

Decreased Growth-Induced Water Potential¹

A Primary Cause of Growth Inhibition at Low Water Potentials

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Cell enlargement depends on a growth-induced difference in water potential to move water into the cells. Water deficits decrease this potential difference and inhibit growth. To investigate whether the decrease causes the growth inhibition, pressure was applied to the roots of soybean (*Glycine max* L. Merr.) seedlings and the growth and potential difference were monitored in the stems. In water-limited plants, the inhibited stem growth increased when the roots were pressurized and it reverted to the previous rate when the pressure was released. The pressure around the roots was perceived as an increased turgor in the stem in small cells next to the xylem, but not in outlying cortical cells. This local effect implied that water transport was impeded by the small cells. The diffusivity for water was much less in the small cells than in the outlying cells. The small cells thus were a barrier that caused the growth-induced potential difference to be large during rapid growth, but to reverse locally during the early part of a water deficit. Such a barrier may be a frequent property of meristems. Because stem growth responded to the pressure-induced recovery of the potential difference across this barrier, we conclude that a decrease in the growth-induced potential difference was a primary cause of the inhibition.

Most plant enlargement results from an increasing water content of the tissues. The water is absorbed by osmosis, and ψ_p builds as the water enters. If the cells are capable of growth, the volume of the cell compartment increases and prevents ψ_p from developing fully (Boyer, 1968, 1993; Maruyama and Boyer, 1994). The lack of full ψ_p keeps the ψ_w lower inside of the cells than outside. This growth-induced ψ_w creates a potential difference between the xylem and the growing cells, which drives water uptake by the cells, and thus is central to the growth process (Boyer, 1985, 1988).

The size of the potential difference depends partly on how readily water can move and thus on the conductance of the flow path. Water uptake was slow in stem elongating regions, which implied that the conductance was low (Molz and Boyer, 1978; Steudle and Boyer, 1985); but growth responded rapidly to changes in the water supply (Acevedo et al., 1971; Cosgrove and Cleland, 1983), which was thought to indicate that the conductance was high

(Cosgrove and Cleland, 1983). When the hydraulic conductivity of individual cortical cells was measured, it was high, but decreased in stems of soybean (*Glycine max* L. Merr.) exposed to a growth-inhibiting water deficit, and the growth-induced potential difference decreased (Nonami and Boyer, 1989, 1990b). The decreased potential difference was correlated with decreased stem growth and is the earliest event identified so far that could account for the inhibition. However, growth is also linked to the synthesis of new cell constituents, and water deficiencies can alter solute transport and many biochemical processes, with possible consequences for growth (Hanson and Hitz, 1982; Kramer and Boyer, 1995). Because of this complexity, it has been difficult to identify the exact causes of the growth inhibition.

In the present work we tested whether the decrease of the growth-induced potential difference could be an early and controlling (primary) cause of the growth inhibition during a water deficiency. Our approach was to reverse the decrease without altering the quantity of water present and to observe whether growth responded. The experiment also gave an opportunity to observe the conductance properties of the growing tissue and explore more fully how the growth-induced potential arose.

MATERIALS AND METHODS

Soybean (*Glycine max* [L.] Merr. cv Williams) seeds were disinfected in a 1% solution of NaOCl for 5 min, rinsed with flowing water for 1 h, and sown in vermiculite with adequate water (5.0 mL of 10^{-4} M CaCl_2 /g of vermiculite, ψ_w of -0.01 MPa). The seedlings were grown at $29 \pm 0.5^\circ\text{C}$ and 100% RH in darkness for 55 to 60 h, then transplanted either to a 200-mL beaker containing similar vermiculite (with the same quantity of water, termed 1X) or to a water-deficient vermiculite (0.63 mL of 10^{-4} M CaCl_2 /g of vermiculite, ψ_w of -0.28 ± 0.01 MPa, termed $\frac{1}{8}\text{X}$) and grown under the same conditions as before transplanting. For the preparation of the $\frac{1}{8}\text{X}$ vermiculite, the vermiculite and CaCl_2 solution were shaken together in a plastic container prior to transplanting to ensure uniform mixing. The

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Abbreviations: D_w , cell diffusivity of water; ψ_w , xylem water potential; ψ_p , turgor pressure; ψ_s , osmotic potential; ψ_w , water potential; $T_{1/2}$, half time of pressure relaxation in a cell.

mixing was repeated until vermiculite ψ_w was -0.27 to -0.29 MPa measured with an isopiestic thermocouple psychrometer (Boyer and Knipling, 1965; Boyer, 1995). This adjustment of vermiculite ψ_w was particularly essential to duplicate conditions used in earlier experiments (Nonami and Boyer, 1989, 1990a, 1990b). All seedling manipulations were carried out under a green safelight (green fluorescent bulb wrapped in a green plastic sheet having maximum transmission at 525 nm and negligible transmission below 475 nm and above 575 nm).

Growth Measurements

The rate of stem (hypocotyl) growth was measured with a radial displacement transducer (Schaevitz, Pennsauken, NJ) by attaching the arm of the transducer to the apical hook with a clamp. A rigid reference bar was clamped to the lower stem. The transducer and bar were mounted onto a microscope frame, the fine adjustment of which could be used to calibrate the instrument without disturbing the seedling. Length was recorded electrically under the growth conditions of $29 \pm 0.5^\circ\text{C}$ and 100% RH in darkness, and the growth rate was determined from the rate of length increase between the transducer and the reference bar.

