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Root responses to soil physical conditions; growth dynamics from field to cell

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Abstract

Root growth in the field is often slowed by a combination of soil physical stresses, including mechanical impedance, water stress, and oxygen deficiency. The stresses operating may vary continually, depending on the location of the root in the soil profile, the prevailing soil water conditions, and the degree to which the soil has been compacted. The dynamics of root growth responses are considered in this paper, together with the cellular responses that underlie them. Certain root responses facilitate elongation in hard soil, for example, increased sloughing of border cells and exudation from the root cap decreases friction; and thickening of the root relieves stress in front of the root apex and decreases buckling. Whole root systems may also grow preferentially in loose versus dense soil, but this response depends on genotype and the spatial arrangement of loose and compact soil with respect to the main root axes. Decreased root elongation is often accompanied by a decrease in both cell flux and axial cell extension, and recent computer-based models are increasing our understanding of these processes. In the case of mechanical impedance, large changes in cell shape occur, giving rise to shorter fatter cells. There is still uncertainty about many aspects of this response, including the changes in cell walls that control axial versus radial extension, and the degree to which the epidermis, cortex, and stele control root elongation. Optical flow techniques enable tracking of root surfaces with time to yield estimates of two-dimensional velocity fields. It is demonstrated that these techniques can be applied successfully to time-lapse sequences of confocal microscope images of living roots, in order to determine velocity fields and strain rates of groups of cells. In combination with new molecular approaches this provides a promising way of investigating and modelling the mechanisms controlling growth perturbations in response to environmental stresses.

Key words: Cell expansion, cell walls, mechanical impedance, root growth, soil compaction, water stress.

Introduction

Plants require a root system that delivers adequate water and nutrients for shoot growth, and to anchor them in the soil. The optimum distribution of root length depends mainly on the distribution of water and nutrients in the soil. In dry seasons plants may require long main root axes to access water stored deep in the soil profile, whilst if abundant water and nutrients are available, only a small fraction of the root length may suffice. Roots of individual plants may experience a wide range of soil conditions, and as much variation has been recorded within 0.5 m of the stem base as across a 100 m^2 field plot (Jackson and Caldwell, 1993). Soil matric potential may be drier than −1.5 MPa (permanent wilting point) at the soil surface on a summer day, but saturated at a depth of 1 or 2 m, if a water table is present. Soil physical stresses may limit root elongation; for example, if the soil is too wet with insufficient oxygen diffusion to the root tip resulting in hypoxia; insufficient water availability if the matric potential is too negative; and mechanical impedance if the soil is too hard due to compaction or soil drying (Taylor and

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Ratliff, 1969; Blackwell and Wells, 1983; Sharp *et al*., 1988; da Silva *et al*., 1994). Soil physical stresses have sometimes been found to interact to decrease root elongation more than predicted from the combination of stresses acting independently. Interestingly, this effect has only been observed in maize roots (Gill and Miller, 1956; Barley, 1962; Mirreh and Ketcheson, 1973; Goss *et al*., 1989) and not, as yet, in other species (Taylor and Gardner, 1963; Taylor and Ratliff, 1969; Greacen and Oh, 1972). It is a considerable challenge to evaluate the most important factors limiting the growth of the crop, and to understand the mechanisms underlying the root growth responses.

Studying the detailed response of roots to physical stresses, in addition to its agronomic application, can also elucidate the fundamentals of root growth processes and their regulation. This should reveal the scope for manipulation of root growth responses to soil physical stresses, via appropriate plant breeding or genetic engineering technologies. The explicit selection of particular root traits has been largely unexploited, although root traits are starting to receive increased interest, for example, with the enhanced ability to capture phosphate (Gahoonia *et al*., 1999, 2001; Liao *et al*., 2001; Bates and Lynch, 2002).

The aim of this paper is to discuss briefly the different soil physical stresses acting on the growth of a crop root system, and to consider the mechanism of root growth and the dynamics of its response to changes in soil physical conditions, in particular, soil strength. The potential of recent image analysis and modelling approaches for unravelling the dynamics of growth will be illustrated. Due to the relatively wide scope of this paper, the coverage of the literature is selective and readers are directed to other recent reviews for more detailed coverage of particular topics (for example, Clark *et al*., 2003, review the mechanics of soil penetration in more detail).

Soil physical factors limiting root growth in the field

A number of soil physical stresses, acting independently or in combination, can limit root elongation. The physical stresses operating vary markedly between different soils with, for example, roots growing in poorly drained clay soils being subject to hypoxia to a much greater extent than on a well-drained sandy loam. Soil strength increases generally as the soil dries, often by an order of magnitude between matric potentials of −5 kPa and −1.5 MPa (permanent wilting point of mesophytic plants). Large strength increases on drying can occur for a wide range of soil textures, including soils that are predominantly sandy, silty, or clayey. Such strength increase is particularly marked in hard-setting soils that slump to a massive structure and can only be cultivated over a narrow range of soil water contents (Mullins *et al*., 1987). A major problem in applying laboratory-based understanding of root growth is that soil water potential in the field changes constantly, and can vary substantially through the soil profile. Thus, the factors limiting root growth will also vary with time and with the local soil water content at any point in the field.

