Plant Physiol. (1990) 94, 1601–1609
0032-0889/90/94/1601/09/$01.00/0

Received for publication November 20, 1989
Accepted May 3, 1990

Primary Events Regulating Stem Growth at Low Water Potentials[1]

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ABSTRACT

Cell enlargement is inhibited by inadequate water. As a first step toward understanding the mechanism, all the physical parameters affecting enlargement were monitored to identify those that changed first, particularly in coincidence with the inhibition. The osmotic potential, turgor, yield threshold turgor, growth-induced water potential, wall extensibility, and conductance to water were measured in the elongating region, and the water potential was measured in the xylem of stems of dark-grown soybean (*Glycine max* [L.] Merr.) seedlings. A stepdown in water potential was achieved around the roots by transplanting the seedlings to vermiculite of low water content, and each of the parameters was measured simultaneously in the same plants while intact or within a few minutes of being intact using a newly developed guillotine psychrometer. The gradient of decreasing water potential from the xylem to the enlarging cells (growth-induced water potential) was the first of the parameters to decrease to a growth-limiting level. The kinetics were the same as for the inhibition of growth. The decreased gradient was caused mostly by a decreased water potential of the xylem. This was followed after 5 to 10 hours by a similar decrease in cell wall extensibility and tissue conductance for water. Later, the growth-induced water potential recovered as a result of osmotic adjustment and a rise in the water potential of the xylem. Still later, moderate growth resumed at a rate apparently determined by the low wall extensibility and tissue conductance for water. The turgor did not change significantly during the experiment. These results indicate that the primary event during the growth inhibition was the change in the growth-induced water potential. Because the growth limitation subsequently shifted to the low wall extensibility and tissue conductance for water, the initial change in potential may have set in motion subsequent metabolic changes that altered the characteristics of the wall and cell membranes.

This work was undertaken to determine the primary events occurring when plants are subjected to growth-inhibiting water potentials (ψ_w[3]) as water is depleted in soil. Knowledge of these events is the first step toward finding the cause of the inhibition. Among the parameters that affect growth, some involve water directly because water occupies the increasing volume of the cells and extends the cell walls. Therefore, the most expeditious way to identify the primary events is to include in the analysis the parameters that determine how the walls extend and how the cells obtain water while water is being depleted around the roots. Many of the parameters affecting growth have already been explored. At low ψ_w, turgor often decreased (2, 9, 18, 36), osmotic potentials generally became lower (18, 25, 28, 30), wall extensibility appeared to decrease (19, 26), and the growth-induced water potential sometimes diminished (34, 42). However, the initial changes were not identified, and the primary events remain unknown.

Recently, a technique became available for measuring each of the component processes contributing to cell enlargement simultaneously (4, 5). In the present work, we used this technique to explore for the first time the sequence of events occurring at growth-inhibiting ψ_w when all the parameters affecting growth could be operating.

The technique, termed guillotine thermocouple psychrometry, depends on a mathematical description of the processes affecting cell enlargement based on the concepts of Lockhart (23) as modified by Boyer *et al.* (5). It was shown (5) that the guillotine psychrometer measures the volume-averaged water potential for intact and excised enlarging tissues. Therefore, for simplicity, each process is characterized as an average for the tissue as a whole. The equations consider turgor (ψ_p, MPa) to bring about steady enlargement (expressed as the relative growth G in s^{-1}) according to the extensibility of the wall (m, $s^{-1} \cdot MPa^{-1}$) as given by Green *et al.* (22):

$$G = m\,(\psi_p - Y) \quad (1)$$

where Y is the yield threshold turgor below which the force on the wall is too small to enlarge the wall irreversibly. Thus, $(\psi_p - Y)$ is the growth-active turgor.

Likewise, steady water uptake (expressed as the relative uptake g in s^{-1}) occurs because a difference in water potential is induced by growth between the water supply (ψ_o) and the interior of the cells (ψ_w) and favors water movement into the cells as given by Boyer *et al.* (5):

$$g = L\,(\psi_o - \psi_w) \quad (2)$$

where L is the hydraulic conductance of the flow path into the cells ($s^{-1} \cdot MPa^{-1}$) that, in shoots, extends from the xylem into the enlarging tissue. The $(\psi_o - \psi_w)$ is the growth-induced

[1] Supported by Department of Energy grant DE-FG02-87ER 13776 and a grant from E.I. duPont de Nemours and Company to J.S.B.

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[3] Abbreviations: ψ_w, water potential (MPa); ψ_p, turgor (MPa); ψ_s, osmotic potential (MPa); m, cell wall extensibility ($s^{-1} \cdot MPa^{-1}$); L, hydraulic conductance ($s^{-1} \cdot MPa^{-1}$); Y, yield threshold turgor (MPa); G, relative growth rate (s^{-1}); ψ_o, water potential of xylem solution (MPa); g, relative water uptake (s^{-1}); V_w, volumetric water content (m^3); V, total tissue volume (m^3); α, proportionality constant relating total volume to the water content of the tissue; $(\psi_p - Y)$, growth-active turgor (MPa); $(\psi_o - \psi_w)$, growth-induced water potential (MPa).

water potential, *i.e.* the difference in potential caused by the yielding of the cell walls during growth. The yielding prevents turgor from becoming as high as it otherwise would and thus ψ_w is lower than ψ_o.

In the original Lockhart (23) treatment, the low ψ_w of the cells was considered to result only from a low osmotic potential outside the cells. However, the apoplast in land plants is frequently under tension and we showed (33) that the low ψ_w of enlarging cells was transmitted to the apoplast mostly as a tension that moved water out of the xylem and into the cells. By expressing the driving force in terms of water potential ($\psi_o - \psi_w$) in Equation 2, we include any effects of negative pressures in the apoplast (5, 33).

