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Mechanical impedance of root growth directly reduces leaf elongation rates of cereals

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SUMMARY

A dry soil is generally a hard soil. Thus, the effects of water stress and mechanical impedance on plant growth are difficult to separate. To achieve this we have developed a growth cell that allows manipulation of the strength of growth media (i.e. mechanical impedance) without altering the availability of water or nutrients. We monitored leaf elongation rates of barley and wheat seedlings before and after the mechanical impedance to root growth was increased. Results show that a large and rapid reduction (within 10 min) of leaf elongation rates occurred after impedance to the roots was increased. The average reductions for barley and wheat, with associated standard errors, were 22·6% (4·84) and 36·2% (5·48), respectively. The data are consistent with the hypothesis that mechanical impedance of roots might have a direct negative effect on leaf growth even where nutrients and water are in plentiful supply to the plant. The implications of the rate of the response are examined with respect to the underlying mechanisms controlling root–shoot signalling.

Key words: Soil compaction, leaf elongation, water stress, cereals, root–shoot signalling.

INTRODUCTION

Plant roots can sense adverse soil conditions and, via some internal signal, transmit the condition of the soil to extending leaves, with the typically net result of a decrease in leaf elongation rates. A variety of soil conditions which produce this effect have been examined in detail (for review see Jackson, 1993), but the interaction of plant development with soil water status has attracted most attention (Tardieu, 1993; Tardieu & Davies, 1993). However, it has been postulated that plant roots might have an ability to sense the strength of the soil (i.e. the mechanical impedance) in isolation from any associated water stress (Masle & Passioura, 1987; Passioura & Gardner, 1989). This is a particularly attractive alternative as it is recognized that root elongation rates are often more dependent on soil strength than on soil water status, within defined boundaries, as measured by soil matric potential (Taylor & Ratliff, 1969; Greacen & Oh, 1972).

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A reasonable body of literature exists supporting the theory that such an effect of mechanical impedance on roots might control the overall growth rate of plant leaves (Masle & Passioura, 1987; Ludlow *et al.*, 1989; Andrade, Wolfe & Fereres, 1993). However, some authors reject such a first order effect of impedance and insist that any change in leaf growth is due to water stress associated with soil compaction (Tardieu, 1993; Tardieu & Davies, 1993; Hartung, Zhang & Davies, 1994). The problem lies in the fact that a dry soil generally tends to be a hard soil with a high mechanical impedance, making it difficult to partition the effects of strength and matric potential. The question remains: can a plant sense the strength of soil *directly*, and does that strength control above-ground development?

To answer this we have adopted an approach which relies on specially constructed growth cells that allow manipulation of the strength of growth media, without altering their water or nutritional status. These cells are similar to those used by Abdalla, Hettiaratchi & Reece (1969), Goss (1977), Veen (1982) and Gordon *et al.* (1992) to examine the effects of soil strength on root growth. We measured

leaf elongation rates on a timescale of minutes, before and after increasing mechanical impedance to roots.

MATERIALS AND METHODS

The work presented was split equally between two laboratories, one in Scotland and one in Australia. Due to the differences in availability of materials and facilities in each laboratory, slight modifications to growth cells and environmental conditions were necessary. However, the essential details remained the same in each laboratory. All pertinent modifications to the experimental apparatus and conditions used are described.

Growth cells

Individual plants were grown in a series of growth cells to which an external confining pressure could be applied. Each cell consisted of acid-washed sand, surrounded by a flexible impermeable plastic or rubber membrane inside a rigid plastic tube, height 0·15 m, internal diameter 0·065 m (Fig. 1). The sand was kept saturated, either by circulating aerated Hewitt's solution, which was pumped in at the base of the cell (2·0 ml min^{-1}), or by slowly flushing aerated nutrient solution through the sand from a constant head. Nutrient solution was circulated through the growth cells at least 24 h before seeds were introduced. Solution pH was monitored daily. Typically, fresh solution was used every 2 d. Tubing ends were covered with a fine mesh to prevent sand blocking the system. Cells were held in place either in a rigid perspex frame, secured with screw rods and sealed with silicone grease and a rubber gasket at either end, or sealed at either end with end-caps.