The Least Limiting Water Range (LLWR) approach taken by da Silva and colleagues (da Silva *et al*., 1994; da Silva and Kay, 1997*a*, 2004) provides an appropriate framework for considering which factors are limiting root growth in a particular soil during a growing season. Limiting water contents are first defined for each physical stress: For example, the lower (drier) bound on soil water content corresponds to the greater (wetter) bound of the permanent wilting point (−1.5 MPa matric potential) and the water content for penetrometer resistance of 2 MPa (typically 2–8 times greater than the root penetration resistance; Bengough and Mullins, 1990). The upper (wetter) bound is determined by the lesser (drier) bound of the soil water content at 10% air-filled porosity, and that at −10 kPa (field capacity). The LLWR is the difference between the lower and upper limiting water contents, and may be thought of as the soil water content range within which root growth is not severely restricted by one of these soil physical stresses. The LLWR decreases with increasing soil bulk density, increasing clay content, and decreasing organic matter content (da Silva and Kay, 1997*b*). To evaluate the stresses experienced by the crop root system during any given period, the number of days the soil water content is outside the LLWR must be determined (da Silva and Kay, 1997*a*); this is illustrated in Fig. 1. In 78–90% of Canadian topsoils investigated, soil strength limited root growth in dry soil (da Silva *et al*., 1994; Topp *et al*., 1994), whereas the air-filled porosity threshold limited crop establishment and yield on the majority of no-till sites (Lapen *et al*., 2004). The approach is still being developed, and has to be evaluated for a wide range of soils and climates, although it was recently applied in Australia and Brazil, and to trees as well as arable crops (Tormena *et al*., 1999; McKenzie and McBratney, 2001).

Difficulties with the LLWR approach concern the choice of depth in the profile to define the LLWR (often 0–20 cm), and the binary nature of the thresholds in contrast to the more gradual onset of stress in reality. Soil management and weather will both affect the LLWR; including tillage, compaction, wet–dry cycles, and freeze–thaw action. The LLWR could therefore be evaluated for each soil horizon, at multiple locations throughout the field, and at several times in the growing season. Mean penetrometer resistance may not always be the best indicator of mechanical impedance to root growth, especially for zero-tillage treatments where networks of continuous channels for root growth may develop within a relatively strong soil matrix. Soil physical measures such as relative density (da Silva *et al*., 1997), shear strength (McKenzie and McBratney,

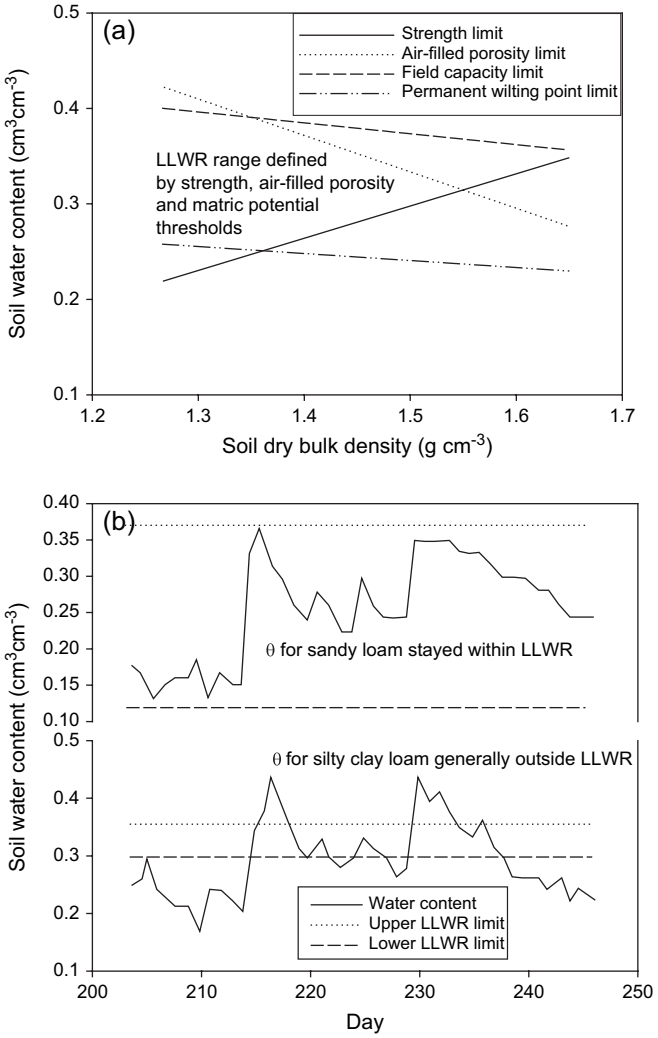

Fig. 1. Least Limiting Water Range (a) for a silt loam soil in relation to soil dry bulk density (redrawn from da Silva *et al.*, 1994 and reproduced with kind permission from the ASA-CSSA-SSSA), and (b) in relation to water content variation and time for two soils (redrawn from daSilva and Kay, 1997*a* and reproduced with kind permission from the ASA-CSSA-SSSA).

2001), and percentages of penetrable soil (Groenvelt *et al*., 1984) are alternatives to mean penetrometer resistance, but require fuller investigation.

Interestingly, Dexter proposed *S*-theory as a measure of soil microstructure and the suitability of soil structure for root growth (Dexter, 2004). The *S*-value is defined as the slope of the soil water release curve at the point of inflection: and so requires fewer parameters than the LLWR approach. It is likely that soils with large *S*-values may also have relatively large values of the LLWR, and the comparison between the two approaches warrants further investigation. *S*-theory may be more suited than LLWR for a measure of soil physical quality, but not as suitable for defining the physical stresses that act on a daily basis in a growing crop. In the next section, the physical factors controlling the elongation of individual root tips will be considered.