The relative growth rate G (Eq. 1) expressed in terms of total volume $(dV/dt)(1/V)$ and the relative water uptake g (Eq. 2) expressed in terms of water volume $(dV_w/dt)(1/V_w)$ are numerically identical because the total volume is proportional to the water volume ($V_w = \alpha V$) and the proportionality constant α cancels in the relative expressions. Also, because the total potential affecting enlargement is $(\psi_o - \psi_w) + (\psi_p - Y)$, these terms in Equations 1 and 2 can be added to give the combined expression:

$$G = (mL/m + L)(\psi_o - \psi_s - Y) \quad (3)$$

where $\psi_s = \psi_w - \psi_p$. Equation 3 is the governing equation for the rate at which tissue enlargement occurs in the steady state. Each of the terms in Equations 1 to 3 were measured in the present work.

MATERIALS AND METHODS

Plant Material

Soybean (*Glycine max* [L.] Merr. cv Williams) seedlings were grown from seeds disinfected in a 1% solution of NaOCl for 5 min and rinsed with flowing water for 1 h. The seedlings initially were grown in vermiculite having adequate water (5.0 mL of 0.1 mM $CaCl_2$/g of vermiculite, *i.e.* $\psi_w = -0.01$ MPa) at 29 ± 0.5°C and 100% RH in darkness for 55 to 60 h. At that time, the seedlings were separated into 1) controls that were transplanted into identical vermiculite (1× treatment) and 2) seedlings that were transplanted to water-deficient vermiculite containing 1/8 of the water supplied to the controls (1/8× treatment). The 1/8× vermiculite had a $\psi_w = -0.28 \pm 0.02$ MPa measured with an isopiestic thermocouple psychrometer (6). The vermiculite and $CaCl_2$ solution were shaken together prior to transplanting to insure uniform mixing. The plants were grown at the same temperature and RH conditions used for germination. All seedling manipulation and experimentation were done under a green safelight in the growth conditions (green fluorescent bulb wrapped in green plastic sheet having maximum transmission at 525 nm and negligible transmission below 475 nm and above 575 nm).

Water Status

Measurements of the terms in Equations 1 to 3 were carried out in a guillotine thermocouple psychrometer (5) in which four whole plants and vermiculite could be sealed. Measurements of stem (hypocotyl) ψ_w were made in the zone of elongation sealed with petrolatum (Vaseline) into a small internal vapor chamber for the thermocouple (5). The apparatus allowed the plants to grow without restriction while ψ_w was measured. A thermocouple bearing a sucrose solution of known water potential was inserted into the vapor chamber to measure 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. In cases where the isopiestic condition was not possible to obtain, it was determined by extrapolating no more than 0.1 MPa. This prevented errors caused by the diffusive resistance of the tissue to water vapor and assured that the tissue neither hydrated nor dehydrated significantly during the course of the measurement (6).

After the ψ_w had been measured, the tissue inside the vapor chamber was excised by sliding a tube enclosing the chamber and having a sharpened edge (guillotine). No cut surfaces were exposed within the chamber, as the excision was made on the outside under the Vaseline. After the ψ_w of the excised tissue was determined, the ψ_s of the tissue was measured by rapidly removing the guillotined sample, placing the segments in a hypodermic syringe, freezing and thawing, and expressing the cell solution under pressure through filter paper in the syringe. The solution was placed on a thermocouple junction and its ψ_s was measured above sucrose solutions by the isopiestic technique (6). No correction was made for dilution of the protoplast solution by water in the apoplast because the volume of the apoplast was negligible (3.9% of cell volume [31]). The ψ_p was calculated from ψ_w in the intact tissue according to $\psi_w - \psi_s$. Because the tissue grew rapidly when intact in the psychrometer, this was the ψ_p present during growth. Likewise, because ψ_p decreased to Y after operating the guillotine (4, 5, 27), the Y was calculated from the ψ_w of the excised tissue according to $\psi_w - \psi_s$.

At the same time that the elongating tissue was removed for the measurements of ψ_s, the nonelongating basal tissue from the stem was sampled to measure the water potential of the xylem (ψ_o). No growth or transpiration occurred there and the water potentials of the xylem and surrounding tissues should have equilibrated (33). These water potentials should have been determined by the solute concentrations and root pressures affecting the xylem solution. Because the solute concentrations were small (33), the pressures were also small and excision did not alter the water potential (5, 27). Four basal hypocotyl segments about 1.5 cm long were obtained from the plants and placed on the bottom of a psychrometer chamber coated with melted and resolidified Vaseline. The water potential was measured by the isopiestic technique (6). All manipulations were carried out in a water-saturated chamber.

In order to test whether this measurement in the basal tissue gave the xylem ψ_o, the pressure in the xylem located in the elongating region was determined with a pressure chamber (37). A single seedling was first transplanted through the lid of the pressure chamber into a 200-mL beaker containing vermiculite and was allowed to grow for several hours. The seedling was transplanted from 1× vermiculite to 1/8× vermiculite by removing the 1× vermiculite and pouring 1/8×

vermiculite around the roots in a new beaker. At various times, ψ_o was measured in the elongating region by sealing the stem in the lid of the chamber, detopping the plant in the middle of the elongating region just above the chamber lid, and applying pressure to the root system. When solution from the xylem appeared on the cut surface, the applied pressure was considered equal and opposite to the pressure in the xylem of the elongating region. Because the solute contribution to ψ_o was small (about −0.04 MPa [33]), it was ignored. The temperature was 29 ± 0.5°C and the humidity inside and outside the pressure chamber was kept at saturation.

Growth

The rate of stem elongation was measured in the same seedlings used to measure the water potential by marking the stem bases with India ink before placing the seedlings in the guillotine psychrometer. The change in length was then noted at the end of the water potential determinations. The average rate was calculated from the four seedlings in the psychrometer. Stem elongation also was determined in seedlings growing in the vermiculite by measuring from a reference position with a ruler at various times.

