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Leaf water status controls day-time but not daily rates of leaf expansion in salt-treated barley

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Abstract. Barley plants were grown in pots that would fit inside a pressure chamber, so that their shoots could be kept fully turgid by applying pressure in the chamber to bring the xylem sap of the shoot to the point of bleeding. Pressurisation increased the growth rate of NaCl-treated plants in the light period but not in the dark. The promotive effect on growth was greatest in the light period of the first day of pressurisation, but disappeared during the first night. Pressurisation promoted growth the next day during the light period, but on the second night the elongation rate was significantly lower than that of unpressurised NaCl-treated plants. This pattern of high day-time and low night-time growth then continued indefinitely. The lower night-time growth counteracted the higher day-time growth, with the result that total growth over 24 h was the same as in NaCl-treated plants that were not pressurised. Levels of total reserve carbohydrates were unaffected by pressurisation, indicating that the slower growth of the pressurised plants during the night was not due to depletion of assimilates. These results are interpreted in the context of hormonal signals controlling growth on a 24-h basis, such that any short-term stimulation of growth arising from unusually high water status during the light period is counterbalanced by slower growth during the night.

Introduction

The aim of this study was to reconcile findings that short-term responses of leaf growth to changes in salinity were due to changed water status with findings that long-term responses were not, but were in fact controlled by hormonal signals from roots.

Leaf growth responds rapidly to a sudden rise in salinity. The elongation rate falls within minutes, to zero if the salinity is strong enough, then partly recovers over an hour or so to a steady rate whose value depends on the salinity level (Thiel *et al.* 1988; Cramer and Bowman 1991*a*). To test the view that this rapid response is due simply to lower leaf water status, in a recent series of experiments we maintained the water status of the shoot at its maximum while the salinity of the soil solution was increased, by automatically pressurising a chamber containing the roots to maintain the xylem of the shoot on the point of bleeding. This is called the 'balancing pressure'. When the soil was irrigated with a saline solution, the balancing pressure rose sharply and prevented any decrease in leaf elongation rate, which remained steady. When the salt was removed after 1–2 h, the balancing pressure fell sharply and again prevented any change in elongation rate (Passioura and Munns 2000). These experiments indicated that the lower water status induced by salinity, rather than any signals from roots, was responsible for the lower leaf elongation rate over this time frame. This is consistent with the results of Cramer and Bowman (1991*b*), who excised 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. They concluded that root signals had a minor role in determining leaf elongation rates in the first few hours after a change in salinity.

The results of these short-term experiments contrast with previous studies on salt-treated plants over a longer time frame, which showed that leaf water status did not affect leaf growth rates on a 24-h basis (Termaat *et al.* 1985; Munns and Termaat 1986). In those experiments, which lasted 5–7 d, the shoot water status was also elevated by pressurising the roots, but with a constant pressure equal to the osmotic pressure of the saline solution. The salinity used was usually 100 mM NaCl, so the pressure applied was 480 kPa. Our aim in the earlier studies was to counteract the osmotic effect of the saline solution to see if the pressurised plants with elevated shoot water status would grow as fast as the unsalinised controls. They did not, apart from an increase in the first day

Abbreviations used: ABA, abscisic acid; DW, dry weight; FW, fresh weight; LVDT, linear variable differential transformer; RWC, relative water content; WC, water content; ψ, water potential; π, osmotic pressure; $\pi_{(100)}$, osmotic pressure calculated for 100% RWC.

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(Termaat *et al.* 1985). There was no lasting effect of pressure on growth rate of salinised plants, over periods of several days, with barley and wheat (Termaat *et al.* 1985). Neither was there a response to elevated water status with species as diverse as white lupin (*Lupinus albus* L.), which is very salt-sensitive, the Egyptian clover (*Trifolium alexandrinum* L.), which is salt-tolerant, and the saltbush (*Atriplex spongeosa* F. Muell.), which is a halophyte that requires salinity for optimal growth (Munns and Termaat 1986; Munns 1993).

A likely explanation for this seemingly discrepant behaviour is that the dominating effect of leaf water status on leaf elongation might be short-lived, and would be overridden by hormonal signals after several hours, or as long as it took for signals arising in roots to accumulate in the growing zone in effective amounts. In case root signals might take several hours to accumulate to high enough levels to influence the elongation rate, in the present study we prolonged the exposure of plants to salinity while maintaining them at balancing pressure. We hypothesised that hormonal signals from the roots might interact with the effects of leaf water status on a diurnal basis. Earlier experience with droughted plants grown at balancing pressure was that they grew markedly faster than unpressurised plants during the day, but slower at night, with the result that the total growth over 24 h was not altered (Passioura 1988).

A preliminary experiment with salinised plants grown at balancing pressure (R. Munns and J. Passioura, unpublished data) indicated there might be a similar pattern of diurnal variation in leaf growth. Here we looked in detail at the effect of salinity and of pressurisation on the light:dark pattern of growth, to see if we could better define how roots signals seemingly override the effects of water status. Our aim was to understand why the short-term responses to changed water status (minutes to hours) might become invisible at time scales of a day or more. We also searched for an explanation of the findings in terms of the carbohydrate status of the plants.

