Root Signals Control Leaf Expansion in Wheat Seedlings Growing in Drying Soil

J. B. Passioura
Division of Plant Industry, CSIRO, G.P.O. Box 1600, Canberra, 2601, Australia.

Abstract
Wheat plants were grown with their roots and soil in pressure chambers, so that the leaves could be kept highly turgid, even when the soil dried, by applying a pneumatic pressure to the roots. The relative leaf expansion rate (RLER) of plants in drying soil eventually fell behind that of well-watered plants, but, remarkably, the fall in RLER was the same whether or not the leaves were kept highly turgid. It is argued that the roots sensed the drying of the soil and sent signals to the leaves that controlled their behaviour, overriding any effects of turgor on the leaves. It is likely that the roots were sensing not only the water potential of the soil but also its hardness, which increased substantially as the soil dried.

Introduction
When a wheat plant is growing in a drying soil, both the water status of its leaves, and their growth rate, eventually fall. It is tempting to conclude, in accordance with the prevailing view of the physiology of water-deficient plants, with its emphasis on turgor as an important physiological variable (e.g. Kramer 1983), that the falling water status is the main cause of the falling growth rate; after all, cells without turgor will not expand. But the connection between turgor and expansion is not so simple as once thought. Although there are examples of plants behaving as expected in relation to water status (e.g. Waldron and Terry 1987), there are also examples in which expansion is unrelated to turgor (Wenkert et al. 1978; Cutler et al. 1980; Michelena and Boyer 1982; Shackel et al. 1987).

The aim of the work described here was to test the proposition that the falling growth rate in a drying soil is caused by the falling water status, by breaking the nexus that normally occurs between the water status of shoot and of root. This nexus can be broken by growing the plants with their roots and soil in a pressure chamber, and applying enough pressure in the chamber to maintain the leaves turgid despite the drying of the soil (Passioura and Munns 1984). If the growth rate of the leaves were controlled by turgor, then plants treated in this way would continue expanding their leaves quickly even though the soil was quite dry. This work is an extension of that of Gollan et al. (1986) who showed, using this technique, that the stomata of wheat and sunflower started closing when the soil in which the plants were growing dried beyond a certain point, even when the leaves were kept highly turgid. Munns (1987) has also shown using this technique that the elongation rate of leaves of barley growing in a drying soil seemed independent of whether or not the leaves were kept highly turgid. The implication is that the roots sense adverse conditions in the soil and send a signal to the leaves that controls their behaviour.
Materials and Methods

Wheat plants (*Triticum aestivum* L. cv. Egret) were grown in cylindrical stainless steel pots. Each pot was capped with an aluminium plate which could serve as the top of a pressure chamber, and which was pierced by a centrally placed hole through which the roots of newly germinated seed could be induced to grow. The hole can be filled with silicone rubber to make a pressure seal. The pots were 45 mm in diameter and 150 mm long, with a volume of 180 cm³. The culture of plants grown in them is described in detail in Passioura (1980) and Passioura and Munns (1984).

The pots were packed with an alluvial silty loam soil (5% coarse sand, 40% fine sand, 31% silt, and 19% clay) to a bulk density of 1.20 g cm⁻³. The soil was fertilised with 189 mg phosphorus as finely ground superphosphate, and 125 mg nitrogen as ammonium nitrate, per kg. Previous experiments with this soil (see Masle and Passioura 1987) showed that these levels of fertiliser allowed the plants to grow unhindered by any nutrient deficiency. The soil was watered to 0.25 g water per g soil at the time of sowing, corresponding to a suction of about 30 kPa, and was maintained close to that water content.

Once the seedlings were established in the pots (3 days after germination), they were moved to a growth chamber having a mixture of fluorescent and incandescent lights (photosynthetic photon irradiance 550 μmol m⁻² s⁻¹) and a photo- and thermoperiod (16°C day/13°C night) of 10 h. The difference in the vapour pressure of water between leaf and air in the chamber was estimated using a thermocouple and a dew point hygrometer (see Masle and Passioura 1987) to be 0.50 kPa.