Stem growth was also measured when the roots were pressurized. A seedling was grown through the seal in the lid of a pressure chamber (Scholander et al., 1965) in 1X or $\frac{1}{8}$ X vermiculite. The young seedling was transplanted into the pressure chamber containing vermiculite and allowed to grow for the times shown. A radial displacement transducer was mounted onto a micromanipulator and attached to the stem hook extending outside of the chamber. A reference bar was connected between the body of the pressure chamber and the basal end of the hypocotyl. The whole system was kept under growth conditions and was calibrated without disturbance. To test the effect of pressure application on the instrument, a steel rod with the diameter of the stem was pressurized in place of a stem.

Water Status Measurements with a Pressure Probe

The ψ_p of cells in the stem was measured directly in the intact seedlings with a pressure probe (Hüsken et al., 1978; Nonami et al., 1987; Boyer, 1995). The stems were exposed to air in the growth conditions, but under bright green light (light emitted from a tungsten lamp was guided through a light pipe, which had a green plastic filter having maximum transmission at 525 nm and negligible transmission below 475 nm and above 575 nm). The stems were coated with petroleum jelly, and the vermiculite surface was tightly covered with plastic film to minimize water loss. Further, the stems were covered with wet tissue paper on top of the petroleum jelly. To use the pressure probe, a small window was cut in the tissue paper and the tip of the microcapillary was pushed into the stem. ψ_p was measured by returning the cell solution/oil meniscus in the microcapillary to its position before entering the cell.

The local diffusivity for water was obtained from the half-time of water exchange between the cells and their surroundings (Steudle, 1992; Boyer, 1995). The D_c (in m^2

s^{-1}) was calculated from $T_{1/2}$ (s) of the ψ_p in each cell using the pressure probe according to:

$$D_c = \frac{a_{cc} \cdot \Delta x^2 \cdot \ln 2}{2A} \cdot \frac{1}{T_{1/2}} \quad (1)$$

where a_{cc} is the cross-sectional area of the cell normal to the flow (m^2), Δx is the width of the cell in the direction of the flow (m), and A is the surface area of the cell (m^2). The factor of 2 in the denominator denotes that two membranes have to be crossed per cell layer. It should be noted that the diffusivity measures the properties for water transport along the cell-to-cell path and incorporates both a symplasmic component via plasmodesmata and a transcellular component across the plasmalemmae. It can be seen that D_c is inversely proportional to the directly measurable half-time of water exchange, according to a factor that depends on cell dimensions and shape. For small and large cells having the same half-time, D_c is less for the small cell because less water is transported during the relaxation due to the small volume of the cell. For applying Equation 1, the soybean cells were assumed to be elongated boxes. Water was assumed to flow from the xylem to the surrounding cells radially, and to flow perpendicularly across the tangential cell surfaces. Cell sizes were measured under a microscope from freehand sections of tissue.

It should also be pointed out that diffusivities at the cellular and organ levels were previously used to describe water transport in soybean stems treated identically to those used here (Molz and Boyer, 1978; Steudle and Boyer, 1985). The diffusivities were assumed to be uniform throughout the stem and did not include the possibility of different diffusivities in different tissues. The above cellular measurements address that possibility. Because of the previous work (Molz and Boyer, 1978; Steudle and Boyer, 1985), measurements at the organ level were not repeated here, but can be summarized by recognizing that the cylindrical geometry of stems causes water flow to be radial in growing regions and gradients in ψ_w to be steeper when the water source is in the center of the cylinder (at the xylem) than when it is at the outer surface (as in roots). Gradients in potential have been measured with cellular detail in these stems (Nonami and Boyer, 1993) and agree with those predicted for cylindrical stems (Molz and Boyer, 1978). Radial flow through these stems also includes an apoplast path in addition to the cell-to-cell path measured here, but flow in the apoplast was small and the cell-to-cell path was shown to predominate (Steudle and Boyer, 1985).

Water Status Measurements with a Psychrometer

Stem ψ_w was measured in the elongating zone in a specially constructed isopiestic thermocouple psychrometer, which allowed four whole seedlings to be sealed into a thermally stable chamber, inside of which was a small vapor chamber for the thermocouple, as described earlier (Boyer et al., 1985; Boyer, 1995). The apparatus was large enough to accommodate the seedlings with the roots outside in 1X or $\frac{1}{8}$ X vermiculite, so that they could grow as intact plants under the growth conditions and be transplanted without restriction. The vapor pressure chamber

was coated with melted and resolidified petroleum jelly to minimize water sorption (Boyer, 1967). A thermocouple bearing a Suc solution of known ψ_w was placed in the chamber for measurement of tissue ψ_w . Wherever possible, the measurement was isopiestic, i.e. the vapor pressure of the solution was the same as that of the tissue, and no net vapor exchange took place. This prevented errors caused by the diffusive resistance of the tissue to water vapor and ensured that the tissue neither hydrated nor dehydrated during the course of the measurement (Boyer and Knipling, 1965; Boyer, 1995).