Co-ordination of root system growth in compacted soil

The growth of an individual root tip depends not only on the local soil conditions, but also on communication with the rest of the plant via the supply of carbon compounds and chemical signals. Communication is particularly relevant when different regions of the same root system are exposed to contrasting soil conditions, such as patches of high nutrient concentrations from fertilizer granules, or locally compacted soil produced by a tractor wheel, or a plough pan. Co-ordination of growth with respect to heterogeneous mechanical impedance is understood relatively poorly, and depends on species, the branching order of root impeded, and whether the impedance is applied horizontally or vertically (Goss, 1977; Montagu *et al*., 2001; Bingham and Bengough, 2003).

First, consider the case of impeding only the main root axes. Barley seedlings exhibited compensatory growth of lateral roots when the main axes were mechanically impeded (Goss, 1977): Seedlings were grown in ballotini within flexible-sided growth chambers subject to an external confining pressure. The ballotini size was increased to impede the seminal root axes, but not the laterals. This resulted in a total length of lateral roots equal to the unimpeded treatment, but with a doubling of the mean lengths of individual lateral roots. Thus, the elongation of individual lateral roots was increased, but no new laterals were initiated from the main axes.

Secondly, consider compensatory root growth resulting from splitting the root system horizontally or vertically. The situation in the horizontal split is similar to that encountered by roots meeting a compact subsoil, when main root axes grow from loose soil into compact soil. In this situation, substantial compensatory root growth occurs in the looser upper layer, maintaining the total root length (Montagu *et al*., 2001). For vertical split root systems, the situation is more akin to that adjacent to a tractor wheeling, where whole seminal roots of an individual plant may grow in either loose or compacted soil. This has only been investigated relatively recently, and data indicate that species may differ in whether compensatory root growth occurs (Montagu *et al*., 2001; Bingham and Bengough, 2003). Whereas for barley growth of laterals in the loose half of the vertical split was enhanced, as compared with loose controls, no such compensation was observed for wheat (Bingham and Bengough, 2003). Similarly, no compensatory root growth was found for broccoli (Montagu *et al*., 2001). The compensatory lateral growth for barley was a result of increased lateral root elongation (30% greater mean lateral length), similar to that found in the results of Goss (1977). It is interesting to note that barley is generally considered more plastic than wheat in response to changes in localized nutrient supply (Robinson, 1994). However, the wheat variety that did not exhibit compensatory root

growth in response to vertical variation in soil strength previously exhibited considerable compensatory growth in response to variation in nutrient supply (Bingham *et al*., 1997). This suggests that the degree of plasticity may depend on the particular stress or localized soil conditions, such that particular species or genotypes will not necessarily show high plasticity to all stresses.

Reasons underlying root growth co-ordination in response to mechanical impedance are still unknown, although they are likely to reflect changes in carbon fluxes and sink strengths within the roots (Thaler and Pages, 1998, 1999). In the following sections the cellular basis of root elongation is considered, as well as root responses to the local soil physical conditions.

Steady-state root elongation

The cellular dynamics of root growth is easiest to analyse for roots that are growing in a steady-state at constant rate (Sharp *et al*., 1988). For a root in this steady-state the elongation rate is simply given by

$$E = l_{\mathrm{mat}} \times f \qquad (1)$$

where l_{mat} [L] is the mature cell length and f is the cell flux [T^{-1}]. Cells are produced in the meristem by cell division, effectively adding more cells on to each cell file. In the meristem the cells expand until their next division, when their length is effectively halved. After undergoing a number of divisions these cells cease dividing and enter a phase of more rapid elongation in the elongation zone. Elongation then continues until the mature cell length is reached and the cell emerges from the proximal end of the elongation zone. The rate of elongation per unit length of tissue (strain rate, [T^{-1}]) is a common and convenient expression of how fast each region of tissue is extending. It can be measured either from analysing longitudinal sections through the root (Silk *et al*., 1989), using an assumption of steady-state elongation, or by time-lapse imaging of the root at a series of time intervals (Sharp *et al*., 1988; Walter *et al*., 2002; Van der Weele *et al*., 2003). For maize and pea roots, the strain rate typically forms a bell-shaped curve (Fig. 2), with a relatively short accelerating region and a longer-tailed decelerating region. High-resolution studies of the velocity field along the meristem and elongation zones of five species including tomato and *Arabidopsis* found a linear increase in velocity with distance in the meristem, followed by an abrupt acceleration with faster linearly increasing velocity in the elongation zone (Van der Weele *et al*., 2003). This may indicate that some previous studies smoothed out the more rapid transition in elongation by averaging data from a number of roots, and by having relatively coarse spatial and temporal resolution on marked roots: this is likely also to have been the case for the data shown in Fig. 2.

