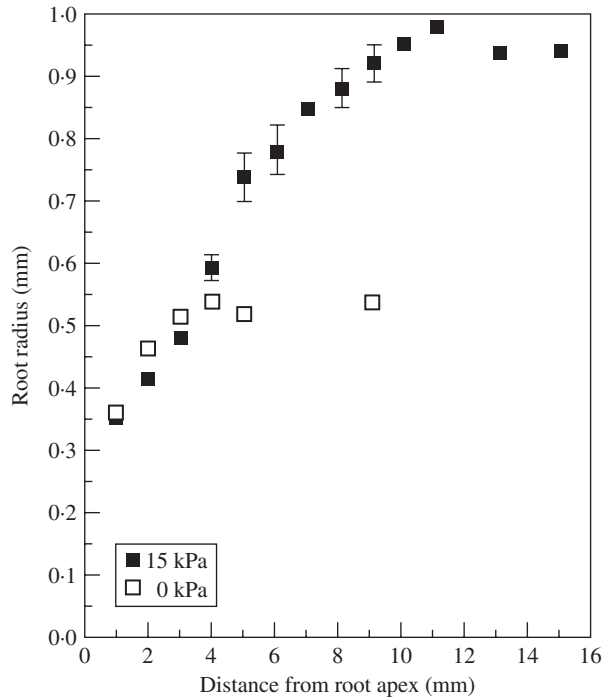
FIG. 3. Local radii of root axes grown in coarse sand unpressurized (0 kPa) and pressurized to 15 kPa for 47 h. Observations are means ± s.e.

first day. Experiments not reported here showed that an equivalent decrease in root elongation was achieved by 30 mm depth, whether roots were observed as they grew into a pressurized matrix or pressure (15 kPa) was applied to the matrix 36 h after roots entered it.

While elongation of roots was restricted by mechanical impedance, radial expansion was typically enhanced in basal regions of the apex (Fig. 3). Segments of root >10 mm basal to the apex were about twice as thick when impeded and all tissues >4 mm from the apex swelled to some degree when impeded. The most distal reaches of the zone of elongation did not swell in response to impedance. Roots were extracted for measurement 47 h after impedance was applied.

The manner in which roots thickened radially in response to mechanical impedance is shown in Fig. 4. Root axes growing normally in unimpeded matrices (A and B) were approx. 50 % thicker as cells were displaced from the apex, while impeded roots (C and D) swelled specifically in response to impedance. The increase in radial dimensions of cortical cells and conservation of the volume of stele is clear from these sections. There is no indication of an increase in the number of cell files as roots swelled, rather that individual cells became larger.

Recovery from impedance

Roots elongated faster once mechanical impedance was relieved (Fig. 5): the extension rate rose rapidly within 1 h to 0·70 mm h^{-1} and then increased more gradually. The initial surge in growth was repeatable in duplicate experiments. Recovery of elongation, when compared with roots growing
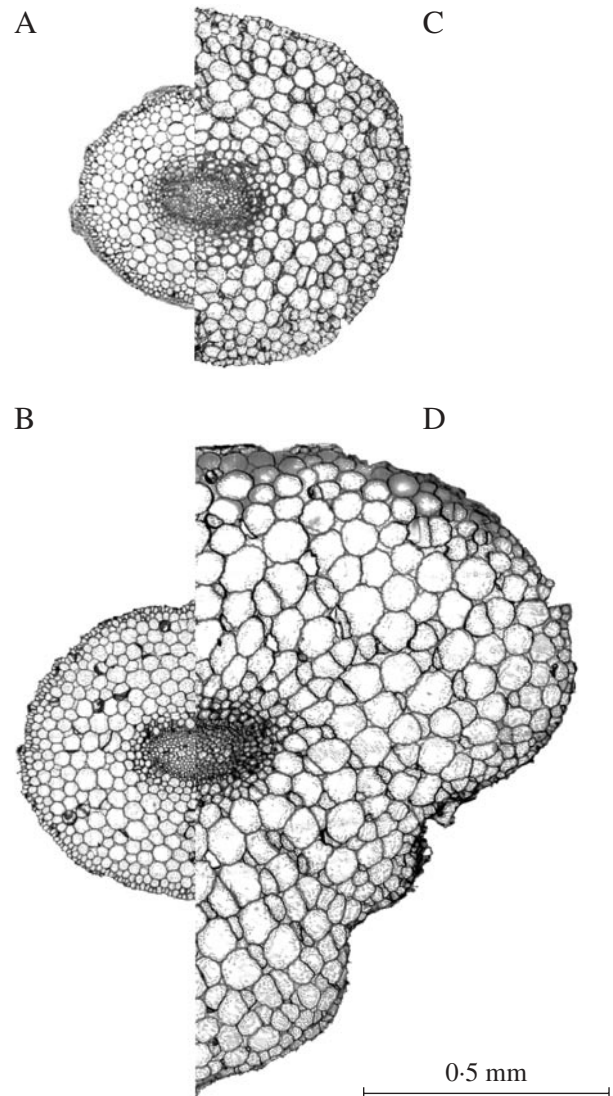