We used balancing pressure (which maintains xylem sap in the shoot at atmospheric pressure), rather than just a constant pressure, to balance the osmotic pressure of the salt as in the earlier studies. Barley was used as a model plant on the basis of previous studies. The salinity treatments used here are essentially water stress treatments. The salt concentration was not applied for long enough to have more than an osmotic effect on the plant (Munns *et al.* 1995). Salt concentrations had not reached toxic concentrations in the fully expanded leaves (no leaves died), and concentrations in the elongating cells would in any case be quite low (Munns *et al.* 1988). The results are therefore indicative of those of drought-affected plants. The temperature of the growing zone of the leaves was held constant, to avoid the complicating effects on leaf growth of the diurnal variation in temperature.

Materials and methods

Experiments lasting 2–24 h (with single plants, using automatically controlled balancing pressure and LVDT measurements of elongation growth)

Barley (*Hordeum vulgare* L. cv. Himalaya) plants were grown in sand in pots made of PVC tubing, 86 mm in diameter and 200 mm long. Plants were watered with modified Hoagland's solution containing 4 mM Ca^{2+} (Termaat and Munns 1986), grown at a photosynthetic photon irradiance of 400–450 µmol m^{-2} s^{-1} with a daylength of 10 h and a temperature of 18°C. The water status of the shoot was raised by placing a pot in a pressure chamber, and balancing pressure was established and maintained automatically by connecting the sensor of a pressure controller to the xylem of the first leaf, as described in Passioura and Munns (2000). The sensor determined whether or not sap was bleeding from the cut xylem, and the controller lowered the pressure if the xylem was bleeding and raised the pressure if it was not.

Leaf elongation was measured with a linear variable differential transformer (LVDT). 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 pressurised, and saline solution was delivered by gravity through the tubing while the whole system was under balancing pressure (Passioura and Munns 2000). The soil was made saline by introducing 600 mL of solution through a porous disc inserted at the top of the pot.

Experiments lasting 2-6 d (with replicate plants, using manually controlled balancing pressure and ruler measurements of elongation growth)

Barley plants were grown in sand mixed with perlite, in a ratio of 4:1 on a volume basis (to allow rapid drainage and prevent compaction of sand) in stainless steel cylindrical pots, 45 mm in diameter and 150 mm long, designed to fit in the small pressure chambers described in Termaat *et al.* (1985) and Passioura (1988). The plants were grown under a photosynthetic photon irradiance of about 400 µmol m^{-2} s^{-1} and a daylength of 8 h. The ambient temperature was set to give a constant growing zone temperature of 17°C, measured by a fine thermocouple inserted into the sheath of leaf 1 at the base of the shoot. A total of six experiments were completed, lasting 2–6 d.

The pots were watered with the modified Hoagland's solution, described above. Seedlings were fed with half-strength nutrient solution until leaf 2 was emerging, then full-strength solution for 1 d before starting salinity treatments. Salinity was increased by replacing the soil solution with a solution of NaCl mixed with full-strength nutrients. About 100 mL (twice the solution contained in the pot) was introduced via a syphon through a tube inserted at the top of the pot, allowed to drain, then placed on cotton towels for 10 min so that excess solution from the bottom of the pot was absorbed. NaCl was added to plants in steps of 25 mM over a period of 1 or 2 d. The final level was 100 mM in all but one experiment, where it was 80 mM.

There were normally three treatments: NaCl with balancing pressure, NaCl without pressure, and control plants without NaCl or pressure, with 5–6 plants per treatment. One experiment included an extra treatment of control plants with balancing pressure.

Plants to be pressurised were sealed in pots and placed in pressure chambers connected in series to a pressure controller operating cylinders of air and of N_2, as described above. The chambers were bled continuously to avoid a build-up of gases produced in the soil, such as ethylene or CO_2. Balancing pressure was established and maintained manually by ensuring that the xylem of the oldest leaves of three of the six replicate plants was just bleeding at all times (visual observation of sap appearing at a cut midrib at the tip of leaf 1). Because balancing pressure varies diurnally, depending on the transpiration rate and hydraulic resistance of the plant, frequent adjustments during the day

and into the first 3 h of the dark period were needed. Thereafter, the pressure remained stable, and was left constant for the rest of each night. Pressurisation started either at the beginning of the night when the final NaCl concentration had just been reached, or at the beginning of the next day. Balancing pressures were about 600 kPa during the dark period, and increased up to 1600 kPa during the light period. Plants that were not pressurised were also placed in pressure chambers to ensure they experienced the same light and temperature as the plants that were pressurised.

Plants were irrigated with new salt or nutrient solution every 2 d; this was done at the end of the dark period as pressure had to be taken off to do this, and we considered this gave the least disturbance to the treatment. The first experiment included an extra treatment of control plants with balancing pressure; these pressures were about 80 kPa during the dark and up to 450 kPa during the day.

