Water relations and leaf expansion: importance of time scale

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Abstract

The role of leaf water relations in controlling cell expansion in leaves of water-stressed maize and barley depends on time scale. Sudden changes in leaf water status, induced by sudden changes in humidity, light and soil salinity, greatly affect leaf elongation rate, but often only transiently. With sufficiently large changes in salinity, leaf elongation rates are persistently reduced. When plants are kept fully turgid throughout such sudden environmental changes, by placing their roots in a pressure chamber and raising the pressure so that the leaf xylem sap is maintained at atmospheric pressure, both the transient and persistent changes in leaf elongation rate disappear. All these responses show that water relations are responsible for the sudden changes in leaf elongation rate resulting from sudden changes in water stress and putative root signals play no part. However, at a time scale of days, pressurization fails to maintain high rates of leaf elongation of plants in either saline or drying soil, indicating that root signals are overriding water relations effects. In both saline and drying soil, pressurization does raise the growth rate during the light period, but a subsequent decrease during the dark results in no net effect on leaf growth over a 24 h period. When transpirational demand is very high, however, growth-promoting effects of pressurization during the light period outweigh any reductions in the dark, resulting in a net increase in growth of pressurized plants over 24 h. Thus leaf water status can limit leaf expansion rates during periods of high transpiration despite the control exercised by hormonal effects on a 24 h basis.

Key words: Leaf growth, water stress, salinity.

Introduction

The role of water relations in controlling leaf growth under stress has been debated vigorously for some time. Some authors have emphasized the role of cell turgor or tissue water status in determining leaf growth rates (Kramer, 1988; Frisch, 1997; Hsiao et al., 1998) while others have emphasized the role of signals from roots (Termaat et al., 1985; Passioura, 1988a; Saab and Sharp, 1989; Gowing et al., 1990). These root signals could be hormones such as abscisic acid (Davies and Zhang, 1991), or chemical signals such as pH (Bacon et al., 1998). The debate has been sustained by a number of apparently contradictory findings on the extent to which leaf growth is controlled by leaf water status. However, it is possible that differences in the time scale over which the treatments were studied may have confused much of the debate.

Sudden environmental changes bring about rapid and often transient changes in leaf elongation rate. Such environmental changes include light intensity (Christ, 1978), humidity (Parrish and Wolf, 1983; Shackel et al., 1987), and soil water potential caused by changes in salinity (Cramer and Bowman, 1991a; Neumann, 1993) or polyethylene glycol (Acevedo et al., 1971). The speed of the response suggests that it is changes in leaf water status that drive the initial changes in elongation rate, presumably mediated by changes in turgor. This conjecture has been explicitly confirmed: when wheat and barley plants are kept fully turgid throughout such environmental changes, by placing their roots in a pressure chamber and raising the pressure so that the leaf xylem sap is maintained at atmospheric pressure, the changes in leaf elongation rate essentially disappear (Passioura and Munns, 2000). Furthermore, with salinized plants, pressurizing the roots also prevents the persistent fall in elongation rate that accompanies the application of salinity, at least for many hours (Passioura and Munns, 2000).
However, at a time scale of days, root pressurization fails to maintain high rates of leaf elongation of plants in either saline or drying soil, indicating that messages from roots are overriding the effects of shoot water relations (Termaat et al., 1985; Passioura, 1988b; Passioura and Gardner, 1990; Munns et al., 2000). Also ‘split root’ experiments, in which shoot water status is maintained high by having roots split between two pots, one of which is kept well-watered while the other is allowed to dry, show that shoot growth slows down even though the shoot water status is maintained to a high degree through access to the wet soil (Saab and Sharp, 1989; Gowing et al., 1990).

This paper attempts to reconcile these apparently inconsistent responses by exploring the possibility that the dominating effect of leaf water status on leaf elongation might be short-lived, and be overridden by root signals after several hours, or as long as it took for signals arising in roots to accumulate in the growing zone in effective amounts. The aim here is to understand why the short-term responses to changed water status (minutes to hours) may become invisible at time scales of a day or more. This paper examines the transition between the short-term and the long-term responses to water status. Working on the hypothesis that root signals might take several hours to accumulate to high enough levels to influence the elongation rate, this present study prolongs the exposure of plants to salinity while maintaining them at ‘balancing pressure’, the pressure in the root chamber required to keep the leaf xylem at atmospheric pressure. The behaviour of shoots whose roots had been removed before exposure to salinity is also examined. Previous work with wheat and barley is extended to include experiments with maize. Longer-term experiments with barley also look at the effects of high transpirational demand on the relative role of leaf water status and root signals in controlling leaf elongation rates.