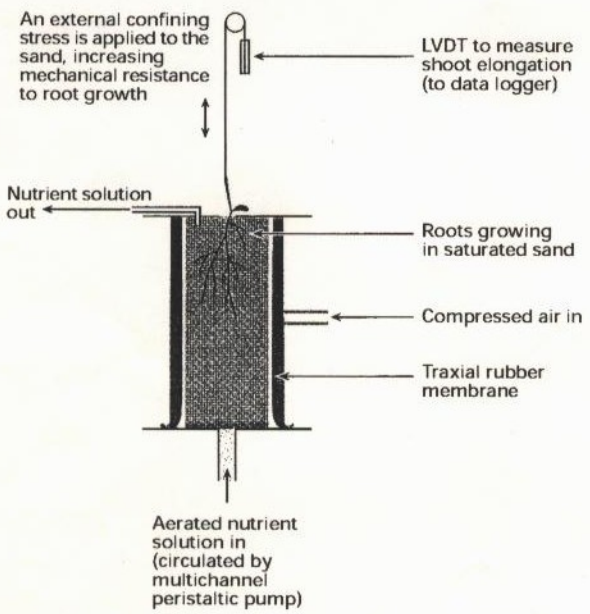

Figure 1. Schematic diagram of the growth cell apparatus used to regulate mechanical impedance to roots.

All cells were connected via tubing through the rigid plastic tube to a compressed air supply. Increased mechanical impedance to root growth was achieved by introducing compressed air via a pressure manifold to the air space between the impermeable membrane and the rigid plastic tube. This increase in pressure packed the sand grains more tightly increasing the mechanical impedance to subsequent root growth. It is important to note that the applied pressure was borne entirely by the sand matrix and did not affect the hydraulic potential of the nutrient solution, which remained at *c.* 0 kPa.

Seedling growth conditions

Wheat (*Triticum aestivum* L., cv. Hartog) and barley (*Hordeum vulgare* L., cv. Markina) seedlings were used in Australia and Scotland, respectively. All caryopses were surface-sterilized with a saturated solution of calcium hypochlorite, rinsed thoroughly in deionized water then imbibed in deionized water for 2 h. The caryopses were placed between two layers of blotting paper previously moistened with Hewitt's nutrient solution, and kept at 15 °C, in the dark, until approx. 5 mm of the first seminal root had emerged. Seedlings were then transplanted to seed holders at the top of the growth cell and covered with sand moistened with Hewitt's solution. The dimensions and shape of the seed holders were such that the seed was held slightly above the out-flow tube in moist, but not saturated, sand. To reduce evaporation and minimize heat exchange, the top of the growth cells and the connectors were covered with aluminium foil. Typically, shoot emergence occurred 2 d after transplanting.

This experimental approach meant that plant roots grew in a granular, saturated environment where nutrients, aeration and water were non-limiting, and mechanical impedance to root growth could be increased without altering the water or nutritional status of the sand.

All experiments were performed under controlled environmental conditions. The barley plants were grown at 21 °C with an 8 h dark and 16 h light phase at an irradiance of 500 μmol m^{-2} s^{-1}. The wheat plants were grown at 23·5 °C with an 11 h dark and 13 h light phase at an irradiance of 900 μmol m^{-2} s^{-1}.

Plant measurements and penetrometer resistance

The reduction in root growth was determined by measuring the length of the seminal roots from growth cells which were compressed continuously, whereas seminal roots were grown in non-compressed sand. Root lengths were measured with a ruler after carefully washing. Penetration resistance was measured using a 2 mm diameter 15° semi-angle probe with a non-relieved shaft at a rate of penetration of 10 mm min^{-1}. The resistance was

calculated after subtracting the resistance to withdrawal of the penetrometer probe (i.e. the shaft friction).