Leaf elongation was measured from the ligule of leaf 1, which was fully elongated at the start of the measurements, at the beginning and end of the light period.

Water relations were measured in both fully expanded leaves and growing tissue, at two time points — at the end of the night period, and in the light period mid-morning. For the fully expanded leaf (leaf 2) the blade was cut 1 cm from the ligule and the water potential (ψ) was measured with a pressure bomb (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Osmotic pressure (π) was measured in the same material frozen in liquid N_2 and stored at –20°C until analysis with a freezing point depression osmometer (Micro-Osmometer Model 3MO, Advanced Instruments Inc., Needham Heights, MA, USA). Turgor was estimated as $\psi + \pi$. Relative water content (RWC, % of maximum water content) was measured on the remaining basal 1 cm of the blade, from the weight before and after floating on water in a Petri dish for 4 h, and the dry weight after 16 h at 70°C. For the growing tissue (basal 20 mm of the growing leaf), only the fresh and dry weights were measured, because the RWC of elongating tissue cannot reliably be measured due to water uptake associated with continued growth of the tissue.

Water use per unit leaf area was measured from the weight loss of pots. Leaf area was estimated from the length and width (maximum width × 0.75) of the blade of each emerged leaf.

Stomatal conductance was measured with a porometer (AP4, Delta-T Devices, Burwell, UK) in the middle of the light period (1100–1300 h).

Total reserve carbohydrates were measured in the whole shoot. Shoots were frozen in liquid N_2, and the soluble carbohydrates extracted with boiling 80% ethanol followed by 50% ethanol then hot water. Sugars in the combined extracts were assayed by the anthrone method, using glucose as standard (Yemm and Willis 1954). Starch in the residue was digested with amyloglucosidase and α-amylase according to Lunn and Hatch (1995) and glucose again assayed by the anthrone method. Results are expressed as mg glucose mg^{-1} FW of shoot. The FW:DW ratio was measured only in the light, and the same ratio was also used for the calculation of plants in the dark, being 9.71 for the controls and 8.69 and 9.65 for the unpressurised and pressurised salt-treated plants, respectively.

Results

Effect of elevated water status on leaf elongation rates during addition of salt

Replacing the soil solution with 100 mM NaCl caused a rapid decrease in leaf elongation rate: growth ceased for 30 min, then gradually recovered to a rate about half of the original one (Fig. 1). If the plant was maintained at balancing pressure throughout the change in salinity, the effects of the sudden increase in salinity on leaf elongation were completely removed (Fig. 2). The pressure required to keep the plant at balancing pressure rose by about 0.5 MPa, the amount expected to balance the osmotic pressure of the NaCl. Balancing pressure caused the plant to maintain a high elongation rate for the duration of the treatment, which was 16 h in this case.

Experiments adding NaCl to plants under balancing pressure were repeated many times, for periods up to 24 h in continuous light (data not shown). Plants were kept under continual light to avoid the large transient effects on leaf elongation rate caused by sudden changes in illumination (Passioura and Munns 2000). In no case was there evidence of a decrease in leaf elongation rate. This indicated that water relations were largely responsible for the decrease in leaf elongation rate shown in Fig. 1, and that root signals had little role in determining growth rates. There might have been small growth reductions of the order of 10% after several

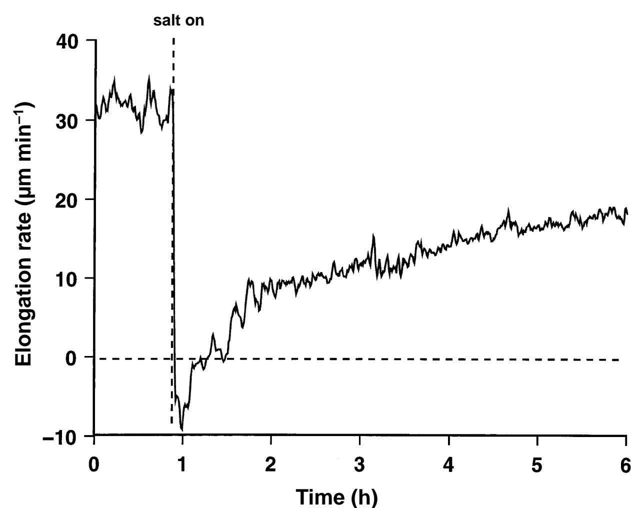

Fig. 1. Effect of the addition of 100 mM NaCl on leaf elongation rate of barley without pressurisation, and in the light. The dotted horizontal line shows an elongation rate of zero.