FIG. 4. Transverse root sections taken either 4–5 mm (top pair) or 10–11 mm (bottom pair) from the root apex. Sections A and B are from unimpeded roots (0 kPa) and sections C and D from impeded roots (15 kPa). Impeded roots were pressurized to 15 kPa for 47 h.

without restriction, was approx. 50 % within 3 h and finally reached approx. 75 % 2 d later. By this time (>90 h after impedance was initially imposed), elongation rates of roots that had earlier been mechanically impeded were at least three times faster than in roots that remained impeded (approx. 0·9 mm h^{-1} *vs.* 0·25 mm h^{-1}).

Effect of oxygen concentration on root extension

The fitted curves in Fig. 6 show a distinct and large difference between the O_2 concentration at which root elongation was slowed (i.e. COP_e): impeded roots had a COP_e of ≈21 % O_2 compared with a COP_e of ≈11 % in unimpeded roots. COP_e values were defined as the O_2 concentration at which 95 % of the maximum elongation rate was reached.

15 kPa applied to 0/15 and
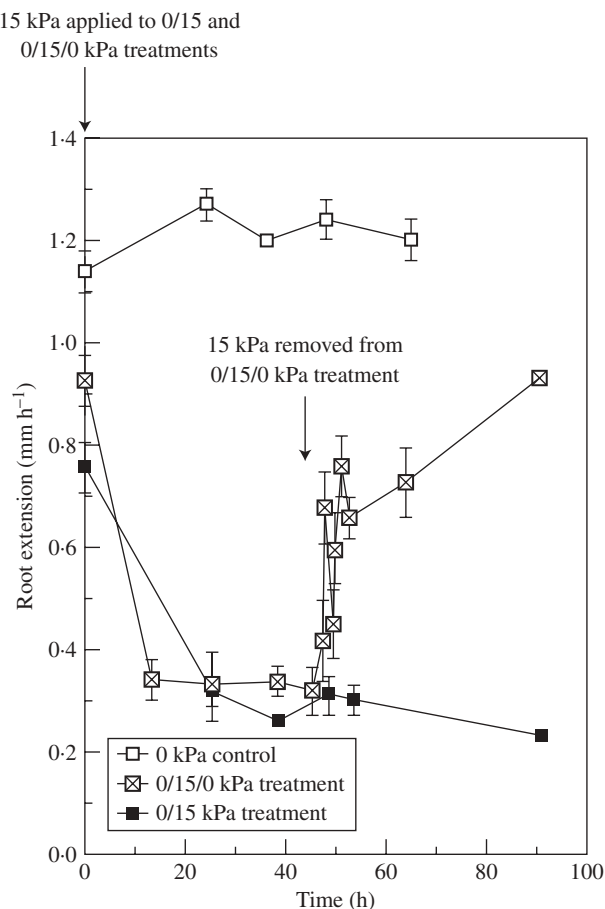0/15/0 kPa treatments

FIG. 5. Extension of lupin roots that have grown in coarse sand that were either unpressurized (0 kPa) or pressurized to 15 kPa for 47 h. Half the roots that were growing under pressure were returned to unpressurized conditions for a further 43 h. Observations are means ± s.e.

Cell biology of impeded roots

Epidermal cell lengths were measured in the distal 20 mm of unimpeded and impeded root axes so as to include the zones of elongation and differentiation (data not presented). Mechanical impedance did not affect epidermal cell length significantly in the terminal 4 mm of axes where cell division and the early stages of cell elongation occur. However, epidermal cells in mechanically impeded roots were up to 50 % shorter in zones basal to the terminal 4 mm, a trend that persisted through the elongation zone into the zone of differentiation where cell growth had ceased. Final length of epidermal cells was $40 \pm 2 \, \mu m$ for impeded roots compared with $95 \pm 4 \, \mu m$ in the unimpeded roots.