In much of the work described here, salinity is used to induce water stress. Salt is considered preferable to mannitol or PEG because of specific inhibitory effects of the latter compounds on plant growth (Yeo et al., 1991; Chazen et al., 1995; Verslues et al., 1998). There would have been no salt-specific effect on leaf growth caused by NaCl over the period of experimentation (Munns et al., 1995). Possible artefacts induced by the pressurization technique used to maintain shoots at high water status, such as infiltration of air spaces in the roots, and oxygen toxicity resulting from pressurization with compressed air, have been previously examined and overcome (Passioura and Munns, 1984; Termaat et al., 1985).

**Materials and methods**

**Short-term responses to rapid changes in environmental conditions (time scale of minutes to hours)**

**Rapid changes in pressure in the root chamber or in humidity:** For the experiments manipulating humidity or pressure, barley (Hordeum vulgare L. cv. Himalaya) or wheat (Triticum aestivum L. cv. Hartog) seedlings were maintained at a photosynthetic photon irradiance of about 400 μmol m⁻² s⁻¹ in a growth cabinet with a daylength of 10 h, and an ambient temperature of 18 °C. The plants were grown in a sandy loam soil in pots that could be placed in a pressure chamber, essentially as described previously (Stirzaker and Passioura, 1996). The pots were made of PVC tubing, 86 mm in diameter and 200 mm long, and held 1.6 kg soil.

Leaf elongation was measured with an LVDT (linear variable differential transformer, HP Model 7DCDT-500, Andover MA, USA). The LVDT was mounted vertically above the growing leaf (usually leaf 4) and the core was attached to the tip of the leaf with a fine gold chain and a smooth-jawed lightly-sprung clip. Suction was applied to the top of the LVDT to levitate the core and to generate a pull of 2 g weight on the leaf. Data were collected at 30 s intervals by a data logger (HP 3421A), converted to elongation rates by dividing the increment in leaf length by the time interval, and smoothed with Fig.P software by using a running quintic equation covering 5 min of data. The basal 50 mm of the shoot was enclosed in an aluminium sleeve whose temperature was controlled at 20 °C by a Peltier-block.

Pressure was supplied as an automatically controlled mixture of air and N₂ to ensure that the partial pressure of O₂ remained at about 21 kPa, its value in normal air (Termaat et al., 1985; Passioura, 1988b). Step changes in pressure of 100 kPa were applied in the root chamber and the leaf elongation was measured with an LVDT. Another LVDT was used to measure the thickness of a mature leaf, as a surrogate for changes in leaf water status (Malone and Stankovic, 1991).

Changes in humidity were induced by enclosing the shoot in a glass cuvette and controlling the humidity of the air entering the cuvette by injecting water at a constant rate into a previously dry air stream.

**Rapid changes in salinity:** For the salinity experiments, maize (Zea mays L. hybrid ‘PB8099’ or barley (cv. Himalaya) seedlings were grown in pots were filled with coarse river sand in order to allow rapid changes in soil solution. Plants were watered with a modified Hoagland’s solution (Termaat and Munns, 1986) containing 4 mM Ca²⁺ at full strength. Barley was grown as described above, maize was grown at temperatures of 25 °C during the day and 20 °C during the night. Changes in salinity were made by flushing the soil with the nutrient solution containing NaCl with enough supplemental Ca²⁺ added as CaCl₂ to ensure that the ratio of Na⁺:Ca²⁺ was no more than 15:1.

Balancing pressure was established and maintained by connecting the sensor of a pressure controller to a cut in the xylem of the first leaf. The sensor determined whether or not sap was bleeding from the cut xylem, and the controller lowered the pressure in the chamber containing the pot if the xylem was bleeding and raised the pressure if it was not. The details of this procedure have been described previously (Stirzaker and Passioura, 1996; Passioura and Munns, 2000). To enable the balancing pressure to be automatically maintained while the soil solution was changed, the pot within the pressure chamber was connected with pressure tubing to a raised reservoir that could also be pressurized, and saline solution was delivered by gravity through the tubing while the whole system was balancing pressure. Soil solution was changed by flushing with 600 ml (twice the volume of resident solution) introduced through a porous disc at the top of the pot.