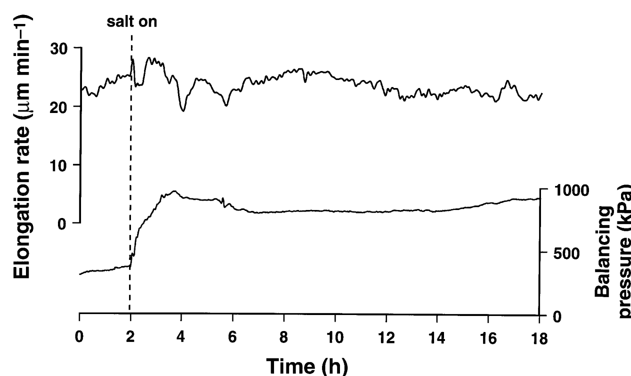

Fig. 2. Effect of the addition of 100 mM NaCl on leaf elongation rate of barley plants while maintained at balancing pressure in the light. Balancing pressure is shown on the lower trace.

hours that we could not detect because the elongation rate of any one leaf was not steady over 24 h, and variations or oscillations of 10% or more in the intrinsic growth rate were common over that period of time. But it is clear that any such effects would be insignificant compared to those found in plants whose water status was not elevated artificially, as in Fig. 1.

Effect of elevated water status on leaf elongation, water and carbohydrate status of salinised plants over several days

In order to measure the effect of pressurisation over several days, and to enable adequate replication (we could run only one plant at a time with automatically controlled balancing pressure), we grew plants in a growth chamber and used a ruler to measure leaf elongation during the light and dark periods. A total of six experiments lasting 2–6 d were carried out with plants already growing in saline soil, all including control treatments without salt and pressure.

Leaf elongation rates

Balancing pressure increased leaf elongation to a rate that was higher than the salt-treated plants without pressure, but growth during the first night was not elevated (Fig. 3). After this first 24-h period, a consistent pattern emerged: pressurisation increased growth during the light periods, but reduced it during the dark. The growth increment during the first light period after pressurisation exceeded that of the control plants without salt. This was due to a growth surge when the pressure was first applied, as described in an associated paper (see Fig. 2, Passioura and Munns 2000). In contrast to the pressurised plants, salt-treated plants without pressure grew faster in the dark than the light (Fig. 3). Control plants without salt or pressure grew at similar rates in the dark and light. Thus, salinity reduced the light:dark ratio of growth, and pressurisation increased it, above that of the control plants (Fig. 4).

On a 24-h basis, the rate of leaf elongation of salt-treated plants was increased by pressure during the first 24 h, but not subsequently (Fig. 5). The elongation rate increased to that of the controls without salt for the first day, due entirely to the effect during the first light period (Fig. 3), but after the first 24 h the rate was indistinguishable from that of salt-treated plants without pressure (Fig. 5). The decrease in elongation during the night in the pressurised plants balanced the increase in elongation during the day, with the result that the total elongation growth over 24 h was the same for pressurised and unpressurised plants.

A similar result was found in another experiment when the pressure treatment started at the beginning of the night period and ran over a 4-d period (data not shown). The growth rate in the first night was increased by pressurisation, and in the first light period, but was reduced in the second night (data not shown). This experiment also included a second control treatment, namely plants without salt but with balancing pressure. There was no significant difference between these two treatments. Previous studies had also shown no effect of balancing pressure on growth of unstressed plants (Passioura 1988).

Five separate experiments confirmed that by the second day, day-time growth was still enhanced by pressure, but night-time growth was not, and was often lower. On this second day of pressurisation, the average in the five separate

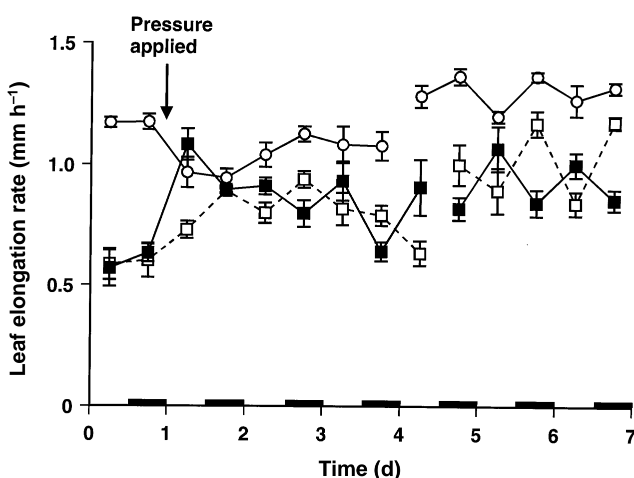

Fig. 3. Effect of salinity (100 mM NaCl) and pressurisation on leaf elongation rates in the light and dark period. Gaps indicate when the measurement changed from leaf 2 to leaf 3. ○, control plants with no salt and no pressure; ■, salinised plants at balancing pressure; □---□, salinised plants with no pressure. Bars on the *x*-axis denote the dark periods. Vertical bars show the s.e.m.