Local velocities of root elongation, relative to apices, were calculated from the measured epidermal cell lengths and root extension rates (Fig. 7). Velocities at each position along root axes revealed that mechanical impedance (15 kPa applied pressure) slowed root elongation at all points 3–10 mm from the root apices, corresponding approximately to the zone of cell expansion in normal lupin roots. Another element of the slower rates of root elongation in mechanically impeded roots is the significantly shorter

zone of elongation compared with roots that grew without restriction. The fitted logistic curves to the velocity data (Fig. 7) indicate that 95 % of maximum velocity (and hence the asymptotic rise in epidermal cell length that is used to calculate velocity) was reached 3·95 mm from the apex in impeded roots and 9·70 mm in the unimpeded roots. That is, even though cells expanded until they were approx. 10 mm from the apex in unimpeded roots, elongation of cells in impeded roots ceased just 4 mm from the apex. Figure 7 reveals that while roots in an impeded medium did not elongate in the region 4–10 mm from the apex, they still expanded (Fig. 3), demonstrating radial swelling in cells that had ceased to elongate. Root hairs initiated immediately basal to the position of maximum epidermal cell length, i.e. in impeded roots root hairs initiated 4 mm from the apex and in unimpeded roots at 10 mm from the apex.

Oxygen consumption by impeded roots

Oxygen consumption rates on a volume basis in the apical 2 mm of roots were about five-fold above the basal rate measured in mature root axes (Fig. 8A). Rates of O_2 consumption decreased rapidly in tissues basal to the apex in all roots, when plotted against absolute distance from the apex; there was no obvious effect of impedance on this decline. The lower rates in fully expanded tissue were similar in both impeded and unimpeded roots. When logistic curves were fitted to the data in a similar fashion to those in Fig. 7, the result was highly significant ($P < 0·001$), the two curves accounting for 90 and 98 % of the variation. Detail of the logistic curves is shown in Fig. 8B and C, representing the respective zones of cell elongation in unimpeded and impeded roots. The shapes of the curves in Fig. 8B and C reflect the differences in O_2 demand between treatments: O_2 consumption was faster over the zone of elongation in impeded roots than in unimpeded roots: the two curves being significantly different ($P < 0·001$) from each other.

The pattern of O_2 consumption was also seen in COP_r, which declined basally (Fig. 8D). Logistic curves were again fitted to the data and were highly significant ($P < 0·001$), accounting for 85 and 89 % of the variation. The detail of the curves is shown for unimpeded and impeded roots in Fig. 8 (E and F, respectively).

The fitted curve in Fig. 8E indicates that in unimpeded roots COP_r was at a minimum for the basal 60 % of the zone of elongation ($\approx$4–10 mm from the apex). However, in the impeded roots COP_r decreased basally throughout the zone of elongation (Fig. 8F). Data in Fig. 7 from unimpeded roots show that the 4- to 10-mm zone, where COP_r was minimal, accounted for 68 % of total elongation. Thus only 32 % of elongation in unimpeded roots occurred in tissues with elevated COP_r, whereas all the elongating cells in impeded roots had a COP_r above that in mature tissues.