Preliminary experiments were carried out to find the linear growth range of the first and second leaves. Experiments were conducted only while plants were within this linear growth phase. Linear variable differential transformers (LVDT) were clamped to either the first or second leaf of plants growing in the growth cells. Cotton wool was placed over the leaf tip at the point of attachment to the LVDT to minimize damage to the plant. The LVDTs were connected to a data logger and measurements were recorded every 10 min. Normally, leaf elongation rates were measured for 24 h before and after raising the impedance. For the wheat plants, the LVDTs available could be used to measure the leaf growth over 2–3 d without repositioning (about 100 mm usable range). On a practical level this has great benefits. However, the voltage output over this distance was such that some resolution was lost. The LVDTs and logger used in the barley experiments had an operating length of 25 mm. The voltage resolution of the loggers resulted in a minimum displacement resolution of *c.* 14 μm.

To increase impedance to root growth, a pressure of 0·04 MPa was applied to the growth cells. The pressure was applied slowly to minimize sudden crushing effects and movement of the sand particles or seeds, and reached 0·04 MPa after 2 min. To calculate the leaf elongation rates we used the first measurement of leaf length after the pressure had been applied fully and there was no movement in the growth cell. Generally, for each run, three growth cells were pressurized and three acted as controls.

Analysis of elongation rates

In the case of wheat and barley, the leaf elongation rates were analysed directly before and after pressure application.

Possible wounding effects

The surface of the wheat roots was examined after the experiment by environmental scanning electron microscopy (E-SEM, Electronscan II). Fresh roots were carefully washed free of sand and transferred to the cold stage of the E-SEM. Manipulation of the stage temperature and chamber water vapour and pressure allowed viewing of the fresh roots for up to 15 min before root hairs showed signs of desiccation. Leakage tests were also performed on unimpeded and impeded roots. For the leakage tests, the plant-sand mixture was immersed in distilled water 15 min after increasing the impedance. Roots were then separated carefully from the sand and washed in tap water for 60 s before being rinsed in distilled water then in distilled deionized water, for 30 s. The roots were then cut from the seed and placed in test tubes with 6 ml of distilled deionized water, covered with Parafilm® and left for 30 min at 25 °C. The electrical conductivity of the solution (EC_{fresh}) was then determined using a conductivity meter (Model 1671, Jenco Electronic Ltd). The solution, still containing the roots, was transferred to a warm water bath, brought to boiling point and held for 30 min before samples were removed and left to cool to 25 °C, when the electrical conductivity of the solution was again measured (EC_{total}). Readings were corrected with a blank, and the percentage of solutes (charge equivalents) leaking out of the roots was calculated by dividing the EC_{fresh} by the EC_{total}.

Stomatal conductance

Stomatal conductance was measured on the first leaf of barley plants at four 15-min intervals before and after pressure application, using a Delta T AP4 Porometer.

RESULTS

Penetration resistance and root responses

The application of pressure to the growth cells, via the impermeable membrane, resulted in a *c.* 40% ($P < 0{\cdot}01$) decrease in root elongation rate, in the case of wheat seedlings. Penetration resistance at the mid-point in the growth cells supporting barley increased from 0·14 MPa, in unimpeded cells, to 3·38 MPa in impeded cells.

Stomatal conductance

Stomatal conductance of barley did not vary significantly before or after impedance (Fig. 2).

SEM and leakage tests

No differences were observed between impeded and unimpeded wheat roots under E-SEM. The application of pressure to the growth cells did not increase the leakage of solutes from the wheat roots (Table 1).

Response of leaf elongation rates to impedance

For both wheat and barley plants, increasing impedance to root growth had a large and rapid negative effect. Figure 3 *a*, *b* shows mean responses observed for barley and wheat, respectively, during the light cycles. Overall, the average reductions for wheat and barley (with associated standard errors, and number of replicates) were 36·2% (5·5, $n = 8$) and 22·6% (4·9, $n = 8$), respectively. The control plants showed no significant change in elongation rates over the same time period. For the barley plants, leaf 1 had a