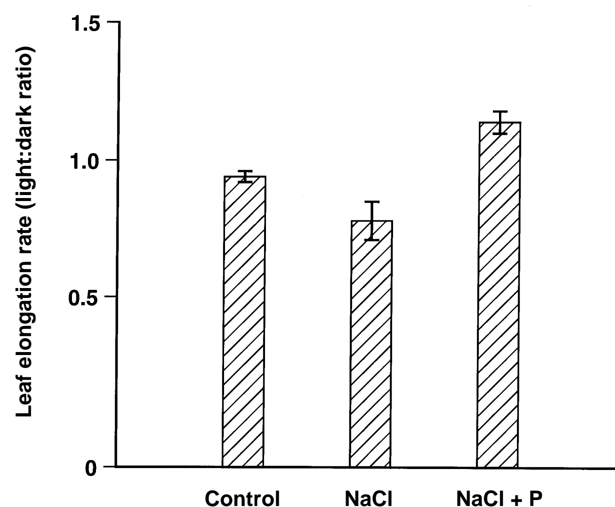

Fig. 4. Effect of salinity (100 mM NaCl) and pressurisation on the ratio of leaf elongation rate during the light and the dark period for the experiment shown in Fig. 3, except for the first day of pressurisation. Vertical bars show the s.e.m.

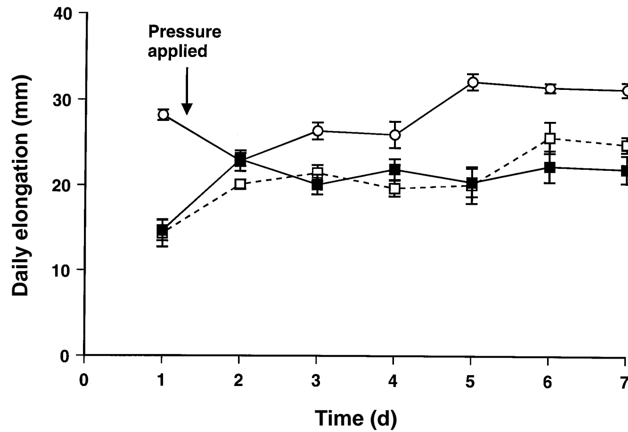

Fig. 5. Effect of salinity (100 mM NaCl) and pressurisation on leaf elongation rates on a 24-h basis for the experiment shown in Fig. 3. ○, control plants with no salt and no pressure; ■, salinised plants at balancing pressure; □---□, salinised plants with no pressure. Vertical bars show the s.e.m.

experiments for the day:night ratio of elongation rate was 0.95 ± 0.06 for control plants, 0.70 ± 0.05 for salt-treated plants, and 1.06 ± 0.08 for pressurised salt-treated plants.

Water relations

Water status during the night was raised substantially by pressurisation. Leaf blades of the plants whose growth is shown in Figs 3–5 were sampled at the end of the last night. In fully expanded tissue, the total water potential was as expected, i.e. close to zero in the pressurised plants and about –0.5 MPa in the unpressurised salt-treated plants, similar to the osmotic pressure of the soil solution (Table 1). The calculated turgor was 0.3 MPa higher for the pressurised than unpressurised salt-treated plants (Table 1). Turgor for the NaCl-treated plants could be overestimated, as there could be 0.1 MPa of salts in the xylem (Munns and Passioura 1984). In that case, the pressure bomb measurement would overestimate ψ by 0.1 MPa, and the calculated turgor would be overestimated by the same amount. However, the same error would apply to plants with or without pressure, as balancing pressure did not alter the transpiration rate (Table 2) so would not be expected to alter the concentration of solutes in the sap. The RWC would not be subject to this error, and was much higher in the pressurised plants than in the unpressurised, and similar to the controls.

In the growing zone, the only parameter of water relations measured was the water content (g H_20 g^{-1} DW), as we had no way of measuring the total water potential of this small tissue, and RWC cannot be measured in rapidly growing tissues. The water content of the growing zone was much higher in the pressurised plants than in the salt-treated plants without pressure, even higher than the controls without pressure (Table 1).

Water status during the day was also substantially raised by pressurisation. Similar trends were found as in the dark; that is, the turgor and RWC of the expanded leaves was higher with pressurisation, and the water content of the growing tissue was still much higher than that of the plants without pressure, by about 50% (Table 1).

There was some osmotic adjustment due to salinity — the $\pi_{(100)}$ (the osmotic pressure calculated for 100% RWC) was higher in the salt-treated plants than in the controls without salt by about 0.12 MPa (calculated from data in Table 1). However, there was little evidence of osmoregulation, i.e. a regulation of the osmotic pressure to maintain turgor. If there were osmoregulation, the levels of solutes would have been down-regulated in the pressurised plants. Instead, the turgor of the pressurised plants was 0.66 MPa higher than the unpressurised ones during the day, and 0.32 MPa during the night. That the pressurised plants had lower osmotic pressures than the unpressurised ones (Table 1) was due simply to the increased water content. The difference in osmotic

Table 1. Effect of salinity (100 mM NaCl) and pressurisation on leaf water relations
Tissues were sampled while still under balancing pressure. The fully expanded tissue was the blade of leaf 2. The growing tissue was the basal 20 mm of leaf 3 for salt-treated plants, and leaf 4 for controls