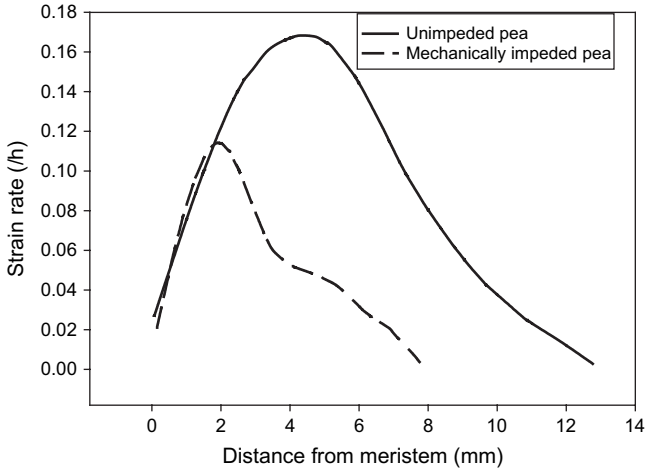

Fig. 2. Relative root elongation rate (mean strain rate) as a function of distance behind the root tip. Pea roots grown in loose or compact sand (data from Croser *et al*., 1999).

Recent attempts to describe the process of root elongation using a model of cell division and expansion promise to yield new insights into the growth process at the cellular level (Chavarria-Krauser and Schurr, 2004). In their model, cell division occurs at a constant rate in the meristem; during this time the cell extension rate is increasing linearly with cell length (Fig. 3). Cells then leave the meristem after several divisions, when their length reaches l_{el}. The cells entering the elongation zone extend at a rate that increases linearly, but more rapidly than within the meristem, with cell length until they reach a saturating length, l_{sat}. This is when the cell wall begins to stiffen and the cell elongation rate decreases linearly with cell length, until it reaches its final value of l_{mat}. Boundaries between growth regions that determine values of l_{el}, l_{sat}, and l_{mat} were determined by the relative concentrations of phytohormones diffusing from the root tip and shoot, but this is more detail than required for our consideration of the model here. The surface velocity curves produced by the model match experimental values of velocity as a function of distance from the root tip very closely for *Arabidopsis*, tobacco, and pea roots. The fit is poorer for strain rate as a function of distance from the root tip, with a faster transition in the accelerating region of the strain rate curve than is found in practice (experimental errors are also relatively more important in strain rate calculations).

The Chavarria-Krauser and Schurr (2004) model offers interesting potential to analyse changes in root elongation that occur in response to soil physical stresses, and the effects on the expansion of individual cells. To illustrate this, how the relation between cell growth rate and cell length given in Fig. 3 could be applied to mechanically impeded roots, will now be explored using data for peas taken from the literature (Croser *et al*., 1999). In Croser *et al*. (1999) the roots were subject to mechanical impedance by growing in compressed sand; when the roots were

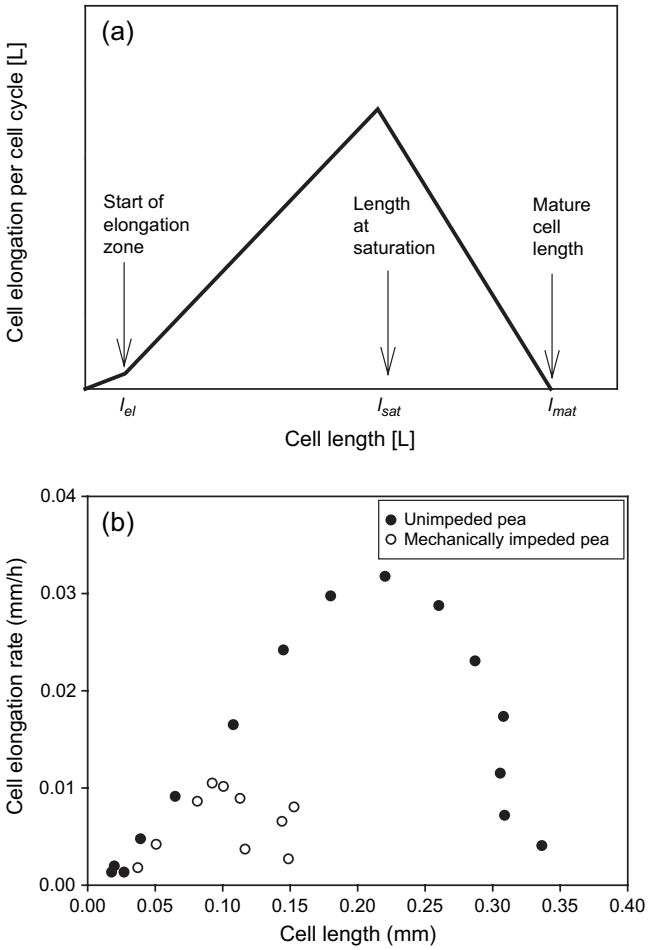

Fig. 3. Elongation rate of an individual cell as a function of its length: (a) simplified linear assumptions of growth for model from Chavarria-Krauser and Schurr (2004); (b) absolute elongation rate calculated for pea roots grown in loose and compacted soil from data of Croser *et al*. (1999).

removed from the sand, they took 3 d to return to their unimpeded elongation rate. The roots, after removal from sand, were marked with graphite particles, and photographed to calculate strain rates. The same strain rates as found in unimpeded roots were maintained in the accelerating region of the elongation zone, but the maximum strain rate was decreased and the length of the elongation zone was shortened (Fig. 2). The maximum strain rate and the corresponding cell length at which it occurred, the final cell length, and the cell length on entry to the elongation zone were estimated graphically for unimpeded and impeded pea roots (Croser *et al*., 1999, 2000). The elongation rate for a single cell was then hypothesized as a function of cell length according to the model in Fig. 3 and the results are shown in Fig. 4.