**Effects of rapid changes in salinity on plants with excised roots:** For the excised root experiments, maize or barley were grown...
in solution culture in plastic containers containing half-strength Hoagland’s solution aerated with compressed air. Four plants were selected of the same age and leaf elongation rate and placed singly in 2 litre plastic boxes containing half-strength Hoagland’s solution. Roots were excised below the surface of the solution, about 1 cm from the root tip, and their leaf elongation rates monitored daily with a ruler. The next morning, two plants with similar leaf elongation rates were attached to LVDTs and the growth rate monitored for a few hours to check that their leaf elongation rate matched before the start of the pressure treatment. One plant was brought to balancing pressure, and this treatment was maintained over a diurnal cycle of 8 h light (25 °C) and 16 h dark (20 °C) for up to 3 d.

Barley under constant temperature and low evaporative demand (replicated treatments): For this experiment with replicated treatments, barley seedlings (cv. Himalaya) were grown in sand mixed with perlite (4:1 on a volume basis) in small stainless steel cylindrical pots (45 mm in diameter and 150 mm long) designed to fit in 18 small pressure chambers (as described in Termaat et al., 1985; Passioura, 1988b). The plants were grown in a cabinet with a photosynthetic photon irradiance of 400 µmol m⁻² s⁻¹ and a photoperiod of 8 h; ambient temperature was 13 °C during the day and 17 °C at night, giving a constant growing zone temperature of 17 °C, which was measured by inserting a fine thermocouple inside the sheath of leaf 1 at the base of the shoot. The pots were initially watered with full-strength Hoagland’s solution until leaf 2 was emerging, then the salinity was increased to 100 mM NaCl in steps of 25 mM twice a day. Pressurization started at the beginning of the next day. Plants were placed in pressure chambers that were connected in series to a pressure controller. The chambers were bled at a rate that would prevent build-up of gases produced in the soil such as ethylene or CO₂. An average balancing pressure was maintained manually by ensuring that the xylem of the oldest leaf of at least half the plants was just bleeding at all times; this required regular observation during the day and into the first 3 h of the dark period; then the pressure was fixed at that value for the rest of the night. There were three treatments: 100 mM NaCl with balancing pressure, 100 mM NaCl without pressure, and control plants without NaCl and without pressure, six plants per treatment. Length of leaves 3 and 4 were measured with a ruler at the start and end of the light period.

Results and discussion

Short-term responses to rapid changes in environmental conditions (time scale of minutes to hours)

Rapid changes in pressure in the root chamber or in humidity: Rapid increases in leaf water status were made to wheat or barley leaves by applying step changes in pressure (complete within a few seconds) to the root chamber. These treatments caused large but transient changes in leaf elongation rate, the rate returning after 20–30 min to near the original level (Fig. 1). That the applied pressure rapidly increased the water status of the shoot was shown by the concurrent increase in thickness of a mature leaf (Fig. 1).

With these fairly minor changes in leaf water status, the steady rate of elongation after the transients were complete returned to a rate that was very close to the original one. In other words, the steady elongation rate was independent of leaf water status, at least to a first
Fig. 2. Effect of changes in humidity on the elongation rate of a barley leaf. The vertical broken lines mark the times at which the humidity was changed. The accompanying values of the difference in vapour pressure (vpd, kPa) between leaf and air, in the steady state, are shown on the graph.

approximation. A possible explanation for this is that the turgor of the growing cells had adapted to the increase in tissue water content—that solute fluxes across the plasma membrane changed to restore cell turgor to its original value. Evidence for this has been found in mature leaves (Tomos and Leigh, 1999), and suggested for growing tissues (van Volkenburgh, 1999). However, direct measurement of turgor in growing leaves of dicotyledonous species (grapevine and begonia: Shackel et al., 1987; Serpe and Matthews, 1992) showed that step changes in water status produced stable step changes in turgor—there was no relaxation of turgor towards its initial value. In that case, the properties of the expanding walls must have changed. A molecular explanation for turgor-dependent changes in wall rheology is proposed in the quantitative molecular model of Passioura and Fry (Passioura and Fry, 1992).