		NaCl	NaCl plus pressure	Control
End of the night				
Fully expanded tissue	ψ (MPa)	–0.51 ± 0.06	–0.05 ± 0.01	–0.13 ± 0.06
	π (MPa)	1.08 ± 0.02	0.94 ± 0.01	0.82 ± 0.01
	Turgor (MPa)	0.57 ± 0.05	0.89 ± 0.02	0.69 ± 0.06
	RWC (%)	84.0 ± 0.6	97.4 ± 0.7	97.4 ± 0.4
Growing tissue	WC (g H_2O g^{-1} DW)	7.0 ± 0.2	11.8 ± 2.5	8.5 ± 0.3
During the light period				
Fully expanded tissue	ψ (MPa)	–1.04 ± 0.02	–0.09 ± 0.04	
	π (MPa)	1.47 ± 0.03	1.18 ± 0.02	
	Turgor (MPa)	0.43 ± 0.05	1.09 ± 0.04	
	RWC (%)	82.1 ± 1.3	97.2 ± 0.04	
Growing tissue	WC (g H_2O g^{-1} DW)	7.0 ± 0.2	10.3 ± 0.1	

pressure disappeared when normalised for the different water content. For plants harvested in the dark period, the $\pi_{(100)}$ was 0.91 MPa for the salt-treated plants without pressure and 0.92 MPa with pressure (calculated from data in Table 1). For the plants harvested in the light period, the $\pi_{(100)}$ was 1.21 MPa for the salt-treated plants without pressure, and 1.15 MPa with pressure (calculated from data in Table 1). These data indicate that there was no system of osmoregulation operating, i.e. that turgor was not regulated by this means.

Water use

Transpiration was reduced by one third by salt treatment, and was not significantly affected by pressurisation except for the first day (Table 2). Stomatal conductance was also reduced by one third by salt treatment, and was also affected by pressurisation on the first day but not thereafter (data not shown).

Carbohydrate status

It is possible that the reduced growth of the pressurised plants during the night might be due to insufficient carbohydrate reserve, if the enhanced growth during the day consumed part of the normal reserves, and stomatal conductance and hence photosynthesis were not higher in the pressurised plants. Total reserve carbohydrates (total soluble sugars, fructans and starch) were measured at the end of the day and the end of the night. The whole shoot was measured, as reserve carbohydrates utilised by the growing leaf could be stored in older leaf blades or sheaths.

At the end of the light period, all salt-treated plants had a higher concentration of total reserve carbohydrates than the controls, due to increases in both soluble sugars and in starch (Fig. 6*a*). The pressurised plants had the same soluble sugar level but a little more starch than the unpressurised plants (Fig. 6*a*). At the end of the dark period, the carbohydrate level was lower in all treatments than at the end of the light period (Fig. 6*b*). The salt-treated plants again had a higher carbohydrate concentration than that of the control, and there was no significant effect of pressurisation (Fig. 6*b*).

We did not measure mono-and di-saccharides separately, so cannot calculate the osmotic pressure due to the sugars, but the fact that the pressurised plants here had the same high levels of monosaccharide equivalents as did the unpressurised plants suggests that the increase in sugars was associated in some way with the lower utilisation of assimilate in the salt treatment, rather than with putative osmoregulation. If there were osmoregulation, the levels of sugars would have been down-regulated in the pressurised plants.

The carbohydrate levels were also calculated on a DW basis, as the water contents of the treatments was different (Table 1). The total carbohydrates on a DW basis, along with the growth rates in the period preceding the harvest time, are shown in Table 3. The carbohydrate content of the salt-treated plants on a DW basis was not significantly higher than that of the controls at the end of the day, and only a little higher at the end of the night. The carbohydrate content of the pressurised plants was a little higher than that of the unpressurised plants, particularly at the end of the day, indicating that the subsequent lower growth at night of the pressurised plants was not due to insufficient assimilate.