The COP_r of the terminal 2 mm of both root types was close to the O_2 concentration at full aeration. Hence, O_2 supply was compromised by even the mildest hypoxic conditions in the most distal tissues of all roots.
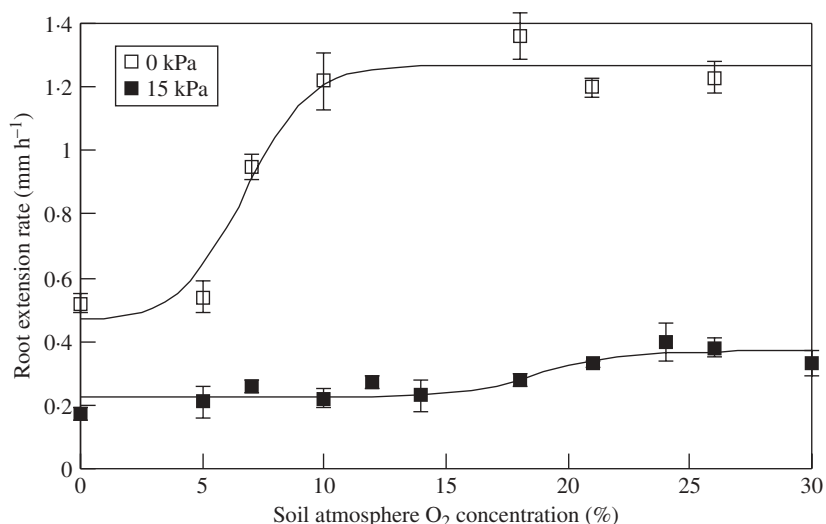
FIG. 6. Rates of root extension of unimpeded (0 kPa) or impeded (15 kPa) roots at O_2 concentrations ranging from 0 to 30 % in the root environment. Observations are means ± s.e. The non-linear regression of logistic curves fitted was highly significant ($P < 0.001$); the two fitted logistic curves (for 0 kPa and 15 kPa roots, respectively) were significantly different ($P < 0.001$) and explained 97 % of the variance.
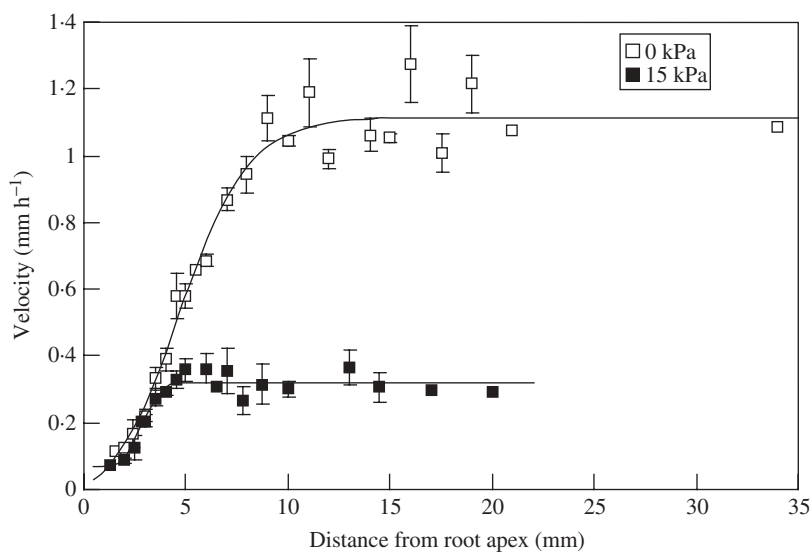

FIG. 7. Local longitudinal velocities throughout the distal zone of lupin roots, relative to the root apex. Roots were either unpressurized (0 kPa) or pressurized to 15 kPa for 47 h. Terminal velocities observed at points further than 10 mm from the root apex are thus equivalent to the elongation rate of the root axis. Observations are means ± s.e. The non-linear regression of logistic curves fitted was highly significant ($P < 0.001$); the two fitted logistic curves (for 0 kPa and 15 kPa roots, respectively) were significantly different ($P < 0.001$) and explained 98 % of the variance.

The O_2 demand to sustain growth was calculated as follows. Root elongation rates were derived from the mean of all root extension experiments conducted at atmospheric O_2 concentration (21 % O_2). Local longitudinal velocities (Fig. 7) and root radii (Fig. 3) were used to calculate rates of volume expansion in these roots. Oxygen consumption of the terminal 10 mm of roots was estimated from measured O_2 uptake rates by segments (Fig. 8A). The oxygen required to sustain root growth was then calculated (Table 1). Oxygen demand for elongation compared only elongating zones (0–10 mm in unimpeded roots and 0–4 mm in impeded roots) while O_2 demand for expansion compared

the expanding terminal 10 mm of all roots. Using either criterion, impedance caused a 50–80 % increase in oxygen demand for growth.