ψ_s of the tissue in the elongating zone was measured by excising the sample from other seedlings grown at the same time as the seedlings used for measurements of the ψ_w . The segments were placed in a hypodermic syringe, frozen, thawed, and pressed against tissue paper in the base of the syringe. The extracted solution was placed on a thermocouple junction, and its ψ_s was measured above Suc solutions by the isopiestic technique (Boyer, 1995). The ψ_p was calculated from $\psi_w - \psi_s$.

The nonelongating basal tissue of the stem was sampled from seedlings grown at the same time as those used to measure the ψ_w of the elongating zone. Four segments about 1.5 cm long from the basal region were placed on the bottom of a psychrometer chamber that was coated with melted and resolidified petroleum jelly. The ψ_w was measured using the isopiestic technique. Because no growth or transpiration occurred in this part of the stem, the ψ_w of the xylem and its surrounding tissues were equilibrated in the intact plant, and the ψ_w measured in these samples was ψ_o (Nonami and Boyer, 1987, 1993). Nonami and Boyer (1990a) showed that these ψ_o were similar when measured with the psychrometer or a pressure chamber that determines the tension on water in the xylem (Scholander et al., 1965). Solute concentrations were low in the xylem (Nonami and Boyer, 1987). Under steady conditions, the ψ_o was the same as the ψ_w in the vermiculite, as expected for equilibrium conditions (Nonami and Boyer, 1990a). All manipulations were carried out in a humid chamber under the green safelight in the growth conditions.

Stem Anatomy

Seedlings were grown for 55 h in 1X vermiculite as described above, but, instead of transplanting, the stems were excised and placed with their ends in a solution of safranin (0.05%, w/w) for 5 h in the growth conditions. At this time, they were the same age as the plants with pressurized roots, and the anatomy should have represented both the 1X and $\frac{1}{8}$ X seedlings, since only 5 h had elapsed after excision or transplanting. After 5 h, the safranin was absorbed by the water-conducting xylem, where it stained lignin in the walls. Freehand sections were made 1 cm below the cotyledons and 1 cm above the root/shoot transition. These positions were the same as the elongating and basal regions used for the psychrometer and pressure-probe measurements. The fresh sections were mounted in water and viewed under the light microscope. The conducting xylem appeared red in otherwise unstained tissue.

The number and diameter of the xylem vessels were determined from micrographs.

RESULTS

Decrease of Xylem ψ_o during Growth at Low ψ_w

Before the transplanting treatment the seedling stems grew actively (Fig. 1A). The xylem ψ_o was -0.01 MPa, the same as the ψ_w of the vermiculite (Fig. 1B). The ψ_w of the elongating zone was lower and stable at -0.26 MPa in the intact plant (Fig. 1C). The lower ψ_w indicated that a gradient of decreasing ψ_w extended outward from the xylem to the surrounding, expanding cells during active growth, i.e. a growth-induced ψ_w was present (Molz and Boyer, 1978; Nonami and Boyer, 1993). When the seedlings were transplanted to water-deficient vermiculite (-0.28 MPa), growth began to be inhibited within a few minutes (Fig. 1A). The water potential decreased in the xylem, but not in the elongating tissue (Fig. 1, B-C), so that the difference between them ($\psi_o - \psi_w$) became less, indicating that the

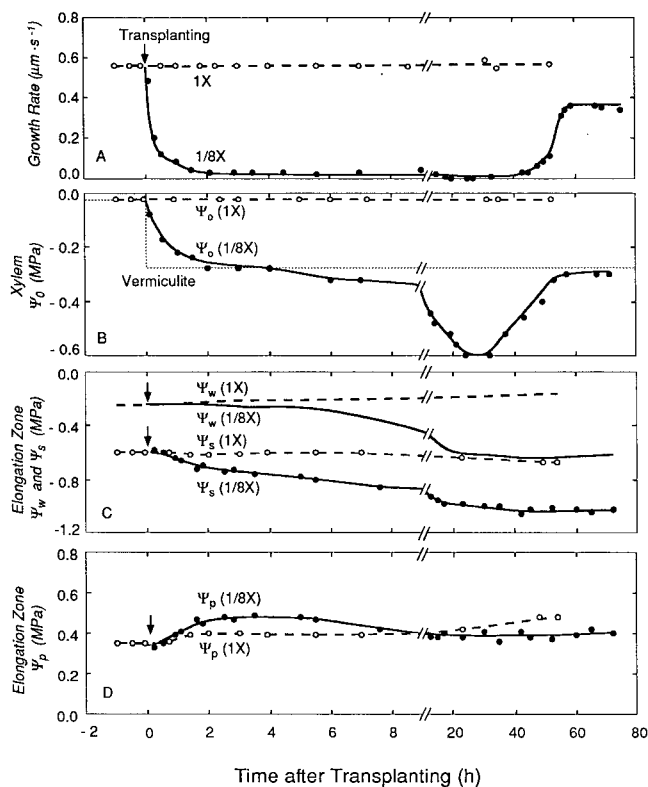


Figure 1. Growth and potentials in soybean seedlings after transplanting to 1X or $\frac{1}{8}$ X vermiculite. A, Growth rate; B, xylem ψ_o and vermiculite ψ_w for $\frac{1}{8}$ X vermiculite (dotted line); C, stem ψ_w and ψ_s in the elongating zone; D, stem ψ_p in the elongating zone. Detailed kinetics during the first 8 h are shown on the left side of the figure and long-term kinetics are shown on the right. In A, growth is for a single representative seedling on the left and a separate seedling on the right. Measurements were repeated three times with similar results. In C, the ψ_w are shown as a continuous recorder trace for four intact seedlings. ψ_s and ψ_o were measured in excised tissue from other seedlings grown in parallel in the same vermiculite at the same time. In D, ψ_p is calculated from $\psi_w - \psi_s$.