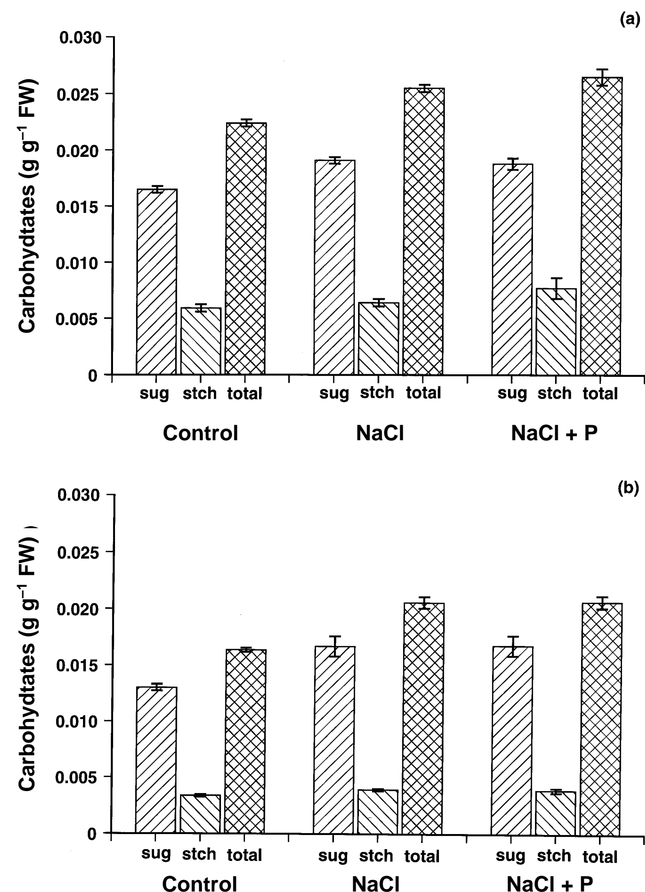

Fig. 6. Effect of salinity (100 mM NaCl) and pressurisation on reserve carbohydrate levels in the whole shoot (*a*) at the end of the light period and (*b*) at the end of the dark period. Values shown are for water-soluble sugars including fructans ('sug'), starch ('stch'), and the total of these ('total'). Plants were harvested at the end of the second day or second night after pressurisation started.

Table 2. Effect of salinity (100 mM NaCl) and pressurisation on transpiration on the first and second day of pressurisation

	Transpiration (mmol m^{-2} s^{-1})	
	First day	Second day
Control	2.17 ± 0.06	2.04 ± 0.02
NaCl	1.42 ± 0.02	1.33 ± 0.02
NaCl plus pressure	1.50 ± 0.08	1.33 ± 0.20

Table 3. Effect of salinity (100 mM NaCl) and pressurisation on leaf elongation rate and total shoot reserve carbohydrates for plants harvested at the end of the light or dark period
Elongation rate is the average for the light or dark period, respectively, preceding the harvest

	Light		Dark	
	Elongation rate (mm h^{-1})	Total reserve carbohydrates (mg g^{-1} DW)	Elongation rate (mm h^{-1})	Total reserve carbohydrates (mg g^{-1} DW)
Control	1.48 ± 0.05	217 ± 3	0.98 ± 0.02	159 ± 2
NaCl	1.00 ± 0.02	222 ± 2	0.94 ± 0.04	179 ± 4
NaCl plus pressure	1.47 ± 0.06	256 ± 6	0.78 ± 0.06	200 ± 6

Discussion

In this study we sought to explain the discrepancy between the short-term promotive effects of water status on leaf growth rate shown by Passioura and Munns (2000) and the lack of effects in the long-term found by Termaat *et al.* (1985).

Previous experiments had shown that balancing pressure prevented a fall in leaf elongation rate as the soil was made saline (Passioura and Munns 2000), but those experiments lasted only 1–2 h. We thought it likely that root signals might take more than 2 h to accumulate to high enough levels to influence the elongation rate, so we prolonged the exposure of plants to salinity while maintaining them at balancing pressure. We hypothesised that the promotive effects of water status on leaf elongation would diminish with time, as inhibitory signals coming from roots exposed to saline solutions started to accumulate in the shoots, so we ran some experiments for up to 24 h. However, despite the long times, the leaf elongation rates remained high. Plants were kept under continual light for these treatments, to avoid the sudden and large effects on leaf water status caused by sudden changes in illumination, and the accompanying transient but large changes in leaf elongation measured in plants whose water status was not controlled (e.g. Pardossi *et al.* 1994; Passioura and Munns 2000). Rapid elongation was maintained by pressurisation as long as the plant was held under continuous light. However, the introduction of a dark period diminished the promotive effect of pressurisation.

It became clear from longer term experiments that, after the first 24 h, pressurisation increased the rate of leaf elongation only during the light period. Faster elongation at night occurred only on the first night, and thereafter elongation was usually slower at night, so that the overall growth over 24 h was independent of the pressure treatment. Earlier work with wheat plants in drying soil (Passioura 1988) also indicated that pressurisation increased the leaf elongation during the light period but reduced it at night. What might cause this shift in the light:dark growth pattern?

One factor that we considered might have caused the lower growth in the dark of the pressurised plants was a differential effect of pressurisation on cell elongation and cell division, i.e. cell elongation might have responded more to elevated water status during the light period than did cell division, with a consequent lag due to new cell production in the dark. However, we found the same effect of pressure on the light:dark pattern of growth in leaves at all stages of development. Figure 3 shows that the same pattern occurred when leaves were less than 30% of their final length, during which time cell division would have been maximal, and also when they were nearing full expansion, by which time cell division would have finished (Schuppler *et al.* 1998). A more likely explanation for the diurnal effects on growth lies in water relations, carbohydrate levels or hormonal control.

Water relations

We had expected to find that the faster growth of unpressurised plants at night than during the day would be associated with higher turgor at night. We were unable to measure the turgor of the growing cells, but the water relations variables we did measure showed that, contrary to our expectation, the turgor and RWC of the fully expanded leaves of the unpressurised plants were essentially the same, and the water content of the growing tissues was exactly the same, day and night (Table 1).

However, pressurisation with its consequent high leaf water status did increase the growth rate of plants during the light period, which suggests that inadequate turgor was inhibiting growth of the unpressurised plants during the light, despite their showing no evident diurnal differences in turgor or RWC. Possibly there were diurnal changes in the rheological properties of the expanding cell walls — that is, in terms of the classical Lockhart model, there were changes in extensibility or yield threshold.