DISCUSSION

Dynamics of root growth during exposure to impedance and hypoxia

Elongation of primary lupin roots was slowed by approx. 75 % when a mild external pressure was used to generate mechanical impedance. This level of impedance of root
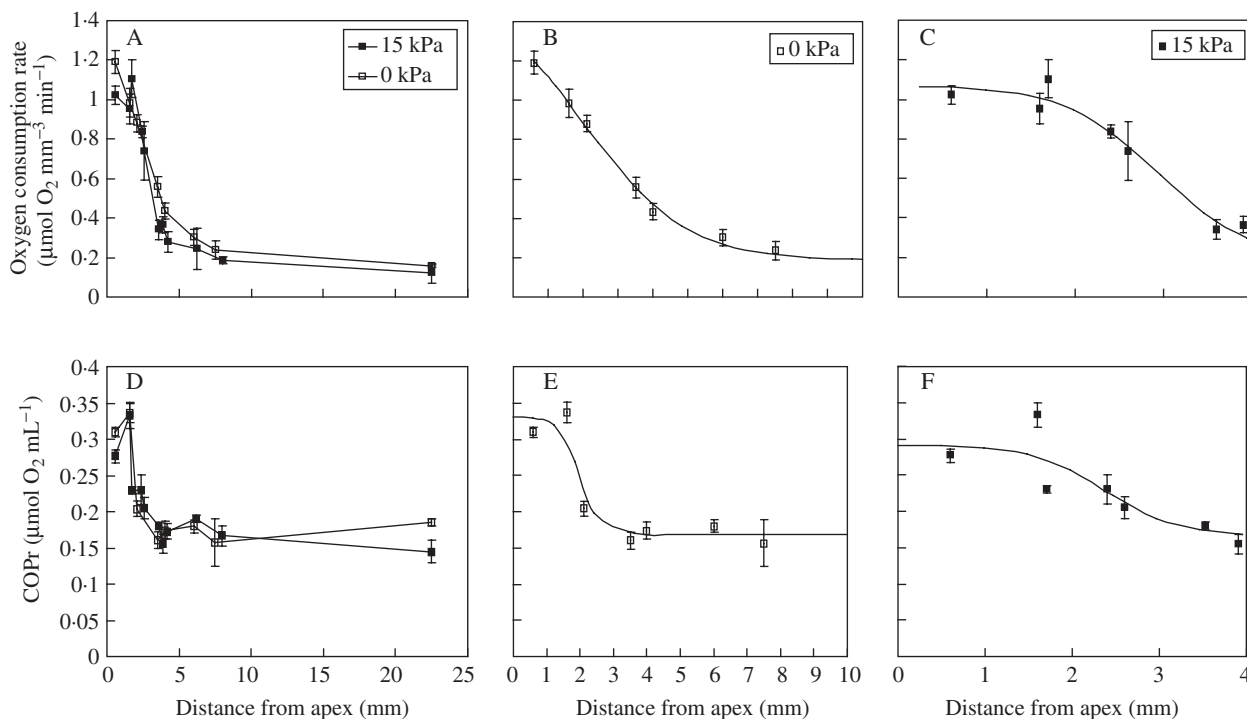
FIG. 8. (A) Local O_2 consumption rate (μmol mm^{-3} min^{-1}) in the terminal 22 mm of unimpeded or impeded roots and (D) COP_r values of roots over the same region. (B) and (E) show data from the unimpeded roots while (C) and (F) show data from impeded roots. (B) and (E) present data for the 10-mm-long elongating zone of unimpeded roots, while those in (C) and (F) present data for the 4-mm-long elongating zone of impeded roots. (B) and (C) are derived from (A) with fitted curves included, while (E) and (F) are derived from (D) (note symbols within the figures). The non-linear regressions of logistic curves fitted to (B), (C), (E) and (F) were highly significant ($P < 0.001$): in each case the fitted logistic curves were significant ($P < 0.001$) and explained between 85 and 98 % of the variance. Observations are means ± s.e. Straight lines connecting points in (A) and (D) are for ease of distinguishing treatment type.

TABLE 1. *Oxygen consumption per unit of elongation and volume expansion of unimpeded (0 kPa) and impeded (15 kPa) roots, using data from root growth and oxygen consumption experiments*

Root characteristic	0 kPa	15 kPa	% increase
Extension rate (mm h^{-1})	1·1	0·3	
O_2 consumption of growing tissues* (μmol h^{-1})	200	100	
O_2 consumption per unit extension (μmol mm^{-1})	180	330	80
Rate of volume growth (mm^3 h^{-1})	0·93	0·83	
O_2 consumption of growing tissues[†] (μmol h^{-1})	200	270	
O_2 consumption per unit volume growth (μmol mm^{-3})	215	325	50

 * 0–10 mm in unimpeded roots (0 kPa); 0–4 mm in impeded roots (15 kPa).
 [†] 0–10 mm zone in both treatments.