Rapid changes in salinity: Irrigation with saline solution reduced leaf elongation rate of unpressurized plants, which partly recovered after 2–3 h to about half the rate before the salt solution was applied. This is shown for maize irrigated with 80 mM NaCl (about 400 kPa) in Fig. 3a. Irrigation with nutrient solution without salt reversed this behaviour; after a transient and very large increase in growth rate, the rate returned to that before the salt solution was applied (data not shown).

The effects of salinity differed from those of humidity in that the original elongation rate was not regained, however, this is probably due to the extent of the water deficit caused by the respective treatments: salinity lowered the leaf water potential more than low humidity (400–500 kPa for salinity compared to only 100–150 kPa for humidity). If a larger change in humidity had been imposed, it would probably have been found that the elongation rate did not fully recover. This is indicated by the results of Ben Haj Salah and Tardieu with maize (Ben Haj Salah and Tardieu, 1996); they found that an increase in vpd causing a decrease in leaf water potential of about 700 kPa caused a decline then partial recovery of leaf elongation rate. The effect of salinity on growth is not due to a specific effect of the NaCl, as similar responses have been found with other osmotica such as PEG, mannitol or KCl (Acevedo et al., 1971; Cramer and Bowman, 1991a; Yeo et al., 1991; Chazen and Neumann, 1994).

When the water status of the shoot was maintained at its maximum, under automatic control, while the salinity of the soil solution was increased, the balancing pressure rose sharply and prevented any decrease in leaf expansion rate (Fig. 3b). The increase in balancing pressure of 400 kPa equalled the osmotic pressure of the saline solution (Fig. 3b). When the salt was removed the balancing pressure fell sharply and again prevented any change in elongation rate (Fig. 3b). That is, rapid changes in salinity had no effect on the elongation rate of plants maintained at balancing pressure.

The experiment shown in Fig. 3 for maize seedlings at 80 mM NaCl was repeated many times with both maize and barley seedlings over a range of 50–100 mM NaCl. These all gave the same results, namely, that balancing pressure prevented both the transient and persistent effects of salinity on leaf elongation rate, suggesting that changes in leaf water status were responsible for the decrease in leaf elongation caused by soil salinity, and that root signals had no detectable role over the first few hours. A different type of experimentation had led Cramer and Bowman to the same conclusion (Cramer and Bowman, 1991b). They excised a large fraction of the roots of
maize plants before increasing the salinity of the solution and found that the leaf elongation rate decreased to a similar extent to that in intact plants, indicating that root signals did not affect elongation rates for several hours. Therefore, it was decided to extend these experiments for a longer period of time, and with barley as well as maize.

**Effects of rapid changes in salinity on plants with excised roots:** Excised root experiments were done with both barley and maize. When roots were first cut, there was a transient rise in growth rate (Fig. 4a), consistent with a release of xylem tension and a sudden increase in shoot water status as in the sudden changes shown in Fig. 1. When salt was added to the nutrient solution, the leaf elongation rate of intact plants became negative, i.e. the leaf shrank, and continued to shrink for an hour. Then the shrinkage stopped, but the leaf did not grow for at least another hour. In contrast, the leaf elongation rate of plants whose roots were excised shrank only briefly and recovered relatively quickly (Fig. 4b). This experiment was repeated for up to periods of 6 h, with both barley and maize. Leaves that were cut but not exposed to saline solution maintained a normal rate of elongation for 4–6 h, but after this the rate declined, so these experiments were terminated at this time. Addition of 30 mM up to 100 mM NaCl produced the same pattern of response.

The greater effect on intact than de-rooted plants is surprising. It may have been due to differential effects on the hydration of the walls of the growing leaf cells. With intact plants, the increased tension in the xylem following application of NaCl to the solution around the roots will increase matric suction in the walls of the leaf cells, which will cause the wall to shrink and the pore size to decrease so that the movement and hence activity of proteins may become impeded. If the pores in the wall are 4 nm or less, shrinkage could affect the movement of proteins of molecular weight of 30 kDa such as expansin or XET (Passioura, 1994). With de-rooted plants, the salt solution will travel up the xylem and though causing the protoplast of leaf cells to shrink, will not dehydrate the walls and so not impede enzyme movement. The more rapid recovery of elongation rate in plants with excised roots is consistent with this theory. Alternatively, the ready availability of NaCl in the xylem of the leaves of plants without roots may have provided a supply of solutes for more rapid osmotic adjustment and hence recovery of turgor in the growing cells.