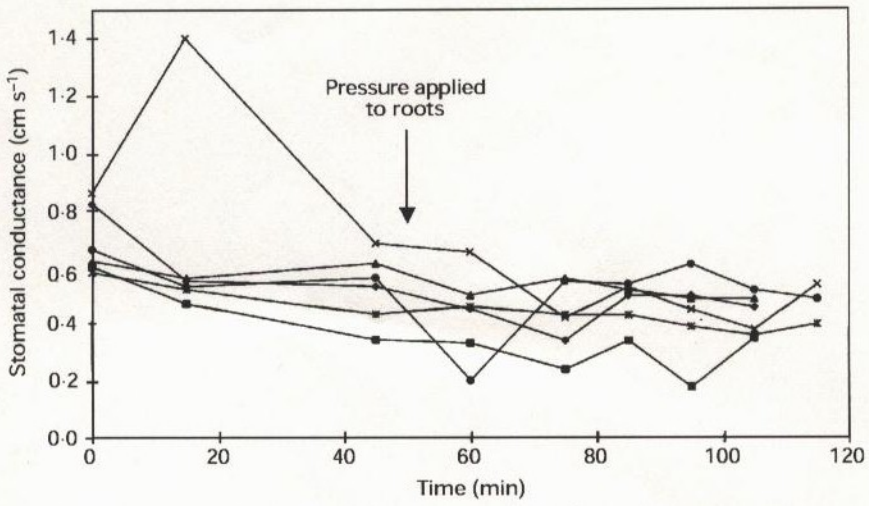

Figure 2. Stomatal conductance of six barley plants, before and after mechanical impedance was increased in the root zone.

Table 1. *Solute leakage, measured as the electrical conductivity (EC) of the bathing solution, from unimpeded (control) and mechanically impeded wheat roots*

Treatment	EC^*_{fresh}	EC_{total}	Leakage (%)
Control	41 (17)	1148 (110)	3·6 (3)
Impeded	35 (23)	948 (152)	3·7 (1)

Values are means with ± one SE in parentheses.
* See text for details.

29·6% (3·8) reduction in elongation rate compared with a 16·4% (2·9) reduction for leaf 2. During the dark cycles the leaf elongation rate of the wheat plants was unaffected by increasing impedance in the root zone (0·87 ± 0·09 mm h^{-1} and 0·96 ± 0·05 mm h^{-1} in unimpeded and impeded roots, respectively).

DISCUSSION

The dynamic method adopted to increase mechanical impedance of the root would have undoubtedly resulted in some compression of the mature root tissue and possible wounding, but we do not consider that this played a major role in the subsequent response of the leaf. The external pressure experienced by the root system would have been relatively small, approximately equal to the confining pressure. By comparison, the pressure a root must exert to grow through the sand would be five to nine times greater than the confining pressure (Bengough & Mullins, 1990). This increase in confining pressure caused no obvious damage to either the root surface or the integrity of the root cell membranes (Table 1). In a similar growth cell apparatus, Sarquis, Jordan & Morgan (1991) reported the absence of root damage, even though their roots were subjected to impedances higher than those used in our experiment.

Hydraulic and electrical signals, due to wounding, (with any corresponding change in growth rates) have been shown to last only 10–15 min in damaged leaves/shoots (Boari & Malone, 1993; Stahlberg & Cosgrove, 1995). Transient (10 min) *increases* in leaf elongation rates due to scorching of neighbouring leaves, were shown to be caused by increases in leaf turgor (M. Malone, unpublished). This is in contrast to the longer term *reduction* in leaf elongation rate observed in our experiments. The balance of evidence suggests strongly that wounding of roots did not play a significant role in decreasing the leaf elongation rates.