The simulated strain rates (Fig. 4b) calculated for the unimpeded model parameters and for stress model 1 parameters (Fig. 4a) are similar to the experimental measurements from which the model input parameters were derived (Fig. 2). This is also qualitatively similar to the

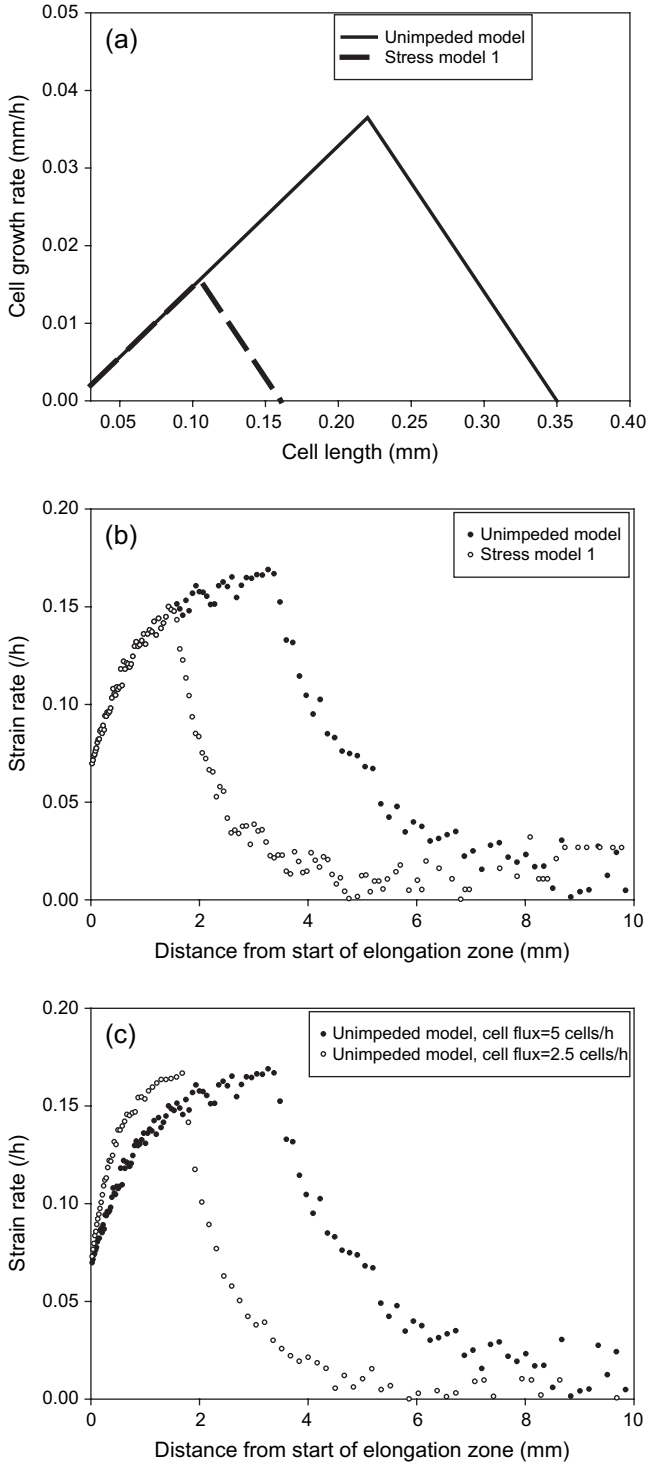

Fig. 4. Simulations of cell elongation for different patterns of cell growth. (a) Input functions of cell extension rate versus cell length for unimpeded roots and a hypothetical stress condition; (b) simulated strain rate versus distance into elongation zone for unimpeded model, and stress model 1 (similar to mechanical impedance and water stress); (c) simulated strain rate versus distance into elongation zone for the unimpeded model at two cell flux rates (2.5 and 5 cells h^{-1}).

strain rate effects on cell extension produced in response to water stress (Sharp *et al*., 1988). The detailed form of the strain rate curve is rather different from that of the experimental data, however, as it arises from the simplified linear model for cell growth versus cell length.

Although, in reality, cell flux and cell expansion are not independent processes, it is interesting to simulate some hypothetical scenarios in order to improve understanding of how the Chavarria-Krauser and Schurr model works within the elongation zone. Halving the cell flux was simulated, whilst maintaining the unimpeded model parameters for cell growth versus cell length (Fig. 4c). The elongation zone produced was approximately halved in length for the halved rate of cell flux, although the maximum strain rate was largely unaffected. The form of the strain rate distribution can be investigated with respect to other stresses, such as cool temperatures where, experimentally, it has been found that the length of the elongation zone is maintained but the maximum strain rate is decreased (Pahlavanian and Silk, 1988). It would also be possible to simulate strain rate and cell length distributions in roots responding to changing stress. The next section considers how roots respond to changes in soil physical conditions, with particular emphasis on what happens when the stress is removed.