growth is not unusual in field conditions, where soil strength slows root growth up to 5-fold (Bennie, 1991) and even more stringent conditions have been applied in laboratory studies of mechanical impedance (Materechera *et al.*, 1991). The experiment reported here demonstrates the dynamics of root response to mechanical impedance that are not evident from steady-state observations. For example, the slowing of elongation is not instantaneous in the externally pressurized

matrix used in the experiment, probably in part because local displacement and compression of sand particles by root apices is necessary before external pressure is expressed as an impeding force on roots (Farrell and Greacen, 1966). The observation that 10 h elapsed before root elongation declined to its final rate could reflect a steady rise in external pressure (σ_N) perceived by roots as they penetrate a medium (Greacen and Oh, 1972). By contrast, when roots of barley made contact with ballotini beads (Goss and Scott Russell, 1980), growth slowed abruptly (tens of minutes). This essentially instantaneous inhibition of root elongation suggests that the manner in which mechanical resistance is imposed on roots influences the biological response. Solid, incompressible matrices (e.g. rock) or a change of phase from a fluid to a solid matrix (e.g. the Goss experiment cited) both contrast with these lupin experiments, where roots grew steadily through a granular matrix, as they commonly do in field conditions. The recovery phases after mechanical impedance was relieved reveals more about the internal root processes, with an initial rapid elongation followed by a slow phase that continued over several days. The initial surge is probably due to relaxed turgid cells expanding (Atwell and Newsome, 1990; Croser *et al.*, 2000), with events such as the recovery of cell division underpinning the lag phase (see below).

Root dimensions are affected by mechanical impedance beyond the obvious inhibition of axial growth (Taylor and Ratliff, 1969; Bengough and Young, 1993). Experiments

with lupin show that the commonly reported radial expansion of mechanically impeded roots is a highly controlled process rather than a generalized anisotropic growth response. The terminal 4 mm of lupin root axes was not thicker during impedance, a phenomenon that has not been commonly reported as part of the swelling response when roots are mechanically impeded (Wilson *et al.*, 1977; Croser *et al.*, 2000). Bengough *et al.* (1997) generalized that radial swelling encroached to within 2 mm of the apex of impeded roots. Even though impeded roots only elongated at 0·3 mm h^{-1} in a well-aerated matrix, growth was slowed by a further 50 % when O_2 levels fell below 21 % O_2 (full aeration). On the other hand, unimpeded roots grew optimally at moderate levels of hypoxia (10–20 % O_2). Precisely why impeded roots were more sensitive to hypoxia than unimpeded roots is not evident from the present data. He *et al.* (1996) found that mechanical impedance and hypoxia synergistically enhance ethylene release, increasing the chance that ethylene-related effects influence roots in compacted soil. Radial swelling, a conspicuous response to impedance (Fig. 4), increased diffusive resistance to O_2 flux (see analysis below) but this study does not determine what role ethylene might play in the swelling response. Because impeded roots swelled basal to the zone of elongation, radial swelling could not be invoked as a direct factor in the heightened sensitivity of elongation to hypoxia. A range of structural and metabolic changes could be induced in mechanically impeded roots that exacerbate O_2 deficits. When the swollen mature axes of impeded roots became hypoxic, they would develop anoxic steles (Armstrong and Beckett, 1985; Thomson and Greenway, 1991), affecting metabolism throughout the root axis.

Differential growth in root tissues

When lupin roots were impeded, the basal boundary of the elongation zone shifted from approx. 10 mm to 4 mm from the apex (matching closely the observed sites of root hair initiation in both impeded and unimpeded roots) while the boundary of radial expansion moved from 4 mm (unimpeded roots) to 10 mm (impeded roots). A similar shortening of the elongation zone was observed in peas, where the basal boundary was 6 mm from the apex when roots were removed from impedance but moved to 12 mm over the following 2 d (Croser *et al.*, 2000). Thus, the axial and radial growth vectors that describe cell expansion responded independently to external pressure. It remains to be seen whether this developmental uncoupling is observed in all species. It is conceivable that the extent to which root swelling encroaches into the apical zone could account for reported differences between species in tolerance to impedance reported by Materechera *et al.* (1991). This is significant because the calculation of root growth pressures relies on estimates of a cross-sectional area of the advancing root apex and should thus take into consideration whether the elongating cells also swell when impeded (see Abdalla *et al.*, 1969).

The implications of altered growth distribution in impeded roots are complex because they simultaneously change the demand for internal resources (e.g. carbohydrates for cell wall synthesis) and the pathway of diffusion through metabolically active root apical tissues (e.g. O_2, ethylene). Increments in tissue volume in impeded root axes are generally smaller than in unimpeded axes (Atwell, 1988; Table 1), resulting in a diminished demand for new cell constituents for growth and potentially smaller demand for O_2 (Atwell, 1990; Bidel *et al.*, 2000). However, the doubling of root diameter through mechanical impedance also restricts the delivery of O_2 from the soil atmosphere to cells deep within root axes by increasing the length of the diffusion pathway. Estimates of resistance to O_2 flux into unimpeded and impeded roots can be made by treating the root as defined by the epidermis as one cylinder and surface of the stele as a second concentric cylinder, these cylinders having radii of r_o and r_i, respectively (see Armstrong *et al.*, 1994). Assumptions made in these calculations are referred to in the text below. In the roots shown in Fig. 4 ($r_i \approx$ 0·01 cm), r_o increased from $\approx$0·038 to $\approx$0·078 cm as roots swelled, increasing the resistance to O_2 flux through the cortex from $1·04 \times 10^2$ s cm^{-3} to $1·59 \times 10^2$ s cm^{-3}. Thus a doubling of root diameter caused a modest 50 % increase in resistance to O_2 flux. This is relevant to the interpretation of O_2 demand by roots (see the next section).