Leaf elongation rates

We see a large and significant reduction in leaf elongation rates once root elongation is physically impeded. Since water and nutrient availability remained constant during the experiment, it is clear that the response is related directly to the increased mechanical impedance experienced by the roots, in agreement with studies which reported that the leaf growth of wheat seedlings was reduced when plants were grown in compact soil (Masle & Passioura, 1987; Passioura & Gardner, 1989). In these studies measurement of leaf nutrient, water and carbohydrate status failed to explain the reduced leaf growth. However, when plants are grown in compact soil it is difficult to rule out completely transient shortages in water or nutrients due to reduced root extension. Such shortages could cause a down regulation of shoot growth resulting in the shoot maintaining critical nutrient and water status. Since in our experimental system the sand was saturated and there was always a plentiful supply of water and nutrients to the root system, we can confidently rule out decreased water or nutrient availability. Such a reduction in leaf elongation could arise if compression somehow decreased the hydraulic con-

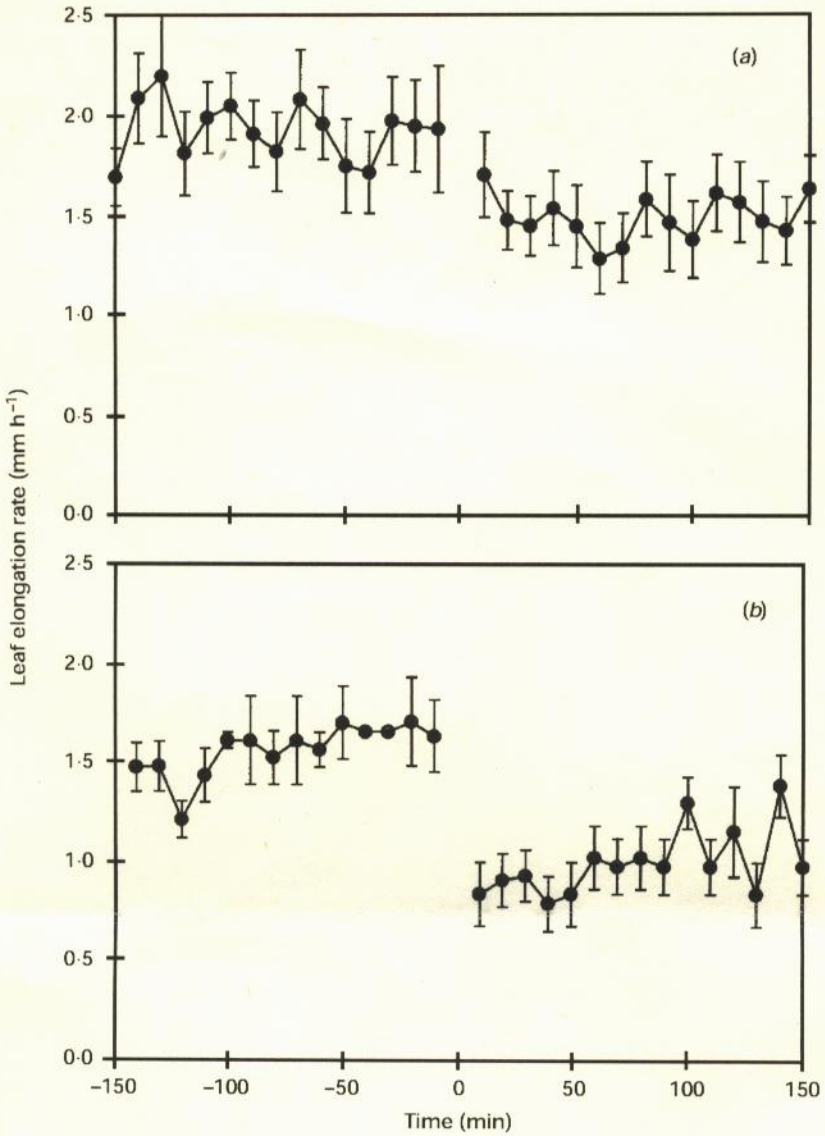

Figure 3. Mean leaf elongation rates as a function of time. (*a*) Barley. (*b*) Wheat. An external pressure was applied to the growth cell at time zero. Error bars indicate ±SE.

ductivity of the root. However, such a decrease in hydraulic conductivity should have caused a decrease in stomatal conductance, which was not observed (Fig. 2). Furthermore, if water uptake by the roots was reduced by increases in mechanical impedance then a decrease in stomatal conductance could be expected. Clearly this did not occur (Fig 2). Our results, therefore, provide strong supportive evidence for the existence of shoot-inhibiting signals being generated by roots growing in a medium of large mechanical impedance.