Root elongation in response to changes in physical conditions

Modified Lockhart equation in relation to cell expansion

The Lockhart equation has long been used as a framework for interpreting regulation of root growth rate in response to soil physical stresses (Greacen and Oh, 1972). For root elongation in soil the equation was modified (Greacen and Oh, 1972; Greacen, 1986) to include the root penetration resistance of the soil (σ) [$ML^{-1}T^{-2}$]:

$$l^{-1}\left(\frac{dl}{dt}\right) = m(\sigma)(P - Y(\sigma) - \sigma) \qquad (2)$$

where l is the length [L] of elongating tissue under consideration, P is the turgor pressure [$ML^{-1}T^{-2}$], Y is the yield threshold [$ML^{-1}T^{-2}$], and m is the extensibility of the cells walls [$M^{-1}LT$]. Y and m are written as functions of σ to emphasize that they are not constants but are physiological variables that depend on the root penetration resistance of the soil. Passioura and Fry (1992) revisited this model and interpreted m and Y in terms of the activities of enzymes that make and break cross-linking bonds in the cell wall. Their model suggested that m and Y were related physiological variables that can rapidly respond to changes in the root environment.

An interesting prediction of the Passioura and Fry model is that cells will show self-regulating behaviour in response to perturbations in turgor. This seems to be at least partly correct for changes in leaf turgor brought about by pressurizing the root system, and for the roots in hydroponics subject to perturbations in the osmotic potential of their bathing solution. Similar behaviour is also apparent for short-term (e.g. 10 min) adjustment in the elongation rate of roots that have been subjected to an externally applied mechanical pressure (Bengough and Mackenzie, 1994).

Roots subjected to mechanical impedance for a period of days take a further 2–5 d to return to their unimpeded rate once they grow into loose soil (Bengough and Young, 1993). This is because the strain rate at the basal end of the root elongation zone does not recover immediately (Fig. 2). This is probably due to the time taken for the cell flux rate to recover, and for a new batch of cells (which have not reached their saturation length; Fig. 3) to enter the elongation zone. The modelling approach of Chavarria-Krauser and Schurr (2004) again offers considerable potential to investigate the dynamics of cell growth as soil physical conditions change: the cell growth versus cell length function, and the cell flux from the meristem could both be adjusted dynamically and compared with measurements of strain rates and cell length profiles at a range of time points.

Control of cell wall stiffness in relation to growth anisotropy

Cortical cells of roots grown in strong soil are generally shorter and fatter than those grown in loose soil (Wilson *et al*., 1977; Croser *et al*., 2000). Growth anisotropy in these root cells arises from the balance between turgor pressure, that acts isotropically, and the pressures arising from forces that restrain the cell anisotropically. These forces include the tension in the expanding cell wall, the forces exerted by the neighbouring cells, and the force required to deform the external growth medium. For roots growing in hard soil, the pressure required to deform the soil by longitudinal cell growth (causing a more spherical mode of soil deformation) may be one-third greater than that required for cylindrical deformation of the soil (Farrell and Greacen, 1966; Greacen *et al*., 1968). The radial confining pressures exerted by neighbouring cells are probably greatest towards the middle of the root, and, indeed, the increase in cell diameter in response to mechanical impedance was smaller for the inner cortical cell layers of barley roots than for the outer cortex (Wilson *et al*., 1977). Anisotropy in the stiffness of the expanding cell wall must arise from anisotropy in the cell wall structure and yielding properties. Indeed, the cortical cells of slow-growing mechanically impeded roots of maize contain more cellulose microfibrils oriented longitudinally than unimpeded roots (Veen, 1982). However, in this study, the distance behind the root tip of the cells examined was not recorded, and the cells simply noted as mature, hence, presumably not expanding. More recent studies have

investigated the link between microfibril orientation, microtubule orientation, and the orientation of cell growth in detail (see review by Baskin, 2001). Reverse genetics has been used to reduce the expression of α-tubulin genes in *Arabidopsis*, causing abnormal microtubular structures (Bao *et al*., 2001): Cell expansion and division were both affected, with enhanced radial cell expansion and decreased root elongation. Negative mutations of α-tubulins can cause right-handed oblique arrays of microtubules in the root epidermis, and left-handed helical (twisted) growth of roots in *Arabidopsis* (Thitamadee *et al*., 2002). Whilst microtubules and microfibrils are aligned and transverse in the accelerating region of the elongation zone (Baskin *et al*., 1999; Sugimoto *et al*., 2000; Granger and Cyr, 2001), they are often unaligned in the decelerating region (Baskin *et al*., 1999; Sugimoto *et al*., 2000). In fact, decrease in elongation rates associated with the distal end of the elongation zone precedes the change in microfibril and microtubule orientation (Baskin *et al*., 1999; Sugimoto *et al*., 2000). Whilst microfibrils provide a clear anisotropy in the cell wall structure, it is not clear, however, that their orientation controls the direction of cell expansion. An alternative model for the control of cell expansion is that regulation of cell wall stiffness in the longitudinal and transverse directions may be regulated by cell wall components that independently control the rate of separation of microfibrils, and the rate of shear between microfibrils (Baskin *et al*., 1999).