Table 1. Leaf area at the beginning (upper value) and end (lower value) of the experiment, and root and shoot dry weight and osmotic pressure of expanding and fully expanded leaf tissue, at harvest, for the various treatments (means ± s.e.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf area (cm²)</th>
<th>Dry weight (mg)</th>
<th>Osmotic pressure of cell sap (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>Shoot</td>
<td>Expanding</td>
</tr>
<tr>
<td>Wet, no pressure</td>
<td>13.6 ± 0.5</td>
<td>76 ± 6</td>
<td>189 ± 3</td>
</tr>
<tr>
<td></td>
<td>44.4 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet, pressure</td>
<td>14.1 ± 0.8</td>
<td>79 ± 7</td>
<td>191 ± 7</td>
</tr>
<tr>
<td></td>
<td>43.2 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry, no pressure</td>
<td>14.5 ± 0.4</td>
<td>103 ± 10</td>
<td>186 ± 6</td>
</tr>
<tr>
<td></td>
<td>39.4 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry, pressure</td>
<td>14.8 ± 0.5</td>
<td>95 ± 8</td>
<td>196 ± 5</td>
</tr>
<tr>
<td></td>
<td>41.6 ± 0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When the plants were 12 days old they were arranged into four groups of six to eight plants, matched for size. The pots were then rewatered to 0.25 g g⁻¹, and the treatments were begun: two groups received no more water ('dry'), while the other two ('wet') were watered frequently to 0.25 g g⁻¹; two groups (one wet, one dry) were placed in pressure chambers so that the leaves could be kept turgid whether or not the soil was allowed to dry, while the other two had no pressure applied. After the pressure was first applied some rearrangement of the plants within the groups was necessary, because of a few faulty pressure seals where the roots passed through the aluminium caps; this rearrangement resulted in some differences in mean leaf area among the treatments exceeding the standard errors (Table 1), but these differences largely faded when the results were analysed in terms of relative leaf expansion rate.

The chambers within each of the two pressurising treatments were connected in series with pressure tubing. The pressurising gas was a mixture of air and pure nitrogen whose composition was varied according to the working pressure so that the partial pressure of oxygen within the chambers was maintained fairly close to its value in normal air (21 kPa), i.e. within the range of about 18–25 kPa. (Earlier experiments using air alone as the pressurising gas had shown that the plants became necrotic after several days in the chamber, presumably because of the high partial pressure of oxygen they were experiencing; adjusting the partial pressure of oxygen to its value in normal air overcame this problem (Termaat et al. 1985).) The gas within the chambers was allowed to bleed off through a needle valve attached to the final chamber in the series at a rate of about 1 cm³ s⁻¹ to prevent respired carbon dioxide accumulating in the soil.
The pressure in the chambers was adjusted so that at least one plant in each treatment was at balancing pressure: that is, that xylem sap was at atmospheric pressure and on the verge of exuding from the leaves. When the soil was wet, the range of balancing pressure among the plants within a treatment was generally about 50 kPa (determined by raising the pressure until all plants were bleeding), so that although some plants were not quite at balancing pressure they were not far off. When the soil was becoming dry, variation in soil water content, and hence soil water suction, among the pots increased the range in balancing pressure to possibly 300 kPa, although it was hard to determine the range directly because that would require applying a substantial overpressure to some of the plants. Thus in the dry pressurised treatment, while some of the plants were at balancing pressure, others may have been 300 kPa below. Nevertheless, by the time this discrepancy became large, the balancing pressure was high (>1000 kPa) and the plants had a much higher water potential in their leaves than the corresponding plants in the unpressurised treatment; all pressurised plants remained palpably highly turgid at all times.

The gas bleeding from the chambers was monitored several times a day (Hanna dissolved oxygen meter, model HI8543) to ensure that the partial pressure of oxygen was within the range 18-25 kPa. It was particularly important to monitor the oxygen level at the end of the light period, when the balancing pressure was falling rapidly, for there was the danger then of inducing very low partial pressures of oxygen within the chambers. During this time the chambers were flushed rapidly with pressurising gas of appropriate oxygen concentration to ensure that the oxygen level did not fall below about 10 kPa. This transition took about 15 min.