For one experiment, we used a radial displacement transducer (Schaevitz, Pennsauken, NJ) to measure the length of the stems under growth conditions. The arm of the transducer was clamped to the upper part of the stem and a rigid bar was attached below the elongating region. The transducer and bar were mounted on a microscope body, the fine-adjustment of which could be used to calibrate the instrument without disturbing the tissue. The rate of elongation was determined from the change in stem length at least every 15 min.

RESULTS

Figure 1A shows that stem elongation was rapid in plants growing in control vermiculite (1× treatment) but inhibited in plants in drier vermiculite (1/8× treatment). In the guillotine psychrometer, stem elongation behaved similarly (Fig. 1B). The 1/8× seedlings always showed a pronounced inhibition lasting about 40 h followed by a modest recovery.

The water potential in the xylem (ψ_o) changed in response to the dry vermiculite. Before transplanting, ψ_o was high (−0.01 MPa) and in equilibrium with the water potential of the vermiculite (Fig. 2). After transplanting, ψ_o decreased to water potentials below those in the vermiculite but eventually came once again into equilibrium with the vermiculite at a lower potential (−0.3 MPa, after about 30–45 h). The decrease in ψ_o occurred because of a transient dehydration of the mature tissue as water was withdrawn to sustain the growth of the stem after transplanting (R. Matyssek, A.-C. Tang, J. S. Boyer, unpublished data). Regardless of whether ψ_o was measured in the mature tissue psychrometrically or in the elongating tissue by applying pressure to the roots, the results were the same except for the period between 25 and 40 h when measurements differed probably because of slight kinetic differences between experiments (Fig. 2). Thus, the xylem was nearly in equilibrium with the mature tissue, the xylem had virtually the same potential in the elongating

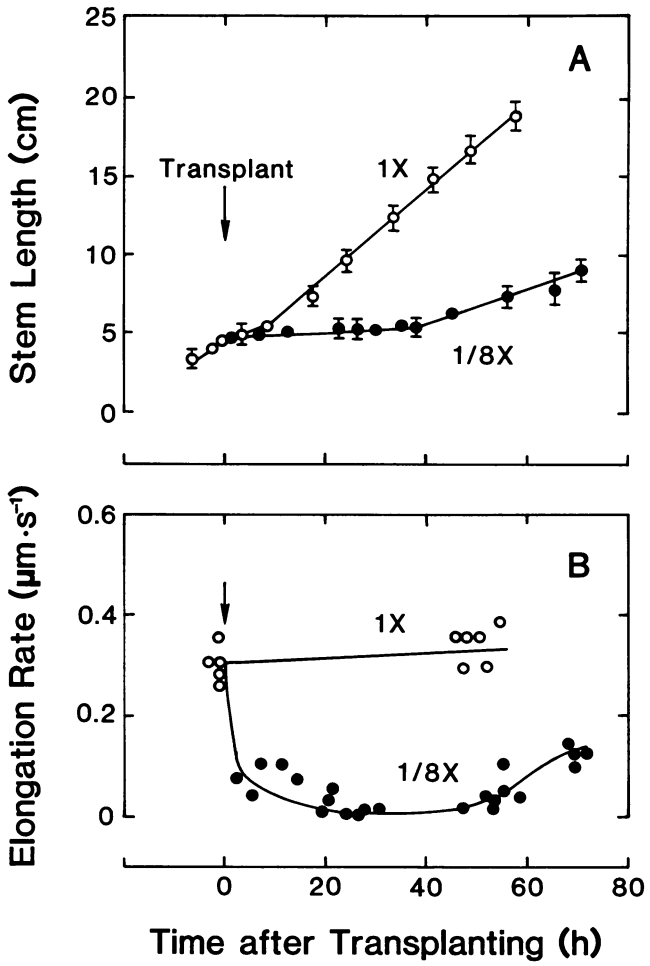

Figure 1. Stem length of soybean plants growing under culture conditions (A), and elongation rate of soybean stems in intact plants growing in the guillotine psychrometer (B) in 1× or 1/8× vermiculite. Data in (A) represent means ± 1 SD.

tissue, and ψ_o could be measured by either technique. In all subsequent measurements, ψ_o was measured with the psychrometer.

In contrast, the water potential of the elongating stem tissue (ψ_w) was not in equilibrium with the water potential in the xylem or vermiculite. This can be seen in Figure 3A showing that intact plants grew rapidly (0.36 $\mu m \cdot s^{-1}$) with their roots in 1× vermiculite in the apparatus but had a ψ_w in the elongating region that was 0.18 MPa lower than the ψ_o of the xylem (*cf.* Fig. 2). This indicates that there was a gradient in potential decreasing radially from the xylem into the elongating tissue. This gradient, induced by cell wall yielding as the cells enlarged (2, 33), was likely to be the one moving water into the enlarging tissue. The ψ_w was measured first by determining the temperature of the vapor chamber with a dry thermocouple, then placing a sucrose solution on the thermocouple to give an output as close as possible to the dry reading (Fig. 3A). The matching outputs indicated that no vapor was exchanged between the thermocouple and the tissue, and thus the vapor pressure of the solution was iso-