**Longer-term responses to salinity (time scale of hours to days)**

These data above showing that elevated water status prevents the effects of salinity on leaf elongation rate, and that prior excision of roots had no effect, seemingly contradict previous publications that showed that growth in saline soil over several days was not affected by water status (Termaat et al., 1985; Munns and Termaat, 1986; Munns et al., 2000). Therefore, the transition between short-term and long-term responses and, in particular, the diurnal behaviour of salinized plants, was looked at closely.

**Maize at the day-night transitions:** Two salt-treated maize plants were chosen whose growth rate was the same. Balancing pressure was applied to one plant, while the other was left unpressurized. On the first day, pressurization increased the growth rate dramatically while the light was on, but when the light was turned off, the growth rate of the pressurized plant fell to about half that in the light (Fig. 5a). At the same time, the growth rate of the unpressurized plant increased transiently, and settled down at a rate similar to that in the light, and only a little lower than that of the pressurized plant in the dark (Fig. 5a). When the light came on the next morning, the reverse occurred, i.e. there was an increase in growth rate of the pressurized plant, and the usual transient decline then recovery of the plant without pressure (data not shown). During this second day, the pressurized plant grew faster than the unpressurized one in the light period, but during the night it grew slower (Fig. 5b). This relationship between pressurized versus unpressurized plants, namely the growth rate being higher during the daylight period, and lower during the night, continued for third day, at which time the experiment was terminated as the leaves had reached the end of their linear elongation phase of growth. The experiment was repeated with two more pairs of plants, with similar results to that shown in Fig. 5b.

The total growth of the plants shown in Fig. 5 was calculated over the experimental period. Over the first day, the pressurized plant elongated more than the unpressurized one, but over the next 2 d, the elongation of the two plants was similar. However, without replication (the automatic balancing technique could be used with only...
Fig. 5. Effect of pressure on diurnal changes in leaf elongation of salt-treated maize seedlings growing in the light for 8 h at 25 °C and in the dark for 16 h at 20 °C. Two plants were previously grown in 80 mM NaCl for 48 h, and pressure was applied to one, 4 h before the end of the first day. (a) Leaf elongation before and after the first light-dark transition, marked with a vertical broken line. (b) Diurnal behaviour of leaf elongation of the pressurized (●) and unpressurized (○) plant, measured continuously with LVDTs and averaged over the light and the dark periods. Thick lines on the x-axis denote night-time.

Barley under constant temperature and low evaporative demand: To avoid the confounding effects of different day and night temperatures on leaf growth as in the maize shown in Fig. 5, plants were grown in a controlled environment cabinet and the temperature of the cabinet was adjusted so that temperatures of the growing zone were constant in light and dark (17 °C). The salinity was raised gradually to 100 mM NaCl before the pressurization treatments started. A control treatment, without salt and without pressure, was included. Not included was a pressurized control treatment, without salt, as previous experiments had shown that there was no effect of pressurization on control or unstressed plants (Passioura, 1988b).

Figure 6 shows that the pressurized plants grew better than the unpressurized plants during the day but not during the night. On the first day of pressurization, the leaf elongation rate of pressurized plants in the first light period substantially exceeded that of unpressurized salt-treated plants, but was below that of the controls that were without salt and without pressure (Fig. 6). In the first dark period, it became the same as that of unpressurized salt-treated plants. In the second light period it was again greater than unpressurized salt-treated plants and in the second dark period was less (Fig. 6). That is, the same pattern occurred as with maize. These plants were harvested at this stage, but in other experiments that were continued for another 2 or 4 d (data not shown), the same pattern occurred, i.e. that pressurized plants grew faster during the light period and slower during the night. When the total elongation over 24 h was calculated, it showed that pressurization raised the growth rate only on the first day, but had no significant effect on the second day. Growth rates of the pressurized plants were $23 \pm 1 \text{ mm d}^{-1}$ on the first and $20 \pm 1 \text{ mm d}^{-1}$ on the second day, while those of the unpressurized plants were $20 \pm 1 \text{ mm d}^{-1}$ on both days (data derived from Fig. 6). There was no net effect of pressurization over 24 h in this experiment, or in other similar experiments (Munns et al., 2000). The data thus confirm the result with the maize plants shown in Fig. 5. Water relations measurements (Munns et al., 2000) had confirmed that the water content of both growing and expanded tissues of the salt-treated plants were indeed raised by pressurization, and similar to the control plants without salt.