gradient began to flatten at the xylem side. The ψ_s also gradually decreased (Fig. 1C), as osmotically active solutes accumulated in the cells of the elongating zone (osmotic adjustment). The ψ_p increased slightly (Fig. 1D).

By about 5 h after transplanting, the xylem ψ_o had decreased to the ψ_w of the stem elongating zone, and the growth-induced potential difference was near zero; stem growth was also near zero. The roots had been disconnected from the external water supply during transplanting, and the xylem and elongating zone continued to decrease in ψ_w for the next 20 h, as water was withdrawn from the mature tissue by the elongating tissue (Matyssek et al., 1991a, 1991b), eventually reaching -0.6 MPa. By then, the ψ_s of the elongating zone had decreased to -1.0 MPa (Fig. 1C) and the xylem began to rehydrate as the roots reconnected with water in the vermiculite (Fig. 1B). By 50 h the xylem ψ_o had increased until it became the same as in the vermiculite, reestablishing the growth-induced potential difference but at lower potentials ($\psi_o = -0.3$ MPa, Fig. 1B, and elongating zone $\psi_w = -0.6$ MPa, Fig. 1C). During this rehydration, growth resumed at a moderate rate (Fig. 1A), where it continued for several days (data not shown; for an example, see Kramer and Boyer [1995]). The recovery of xylem ψ_o and growth indicates that water could again be absorbed from the vermiculite and move from the xylem into the surrounding cells.

Growth Recovery Induced by Pressure Application

The association of decreased growth with decreased xylem ψ_o suggested that pressure could be applied to the root system to independently alter xylem ψ_o and observe the growth response. Pressure of 0.28 MPa was applied to the root system of the $1/8X$ plants 5 h after transplanting, when the growth-induced potential difference approached zero. Before pressurizing, stem growth was 0.04 m s^{-1} or about 6% of that in the $1X$ plants (Fig. 2). Applying pressure increased the rate 4-fold to about 24% of that in the $1X$ plants. Releasing the pressure caused the rate to return immediately to the earlier inhibited level. The actively growing $1X$ plants did not change growth rates significantly with this pressure, although a transient increase in stem length was observed ($1X$ in Fig. 2). The release of pressure gave the reverse transient. If a metal rod or mature stem was sealed in the pressure chamber in place of the growing stem, the same transient was observed, but it was smaller, indicating that the extension must have been caused mostly by stretching of the rubber seal in the chamber top. Thus, some of the transient extension and shrinkage was associated with the elasticity of the pressure chamber system and not the growing stem.

ψ_p in the Growing Cells during Pressure Application

The applied pressure of 0.28 MPa should have been large enough to recover most of the growth-induced difference in ψ_w . We tested whether recovery occurred by measuring cell ψ_p with the pressure probe. When seedlings were growing actively in $1X$ vermiculite, the ψ_p in cells next to the xylem (Inner Cells, Fig. 3) was similar to that of cells

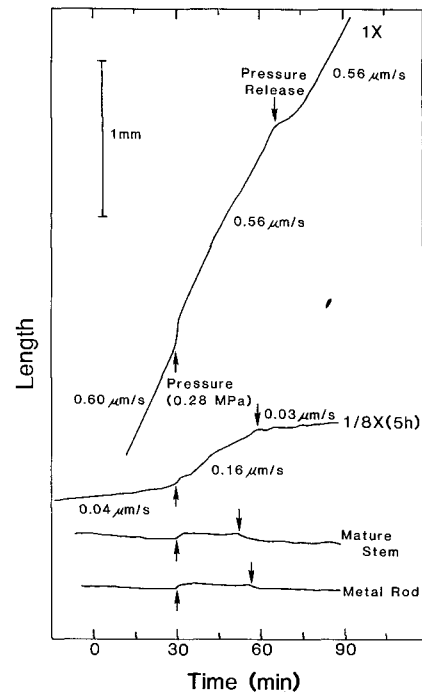


Figure 2. Length of the total stem of soybean seedlings transplanted 5 h previously to vermiculite of high water content ($1X$) or low water content ($1/8X$) showing the effect of pressure applied to the roots. Growth rates are shown alongside each tracing. The mature region of the stem and a metal rod were controls. Pressure was 0.28 MPa. The growth and water status of seedlings grown under similar conditions is shown in Figure 1.

near the epidermis (Outer Cells, Fig. 3) in the elongating zone. About 1.5 h after transplanting, the ψ_p had decreased in the inner cells but not in the outer cells. When pressure was applied to the root system, ψ_p in the inner cells increased 0.2 MPa and became the same as in the $1X$ plants prior to transplanting. When pressure was released, ψ_p in the inner cells returned to the low level in the $1/8X$ plants. This rapid ψ_p response indicated that water flow could be rapidly reversed and reestablished between the xylem and the surrounding inner cells.