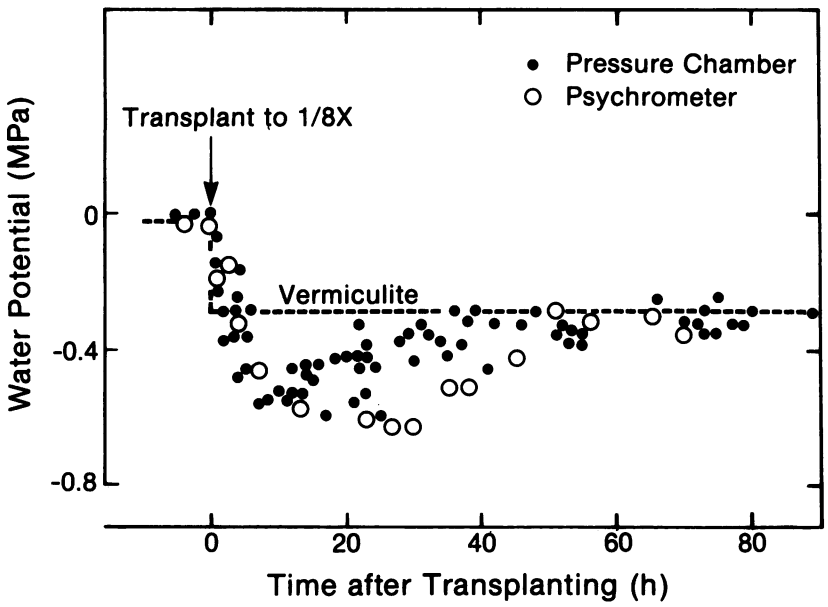

Figure 2. Xylem water potential (ψ_o) measured with a pressure chamber and isopiestic psychrometer before and after transplanting to 1/8× vermiculite. The pressure chamber was used to pressurize the roots sufficiently to maintain the xylem solution at the cut surface in the elongating region after detopping the seedlings. The psychrometer was used to measure the water potential of the mature stem tissue. The vermiculite water potential (dashed line) was measured with the isopiestic psychrometer.

piestic (the same as that of the tissue). Because the ψ_w of the solution was known, the ψ_w of the tissue was known.

When tissue in the zone of elongation was excised with the guillotine, the ψ_w decreased 0.06 MPa ($\Delta\psi_{relax}$, shown as the increase in output upon excision, Fig. 3A). The decrease occurred only in rapidly enlarging tissue (5, 27) and was caused by a decrease in ψ_p as the wall yielded and relaxed without a water supply. Because growth became zero in about 1 min (5, 11), the ψ_p had decreased to Y. Therefore, $\Delta\psi_{relax}$ represented the growth-active turgor ($\psi_p - Y$) and could be measured very precisely. In this experiment (Fig. 3A), $\Delta\psi_{relax}$ = ($\psi_p - Y$) was 0.06 MPa. Calculating the turgor from $\psi_w - \psi_s$ also gave 0.06 MPa (0.45 MPa before excision minus 0.39 MPa after excision) thus confirming $\Delta\psi_{relax}$.

The same measurements in seedlings transplanted to 1/8× vermiculite for 24 h showed that growth had decreased to 0.02 $\mu m \cdot s^{-1}$ (Fig. 3B) because of the low water content (1/8×) of the vermiculite around the roots in the apparatus. The ψ_w in the stem elongating region was low (−0.68 MPa, Fig. 3B) and the ψ_s also was low. After operating the guillotine, the ψ_w decreased only slightly ($\Delta\psi_{relax} = \psi_p - Y = 0.02$ MPa, Fig. 3B). Although ψ_p (0.30 MPa in Fig. 3B) was lower than

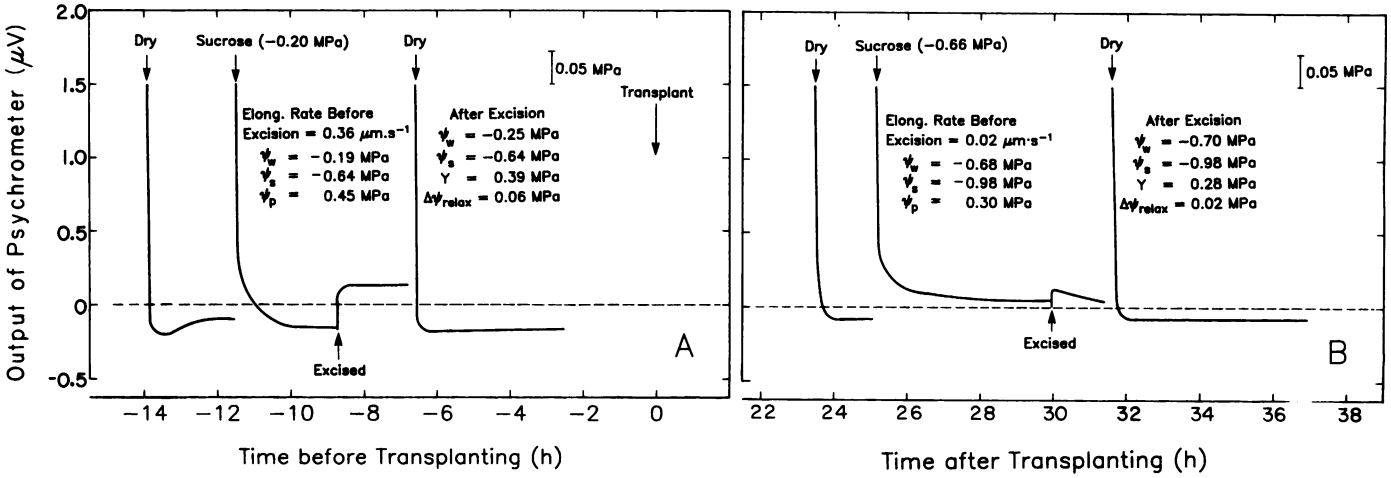

Figure 3. Stem elongation rate, water potential (ψ_w), osmotic potential (ψ_s), turgor (ψ_p), yield threshold turgor (Y), and growth-active turgor ($\Delta\psi_{relax} = \psi_p - Y$) measured simultaneously in the guillotine psychrometer in the elongating region of stems of soybean seedlings. The tracing is from a strip chart recorder indicating the output of a thermocouple that was monitoring ψ_w. The seedlings were initially intact and the guillotine was operated at 'excised,' which removed the elongating tissue from the rest of the plant. The ψ_w was monitored in the elongating tissue continuously during the excision. The ψ_w is the potential of the sucrose solution that gave a thermocouple output matching that of the thermocouple when dry. $\Delta\psi_{relax}$ is shown by the rise in output of the thermocouple after excision. The ψ_s was measured in the elongating tissue immediately after the ψ_w measurements were completed. Turgors ψ_p and Y were calculated from ψ_s and ψ_w before and after excision. (A) 1× vermiculite prior to transplanting; (B) transplanted for 24 h to 1/8× vermiculite.