Figure 6 also shows that the unpressurized salt-treated plants grew better at night than in the day. As the
temperature of their growing zone was constant day and night, it would be tempting to think that better water relations was responsible for their better growth at night. However, when the water relations were measured, it was found that the salt-treated plants had similar water relations day and night (Munns et al., 2000).

In all the experiments done at a constant growing zone temperature of 17 °C, the reduced growth at night in the pressurized plants counteracted the increased growth during the day, so that the total elongation over 24 h was not affected by pressurization. However, it is possible that this near-perfect counteraction would not occur at very high daytime water deficits, as indicated by recent results (Ben Haj Salah and Tardieu, 1997), which proposed that during such times there was a superposition of water status effects on hormonal regulation of leaf expansion.

Barley under high day:night temperature and high evaporative demand: Plants were grown at a higher daytime temperature to raise the vpd (25 °C in the growing zone) with lower night-time temperature (15 °C), and balancing pressure was applied to plants as the salinity was increased to 75 mM and held there for 4 d. As shown before, balancing pressure enhanced growth only during the light period (Fig. 7a), and not in the dark, and often there was a depression although this was not statistically significant. In this experiment, pressurization brought the growth rate during the light period very close to that of controls, a phenomenon that was not seen with a higher salinity (this was the only long-term experiment done at 75 mM NaCl; the others were at 100 mM NaCl). The total growth increment over 24 h was significantly increased by pressure, although not to the level of the control plants without salt (Fig. 7b). Thus the plants at the end of the experiment had a significantly larger leaf area (data not shown).

Longer term responses to drying soil

Previous experiments with wheat in drying soil had also shown that pressurization did not affect growth rate on a 24 h basis, leading to the conclusion that hormonal signals from roots were controlling leaf expansion rates (Passioura, 1988b; Passioura and Gardner, 1990). In those experiments, when growth was dissected into increments during the light and the dark period, the same phenomenon described above was noted: that in water-stressed plants, pressurization increased the growth rate during the light period but decreased it during the dark (Fig. 8). Pressurization had no effect on well-watered plants in either light or dark periods (data not shown), but as the soil became drier, a differential effect in the light and dark periods became more evident (Fig. 8). Growth on a 24 h basis was not altered by pressurization.

General discussion

The findings presented here help to resolve many of the apparently contradictory reports on the extent to which leaf growth is controlled by leaf water status, by following responses over different time scales. When plants were pressurized (kept fully turgid) throughout sudden environmental changes, both the transient and persistent changes in leaf elongation rate disappeared, indicating that water relations are responsible for the sudden changes in leaf elongation rate resulting from sudden changes in water stress and putative root signals play no part. These findings are consistent with those of other authors who have emphasized the role of cell turgor or tissue water status in determining leaf growth rates (Kramer, 1988; Frensch, 1997; Hsiao et al., 1998). However, when
pressurization was maintained over a number of days, the negative response during the dark periods, which in some cases completely counteracted the growth promotion during the light periods, showed that water relations alone does not determine the elongation of a leaf over the time scale of 24 h. This is consistent with those who have emphasized the role of signals from roots in controlling growth in dry or saline soil (Termaat et al., 1985; Passioura, 1988a; Davies and Zhang, 1991).

The data presented here for the longer-term experiments show that shoot water status can regulate leaf expansion during the light period, particularly when a low soil water potential is coupled with a high evaporative demand. This interpretation is consistent with that of Ben Haj Salah and Tardieu, who proposed that chemical messages from roots in dry soil were regulating growth in both light and darkness, but that during periods of high evaporative demand, a low leaf water status could reduce growth further (Ben Haj Salah and Tardieu, 1997). These authors proposed that the chemical message was abscisic acid (ABA) produced by the roots and carried in the xylem sap. However, the behaviour of the pressurized plants, which grew slower at night, cannot entirely be due to ABA produced by roots. If it was, pressurization would have had to increase the flux of ABA from the roots at night, so it was higher than in the unpressurized plants. This is very unlikely as pressurization does not alter the flux of water in the xylem; it did not alter the stomatal conductance or transpiration rate of plants in saline or drying soil (Termaat et al., 1985; Gollan et al., 1986; Passioura, 1988b). Further, earlier experiments with salt-treated barley showed that pressurization did not affect the ABA concentration in roots, leaves or xylem sap (Zhao et al., 1991).