Similarly, it is hard to explain the lower growth of the pressurised plants at night in terms of water relations: even though the turgor of their fully expanded leaves was a little lower at night, the RWC of expanded leaf tissue of the pressurised plants exceeded 97% both day and night, and the water content of the growing tissues was large and similar day and night (Table 1). Neither can the fact that the pressurised plants grew slower than the unpressurised plants at

night be explained in terms of water relations: the pressurised plants had a much higher water status than the unpressurised plants (Table 1). Other controls must be operating.

Carbohydrates

Carbohydrate supply could conceivably limit growth at night, especially in the pressurised plants. The increased growth during the day caused by pressurisation, in the absence of a significant increase in stomatal conductance and presumably photosynthesis, might not have allowed the same build-up of carbohydrate reserves by the end of the day as in the unpressurised plants, and the supply of assimilate during the night might have limited growth.

However, we found that there was no reduction in the reserves of the pressurised compared to unpressurised plants, even at the end of the day when the plants had been growing faster than their unpressurised counterparts. Further, monitoring of growth rates during the night using displacement transducers (data not shown) indicated that the growth rate was steady throughout, and showed no sign of decreasing towards the end of the night, which might reflect a depletion of carbohydrate or nutrient reserves. On some occasions, LVDTs were connected to plants in the growth cabinet and the elongation rate monitored throughout the light and dark periods. The change from the rate in the light to that in the dark occurred at the light:dark transition, and the new rate became apparent within about 30 min of the lights going on or off. (It was difficult to ascertain what was happening within 30 min of the change in illumination, because of the transient surges or pauses that occurred (Passioura and Munns 2000), and the oscillations that usually followed.)

Hormonal regulation

In considering the effect of salinity alone (without pressurisation) on growth rates in the light versus the dark, it would be reasonable to consider the lower rate in the light as due to leaf water deficit superimposed on an inhibitory signal generated by the saline soil over the 24 h, as suggested by Ben Haj Saleh and Tardieu (1997) for plants in dry soil. If that were so, pressurisation should increase the growth rate in the light without affecting that in the dark. However, in the experiments reported here, it increased growth in the light, but depressed it in the dark, so an additive effect of water relations plus a constant supply of growth inhibitor does not offer a satisfactory explanation for the control of growth. A more satisfactory explanation is that there are control systems that regulate growth over 24 h such that faster growth during part of the day is counterbalanced by slower growth during the rest of the day.

Growth regulators induced by the saline soil might determine leaf expansion rate on a 24-h basis. These growth regulators would operate on diurnal patterns that are directly or indirectly influenced by water relations, and together control the daily elongation rate. There is little information on the effects of water stress on diurnal patterns of hormonal synthesis or turnover. In unstressed plants, ethylene production in shoots shows a marked diurnal fluctuation (Machácková *et al.* 1997; Finlayson *et al.* 1998), and is controlled by the phytochrome system (Finlayson *et al.* 1998). Gibberellin levels in the growing zones of unstressed sorghum leaves also show a diurnal fluctuation (Foster and Morgan 1995). There is little diurnal variation in abscisic acid (ABA) levels in unstressed plants, but there is strong diurnal variation in the level of transcripts of genes in the ABA biosynthetic pathway (Audran *et al.* 1998), which may be under phytochrome control (Weatherwax *et al.* 1996). Water stress can decrease the level of gibberellins in growing tissues (Benson *et al.* 1990) and increase the level of ABA (Benson *et al.* 1990; Chazen *et al.* 1995; Dodd and Davies 1996). Diurnal changes in the growing zone have not been measured.

Although ABA is the hormone most studied in relation to water or salinity stress, it is difficult to envisage a relationship between diurnal patterns of growth and of ABA levels. It is unlikely that pressurisation itself affects xylem or leaf ABA levels, either day or night. Pressurisation did not affect transpiration rates of the salt-stressed plants (Termaat *et al.* 1985), and our earlier experiments with salt-treated barley showed that pressurisation did not affect the ABA concentration in roots, leaves, or in xylem sap (Zhao *et al.* 1991), so the role of ABA in the light:dark regulation of growth is not clear.

Conclusions

Our results serve to re-interpret previous results that looked for coincidence between growth and hormonal changes in the short-term responses to stress. For example, Benson *et al.* (1990) concluded that gibberellins were not primary regulators of elongation in water-stressed soybean seedlings because growth recovered within 30 min of rewatering, whereas gibberellin (GA_1) levels showed a significant increase only after 2 h, and recovery to pre-stressed rates took the order of a day. However, our data show that any change in water relations will cause a rapid change in growth, and will dominate any effect of hormones for many hours. A full diurnal cycle is evidently needed to allow hormonal regulation to fully exert itself, and once exerted it seemingly controls growth rate on a 24-h basis, counterbalancing any short-term stimulation of growth arising from unusually high water status during the light period.

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