It should be noted that the ψ_p of the outlying cortical cells was unmodified before, during, and after pressure application (Fig. 3), which implies that they were somewhat isolated from the xylem hydraulically. The isolation was detectable for the entire time of pressure-probe measurements, judging from the high ψ_p of these cells 8 h after transplanting (Fig. 3). These cells account for 60% of the volume of the elongating zone (Nonami and Boyer, 1993), whereas the inner cells occupy a much smaller volume (19%), which probably explains why the isopiestic psychrometer detected no decline in ψ_p in the elongating zone after transplanting (Fig. 1D).

Diffusivity of Cells Adjacent to the Xylem

We measured the ability of water to move through cells in the elongating region by determining its diffusivity from

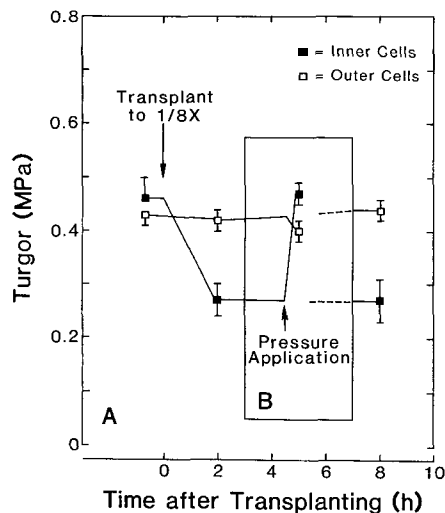


Figure 3. ψ_p of the outer and inner cells in the stem elongating zone of soybean seedlings after transplanting to $1/8X$ vermiculite and during pressure application to the roots. Pressure of 0.28 MPa was applied for 1 h beginning 4.5 h after transplanting (inset box B encloses data measured when roots were pressurized). The outer cells (\square) were located 70 to 320 μm from the hypocotyl surface (i.e. 830 to 1080 μm from the center), and the inner cells (\blacksquare) 380 to 575 μm from the surface (i.e. 575–770 μm from the center). Bars indicate 95% confidence intervals calculated from the Student's *t*-distribution. Refer to Figure 4A for cell size and location.

the half-time for ψ_p relaxation using the pressure probe. The average half-time was 2.13 s in cells close to the epidermis (i.e. 1100 μm from the center of the stem) and 5.08 s in cells adjacent to the xylem (i.e. 630 μm from the center of the stem; Fig. 4B). When cell dimensions were considered (Fig. 4A), the local diffusivity for water could be calculated according to Equation 1 and showed significant differences, depending on the position in the tissue. For the pith cells (in the center of the stem) the average diffusivity was $1.11 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ (Fig. 4C), and for the outlying cortical cells (850–1150 μm from the center of the stem) the average diffusivity was 0.30 to $0.99 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$. Situated between these two groups of cells, the small cells nearest the xylem had an average diffusivity of $0.58 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$ (range $0.35\text{--}0.84 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$), which was an order of magnitude less than for the pith and outlying cortical cells on either side.

Stem Anatomy

The safranin dye required 3 to 5 h to reach the xylem in the elongating tissue. The dye indicated that conducting xylem was located in a band of small cells forming a cylinder in the stem. In the elongating zone the cylinder had four quadrants, and Figure 5A shows one of these with its conducting protoxylem, mostly scattered individual vessels with small diameters (about 10 μm ; Table I). In the base of the stem this protoxylem had mostly disappeared (Fig. 5B), and conducting metaxylem elements had differentiated to form a continuous band along the inner side of the cylinder of small cells. These vessels were more than

twice as numerous as in the elongating region (2.6X, Table I), and their diameters were twice as large (Table I).

DISCUSSION

Many physical and biochemical changes occur when plants are exposed to water-deficient root media, and any of them could be a cause of the growth inhibition that ensues. To identify a cause it is necessary to reverse a particular change and observe whether the inhibition also reverses. If the change is not limiting, the growth inhibition will not respond. If it is limiting, growth will improve until the next most limiting factor takes over control, and thus the reversal is diagnostic for causality. The results show that exposing the root system to a low ψ_w collapsed the ψ_w difference between the xylem and the growing cells in the stem, and pressurizing the roots reversed the collapse and reversed the inhibition of growth in the stem. Therefore, the collapse of the potential difference was a primary cause of the growth inhibition.