The effective radial fractional porosities of roots from each treatment and tissues from individual roots are likely to vary significantly, with O_2 entering meristematic cells as a solute in the liquid phase and mature segments as a gas diffusing through air-filled pore spaces (Bidel *et al.*, 2000). Thus important assumptions can be made that fractional porosities and cell packing arrangements in the two treatments are similar, acknowledging that they could modify O_2 diffusion rates if they differed significantly. It has been established that impeded roots swell through radial and tangential cortical cell expansion while the number of cortical cell files increases by only 0–30 % (Wilson *et al.*, 1977; Atwell, 1988; Croser *et al.*, 1999; this study). Thus, while the contribution of vacuoles to the radial pathway increases, there was little addition of new cell walls that offer higher impedance to O_2 diffusion (Armstrong *et al.*, 1994).

Does hypoxia limit growth?

The interaction between O_2 supply to roots, particularly apices, and mechanical impedance was the subject of several early studies (Gill and Miller, 1956; Eavis, 1969), driven by the rationale that compacted soils are likely to impede both root extension and diffusion of an adequate supply of O_2 to root surfaces. However, other factors that can exacerbate the supply of O_2 to cells deep within root tissues are the depth of root tissue that O_2 must penetrate (hence radial root dimensions) and, potentially, the effect of changes in root anatomy (e.g. cell packing arrangements, porosities) on diffusivity of O_2 through bulk tissues. In the experimental system reported here, local rates of root elongation were observed in a uniformly impeded matrix, then roots were rapidly removed to assess O_2 uptake independently of the growth medium. Interpretation of these data is complex: the rate of root expansion as well as the morphology of impeded roots affects O_2 demand through

energy requirements (Bidel *et al.*, 2000) and diffusive limitations (Armstrong *et al.*, 1994), respectively. Removing roots from impeded soil had the advantage that intrinsic properties of O_2 uptake into local root zones (e.g. critical O_2 pressure of root apices) could be assessed independently of the permeability of the soil matrix, while it is conceded that the pathway for O_2 movement was shorter via exposed cut surfaces.

The kinetics of O_2 uptake show that the terminal apices of all roots were O_2-limited, even after excision. Such an O_2 limitation has been observed in other species (Berry and Norris, 1949; Atwell *et al.*, 1985; Armstrong *et al.*, 1994); paradoxically, this suggests that apical cell proliferation always occurs in hypoxic conditions. It also precludes O_2 as the sole factor in the lower cell flux rates that are characteristic of impeded roots.

There were fundamental differences in the expanding tissues zones that lie basal to the zone of cell division, with contrasting O_2 requirements under impedance (Fig. 6) and differential tissue responses to hypoxia (Fig. 8). All the elongating cells in impeded roots were located in the zone of rapid O_2 consumption and high COP_r, unlike unimpeded roots where the basal portion of the elongating zone consumed O_2 more slowly and had a low COP_r. It is postulated that this is linked to the high COP_e measured in impeded roots. That impeded roots consumed O_2 faster in the expanding zone as a whole (270 cf. 200 μmol O_2 h^{-1}) and particularly per unit volume growth (325 cf. 215 μmol O_2 mm^{-3}) emphasizes the vulnerability of impeded roots to hypoxia (high COP_e). The sensitivity of impeded roots to hypoxia is illustrated by their slow extension rates: they consumed 80 % more O_2 per unit of elongation growth than unimpeded roots. Atwell (1990) observed a doubling of demand for O_2 per unit of elongation in wheat under field conditions.