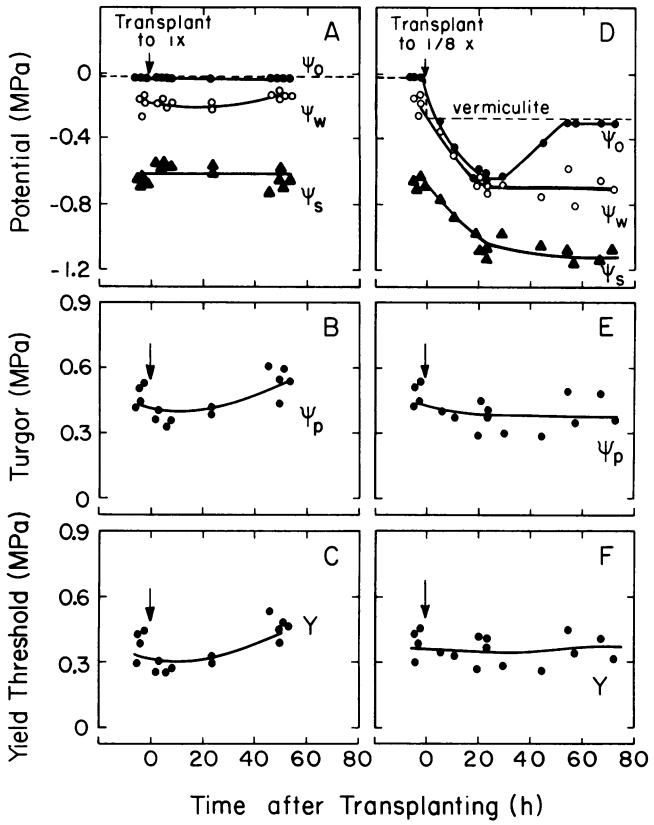

Figure 4. Kinetics of changes in ψ_w, ψ_s, ψ_p, and Y measured at various times in the guillotine psychrometer in stems of soybean seedlings transplanted to 1× (A, B, and C) or 1/8× vermiculite (D, E, and F). Also shown is the water potential of the xylem (ψ_o) measured in the mature stem from the same seedlings. Vermiculite water potential is shown by the dashed line. Symbols are defined in Figures 2 and 3.

before transplanting (0.45 MPa in Fig. 3A), subsequent measurements (Fig. 4) indicated that this difference was caused by random variation between plants and not a generally decreased ψ_p.

These data show many of the changes in the elongating stem tissue after transplanting. To investigate the detailed behavior of the plants over an 80 h growth period, we operated the guillotine at various times before and after transplanting. Figure 4, A–C, shows that the ψ_o, ψ_w, ψ_s, ψ_p, and Y changed little in the 1× controls during the experiment. Y was always close to ψ_p and therefore the growth-active turgor ($\psi_p - Y$) was small. In the 1/8× plants, ψ_o decreased and later increased (Fig. 4D) as was observed earlier (Fig. 2). The ψ_w decreased but stayed low. As a result, the growth-induced water potential gradient ($\psi_o - \psi_w$) virtually disappeared by about 2 h, but began to reappear at about 30 h (Fig. 4D). The ψ_s gradually decreased as solutes accumulated unused (29) in the elongating tissue, but ψ_p and Y (Fig. 4, E and F) did not differ from the controls, which showed little detectable change. The lack of change in ψ_p confirms our recent report of constant ψ_p in most of the cells of the enlarging region after a similar treatment (34).

Figure 5A shows the relative growth rate G obtained in the intact plants in the psychrometer while ψ_w was determined. G was rapid initially but decreased to about 25% of the control level in the first 5 h (Fig. 5A). By 24 h, G had decreased to zero. Although ($\psi_p - Y$) changed early, the change was much less than in G (Fig. 5B). Measured as $\Delta\psi_{relax}$ (which gave a more accurate indication than could be obtained by measuring ψ_p and Y separately), ($\psi_p - Y$) decreased only to 60% of the initial level by 5 to 10 h after transplanting (Fig. 5B). By comparison, the (ψ_o - ψ_w) decreased rapidly and to a much larger extent (Fig. 5C), becoming almost zero within 5 to 10 h. It began to recover at about 30 h. This was followed by a recovery of ($\psi_p - Y$) that began at about 50 h (Fig. 5B), and growth started to recover after about 55 h (Fig. 5A).