The effect could be seen without a direct treatment of the stem, indicating that the growth reversal arose from a signal in the roots. In these plants 3 to 5 h was required for dye to move from the base of the stems to the elongating

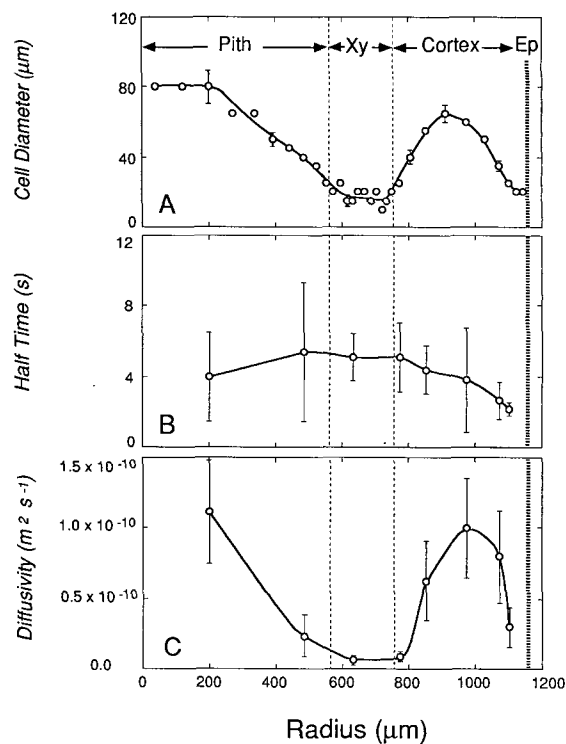


Figure 4. Average cell diameters (A), $T_{1/2}$ measured with the pressure probe (B), and diffusivity (C) at various positions along the radius of the soybean stems in the elongating zone 5 h after transplanting. Pith, Xy, Cortex, and Ep indicate, respectively, the pith region defined as 0 to 575 μm from the center, the xylem region defined as 575 to 770 μm from the center, the cortex region defined as 770 to 1110 μm from the center, and the epidermal surface, which was located at 1150 μm from the center of the stem. Bars indicate 95% confidence intervals calculated from the Student's *t*-distribution.

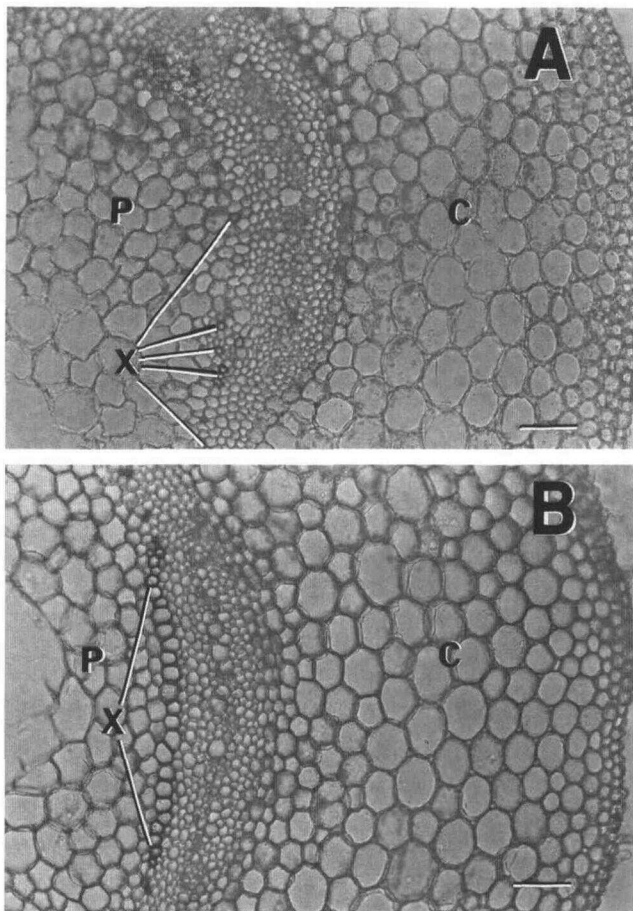


Figure 5. Fresh cross-sections of the stem elongating region (A) and basal region (B) showing the conducting xylem vessels (black) 5 h after transplanting. A, Lines extending from X point to individual protoxylem vessels. B, A continuous layer of metaxylem is shown between the tips of the lines. The metaxylem has differentiated since A. There are also a few protoxylem vessels at the ends of the metaxylem layer, but many of the protoxylem vessels in A are not seen in B and probably have been obliterated during development. Note that the xylem is more extensive in B than in A and has larger vessels, indicating that water movement axially and radially encounters less resistance in mature tissue than in elongating tissue. P, Pith; X, xylem; C, cortex. Bars = 100 μm .

region, because the xylem solution moved slowly in the absence of transpiration. As a consequence, most molecular signals were too slow to account for the root signal, which has been detected in 1 min (Matyssek et al., 1991a). The signal was perceived in the stem as a change in ψ_p of the growing cells next to the xylem. The speed of the signal, its local perception next to the xylem, and its effect on the ψ_w difference between the xylem and surrounding cells indicates that the root signal was hydraulic and traveled to the stem via the xylem.