Control of root elongation in response to change in mechanical stress

Role of the root cap, border cells, and mucilage

Finite element analysis techniques using constitutive models from soil mechanics have been used to predict the stress distribution around growing roots (Richards and Greacen, 1986; Faure, 1994; Kirby and Bengough, 2002), and from these it appears that the peak stress occurs in the soil adjacent to the apex of the root cap. The root cap must, therefore, play an important role in both protecting the root meristem from damage, but also in determining the mechanical interaction between the root and the soil, namely the mode of soil deformation and the root–soil friction. Removal of the root cap has recently been shown to halve the elongation rate of maize roots grown in compacted sandy loam soil (penetrometer resistance 1.0 MPa), whereas root elongation rate was unaffected in loose soil (penetrometer resistance 0.06 MPa; Iijima *et al*., 2003). The reason for the slowing of root elongation was the associated increase in the root penetration resistance, equal to the force exerted per unit cross-sectional area of root tip, from 0.31 MPa to 0.52 MPa. This demonstrates that the intact root cap and its associated border cells and mucilage facilitate root penetration by decreasing the coefficient of friction between the root surface and soil particles (Bengough and McKenzie, 1997). Direct measurements of the frictional forces suggest that, in wet soil, the coefficient of root cap–soil friction is approximately 0.03 (Kirby and Bengough, 2002), with the border cells forming a low-friction sleeve around the root cap (Bengough and McKenzie, 1997). In soil drier than −60 kPa matric potential, however, the coefficient of friction may well be significantly greater, depending on the lubricating action of the less-hydrated mucilage between border cells and the root cap (Guinel and McCully, 1986; Read *et al*., 1999). The relative contributions of border-cell sloughing and mucilage to decreasing root penetration resistance have been estimated as 58% and 42%, respectively, for maize roots grown in loamy sand soil (Iijima *et al*., 2004), although the water potential around the root tip was not known precisely.

Growth control by individual cell layers

Cells in roots, with the exception of border cells, adhere firmly to their neighbours and columns of cells do not obviously slide past each other as the root grows (slow relative creep of adjacent cells under the influence of intracellular stresses, however, would be a relatively much more subtle effect and has not, to our knowledge, been definitively investigated and excluded). Due to this adhesion between cell layers, it is possible for individual cell layers to control root extension. Reviews of the cellular basis of root growth suggest that the inner root cell layers, rather than the epidermis and outer cortex, are more likely to limit root elongation (Barlow, 1989; Pritchard, 1994). This is in marked contrast to shoot elongation, in which the epidermis plays a dominant role in constraining extension (Kutschera *et al*., 1987). The evidence for the inner root tissues controlling growth are based largely on studies of the roots of maize (Bjorkman and Cleland, 1988) and wheat (Burstrom, 1949). Three types of evidence exist: experiments in which the root epidermis and cortex are dissected out or the epidermis is treated with *n*-diamylacetic acid (at a concentration that stops epidermal elongation) with no effect on whole root elongation or gravitropic response; observations of the way the root bends inward when divided longitudinally (Pritchard, 1994); and measurements of the mechanical stiffness of the stele versus the cortex and epidermis (Pritchard and Tomos, 1993). When taken as a whole, these experiments suggest the endodermis or inner cortical cell layer are likely to be important tissues for growth regulation of maize and wheat roots.

Recently, however, there has been renewed interest in studying the possible role of the epidermis in restraining the growth of the inner root tissues in *Arabidopsis* roots (Ubeda-Tomas *et al*., 2005). It is also possible that the transition to more rapid cell elongation between the meristem and the elongation zone of *Arabidopsis* may be influenced by the presence of the outer root cap cell layer (J Haseloff, personal communication). It could be that there are significant species

differences: *Arabidopsis* roots have an outer root cap cell layer that extends a distance of 3–4 times the root diameter from the apex, whereas, in maize, the outer cap cell layer may only reach 1.5 times the root diameter from the apex. Only a single cortical cell layer is present in *Arabidopsis*, in comparison to the many cell layers in the maize and wheat root cortices. In maize and wheat the epidermis may account for only a relatively small percentage of the root cross-sectional area, perhaps 5–10%, compared with 45–50% in *Arabidopsis*. In maize or wheat, therefore, to impose the same force limitation on the extension of the inner root tissues would require >5-fold greater average tensile stress in the epidermis, than in the *Arabidopsis* epidermis. In addition, there are preliminary observations that for pea roots grown in compacted sand the epidermal cell layer may collapse within a few mm of the root tip (Dr Trudi Gillespie, personal communication), suggesting that the epidermis is unlikely to regulate cell extension in this case.

Potential to combine modelling and image analysis studies at high resolution

To elucidate the tissues that control the root elongation rate requires spatial and temporal resolution adequate to discern the expansion rates of individual cells with a time-scale of minutes. One promising approach is the recent application of optical flow techniques to root growth kinematic analysis (Walter *et al*., 2002; Van der Weele *et al*., 2003). In preliminary experiments two optical flow techniques have successfully been applied to *in vivo* images of *Arabidopsis*