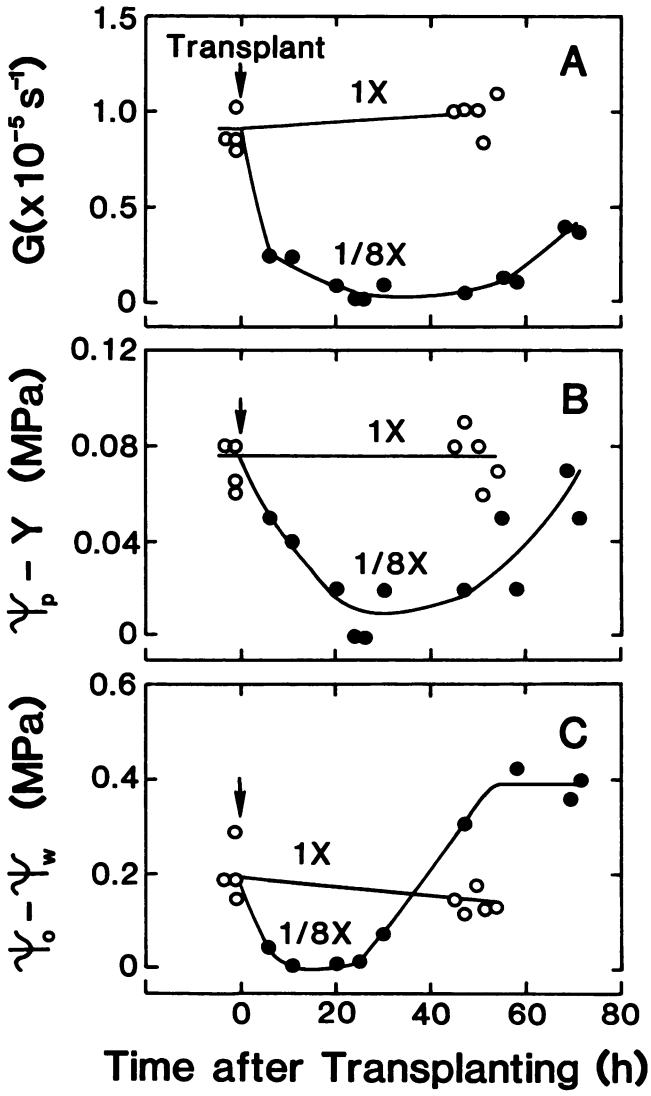

Figure 5. Kinetics of changes in (A) relative growth rate (G), (B) growth-active turgor ($\Delta\psi_{relax} = \psi_p - Y$), and (C) growth-induced water potential ($\psi_o - \psi_w$) at various times in stems of the same soybean seedlings in the experiment shown in Figure 4. Symbols are defined in Figures 2 and 3.

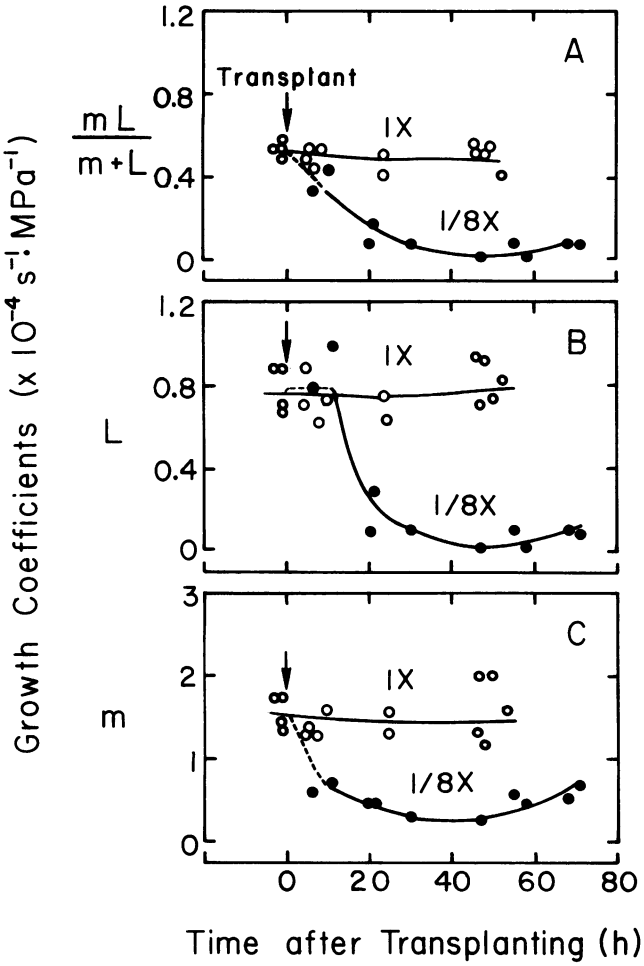

Figure 6. Kinetics of changes in (A) growth coefficient $mL/(m + L)$, (B) tissue conductance to water L, and (C) cell wall extensibility m at various times in stems of the same soybean seedlings in the experiment shown in Figure 4.

The growth coefficient $mL/(m + L)$ and the wall extensibility m and tissue conductance to water L were determined from the data of Figure 5 according to Equations 1 and 2 and showed that, prior to transplanting, L was smaller than m (Fig. 6). This indicates that the growth rate was slightly more limited by the conductance to water than by the extensibility of the walls when water was readily available. After transplanting, each coefficient decreased gradually (Fig. 6, A-C). The decrease in L was apparent after 10 h and in m after 5 h, but L decreased more than m. L and m reached their lowest levels at about 40 h. A modest recovery eventually was observed in both parameters but was delayed until the growth parameters ($\psi_o - \psi_w$) and (ψ_p - Y) had recovered (*cf.* Figs. 5, B and C, and 6, B and C). The partial recovery of L, m, and $mL/(m + L)$ coincided with the partial recovery of G.

These results indicated that the earliest events in the growth inhibition probably took place in the first 5 h and involved ($\psi_o - \psi_w$). We increased the resolution of our measurements during this time by monitoring ψ_w continuously in intact seedlings having their roots outside the guillotine psychrometer. The seedlings were transplanted by replacing the 1× vermiculite with 1/8× vermiculite, and G, ψ_o, and ψ_s were measured frequently in other seedlings treated identically at the same time. Figure 7A shows that G measured with a displacement transducer was markedly inhibited at 2 h. The ψ_o decreased immediately upon transplanting, paralleling the decrease in G (Fig. 7B). The ψ_w was virtually unchanged (Fig. 7C). Therefore, ($\psi_o - \psi_w$) decreased simultaneously with the changes in G. Because ψ_w was unchanged during this time, ψ_p also was constant. This was confirmed by calculating ψ_p from $\psi_w - \psi_s$ (Fig. 7D), which showed no change after transplanting. The experiment required seedlings that were continuously intact for the measurement of ψ_w and it was not possible to evaluate m or Y.