The growth recovery during root pressurization was not complete, probably because other factors began to limit the rate or perhaps because pressurization did not completely reverse the collapse of the ψ_w difference. Passioura (1988) subjected wheat plants to water deficiency, but continuously applied pressure to the roots. Leaf growth eventually

slowed, and it was concluded that the inhibition was caused by metabolic signals rather than hydraulic ones. The continuous application of pressure probably maintained the potential in the xylem while the soil dried, and growth effects would be visible only after molecular signals became important. Similarly, in the soybean seedlings we used, the collapse of the potential difference was followed by many molecular changes. After the potential difference collapsed fully at 2 h, the walls were beginning to be less deformable and the hydraulic conductivity was smaller in the cortical cells (Nonami and Boyer, 1990a, 1990b), ABA was beginning to accumulate (Bensen et al., 1988; Creelman et al., 1990), and protein synthesis was inhibited (Mason et al., 1988). By 24 h gene expression increased for certain wall proteins (Bozarth et al., 1987; Surowy and Boyer, 1991; Mason and Mullet, 1990) and the cells had completed osmotic adjustment (Meyer and Boyer, 1972, 1981). Nonami and Boyer (1990a) showed that the collapse of the potential difference was sufficiently early and large to have been a possible cause of the growth inhibition, but after 40 h the potential difference had been restored and growth could have been controlled by other factors.

The other possibility, that the incomplete growth recovery resulted from an incomplete restoration of the potential difference, is suggested by the incomplete recovery of ψ_p (0.2 MPa) in the inner cells when the roots were pressurized (0.28 MPa). The incomplete recovery may have been caused by the transplanting, which broke the liquid continuity between the roots and the surrounding medium. Continuity was not restored for about 40 h, and pressure applied at 5 h would have moved water from the roots into the stem without moving water from the vermiculite into the roots. Moreover, at 5 h a small amount of osmotic adjustment had occurred, increasing the amount of ψ_p necessary to completely recover the difference in ψ_w between the xylem and the growing cells. There also may have been time lags between the application of pressure and the reestablishment of steady conditions in the flow path. Regardless of these points, however, growth clearly responded to the pressure and thus identified the collapse of the ψ_w difference as a primary cause of the inhibition.

Local Gradients in ψ_w

The ψ_w difference between the xylem and surrounding growing cells was the manifestation of a gradient in growth-induced ψ_w extending outward from the xylem in the stem elongating zone (Nonami and Boyer, 1993). When the po-

Table 1. Number and diameter of conducting xylem vessels in the elongating and basal regions of soybean stems grown in 1X vermiculite

Data are means \pm 1 SD.		
Region	No. of Conducting Vessels	Average Vessel Diameter
μm		
Elongating	41.2 \pm 2.1	10.0 \pm 3.0
Basal	106.0 \pm 9.6	20.7 \pm 0.8

tential difference decreased and ψ_p decreased in the cells next to the xylem, the gradient must have flattened and then reversed next to the xylem, judging from the amount of ψ_p loss in the inner cells but constant ψ_p in the outer cells. This gradient is the driving force for water uptake for growth, and the flattening would inhibit water movement from the xylem into the growing cells.

It is noteworthy that the collapse in the potential difference was confined to a few cells occupying only 19% of the volume of the stem. The rapid response of growth was thus explained by the rapid response of a few cells rather than by a change in the water status of the whole growing tissue. The reason that a few cells could inhibit the growth of the whole tissue is most likely because a smooth, downward sloping gradient in ψ_w must exist to supply the force for water to move to the outlying cells, and collapsing the gradient in a local region next to the xylem reversed that part of the gradient and reversed the force, preventing water from moving outward across that part. This would block the flow to the outlying cells, even though their ψ_w was unchanged and would otherwise favor water uptake. The result was an immediate growth inhibition for the whole region.

Others (Acevedo et al., 1971; Cutler et al. 1980; Cosgrove and Cleland, 1983; Matyssek et al., 1991a, 1991b; Chazen and Neumann, 1994) also saw rapid growth changes when water availability was altered around roots. In light of the present experiments such responses might be explained as shown in Figure 6, where the ψ_w gradient (Fig. 6A), its collapse (Fig. 6B), and the probable effect of root pressurization alter only the inner cells (Fig. 6C), but growth responds rapidly. However, it is important that the rapid response does not indicate that the tissue is highly conductive to water, which demonstrates that transport properties cannot be inferred for the whole tissue from rapid growth kinetics.

The remnants of the old gradient in the outlying cells in Figure 6, B and D would gradually disappear as water was redistributed among the growing cells. The redistribution took longer than 8 h, according to the pressure-probe findings. As water was redistributed stem growth would be negligibly affected because elongation must occur in all the cells as a unit, and the constraint of the cells next to the xylem would inhibit elongation of the whole stem.

Although growth was markedly inhibited by transplanting, it did not cease entirely. The residual growth probably depended on the redistribution of internal water from other tissues. Matyssek et al. (1988, 1991a, 1991b) showed that internal water could be moved slowly from nearby tissues to growing tissues having slightly lower ψ_w . Roots continue to grow with this mobilized water, and they eventually reconnected with external water in the new medium (Matyssek et al., 1990a). The reconnection began at about 30 h in the present work, judging from the recovery of ψ_o . If stem growth had been inhibited completely because both internal and external water uptake were prevented, cell wall relaxation would have been observed (Boyer et al., 1985) and ψ_p would have rapidly decreased in the whole tissue (Boyer et al., 1985; Matyssek et al., 1988). There was no general decrease in ψ_p because slow growth with internal water prevents relaxation (Matyssek et al., 1988).