The length and breadth of each leaf was measured every 2 days with a ruler, just after the start of the light period. Leaf area was estimated as 0.84 times the product of length and breadth, summed over each plant (Masle and Passioura 1987). From these data the relative leaf expansion rate (RLER) between successive measurements was calculated, by taking the natural logarithm of the ratio of the successive measurements of leaf area and dividing by the elapsed time. The lengths of the expanding leaves on the main stem were also measured at the beginning and end of the light period because there was reason to believe that the diel pattern of leaf growth might vary among treatments (Munns 1987).

The relation between the suction and the water content in the soil was determined using a pressure plate. The penetrometer resistance of the soil as a function of water content was measured, using a 60° cone penetrometer of diameter 2·0 mm, on samples of soil packed to a bulk density of 1·20 g g\(^{-1}\) into aluminium rings 50 mm in diameter and 40 mm deep, which were allowed to dry slowly from an initial water content of 0·25 g g\(^{-1}\).

Results and Discussion

Fig. 1a shows the RLER for the dry treatments as a function of soil water content during the drying of the soil. RLER for the wet treatments is also shown on the figure, as short horizontal bars, whose abscissae denote not water content but the fact that they correspond in time to the dry treatments with similar abscissae. The numbers adjacent to these bars denote the four successive periods of measurement during the experiment. The wet treatments (pressurised and not) are combined because at no stage did they differ significantly. The main point to note in the figure is that the RLERs of both dry treatments fell as the soil dried, whether or not the plants' leaves were kept highly turgid, and were significantly lower than that of the well-watered plants once the water content had fallen below about 0·19 g g\(^{-1}\). There was a small separation of the two dry treatments at the lowest water content, but even this may have been more apparent
Fig. 1. (a) Relative leaf expansion rate (RLER) as a function of soil water content during the drying of the soil. ○: unpressurised plants; ●: pressurised plants; horizontal bars: RLER's of watered plants, whose abscissae denote not water content but the fact that they correspond in time to the points for the dry treatments having similar abscissae. The numbers adjacent to these bars denote the successive periods of measurement during the experiment. Vertical bars denote ± s.e.m. (b) Daily transpiration rate as a function of soil water content. The symbols have the same meaning as in (a). (c) Balancing pressure in the pressurised treatments, soil water suction, and soil penetrometer resistance, as functions of soil water content. ●: balancing pressure for the pressurised dry treatment; horizontal bars: balancing pressures for the pressurised wet treatment; the abscissae denoting not water content but correspondence in time as in (a); □: penetrometer resistance; △: soil water suction.
than real, for by this time the unpressurised plants were wilting, and there was some slight shrinkage of the fully expanded leaves, which would have influenced the calculated RLER.

Fig. 1b shows daily water use as a function of soil water content, with the symbols denoting the same treatments as in Fig. 1a. In accordance with the experience of Gollan et al. (1986), the dry treatments behaved similarly, with a slight tendency for the pressurised plants to transpire faster than the unpressurised ones. There was no clear separation between the wet and dry treatments until the last period of measurement, when the soil was quite dry. Evidently, RLER was substantially more sensitive to the drying of the soil than was stomatal conductance.

Table 1 shows the leaf areas at the beginning and end of the experiment, and the dry weights of roots and shoots at final harvest. The leaf areas of the wet treatments exceeded those of the dry treatments at the end of the experiment despite being somewhat smaller at the beginning, although the differences were not great because the relative leaf expansion rates were similar among the treatments in all but the last few days of the experiment. The dry weights show no differences among treatments except that the roots of the dry treatments were larger than those of the wet, and, somewhat puzzlingly, that the total dry weight (roots plus shoots) of the dry plants was greater than that of the wet. The latter may be a result of the plants of the dry treatments having been about 6% larger than those of the wet at the start of the experiment, and the fact that there was no substantial fall in either RLER or transpiration rate of the dry plants (and therefore in stomatal conductance and probably assimilation rate) until the last 2 days of the experiment.