DISCUSSION

There are many ways that cell enlargement might become inhibited at low ψ_w. To sort out which ones regulate the

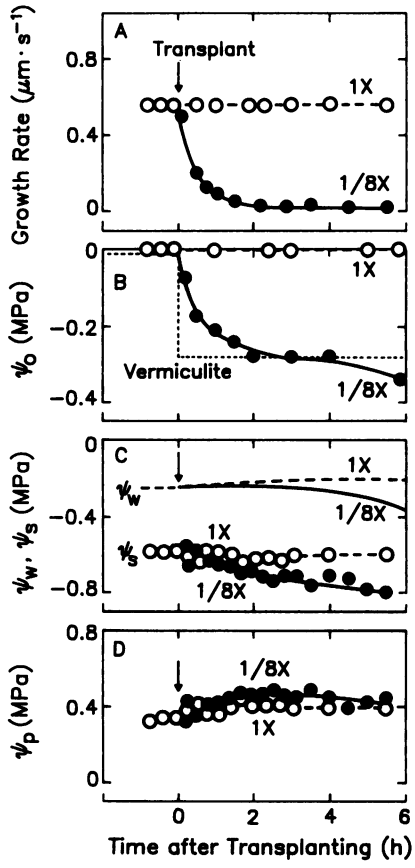

Figure 7. Detailed kinetics during first 5 h after transplanting for changes in (A) growth, (B) ψ_o, (C) ψ_w and ψ_s, and (D) ψ_p in the elongating region of stems of soybean seedlings after transplanting to 1× or 1/8× vermiculite. Vermiculite water potential is shown as a dashed line. The ψ_w are shown as a continuous recorder trace for intact seedlings. The ψ_s and ψ_o were measured in excised tissue from other seedlings grown in parallel at the same time. The ψ_p were calculated from $\psi_w - \psi_s$. Growth was measured in intact plants grown at the same time under identical conditions. Symbols are defined in Figures 2 and 3.

process, the first step is to identify those parameters undergoing change. Those changing earliest and by the largest amounts are the most likely candidates, and to be considered regulatory the kinetics must coincide with the kinetics of the inhibition. To establish causation, the second step is to vary the putative regulatory parameters to ascertain whether the inhibition varies in response. The present study attempts the first part of this process. We used the guillotine psychrometer to identify the sequence of events because it is the only technique so far that allows virtually all of the parameters affecting growth to be measured. Each parameter could be evaluated rapidly in the intact plant or within a few minutes of being intact.

The results show that most of the growth parameters underwent early changes but only the gradient in water potential extending from the xylem into the enlarging tissue ($\psi_o - \psi_w$) declined to a low enough value to be growth limiting. Growth and ($\psi_o - \psi_w$) decreased together, approaching zero at the same time. Thus, the change was not only a primary event but also a candidate for the initial cause of the inhibition. It should be noted that the gradient ($\psi_o - \psi_w$) was determined by the high potential of the xylem (ψ_o) and the low potential of the surrounding tissues (ψ_w). Thus, ψ_o could decrease to ψ_w without altering ψ_w. The consequence was a decreased water movement into the surrounding cells and a decreased growth. The decrease in ($\psi_o - \psi_w$) imposed by the decrease in ψ_o in effect starved the cells for water.

Because ψ_w remained constant while growth was changing, measuring ψ_w alone gave no indication of the effect. Only when the difference between ψ_o and ψ_w was known could a rationale for the inhibition be seen. Therefore, the change in ψ_o represented a hydraulic signal from the roots indicating that the vermiculite water potential had changed, a conclusion we also reached after measuring turgor near the xylem (34). Changes in ψ_w began only when growth had been inhibited for more than 2 h.

Figure 8 shows a comparison between each parameter and the rate of growth. The coincidence between changes in G and ($\psi_o - \psi_w$) is apparent. Eventually, ($\psi_o - \psi_w$) recovered as the result of a recovery of ψ_o and osmotic adjustment (28), ($\psi_p - Y$) recovered, and a moderate growth recovery ensued. However, the incomplete recovery of growth indicates that other parameters became limiting once any limitation by ($\psi_o - \psi_w$) and ($\psi_p - Y$) had been relieved. The only other parameters remaining low were the wall extensibility m and tissue conductance L. Thus, m and L are likely candidates for the growth-limitation late in the experiment.

Both m and L are under metabolic control, and their low levels indicate that substantial metabolic change had occurred in the enlarging cells. Wall extensibility probably is determined by the action of wall enzymes on the polymerization of wall constituents and the cross-linking of structural proteins with wall constituents (10, 20, 39). In seedlings similar to those used here, polyribosome and mRNA levels decreased a few hours after transplanting (24). Increased amounts of proteins were extractable from the walls (7) and a 28 kD protein present in the cytoplasm accumulated in the walls (7). Hormone changes could have triggered some of these changes. Abscisic acid began to increase within 0.5 h after transplanting

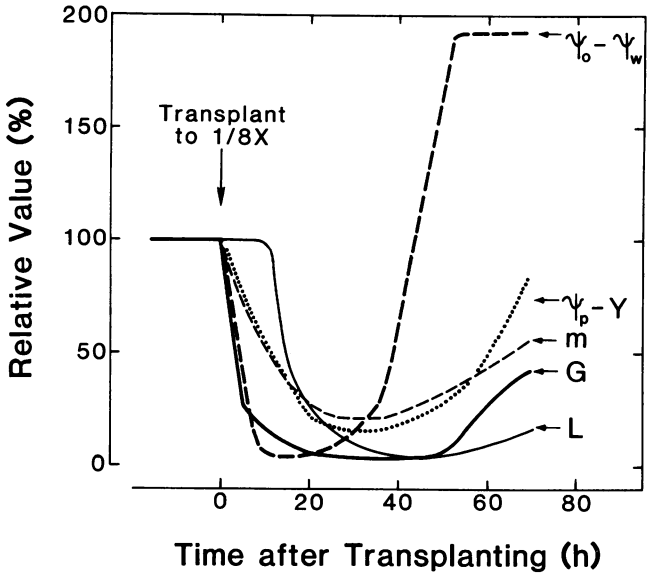

Figure 8. Relative values of the stem elongation rate (G), growth-induced water potential ($\psi_o - \psi_w$), growth-active turgor ($\Delta\psi_{relax} = \psi_p - Y$), wall extensibility ($m$), and tissue conductance for water (L) at various times in stems of intact soybean seedlings transplanted to 1/8× vermiculite, compared with the control (100%, before transplanting). Based on data from Figures 4 to 6.