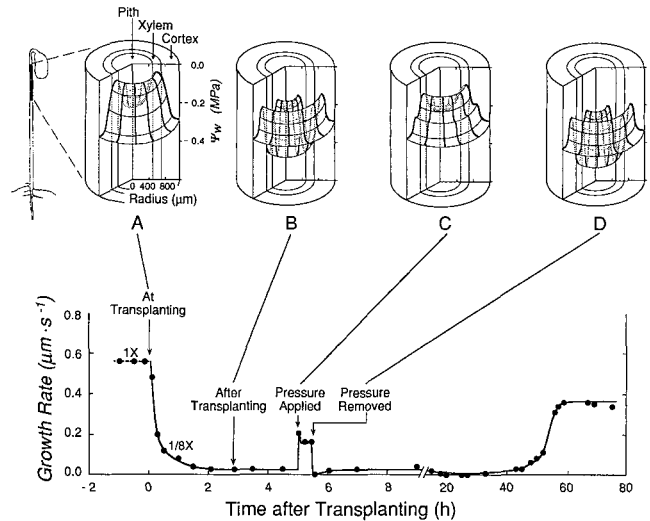


Figure 6. Summary of growth rates of soybean stems during these experiments, and the likely gradients in ψ_w at various times. A, Gradient in ψ_w during rapid growth before transplanting; B, local collapse of gradient in cells close to the xylem, which blocks water flow to outlying cells; C, pressurizing roots reestablishes local gradient around xylem; D, releasing pressure around roots causes gradient to return to locally collapsed condition. Growth response to root pressurization is from Figure 1 and slope of $1/8X$ trace in Figure 2. Gradient of ψ_w in A was directly determined from detailed cell measurements of ψ_p and ψ_s across soybean stems under conditions identical to those used here and published previously (Nonami and Boyer, 1993). Gradients in B, C, and D were based on modifications to the detailed gradient using data for ψ_p from Figure 3, and ψ_s from Figure 1 (assuming ψ_s gradient directly measured by Nonami and Boyer [1993] remained unchanged, but was displaced downward during osmotic adjustment).

Meristem Barriers to Water Movement

The above results raise the questions of why growth with internal water is not more rapid and why the water status of the cells next to the xylem was not transmitted rapidly to the outlying cells. In the elongating tissue the reversed local gradient existed for long times, implying that there was a substantial hydraulic resistance between the conducting xylem and the outlying cells, and, indeed, there was a low diffusivity to water in these intervening small cells. This barrier would require a substantial growth-induced ψ_w to be present during rapid growth to meet the demand for water for enlargement, but it also would make water movement slow to outlying cells during xylem transients in water availability. As a result, gradients in growth-induced ψ_w could be altered in just a few cells next to the xylem without altering the outlying cells. Similar reasoning applies to the mobilization of internal water to the growing region after transplanting, because the differences in ψ_w between the growing and nongrowing cells were small and water had to traverse significant barriers to flow.

The barrier to radial transport resulted, in part, from the small cell diameters (10–25 μm ; Figs. 4A and 5A), which allowed tight packing of 8 to 10 cell layers around the xylem. During water movement many walls and membranes needed to be crossed over short distances, which

would tend to resist flow. Such small cells worked well for measuring diffusivities, because the half-times for water exchange were simply determined, but they presented experimental difficulties for other measurements such as membrane hydraulic conductivities, where there is a need to know the elastic modulus of the cells (Hüsken et al., 1978). Measurements of elastic modulus involved removing a volume of water larger than the volume of the small cells and would have led to large errors. The diffusivity determinations did not have this limitation and provided a direct indication of water-exchange properties. By contrast, the large, outlying cortical cells showed high diffusivities in agreement with the high diffusivities and hydraulic conductivities measured previously in these and similar cells (Cosgrove and Cleland, 1983; Steudle and Boyer, 1985; Nonami and Boyer, 1990b).

In the basal stem tissues the barrier to water movement in the small cells next to the xylem was less than in the elongating tissues. This was apparent after transplanting, when basal ψ_w decreased rapidly, whereas the potential in the elongating tissues did not. Because these potentials were for the whole tissue (measured in the psychrometer), they indicated that water was readily transmitted to the outlying basal tissues, but not the outlying elongating tissues. The likely reason is that the xylem vessels were larger in the basal region than in the elongating region and were more numerous. The diameter advantage gave an axial conductance about 18X larger in the basal vessels than in the elongating vessels (2.1X larger radius raised to the fourth power). Because the basal vessels were more numerous and formed a continuous layer of water-supplying tissue, the conductance for radial flow to the outlying cells also was much higher than in the elongating tissues, where vessels were few, individually scattered, and a small source for radial flow.

It may be relevant that poor vascularization and small, undifferentiated cells are characteristic of meristems. Often, the smallest undifferentiated cells are situated between the protoxylem and the outlying regions. Some of them differentiate into new vascular elements after elongation ceases. If they have a low diffusivity for water before differentiation, as in soybean stems, a barrier to water flow around the early xylem is likely to be present, and large, growth-induced ψ_w will be frequent. An important consequence is that growth may be influenced by changes in the water status of only these few cells and may not be detected from measurements of whole tissues. Increased attention may need to be directed toward gradients and transport properties of growing organs.

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