Tardieu *et al.* (1991), Tardieu, Zhang & Davies (1992), and Tardieu & Davies (1993), however, could find no evidence of root messages which affected stomatal conductance being generated by roots growing in compact soil. Instead, the shoot/root water relations of plants grown in partially compact soil were affected by a more rapid decrease in root water potential and water flux arising from the altered root spatial arrangement. It is perhaps not suprising that only second order effects of compaction were observed in their studies, since a relatively small portion of the root system was growing in the compacted soil. Shoot parameters are often unaffected when plants are grown in a heterogeneous mechanical impedance environment (Kirkegaard, So & Troesdson, 1992; Hartung *et al.*, 1994). Interestingly, we did not observe any change in stomatal conductance despite the large reduction in leaf elongation. Consequently, leaf elongation rates, in the first instance, were far more sensitive to the increase in mechanical impedance than was stomatal conductance, as suggested by Ludlow *et al.* (1990).

Mechanism of response

It is not our intention to speculate on which specific signals might be involved in this response, but simply to give some clues. The speed of the response

in our experiment might suggest that an internal hydraulic signal not related to external water relations is responsible for the decreases in leaf elongation rates. This is consistent with some preliminary data of impedance imposed during the dark phase when leaf turgor would be high. Only when transpiration began, during the light phase, was any reduction in leaf elongation observed. A qualitatively similar response in leaf elongation rates has been observed when corn roots (Barlow & Boersma, 1972) or French beans (Sattin *et al.*, 1990) were cooled rapidly also causing the leaf elongation to decline rapidly. Both groups of authors considered that a fall in leaf turgor was the prime cause of reduced leaf elongation, with decreases in root temperature reducing the hydraulic conductivity of the roots. In other work, however, leaf growth responses of barley and sorghum to decreasing root temperature have been reported to be uncoupled from water transport properties (Bassirirad, 1991). In our work, no decrease in stomatal conductance, which might be expected to be associated with a negative hydraulic signal, was observed. The most likely alternative is that a chemical signal transported in the transpiration stream is involved.

We recognize that ABA has been supported strongly as a signal in relation to water stress and compaction (Morgan, 1990; Tardieu *et al.*, 1991; Jackson, 1993; Hartung *et al.*, 1994). The studies that implicate ABA generally argue that any ABA response is associated with water stress that accompanies compaction, rather than with roots sensing the soil strength directly. However, for ABA, or other phytohormones, to be a candidate as the signal in our experiments, the process from sensing of the mechanical impedance in the root through to a reduction in leaf elongation rate, must occur within 10 min. At present, the dynamics of ABA are poorly understood. In the case of another hormone, ethylene, production in response to increased mechanical impedance only became measurable 30–60 min after the increase (Sarquis *et al.*, 1991).

Our data, and those of Masle & Passioura (1987), refer to young cereal plants, whereas other authors examined plants at maturity (e.g. Tardieu *et al.*, 1991). The overall effect of impedance on plant growth might be expected to be greatest when a young root system is establishing itself and its entire root system is in hard soil. For a mature plant with a deep, extensive root system which has expanded in soil with a wide range of impedance regimes, the growth of only a portion of the root system in compact soil might affect plant development to a lesser extent, as in the case reported by Tardieu & Davies (1993). The importance of the timing and proportion of roots exposed to compact soil, to subsequent shoot behaviour, warrants further investigation. It seems doubtful that 10 min is sufficient for the synthesis of a chemical message in the root, its transport to the shoot and its action on leaf elongation. Further possibilities are that impedance modulates the rate of export of a pre-existing chemical to the shoot or activates a chemical already present in the leaf. This would permit the faster response time observed in our experiments. As yet we can not draw any definite conclusion as to the nature of the signal involved.

To conclude, we point to a direct initial effect of impedance of root growth on the leaf elongation rate, even where water and nutrients are plentiful. We support the original hypothesis of Masle & Passioura (1987) that plants, in some way, are able to sense the strength of the soil in isolation from the water status of the soil.

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