So, what is regulating leaf expansion at night? A depletion of carbon or nutrient reserves is unlikely to be limiting. The lower growth rate at night did not occur as a gradual drop throughout the dark period; the rate changed within 30 min of the light going off, and stayed steady during the night (Fig. 5). This was also found with the barley plants whose growth data are shown in Fig. 6; many individual replicates with LVDTs were monitored (data not shown) and it was found that growth during the night was steady. This suggests that the pressurized plants were not running out of nutrients or carbon substrates. To test the possibility that the higher growth rate during the day of the pressurized plants might have depleted carbohydrate levels, and this might decrease the growth rate during the night, plants were harvested at the beginning and end of the light period for measurements of total reserve carbohydrates. Levels of reserve carbohydrates (sugars, fructans and starch) were similar in pressurized and unpressurized salt-treated plants, and were a little higher than those in plants without salt (Munns et al., 2000). Thus the lower growth at night of the pressurized plants was not due to insufficient carbon substrate.

The most likely control of leaf expansion in water-stressed plants during the night would be exerted by hormones, arising either from the roots, or from the mature leaves. This control would appear to integrate growth on a 24 h basis, so that the faster growth in the light period of the pressurized salt-treated plants was counteracted in the dark period. The regulation of this is obviously complex, and is unlikely to be due to the action of a single hormone alone. The rapid change to a new elongation rate at the start of the dark period (Fig. 5a) is consistent with a phytochrome-mediated regulation of hormonal function. In unstressed plants, the levels of gibberellins (GA) in leaf growing zones vary diurnally (Foster and Morgan, 1995). The levels of ethylene (Finlayson et al., 1998) and ABA (Weatherwax et al., 1996; Audran et al., 1998) in whole shoots also vary diurnally; growing tissues have not been measured. The synthesis or accumulation of ethylene is clearly under phytochrome control (Finlayson et al., 1998), and it is possible that GA and ABA are also (Lee et al., 1998; Weatherwax et al., 1996). Little is known about the effects of water stress on diurnal patterns of hormonal control; the only published data are for ABA, and only for whole shoots or xylem sap. The flux in the xylem from roots of water-stressed plants is higher during the day than the night (Loveys, 1984; Schurr and Schulze, 1995; Ben Haj Salah and Tardieu, 1997), and the level of ABA in the shoots of water-stressed plants increases towards the end of the day, often dropping gradually over the night (Henson et al., 1984; Loveys and Düring, 1984). However, it is not known whether there are diurnal patterns in the levels of ABA in the growing cells, or whether these cells synthesize their own ABA, or whether water stress affects the compartmentation or receptivity to ABA within these cells.
Water stress decreased the level of gibberellins in growing tissues of soybean hypocotyls, while increasing the levels of ABA (Benson et al., 1990). These authors tried to distinguish between the roles of GA and ABA in controlling growth rates by following changes through time in the levels of these hormones shortly after a water stress was imposed or removed. They found that growth rates recovered faster than did GA levels, but coincided with reductions in ABA levels, and so concluded that growth is more likely to be responding to ABA than GA. However, the results presented here suggest that the rapid recovery of growth on relief of stress was due entirely to the increase in water status, and that a regulatory role of GA in the long-term or steady state cannot be ruled out.

The biochemical mechanism by which hormones might be controlling cell expansion is still unclear. Whether or not turgor pressures are higher in the light or the dark, or are affected by water stress or by pressurization, is difficult to know. Measurements of the turgor of growing cells in leaves of rapidly-transpiring cereal seedlings is difficult if not impossible to measure with confidence. Cell turgor might adjust to environmental changes by changes in ion transport across the plasmalemma (as discussed by van Volkenburgh, 1999), but how this adjustment might be signalled and regulated is unknown. Is it under hormonal control?

In conclusion, there is much evidence that shoot water status or cell turgor can regulate leaf growth during periods of high transpiration rate, particularly when a soil water deficit due to drought or salinity is coupled with a high evaporative demand. However, at night, shoot water status is not regulating leaf expansion, and hormones probably are. The control of diurnal patterns in hormonal synthesis or transport to growing tissues may explain this complex regulation.

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