This experiment was one of several similar ones, all of which showed that the RLER of plants in drying soil eventually fell below that of well-watered controls, whether or not their leaves were kept highly turgid. In trying to explain such results it is hard to avoid the conclusion that the roots must have been sensing conditions in the soil, and transmitting controlling messages to the leaves, that overrode the effects of turgor. That turgor may have had some influence in the short term (hours) but not in the long (days) is illustrated in Fig. 2, which shows the elongation rate of the most rapidly growing leaf on the main stem for the two dry treatments during the last few days of the experiment. The leaves on the pressurised plants grew faster than those on the unpressurised plants.
during the light period, suggesting that their rate of expansion may have been limited by turgor then, but slower during the dark, so that there was little difference between the treatments over a full day, particularly in the last 2 days when the soil was becoming quite dry.

It is faintly conceivable that, despite the xylem sap in the shoot being at atmospheric pressure, several hundred kPa above that in the unpressurised controls, the turgor in the leaves was not raised substantially by the pressurising treatment (although it certainly was macroscopically). For the turgor not to have risen, either the osmotic pressure of the cell sap would have fallen substantially, or the osmotic pressure in the cell walls would have risen substantially. I have no information on the osmotic pressure in the cell walls, but measurements on another species (Nonami and Boyer 1987) have shown it to be small (<50 kPa), at least in unpressurised plants. The average osmotic pressures in the leaves harvested at the end of the experiment are shown in Table 1. The pressurised treatments did have slightly lower osmotic pressures than did the unpressurised ones, in both expanding and fully expanded tissue, but these small differences were probably a result of the pressurised plants having turgid leaves, while the others did not. Thus the turgor pressure must have been very high in the pressurised plants, approximately equal to the osmotic pressures as long as the apoplasm had a low osmotic pressure. Certainly, the leaves of the pressurised plants were always highly turgid to touch.

What in their environment could the roots be sensing? Fig. 1c shows the soil water suction, and the penetrometer resistance, as functions of soil water content. If the soil water content remained uniform throughout each pot as the soil dried, then the soil water suction would have risen to about 160 kPa by the time that the expansion rates of the leaves were affected, which was about 80 kPa greater than during the previous measurement interval when there was little difference in expansion rates among the treatments. Such changes seem rather small for the roots to be responding to. Suppose, however, that the soil water content did vary within the pots perhaps because the roots had more thoroughly colonised the soil in the tops of the pots than in the bottoms, or because substantial gradients of suction had developed in the rhizosphere. If the drying of the soil had little effect on the hydraulic conductivities of the plants, as seems likely (Passioura 1980), then the difference in balancing pressure between the wet and dry treatments (Fig. 1c) must have arisen because of a difference in water potential of the roots. This difference was about 300 kPa in the middle of the period in which changes in RLER were first evident. Perhaps roots do respond to fairly small differences such as this, but if so they are very different in response from the shoots, which seem to be insensitive to changes in turgor of at least 1000 kPa.

An alternative possibility is that the roots were responding to the hardening of the soil as it dried. Fig. 1c shows the penetrometer resistance of the soil as a function of soil water content. Masle and Passioura (1987), working with the same variety of wheat in the same soil, found a discernible effect of soil strength on the expansion rate of the leaves once the penetrometer resistance exceeded about 2 MPa. In this experiment also, as Fig. 1 shows, falling soil water content significantly affected relative leaf expansion rate once the penetrometer resistance had exceeded 2-0 MPa. However, the effect of soil strength on the RLER in the experiments of Masle and Passioura (1987) was never as great as the effects of soil drying on RLER evident in Fig. 1. Perhaps the effects of falling water potential and increasing soil strength are additive, or even synergistic, in their effects on the roots. Experiments are in progress, with soil packed to various bulk densities, that aim to explore the interaction between the two.

The signal(s) that the roots send to the leaves when they experience adverse conditions in the soil is at present unknown. Because of the effect on the stomata, an obvious possibility is that it is abscisic acid, and that it is carried in the xylem sap from the roots to the leaves (Zhang et al. 1987). However, there is strong evidence that, in wheat at least, it is not abscisic acid (Munns and King 1988).
Acknowledgments

I thank Rana Munns for many stimulating discussions, Anne Gardner for excellent technical assistance, and Murray Long for drawing the figures.

References


Manuscript received 20 July 1988, accepted 20 September. 1988