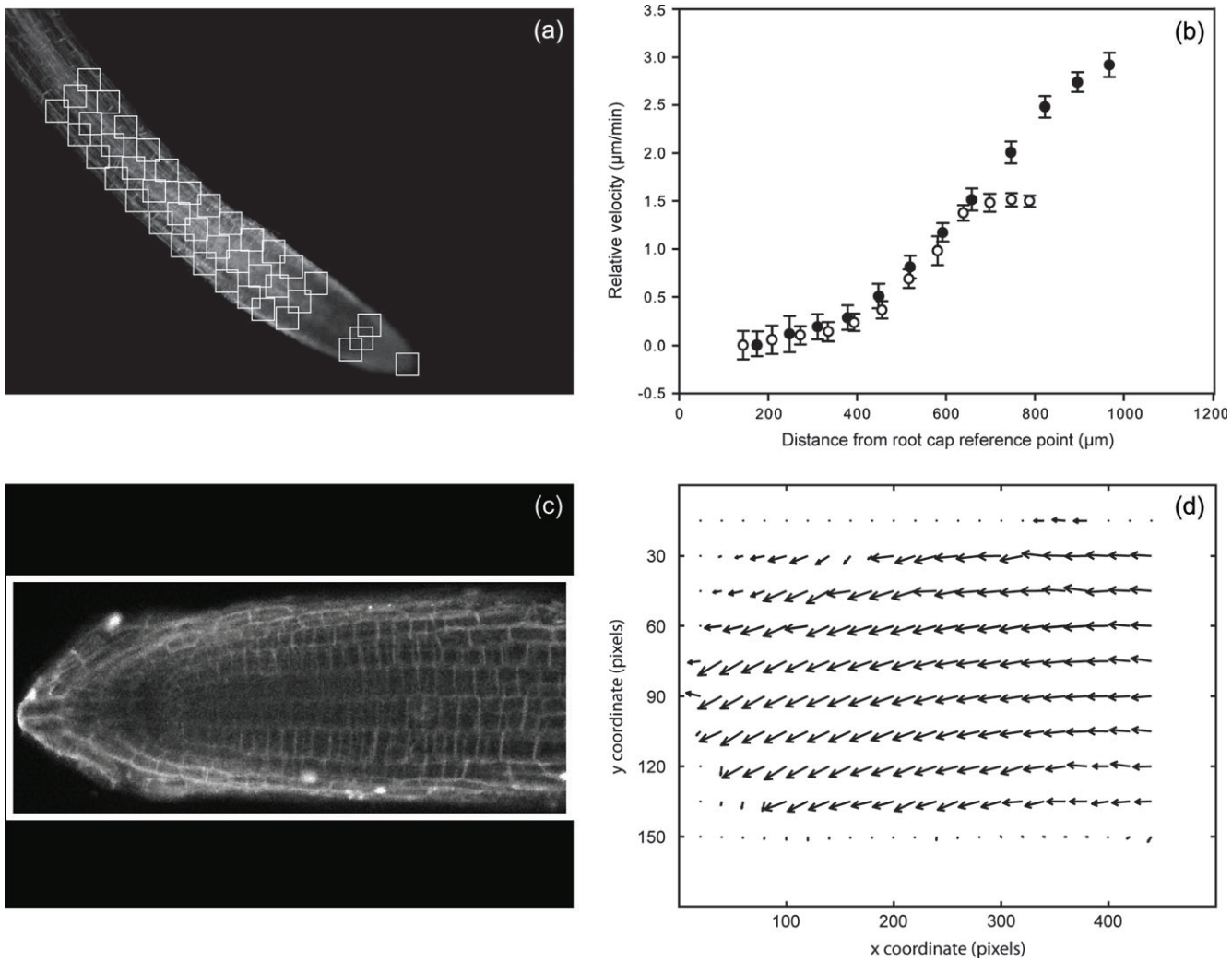

Fig. 5. Growth displacements between sequential confocal microscope images of living *Arabidopsis* roots. (a) Image of 35S ER-GFP *Arabidopsis* root with overlaid patches used for correlation-based optical flow analysis (method of White *et al*., 2003) and (b) resulting longitudinal velocities for two individual 14-d-old roots (hollow and filled circles). Means and standard errors are shown, each representing the velocities of three columns of cells monitored over ten 2 min time intervals. (c) Image of a WT *Arabidopsis* root stained with propidium iodide and (d) analysis of the boxed area by a robust optical flow estimation (based on the method of Black and Anandan, 1993). Arrows represent relative displacements between subsequent images (maximum displacement is approximately 5 pixels). The *Arabidopsis* roots are approximately 130 μm in diameter.

roots (Fig. 5), labelled with Green Fluorescent Protein (GFP) markers or fluorescent stain. It was found that it was possible to track changes in growth for groups of cells with a temporal resolution of minutes. Higher temporal resolution (e.g. tens of seconds) would depend on there being sufficient spatial displacement between sequential images and so would only be achievable at relatively high magnification. The development of methods for tracking cells and cellular structures, alongside optical flow techniques, offers the opportunity to develop more sophisticated three-dimensional models of root cell growth than has been possible previously. This approach will add dynamics to the visualization of cell morphogenesis (Haseloff, 2003) and, alongside targeted molecular approaches to manipulating cell properties, enable the complex process of root growth to be analysed. It is evident that many practical hurdles exist on the way, such as analysing and accommodating the growth rate changes due to root nutation: Such nutational movements are relatively large at high magnification, and increase the movements around the main axis of extension (Shabala and Newman, 1997; Walter *et al*., 2003). Further care must also be taken to check lens calibration within the field of view, translating a rigid object across a distorting lens will give rise to apparent strain (growth) as an artefact. The method of staining or marking the internal structure of the root requires further investigation to optimize image texture and contrast of the cell walls, without interfering with the natural growth processes.

Conclusions

The LLWR, when used in conjunction with monitoring the soil water status, provides a method of determining the soil physical stresses acting on a crop during a growing season. Root system growth and the elongation of individual roots are often limited by soil strength, and roots have evolved ways of penetrating and exploiting compacted soils. Modelling and image analysis methods, in combination with confocal microscopy and molecular methods, are promising techniques for giving new insights into the control of root elongation at the cellular level, and how roots respond to changes in environmental stress. This should ultimately enable more targeted approaches to selecting for root traits beneficial to crops growing in problem soils with relatively narrow LLWR.

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