The problem of O_2 supply is further exacerbated in the field, where hypoxia can occur because O_2 diffusion through compacted bulk soil limits the diffusive flow into roots (Eavis, 1972). While the proportion of root surface that is occluded by soil particles when the system is under pressure is not necessarily greater than in roots from friable soil (Barley and Greacen, 1967), any localized soil compression around impeded root axes potentially restricts O_2 flow into roots (Armstrong and Beckett, 1985) and inhibits growth. Thus, hypoxia could conceivably affect metabolism in most of the growing zone of impeded roots *in situ*. Contrasting with the compressed zone of elongation in impeded root axes, unimpeded roots elongated up to 10 mm from the apex and would be much less susceptible to hypoxic conditions in friable soil. These two factors (restricted O_2 flow through soil and high COP_r values in elongating tissues) support the *a priori* case that impeded root axes are susceptible to hypoxia.

While a case is made for O_2 affecting elongation of impeded roots, carbohydrate import has been cited as a potential limitation on root metabolism. However, earlier reports have provided no evidence that carbohydrate import limits growth in apices of impeded roots (Atwell and Newsome, 1990; Masle *et al.*, 1990). Indeed, the acute limitations that apical root tissues impose on O_2 diffusion

in *Prunus* (Bidel *et al.*, 2000) and lupin suggest that carbohydrates are probably rarely limiting, in spite of the high marginal demand for carbohydrates as roots penetrate hard soils (Atwell, 1990). Under most conditions it remains likely that the physical impedance that soils generate directly restricts root elongation through opposing turgor-driven expansion (Atwell and Newsome, 1990; Bengough *et al.*, 1997) and restricting O_2 diffusion. To the extent that root apices are dense and rapidly respiring, these are the most vulnerable sites for O_2 deficits, whereas mature, vacuolated root tissues have more modest O_2 demand (Berry and Norris, 1949) and experience less acute deficits of O_2. All but the most basal cells of the elongating zone of impeded roots are hypoxic, even when roots are in an aerated medium (Fig. 8).

Control and dynamics of cell expansion

Cell formation in impeded root apices changed in a complex manner; epidermal cells were almost unaffected by impedance in the earliest stages of root expansion (0–4 mm from the apex) but older cells did not continue to elongate beyond about 40 μm in length. The wall loosening events described by Cleland (1977), Passioura and Fry (1992) and Frensch and Hsiao (1995) that sustain cell expansion in turgid cells are clearly reversed or opposed in the final phase of cell extension in impeded roots. The differential radial and axial growth observed along the axis of mechanically impeded lupin roots can be reconciled with the trivial variance in turgor pressure in these tissues (Atwell and Newsome, 1990; Croser *et al.*, 2000) through fine control of cell wall loosening. In cases where turgor pressure has been shown expressly to respond to mechanical impedance (Clark *et al.*, 1996), wall properties presumably play a role alongside turgor pressure in generating axial force on the soil. The data from lupin and a previous study with peas (Croser *et al.*, 2000) confirm that local velocities of epidermal cell elongation (cf. overall axis elongation rates) vary with impedance, making a more compelling case for positional control of cell wall properties in axial and radial walls. The possibility that either wall extensibility or yield threshold turgor are affected by mechanical impedance, thereby changing irreversible expansion rate, are not separated in these experiments. Challenging roots with osmotic drought (Pritchard *et al.*, 1991; Frensch and Hsiao, 1995) induced measurable changes within minutes in wall properties but equivalent experiments in impeded roots are lacking.

Cell flux through individual epidermal cell files was suppressed by 35 % when lupin roots were exposed to mechanical impedance, from 12 to 7·5 cells $file^{-1}$ h^{-1}. A number of authors from Barley (1965) to Croser *et al.* (1999) reported up to a 50 % decrease in cell flux when roots of cereals and pea were impeded, with smaller effects of impedance on the number of cell files (Fig. 4; Atwell, 1988; Croser *et al.*, 1999). Thus lupin is typical of species in which cells of impeded roots exit their meristems more slowly, with implications for recovery when plants grow into loose soil. This is not easily reconciled with the fact that impedance did not affect growth in the terminal 3 mm of root axes (where the

zone of cell division is located). The inhibition of cell fluxes by steady-state mechanical impedance illustrates that there are either subtle, direct effects of mechanical forces on meristems or that signals were acropetally transduced to apices from basal tissues (>3 mm from the apex). The 10–80 h recoveries in root growth that follow instantaneous release of mechanical impedance in the present and previous studies (Goss and Scott Russell, 1980; Croser *et al.*, 1999) suggest that cell production was repressed through the action of metabolic events (e.g. cytological) rather than direct and immediate effects mediated by external pressure.

ACKNOWLEDGEMENTS

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