(1). Bray (8) showed that high abscisic acid can enhance the abundance of mRNAs similar to those increased by dehydration in wilted tomato leaves although in elongating soybean stems under our conditions, the mRNAs enhanced by abscisic acid were not the same as those at low ψ_w (17). Although protons are secreted into walls (12, 13) and are secreted more slowly at low ψ_w (40), recent evidence indicates that wall acidification may have less effect on wall extensibility (38) than once was thought (12, 13). The response of m and L to these changes is unknown but could be a manifestation of some or all of the early biochemical changes inside the elongating cells and walls.

The time lag between the inhibition of growth and the change in character of the cell walls and membranes is important. Under natural conditions, low ψ_w often occur diurnally but only last a few hours. These times at low ψ_w would be too short to bring about most of the wall and membrane changes, and growth could resume. On the other hand, low ψ_w for 24 h or more would be of long enough duration for walls and membranes to be altered. This may explain previous observations of completely reversible oscillations in leaf growth that occurred diurnally (2, 21) but nonreversible inhibition of growth when plants were rewatered after longer times (3, 26).

The seedling system used in the present work is advantageous for studying the sequence of events affecting growth because the growing region of the stem is exposed, allowing ψ_w to be measured in the same plants used to measure ψ_o and the other growth parameters. Because transpiration was virtually absent, ψ_o represented the water potential in the xylem and it was possible to compare changes in ψ_o and ψ_w as water

was withheld from the plants. If transpiration had occurred, ψ_o and ψ_w would have been affected by the additional water flow through the plant. Westgate and Boyer (41) studied this situation in rapidly growing maize at high ψ_w and observed that, given enough time, $(\psi_o - \psi_w)$ during rapid transpiration became about the same as when transpiration was slow.

The vermiculite transplant method provided a way to rapidly change the water status of the root medium without resorting to osmotica that can cause side effects. Although vermiculite is a soil parent material and should adequately simulate conditions in dehydrating soils, the transplanting necessary to achieve a rapid step down in water content required breaking the continuity between the root surfaces and the surrounding water. For a time, the seedling was forced to utilize internal water stores to sustain stem and root growth (R. Matyssek, A.-C. Tang, J. S. Boyer, unpublished data). The mature stem tissues were an important source of water during this time, and the rapid decrease in ψ_o to water potentials below those in the vermiculite was attributable to dehydration of the mature stem tissues. Upon recontact with water in the 1/8× vermiculite, ψ_o recovered to equilibrate with the vermiculite water potential. Under natural conditions, soil water depletion is a more gradual process and the reliance on internal water stores is less apparent.

The psychrometer method allowed all the measurements to be accomplished in the same plants, minimizing interplant variation. This proved especially important for the growth-active turgor $(\psi_p - Y)$ which was always less than 0.1 MPa. This difference was so small that changes in $(\psi_p - Y)$ could not be detected when each parameter was observed separately. Changes in $(\psi_p - Y)$ could be detected only from continuous traces of $\Delta\psi_{relax}$ after excision. Because of this, the data do not indicate whether the changes were attributable to ψ_p or Y. However, in a similar experiment, ψ_p measured continuously at high resolution (34) showed no change in the enlarging cells except for a few close to the xylem during transplanting. Moreover, ψ_w measured continuously during the first 2 h showed no change (Fig. 7C), confirming that ψ_p was stable in most of the cells. Thus, it is likely that changes in $(\psi_p - Y)$ were caused mostly by increases in Y.

It is important in this respect that ψ_p and Y had values similar to those measured in intact plants with a pressure probe (27, 32, 34). Moreover, $(\psi_p - Y)$ in the intact 1× plants were virtually the same as measured earlier in 1× plants (27). Matyssek *et al.* (27) showed that the $(\psi_p - Y)$ were smaller than reported by Cosgrove and co-workers (14–16) because the Cosgrove measurements were affected by mature or slowly growing tissue that delayed wall relaxation for several hours. By then, Y had decreased below its level in the intact plant (5, 22), and the measurements (14–16) had become unrepresentative.

In our experiments, this problem was avoided by determining Y rapidly. Green *et al.* (22) demonstrated that Y can change in times as short as 20 to 30 min in *Nitella*. In soybean, Y changed in 1 to 2 h (5). By completing our determinations in 5 to 10 min, Y should not have changed and should have reflected the condition in the cell walls in the intact tissues.

Apart from these considerations, the only other parameter to undergo change was ψ_s, which decreased after transplanting as the plants adjusted osmotically to the drier vermiculite (28). The adjustment was caused by solute that accumulated unused after growth was inhibited (29). The resulting adjustment was sufficient to fully maintain ψ_p and cell water content (7) in the elongating tissue. Others observed a similar osmotic adjustment in leaves (18, 25, 26, 30). Matsuda and Riazi (25) and Michelena and Boyer (30) observed continued growth inhibition despite osmotic adjustment and concluded that there was an unexplained remaining inhibition. The present results suggest that the remaining inhibition could have resulted from a decreased $(\psi_o - \psi_w)$, $(\psi_p - Y)$, or low m and/or L.

We conclude that the kinetics of the initial change in the growth-induced water potential $(\psi_o - \psi_w)$ are consistent with but do not prove that growth was limited by this event. Taken together the results suggest that the inhibition of cell elongation could have been caused first by a physical parameter $(\psi_o - \psi_w)$ followed several hours later by metabolically controlled parameters (m and L) that probably became limiting as the physical parameter recovered. Because early changes in m and L were apparent but became large only about 5 to 10 h later, the metabolic changes leading to altered cell walls and membranes required some time to be set in place. In a companion study (35), wall extensibility and hydraulic conductance are measured by independent methods to test the validity of these conclusions.

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